Studies On Some Parasites In Relation To Public Health importance In Market Fish With Trial To Its Control

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ABSTRACT

A total of 500 fish (250 Tilapia sp. and 250 *Clarias lazera* were collected from fish markets at Zagazige city in Sharkia governorate. The encysted metacercariae were detected in Tilapia sp. (84.8%) and in *Clarias lazera* (86.8%) of the examined fish. The adult worm were collected after experimental infection of puppies with encysted metacercariae as *Heterophyes heterophyes, Heterophyes aequalis, Prohemistomum vivax, Mesostephanus appendiculatus* and *Mesostephanus flapi*. The control of these metacercariae were attempted by freezing at-18◦C for 48 hour, grilling for 10 minutes and frying for 5 minutes. The aim of the present investigation is to study the public health importance of encysted metacercariae in *Tilapia sp.* and *Clarias lazera* by experimental infection of puppies and identification of recovered adult. Moreover, the effect of some treatment rendering the infected fish safe for consumption.

INTRODUCTION

Fish serve as a primary source of proteins allover the world especially to overcome the dietary need of the expanding population but fish may harbor many pathogens especially parasites, which could interfere with the aqua culture industry and constitute a zoonotic threat, especially in developing countries *(1,2)*. Different species of fresh water fish are known to serve as second intermediate hosts of wide variety of parasites in Egypt *(3,4)*. Human infections with several fish-borne trematodes were reported *(5)*. Symptoms of human infections are usually mild; heterophyiasis can be accompanied with abdominal pain and diarrhoea in the infected humans, and their egg, may produce easinophilic granuloma in the heart, brain and spinal cord in humans and animals after being transferred by the blood stream *(6-8)*. In Egypt, heterophyids, agents of human heterphyiasis, are transmitted in fresh, saline inland, and marine waters, where the mollusc and fish intermediate hosts are abundant *(9-11)*.

MATERIAL AND METHODS

I Fish samples

Five hundred fish samples represented 250 from each of Tilapia sp. and *Clarias lazera* fish were collected from fish markets at of Zagazige city in Sharkia governorate. The collected fish samples were identified and transferred in an ice-box to lab., and were examined for parasites of public health importance.

II Examination of fish for detection of the encysted metacercariae

A. Macroscopic examination: Carried out either by naked eye or by the magnifying hand lens (2x and 4x) detect macroscopic encysted metacercariae *(12)*.

B. Microscopic examination : Fish sample were examined microscopically after compression a very small pieces taken from different parts of each fish including the head, dorsal and tail region, visceral organs, fins and gills. Each piece was compressed between two microscopic glass slides and examined for presence of encysted metacercariae *(13)*.

C. Tissue digestion technique: Using digestive fluid (5gm pepsin,7ml conc HCL and 1000ml distal water) at rates of 1part tissue to 20 parts fluid in an flask and incubated at 37°C for 12-24hours, the digested material was filtered through a sieve in clean Petri dish and washed several times with 0.85% physiological saline, the free metacercariae were picked up individually by a fine pipette and kept for further study *(14)*.

III Experimental infection of puppies with encysted metacercariae *(15)*

1.Preparation of puppies for infection: Twenty puppies (4weeks old) were used for experimental infection. Anthelmintic drugs were given a week before experimental infection as further care. All puppies were reared on dry ration and wheat bread. These prepared puppies were divided into 2 groups. Each one was infected by feeding on separated species of the infected fish sample muscles with encysted metacercariae .

2.Detection of eggs after experimental infection: After experimental infection of puppies with encysted metacercariae, direct examination, sedimentation and floatation techniques were carried out *(16)*. Daily feacal samples were collected and examined for dectection of eggs.

3.Worm collection and detection of the predilection seat of the parasites

The experimentally infected puppies were sacrificed. The small intestine was separated and divided into duodenum, jejunum and ileum where each part was opened in separated Petri dish containing saline *(17,18).* Each part was scarped by scalpel. Then the sediment was examined with steriobinocular microscope. The worms were collected and washed by normal saline and left in the refrigerator at 4°C until the specimens was completely relaxed.

4.Preparation of adult trematodes for morphological studies

The collected worms were compressed by placing them between two slides tightly compressed and fixed in 4% formalin for 24 hours. After that the specimens were washed in running tap water, stained with acetic acid alum carmine 3% for few hours according to the size and the thickness of the worm .Then dehydration in ascending grades of alcohol 70%,80%,90% then absolute alcohol. Every change for about 20 minutes or more was according the thickness of the worms. After that the specimens were cleared in clove oil and xylol and mounted in Canada balsam *(19)*.

