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PREVALENCE OF METACERCARIAL INFECTION IN SOME MARKETED FISH IN GIZA GOVERNORATE, EGYPT

BY

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Abstract

A total of 180 *Oreochromis niloticus* and 120 *Clarias gariepenus* of different weight and length were randomly collected from three different markets (El-Moneib, El-Ayiat and El-Badrashin) in Giza Governorate from January to June 2012. Muscles and organs of the fishes were examined for infective zoonotic parasites. The total prevalence of encysted metacercariae (EMC) among examined *O. niloticus* was 82.8%, the highest prevalence was in the tail (75%), while the lowest one (17%) in skin, and in the gills was (57.2%). Total prevalence of EMC was 35.8% in the *C. gariepenus*, the highest was in trunk (27.5%), then head & tail (25.8% &25%) and the lowest one (4.1%) was in the skin but none in the gills. There was an indirect relationship between the EMC prevalence and the fishes weight and length. The recovered EMC from *O. niloticus* were*; Diplostomatidae, Cyathocotylide* and *Heterophyidae*. Besides, *Cyanodiplostomatidae* and *Cyathocotylide* were recovered from *C. gariepenus*. These EMC were successfully developed to adults after experimental infection to pigeons and rats. The emerging adult flukes were *Prohemistomum vivax*, *Mesostephanus appendiculatus* and *Centrocetus armatus*.

Key words: Freshwater fishes, *Clarias gariepinus*, *Oreochromis niloticus*, Zoonotic trematodes, EMC.

Introduction

The expansions in the production of fishes from both natural water resources and fish cultures have been widely spread all over the world to compensate the shortage of good quality animal protein. So ,as fish considered one of the most valuable nutritive ,tasty palatable and easily digested protein ,it also act as host for many parasitic diseases which may be harmful for man and fish eating mammals if it consumed raw or lightly cocked (El- Naffar *et al*, 1985). The consumption of encysted metacercariae developed diseases to human consumer (Hernandez *et al*, 1998). The WHO (1995) estimated that the number of people currently infected with fish-borne parasite trematodes exceed 18 million, but worldwide the number of people at risk, including those in developing country is more than half a billion (Chai *et al*, 2005). In Egypt, metacrcarial infections are the main larval digenean causing severe economic loss among fishes in both open water resources and fish culture. Also, many of metacercaria in fish have public health importance (Park *et al*, 2009). In Egypt, the endemic of encysted metacercarial infection in *Oreochromis niloticus* and *Clarias gariepenus* fishes were reported (Abdallah *et al*, 2009).

The present study, clarified the role of *Oreochromis niloticus* and *Clarias gariepenus* as a source of parasitic infection of public health importance.

Material and Methods

A total of 300 fishes (180 *O. niloticus* and 120 *C. gariepenus*) of different weight and length were randomly collected from three different located markets (El-Moneib, El-Ayiat and El-Badrashin) in Giza Governorate from January to June 2012. The collected fishes were put in ice-boxes, and immediate transported to the laboratory.

They were identified by species, and then weight and length were determined. They were clinical examined (Burgess *et al*, 1999).

Table 1: Number and average weight of fishes.

Fish weight	*O. niloticus*	*C. gariepenus*
< 50gm	81	0
50-100gm	65	12
100-150gm	11	45
150-200gm	13	13
200-250gm	10	16
250-300gm	0	25
300-400gm	0	9
Total	180	120

Table 2: Number and average length of fishes.

Length	*O. niloticus*	*C. gariepenus*
< 10cm	7	0
>10-15cm	126	2
>15-20cm	31	2
>20-25cm	16	34
>25-30cm	0	44
>30-35cm	0	35
>35- 4 cm	0	3
Total	180	120

Macroscopical examination was done (Mahdy *et al*, 1995) to detect any abnormalities using a hand lens. Microscopic examination was done after compression between 2 slides, fixation and staining (Garcia, 2001) to detect EMC lodged in/or attach to different organs and tissues, which were gently removed (Srisawangwong *et al*, 1997).

Clean laboratory bred ten rats of 3-weeks old males and ten pigeons of 2-weeks old males were divided into 2 groups of 5 each. G1 was fed on EMC isolated from *O. niloticus* and G2 was fed on EMC from *C. gariepenus* (Tab. 3). G3 included 2 rats and 2 pigeons without infection as control negative group. All were kept in separate cages under laboratory conditions.

