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Study on Metacercarial Infection in *Clarias lazera* and Their Public Health Importance in Assiut City, Egypt

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Abstract

A study of helminth parasites of *Clarias lazera* in Assiut city, Egypt was carried out. A total of 50 *Clarias lazera* were collected live from Nile River at Assiut city, Egypt in the period between April 2014 to April 2015. Adult flukes were obtained from Albino rats experimentally infected with the metacercariae at day 7 post-infection. Overall prevalence of infection was 92%.The highest incidence percentage of encysted metacercariae in examined *Clarias lazera* fishes was detected in the posterior region (93.47%), moderate distribution was detected in the middle part with a percentage of 71.73% while the lowest distribution was recorded in the anterior part (43.7%); these data were found statistically significant (p level<0.01). The prevalence of encysted metacercariae (EMC) was higher during Summer (81.8%), followed by Spring (78.1%), Autumn (60%) and Winter (53.4%). In Conclusion, 92% of examined *C. lazera* fishes were infested with EMC.

Keywords

Freshwater Fishes, *Clarias lazera,* Encysted metacercariae (EMC), Egypt

1. Introduction

Fish is important as a source of protein with low cholesterol level in the diets of the human and economically as a source of subsistence income [1]. Parasitic diseases are considered to be serious problem rather than bacterial diseases in warm water fishes [2]. From the public health point of view, the consumption of fishes infected with the larval stages of some parasites caught from local water, constitutes a human hazard. Fresh water fishes are considered as one of the important sources of parasitic infection to man and fish-eating mammals particularly after the increased pollution of rivers and lakes in Egypt [3]. 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 [4]. 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 [5]. Therefore, the current study investigated the prevalence and distribution of EMC in different parts of C. Lazera and effect of seasonal variations on metacercarial infection.

2. Material and Methods

2.1 Collection of fish

A total of 50 Clarias lazera fishes were collected from April 2014 to April 2015. The fishes were caught from different localities of the River Nile at Assiut city. The fishes were brought in plastic bags to the laboratory where they were kept on the refrigerator (10oC). They were examined immediately.

2.2 Examination of the fish

a) Examination of the fish for the presence of different larval helminth parasites:

The fishes were examined first by the naked eye to find out any macroscopic metacercariae. Then the examination was carried out by taking small snips of the muscles from head and trunk (mainly near the dorsal fins) and tail regions. Each snip was compressed between two slides and examined under binocular dissecting microscope looking for metacercariae.

b) Making permanent slides of metacercaria:

Fixation, staining, dehydration, cleaning, and mounting.

2.3 Experimental infection of laboratory animal

In a trial to get the adults of the obtained metacercaria, viable cysts were fed singly to parasites-free laboratory animals namely rats 6-8 weeks old.

2.4 Statistical Analysis

All experiments were carried out in triplicates. Data obtained were subjected to analysis of variance (ANOVA). Differences among means were determined using Duncan's multiple range test. Using SPSS 16.0 statistical software (SPSS, 2001).

3. Discussion

3.1 Prevalance of encysted metacercariae in tissues of *Clarias lazera*:

In the present study, the metacercarial infestation rate in the muscles of *Clarias lazera* was 92% (46 out of 50) as shown in Table 1.

Lower results were recorded by many investigators. El-Mossalami and Sherif recorded 73% rate of EMC infestation in *C. lazera* caught from Maryot Lake [6] , while, Derwa et al. observed that the rate of infestation with different species of encysted metacercariae in *Clarias lazera* in Ismailia Governorate was 80% [7].

Table 1. The incidence of encysted metacercariae in *Clarias lazera*

Fish species	No. of examined fish	No. of infected fish	Incidence % of infestation
Clarias lazera	50	46	92%

3.2 Distribution of encysted metacercariae in different parts in tissues of *Clarias lazera:*

During the present study, it was found that the highest incidence percentage of encysted metacercariae in examined *Clarias lazera* fishes was detected in the posterior region (93.47%), moderate distribution was detected in the middle part with a percentage of 71.73% while the lowest distribution was recorded in the anterior part (43.7%); these data were found statistically significant (p level<0.01) as shown in Table 2.

Similar pattern of metacercarial distribution was obtained from Assiut city by Riad [8], although his percentages in different fish parts were slightly lower than the present data as he recorded the highest prevalence and distribution of microscopic encysted metacercariae in the posterior region of *C. lazera* (89.5%), while moderate incidence was found in middle region (59.5%) and the lowest incidence was recorded in the anterior region (38.5%). Also, El-Shahawy et al. showed that the highest distribution of EMC was in the tail region (100%) [9], followed by trunk region (93.8%), and finally head region.

With respect to the data recorded from Cairo by El-Sherbiny [10], he observed that the higher rate of EMCs

infestation found in the middle third of *Clarias lazera* (80%), while the lower infestation rate was observed in the anterior third (74%). Saad et al. recorded that the highest rate was in trunk (27.5%) [11], then head & tail (25.8% &25%). Also, Abdallah et al. showed that the highest distribution of metacercarial infection among *Cl. gariepinus* was in the middle third muscles followed by the posterior third and lastly anterior third [12]. While, Kirrella et al. reported that the highest infection rate was in dorsal region (100%) and trunk (100%) followed by tail region (83%) [13].

Table 2. Prevalence and distribution of average number of encysted metacercariae in different parts of infected *Clarias lazera* (n= 46)

Different tissue parts	Head region (Anterior Part)	Trunk region (Middle Part)	Tail region (posterior Part)	P. value
No. of encysted metacercariae	20	33	43	<0.001**
Incidence %	43.47%	71.73%	93.47%	

** Significant difference (p level <0.01)

3.3 Seasonal variation & prevalence of encysted metacercariae in tissues of *Clarias lazera*:

The prevalence of microscopic encysted metacercariae in tissues of *Clarias lazera* during Summer, Spring, Autumn and Winter seasons were in descending order 94.4%, 91.6%, 90.9% and 88.8%, respectively, with non-significant difference (p level >0.05) as shown in Table 3.

