SAFETY AND QUALITY OF FISH INFESTED WITH ENCYSTED METACERCARIAE OF DIGENETIC TREMATODES

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Abstract

A total of 600 fresh water fish (Mugil sp., Tilapia sp. and Carp sp.) were collected from Abbassa aquaculture (Sharkia province), Qaroun and Wadi Al-Raiyan lakes (Fayom province). The prevalence of encysted metacercarial infestation in fishes from Abbassa aquaculture, Qaroun and Wadi Al-Raiyan lakes were 59.5%, 5% and 53% respectively. Eight types of encysted metacercariae were detected in fishes. The isolated metacercariae were experimentally fed to fish eating mammals (cat and rat) and birds (pigeon and heron) producing fourteen types of trematode species, where, all of them are of zoonotic importance except the *Diplostomum Tilapi*. Concerning the estimation of bioaccumulation of some heavy metals as lead (Pb), Cadmium (Cd), Mercury (Hg), Copper (Cu) and Zinc (Zn) in fish flesh recommended in the study were higher in heavily infested fishes, where, the levels of lead and mercury residues in examined fishes from Abbassa aquaculture and Qaroun lake were higher than those of WHO limits (1984). The detectable residues of other heavy metales were within the permissible limit. A negative correlation between the rate of metacercarial infestation and the native score of accepted sensory evaluation was also demonestrated. Application of physical control for killing the metacercarial parasitic encystation in fish with thermal procedures as frying, grilling and microwaving revealed 5 minutes, 10 minutes at 60-80^{o}C and 5 minutes at high power, respectively, while, freezing for 72 hours at - 4^{o}C and 48 hours at - 18^{o}C were sufficient to kill all encysted metacercariae in the tested fish flesh.

INTRODUCTION

Reviewing the avialable literature, it was found that the incidence of encysted

metacercariae in Tilapia sp. was 21% (in Qaroun lake) as recorded by Mahmoud (1990), while, Ebtsam (1993) and Olfat *et al.* (1995) reported that the metacercarial infestation rate in Tilapia sp. was 78% and 41.6%, respectively, in Cairo and Giza markets. Regarding the infestation rate in Mugil sp., it was found to be 82% and 88%, as recorded by El-Dally (1988) and El-Sherbiny (1988), respectively. Inaddition, Ramadan (1994) revealed that the rate of infestation with encysted metacercariae in Carp sp. was 49%. In Egypt, some Heterophyid parasites were recorded to infect man as *Heterophyes heterophyes*, *H.aaequalis* and *H.dispar* (Wells and Randall 1956). Moreover, *Pygidiopsis genata* and *Clinostomum tilapi* have a public health hazard. On the other side, Paperna (1980), Joo (1988) and Sinderman (1990) reported that there is a synergistic effect between the parasitic infestation and bioaccumulation of heavy metals in fish. Parasites play an important role in determining the quality of fish (Shahidi and Bota, 1994). Moreover, El-Sherbiny (1988) reported that the physical procedures as thermal and freezing (at - 4°C and - 18°C) were sufficient to kill all encysted metacercariae in the tested fish flesh.

MATERIALS AND METHODS

A total number of 600 fishes of (*Tilapia sp., Mugil* sp. and *Carp sp.*) were collected from different localities [Central laboratory Aquaculture Research in Abbassa at Sharkiya province, Qaroun and Wadi Al-Rayian lakes at Fayoum province]. Each fish sample was rolled in aluminum foil and kept in a clean plastic bag and transferred to the laboratory in ice box container with minimum delay. The organoleptic examinations were done for fishes as described by Braumuller (1958), then, examined microscopically for the presence of the encysted metacercariae in muscles and organs, with the application of the digestion technique according to Oshima *et al.* (1966). The isolated metacercariae were experimentally fed to parasite free mammals and birds (cat, rat, heron and pigeon) to identify the metacercariae based on the revealed adult worms (Paperna, 1980). The encysted metacercariae and adult fluks obtained, fixed and stained with Lucky technique (1977). Estimation of the bioaccumulation of some heavy metals as [pb), Cadmium (Cd), Mercury (Hg), Copper (Cu) and Zinc (Zn)] in fish muscles by using the Atomic Absorption Spectophotometer was done according to Chapman and Pratt (1979). Thermal procedures as frying (for 3 and 5 minutes on each side), grilling (at 60 - 80°C for 5 and 10 minutes on each side) and cooking in microwave oven (at high power for 3-5 minutes) were applied. Moreover, freezing procedures (at-4°C for

24h, 48h and 72 h) and (at-18°C for 24h and 48h) were carried on. The viability of the encysted metacercariae recommended by using the digestive solutions (Oshima *et al.* 1966) was also checked.

