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*Original Research Article*

# Impact of fish infected with encysted metacercariae on the public health, at Cairo District, Egypt

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**Examination of 452 fresh and brackish water fish, from Cairo district, Egypt, representing *Clarias gariepinus, Lebeo noliticus* and *Mugil cephalus*, revealed the occurrence of nine species of encysted metacercariae in *Cl. gariepinus*, belonging to family *Heterophyidae,* two unidentified digenean metacercariae in *L. niloticus* and two unidentified digenean metacercariae in *M. cephalus*. The relationship between parasite prevalence and fish species, seasonal prevalence and fish weight was investigated. The highest rate of infection (73.68%) was recorded in autumn, in *Cl. gariepinus*, while the lowest (54.05%) was observed in summer. Whereas, in *M. cephalus* and *L. noliticus*, the highest prevalence of infection (56.67 and 26.09%, respectively), was recorded, in spring. But, in *M. cephalus* the lowest infection rate (25.82%) was detected in winter, while in *L. niloticus* (12.50%), it was recorded in autumn. The intensity of infection in *Cl. gariepinus* increased with fish weight till 150g while, it decreased in fish weighing more than 200g. Similarly, in *M. cephalus*, the infection rate increased with increase of fish weight till 100g, but was lower in larger fish. In *L. niloticus*, there was no relationship between rate of infection and fish weight; the highest intensity of infection was recorded in fish weighing 100- 150g. The identification of metacercariae was assessed by feeding experimentally two dogs with flesh of *Cl. gariepinus*, containing encysted metacercariae and the recovery of flukes from their intestines. The impact of infection of humans and animals is discussed**.

**Key words**: Trematodes, prevalence of infection, *Heterophyidae*, *Heterophyes heterophyes*, *Pygidiopsis genata*, fish, *Clarias gariepinus, Lebeo niloticus, Mugil cephalus*.

## INTRODUCTION

Fish–born zoonotic trematodes cause great public health problems worldwide. An estimate of 18- 40 million infected people was reported (WHO, 1995, 2004). Meanwhile, there are unknown million infected with intestinal flukes (WHO, 2004; Chai et al., 2009; Melhorn, 2015).

Most fish living in fresh or brackish water contain encysted trematode metacercariae in their flesh (Khalil, 1933; Martin, 1959; El Assal, 1974; Riffaat et al., 1980; Seo et al., 1980 and others). Thus, man and animals may acquire a great variety of digenetic trematodes when ingesting undercooked, poorly salted or raw fish (Kuntz and Chandler, 1956; Kuntz et al., 1958; Nagaty and Khalil, 1961; Sheir and Abou Einen, 1970; Morsy et al., 1981). Hence, fish may play a significant role in transmitting diseases to humans and animals.

Heterophyiasis, an intestinal parasitic disease caused by the trematode fluke *Heterophyes heterophyes* (Siebold, 1852) and other heterophyids, is considered as a zoonotic problem in several countries (Well and Blagg, 1956; Kuntz et al., 1958; Nagaty and Khalil, 1964; Moravec, 1977; Sheir and Abou- El Enein, 1979; Seo et al., 1980, Massoud et al., 1981; Park et al., 2007; Chai et al., 2015; Doi et al., 2017).

Although, most human infection are asymptomic or unrecognized, heavy infection may cause damage to the

intestinal mucosa, abdominal pains, diarrhea sometimes with blood and when some eggs enter the blood or lymph vascular system and migrate to the various organs, they cause granuloma and fibrosis ( Abou- Basha et al., 2000; Macpherson, 2005; El- Sheikha, 2007; Lobna et al., 2010).

Egypt enjoys great water sources which provide people with great amount of fish. Among the fresh and brackish water fish species, *Clarias gariepinus* (Burchell, 1822), *Labeo noliticus* Geoffrey, 1827 and *Mugil cephalus* Linnaeus,1758, constitute an important source of food supply for Egyptian people, especially in low socioeconomic areas. These species have been found to harbour several species of trematode encysted metacercariae (Martin, 1959; Fahmy and Selim, 1959; El Assal, 1974; Moravec, 1977; Rifaat et al., 1980; Abdallah et al., 2009; Ibrahim et al., 2010; Hegazy et al., 2014). Thus, fish infected with encysted metacercariae may cause serious diseases to humans. Heterophyiasis, an intestinal parasitic disease, caused by the infection with *Heterophyes heterophyes* and other heterophids, is considered as a zoonotic problem in some areas of Egypt (Mohamed et al., 2010). Also, in several countries, heterophyiasis is found to be endemic (Chai et al., 2004; Park et al., 2007; Mahanty and John, 2011; Chai et al., 2015; Traub and Dalsgaard, 2016; Doi et al., 2017).

Therefore, this study aims to investigate the incidence of infestation of three freshwater and brackish water fish (*Cl. gariepinus, L. noliticus and M. cephalus)* with encysted metacercariae, the seasonal infection prevalence and relation of fish weight and degree of infection and discuss its impact on human health and domestic animals.