Table 3. Seasonal variations & prevalence of encysted metacercariae in tissues of *Clarias lazera*

	Clarias Lazera		
	No. of fish examined	**No. of fish infected**	**% of infestation**
Summer	18	17	94.4%
Autumn	11	10	90.9%
Winter	9	8	88.8%
Spring	12	11	91.6%
P. value		0.962^{ns}	

** Significant difference (p level <0.01)

The same results were reported by Saleh et al. who recorded that the highest infection rate of zoonotic trematodes in fresh water fishes in Port Said Province was in Summer (95.9%) followed by Spring (84.4%) then Autumn (80.6%) and the lowest infection rate was in Winter (94.2%) [14]. Also, Elsheikha and Elshazly recorded that peak prevalence of metacercaria infestation was during the summer season 38.2% [15] , followed by spring 26.6%, and autumn 19.3% seasons, whereas the lowest prevalence was recorded in the winter 8.7%.

On the other hand, the present findings disagree with observations obtained by other researchers. El-Shahawy et al. recorded that the highest incidence of encysted metacercariae in *C. lazera* in Southern Egypt was in the Winter (55.6%) [9] , followed by summer (44.4%), then spring (33.9%), whereas the lowest was in autumn (33.3%). In addition, Derwa et al. observed that the highest incidence rate of EMC in *C. lazera* in Ismailia Governorate was in winter (95%) [7], followed by spring (87.5%), then summer (75%) and the lowest was in the autumn (62.5%).

3.4 Identification of adult worms in the intestine of experimentally infected laboratory albino rats:

In the present study the encysted metacercariae which were found in the muscles of *Clarias lazera* were fed singly to parasite free laboratory rats. Sacrefication of rats seven days post infection revealed the adult flukes in their intestine. These digenic trematodes were fixed, stained and mounted in Canada balsam. Microscopical morphological identification of these adults revealed the adult worms of *Prohemistomum vivax*, *Mesostephanus sp.* and *Cynodiplostomum azimi* (Fig. 1&2).

Although most of the encountered metacercariae could be referred to its trematode genus, it was impossible to get the species of these parasites without developing adults experimentally in albino rats [16, 10].

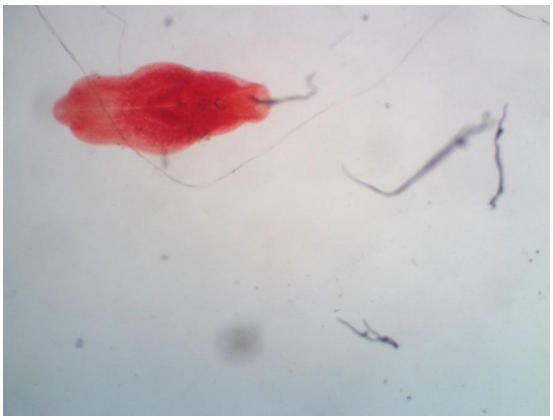
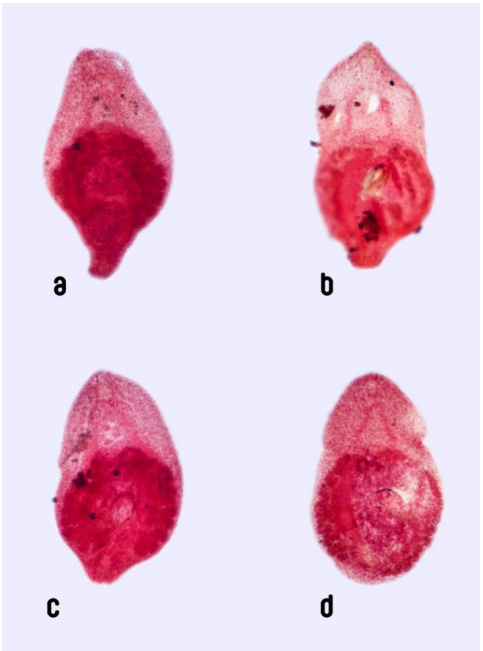


Fig. 1. Adult worms of *Cynodiplostomum azimi* recovered from small intestines of experimentally infested rat with microscopic encysted metacercaria form the muscles of *Clarias lazera.*



(d: Adult worm of *Mesostephanus* sp. recovered from small intestine of experimentally infested rat with microscopic encysted metacercariae from the muscles of *Clarias lazera.*)

Fig. 2. a & b & c: Adult worms of *Prohemistomum vivax* recovered from small intestines of experimentally infested rat with microscopic encysted metacercaria form the muscles of *Clarias lazera.*

4. Conclusion and Recommendations

Metacercariae of different trematode parasites are still abundant in the muscles of *Clarias lazera* fishes in Assiut while they were not infested by metacestodes and larval nematodes.

EMC and adults of *Prohemistomum vivax*, *Mesostephanus sp.*, *Cynodiplosomum azimi* were identified, some of which may have severe symptoms in heavily infected humans.

In spite of the absence of *Capillaria philippinensis* larvae in the fishes examined during the present study, there were many records of human infection with that highly human pathological parasite, hence, the present worker recommend future studies on different fish species in Egypt to confirm this finding as these larval stages are so

small in size and may not be easily detected through ordinary microscopy and it is advised to undergo these studies by using molecular identification techniques for detection of *Capillaria philippinensis* coproantigen in fish feces.

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