THE OCCURRENCE OF PROHEMISTOMATIDAE METACERCARIAE AMONG CULTURED TILAPIA IN EL-ABBASSA FISH FARM WITH SPECIAL REFERENCE TO ITS CONTROL

Amer O. H. and El-Ashram A. M.M.*

Dept. of Parasitology, Fac. Vet. Med. Zagazig University

* Fish Disease Dept., Central Laboratory for Aquaculture Research (El-Abbassa), Agricultural Research Center

ABSTRACT

Encysted metacercariae were encountered in the muscles of cultured tilapia of fish by the compression technique and artificial tissue digestion beside histopathologic examination of H & E stained 5 micron paraffin sections, obtained from infested fish in El-Abbassa farm. After experimental infection of parasite free puppies, 3 Prohemistomatidae adult worms (Prohemistomum vivax, Mesostephanus appendiculatus and Mesostephanus melvi) were recorded.

Trials for the control of these metacercariae were attempted in this study. The freezing at - 20 C for 72 hrs., frying or praziquantel (1 mg/L water) killed the encysted metacercariae in the fish flesh; but the 15 salinity failed to do so. The death of encysted metacercariae was proved by their failure to infect puppies.

INTRODUCTION

Fish represent a good source of animal protein due to its high nutritive value and relative cheap price. On the other hand, fish consumption was sometimes associated with public health hazards due to the role they may play as an intermediate host of some parasites involving man, beside fish eating mammals and birds which act as definitive hosts (**Murrell 1995, Paperna 1996 and El-Leathy 1997**).

The fresh water fish tissues parasites are more important than those which merely attach to the fish surface (**Paperna, 1996**). The rising concern for food safety is causing a reappraisal of the significance of food borne parasites and control strategies. Moreover, the development of more effective safeguards requires a great understanding of the nature and epizootiology of these zoonoses (**Murrell, 1995**).

The current investigation was planned to study the Prohemistomatidae cysts and the induced lesions in the musculature of tilapia species and recovering of adult parasites from the intestine of the experimentally infested puppies for identification. The effect of freezing, frying and chemotherapy on the infectivity and viability of the metacercariae were evaluated.

MATERIAL AND METHODS

Three hundred apparently healthy tilapia species of various weights were collected from El-Abbassa fish ponds and transferred alive to the laboratory. Fish were examined for the presence of viable encysted metacercariae in the muscle by using the compression technique and artificial tissue digestion (**Jackson et al. 1981**).

I. Experimental infection :-

Five puppies (four weeks old, reared on boiled cow milk and wheat bread) were orally given 50 mg/kg. B.wt. praziquantel and examined twice weekly to exclude any intestinal parasites.

The puppies which were parasite free, were orally given 20 ml saline containing 50 viable encysted metacercariae one week after treatment with the anthelmintic (**Shibahara and Nisheda, 1986**). After one week of infestation, daily fecal samples, from each infested puppy, were examined by direct and simple sedimentation technique till the demonstration of eggs. (**Faust et al, 1976**).

The experimentally infested puppies, which began to shed trematode eggs, were sacrificed and necropsied. Their intestines were divided into 3 segments in Petri dishes containing saline solution where each segment was separately opened. The worms were collected by gentle scraping of the intestinal mucosa. Collection, staining and mounting of the worms were performed according to the technique described by **Kruse and Pritched (1982)**. The trematode worms were identified according to **Yamaguti (1958) and Raef (1994)**.

II. The effect of freezing, frying and chemotherapy on the infectivity and viability of encysted metacercariae :

A] Freezing : The Tilapia fish were preserved by freezing at -20 C for a period of 24-72 hours, then fish were examined for infectivity of metacercariae after each period by feeding it to experimental puppies as described before.

B] Frying : The infected eviscerated Tilapia species were fried in cotton seed oil until its skin became brownish, then they were fed to experimental puppies as mentioned before.

