Field Studies Encysted Metacercariae infested Natural Male Tilapias and Monosex Tilapias in Kafr El-Sheikh Governorate Fish Farms

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Abstract: The present study was carried out on 1800 specimens of Tilapia fishes *Oreochromis niloticus (O.niloticus)* (*phenotypic, hybrids and monosex O.niloticus* of different size and body weight. They were randomly collected at different seasons from Kafr El- Sheikh Governorate cultured fish farms. The clinical signs of most examined fishes revealed no pathognomonic abnormalities on the external body surface except black spots were detected on skin and fins. Tilapia fishes were shown emaciation. The postmortem findings the black spots were detected on skin and fins. Encysted metacercariae including *Euclinostomum heterostomum, Posthodiplostomum cuticola*, *Heterophidae* and *Haplorochoidae* were investigated and recorded. The highest prevalence possessed in hybrids while monosex *O.niloticus* occupied the last position. The histopathological examination in different organs of infested fish revealed pathological changes in gills and musculatures.
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1. Introduction:

Encysted metacercariae parasitic diseases have the upper hand in fish parasitic diseases regarding the low body gain, high mortality. In addition, such diseases lead to gastrointestinal abrasions which facilitate the invasion of the opportunistic microorganisms. Where unfavourable environmental conditions contribute to stress, which weakens immunity and opens the pathway to pathogens Kabata, (1985), Eissa, (2002). The prevalence rate of encysted metacercariae in *Oreochromis sp* collected from the River Nile at Al-Monib area was considered the highest in egypt and the prevalence of encysted metacercariae was higher in females than that of males Taghreed B.El-Deen (2005). In addation , the prevalence of digenea in summer and spring followed by winter while the lowest one was in autumn Osman (2001) ,while, Tawfik (2005) recorded a prevalence of digenia was in winter . Histopathological alterations of infested male phenotypic, hybrids and monosex *O. niloticus*. It was concluded that monosex *O. cniloticus* are less exposed to parasitic diseases than hybrid and phenotypic *O. niloticus*.

2 Materials and Methods

2.1 Fish

A total number of 1800 cultured *Oreochromis niloticus (O.niloticus)* of various life stages; fry, fingerling and adult Tilapia of different Male fish types (phenotypic *O. niloticus*, hybrids and monosex (hormone treated) were 100, 200 and 300 for each type fish, respectively. The length of fry, fingerlings and adult specimens were ranged from 1- 1.5, 2 - 8 and 20 - 30 cm . Body weights ranged from 0.9 – 15.0, 17 – 28.7 and 105 – 220 g respectively.

2.2 Clinical examination:

The collected fishes were examined clinically according to the methods described by **Noga (1996).**

2.3 Experimental infection

2-3-1- Experimental puppies.

Six puppies, 4-6 weeks old, weighing 1.750-2.050g; were hygienically caged in suitable steel cages with wire – mesh floor. The puppies were fed on bread soaked in pasteurized milk and supplied with clean water according to **Farris (1967).** The dogs were proved to be free from any parasitic infestations by three successive fecal examinations with one-week interval. Moreover, they were given orally dose of broad-spectrum anthelmintic; Yomesan® (*Niclosamid*e) "*Bayer*" at a rate of 0.25 g. / Kg body weight. A frequent removal of the feces and washing the floor of the cages with hot water and soap was adopted.

2-3-2- Experimental ducklings

Eighteen ducklings, 3-weeks old, weighing 120-130 g; were reared conventionally in separate

special cages. They were fed on a ration of 25% protein content and provided with clean water. Their feces were examined daily for three successive days to ensure their clearance of parasitic infestations. In deed, they were provided with a dose of broad-spectrum anthelmintic drug, Ban-minth (*Pyrantel Tartrate, 12.5 %*) "*Pfizer*" at a rate of 0.15 g. / Kg body weight. A frequent removal of the faeces and washing the floor of the cages with hot water and soap was adopted.

2-3-3- Preparing fish musculature for experimental infection:

From heavily infected regions of the examined fish; the musculature and gills containing viable unidentified metacercariae cysts were collected and weighed and given orally to the parasites-free experimentally infected puppies and ducklings.

