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STUDY OF SOME TISSUE ENCYSTATIONS WITH REFERRING TO EPITHELIOCYSIS (CHLAMYDIOSIS) IN THE GILLS OF OREOCHROMIS NILOTICUS IN EGYPT

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SUMMARY

In the present study, attempts are made to detect the parasitic tissue encystations (Haplorichid and Echinotsomatid encysted metacercaria, myxosporean cysts and non parasitic tissue encystations Epitheliocystis, "chlamydial infections of fish"), in gills of *Oreochromis niloticus* fish. The clinical signs of naturally infected freshwater cultured *Oreochromis niloticus* are recorded. The prevalence of tissue encystations (encysted metacercariae, Myxosporean cysts and Epitheliocystis) was 80%, 46.6% and 69.3%, respectively. The isolated parasites identified as Haplorichid, Echinostomatid encysted metacercariae and 2 types of myxobolous spp. In addition, Epitheliocystis was reported in the present work for the first time from Egyptian freshwater fish. Light microscope detect intracellular granular inclusion of Epitheliocystis, while electron microscopic examination revealed that the pathogen are rod shaped and situates at the center and had an electron dense core with electron lucent vesicles on its sites.

INTRODUCTION

Gill encystations were identified as metacercariae, myxosporidans and Epitheliocystis which, cause severe gill damage, lower respiratory efficiency and consequent mortalities (Paperna, 1996). Gill infection with the Heterophyids Centerocestus spp. and Haplorchis spp. occur in all young- of the year cichlids (Farstey, 1986). The only apparent tissue response to the presence of the metacercariae is the formation of a dense fibrous layer around the encysted metacercariae. The cercariae penetrate the gill filaments and encyst in the connective tissue adjacent to the cartilage rags of the gill filaments (Farstey, 1986 and Xiao et al., 2005). Massive Heterophyid

metacercariae infections in juvenile fish have been incriminated as an important cause of natural mortalities (Lemly and Esch, 1984 b). Myxosporidia are also important parasites of the gills of freshwater fish (Roberts, 1989). Landsberg and Lom (1991) counted 450 species of the genus myxobolus common in cichlids and cyprinids. Infection is usually subclinical and undetectable unless fish die, tissue infection are whitish cysts with a milky substance containing microscopic spores (Paperna, 1980). Whitish nodules were concentrated in the gill filaments, causing destruction and necrosis of gill tissue, leading to haemorrhages, anemia and death (Lom and Dykova, 1995 and Morris et al., 2006).

A number of species rickettsiae and chlamydiae are pathogens of humans and other animals.

The rickettsia that cause piscerickettsiosi (Fryer et al., 1990) and the chlamydie- like organism (CLO) that causes Epitheliocystis (Hoffman et al., 1969) the disease has been reported in fish from both warm and cold- water habitats and from a variety of hosts including marine andromous and fresh water species Epitheliocystis was discussed as a new disease of fish by Hoffman et al., (1969). After their observation of cyst- like lesions on gills and fins of the bluegille fish (lepomio macrochirus). It is new recognized as a cytopathological condition responsible for metabolites in a number of fish species held in captivity (Paperna, 1977; Paperna and Sabnai, 1980).

The term of Epitheliocystis is derived from the appearance of epithelial lesion that are mean infested secondary to infection. Development of the organism is similar to that of the Chlamydiae and proceeds from small, rigid infection forms elementary bodies (EB) to larger, pleomorphic non infectious forms reticulate bodies that divide by fission to produce a new generation of infectious daughter cells (Joseph et al., 1996 and Kim et al., 2005).

The clinical disease has been attributed to respiratory distress secondary to gill epithelial hyperplasia and excessive mucus production (Joesph et al. 1996). Infection is apparently transmitted directly from fish to fish hyper infection and consequen mortalities may develop in young fish stocked i culture (less than 60 mm or less than 2 gm) withi four to six weeks after initial contaminatic (Paperna, 1996).

