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SHORT COMMUNICATION

Prevalence of heterophyiosis in *Tilapia* fish and humans in Northern Egypt

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Abstract A total of 100 *Tilapia* fish samples were collected from brackish water (n=50) and fresh water (n=50) resources, Northern Egypt, and examined for heterophyid encysted metacercariae (EMC) during the period from August 2007 to July 2008. The overall prevalence of infection was 32%; 22% for brackish water fish and 42% for fresh water fish. Significant differences in parasite occurrence among body regions were found, with muscles 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 the lowest infection (21.9%). The prevalence was highest in summer season (46.4%) followed by spring (37.5%) and autumn (27.3%), and was lowest in winter (15.4%). The prevalence of infection decreased as fish size increased. Adult heterophyids, *Heterophyes heterophyes*, *Heterophyes aequalis*, *Pygidiopsis genata*, *Haplorchis yokogawai*, and *Ascocotyle* (*Phagicola*) *ascolonga* were recovered from EMC-feed puppies. Eggs of heterophyid type were detected in 10 (13.3%) out of 75 human stool specimens from local residents. An association exists between being a female (odd ratio [OR] 1.59 and 95% confidence interval [CI] 0.42–6.04), a fisherman (OR 1.39 [95% CI 0.26–7.48]), a housewife (OR 1.24 [95% CI 0.29–6.28]), 15–45 years old (OR 2.22 [95% CI 0.58–8.53]), or aged 5–14 years (OR 1.29 [95% CI 0.30–5.58]) and heterophyid infection. Measures should be implemented to reduce the risk of transmission of heterophyids to human and fish-eating animals.

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Introduction

Heterophyiosis is an intestinal illness caused by infection with the heterophyid digenetic flukes. The life cycle of heterophyid parasites includes snails as a first intermediate host, several fish species as the second intermediate host, containing the larval encysted metacercariae (EMC), and fish-eating birds and mammals including humans as a definitive host, harboring the adult fluke causing heterophyiosis (Elsheikha and Elshazly 2008a; Simões et al. 2010). Humans become infected via ingestion of infective EMC in a fish eaten raw or improperly cooked (Hamed and Elias 1969; Pica et al. 2003; Massoud et al. 2007). Human heterophyiosis causes a variety of intestinal symptoms, most commonly aches and diarrhea due to adult flukes in the intestine. In rare occasions, heterophyid embryos migrate using mechanisms not yet fully known to abnormal sites producing eosinophilic granuloma in the heart, brain, or spinal cord (Cho et al. 1985; Elsheikha 2007).

Heterophyiosis is prevalent in humans and fish in many developing countries of Asia and Africa, where its life cycle is sustained because of many reasons, such as the coexistence of poor sanitary conditions, lack of awareness, poor recognition of trematode infection by public health authorities, and/or inadequate fish inspection. These conditions are favorable for the maintenance and spread of heterophyid parasites. Heterophyiosis has been reported as endemic in the Nile Delta of Egypt, where favorable conditions exist for parasite propagation including the availability and abundance of the intermediate hosts, increasing production of fish in unhygienic conditions due to frequent disposal of human wastes directly into rivers, shore marine water, and lakes (Abdusslam et al. 1985; Abou-Basha et al. 2000). Heterophyiosis is common in both urban and rural communities owing to the habit of consuming salted or inadequately processed fish. Populations at risk and the geographical range of the infection are expanding because of the rapid growth of the fish industry, improved transportation systems, and demographic changes such as population movements (Chai et al. 2005).

In Egypt, heterophyiosis constitutes a serious but under-recognized public health problem. The presence of heterophyiosis has been documented in studies carried out in humans (Abou-Basha et al. 2000; Massoud et al. 2007). The occurrence of heterophyiosis in fresh and brackish water fish has been documented from limited geographic areas (Elsheikha and Elshazly 2008a,b). However, the epidemiology of heterophyiosis in fish from other geographic regions in Egypt is unknown, and the predictive factors of heterophyiosis in human population are poorly characterized. Therefore, the current study was conducted to investigate the occurrence of heterophyid EMC in *Tilapia* fish, and determine the prevalence and associated risk factors for heterophyiosis in humans residing in the same geographic region.