RESULTS AND DISCUSSION

In the present study, the total incidence of the encysted metacercariae in the investigated fishes (Table 1), showed that, 235 out of 600 samples (39.1%) were found to be infested with encysted metacercariae. The rate of encysted metacercarial infestation in the present study revealed that *Tilapia* sp. at Abbassa aquaculture and Wadi Al-Rayian lake were 89% and 82%, respectively. These results were found to be higher than that recorded in Qaroun lake, which was 10%. In contrast, Mahmoud (1990) recorded a higher rate of infestation in *Tilapia* sp. (21%) among the samples collected from Qaroun lake. This might be attributed to the gradual increase in the water salinity of the Qaroun lake, pronounced in unadaptability of the snail due to the new environment (Ginetsinskaya, 1988). The variations in the rate of the metacercarial infestation from locality to another might be attributed to the water pollution with human and animal excreta and/or to the difference in the adverse environmental factors: as the temperature, salinity, density of the snails and the migratory birds, (Kenned, 1982).

Table 1. Incidence of the encysted metacercariae in the examined fishes from different lacalities.

Fish	Localities	Total number of fishes		Percentage
		Examined	Infected	
Tilapia sp.	Abbassa aquaculture	100	89	89 %
	Qaroun Lake	100	10	10 %
	Wadi Al-Raiyan	100	82	82 %
Mugil sp.	Qaroun Lake	100	0	0 %
	Wasi Al-Raiyan	100	24	24 %
Carp sp.	Abbassa aquaculture	100	30	30 %
Total		600	235	39.16%

Experimental feeding of fish eating mammals and birds on fish flesh containing the viable encysted metacercarae (Table 2 and 3) revealed that, more or less all the detected and identified parasites (except the *Diplostomum tilapi*) were transmissible

Table 2. Experimental infestation of mammals and birds with the fish flesh containing the viable encysted metacercariae.

Loeality & Fish sp.	Experimen-tal hosts	Amount of fish consumed/gm	Number of E.M.C. consumed	Prepatent period/day	No.of isolated trematodes	Recovery rate	Species of recovered worms
A.Abbassa aquaculture 1- Tilapia sp.	Cat	10	300	3	25	8.3	- Centrocestus aramatus
	Cat	15	375	4	20	5.3	- Pygidiopsis genata
	Rat	10	300	7	60	20.0	- Haplorchis pumilio
	Rat	15	375	10	58	15.4	- Prohemistomum vivax
	Heron	10	300	5	240	80.0	- Mesostephanus appendiculatus
	Heron	15	375	7	350	93.3	- Echinochasmus perfoliatus
	Pigeon	5	150	4	100	66.6	- Diplostomum tilapi
	Pigeon	8	200	6	140	70.0	- Clinostom tilapi
A.Abbassa aquaculture 2- Carp sp.	Cat	10	30	5	2	6.6	- Echinochasmus pefolatus
	Cat	15	15	7	--	--	
	Heron	10	30	5	3	10.0	
	Heron	15	15	10	2	13.3	

E.M.C. = Encysted metacercariac.

Table 3. Experimental infestation of mammals and birds with the fish flesh containing the viable encysted metacercariae.