## MATERIAL AND METHODS

Four hundred and fifty two freshwater and brackish fish( *Cl. gariepinus, L. noliticus and M. cephalus*) collected from several fish markets, at Cairo district ( El Sayeda, Boulak El Dakrour, Giza, El Zawya El Hamra and El Kanater), were transported to the laboratory, in ice-boxes, in the early morning, during one year. Fish were identified according to Bailey (1994) and Bishai and Khalil (1997). To estimate the number of metacercariae, pea sized pieces of fish flesh were dissected out from the right and left sides of the head, trunk and tail regions of each fish. The dissected pieces, stained with vital stain, were compressed between two glass slides and examined under a light microscopy, to search for encysted metacercariae. The highest intensity of metacercariae was observed in the caudal region. Metacercariae were counted and identified. Metacercariae were identified on basis of their morphological characterstics and sizes (Sohn et al., 2009; Elsheikha and El- Shazly, 2008). The prevalence of metacercariae was estimated according to Bush et al. (1997). Fish weights were measured and classified into four classes (1, 2, 3 and 4) of 50g, 50-100g, 100-150g and more than 200g. Comparison between different percentages of infection prevalence, fish species and seasons was performed using Chi-square test. Pearson`s correlation coefficient (r) was used to verify the correlation between fish weight, fish species and infection prevalence. Significant difference was set at $P< 0.05$. All the statistical tests were performed using SAS (Statistical Analysis System) version 9.1. SAS/STAT User`s Guide, SAS Institute Inc.. Cary, NC, USA. Flesh of *Cl. gariepinus*, containing encysted metacercariae, were fed experimentally to two dogs which proved to be free from any worm infection, by three successive faecal examinations and no trematode ova were detected. Moreover, they were given broad spectrum antihelminthic drug (yomesan). Detection of eggs in dog faecal samples were made using the floating sedimentation technique of Soulsby (1968). Recovery of the adult flukes were obtained from intestines of dissected dogs, after the observation of ova in their stool. The intestines were opened longitudinally in a petri dish, washed with saline solution and examined under a stereomicroscope. Worms were collected from intestinal contents and scraped mucosae then put in small bottles containing 10% formalin. The number of metacercariae in *L. noliticus* and *M. cephalus* was insufficient to infect dogs. Therefore, results on flukes are only for those recovered from *Cl. gariepinus* fish. The Carleton technique (1967) was used to prepare permanent stained mounts of encysted metacercariae and adult flukes.

## RESULTS

The total encysted metacercariae prevalence of infection was 42.9%. The infection prevalence differed significantly by fish species ( $x^2 = 33.6528$; P = 0. 008). *Cl. gariepinus* showed the highest infection prevalence (60.66%), when compared with that of *L. niloticus* and *M. cephalus* (36.36%, in both fish species) (Table 1).

The infection prevalence in *Cl. gariepinus*, reached the highest value (73.68 %) in autumn and the lowest in summer (54.05%). While, in *L. noliticus* and *M. cephalus*, the highest prevalence (26.09 % and 56.67%, respectively) was recorded, in spring, in both species. Whereas, in *L. niloticus* the lowest infection rate (12.50%) was reported in autumn and in *M. cephalus* (25.82%), it was observed in winter (Table 2). No significant differences were found among seasons in prevalence of infection of *Cl. gariepinus, L. niloticus* and *M. cephalus*.. The results of the Chi- square test were not significant at $P< 0.05$ ($x^2 = 0.155$, p-value = 0.921; $x^2 = 2.422$, p-value = 0.489; $x^2 = 3.515$, p-value = 0.318, in the three fish species, respectively).

The prevalence of infection in *Cl. gariepinus* increased proportionally with fish weight up to 150g, but was lower in fish weighing more than 150g. In *M. cephalus,* it increased with the increase in fish weight till 100g and was lower in larger fish. While, there was no relationship between the infection rate of *L. niloticus* and fish weight, and the highest rate of infection occurred in fish weighing 100- 150 g. Positive correlations ($P< 0.05$) were found between fish weight classes and prevalence of infection (Table 3).

**Table 1.** Prevalence of infection with encysted metacercariae, in fresh and brackish water fish, from fish markets, Cairo district, Egypt, for one year

| Species of fish | No of examined fish | No of infected fish | % infection |
|---|---|---|---|
| *Cl.gariepinus* | 122 | 74 | 60.66 |
| *L.niloticus* | 176 | 64 | 36.36 |
| *M. cephalus.* | 154 | 56 | 36.36 |
| Total | 452 | 194 | 42.9 |

**Table 2.** Seasonal Prevalence of infection with encysted metacercariae in fresh and brackish water fish, from fish markets, Cairo district, Egypt.

| Season | Fish species | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Cl gariepinus..* | | | *L. niloticus* | | | *M. cephalus.* | | |
| | No Exam. | No, Infect | % infection | No Exam | No Infect | % infection | No Exam | No Infect | % infection |
| **Spring** | 34 | 21 | 60.76 | 46 | 12 | 26.09 | 30 | 17 | 56.67 |
| **Summer** | 37 | 20 | 54.05 | 50 | 9 | 18 | 53 | 17 | 32.08 |
| **Autumn** | 19 | 14 | 73.68 | 56 | 7 | 12.5 | 28 | 11 | 39.29 |
| **Winter** | 32 | 19 | 59.38 | 24 | 6 | 25 | 43 | 11 | 25.58 |

