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Original Article

Update status of *Pygidiopsis genata* (*Trematoda*: Heterophiidae) prevalence in Alexandria (Egypt) Lakeland *Tilapia zillii* fish and its role in human infection

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Abstract : The early half of the last century has witnessed a great attention to family Heterophyidae especially in Middle and Far East since infecting both man and animals. Seasonal variation concerning prevalence and intensity of fish infection. Fish specimens were collected from highly endemic region in Alexandria governorate during summer and spring seasons. Encysted metacercariae were identified and used in subsequent experimental infection of laboratory mice was carried out to allow morphological identification of eggs and adult worms. The prevalence of infection was much higher in summer (90.10%) than in spring season (54.76%).*P.genata* has proved highly infective, since all experimental mice had acquired infection. Morphological characteristics may help distinguish *P.genata* stages from other heterophyids.

Key words: encysted metacercariae, *Pygdiopsis genata*, *Tilapia zilli*.

Introduction:

More than 18 million people are reported to be affected with fish borne heterophyids. These flukes induce noteworthy morbidity and remarkable deterioration to aquaculture **(Sohn *etal*., 2009**). *Pygidiopsis genata* belonging to family Heterophyidae was first reported in Egypt from a pelican (*Pelicanusonochrotalus*) **(Looss, 1907;Ransom, 1920).** It is endemic in many global areas, particularly the Nile Delta in Egypt (**Graczyk and Fried, 2007).**

The parasite is characterized by having a three-host life cycle with snails as first intermediate hosts (I.H), fish as 2nd I.H and fish-eating birds and mammals including human as the final hosts **(Hamed and Elias, 1969).**Eggs passing in stool of final hosts are swallowed by a suitable snail intermediate host (*Melania tuberculata*) snails **(Yamaguti, 1958;Youssef *et al*., 1978)** and *Melano psiscostata***(Dzikowski*etal*., 2004)**. The pleurolophocercouscercariae are released to penetrate and encyst in fish with considerable seasonal variation in prevalence of E.M.C in fish **(Elsheikha and El Shazly, 2008).**

The present study aimed to detect

seasonal variation in prevalence of *P.genata* infection in *Tilapia zillii* fish and giving a precise morphological identification of E.M.C and adult flukes and discuss the role of the parasite in human infection and disease.

Materials and Methods:

1-Collection and examination of fish specimens:

A total of small-sized 133 fish specimens, *T.zillii* (Gervais, 1848) **(Fig.1)** were collected randomly from Souq Alhadara (Bab Sharq district), Alexandria Governorate in the period from August 2017 to May 2018. Their weight range was 14 to 37 grams each. After transport in ice boxes, they were kept in the refrigerator to be examined within 24 hour.

From each fish, snips (about one gram each) were obtained from muscles near the tail, dorsal fins, and viscera as well. According to **Morishita *et al*., (1965) and Sohn(2009),**snips were compressed between two glass slides and examined under binocular dissecting microscope using 10x ocular lens by 4X objective lens to detect lodged heterophyid EMC and their characteristic rotatory motility. If viable, infected fish were skinned, eviscerated, chopped and each 100 grams were added to artificial digestive juice prepared according to **(Yokogawa and Sano, 1968).**

2-Experimental infection of mice with viable E.M.C:

A total of 54 parasite-free 28-35 days laboratory-bred male albino mice were infected experimentally, in accordance with local Institutional Animal Care and Ethics Committee (Assiut University, Egypt). Each one received infective dose 300 EMC per 0.3 ml (infective dose) by introduction of insulin syringe fitted with a blunt tipped gauge into mouse stomach.

Rapid decapitation was done 6-10 days post infection, after detection of *P.genata* eggs in mice stool. In order to obtain adult flukes,resection of small intestine, from pyloric sphincter to caecum was done in petri dish containing 0.9% saline to be opened and scraped by slide edge**(El Assal, 1974).**

For precise morphological identification, adult worms were flattened between glass slides and covers to be fixed with formalin for 30 min**.** Then, they were washed from fixative and stained in diluted acetic acid alum carmine solution (2% conc.) for 10 min.. After that, dehydration using ascending grades of alcohol (30%, 50%, 70%), for two minutes in each grade, was applied. Complete dehydration was done using ascending grades of alcohol, (80%, 90%, 95% and absolute alcohol) for 2 minutes in each grade. Finally, stained worms were cleared in clove oil, mounted in DPX and left to dry **(Kuntz and Chandler, 1956).**

Results:

105 fish had been found infested by *P. genata* EMC with overall prevalence (78.94%).In summer, 82 out of the 91 fish samples were found to be infected (90.10%), while in spring, 23 out of 42 fish specimens were proved to be infected (54.76%). Spheriodal E.M.C **(Plate 1: A, B)** were found abundantly in the striated muscles of *T. zillii*, especially caudal region. They measured 178-183 µm with thinner outer cyst wall. After tissue digestion, freed encysted metacercariae**(Plate 2: A)** were characterized by obscure internal contents except for a Y-shaped excretory bladder and plenty of excretory granules **(Plate 2: C).** Some of them were found starting their

excystation **(Plate 2: B)** illustrating the metacercarial viability.