The present studies are planned to clarify the encystations of ***Oreochromis niloticus*** regard its clinical signs, prevalence, isolation and id fication of the parasitic agents (encysted me rariae and myxosporean) and non parasitic a Epitheliocystis infections. The last one wa tected for the first time from Egyptian fresh fish and confirm this identification by ele microscope.

MATERIALS AND METHODS

Fish:

A total number of 150 freshwater cultured ***Oreochromis niloticus*** of different size (up to 100 gm) were collected alive from Manzala hatcheries and fish cultured ponds. The fishes are transported in plastic bags supplied with battery air pumps. Tricaine methanesulfonate (MS- 222) are used as a tranquilizers to the fish (10- 40 mg of tricaine/ L) (Noga, 1996). Fish are transported to Fish Disease Department in Animal Health Research Institute. The freshly dead fishes are labeled and packed in clean plastic bags and put in ice-box at 4°C until delivered to the laboratory for immediate examination. A live fish put in a suitable glass aquariums (1:3 by volume) and supplied with oxygen and feeding in a ratio of 3% of biomass maintenance ration and change the water periodically according to the quality of aquarium water. The fish are examined clinically for any abnormal lesions according to Noga (1996).

Light microscopical examination:

1- The encystations in tissues of gills are selected, isolated and detected by flat and pressing techniques according to Lucky (1977) (Squash preparation) and examined by dissecting microscope (X 4 & X 8).

2- The isolated cysts are dealing with digenetic metacercaria are identified according to Papena (1996) through fixation with formal saline 10% and staining with semichon's acetocarmine (Kruse and Pritchard, 1982), examined by X 10 & 40 of ordinary light microscope.

3- The isolated Myxosporeans and Epitheliocystis are isolated and identified according to Shulman (1984) and Noga (1996) through fixation with methyl alcohol and staining with Giemsa stain and examined by (X10, X40 and oil immersion lens) of ordinary microscope.

4- The confirmation of Epitheliocystis in gills of Oreochromis niloticus are done through electron microscope by transmission electron microscopy, JEOL 100S (Japan) was applied on selected areas of gill tissues according to Joseph et al. (1996). Samples were prepared in VACSERA (Ministry of Health, Egypt).

RESULTS

Gross pathological findings:

Gross examination of the infested fish revealed the presence of congestion, hemorrhage (Fig., 1) and damage of gills resulted in lower respiratory efficiency.

Microscopical examination:

The microscopical examination revealed the infestation of ***Oreochromis niloticus*** with mixed infection of parasitic; digenetic encysted metacercaria (Haplorichyidae and Echinostomatidae), protozoa myxosporean cysts (2 types of myxobolous spp.) and Epitheliocystis (Chlamydial infections), Table, (1).

1. Parasitic cysts:

Each type of cysts isolated and recorded the main morphological difference between them (Table, 2).

1- Encysted metacercariae:- (Fig., 2 a)

A- Haplorchid metacercariae (Fig., 2 b)

The encysted metacercariae were spherical to oval in shape and measured 0.6-0.98 mm. in length and 0.4-0.45 mm in width. The cyst wall was double layer and transparent. The ventral suckers was seen difficulty. It is characterized by possessing one testis and sometimes appear two eye spots in young stage. The cyst contain characteristically light spots.

B- Echinostomatid metacercariae (Fig., 2 c)

The encysted metacercariae was oval in shape and measured 0.2-1.5 mm. in length and 0.1-0.5 mm in width. The cyst has transparent cyst wall. The metaceraria was elongated, with prominent head collar contained spins, arranged on each side. The oral sucker was subterminal and the ventral sucker was nearly situated at the middle half of the body and the ovary was situated posterior to the ventral sucker.

2. Myxobolous protozoal cysts:

A- Myxobolous sp. (Fig., 3 a).

B- Myxobolous Oreochrome (Fig., 3 b).

Squash preparations from cysts revealed spores of Myxosporea parasites belonged to the Genus Myxobolous as two different species, having the same general morphological characters but differ in morphometeric features. Stained smear showed one spp. of ovoid spores measuring 11-12 µm (Fig. 3a), and the other spp. (Myxobolous Oreochrome) is oval in shape, larger in size (9-10 X 17-19 µm), slightly pointed anteriorly (Fig., 3b).