A. Effects of some treatment on the viability of the encysted metacercariae The infested fish samples were classified into five groups *(20,21)*each one contain 30 fish. The first and second groups were frozen at -18°C (1st for 24hour and 2nd for 48 hours). The 3rd one was fried in boiled cotton seed oil (170°C) for 5 minutes on each side. The fourth and fifth groups were grilled 60-65°C (4th for 5 minutes and 5th for 10minutes) in each side.

RESULTS

Examination of 500 of Tilapia sp. and *Clarias lazera* fish revealed that the infestation rates with encysted metacercariae are 84.8%, 86.8% respectively.

Table 1. Prevalence of the encysted metacercariae in the examined fish

Fish sp.	No. examined	No. of infected fish	Percentage of infection
Tilapia sp.	250	212	84.8%
Clarias lazera	250	217	86.8%

Macroscopic examination of the encysted metacercariae in the infected fish of Tilapia sp. displayed either black spots or less distinct grayish white spots on the skin that varied in size, shape and number, while in *Clarias lazera* skin showed yellowish white nodules of varying size, distributed in the muscle of abdomen.

Microscopic examination of the encysted metacercariae revealed that, two types of the isolated encysted metacercariae were obtained. Heterophyids were globular to elliptical in shape measures 440x329µ, it had two layers of cyst wall, the outer was thin and transparent but the inner layer was homogeneous in structure with bright bluish color with pigment granules distributed all over the body. While in

prohemistomatid they were spherical in shape and it had double wall; the outer one was fragile and easily ruptured and the inner layer was hyaline and separated from the metacercaria by a potential space containing fluid in which the metacercaria was moved. The cyst measure 270u in diameter.

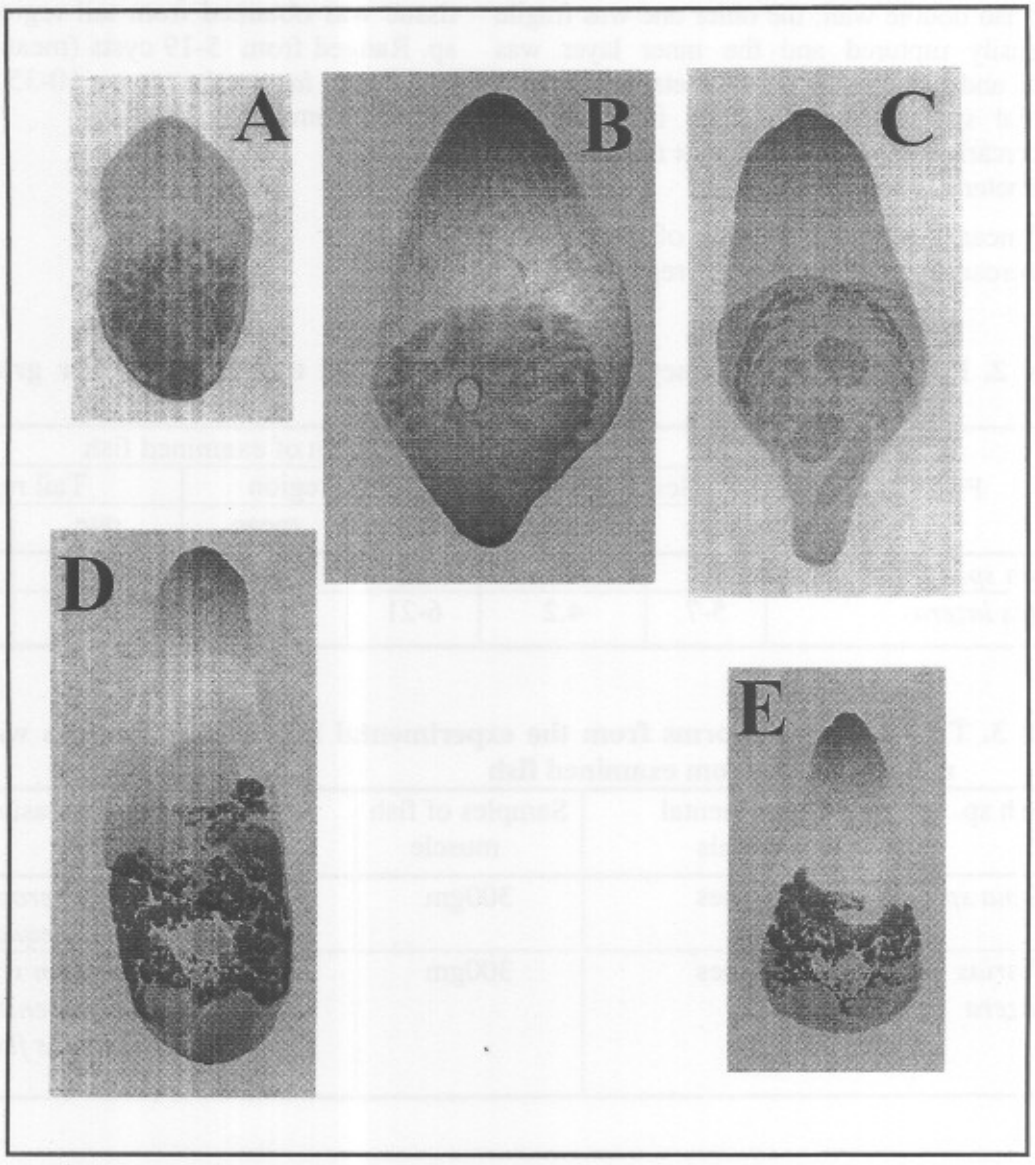
Concerning the distribution of the encysted metacercariae in the different regions of fish body, the highest number of cysts per gram of tissue was obtained from tail region in Tilapia sp. Ranged from 5-19 cysts (mean 9.7). While in *Clarias lazera* they were 10-35 cysts (mean 19) per gram (Table 2).