Materials and methods

Sampling areas

The fish used in this study are collected from River Nile resources of El-Qanater, Qalyobia, and brackish water of Manzala Lake, Dakahlyia. Qalyobia and Dakahlyia are relatively highly populated governorates located in the Nile Delta of Egypt. Qalyobia comprises 1,001.09 km^2 with 4,686,804 inhabitants, which means a density of population of 4,681.7 inhab/km^2. Dakahlyia comprises 3,459 km^2 with 4,985,178 inhabitants, which means a density of population of 1,441.2 inhab/km^2. Most people in both governorates live in rural villages and are dedicated for farming and agricultural practice. The climate is subtropical, with mild temperatures and seasonal precipitations.

Specimen collection

A total of 100 fish samples of *Tilapia* species (*Tilapia nilotica* and *Tilapia zilli*) were wild caught by fishermen from brackish water (n=50) or fresh water resources (n=50) at Qalyobia or Dakahlyia governorate, respectively. The study extended from August 2007 to July 2008. The collections of fish were made on a monthly basis (8–10 fish/month) for studying seasonality of infection. The samples were packaged separately in clean plastic bags, labeled, and transferred within 2 h on ice to our laboratory for detection and isolation of heterophyid EMC.

Detection of metacercariae

Small snips were taken from different parts of fish mainly from the head (gills and branchial cavity) and muscles of different body regions. Each piece was compressed between two microscopic glass slides and examined for the presence of EMC of heterophyid parasites as described (Morishita et al. 1965). Fish samples found to be infected were wholly subjected to artificial digestion method (Yokogawa and Sano 1968) using acidified pepsin solution (1 g pepsin, 5 ml concentrated HCl, and 100 ml saline) to separate metacercariae. The digested material was filtered through a sieve and washed several times with 0.85% physiological saline. Different types of metacercariae were segregated and collected from the sediment using a binocular stereo microscope. Recovered metacercariae were categorized according to their phenotypic features into distinct groups before they were administered to the dogs as described (Paperna 1980; Elsheikha and Elshazly 2008b).

The tissue distribution of heterophyid EMC in different parts of the fish body (i.e., tail and caudal third, middle third, anterior third, or head region) was examined. Also, the effects of fish size (small, medium, or large) on the prevalence of EMC in the examined fish were evaluated. For this purpose, fish smaller than 100 g, weight 100–150 g, or larger than 150 g were considered small size, medium size, or large size, respectively.

Experimental Infection

This experiment had the approval of the Research Ethics Committee at the Faculty of Veterinary Medicine, Benha University, Egypt. The experiment was performed to obtain adult heterophyid flukes for confirmation of the morphologically distinct EMC harvested from infected fish. The study was conducted with 15 2-week-old laboratory-reared puppies. The puppies were naive for parasite exposure based on the lack of any detectable parasite eggs as determined by fecal examination before challenge. Puppies were randomly allocated into five groups (three puppies per group) that were fed and watered ad libitum and reared in different isolation units in a biosecurity animal building throughout the experimental period. The puppies in each group were each orally inoculated with 10 ml saline containing about 50 viable EMC representing a distinct phenotype. One week post-infection (PI), daily fecal samples from each infected puppy were examined by direct and sedimentation techniques for the demonstration of eggs. Puppies that began to shed eggs were killed (usually within 2 weeks PI), and the small intestines were resected, opened, and washed several times with 0.85% saline to remove the coarse particles of the intestinal contents. All worms were collected from the intestinal content using a stereo microscope and washed several times with saline to remove mucus that may be attached to them. Recovered worms were then fixed in 10% neutral buffered formalin, stained with Semichon's acetocarmine, and mounted in Canada balsam as described by Garcia (2001). Parasite identification was carried out using key references (Witenberg 1929; Yamaguti 1971).