Exam. = examined Infect. = Infected

**Table 3:** Prevalence (%) of infection with encysted metacercariae and fish weight, in fresh and brackish water fish, Cairo district, Egypt

| Species of fish | Weight of examined fish (g) | No of examined fish | No of infected fish | % infection | Pearson's correlation coefficient (r) |
|---|---|---|---|---|---|
| *Cl. gariepinus* | 50- 100g | 35 | 15 | 42.86 | 0.72* |
| | 100– 150 g | 29 | 21 | 72.41 | 0.63* |
| | More than 200 g | 58 | 38 | 65.97 | 0.31 |
| *L. niloticus* | Up to 50 g | 58 | 11 | 18.97 | 0.72* |
| | 50 – 100 g | 56 | 8 | 14,29 | 0.56* |
| | 100 – 150 g | 31 | 8 | 25.80 | 0.07 |
| | More than 150 g | 31 | 7 | 22.58 | 0.74* |
| *M. cephalus.* | Up to 50 g | 32 | 13 | 40.63 | 0.78* |
| | 50 – 100 g | 66 | 31 | 46.97 | 0.93* |
| | 100 – 150 g | 31 | 11 | 35.48 | 0.96* |
| | More than 150 g | 25 | 1 | 4 | 0.54* |

* significant correlation at $P<0.05$.

Among metacercariae recovered, herein, in the flesh of examined fish, nine types of metacercariae, belonging to family *Heterophyidae* (Odhner, 1914), were found in the flesh of *Cl. gariepinus*. While, two unidentified digenean metacercariae were recovered from *L. niloticus* and two unidentified digenean metacercariae were found in *M. cephalus*. In *Cl. gariepinus*, two types of heterophyid metacercariae were distinguished. The first one possesses a single wall cyst and represents seven forms, while the second type has a double walled cyst, the inner being fibrous and the outer hyaline and represents two forms (Figure 1). These two types of metacercariae are divided into three shapes. round, oval and ovoid (Tables 4a, b). The round shaped metacercariae and with single wall exhibit two sizes, one small and the other large. The oval shaped metacercariae have three sizes, a large, a medium and a small one. The ovoid metacercariae show two shapes, differentiated according to the enclosed larva, the first with nearly round larva and the second with almost oval one.

Metacercariae recovered from *L. noliticus* were Digenean I and Digenean II (Table 5; Figure 2). The first one is ovoid with a thin granular wall and ovoid larva, while the second is spherical as well as the encysted larva. In *M. cephalus,* two digenean metacercariae were recovered, Digenean III is ovoid in shape and containing an oval larva. While, Digenean IV is spherical as well as the enclosed larva.

The identification of metacercariae found in the flesh of *Cl. gariepinus* was assessed by experimentally feeding dogs with fish containing the metacercariae and the recovery of adult worms. Two dogs fed on *Cl. gariepinus* infected with

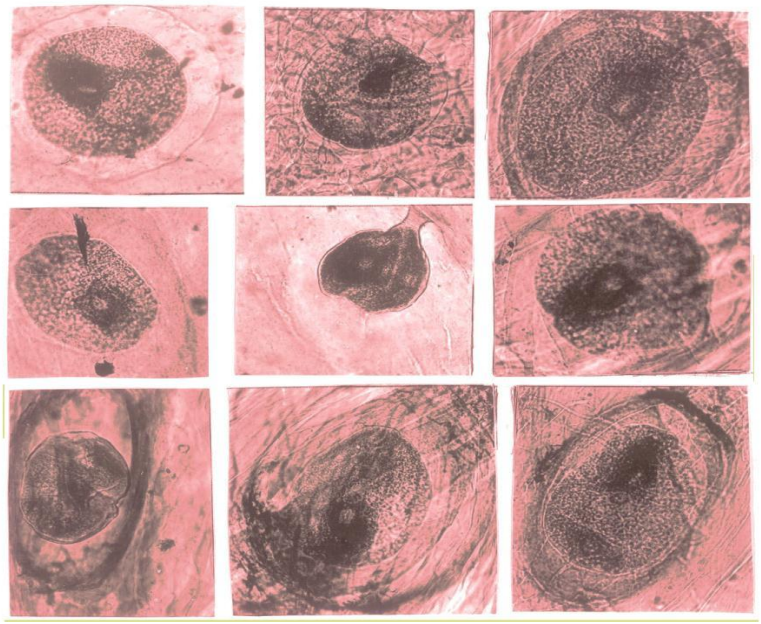

**Figure 1.** Heterophyid metacercariae infecting *Clarias gariepinus* collected from fresh and brackish water, Cairo district, Egypt.

*1)- Heterophyes heterophyes I; 2)- H. heterophyes II; 3)-H. heterophyes III; 4)- H. heterophyes IV; 5)-H. heterophyes V; 6)- H. heterophyes VI; 7)- H. heterophyes VII; 8)- H. heterophyes VIII and 9)- Pygidiopsis genata.* ( X600).

