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Fish as a Potential Source of Parasites of Public Health Importance in El-Minia Governorate, Egypt

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Abstract:
This study was conducted to estimate the infection with larval helminth parasites of freshwater fishes. For this purpose, a total of 200 freshwater fish of different body weights represented as 100 *Clarias lazera* and 100 *Tilapia nilotica* were collected randomly, from May 2017 to April 2018 from fish markets in El Minia city. The overall incidence of metacercariae infestation (60%) was significantly higher in *C. lazera* 70.0%, than *T. nilotica* 50.0%, while the incidence of infestation with 3rd larvae of *Contracaecum sp*. was found significantly higher in *C. lazera* 20.0% than in *T. nilotica* 5.0% at *(P*≤ 0.05). Three types of encysted metacercariae were detected in *T. nilotica* namely metacercaria of *Prohemistomum vivax, Clinostomum phalacrocoracis* and *Haplorchis species,* while *P. vivax* was found only in *C. lazera*. It was observed that the highest distribution percentage of the metacercariae in the tail region in both fish species with a percent of (50%) in *T. nilotica* but with (57%) in *C. lazera* and it was absent in the head region. Seasonally; the highest incidence with metacercariae in *T. nilotica* was found in summer and spring which was 65% and 60% respectively. The lowest incidence was found in winter (28%), but in *C. lazera*, the highest incidence was found in summer (87%) and spring (66%), while the lowest incidence was found in winter (38%). The 3rd larvae of *Contracaecum* were found in *T. nilotica* during summer (12.5%) and in *C. lazera* during summer (37.5%) and spring (16.6%). The higher incidence of parasites in fish represents the potential public health risk, therefore public awareness creation activities should be conducted on the zoonotic nature of fish parasites and danger of consumption of raw or undercooked fish.

Keywords: *Clarias lazera*, *Tilapia nilotica*, metacercariae, public health, fresh water fish.

INTRODUCTION

Fishes are considered as one of the most valuable nutritive, tasty, palatable and easily digested protein for human. Also considered as one of the main source of animal protein with low cholesterol level in human diets (Hadyait *et al.,* 2018). But it may harbor many pathogens which constitute great problems either in cultured or wild fishes as they limit the fish production especially in subtropic countries like Egypt (Elamei, 2001).

Various diseases including parasitic infections pose a threat to fish cultivation which is a valuable source of food and employment in developing countries (Yooyen *et al.,* 2006; Ali *et al.,* 2016). In addition to the economic loss to farmers, many of the parasites, particularly trematodes, are also of zoonotic importance (Iqbal and Ashraf, 2017). Eating raw or improperly cooked or processed fish is the main source of these infections for humans, and this has been reported from various geographical regions (Shamsan and Al-Jobory, 2018). The World Health Organization (WHO) has estimated that the number of people infected with fish-borne trematodes exceeds 18 million, and many more are at risk (WHO, 1995). In Egypt, parasitic diseases represent about 80% of fish diseases (Eissa, 2006).

Some internal parasitic diseases affecting *Tilapia nilotica* have a public health importance such as, yellow grub which can be transmitted to human as a result of ingesting raw or improperly cooked fish and causing Halzoun like disease leading to laryngopharyngitis (Wiliams and Jones, 1976).

Fishes act not only as final host but also as intermediate host for larval stages of many parasites like encysted metacercariae of different species of trematodes. These metacercariae which affect fish may cause retardation of growth especially for young fish and increasing the possibility of secondary infections by decreasing fish immunity, moreover, massive infection with metacercariae may be lethal to fish especially young *Tilapia nilotica* (Elamei, 2001).

In view of the importance of these parasites, the encysted metacercariae have the upper hand in fish parasitic diseases. The presence of metacercariae is very common especially in freshwater fish as that may cause public health problems (Eissa, 2002). Although nematodes may be important pathogens of fish, their role in transmitting parasites to humans, who become accidental hosts, are of greater concern. Among the zoonotic nematodes species that have been found are *Capillaria spp.* and *Anisakis* which can be transmitted to human through unhealthy consumption of infected fish species (Amer, 2014). Hence this study was conducted to identify and estimate the prevalence of the larval helminth parasites of public health importance in freshwater fishes such as *Tilapia nilotica* and *Clarias lazera* in relation to distribution of the larval helminth parasites in infected fishes and their seasonal dynamics.