Figure (1): showing***Tilapia zillii***fish. Body is Brownish –olivaceous in color with a number of dark olive transverse bands.

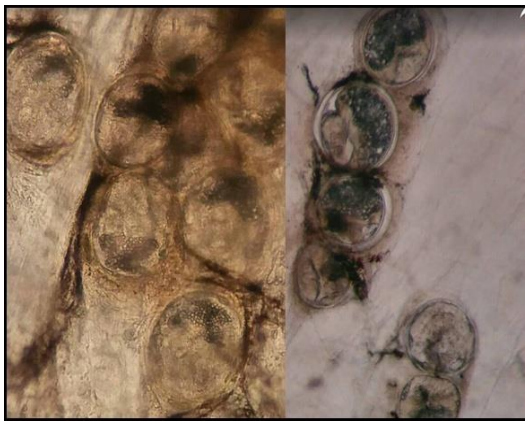


Plate (1): Showing: unstained E.M.C (200X) aggregated between fish muscles (A, B) a: Y-shaped excretory vesicle, b: excretory granules.

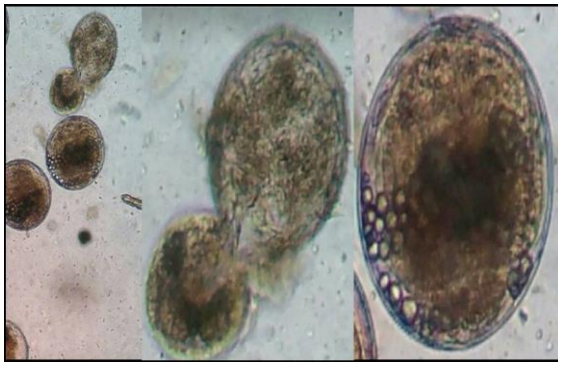
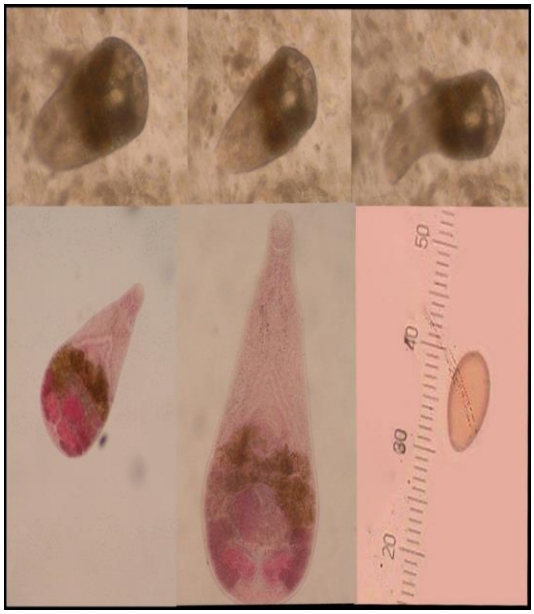


Plate (2): Showing EMC after acidic and alkaline digestion (A) showing beginning excystation of one metacercaria (B) showing one encysted metacercaria after muscle digestion illustrating the numerous excretory granules (C).

The egg measured 20-22um in length and 10-12um in width, embryonated, yellowish in color, thick–shelled, possessing an inconspicuous operculum at its anterior extremity and a knob thickening at the opposite side **(plate 3,F).**The adult worm was light brown and showed continuous state of contraction and retraction with changeable shape **(plate3, A, B, C).**The mature worm is pyriform with average body size 400-410um in length and 200-203um in width. The body is covered with anteriorly arranged, dense scales. They are progressively widely spaced and disappear near mid-testicular level **(Plate3, D,E).**