C] Chemotherapy : Sixty tilapia species fish, with encysted metacercariae in their muscles, were divided into three equal groups in glass aquaria supplied with dechlorinated tap water and continuous aeration. Group (1) was treated with salinity by increasing its concentration in the aquaria water gradualy till reaching 15 and examined weekly for one month to detect any viabile metacercariae. Group (2) was subjected to 1 mg/L praziquantel (Bayer, Germany). Group (3) was left as a control group. Then all fish were examined for the presence of any viabile of metacercariae.

III. Histopathologic Examination:-

The specimens from infected tilapia muscle were trimed and fixed in 10% phosphate b..ffered formalin. Five micron thick paraffin sections were prepared, stained with H & E. and examined microscopically (**Robert 1978**).

RESULTS & DISCUSSION

Examination of 300 tilapia fish from El-Abassa in Sharkia, Egypt, revealed the presence of Prohemistomatidae metacercariae. The encysted metacercariae, in muscle were spherical with double wall and dimensions of 0.35-0.39 X 0.22-0.28 mm. The eggs in fecal sample, appeared large greenish- yellow. They measured 70-75 X 50-60 . The eggs in feacal sample, it appeared large in size grecnish, yellow in colour it measured 70-75 X 50-60 u and after experimental infection of puppies revealed the presence of the adult worms, Prohemistomum vivax, Mesostphanus appendiculatus and Mesostephanus melvi, table (1) and Fig. (1).

Preservation by freezing, (table2) showed that the freezing for 24 hrs. was not sufficient for killing the encysted metacercariae in the muscle of tilapia species. While, the good freezing at -20 C for 72 hrs. was sufficient to kill all metacercariae in the muscle which was indicated by failing to recover adult worms from the experimentally fed puppies. Table (2) shows that frying of fish till the appearance of brownish colour on the skin was able to kill all metacercariae. Moreover, table (2) shows that the treatment of fish with salinity did not kill the encysted metacercariae, while praziquantel killed the encysted metacercariae in tilapia muscles.

Microscopically, the parasitic cysts were embeded within the skeletal .mus.les and surrounded by two fibrous tissue layers. The inner layer was usually edematous fibrous tissue and the outer layer was dense fibrous tissue. The surrounding muscles suffered from hyaline degeneration and coagulative necrosis. Sometimes, inter and intra muscular edema was evident (fig. 2).

Tilapia is an important cheap protein source for human consumption, such food fish may serve as intermediate hosts for parasites of public health importance in Egypt. **Nasr (1941)** described a human infection with Prohemistomum vivax in Egypt. During the past decade, more public attention has been directed to control disease transmitted from fish to human. **Gopalakrishnan (1968)** indicated that studies of the life cycle of parasites is of value in controlling the parasite.

Concerning the effect of some measurments on killing the encysted metacercariae in the muscle of Tilapia fish, the results showed that freezing at -20 C for at least 72 hrs. was sufficient in killing the encysted metacercariae and the frozen fish became safe for human consumption. Similar results were recorded by **Nada et al. (1989), Tantawy (1993) and El-Leathy (1997)** who indicated that freezing temperature for a period more than 2- days is sufficient for destroying metacercariae in frozen fish. On the contrary, **El-Bouhy et al. (1988) and Paperna (1996)** found that freezing of infested fish destroyed the cyst after 7-days.

Also, our results reported that frying of infected fish is efficient for killing of encysted metacercariae. Similar results were reported by **Nada et al. (1989), Tantawy (1993) and El-Leathy (1997)** who recorded that frying for 5 minutes were sufficient to destroy all encysted metacercariae. **Ogawa (1996)** mentioned that human infection with parasites are closely related with Japanese tradition of eating raw fish. Another practicable preventive method of controlling digenean trematode infection in farmed fish is the elimination of the snail. The available measures include the use of chemical molluscicides, environmental manipulation and the use of molluscophagous fish (**Paperna, 1996**). Moreover, the treatment should not exert any noticeable effect on the marketing and taste of the fish.