MATERIALS AND METHODS

Samples collection, examination, and metacercariae isolation

A total of 200 freshwater fish of different body weights represented as 100 *Clarias lazera* and 100 *Tilapia nilotica* were collected randomly during the period from May 2017 to April 2018 from fish markets in El-Minia city and brought separately in plastic bags to the laboratory where they were kept on the lower shelf of the refrigerator (10ºC) and examined within 24 hrs, samples were carefully examined by the naked eye for detection of any macroscopic encysted metacercariae in musculature and gills, then; microscopic examination was carried out by taking small snips of muscles from different regions of the body (head, trunk, and tail), each snip was compressed between two glass slides and examined under binocular dissecting microscope (Morishita *et al.,* 1965). Encysted

metacercariae were isolated by dissecting needles under a binocular dissecting microscope after tissue digestion by pepsin HCL (Yokogawa and Sano, 1968).

Identification of metacercariae

A total of 10 laboratory reared albino mice 6-8 weeks old weighing 200-300 gm were fed singly with large number of collected viable metacercariae, in a trial to get the adults of the obtained encysted metacercariae, the stool of the previously mentioned hosts was examined daily from the 3rd day after infection for detection of eggs by formalin-ether sedimentation concentration technique (Bleding, 1965). After detection of the eggs in the stools, mice was sacrified, intestines were cut longitudinally from the pylorus to caecum, placed in petri-dish containing 0.5% saline, opened and mucosa scraped by the edge of a glass slide (El-Assal, 1974).

Identification of collected nematode larvae

Muscles as well as gills, body cavity, alimentary tract, and liver of collected fishes were taken out and examined in physiological saline on a Petri dish and washed several times by 0.7% saline solution to get rid of any adhered mucous (Baron *et al.,* 2007; Roberts *et al.,* 2012). Samples were examined for the presence of any nematodes larvae for identification and characterization according to Scholz et al. (2009).

Statistical analysis

The obtained data was subjected to analysis of variance (ANOVA). Duncan's multiple range tests was used to determine significance among means and result was considered significant at $P < 0.05$.

RESULTS

Results of the microscopic examination revealed that at 10 X, the viable metacercariae was elliptical in shape, lying in between the muscle fibers, size ranged from 300-320±0.023 µm in length and 310-350 ±0.054 µm in width (Figure 1a). The metacercaria is usually folded inside its cyst wall, the encysted metacercaria in tail muscle is surrounded by two thick cyst walls, and the outer one is thicker than the inner one at 40 X. Figure 1b,c showed the metacercaria of *Prohemistomum vivax* at 40 X is surrounded by two thick cyst walls, the outer one is thicker than the inner one as in Figure 1d, and it is surrounded at either pole by an adipose tissue around the cyst wall either completely or partially while other metacercariae were surrounded by masses of small fat globules at either poles of the cyst. Figure 1e revealed the adult of *Prohemistomum vivax*that was recovered from jejunum of experimentally infected mice X40. Figure 1f showed the adult of *Clinostomum phalacrocoracis* which is characterized by its elongated body, fleshy and tongue shaped, and it measures 1.2±0.063 - 1.4±0.012 cm in length and 2±0.094 - 3 ± 0.014 mm in maximum width. The oral sucker is surrounded by a collar like fold. It is subterminal, rounded and smaller than the ventral sucker. It measures 0.7- 0.8 mm in diameter. The ventral sucker is large and situated in the anterior fifth of the body. It measures 1.2-1.3 mm X 1.4-1.6 mm. Oesophagus is short. There is a small pharynx. The intestinal caeca are long, end blindly and extend to the posterior end of the body. The intestinal caeca run midway between the midline and lateral margins of the body. Figure 1g represents *Clinostomum phalacrocoracis* metacercariae, these macroscopic metacercariae were found in the branchial and pharyngeal regions. It was also found free between muscle bundles and tissues around gills of *Tilapia nilotica.* Figure 1h represent *Haplorchis species* encysted metacercariae, it was ovoid in shape and small in size measuring 150±0.061-170±0.013 µm in length and 130±0.053 – 150±0.066 µm in breadth. The cyst wall thickness was 1.4 µm. The metacercariae are characterized by the presence of a dark excretory vesicle filled with the excretory granules at the caudal end. Figure 1i,j,k showed