The two spp. have two polar capsules of equal size situated in anterior end of spore and the iodinophilous vacuole was not clearly detected.

II- Non parasitic cyst:

Epitheliocyst:

Impression smears were made from infested gi of Tilapia spp. and stained with Giemsa stain examined microscopically to detect intracell granular inclusion of epitheiocystis.

The result was 104 out of 150 were positive tc itheliocystis in percentage of 69.3% (Fig., 4 b). while the electron microscopic examin revealed that in the longitudinal section the p gen were rod shaped and situated at the cer the cyst and had an electron dense core wit tron-lucent vesicles on its sides where pathogen in more peripheral position ha regular shape and did not have the el lucent vesicles (Fig., 5 a & b).

Table (1): Prevalence of gill encystations recovered from *Oreochromis niloticus* fish.

Parasitic infestation	No. of examined fish	No. of infested fish	
		No.	%
* digentic infestation Encysted metacercarea (Haplorichid and Echinostomatid)	150	120	80
Protozoal infestation Myxobolus cyst	150	70	46.6
Non parasitic infection Epitheliocystis	150	104	69.3

Table (2): The main morphological difference between the encysted metacercariae, Myxobolus cyst and Epitheliocystis recovered from gills of *Oreochromis niloticus*.

Types of cyst	Encysted metacercare		Myxobolus cyst	Epitheliocystis
	Haplorichid	Echinostomatid		
Color	Light grayish	Grayish	Whitish	Transparent white to yellow
Shape	Spherical to oval	Oval	Pen head	Round
Size	0.6 - 0.9 X 0.4 -0.45mm	0.2 - 1.5 X 0.1- 0.5 mm	0.5 - 1.5 mm	0.6-0.8 mm
Cyst wall	Thick	Thin and transparent	Fragile	Thick
Contents	Motile larva, have oval and ventral suckers		Milky substance containing spindle - shaped spores (9-11X12-19 µm) they are uniform size and shape	Intracellular coccus shaped pathogen (0.3 - 1 µm)

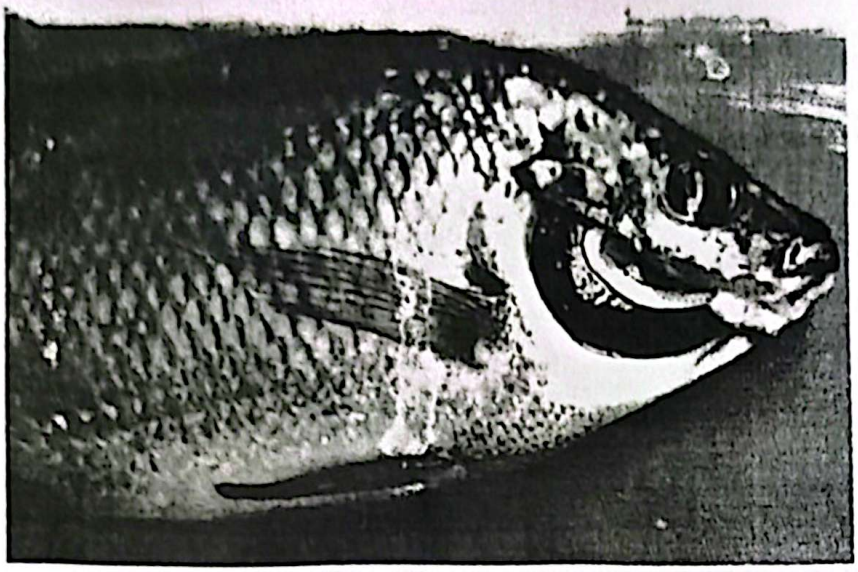


Fig. (1): Congestion, haemorrhage and mucoid gills of *O. niloticus*.