Table 2. Prevalence of the encysted metacercariae and their number per gram of fish muscles

Fish sp.	No. of EMC / gm of examined fish					
	Head region		Trunk region		Tail region	
	rate	mean	rate	mean	rate	mean
Tilapia sp.	1-3	2.1	1-5	3.2	5-19	9.7
Clarias lazera	5-7	4.2	6-21	11.3	10-35	19

Table 3. The recovered worms from the experimental infection of puppies with encysted metacercariae from examined fish

Fish sp.	Experimental animals	Samples of fish muscle	Recovered parasites.
Tilapia sp.	Puppies	300gm	*-Heterophyes heterophyes.* *-Heterophyes aequalis.*
Clarias lazera	Puppies	300gm	*-Prohemistomum vivax.* *-Mesostephanus appendiculatus.* *-Mesostephanus flapi.*



A-*Mesostephanus flapi*. (x200) **B -*Prohemistomum vivax*. (x100)**

C-*Mesostephanus appendiculatus* .(x100) **D-*Heterophyes heterophyes*. (x100)**

E-*Heterophyes aequalis*.(x100)

Table 4. Effect of freezing on the viability of the encysted metacercariae of the fresh water fish

Fish sp.	Experimental animal for prov viability	Fish musc sample	Duration of freezing and viabilit of the encysted metacercariae	
			24hour	48hour
Tilapia sp.	Puppies	300gm	Viable	Died
Clarias lazera	Puppies	300gm	Viable	Died

Table 5. Effect of frying on the viability of the encysted metacercariae of the fresh water fishes

Fish sp.	Time of exposure	Experimental animal for prove viability	Amount of Fish muscle	Viability
Tilapia sp.	5minutes	Puppies	300gm	died
Clarias lazera	5minutes	Puppies	300gm	died

Table 6. Effect of grilling on the viability of the encysted metacercariae of the fresh water fishes

Fish sp.	Experimental animal for prove viability	Amount of Fish muscle	Time of exposure	
			5 minutes	10 minutes
Tilapia sp.	Puppies	300gm	Viable	Died
Clarias lazera	Puppies	300gm	Viable	Died

DISCUSSION

In the present study the prevalence of the encysted metacercariae in *Tilapia sp.* was 84.8%, similar ***(22-26)*** and lower ***(15,20, 27, 28)*** findings wee recorded. In *Clarias lazera* it was found to be 86.8% this result is in agreement with obtained by several authors ***(20, 24, 25, 29, 30)*** and it was higher than that reported by others ***(31,32).*** The variety in the prevalence rate of the encysted metacercariae may be due to variation of the distribution of the snails in different localities and also the pollution of Nile by migratory bird excreta .

In the present study the average number of the encysted metacercariae in head region, trunk and tail muscles of *Tilapia.sp.* was 2.1,3.2and 9.7/gm and in *C. lazera* was 4.2,9.3 and10/gm these results was coincide with those reported previously ***(23, 33, 34)*** . The highest average number of encysted metacercariae infection was 47/gm in anterior third of *Tilapia sp.(30)* , and the anterior third of *Tilapia sp.* had the highest number of encysted metacercariae 43/gm ***(22)*** such variations among different type of infected fish may attributed to the host specificity histological structure of the skin and behaviour of living of *C.lazera* and *T.sp.* It was noticed that the average number of EMC/gm in marine water fish was fewer in number than in fresh water fish. This might be due to the high salinity of sea water leads to

rapid decline in intensity of cercarial emergence from snail intermediate host ***(35)***.