2-3-4-Experimental infection of puppies:

The puppies were divided into three groups; two puppies for each. The puppies from each group were fed on 50 grams of infested musculatures and gills for three successive days according to **(Shaapan, 1997).**

2-3-5- Experimental infection of ducklings:

The ducks were divided into three groups; six ducklings for each. The ducklings from each group were fed on two grams infested musculatures and gills for three successive days according to (Amany Abbass, 1997).

2-3-6- Detection of the eggs after experimental infection:

The stools of the infected puppies and ducklings were daily examined for detection of trematodes eggs using the flotation sedimentation technique described by **Soulsby (1978)** in order to determine the prepatent period, which is the first day of egg appearance after the last day of infection was calculated.

2-3-7– Collection of the adult flukes:

When the number of eggs in the stools of infected puppies and ducklings began to decrease; all puppies and ducklings were killed .The small intestine was subdivided into 3 parts (duodenum. jejunum, and ileum), where each part was separately opened and the contents and the scraped mucosa were collected in suitable jars containing normal saline. Several washings with normal saline were carried out to remove the coarse particles of intestinal contents and mucous that may be attached to the parasites. The sediments were examined with binocular microscope, then the flattened trematodes were picked up in a small bottles containing 10% formalin using Pasteur pipette. Permanent mounting of the collected trematodes and metacercariae; were carried out using

Drury and Wallington (1980), to prepare permanent stained mounts of metacercariae and adults.

2-3-8- Relaxation of trematodes:

The trematodes were mounted on a glass slide and covered with a thin glass slide. Care was necessary to avoid the use of strong pressure on delicate helminthes parasites.

2-3-9- Fixation of obtained trematodes:

It was carried out using formalin saline 5 % as a fixative and left overnight.

2-3-10-Washing:

The specimens were then washed with tap water for 15 min; to get rid of any traces of formalin solution.

2-3-11- Staining:

The specimens were stained with acetic acid alum carmine (Kruse and Pritchard, 1982).The carmine powder, acetic acid and 100 ml of distilled water were put in a mortar to be soaked for 20 minutes. Then they were boiled gently for 1 hour, at the same time the alum was dissolved in the distilled water (900 ml), and then added to the cooled carmine solution. The solution composite was heated again for 1 hour, and cooled filtered. After filtration, 1 g of salicylic acid was added to the filtered solution to inhibit the growth of molds.

2-3-12- Differentiation:

It was done as slowly as possible using a dilute solution of acid- alcohol. It is much better to carry out this process under the dissecting microscope; in order to determine accurately the out most differentiation of internal structures of the trematodes.

2-3-13- Dehydration:

It was carried out in ascending grades of ethyl alcohol (30%, 50%, 70%, 90% and absolute alcohol).The time of dehydration depending on the size of the specimen.

2-3-14- Clearing:

This process was carried out with clove oil as long as, to complete the clearing of the adult trematodes.

2-3-15- Mounting:

It was proceeded with Canda balsam and then the specimens were covered with cover slide and left to dry at 35- 38˚C in an incubator.

3-Identification of parasites:

The identification of the encysted metacercariae was undertaken according to Kabata (1985).

4-Histopathological examination:

It was carried out for the naturally infested fish. Specimens from skin, gills and musculatures were taken in different grades of alcohol, cleared in xylol then embedded in paraffin wax. Sections of 4-5 microns were obtained and mounted on glass slide and stained with Haematoxyline and

Eosin (H & E), according to Drury and wallington (1980).

3. Results:

Clinical examination:

The infested fishes showed no pathognominic lesion except black spots were detected on skin and fins (Fig, 1).

Parasitological examination:

Microscopic smears were taken from skin of examined fish, showed identified as the examined black cyst in stained smears taken from skin and orbital cysts were identified as *Myxobolus dermatobia* , cysts were found inside the skin and fins were identified as *Posthodiplostomum cuticola* metacercariae, Cysts were embedded in gill lamellae and musculature were related to *Heterophid* metacercariae and *Haplorchoid* metacercariae .