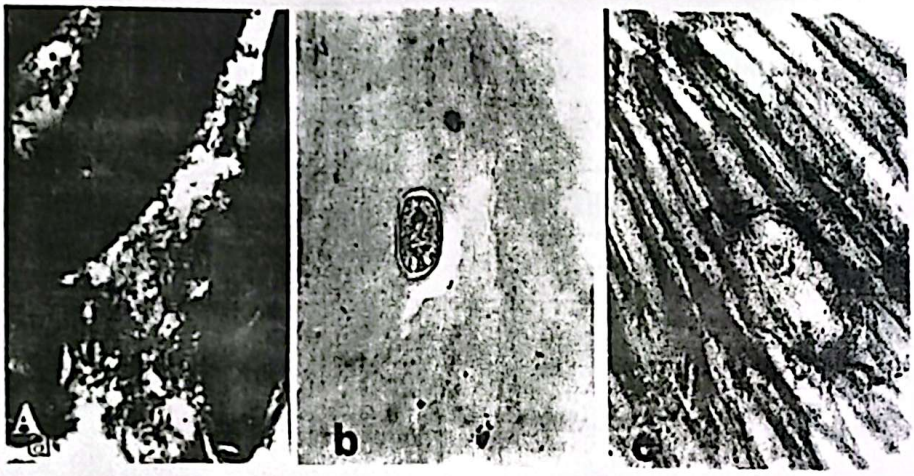
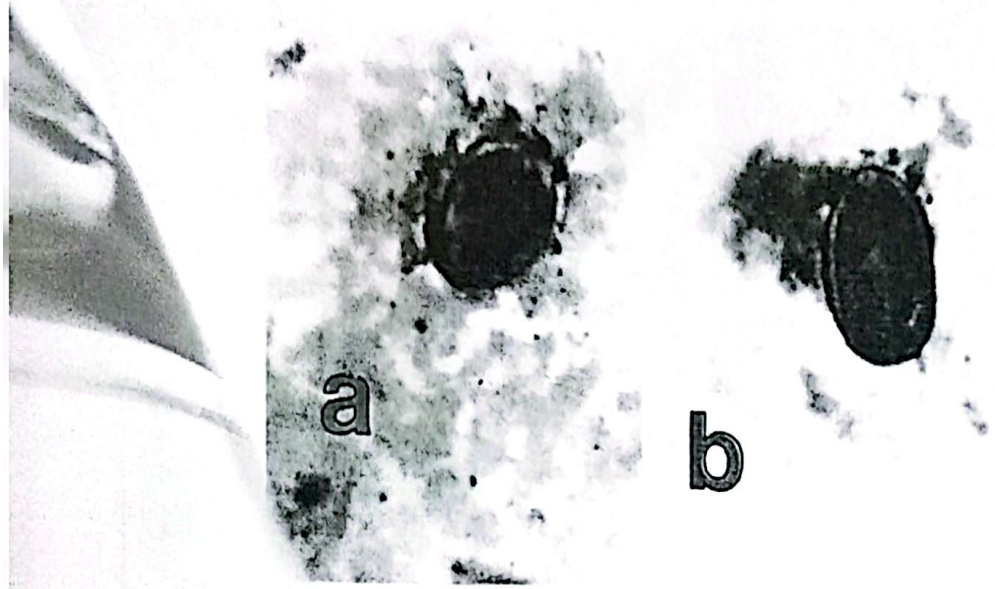


Fig. (2): a: Encysted metacercariae in gills of *O. niloticus* (X 40).
b: Haplorichid encysted metacercariae (X 160).
c: Echinostomatid encysted metacercariae (X 100).

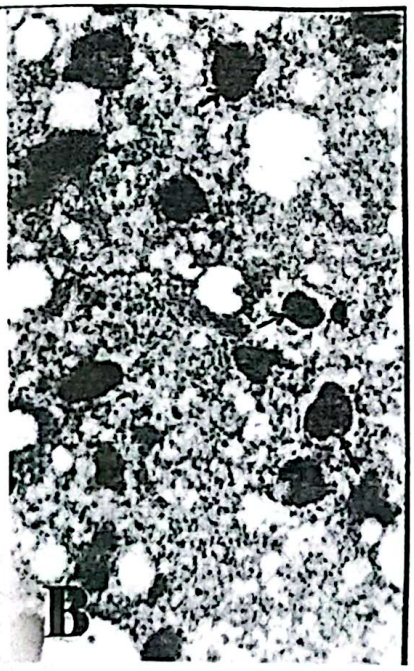


: Myxobolus spp. spores from gill filaments of *O. niloticus*
round in shape (X 400).

yxobolus Orecochrome. spores from gill filaments of *O.*
ticus (oval in shape (X 400).