Assessment of human infection

Naturally voided fresh stool samples were obtained from 75 human subjects randomly recruited from villages of the same locality of fish collection at Qalyobia governorate. No attempts were made to specifically collect samples from diarrheic or non-diarrheic individuals. The specimens were collected into screw-capped plastic containers and transported to the laboratory for examination within 6 h of collection to detect parasitic eggs by using the standard direct wet mount and centrifugational sedimentation techniques. Some potential demographic risk factors were evaluated by a questionnaire administered to the participants. These include gender (male or female), age (>5, 5–14, 15–45, or <45 years), and occupation (school children, housewives, fishermen, or adult unemployed).

Statistical Analysis

Student's *t* test was used for comparison of continuous variables between groups using the SPSS package for Windows (release 10.1.0; SPSS Inc). Categorical variables were compared using chi-square analysis. A *p* value of 0.05 was considered significant. Univariate association between the probability of fecal shedding and individual characteristics were described. Because some categories had a small number of observations, 0.5 was added to the number of observations in each category for the calculation of odds ratios (OR) as described (Agresti 2002), and 95% confidence intervals (CI) were calculated. When one was included in the 95% interval, the risk of shedding in the category of interest was not different from the rest of the dataset. As the sample only contained 75 observations, it was not possible to conduct a multivariate analysis.

Results

Overall prevalence

The overall prevalence of EMC in both *Tilapia* species was 32%. Fish obtained from fresh water resources had significantly ($P<0.05$) more EMC (42%) compared with those obtained from brackish water (22%). However, the prevalence was similar with *T. nilotica* and *T. zilli*: 30% (12 of 40) versus 33.3% (20 of 60), respectively ($p=0.361$, Table 1).

Tissue distribution

The tail and caudal third contained the highest number of EMC (93.4%), followed by the middle third (84.3%), and anterior third (75%) of the body (Fig. 1). On the other hand, 21.9% of the head region in examined fish including branchial cavity and gills had EMC. There was no significant difference between *T. nilotica* and *T. zilli* in the body distribution of EMC ($P<0.661$). Prevalence of the EMC was inversely related to the fish size. Small-sized fish had the highest occurrence 27 of 78 (34.6%), followed by medium-sized fish four of 12 (33.3%). Large-sized fish recorded only 10% (one of 10).

Seasonal variation

The prevalence of EMC fluctuated with season (Fig. 2). Overall, the highest prevalence was recorded in summer (46.4%), followed by spring (37.5%), and autumn (27.3%). The lowest prevalence was recorded in winter (15.4%). The season failed to cause any significant differences ($P<0.45$) in the prevalence rate of EMC between *T. nilotica* and *T. zilli*.

Results of experimental infection

Identification of the five morphologically distinct EMC groups was based on the morphological characteristics of adult flukes recovered from the experimentally infected puppies. These flukes belong to five heterophyid species, namely *Heterophyes heterophyes*, *Heterophyes aequalis*, *Pygidiopsis genata*, *Ascocotyle* (*Phagicola*) *ascolonga*, and *Haplorchis yokogawai*. All the five heterophyids were obtained from brackish water fish. In contrast, only *P. genata*, *A. ascolonga*, and *H. yokogawai* were obtained

Table 1 Prevalence of infection with heterophyid EMC in the examined fish samples

Tilapia spp.	*T. nilotica*		*T. zilli*		Total	
	n	Positive (%)	*n*	Positive (%)	*n*	Positive (%)
Brackish water fish	25	6 (24)	25	5 (20)	50	11 (22)
Fresh water fish	15	6 (40)	35	15 (42.9)	50	21 (42)
Total	40	12 (30)	60	20 (33.3)	100	32 (32)