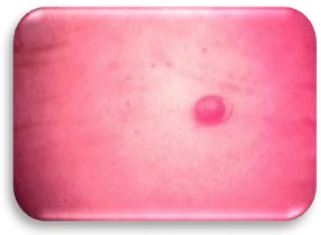
the 3rd stage larvae of *Contracaecum sp* found free in pericardial cavity and gills, they were reddish-yellow in color. The body was long, cylindrical and yellowish in color with the anterior and posterior end red in *Tilapia nilotica* and *Clarias lazera*. The cuticle was smooth and transversely striated with regular and irregular ring forms which were narrower anteriorly and became wider as extended posteriorly.

Statistical analysis of results revealed that; only 60% of examined samples were infected with one or more species of encysted metacercariae of *Prohemistomum vivax, Clinostomum phalacrocoracis* and *Haplorchis species*, and 25% were infected with 3rd stage larvae of *Contracaecum* sp. The overall incidence of metacercariae infestation was significantly higher in *Clarias lazera* 70.0%, than *Tilapia nilotica* 50.0%, similarly, the incidence of infestation with 3rd stage larvae of *Contracaecum sp*. was found significantly higher in *Clarias lazera* 20.0% than in *Tilapia nilotica* 5.0% at *(P*≤ 0.05).

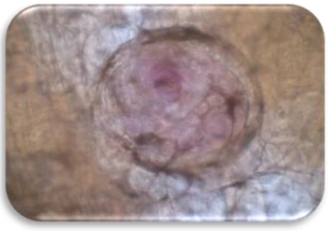
Table 1. Microscopic examination and seasonal prevalence of parasitic infestation from collected *Tilapia nilotica* and *Clarias lazera* fish.

Fish species	Season	Ex.	Infected						S.E	P value
			Encysted Metacercaria			% Infection	Nematode larvae	% Infection		
			a	b	c		d			
Tilapia nilotica	Summer	40	7	13	6	65	5	12.5	0.04	0.034*
	Spring	15	3	5	1	60	00	00		
	Winter	25	2	1	4	28	00	00		
	Autumn	20	3	2	3	40	00	00		
	Total	100	15	21	14	50%	5	5%		
	Head	100	00	10	1	11	00	00	0.034	0.011*
	Trunk	100	9	4	3	16	00	00		
	Tail	100	13	3	6	23	00	00		
	Abd.cavity	100	00	00	00	00	5	5		
Clarias lazera	Summer	40	35	00	00	87	15	37.5	0.023	0.009*
	Spring	30	20	00	00	60	5	16.6		
	Winter	13	5	00	00	38	00	00		
	Autumn	17	10	00	00	58	00	00		
	Total	100	70	00	00	70%	5	5		
	Head	100	00	00	00	00	00	00	0.02	0.001*
	Trunk	100	23	00	00	23	00	00		
	Tail	100	47	00	00	47	00	00		
	Abdominal cavity	100	00	00	00	00	20	20		

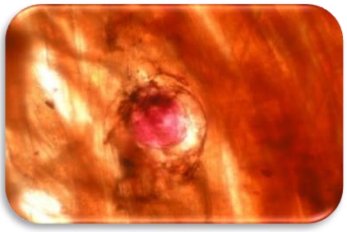
[a]*Prohemistomum vivax*, [b]*Clinostomum phalacrocoracis*, [c]*Haplorchis* species, [d] larvae of *Contracaecum* species.
*significant at (*P* value ≤ 0.05)



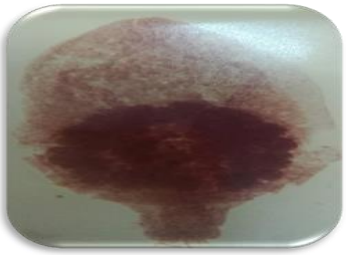
(a)