The sub terminal oval oral sucker (a) is followed by a well-developed muscular pharynx (b) that divides the esophageal tube into a pre pharynx followed by an esophagus, which bifurcates into two intestinal caeca (c) at the level of junction of the anterior and middle thirds of the body. Short caeca end midway between the ventral sucker and the testes. The ventral sucker (d) is well developed, transversely oval and larger than the oral sucker (Plate3, D, E).The tow testes (e) are smooth in outline, transversely oval and located near the posterior end of the body, with the right one is the larger. They measure 30-38um by 40-50um and 22-34um by 37-42 um, respectively. Anterior and medial to the right testis, the rounded ovary (f), measuring 39-44um in length and 30-33um in width, is situated. The vitelline glands (g) are large varying from 5-7 pairs, lateral to both testes. The uterus (h), full of eggs, is located between ventral sucker and testes, covering intestinal caeca laterally fig. (6. B). The ventrogenital sac (i) consists of a ventral sucker, two

genital pores and a gonotyl**(Plate3,D,E).**



Plate(3) (A,B,C): Photomicrograph of *P.genata* adult fluke (100X) recovered from the intestines of mice 7-10 days after oral infection **(D):***P.genata* adult fluke (100X) stained with Alum Carmine.**(E):** at (200X)

Discussion:

This study is concerned with the fish borne intestinal heterophyid flukes. Through the course of this study, fish specimens belonging to *Tilapia zilli* were collected for obtaining heterophyid encysted larvae but only *P.genata* EMC were commonly found in abundant numbers **(Elsheikha& El Shazly, 2008a; Abdallah*et al*., (2009)**.

Makhlouf*et al*. (1987) also reported that the highest prevalence and intensity of *P. genata* EMC were recorded in all Mansoura Nile *Tilapia sp.* fish; (26.6 %, 6 cyst/gm) in *T. nilotica* and (16.9 %, 6 cyst/gm) in *T. zilli*. Although it was reported that *P.genata* may rarely induce intestinal heterophyiasis in humans **Boulos *et al.*, (1981**), the abundance of EMC in infected fishes indicates that the parasite is a very common inhabitant of fish eating birds as pelicans **(Youssef *et al.*, 1978)** or-in our opinion-sea-gulls and herons.

Through the present study, only small sized fish were used which had shown high incidence of infection. **Salem *et al*., 2010** reported that small-sized fish usually have the highest occurrence of encysted larvae. It is suggested that infection had led to stunted growth, which caused the apparently high prevalence in small-sized fish. An alternative explanation could be that small-sized fish had thin skin that was easily penetrated by cercariae, leading to a higher infection rate **Paperna and Overstreet** (**1981).** In the present workers ' opinion the small-sized fish are usually swimming nearer to the lake shore where the infected snails are usually crawling or attached to the sea-side vegetations; shedding their cercariae which have greater opportunity to invade fish due to slow water and fish fin vibrations.

In the present study, the overall prevalence of EMC infection in fish was 78.94% during summer and spring seasons. This finding is higher than that recorded by **El Sheikha and El-Shazly (2008b)** ~70% in both seasons in *T.zilli* from Manzala lake. The present study suggests that such variation in prevalence may be attributed to the variation in chemical composition of water, degree of salinity, water fecal contamination, and abundance of both snails and aquatic birds, which play the main role to complete the life cycle.

In the current study, the higher prevalence of infection was in summer-month period (90.10%), followed by spring (54.76%) with a significant seasonal variation. This coincides with **Salem *et al*., (2010)**, who reported that the highest

prevalence was in summer followed by spring season. Season-dependent variations in the occurrence of heterophyid EMC were previously reported by being the highest in summer **Abou-Zakham *et al*., (1990); Elsheikha &Elshazly (2008b)**. The reason for this phenomenon may be due to temperature-dependent release of cercariae **Oshima& Nishi (1963); Makhlouf *et al*., (1987); Elsheikha& Elshazly (2008b)**. Additional factors including death of the cercariae/metacercariae, may cause significant variation in the observed manner **(Taher, 2009).**

During the examination of tissue distribution of the heterophyid EMC, they were detected in striated muscles only and not in liver nor kidney. This finding coincides with **Elsheikha and Elshazly (2008b)** who found EMC in muscles only. In contrast, **Ibrahim and Soliman (2010)** detected them in muscles and viscera.

In this study, it was observed that the muscles of the posterior third near the tail of the fish were the maximally infected regions with EMC which is in accordance with the findings of **Abdallah *et al*. (2009)**and **Salem *et al*., (2010).**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 **Shalaby *et al*., (1989).**

Laboratory bred mice were used to obtain adults in this study, the life cycle of *P. genata* has been worked out experimentally in hamsters **Boulos *et al*., 1981** , mice, rats, and hamsters **Mansour *et al*., 1981** and puppies **ElShazly *et al*., 2008**.

A high level of infectivity was confirmed in the present study by the ability of the EMC collected to initiate infection in all studied mice. Also, **El-Sayad *et al*., (2014)** reported infection of all studied rats by EMC found in fish samples collected from El Max Bay, Alexandria Governorate. Thus it is clear that the parasite can successfully infect all laboratory experimental animal models illustrating the host non-specificity of the parasite infecting all fish-eating birds and animals as well as man; a common feature in all heterophyids.