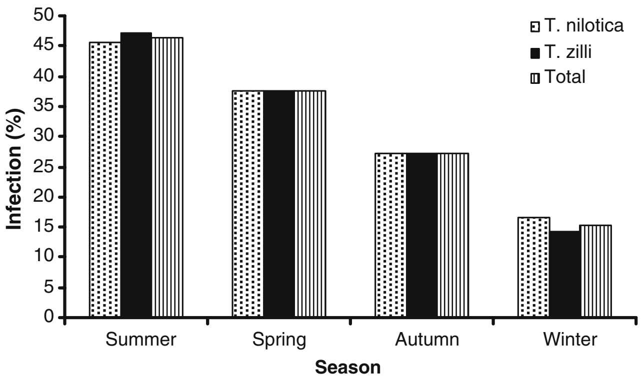

Fig. 1 Tissue distribution of heterophyid EMC in various body regions of the *Tilapia* fish

after feeding dogs EMC of fresh water fish. The overall recovery rate of adult heterophyids recovered from 50 metacercariae of a "specific species" of metacercariae was 12%. *H. heterophyes* and *P. genata* attained the highest recovery rates 15.3% and 14%, respectively. The recovery rate of other species ranged from 9.3% in *A. ascolonga* and *H. yokogawai* to 12% in *H. aequalis*.

Human heterophyiosis

Heteropyhid eggs were detected in 13.3% of 75 human stool specimens. Heterophyiosis was common in females (16.7%) than in males (11.1%), and the high prevalence was found among fishermen (17.4%) and housewives (14.3%). The highest prevalence was found in the age groups 15–45 years (20%) and 5–14 years (15%), but the lowest prevalence was recorded in the age groups >5 years (7.1%) and <45 years (6.3%). As shown in Table 2, risk factors for the occurrence of heterophyiosis in the examined human subjects were being a female gender (OR 1.59 [95% CI 0.42–6.04]), a fisherman (OR 1.39 [95% CI 0.26–7.48]), a housewife (OR 1.24 [95% CI 0.29–6.28]), 15–45 years old (OR 2.22 [95% CI 0.58–8.53]), or 5–14 years old (OR 1.29 [95% CI 0.30–5.58]).

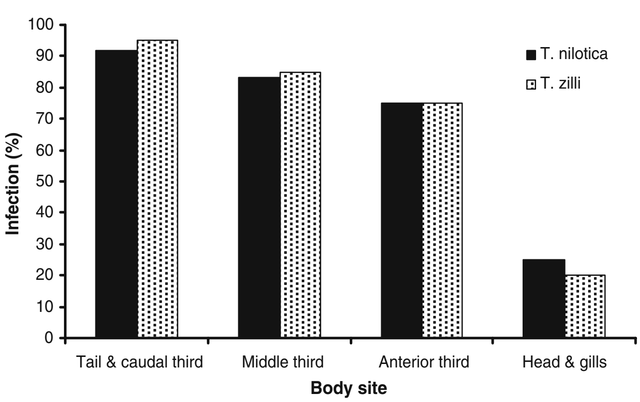

Fig. 2 Seasonal distribution of heterophyid EMC in the examined *Tilapia* fish

Table 2 Factors associated with heterophyid infection in the examined humans

Variable	No. (% of positive)[a]	OR (95% CI)
Sex	75[b]	
Male	45 (11.1)	0.63 (0.17–2.40)
Female	30 (16.7)	1.59 (0.42–6.04)
Age	75	
>5 years	14 (7.1)	0.61 (0.07–5.29)
5–14 years	20 (15)	1.29 (0.30–5.58)
15–45 years	25 (20)	2.22 (0.58–8.53)
45 years	16 (6.3)	0.51 (0.06–4.39)
Occupation	75	
School children	14 (7.1)	0.81 (0.21–3.13)
Housewives	28 (14.3)	1.24 (0.29–6.28)
Fishermen	23 (17.4)	1.39 (0.26–7.48)
Adult unemployed	10 (10)	0.53 (0.12–2.70)
Overall	75 (13.3)	

[a] Number of humans

[b] Total number of humans

Discussion

This study presents data on the prevalence of heterophyid infection in *Tilaipa* fish, and the prevalence and risk factors associated with heterophyid infection in humans. The overall prevalence of heterophyid infection in *Tilapia* fish was 32%, which is in accordance with (Mahdy et al. 1995; Mahdy and Shaheed 2000), but lower than that obtained by Mansour et al. (1987). This variation might be attributed to geographic locality and local conditions that sustain the parasite life cycle. The finding of heterophyid EMC in *Tilapia* fish species collected from fresh water (22%) and brackish water (42%), which are commonly consumed by the local inhabitants, poses a serious zoonotic risk. Adding to that is the practice of people to defecate in the lake shores, river banks, or from their boats while fishing, perpetuating the cycle of infection (El-Sahly et al. 1990).