(b)



(c)



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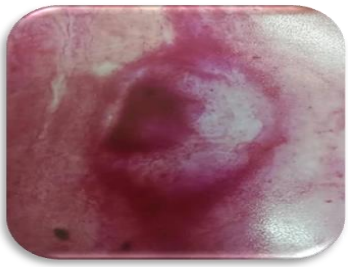
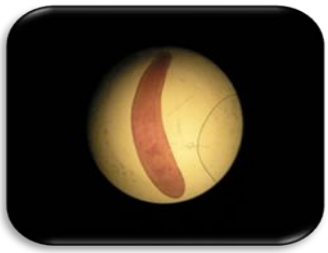
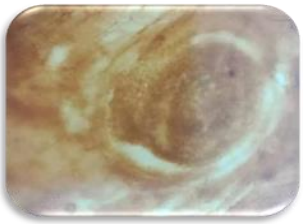


(e)



(f)



(g)

(h)

(i)

(j)

(k)

Fig. 1(a-k). (a) The viable metacercariae at X10. The metacercaria is usually folded inside its cyst wall, the encysted metacercaria in tail muscle is surrounded by two thick cyst walls, the outer one is thicker than the inner one at X40 (b,c). The metacercaria of *Prohemistomum vivax* at X40 (d), (e), the adult of *Prohemistomum vivax* X40. (f) the adult of *Clinostomum phalacrocoracis.* (g) *Clinostomum phalacrocoracis metacercariae in tissues around gills of Tilapia nilotica.* (h) *Haplorchis species* encysted metacercariae,. (i,j,k) showed the 3rd stage larvae of *Contracaecum sp.,* found free in pericardial cavity and gills, in *Tilapia nilotica* and *Clarias lazera.*

Results showed that *Clarias lazera* harbor only one species of metacercariae which was *Prohemistomum vivax* and percent of infestation was 70%, but *Tilapia nilotica* harbor all three recovered species of metacercariae of *Prohemistomum vivax, Clinostomum phalacrocoracis* and *Haplorchis species*it and percent of infestation was 50%, also mixed infection with more than one species was recorded 15%. For the infestation of different body regions with metacercariae, It was observed that the highest distribution percentage of the metacercariae was condensed in tail region in both fish species with a percent of (23%) in *Tilapia nilotica* and (47%) in *Clarias lazera* and it was absent in head region, also it was noted that *Clinostomum phalacrocoracis* only infest head region in muscles around gills and branchial region, on the other hand; *Haplorchis species* was condensed in tail region and this concluding that there was a significant correlation between infestation with metacercaria and tail regions, also between 3rd larvae of *Contracaecum* sp and abdominal cavity at *(P*≤ 0.05). Belonging to the seasonal distribution of parasites infestation; there was a significant correlation between the season and parasitic infestation at *(P*≤ 0.05) and it was found that, the highest incidence with metacercariae in *Tilapia nilotica* was found in summer and spring which were 65% and 60% respectively, but the lowest incidence was found in winter (28%), on the other hand; in *Clarias lazera*, the highest incidence was found in summer (87%) and spring (66%), while the lowest incidence was found in winter (38%). The 3rd larvae of *Contracaecum spp.* was absent in *Tilapia nilotica* except in summer (12.5%). While in *Clarias lazera* it was found in summer (37.5%) and spring (16.6%), and absent in winter and autumn.