Fig. (1): Phenotypic *Oreochromis niloticus* with black spots on the skin and fins.

Fig. (2): Phenotypic *Oreochromis niloticus* showing encysted metacercariae in the posterior kidney.

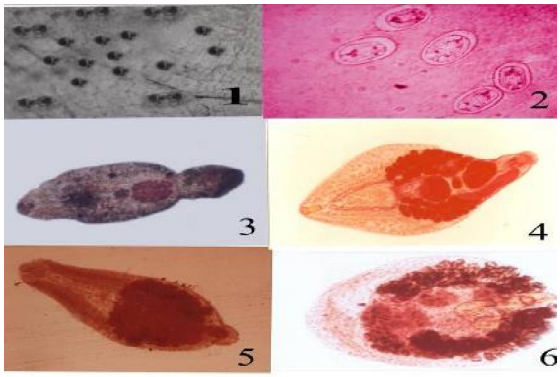

Plate (1):

1- Heavy infestation of *Haplorchoidae* encysted metacercariae in musculature.Wet mount X150.

2- Heavy infestation of Heterophidae encysted metacercariae in musculature. Stain: Acetic acid alum Carmine X40. **3-** Larva of *Posthodiplostomum cuticola*. Wet mount X 40.

4- *Mesostephanus appendiculatis*. Stain : Acetic acid alum carmine X 40.

5- *Prohemostomum vivax*. Stain: Acetic acid alum Carmine X 40. **6-** *Paracoenogonimous ovatus*. Stain: Acetic acid alum Carmine X 40.

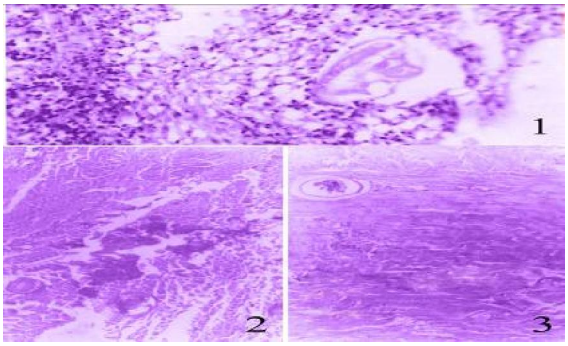

Plate (2): 1- Encysted metacercariae embedded in the primary gill lamellae and surrounded with severe leucocytic infiltrations (arrows). Stain (H& E) X 125. **2-** Dorsal musculature deeply containing cysts invaded with few leucocytes (arrows).Stain (H& E) X 125.**3-** Encysted metacercariae embedded in tilapia musculature and surrounded with Oedema (arrows). Stain (H& E) X 125

Table (1): Unidentified encysted metacercariae (EMC) in experimentally infested puppies.

puppies group	Type of Tilapia	No. of Inf. puppies	Source of EMC	No. of EMC per /g	No. of + ve puppies	No . of recovered Trematodes	Percent of recovered EMC %	Isolated Trematodes	Prepatent Period (days)
Group 1	**Male phenotypic *O.***	1	Musculatures	25 / 10 g	1	153	61.2	*Prohemostomum vivax.*	7-10 days
		1	Gills	4 gill filament contain 5 EMC	1	5	25	*Mesostephanus appendiculatus*	7-10 days
Group 2	**Male hybrids *O. niloticus.***	1	Musculatures	33 /10 g	1	215	65.2	*Prohemostomum vivax* and *Mesostephanus appendiculatus* .	7-10 days
		1	Gills	4 gill filament contain 7 EMC	1	10	35.4	*Mesostephanus appendiculatus.*	7-10 days
Group 3	**Male monosex *O. niloticus.***	1	Musculatures	6 /10 g	1	17	28.3	*Prohemostomum vivax..*	7-10 days
		1	Gills	4 gill filament containing 2 EMC	1	2	25	*Mesostephanus appendiculatus* .	7-10 days

Table (2): Unidentified encysted metacercariae (EMC) in experimentally infested ducklings.