**Table (4a):** Cyst dimensions ± SD of different heterophyid metacercariae recovered from infected *Cl. gariepinus,* from Cairo district, Egypt, in mm

| Heterophyid Metacercariae | | | | External Cyst Dimension | | | Internal Cyst Dimension | | | Cyst thickness. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Min. | Max. | Mean | Min. | Max. | Mean | Min. | Max. | Mean |
| With single cyst wall | I | L | Round | 0.25 | 0.39 | 0.33±0.02 | | | | 0.04 | 0.05 | 0.05±0.01 |
| | | B | | x 0.18 | x0.30 | x0.29±0.01 | | | | | | |
| | II | L | | 0.14 | 0.15 | 0.14±0.01 | | | | 0.02 | 0.03 | 0.02±0.01 |
| | | B | | x 0.11 | x0.14 | x0.13±0.01 | | | | | | |
| | III | L | Oval | 0.45 | 0.60 | 0.52±0.01 | | | | 0.04 | 0.14 | 0.06±0.01 |
| | | B | | x 0.35 | x0.52 | x0.38±0.01 | | | | | | |
| | IV | L | | 0.32 | 0.46 | 0.39±0.03 | | | | 0.02 | 0.09 | 0.05±0.01 |
| | | B | | x 0.28 | x0.39 | x0.32±0.02 | | | | | | |
| | V | L | | 0.37 | 0.49 | 0.44±0.04 | | | | 0.03 | 0.10 | 0.06±0.01 |
| | | B | | x 0.26 | x0.32 | x0.29±0.03 | | | | | | |
| | VI | L | Ovoid | 0.46 | 0.63 | 0.52±0.05 | | | | 0.08 | 0.16 | 0.12±0.01 |
| | | B | | x 0.28 | x0.32 | x0.30±0.02 | | | | | | |
| | VII | L | | 0.24 | 0.39 | 0.34±0.02 | | | | 0.04 | 0.10 | 0.07±0.01 |
| | | B | | x 0.11 | x0.20 | x0.16±0.02 | | | | | | |
| With double cyst wall | VIII | L | Oval | 0.3 | 0.4 | 0.39±0.01 | 0.22 | 0.28 | 0.26±0.02 | 0.03 | 0.06 | 0.04±0.01 |
| | | B | | x 0.23 | x 0.3 | x0.32±0.02 | x0.18 | x0.22 | x0.19±0.02 | | | |
| | IX | L | Ovoid | 0.47 | 0.56 | 0.49±0.05 | 0.32 | 0.42 | 0.36±0.04 | 0.03 | 0.08 | 0.05±0.01 |
| | | B | | x 0.31 | x0.43 | x0.39±0.04 | x0.23 | x0.26 | x0.24±0.03 | | | |

Min= Minimum Max. = Maximum L= length B= breadth SD=standard deviation

metacercariae revealed eight types of *H. heterophyes*, differentiated according to the difference in size and morphological status and *Pygidiopsis genata* (Looss,1907), all from family *Heterophyidae. P. genata* fluke was

**Table (4b):** Mean dimensions ±SD of the different heterophyid metacercariae recovered from infected *Cl. Gariepinus*, from Cairo district, Egypt, in mm.

| Heterophyid cyst | | | metacercariae dimension | Oral sucker | Ventral sucker | Gonotyl dimension |
|---|---|---|---|---|---|---|
| | | | Mean | Mean | Mean | Mean |
| single cyst wall | I | Round | 0.22±0.02 | 0.02 | 0.05±00.01 | 0.01 |
| | | | x0.22±0.02 | x0.02 | x0.05±0.01 | x0.01 |
| | II | | 0.11±0.01 | 0.02 | 0.03 | 0.02 |
| | | | x0.1±0.01 | x0.01 | x0.03 | x0.01 |
| | III | Oval | 0.38 ±0.01 | 0.02 | 0.06±0.01 | 0.01 |
| | | | x0.30±0.01 | x0.01 | x0.05±0.01 | x0.01 |
| | IV | | 0.23±0.02 | 0.02 | 0.05±0.01 | 0.02 |
| | | | x0.18±0.02 | x0.01 | x0.05±0.01 | x0.01 |
| | V | | 0.21±0.01 | 0.02 | 0.05±0.01 | 0.01 |
| | | | x0.20±0.02 | x0.01 | x0.05±0.01 | x0.01 |
| double cyst wall | VI | Ovoid | 0.26±0.02 | 0.02 | 0.06±0.01 | 00.02 |
| | | | x0.22±0.01 | x0.02 | x0.06 | x0.01 |
| | VII | | 0.17±0.03 | 0.02 | 0.06 | 0.02 |
| | | | x0.11±0.1 | x0.02 | x0.03 | x0.01 |
| | VIII | Oval | 0.23±0.01 | 0.03 | 0.05±0.01 | 0.02 |
| | | | x0.19±0.02 | x0.02 | x0.05±0.01 | x0.01 |
| | IX | Ovoid | 0.35±0.04 | 0.03 | 0.09±0.01 | 0.02 |
| | | | x0.23±0.04 | x0.02 | x0.07±0.01 | x0.01 |