Loeality & Fish sp.	Experimen-tal hosts	Amount of fish consumed/gm	Number of E.M.C. consumed	Prepatent period/day	No.of isolated trematodes	Recovery rate	Species of recovered worms
A-Qaroun Lake: -Tilapia sp.	Cat	10	80	3	2	2.5	-*Pygidiopsis summa*
	Cat	15	45	5	1	2.5	
	Rat	10	80	5	12	15	- *Haplorchis pumilio*
	Rat	15	45	7	5	11.1	
B-Wadi Al-Raiyan: Tilapia sp.	Cat	10	1200	4	90	7.5	- *Centrocestus aramatus*
	Cat	15	900	6	43	4.7	- *Pygidiopsis genata*
	Rat	10	1200	7	300	25	- *Pygidiopis summa*
	Rat	15	900	14	140	15.5	- *Haplorchis pumilio*
	Heron	10	1200	5	930	77.3	- *Ascocotyle (Phagicola)*
	Heron	15	900	7	800	88.8	- *Stictodora tridactyla*
	Pigeon	5	600	4	300	50	- *Diplostomum tilapi*
	Pigeon	8	480	7	150	31.2	- *Clinostomum tilapi*
- Mugil sp.	Rat	10	50	7	10	20	- *Heterophys heterophys*
	Rat	15	45	10	11	24.4	- *Heterophyes aqualis*
	Heron	10	50	5	33	66	- *H.dispar*
	Heron	15	45	7	39	86.6	- *Pygidiopsis*
							- *Haplorchis pumilion*

E.M.C. = Encysted metacercariac.

Table 4. Mean values ± S.E. of heavy metals contents in fish flesh with different degrees of encysted metacercariae infestation.

Heavy metal	Permissible limit of who 1984 (ppm)	E.M.C. infestation in Abbassa aquaculture						E.M.C. infestation in Qaroun lake (Tilapia sp.)			E.M.C. infestation in Wadi Al-Raiyan lake					
		(Tilapia sp.)			(Carp sp.)						(Tilapia sp.)			(Carp sp.)		
		uninfested	Moderate infested	Heavily infested	uninfested	Moderate infested	Heavily infested	uninfested	Moderate infested	Heavily infested	uninfested	Moderate infested	Heavily infested	uninfested	Moderate infested	Heavily infested
- Lead:		1.8	2.0	2.6	1.6	2.2	1.6	0.98	1.18	1.24	1.26	1.64	2.80	1.90	1.32	1.02
(ppm)	0.6	±0.37	±0.32	±0.24	±0.24	±0.58	±0.24	±0.24	±0.24	±0.32	±0.45	±0.47	±0.58	±0.52	±0.28	±0.28
-Cadmium:		0.07	0.11	0.14	0.09	0.10	0.13	0.014	0.018	0.042	0.018	0.022	0.034	0.014	0.018	0.040
(ppb)	1.0	±0.007	±0.006	±0.006	±0.004	±0.006	±0.006	±0.002	±0.037	±0.004	±0.003	±0.003	±0.005	±0.002	±0.003	±0.005
-Mercury:		0.68	0.66	0.90	0.46	1.0	1.2	0.30	0.22	0.32	0.18	0.20	0.40	0.20	0.28	0.5
(ppb)	0.5	±0.1	±0.1	±0.04	±0.07	±0.1	±0.1	±0.05	±0.04	±0.05	±0.04	±0.06	±0.05	±0.04	±0.11	±0.04
-Copper:		8.2	8.8	12	7.8	9.2	10.8	10.2	10.0	7.6	10.2	9.6	9.0	5.6	11.6	14.0
(ppm)	20.0	±0.73	±1.16	±1.0	±1.46	±0.70	±0.73	±0.96	±0.70	±0.81	±0.48	±1.6	±1.14	±0.97	±1.07	±1.68
- Zinc:		15.2	14	34.4	17.0	15.6	15.4	17.4	16.8	23.0	18.0	21.0	22.4	15.4	17.0	18.2
(ppm)	50.0	±0.58	±0.44	±1.07	±1.30	±1.02	±0.67	±0.81	±1.06	±1.14	±1.0	±0.70	±0.74	±0.74	±0.70	±0.58

± Standard error

Table 5. Relationship between rates of sensory examination of the examined fish and percentage of the encysted metacercarial infestation.