Regarding to the trials for treatment of the living infected fish, our results showed that salinity by increasing its concentration in the aquaria water gradually till reachig to 15 was unable to kill the encysted metacercariae. The present study agrees with those obtained by **Amlacher (1970), El-Bouhy et al. (1988) and Paperna (1996)** who recorded the existance of the metacercariae of trematodes in muscles of fresh, brackish and salt water fish. Meanwhile, the treatment of infected fish with praziquantel at a dose of 1 mg/L was able to kill the metacercariae and rendering fish safe for human consumption which is in accordance with **Szekely and Molnar s (1991) and Paperna (1996)** who recommended the application of praziquantel as a good effective treatment against the encysted metacercariae in juvenile tilapia.

By the histo-pathologic examination, the parasitic cysts were surrounded by two layers. The surrounding muscles suffered degeneration and necrosis which could be attributed to pressure and toxic metabolites, produced by the cysts. These lesions are in harmony with those described

by **El-Bouhy et al. (1988), Ahmed et al. (1994), Mahdy et al. (1995) and Paperna (1996).** The latter mentioned that the exposure to numerous cercariae may kill fries within a few hours. Cercariae which penetrates and encystes deeper in the tissues of small fish, particularly when large cyst interfered with organ function.

Table 1 : Characteristic feature of Prohemistomatidae sp.

Criterion	Prohemistomum vivax	Mesostephanus appendiculatus	Mesostephanus melvi
Body size	1.4-1.6 X 0.7-0.8 mm	1.6-1.7 X 0.7-0.8 mm	1.6-1.8 X0.9-0.11 mm
Vagini sphincter	absent	present	present
Pre-patent period	9 days	13 days	13 days

Table 2 : Effect of freezing, frying, salinity and praziquantel on viability of encysted metacercariae.

Method treatment		Experimental Host	Effect on viability of encysted metacercariae
Duration of freezing	24 hrs.	+++ ve	+++ ve
	72 hrs.	- ve	- ve
Frying		- ve	- ve
Salinity		+++ ve	+++ ve
Praziquantel		- ve	- ve
Control group		+++ ve	+++ ve