SD= Standard deviation

**Table 5.** Mean dimensions ± SD of the different cysts collected from *Lebeo niloticus and Mugil cephalus*, from Cairo district, Egypt (in mm)

| Fish species | Type of metacerceria | | Cyst | | | Cyst thickness |
|---|---|---|---|---|---|---|
| | | | Length | X | Breadth | |
| *L. niloticus* | Digenean | I | 0.19 ± 0.01 x 0.14 ±0.01 | | | 0.04 ± 0.01 |
| | Digenean | II | 0.16± 0.01 x 0.15 ± 0.01 | | | 0.01 ± 0.01 |
| *M. cephalus* | Digenean | III | 0.25 ± 0.01 x 0.20 ±0.01 | | | 0.06 ±0.01 |
| | Digenean | IV | 0.15 ± 0.01 x 0.18 ±0.01 | | | 0.04 ± 0.01 |

SD= Standard deviation

distinguished from *H. heterophyes* by being shorter and wider and having a longer oesophagus and possessing a quite smaller gonotyl (Table 6 and Figure 3).

## DISCUSSION

Among fish species *Cl. gariepinus* showed the highest infection prevalence (60.66%). Whereas, *M. cephalus* and *L. niloticus* showed 36.36% prevalence rate, and there was a positive correlation between fish weight and prevalence of infection (P< 0.05). The present data of infection, in *M. cephalus,* is much lower than that reported by Fahmy and Selim (1959), studying the role played by fish in transmitting parasitic diseases to humans. These authors found higher infection occurrence of encysted metacercariae (80%), in *Mugil capito* (Cuvier, 1829) infested with different heterophyid metacercariae species, among which *H. heterophyes* metacercariae. While, Riffaat et al. (1980) ) found a 100% of *M. cephalus* infected with *H. heterophyes*, in lakes Manzala and Mataria. Also, 80% of fish was reported by Abdallah et al. (2009) to be infected with heterophyid metacercariae, in freshwater fish from Dakahlia. Moreover, Ibrahim et al. (2010) reported a total prevalence of 95.37% of fish infected with heterophyid metacercariae , in Ismailia province, of which 21.4% were carrying *P. genata* encysted metacercariae. Recently, Hegazi et al. (2014) recorded 57.9% of fish in Dakahlia governorate infected with trematode encysted metacercariae. In other country, fish infected with *H. heterophyes* was reported. In Najran, Saudi Arabia, Khalil et al. (2014) recorded 5% of *M. cephalus* infected with *H. heterophyes* with and a total prevalence of 7.2% of heterophyid metacercariae. Also, in Korea, 96.2% of freshwater fish were found infected with heterophid metacercariae (Park et al., 2007). Variation in the

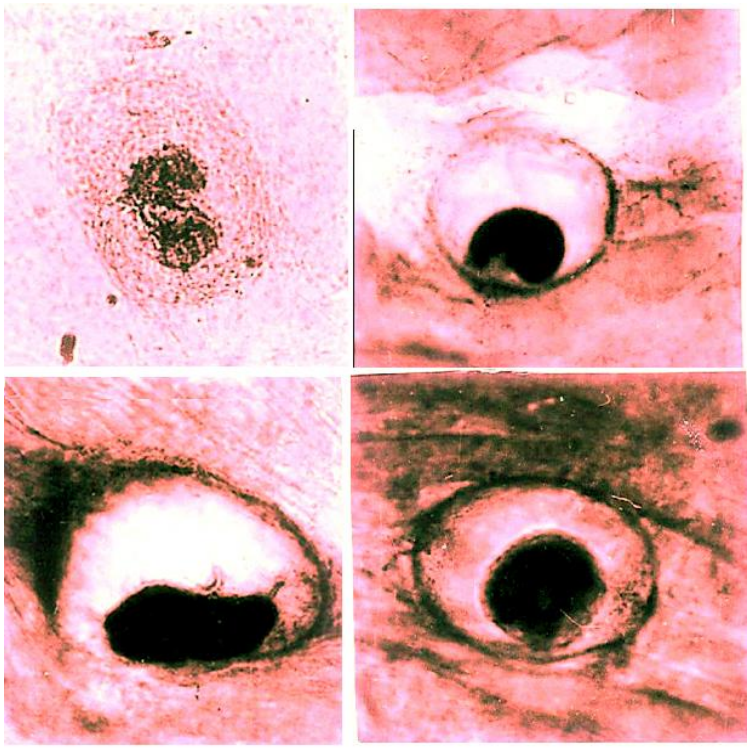

**Figure 2.** Digenean metacercariae infecting *Lebeo niloticus* and *Mugil cephalus* collected from fresh and brackish water, Cairo district, Egypt**.** 1) Digenean I; 2)- Digenean II; 3)- Digenean III and 4)- Digenean IV. (X 600).

prevalence of infection may be related to the locality from which fish where collected, degree of water pollution and the type of water whether fresh or brackish, a higher rate of infection was reported in fresh compared to brackish water (Lobna et al., 2010). Also, the abundance of snail intermediate host is one of the most important factors affecting the infection prevalence (Ibrahim and Soliman, 2010).