The distribution of heterophyid EMC in different parts of the fish body was highest in the posterior third and tail region followed by middle third and anterior third, and the lowest distribution was recorded in the head. The tissue distribution of heterophyid EMC coincides with results reported by Mahdy et al. (1995), but disagrees with findings obtained by Mansour et al. (1987) who reported the highest distribution of EMC to be in the head region (57.5%). The discrepancy in the pattern of distribution of EMC in different tissues may be attributed to the EMC species-specific predilection site within the fish or to the level and extent of water pollution as reported by Shalaby et al. (1989).

This study also found that frequency of infection with EMC is inversely correlated with the size of the fish. It is not clear whether infection has led to stunted growth, which caused the apparently high prevalence in small-sized fish. An alternative explanation could be that small-sized fish have thin skin that is easily penetrated by cercariae (Paperna 1980), leading to a higher infection rate.

Study of the effect of seasonal variation on the prevalence of EMC revealed that the highest infection rate was in summer (46.4%) followed by spring (37.5%) and autumn (27.3%), and the lowest was in winter (15.4%). These findings agree with that reported by Mahdy et al. (1995) and Elsheikha and Elshazly (2008a). The fluctuation of prevalence of infection is probably due to variations in the climatic conditions such as temperature, humidity, and rainfall from year to year. These conditions are important for the growth and reproduction of snails.

Experimental feeding of five separate groups of dogs with five morphologically distinct groups of EMC enabled the identification of five adult heterophyid worms from brackish water fish and three only from fresh water fish. This suggests that the type of water seems to play an important role in infection of fish by certain types of heterophyid parasites. These results are in agreement with previous studies (Makhlouf et al. 1987; Mahdy et al. 1995; Elsheikha and Elshazly 2008b). The recovered heterophyid species in the examined fish have zoonotic importance to Egyptians who reside in the Nile Delta of Egypt. However, because an extraordinary number of heterophyid species are zoonotic (WHO 1995) and have very similar transmission patterns, fish-borne heterophyiosis represent a very significant food safety problem.

In this study, humans showed a prevalence of heteropyhid eggs of 13.3% (Table 2), which is consistent with the 13.5% reported in Egypt (El-Morshidy et al. 1994) and 9.6% in Japan (Kingo et al. 2002). This prevalence is, however, lower than 42.9% reported in Korea (Chai et al. 1994), 33.8% in Egypt (Abou-Basha et al. 2000), and 100% in Vietnam (Dung et al. 2007), but higher that those recorded in Egypt, 5% (Yousef et al. 1987) and 5.1% (Rifaat et al. 1980). The differences in the prevalence rates of human heterophyiosis even within the same country may be attributed to the food habits of people and the heterophyid parasite burden in the local fish (Massoud et al. 1980).

The higher prevalence in females, fishermen, and housewives implies that these groups are more prone to exposure through handling, preparing, and/or tasting of fish during cooking and processing. Also, infection was more common in the age groups 15–45 and 5–14 years. These groups probably had more chances to consuming raw or improperly cooked fish than other age groups.

Summing up, data from the literature and the present study confirm the high frequency of the heterophyid EMC infection in *Tilapia* fish, suggesting a significant role of *Tilapia* fish as a source of zoonotic heterophyiosis if it is ingested raw or partly cooked. Being a female, a fisherman, a housewife, or aged 15–45 or 5–14 years was identified as a risk factor of heterophyid infection in the examined population. The present findings support the rationale for programs aimed at controlling heterophyiosis. These programs should focus on public health education especially among populations at a high risk to discourage eating of raw or insufficiently cooked fish especially in endemic areas, and encourage control of snails with molluscicides where feasible.

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