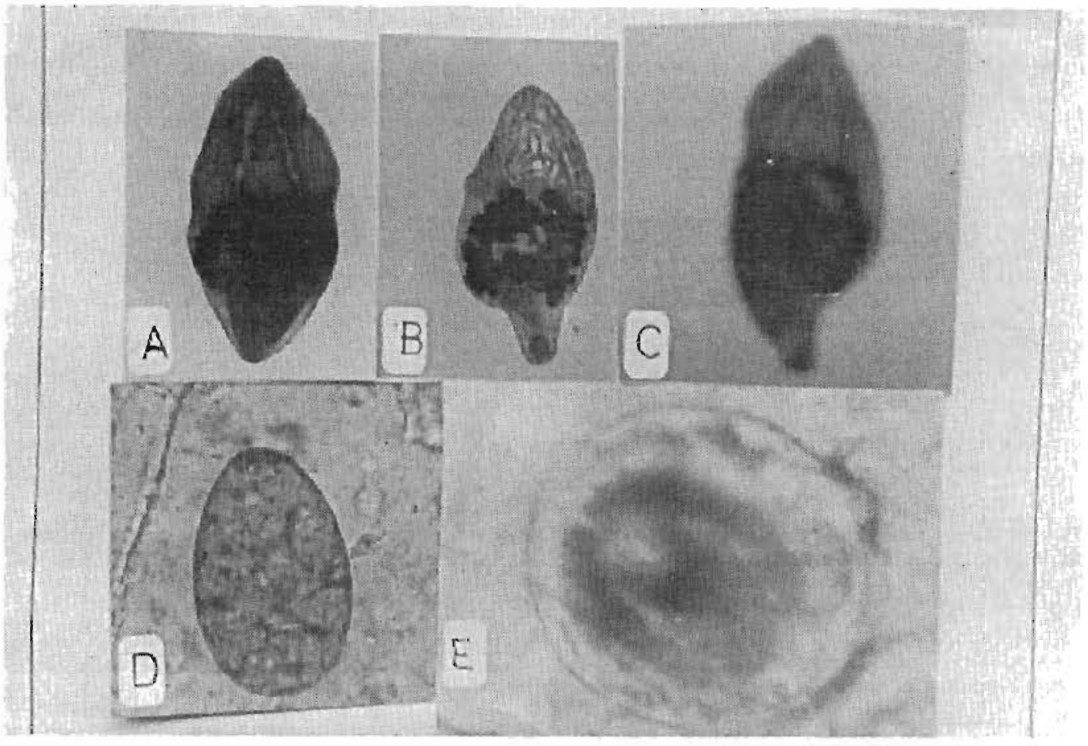

Fig. (1) : **A)** Prohemistomum vivax, alum carmine x 60.
B) Mesostephanus appendiculatus, alum carmine x 60. C) Mesostephanus melvi, alum carmine x 60.
D) Prohemistomatidae egg, x 100.
E) Prohemistomatidae metacercaria x 100.

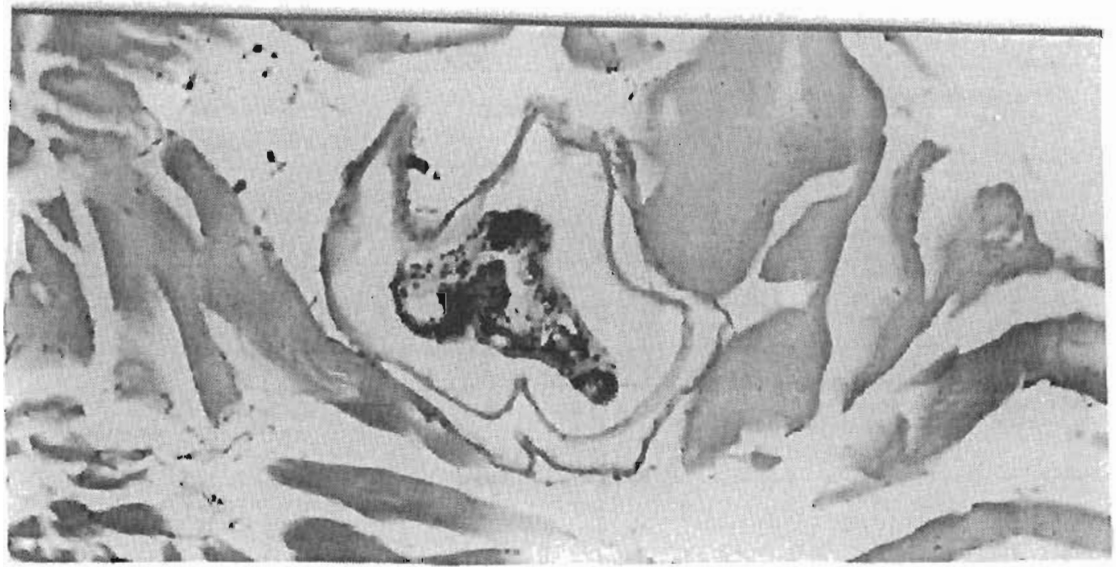

Fig. (2) : An encysted metacercaria with associated edema in the skeletal muscle of tilapia. H & E., X 100.

REFERENCES

Ahmed S. M., Hussein S. Y. and Sadiek A. II. (1994) : Diplostomiasis or black spot disease in Clarias lazera at Assiut. Assiut Vet. Med. J. 31 (61). 216-225.

Amlacher E. (1970) : Textbook of fish diseases. Translated from Germany by Conray D.A. and Herman R.L. Jersy city, T. F.H. publication, 214-217.

El-Bouhy Z. M., Saleh, G., El-Desoky E. A. and Ali A.A. (1988) : Studies on yellow grub infestation in Nile catfish (Clarias Lazer) in Sharkia Province. Zag. Vet. J. XVI (2 B) 166-186.

El-Leathy A. M. (1997) : Prevalence of parasites of public health importance in some fresh water fishes. M.V.Sc. Thesis, Food control Dept., Fac. Vet. Med., Zag. Univ.