The collected trematodes were identified as those of *Pygidiopsis genata* as they were found morphologically very similar to those described by **Youssef *et al.* (1978)** and **Hussein (2007)**.

Heterophyids including *P. genata* are small intestinal parasites of human with mild clinical manifestations except when infection with a huge number of parasites where there are moderate or severe gastrointestinal troubles such as colicky pains and diarrhea, occasionally bloody. However, the small-sized eggs may embolize in the brain, spinal cord, heart and lung leading to epilepsy, neurological manifestations, cardiac insufficiency and pulmonary complications **(Chai and Jung, 2017).**

In an unpublished work in 1964, the senior author fed cats on heterophyid metacercariae in *Tilapia* fishes from the Delta region of Egypt; all of them harbored hundreds of only *Heterophyes heterophyes* adults. It is worth mentioning that most of the studies identified *H. heterophyes* adults in cats or dogs were based on observing adults by intestinal dissection **(Khalifa, 2011)** while human infection with *H. heterophyes* identification was based only on coproscopy**(Abo-Basha *et al*., 2000, Youssef and Uga, 2014).** As eggs of *H. heterophyes, P. genata* could not be differentiated except by micrometry, it

seems very probable that some of these infections might be due to *P. genata* favored by the high incidence of its metacercariae in *Tilapia* fishes. This may be also due to ecological changes that favored the abundance of *Melania tuberculata* snail intermediate host of *P. genata* on the expense of *Pirinella conica* snail intermediate host of *H. heterophyes.*

Conclusion

The encysted metacercariae of a heterophyid trematode harboring the muscles of *Tilapia zillii* fishes in Alexandria lakes (Egypt) were experimentally proved to be those of *Pygidiopsis genata*. Their presence in markedly huge numbers represents a risk factor and hazard for human infection in people fond of eating insufficiently cooked or salted fish.

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الملخص العربي

دراسة حديثة لمدي انتشار طفيل البيجديوبسيس جيناتا في أسماك التيلابيا زيللي من بحيرات محافظة الاسكندرية ودورها في نقل للعدوي للانسان

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ينتمي طفيل البيجديبوبسيس جيناتا الي عائلة الهتروفيد والتي تتسبب في حدوث اصابة للانسان من خلال تناول أسماك غير مطهية أو مملحة بشكل جيد وذلك لاحتوائها علي السركاريا المتحوصلة (الطور المعدي).

تتسبب الطفيليات المنتميه لهذه العائله في اصابة الملايين من البشر علي مستوي العالم وخصوصا في الشرق الوسط . تستوطن هذه الطفيليات منطقة الدلتا في مصر.

تهدف هذه الدراسة الي معرفة مدي انتشار البيجديوبسيس في أسماك التيلابيا زيللي (البلطي الأخضر) في المياه الشبه عذبة بمحافظة الاسكندرية. كما تهدف الي معرفة التوصيف الدقيق للديدان االيافعه والبويضات (الطور التشخيصي).

تم جمع عينات الأسماك وعددها (133) في فصلي الصيف والربيع خلال الفتره بين أغسطس 2017 الي مايو 2018 لنجد أنها تحتوي علي السركاريا المتحوصلة للبيجديوبسيس جيناتا فقط. سجلت الاصابة في الأسماك المجمعه بفصل الصيف نسبة (90.10%) بينما سجل فصل الربيع انخفاض ملحوظ ليصل الي (54.76%). تم استخدام عدد من الحويصلات لاحداث عدوي في الفئران المعملية وقد لوحظ اصابة جميع الفئران بها مما يؤكد علي احتمالة ارتفاع نسبة الاصابة لدي الانسان.

نظرا للتشابه الشديد بين بويضات البيجديوبسيس والهتروفيس(واحده من الهتروفيد) ولأن التشخيص يعتمد علي وجود البويضات مع عدم توفر الديدان اليافعه فان الدراسة الحالية تقترح بشدة أن البيجديوبسيس هي المسئلة عن أغلب حالات الاصابه بالهتروفيد بالمحافظه .

كما أن وجود دراسات سابقة منذ أعوام عديده تؤكد ارتفاع نسبه السركارياالمتحوصلة للهتروفيس في أسماك البلطي في الاسكندريه مع عدم وجود السركاريا الخاصة بالبيجديوبسيس فان هذه الدراسه تعتقد باحتماليه حدوث تغيرات بيئيه أدت الي زياده انتشار العائل الوسيط الاول للبيجديوبسيس(ميلانيا تيوبركيولاتا) مع انخفاض حاد في أعداد (البيرينيلا كونيكا) الخاصة بالهتروفيس.