DISCUSSION

The overall prevalence of parasitic infestation in tissues of *Tilapia nilotica* and *Clarias lazera* in the present study was relatively low. The obtained results were lower than that obtained by some researchers as Sahar et al. (2009) in Sharkia (Egypt), who reported 84.8% of examined *Tilapia sp*. Moreover, Taher (2009) recorded that the total prevalence of infected *Oreochromis niloticus* in Assiut Governorate with different encysted metacercariae was 84.75%. Youssef (2015) observed that the prevalence of encysted metacercariae in muscles of *Tilapia nilotica* was 70.6%. While, the metacercarial infestation rate of *Clarias lazera* was 70%. Sahar et al. (2009) reported 86.8% prevalence of metacercarial infestation in Sharkia (Egypt). Youssef (2015) recorded 92% prevalence of *Clarias lazera* metacercarial infestation. For distribution of encysted metacercariae in different parts in tissues of *Tilapia nilotica* and *Clarias lazera*; belong to *Tilapia nilotica*, according the present study, the highest incidence of encysted metacercariae, was detected in tail region, than trunk region while the lowest was in head region. Similar pattern of metacercarial distribution was obtained from Northern Egypt by Salem et al. (2010) who observed significant differences in encysted metacercaria occurrence among body regions of tilapia fish with muscle of the tail and caudal third being highly affected (93.4%) followed by middle third (84.3%) and anterior third (75%) while the head region had lowest infection (21.97%). Youssef (2015) recorded 84.9% infestation in the posterior region, followed by the middle region (66%) and lowest was in the anterior region (39.6%). In the present study, the highest incidence percentage of encysted metacercariae in the examined *Clarias lazera* was detected in the posterior part followed by the middle part while absent in the anterior part. These results agree with, reported by Youssef (2015) who indicated that the highest infestation in the posterior region (93.47%) followed by the middle region (71.73%) and the lowest infestation was reported in the anterior region (43.47%). Accordingly for seasonal variation and prevalence of encysted metacercariae in tissues of *Tilapia nilotica* and *Clarias lazera*; in the present study, the prevalence of microscopic

encysted metacercariae in the tissues of *Tilapia nilotica* and *Clarias lazera* during different seasons were recorded. The highest incidence of microscopic encysted metacercariae in the tissues of *Tilapia nilotica* was recorded during the summer followed by the spring then the autumn and the winter and this may be due to the suitability of temperature and humidity for reproduction of parasite and susceptibility for infestation, similarly, parasitic infestation rate of *Clarias lazera* during summer, spring, autumn and winter seasons were in descending order. The obtained results were in agreement with Saleh et al. (2009) who recorded that the highest infection rate of zoonotic trematodes in freshwater fishes in Port Said Province was in the summer (95.9%) followed by the spring (84.4%) then the autumn (80.6%) and the lowest infection rate was in the winter (49.2%). Ibrahim and Soliman (2010) observed that the highest infection prevalence of heterophyid metacercariae in Ismalia city was in the summer (98.16%) when compared with that in the autumn (97.63%) and the winter (80.76%). Moreover, Salem et al. (2010) found that the highest prevalence of heterophyid metacercariae in tilapia fishes in Northern part of Egypt was in the summer (46.4%) followed by the spring (37.5%) then the autumn (27.3%) and was lowest in the winter (15.46%). Hegazi et al. (2014) reported that the highest prevalence of heterophyid encysted metacercaria in infected fishes in Dakahilia Governorate was in the summer, followed by the spring and the autumn, while the lowest was in the winter. On the other hand; regarding the prevalence of 3rd larvae of *Contracaecum sp.* of *Tilapia nilotica* and *Clarias lazera* in the present study, it was found that the higher incidence of infestation with 3rd larvae *of Contracaecum sp*. was in *Clarias lazera* (20.0%), while the lower was in *Tilapia nilotica* (5.0%). The determined results were in agreement with some researchers such as Al-Bassel (2003) who recorded that the highest incidence (20%) was reported in *Lates niloticus* and the lowest (6%) was in *Clarias lazera*. Younis et al. (2017) examined nine fish species from Lake Nasser, Egypt and found that the highest prevalence was recorded in *Lates niloticus* (100%).

CONCLUSION

The occurrence and diversity of parasites and metacercaraie of different trematode parasites and larval nematodes in the muscles of *Tilapia nilotica* and *Clarias lazera* fishes in El-Minia governorate highlight the likelihood of disease outbreak in the aquatic ecosystems. This calls for raising awareness in fish health management and the application of appropriate control measures. Additionally, the finding of *Clinostomum sp.*, and larvae of *Contracaecum sp.* represents the potential public health risks, therefore public awareness creation activities should be conducted on zoonotic nature of fish parasites and danger of consumption of raw or undercooked fish.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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