Faust E. C., Russell P. F. and June R. C. (1976) : Craig and Faust,s clinical Parasitology. 8th E.d., Lea and Fegiger; Philadelphia.

Gopalakrishnan V. (1968) : Diseases and parasites of fishes in warm water ponds in Asia and the Far East. FAO- Fish-Report. 44 (5) 319-342.

Jacksan G. J., Bier J. W., Payne W. L. and Mc cluze F. D. (1981) : Recovery of parasite from fish by digestion or elution. Appl. Env. Micra., 912-914.

Kruse G. O. W. and Pritched M. H. (1982) : The collection and preservation of animal parasites. Nebraska Univ. Press, U.S.A.

Mahdy O. A., Manal Essa A. A. and El-Easa M. (1995) : Parasitological and pathological studies on Heterophyid infection in Tilapia species from Manzala Lake, Egypt. Egyptian J. Comp. Path. and Clinical Path. 8 (2) 131-145.

Murrell K. D. (1995) : Food borne parasites. Inter. J. Environmental Health Research, 5 (1) 63-85.

Nada M. S., El-Atabany A. I. and Saleh A. E. (1989) : Effect of some preocessing on the metacercariae of Heterophyid parasites in Tilapa nilotica. Alex. J. Vet. Sci., 5 (1): 277-286.

Nasr M. (1941) : The occurrence of Prohemistomum vivax infection in man, with redescription of the parasite. Lab. and Med. Prog., 2, 135-149.

Ogawa K. (1996) : Marine parasitology with special reference to Japanese fisheries and mariculture J. Vet. Parasitol. 64 : 95-105.

Paperna J. I. (1996) : Parasites, infections and diseases of fish in Africa committe for inland fisheries of Africa. (IFA).FAO Technical papers, No.13:122-143.

Raef A. M. (1994) : Role of marine fish in transmission of some parasites to animals and birds.

Ph.D. Thesis (Parasitol.), Fac. Vet. Med. Zag. Univ.

Robert R. J. (1978) : Fish Pathology. 1st ed. Bailliere Tindall, London.

Shibahara T. and Nishida H. (1986) : Studies on the lung fluke, Paragonimus westermeni-Diploid type in the Northern part of Hyogo prefecture, Japan. VI Experimental oral infection of Bears and Pigs with metacercariae . Jap. J. Parasitology. 35 (4) : 303-313.

Szekely C. and Molnar K. (1991) : Praziquantel (Droncit) is effective against diplostomosis of grass carp (Ctenopharyngodon idella) and silver carp (Hypophthalmichthys molitrix). Dis. Aquat. Org. 11: 147-150.

Tantawy E. A. (1993) : Muscular parasites in market fishes, M. V. Sc., Thesis, Fac. Vet. med., Cairo Univ.

Yamaguti S. (1958) : Systema Helminthum. The digenetic trematodes of vertebrates. Part I. Interscience Pulicher In., New York, V., 865-951.

الملخص العربى

مدى تواجد بروهيميستروماتيدا ميتاسركاريا فى أسماك البلطى المستزرعة بمزارع أسماك العباسة مع الإشارة إلى الوقاية والعلاج منها

المشتركون فى البحث

عمـــر حسـن عامـــر أحمد محمد محمود الأشرم*

أثناء الفحص الروتينى لمزارع أسماك العباسة بمحافظة الشرقية اتضح بالفحص الميكروسكوبى إصابة أسماك البلطى بالسركاريا المتحوصلة فى عضلات هذه الأسماك وبعد العدوى التجريبية للجراء اتضح وجود ثلاثة أنواع من ديدان البروبستومم. كما أجريت محاولات مختلفة للوقاية والعلاج لهذه السركاريا المتحوصلة. كما أوضحت الدراسة أن التبريد لدرجة - ٢٠م لمدة ٧٢ ساعة وكذلك التحمير وعلاج مياه المزرعة بعقار البرازيكوانتيل (١ مجم لكل لتر مياه) أباد السيركاريا المتحوصلة لعدم إحداثها للعدوى التجريبية فى صغار الكلاب.