The highest intensity of encysted metacercariae was recorded in the caudal region of the different fish species. Similar observation was reported by Ibrahim and Soliman (2010) and Khalil et al. (2014). These findings reflect site preference of the parasite.

Seasonal preference and prevalence of infection with heterophyid metacercariae were observed, in this investigation.The highest infection prevalence in *Cl. gariepinus* (73.68%) was recorded in autumn and the lowest (54.05% ) in spring. While, in *M. cephalus* and *L. niloticus*, the highest prevalence of infection (56.68% and 26.09%, respectively) were reported in spring. But, the lowest infection prevalence in *L. niloticus* (12.50%) was found in autumn, whereas in *M. cephalus* (25.58%) it occurred in winter. Similarly, Ghobashy et al. (2010) reported the highest prevalence of heterophyid infection in spring, in the two freshwater fish *Liza auratus* and *Oreochromis niloticus*,. While, they recorded the lowest prevalence in *L. auratus* in autumn and in *O*. *niloticus* in winter. On the contrary, El- Sheikha and El- Shazly (2008) found that the highest heterophyid infection (38.2% ) in brackish fish was reached during the summer season, followed by spring (26.6%) then autumn ( 19.3% ), whereas, the lowest prevalence (8.7%) was observed in winter. Similarly, Abou-Eisha et al. (2008) reported the highest infection rate of *H. heterophyes* (100% ) in *Cl. gariepinus*, in summer, followed by autumn (93.3% ) then spring (90.9% ), and the lowest infection rate (57.1% ) was recorded in winter. Also, El Gayar and Aly (2013) reported 87% of *Cl. gariepinus* infected with *H. heterophyes*, in Sharkia province, with the highest infection rate (82% ), recorded in summer and the lowest (65%) in winter. This was in agreement with the results of Mahdy (1991 ), but disagrees with the findings of Abo- Essa (1993) , who recorded the highest prevalence (91.3% ) in winter. Alteration in seasonal prevalence is most probably due to biotic and climate factors. The influence of these factors, as host condition and seasonal variation on transmission of fish parasites, relative to their prevalence and intensity, is well documented ( Massoud et al., 2007; El-Sheikha and El-Shazly, 2008; Ghobashy et al., 2010). There are numerous biotic and abiotic factors affecting parasite infection including physiological condition of the host ( e.g. host size and species, feeding habit) and environmental factors ( e.g. temperature, salinity, oxygen content) (Iyaj et al., 2009).

The rate of infection among *Cl. gariepinus* and *M. cephalus*

**Table 6.** Mean dimensions ± SD of the different types of *Heterophyes heterophyes* and one type of *Pygidiopsis genata* adult flukes, from Cairo district, Egypt, in mm.

| Aspect | *Heterophyes heterophyes* | | | | | | | *Pygidiopsis genata* |
|---|---|---|---|---|---|---|---|---|
| | I | II | II | IV | V | VI | V | |
| | Mean | Mean | Mean | Mean | Mean | Mean | Mean | Mean |
| **Body length** | 1.05±0.15 | 0.95 | 0.90 | 0.89±0.05 | 0.92 | 0.89 | 0.83 | 0.5 |
| **Body breadth** | 0.42±0.02 | 0.38 | 0.69 | 0.36±0.02 | 0.36 | 0.51 | 0.56 | 0.8 |
| **Oral sucker dimension** | 0.05 X0.04 | 0.05 X0.4 | 0.06 | 0.04±0.01 | 0.06 X0.04 | 0.05 X0.05 | 0.05 | 0.03 0.18 |
| **Ventral sucker dimension** | 0.23±0.03 | 0.17 X0.16 | 0.17 | 0.17±0.01 X0.16±0.01 | 0.16 | 0.23 X0.21 | 0.17 | |
| **Length of oesophagus** | 0.14 | 0.06 | 0.13 | 0.03 | 0.06 | 0.13 | 0.05 | 0.15 |
| **Gonotyl dimension** | 0.15±0.01 | 0.12 X0.11 | 0.14 X0.12 | 0.13±0.01 | 0.14 X0.13 | 0.12 X0.10 | 0.08 | 0.03 |
| **Right Testis dimension** | 0.15±0.01 X0.14-0.02 | 0.15 X0.12 | 0.16 X0.12 | 0.14±0.02 X0.13±0.02 | 0.20 X0.08 | 0.16 X0.16 | 0.16 X0.08 | 0.10 X0.06 |
| **Left Testis dimension** | 0.15±0.01 X0.14±0.01 | 0.15 X0.13 | 0.15 X0.11 | 0.12±0.01 X0.11±0.003 | 0.1 X0.07 | 0.18 X0.16 | 0.17 | 0.11 X0.06 |
| **Ovary dimension** | 0.08±0.01 | 0.10 | 0.10 | 0.04 | 0.07 | 0.08 | 0.13 X0.10 | 0.09 X0.02 |
| **Egg dimension** | 0.02 X0.01 | 0.02 X0.01 | 0.02 X0.01 | 0.02 X0.01 | 0.02 X0.01 | 0.02 X0.01 | 0.02 X0.01 | 0.01 X0.01 |