Stool samples from the three groups were daily examined after being processed for trematode eggs to determine the prepatent periods. On detection of characteristic eggs in feces of experimented with rats and/or pigeons, they were sacrificed and dissected out carefully to recover the adult worms. The small intestine was subdivided into three parts (anterior, middle and posterior) and each part was separately opened. The contents and scraped mucosa of each region were collected in suitable jars containing normal saline, and washed several with normal saline to remove coarse particles of intestinal contents and mucous attached to the worms. Sediments were examined under a binocular microscope; the detected trematodes were collected in normal saline, counted and then picked up by Pasteur pipette in a 10% formalin small bottle.

Adult worms and encysted metacercariae were processed for permanent preparation (Pritchard and Kruse, 1982), and identifications (Yamaguti, 1971).

Results

The results were given in tables (3, 4, 5, 6, 7 & 8) and figures (1, 2, 3, 4, 5, 6 & 7).

Table 3: Infection of rats and pigeons with EMC from *Oreochromis niloticus* and *Clarias gariepenus*

Fish	EMC site	Type of EMC	Rats EMC dose	Pigeons EMC dose
G1: *O .niloticus*	Muscles	*Cyathocotylide & Diplostomatide*	500	500
	Gills	*Cyathocotylide & Heterophyide*	500	500
G2: *C. gariepenus*	Muscles	*Cyathocotylide &Cynodiplostomatide*	1200	1200

Table 4: Prevalence of different types of recovered EMC from examined fish species.

Fish species	No. examined	No. infected	%	Recovered EMC from examined fish
O. niloticus	180	149	82.8	*Diplostomatidae, Cyathocotylidae, Heterophyidae*
C. gariepenus	120	43	35.8	*Cyathocotylidae, Cynodiplostomatidae*
Total	300	192	64.0	Four types

Table 5: Prevalence and distribution of EMC in examined fishes.

Fish species	*Oreochromis niloticus*			*Clarias gariepenus*		
Infection site	No. examined	No. infected	%	No. examined	No. infected	%
	180	149	82.8	120	43	35.8
Tail	180	135	75.0	120	30	25.0
Trunk	180	99	55.0	120	33	27.5
Head	180	87	48.3	120	31	25.8
Gill	180	103	57.2	120	0	0
Skin	94	16	17.0	120	5	4.1
Eye	94	37	39.4	120	14	11.7

Table 6: Statistical analysis of EMC number in examined fishes.

Examined parts or organs	Type of fish	Minimum	Maximum	M±SD
Tail	*O. niloticus*	4	700	35.42 ± 69.49
	C. gariepenus	3	400	22.72 ± 71.99
Trunk	*O. niloticus*	1	700	14.14 ± 57.57
	C. gariepenus	1	350	13.45 ± 48.53
Head	*O. niloticus*	3	700	13.75 ± 56.57
	C. gariepenus	1	300	12.21 ± 41.16
Gill* filament	*O. niloticus*	1	6	1.16 ± 1.30
	C. gariepenus	0	0	0.00 ± 0.00
Skin*	*O. niloticus*	1	80	2.91 ± 10.70
	C. gariepenus	2	50	0.66 ± 4.75
Eye*	*O. niloticus*	1	200	9.86 ± 27.33
	C. gariepenus	4	150	2.93 ± 15.63

*significant, p value ≤ 0.05 between number of EMC in O. *niloticus* and *C. gariepenus*

Table 7: Frequency distribution of EMC in relation to weight of examined fishes

Fish / Weight	*O. niloticus*			*C. gariepenus*		
	No. examined	No. infected	%	No. examined	No. infected	%
< 50 g	81	77	95.1	-	-	-
50 < 100 g	65	57	87.7	12	5	41.7
100 < 150 g	11	8	72.7	45	18	40
150 < 200 g	13	6	46.2	13	3	23.1
200 < 250 g	10	1	10	16	4	25
250 < 300 g	-	-	-	25	12	48
300 < 400 g	-	-	-	9	1	11.1

Table 8: Frequency distribution of EMC in relation to length of fishes.