Concerning the recovered flukes from experimentally infected puppies with encysted metacercariae from *Tilapia sp.*; *Heterophyes heterophyes* and *Heterophyes aequalis* were obtained. The morphological feature of was similar with that obtained in several studies ***(4,12,19,24-27,36-40)***.

Prohemistomum vivax, *Mesostephanus appendiculatus* and *Mesostephanus flapi*. were obtained from the small intestine of experimentally infected puppies with encysted metacercariae from *Clarias lazera*. These result was in agreement with that previously reported by ***(4, 15, 24-27, 39, 41-43).*** The recovered adult trematodes were recorded as fish-borne zoonotic parasites which have public health importance among fresh water fishes so good hygiene measures must be done to avoid infection to the human being.

In the present study some trials were conducted to prevent the infection to human being with encysted metacercariae by the effect of freezing of the infected *Tilapia sp.* and *Clarias lazera* with encysted metacercariae at -18°C for 24 hours. Result, revealed that it has no effect on examined samples which was judjed by recovered adult worms from experimentally fed puppies. While freezing for 48 hours was sufficient to kill all encysted metacercariae in fish muscles which proved by unrecovering of any adult worms from experimentally fed puppies (Table 5). Similar effect was recorded ***(25, 43-47).***

In present study the results indicated that frying for 5 minutes was sufficient for destruction of all encysted metacercariae which provide by unrecovering adult worms from experimentally infected puppies (Table 6). Effect of frying on encysted metacercariae support previous studies ***(21, 25, 43, 48).***

In frying method the temperature of the boiled oil was able to penetrate deeply into the fish flesh and destroy all encysted metacercariae. so it can be considered one of the safest processing methods controlling parasitic hazards which may be associated with fish consumption.

Gilling at 60-65°C for 5 minutes was not sufficient to destroy all encysted metacercariae in fish muscles which was proved by recovering adult worms from experimentally infected puppies (Table 7). Grilling was effective for destruction of encysted metacercariae ***(10, 26, 43,49, 50).***

As temperature of the inner part of fish flesh was unable to kill all encysted metacercariae in different depth of the flesh of fish. However, good grilling which manifested by disappearance of skin striation on its inner surface was sufficient to destroy more encysted metarcercariae in fish fresh sometimes even in the deeply sited ones which proved sometimes by un recovering adult worms from experimentally fed puppies. This result was agreed ***(51)***. Also ***(20, 25, 26, 43-45,52)*** indicated that good grilling encysted metacercariae in fish flesh.

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الملخص العربي

دراسة على بعض الطفيليات في أسماك الأسواق و علاقتها بالصحة العامة مع محاولة السيطرة عليها

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الأسماك مصدر هام جداً من مصادر البروتين الحيواني و لذلك يزداد الإقبال على استهلاكها على الرغم من أنها قد تسبب فى انتقال بعض الطفيليات للإنسان والحيوان والطيور الجارحة إذا تم تناول هذه الأسماك نيئة أو غير مطهية جيداً ولذلك لاحتواء هذه الأسماك على الأطوار اليرقية المتحوصلة للديدان المفلطحة.

لذلك أجريت هذه الدراسة لاستبيان الطور المتحوصل حيث تم فحص ٥٠٠ سمكة من أسماك المياه العذبة (٢٥٠عينه من أسماك البلطي -٢٥٠ عينة من أسماك القرموط) جمعت كلها من أسواق مدينة الزقازيق . وقد أظهرت نتائج الفحص لهذه الأسماك نسبة الإصابة بالطور المتحوصل لهذه الديدان في أسماك البلطي ٨٤,٨% بينما كانت ٨٦,٨% في أسماك القرموط.

وعند عدوى الكلاب تجريبياً بلحوم أسماك البلطي المصابة بالطور اليرقى المتحوصل تم الحصول على الديدان اليافعة للهيتروفيس هيتروفيس- هيتروفس أكواليز أما بالنسبة لأسماك القرموط فتم الحصول على ديدان بروموستومم فايفكس- ميزواستيفانيس ابنديكولاتس- ميزواستيفانيس فلابى.

عند دراسة تأثير بعض الطرق المختلفة والمعدة للأسماك للاستهلاك الآدمي كغذاء صحى للقضاء والوقاية من الأطوار اليرقية المتحوصلة أوضحت الدراسة أن التبريد عند -١٨°م لمدة ٤٨ ساعة والقلي في الزيت المغلي لمدة خمس دقائق لكل جانب وكذلك الشى لمدة ١٠ دقائق عند درجة حرارة ٦٥°م وجد أن ذلك كاف للقضاء على اليرقى المتحوصل في لحوم الأسماك و قد تم التأكد من ذلك بإحداث عدوى تجريبية في صغار الكلاب المغذاة على لحوم هذه الأسماك ولم تتم العدوى لها.