Abbassa aquaculture						Qaroun lake			Abbassa aquaculture					
Tilapia sp.			Tilapia sp.			Tilapia sp.			Tilapia sp.			Tilapia sp.		
A.s.e	pH.	E.M.C. %	A.s.e	pH.	E.M.C. %	A.s.e	pH.	E.M.C. %	A.s.e	pH.	E.M.C. %	A.s.e	pH.	E.M.C. %
78.7	6.3	89	89.3	6.3	30	92.6	6.3	10	80.4	6.4	82	87.3	6.5	24

E.M.C. = Encysted metacercariae.

A.S.E. = Accepted sensory evaluation.

to man, as the members of family *Heterophyidae* (*Heterophyes heterophyes, H. aequalis* and *H. dispare*) and the other genera of family *Heterophyidae* (*Centrocestus armatus, Ascocotyle sp., Haplorchis pumilio, Pygidiopsis gentata*) have been shown to infect human. These results were consisted with those of Wells and Randall (1956) and Yil Chai and Lee (1990). Moreover, families (*Echinostomatidae, Cyathocotylidae* and *Clinostmoatidae*) could also infect man. These reports were similar with those of Paperna (1980) and El-Dally (1988).

The higher levels of heavy metals were recorded in heavily infested fishes with encysted metacercariae (Table 4), where the parasitization in the fish host increased host susceptability to the toxic pollutants of heavy metals (Sinderman, 1990) and (Sorensen, 1991). Table 5 showed that there was a negative correlation between the rate of infestation with encysted metacercariae and the score of accepted sensory evaluation. This was due to that infected fish with encysted metacercariae, even lightly, was rejected by consumers (Paperna, 1980), unsaleable (Hall, 1992) and causes aesthestic problem (Shahidi and Botta, 1994). Either microscopic metacercariae as (Heterophyid which cause Black sport disease, (Fig. 1) or macroscopic metacercariae as (Clinostomatids the cause of yellow Grub disease, (Fig. 2) proved to have public health hazards, where, the quality and marketability of the infested fish will be depreciated. In the present study, the physical methods were including frying, grilling and microwaving not less than 5 minutes on each side, 10 minutes on each side (at 60-80oC) and 5 minutes, respectively. Moreover, the freezing (at-4^{o}C and -18^{o}C) for 72 and 48 hours, respectively, proved to be sufficient to kill all encysted metacercariae in edible fish. These results were consisted with the finding reported by El-Sherbiny (1988) and Hammed and Elias (1970).

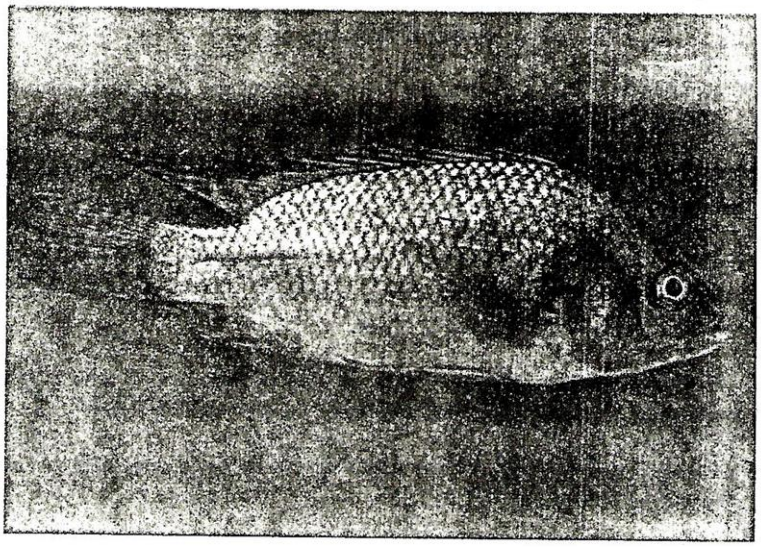

Table 1. Black Spot Disease (Tilapia sp.)