Fish / Length	*Oreochromis niloticus*			*Clarias gariepenus*		
	No. examined	No. infected	%	No. examined	No. infected	%
< 10 cm	7	6	85.7	-	-	-
10 < 15 cm	126	118	93.7	2	1	50
15 < 20 cm	31	20	64.5	2	2	100
20 < 25 cm	16	5	31.3	34	12	35.3
25 < 30 cm	-	-	-	44	16	36.3
30 < 35 cm	-	-	-	35	12	34.3
35 < 40 cm	-	-	-	3	0	0

Table 9: Infection of rats and pigeons with EMC from *O. niloticus* and *C. gariepenus*:

Group No.		G1 (EMC from *O. niloticus)*		G2 (*C. gariepenus)*
Types of EMC		*Cyathocotylidae, Diplostomatidae*	*Cyathocotylidae, Heterophyidae*	*Cyathocotylidae Cynodiplostomatidae*
Rat	No. Examined	2	2	2
	Dose of EMC	500	500	1200
	No. infected	2	2	2
	recovered flukes	360	30	930
	Recovery rat	36%	3%	38.75%
Pi-geon	No. Examined	2	2	2
	Dose of EMC	500	500	1200
	No. infected	2	0	2
	recovered flukes	110	0	600
	Recovery rat	11%	0	25%
Identification of recovered Fluke		*Prohemistomum vivax* *Mesostephanus appendiculatus*	*Centrocetus armatus* *Prohemistomum vivax* *Mesostephanus appendiculatus*	*Prohemistomum vivax* *Mesostephanusappendiculatus*

Discussion

In the present study, the overall EMC infection was 64.0%. These were 82.2% and 35.8% in *O. niloticus* and *C. gariepenus*, respectively. The highest one was (75.0% & 27.5%) in tail and trunk region, respectively. The lowest was found in skin (17.0% & 4.1%), respectively. There was a significant difference in number of EMC between the gill filament, skin and muscles of *O. niloticus* and *C. gariepenus* ($p< 0.05$) but without significant difference in number of EMC recovered from head, trunk and tail of *O. niloticus* and *C. gariepenus* ($p>0.05$).

In the present study, *O. niloticus* weight, prevalence of EMC was higher (95.1%) in weight less than 50gm. Lowest one (10.0%) in fishes ranged from (200-250/g). The highest prevalence of EMC (48.0%) was in fishes' weight from (250-300/g) in *C. gariepenus*, but the lowest (11.1%) was in fishes' weight from (300-400/gm). The *O. niloticus* & *C. gariepenus* highest prevalence of EMC was (93.7% & 100%) in length (10-1\cm & 15-20cm), while lowest one was (31.3% & 0.0%) was in length from (20-25 cm & 35-40cm).

In the present study, the EMC recovered were *Cyathocotylide, Cynodiplostomatidae, Heterophyidae* and *Diplostomatidae* metacercariae recovered from muscles and gills from of examined *O. niloticus* and *C. gariepenus*. By experimental infection trematodes recovered were *Centrocetus armatus, Mesostephanus appendiculatus* and *Prohemistomum vivax*.

In the present study, the overall detected EMC in infected *O. niloticus* was (82.2%) for the totally examined fish. These results agreed with Abo-Esa and Shaheed (2003) who recorded infection of 80%. But, the present results were more or less high than that reported by Abu-El-Ezz *et al*. (2000) and Elsheikha and Elshazly (2008), which were 74.33% &23.2% respectively. The detected EMC was (35.8%) in *C. gariepenus*. This result was lower than those reported by El-Gohry and Samah (1996), and Saleh *et al*. (2009), which were 68% & 87.1% respectively. These differences might be related to the water sources of the studied fishes, habitat whether rural or urban and/or the seasonal time of fishes' collection..

In the present study, highest number of EMC in *O.niloticus* was detected in the posterior body third or tail region (75%) followed by the middle third (55%) and the lowest was in the anterior third (48.3%) with the lowest number in the skin (17%). In *C. gariepenus* the number of EMC was 25.8 % in head region, 27.5 % in trunk and 25% in tail muscles without EMC in gills. These results agreed with El-Naffar and El-Shahawy (1986) and Mahmoud and Sahlab (1993).