Table (2): Th
cyst

Types of cyst	
	Hapl
Color	Light
Shape	Spherical to ov
Size	0.6 - 0.8X 0.4-0.45mm
Cyst wall	Thick:
Contents	Motile larva ventri



gills of *O. niloticus* stained by

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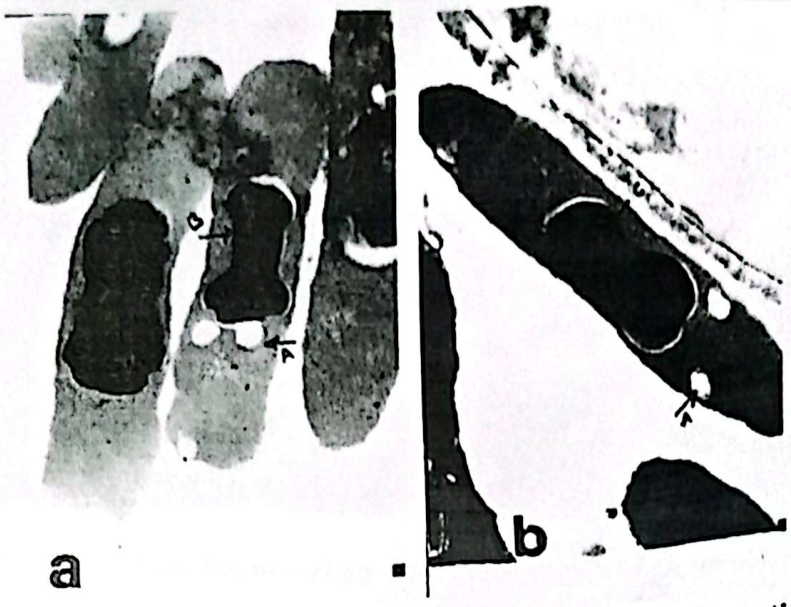

Fig. (5): Transmission electron micrographs of epitheliocystis organisms form gills of *O. niloticus* fish. a: (X 11200). b: (X 1400).
A- Electron-lucent vesicles.
B- Rod shaped pathogen.
C- Electron-dense core.

DISCUSSION

In the present study, ***O. niloticus*** infested with encysted metacercariae, Myxobolus cyst and Epitheliocystis have excess gill mucus and hemorrhages. This was related to host damage occur during cercarial migration and inflammation along the migration path (Sommerville, 1981). The only apparent tissue response to the presence of the metacercariae is the formation of a dense fibrous layer around the encysted parasite. Moreover, hyperinfection cause functional damage when the active tissue is displaced by the encysted parasite (Paperna, 1980).

Fish infested with whitish nodules of Myxobolus in the gill filaments have growth impairment and/or mortalities are due to reduced respiratory capacity of the infested gills (Lom and Dykova, 1995 and Morris et al., 2006).

Moreover, rupture of Myxobolus cysts leads to haemorrhaging, loss of blood and facilitated invasion of secondary opportunistic pathogens (Paperna, 1996). Epitheliocystis occurs as a benign or proliferative disease, in the benign infections, cysts may be surrounded by a layer of squamous or cuboidal epithelial cells. Typically, no host response is apparent, even in the presence of large numbers of cysts. However, in certain cases, the organism induces an extensive host response, with unrestricted proliferation of gill epithelia and extensive mucus production (Lannan et al., 1995). In the present study, the prevalence of