SD= standard deviation

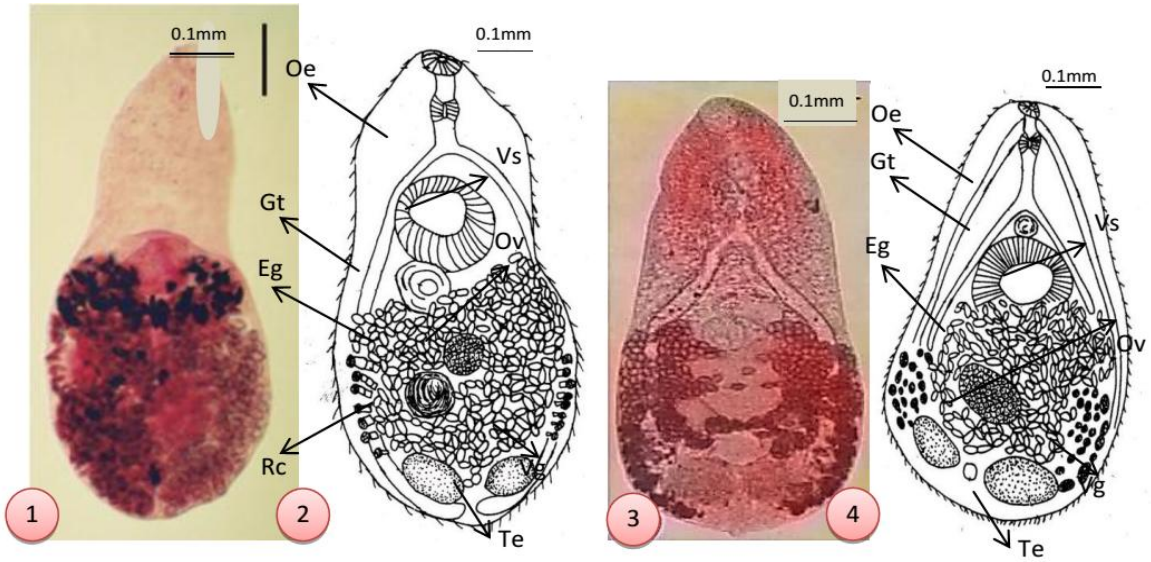


**Figure 3**: *Heterophyes heterophyes* and *Pygidiopsis genata* adults from infected dogs with encysted meracarcarie collected from fresh and brackish water fish, Cairo district, Egypt**.** 1-2 *H. heterophyes;* 3-4 *P. genata*. Eg: eggs; Gt. gonotyl; Oe. oesophagus; Ov. ovary; Rc. seminal receptaculum; Te. testis; Vg. vitellaria; Vs. ventral sucker.

increased with the increase of fish weight till 150g, but decreased when fish weight increased, and there was a postive correlation at P < 0.05. while, Iyaj et al. (2009) reported the occurrence of negative correlation between fish weight and reduction of parasitism. But, Ghobashy et al. (2010) found also that the prevalence of heterophyid metacercariae in *L. auratus* was inversely proportion to weight, it decreases with increase of fish weight, but the correlation was insignificant. The present result coincides with those of Walfgang (1954) and Maksimova (1958) but is contrary to what Khalil et al. (2014) reported. These authors assumed that there was no relationship between

**Table 7**. Human infection with *Heterophyes heterophyes* as descrided by other workers

| Dimensions | Witenberg, 1929 | Taraschewski, 1984 | Chai et al., 1986 |
|---|---|---|---|
| Body L | 0.6 - 2.7 | Not more then 2.0 | 1.36 – 2.06 |
| Body W | 0.2 – 0.9 | --- | 0.06 – 0.086 |
| Oral sucker | 0.05 – 0.18 | 0.063 – 0.092 | 0.083 – 0.145 |
| Pharynx | 0.03 – 0.06 | --- | 0.067 – 0.121 |
| Oesophegus L | 0.08 – 0.43 | --- | 0.067 – 0.214 |
| Ventral sucker | 0.11 – 0.31 | 0.093 – 0.295 | 0.241 – 0.362 |
| Genital sucker | 0.07 – 0.15 | 0.0101 – 0.295 | 0.188 – 0.340 |
| Ovary | 0.05 – 0.29 | --- | 0.072 – 0.308 |
| Right testis | -- | --- | 0.075 – 0.241 |
| Left testies | 0.023 – 0.027 | --- | 0.040 – 0.263 |
| Egg L | 0.013 – 0.015 | 0.0243 – 0.0141 | 0.0230 – 0.0295 |
| Egg W | --- | --- | 0.0134 – 0.0156 |

L= Length W=Width

fish weight and intensity of heterophyid encysted metacercariae infection. This was observed, herein, in *L . niloticus*, which showed irregular infection rate pattern in relation to fish weight and the highest infection rate was among fish weighing 100 -150g, but no significant correlation was recorded. This also agrees with the findings of Vandenbroek (1979).