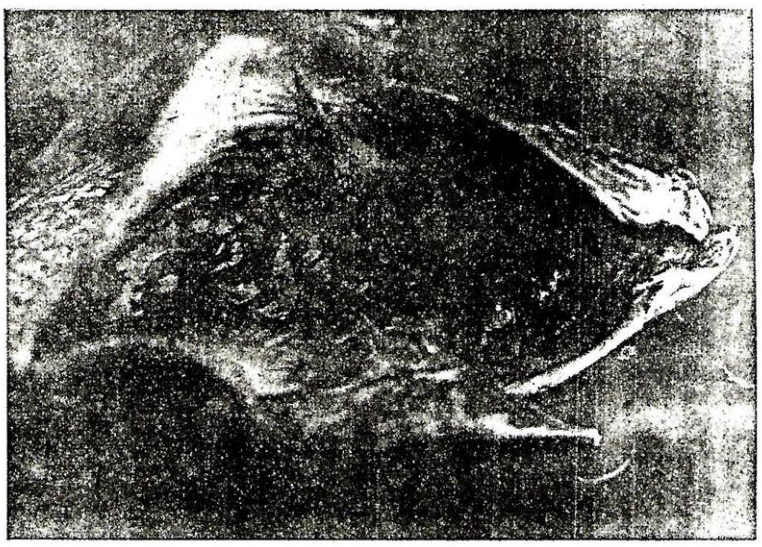

Table 2. Yellow Grub Disease. (dinostomatid metacercariae) in Tilapia sp.

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سلامة وجودة الأسماك المصابة باليرقات المتحوصلة للديدان المفلطحة ثنائية العائل

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اشتملت هذه الدراسة على فحص ٦٠٠ سمكة من الأسماك الطازجة من البورى والبلطى والمبروك وقد جمعت من مزرعة العباسة (بمحافظة الشرقية) من بحيرتى قارون ووادى الريان (بمحافظة الفيوم)ك وكانت نسبة الاصابة بالأطوار اليرقية المتحوصلة لديدان التريماتودا فى اسماك مزرعة العباسة وبحيرتى قارون ووادى الريان ٥٩,٥٪ ، ٥٣٪ على التوالى، ولقد تم عزل ثمانية أنواع من هذه الاطوار اليرقيةك تم اجراء العدوى التجريبية بهذه اليرقات فى بعض الحيوانات الثديية آكله الأسماك مثل (القطط والفئران) وبعض الطيور مثل (ابو قردان والحمام)ك ونتج عن ذلك أربع عشر نوعا من ديدان التريماتودا البالغة جميعها تنتقل للانسان فيما عدا دودة الديبلوستومها تلابى.

وقد تناولت هذه الدراسة ايضا تحديد نسب بعض العناصر الثقيلة مثل الرصاص، الكادميوم، الزئبق النحاس، الزنك فى لحوم الاسماك التى فحصت حيث كانت التركيزات الأعلى لهذه العناصر فى لحوم الأسماك الأكثر، اصابة، وكانت قيم الرصاص فى لحوم العينات التى فحصت من مزرعة العباسة وبحيرة قارون أعلى من الحد المسموح به من قبل منظمة الصحة العالمية (١٩٨٤). بالإضافة الى ذلك كانت قيم الزنك بالأسماك التى تم فحصها من مزرعة العباسة أعلى من الحد المسموح به من قبل منظمة الصحة العالمية (١٩٨٤). بينما سجلت العناصر الأخرى قيما فى الحدود المسموح بها عالميا.

ولقد اظهرت الدراسة ان هناك علاقة عكسية بين معدل الإصابة بالأطوار اليرقية المتحوصلةومعدل الخواص الحسية المرغوب بها. كما تضمنت هذه الدراسة ايضا طرق معالجة لحوم الأسماك المصابة ب الحويصلات اليرقية بالحرارة مثل (القلى والشى والتسخين فى فرن الميكروويف) لمدة ٥ دقائق، ١٠دقائق عند درجة ٦٠ – ٨٠ م ٥ ، ٥ دقائق (عند القوة العالية) على التواليك بينما المعالجة بالتجفيد لمدة ٧٢ ساعة عند درجة – ٤م٥ ولمدة ٤٨ ساعة عند درجة – ١٨م ٥ كانت كافية لموت جميع الأطوار اليرقية المتحوصلة.