cysted metacercariae (Haplorichidae and Echi-stomatidae) in *O. niloticus* gill was relatively gh (80%). This result was in agreement with ose of Papenna (1996) and Ibrahim (2000). gh prevalence might be attributed to the ad-rse environmental factors, as the temperature, linity, density of the snails and the migratory rds (Kennedy, 1982). Moreover, cyst formation probably responsible for the characteristic lack f host to metacercariae (Noga, 1996). The preva-nce of Myxobolus cyst was 45%, this result was igher than those obtained by Abd El Aal et al. 2001) and on the other hand was lower than that nentioned by Paperna (1996). The prevalence of nfestation is variable, this could be due to the life ycle can not be completed in aquaria because of he absence of an essential intermediate host but in natural environment, myxozoans can be seri-ous (Wolf and Markiw, 1984). In this study, the morphological characters of encysted metacercar-ia (Haplorichidae and Echinostomatidae) and Myxobolus spp. cyst infesting *O. niloticus* were coincided with those of Ibrahim (2000) and Abd El Aal et al. (2001).

Epitheliocystis was reported in the present work for the first time from *O. niloticus*, Egyptian freshwater fish. Epitheliocystis previously detect-ed from gill of Tilapia nilotica and Blue/Nile tila-pia hybrid in Israel (Paperna et al., 1981) and cy-prinus carpio in South Korea (Kim et al., 2005). In the present study, the prevalence of Epithelio-cystis in the *O. niloticus* gill was relatively high (69.3%). This result similar to that obtained by Paperna et al. (1981). Natural transmission of the Epitheliocystis from infested fish may occur in culture facilities through contaminated nets and other equipment (Paperna, 1977).

The results of detection of Epitheliocystis by Giemsa stain was 69.3% and that was agreement with what is mentioned by Frances et al. (1997) whose found that the ratio of infected fish by epi-theliocyst was 75%, also, they mentioned that the cytoplasmic inclusions were composed of a fine, homogenous, dense, basophilic granular material suggestive of coccoid to coccobacilary microor-ganisms observed in epitheliocystis infection. While Zimmer et al. (1984) mentioned that the ratio of infested cat fish by epitheliocystis was 46% by microscopic and electron microscope ex-amination.

While the results of electron microscopic examination agreement with that mentioned by Joseph et al. (1996) and Kim et al. (2005).

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بعض الدراسات عن التحوصل النسيجى بالإشارة إلى حويصلات الكلاميديا (الكلاميديوزس) فى خياشيم البلطى النيلى بمصر

إبتسام عبدالغنى أحمد طنطاوى ، هادية عبدالرحيم على

قسم بحوث أمراض الأسماك ووحدة بحوث الكلاميديا ، معهد بحوث صحة الحيوان - مركز البحوث الزراعية - الدقى

تم فى هذه الدراسة محاولات لإكتشاف التحوصل النسيجى شاملاً حويصلات الميتاسيركار (الهابلوركيس والايكينوستوماتيد)، حويصلات الميكزوسبوريا وحويصلات الابيسيليوسيست (عدوى الكلاميدنا فى الأسماك) وذلك بخياشيم أسماك البلطى النيلى المستزرعة والمجمعة من المفرخ السمكى ببحيرة المنزلة. وتم تسجيل العلامات المرضية الناتجة عن إصابة الأسماك بهذه الحويصلات. وكانت نسبة إصابة الأسماك بحويصلات الميتاسيركاريا (الهابلوركس والايكينيوستوماتيد) والميكزوسبوريا والابيسيليوسيست هى ٨٠٪ ، ٤٦.٦٪، ٦٩.٣٪ على التوالى. وتم وصف الطفيليات المعزولة وتصنيفها إلى حويصلات الميكزوسبوريا من جنس المكزوبولس . بالإضافة تم فى هذا العمل تسجيل إصابة حويصلات الإبيسيليوسيست (الكلاميديا) لأول مرة بأسماك المياه العذبة فى مصر. وقد أظهر الفحص الميكروسكوبى وجود الأجسام المحورية للككلاميديا داخل سيتوبلازم الخلايا المصابة. وقد تم تأكيد هذا التشخيص بالميكروسكوب الإلكترونى الذى أثبت إصابة خياشيم أسماك البلطى بحويصلات الإبيسيليوسيست.