The occurrence of various types of *H. heterophyes* metacercariae recovered, herein, substantiates the findings of Martin (1959) on the presence of different types of *Heterophyes* cercariae (Two large ones, of which one is very pigmented, and the third one is small ). When he infected *M. cephalus* with these cercariae, and cats were fed with the infected fish, the small type developed into *H. aequalis* (Looss, 1902) and the two large ones into *H. heterophyes*. Therefore, he suggested that *H. heterophyes* may represents two species or subspecies. Moreover, Kuntz et al. (1958) have earlier discovered an apparent complex of the Egyptian heterophyid. This finding supports the present result. Recently, El Beshbishi (open access) found also various types of the Egyptian *H. heterophyes.* The occurrence of different types of *H. heterophyes* recovered herein may be attributed to difference in the physiological status encountered in the reservoir hosts (birds, cats, dogs or humans) which may pollute fresh or brackish water with their excreta. Comparison of measurements of *H. heterophyes* with other workers (Tables 6 and 7) also supports the occurrence of different types of *H. heterophyes*. In the specimens of *H. heterophyes* described by Chai et al. (1986), the body length (1.36- 2.06 mm) and the oral sucker (0.083- 0.145mm) are larger than those reported in this study (0.83- 1.05 mm and 0.05- 0.06 mm, respectively), while, the oesophagus length, the ovary and the testes dimensions are smaller (Tables 6 and 7). Also, *H. heterophyes* described by Witenberg (1929) is larger (0.6- 2.7 mm) as well as the oesophagus length (0.08- 0.43 mm) than what is reported in the different types described herein (0.83- 1.05 and 0.03- 0.15 mm, respectively). The dimensions reported by Taraschewski (1984) are not complete to make a comparison with the presents *H. heterophyes.*

On the other hand, Mohamed et al. (2010) considered heterophyiasis as a zoonotic problem in some areas in Egypt, particularly in Dakahlia Province, where 13.3% of people showed heterophyid eggs in their stool. Much earlier, Khalil (1937) has found 90% of children and 22% of adults near Lake Manzala infected with *H. heterophyes*. Afterward, heterophyiasis, declined. Hence, Wells and Blagg (1956) detected *H. heterophyes* eggs in 36% of shoolchildren at Baltim and Lake Burullus. Furtheremore, Nagaty and Khalil (1964) reported 10% adults infected with this fluke at Lake Manzala. In 1980, only 2.5% of people at the same area were reported infected with *H. heterophyes* (Riffaat et al., 1980). Thus, it is evident that heterophyiasis occur in people living near lake shores and consuming infected fish. Also, in several countries, heterophyiasis is found to be endemic. Massoud et al. (1981) reported 8% of people infected with heterophyid flukes, in Khuzestan. Chai et al. (2004) found that human infection with *H. nocens* (Onji and Nishio, 1916) *(syn. H. heterophyes* ) was indigenous to the western and southern coastal islands of the Republic of korea. Also, Park et al. (2007 ) reported 40.2% of people infected with *H. nocens*, in a coastal Korean area, most probably due to consumption of raw fish infected with metacercariae. Moreover, Mahanty and John (2011 ) reported 42% of the Korean population infected with *H. heterophyes.* In South Korea, Chai et al. (2015 ) found 23.3% of people infected with heterophyid fluke, 23% with *H. nocens* and 3% with *H. continua* ( Onji & Nishio, 1916). Furtheremore, 21% of schoolchildren, in Sakon Nakhon Province, in Thailand, were reported to be infected with *H. heterophyes* ( Doi et al., 2017 ).

Cats and dogs may play an important role as reservoir host in endemic areas of zoonotic trematodes (Chai et al., 2013). In Egypt, Kuntz and Chandler (1956) detected many trematode species in stray cats, among which *H. heterophyes, H.aequalis* and *P.genata* were common. Recently, El Azazy et al. (2015) found that 15.8% and 10.8% of stray cats, from the municipality, in Kuwait, were infested with *H. heterpohyes* and *H. dispar* (Looss, 1902),

respectively. Also, Shin et al. (2015) reported 92.6% of stray cats, from the riverside areas of the Republic of Korea, infected with *H. nocens* and *P. summa* (Looss, 1907).

Variation in prevalence and intensity of infection in fish and humans and reservoir hosts, worldwide, may be due to several factors among which the level of water pollution by human , animal and birds excreta, species of fish, locations from which fish are caught and the local climate (temperature, humidity, rainfall ). Physiological factors of hosts may also be responsible of changes in the degree of infection of the different species of parasites ( Shalaby et al., 1989).

Hence, heterophyid encysted metacercariae in fish, commonly consumed by local inhabitants, may cause a serious zoonotic risk. Infection may cause loss of fish in ponds and rivers. Also, these flukes may provide morbidity and cause serious damages to aquacultures. To prevent and control hazard associated with fish infections, people should avoid consuming insufficient cooked, poorly salted or raw fish. Also, sufficient heat processing of fish before consumption or simple freezing of fish, for appropriate periods, should be done (El Sayad et al., 2018). First of all, defecation on the lake shores or river banks should be avoided and incriminated by governments. Moreover, control of snail vector of the disease by using biological methods, to conserve a clean and safe environment, should be proceeded. Furthermore, control has to be integrated. education; more knowledge on basic epidemiology; treatment of humans and animal reservoirs; sanitation; aquaculture management practices.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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