In the present study, the highest average number of EMC in *O. niloticus* was in muscles of tail region (35.42±69.49)/g followed by trunk region (14,14±57,57) and lastly head region (13,75±56,57). Average numbers of EMC in different organs were (1.16±1.30)/gm gill filament, (2.91±10.7)/ gm skin, and (9.86±27.33)/eye. The result agreed with Mahmoud and Sahlab (1993), but disagreed with Waheb and Abo-Esa (2002) and Taher (2009). In *C. gariepenus*, highest EMC average numbers were detected in tail muscles (22.72±71.99)/gm, followed by trunk muscles (13.45±48.53)/gm and head muscles (12.21±41.16)/gm. EMC average number was (0.66±4.75)/gm in skin and (2.93±15.63)/eye but, none in gills. These results differed from those recorded by Saleh *et al*. (2009). There was a significant difference of the number of EMC between (gill filament, skin and eye) of *O. niloticus* and *C. gariepenus* ($p< 0.05$), without significant difference in number of EMC between head, trunk & tail in *O. niloticus* and *C. gariepenus*. Variation in number and pattern of EMC distribution might be due to size difference and/or immunological response.

In the present study, number of EMC and weight and length of *O. niloticus* & *C. gariepenus*, EMC was higher (95.1% & 48.0%) in weight less than 50gm & 250-300/gm and

the EMC in relation to length was (93.7% & 100%)) in fish with length ranged from (10-15cm & 15-20cm). These results more or less agreed with El-Bouhy *et al*. (1988).

In the present study, the EMCs were *Diplostomatidae, Heterophyidae, Cyathocotylide* and *Cynodiplostomatid*ae recovered from muscles and gills of *O. niloticus* and *C. gariepenus*. The EMCs were previously recorded from the same fish spp. in Manzala Lake and River Nile in Upper Egypt by Mahdy *et al*, (1995) and Mousa *et al*. (2000) respectively. The trematodes recovered were *Prohemistomum vivax, Mesostephanus appendiculatus* from small intestine of rats and pigeons and *Centrocetus armatus*, from small intestine of rats. These results agreed with Mahdy *et al*. (2000), Saleh *et al*. (2009) and Abu El- Ezz *et al*. (2000).

In the present study, *P. vivax* and *M. appendiculatus* adult worms were detected in infected pigeons and rats with EMC from muscles of examined fish species. This result agreed with Saleh *et al*. (2009). Paperna (1980 and Abo-Shady (1980) recorded *P. vivax* in both man and animals and Williams and Jones (1976) reported fatal zoonotic infection due to *M. appendiculatus.* Besides, El-Azazy *et al.* (2015) in Kuwait recovered *M. appendiculatus* from stray cats and concluded that cats are good indicators of fish-borne trematodes in the environment.

In the present study, adult of *Centrocetus armatus* were obtained from small intestine of experimentally infected rats with EMC from the gills of examined *O. niloticus*. This result agreed with Abu El-Ezz *et al.* (2000). Hong *et al*. (1988) in Korea detected the first human zoonotic *C. armatus* and Chai and Lee (1990) stated that the *C. armatus* have been proven to be the main source of zoonosis by eating raw infected fish.

Conclusion

The presence of four types of encysted metacercariae from fishes used for human consumption is risky health problem. This is especially true with consumption of raw or insufficiently cooked fishes.

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Explanation of figures

Fig. 1: a-Cyathocotyidae EMC in *O. niloticus*, b- Cyathocotyidae EMC of *C. gariepenus* (scale bar 0.1mm)
Fig. 2: Cynodiplostomatidae in muscles of *C. gariepenus* (scale bar 0.1mm)
Fig. 3: Heterophydae EMC in gill filaments of *O. niloticus* (scale bar 0.1mm)
Fig. 4; *Diplostomatidae EMC in O. niloticus* (scale bar 0.2mm),
Fig. 5: Adult *Centrocetus armatus* recovered from experimentally infected rat (scale bar 0.1mm),
Fig. 6: Adult *Mesostephanus appendiculatus* recovered from experimentally infected pigeon & rat (scale bar 0.2mm),
Fig. 7: Adult *Promehistomum vivax* recovered from experimentally infected pigeon & rat (scale bar 0.2mm),