Ducks group	Type of Tilapia	No. of Infected ducks	Source of EMC	No. of EMC per /g	No. of + ve. ducks	No. of recovered Trematodes	Percent of recovered EMC %	Isolated Trematodes	Prepatent Period (days)
Group 1	**Male phenotypic *O. niloticus.***	2	Musculatures	25/ 10 g	1	5	2	*Paracoenogonimus ovatus.*	7-10 days
		2	Gills	4 gill filament containing 5 EMC	0	0	0		7-10 days
Group 2	**Male hybrids *O. niloticus.***	2	Musculatures	23 / g	2	9	2.7	*Paracoenogonimus ovatus.*	7-10 days
		2	Gills	4 gill filament containing 7 EMC	0	0	0	0	7-10 days
Group 3	**Male monosex *O. niloticus.***	2	Musculatures	5 /10 g	0	0	0	0	7-10 days
		2	Gills	4 gill filament containing 2 EMC	0	0	0	0	7-10 days

Experimental infestation of puppies and ducklings with unidentified encysted metacercariae form different types of Tilapia fishes *O.niloticus* (phenotypic (group 1), hybrid (group 2) and monosex (group 3): **(Tables 1** and **2).** The obtained adult flukes from the upper part (duodenum) of the small intestine of experimentally infested puppies with unidentified metacercariae encysted in phenotypic *O. niloticus* musculatures was identified as *Prohemostomum vivid, Mesostephanus appendiculatus,Paracoenogonimus ovatus* and *Euclinostomum heteroatom from* midgut of the small intestine of experimentally infested puppies. Gills infested with encysted metacercariaes revealed multiple parasitic cysts in primary and secondary lamellae the parasitic cysts appear surrounded with connective tissue proliferation .Oedematus musculature and infested with encysted metacercariaes were appear surrounded with serous fluid which contains a network of fibrin (plate, 2).

4. Discussion

The present study deals with most of different encysted metacercariae parasitic diseases among naturally infested the cultured Tilapia sp *O. niloticus* (phenotypic, hybrid and monosex) in Kafr El- Sheikh fish farms. The internal organs of naturally infested fish appeared pale, anemic with enlargement and congestion of spleen, liver with distended gallbladder. Signs of emaciation with petechial haemorrhage on the surface of abdomen and slight bulging of stomach was observed.

Concerning the identified encysted metacercariae, they were *(Euclinostomum heterostomum, Posthodiplostomum cuticola, Heterophidae* and *Haplorchoidae*). Such results are nearly similar to those of the original descriptions of Yamaguti (1985), Eissa *et al.*, (1996), Shaapan (1997), Gado and El-Bahy (1999), Ibrahim, (2000), Mousa *et al*.,(2000) and Eman Bazha (2003). The identification of *Prohemostomum vivax, Mesostephanus appendiculatus* and *Paracoenogonimous ovatus* was dependent on the morphological characters of the obtained adult trematodes recovered from the experimental infestation in puppies and ducklings, these results nearly similar to that recorded with Olfat Mahdy and Shaheed,. (2000): (1991) who recovered them from ducks fed on *Tilapia sp* and Shaapan (1997) who recovered them from dogs.

Regarding to encysted metacercariae (EMC) infestation in Tilapia sp show moderate to severe hyperplasia of gill epithelial of the primary gill lamellae also the cysts were surrounded with multiple cellular reactions included mononuclear inflammatory cells. These may be attributed to displacement of gill filament tissues by the EMC which accompanied by loss of the fine lamellar structure deformation and even atrophic degeneration of gill filament. These results nearly similar to that recorded agreed with that finding by Badran *et al.*(1996), Osman (2001) , and Ibtsam (2004).

Concerning musculature oedematous and infested with encysted metacercariae was appear surrounded with serous fluid which contain a network of fibrin. It may be attributed to irritation of infective parasite and their product. These nearly were similar to that recorded by Soliman (1997). It was concluded that monosex were less exposed to encysted metacercaraie tha hybrid and phyenotypic *o.niloticus.*

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