PREVALENCE OF ENCYSTED METACERCARIOSIS AMONG OREOCHROMIS NILOTICUS IN DAKAHLIA PROVINCE WITH SPECIAL REFERENCE TO TREATMENT

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ABSTRACT

A total number of 827 fish samples (Oreochromis niloticus) were collected from different fish farms at Dakahlia province during a period extended from December 2001 to November 2002. The prevalence varied from 61.44% in May to 17.46% in December. Infection rate seemed to be increased with the increases of water temperature. The obtained encysted metacercariae were isolated and identified as Clinostomatid (in branchial cavity), Diplostomatid (in the eyes), Euclinostomatid (in the kidney tissue) and Heterophyid (in the muscular tissue, gills, liver and heart).

There were no pathognomonic clinical signs associated with encysted metacercariae infestations except in Diplostomatid, which infested fish represented as loss of body weight, dark discoloration of the skin, uni or bilateral eye cataract and black spots on the skin in case of Heterophiid infection. Histopathological alterations were detected in fish samples especially in liver, eyes, heart, kidney, gills and branchial cavity of O. niloticus.

Treatment trials of Nile tilapia (O. niloticus) naturally infected with black spot disease (Heterophyid) cultured in earthen ponds with heavy loaded with fresh water snails at their bottoms. Copper sulphate and Bayluccid, 73 were used as chemical molluscicides at a dose of 0.25 mg/liter of ponds bottom after drainage. The obtained results showed that Bayluccid was more effective in eradication of snails and lowering black spots numbers of infected fish from 73±8 to 31±10 than copper sulphate, which reduces black spots from 78±5 to 57±9, but Bayluccid had a negative effect on fish viability and reflexes post-exposure.

INTRODUCTION

Oreochromis niloticus fish are fast growing, resistant to disease and handling, easy to reproduce in capitivity and able to tolerate a wide range of environmental conditions. They are widely cultured in tropical and subtropical regions of the world and constitute the third largest group of farmed finfish, with an annual production growth rate about 11.5% **(El-Sayed, 1999)**.

Parasitic diseases, especially that caused by adult or encysted metacercariae of digenetic trematode have their own harmful effects on affected fish populations, which are exhibited clinically by loss of body weight, weak performance, decrease fertility and reproduction, as well as increase morbidity and mortality rates with the resultant high economic costs **(Paperna, 1991& 1996 and Mohan et al., 1999)**.

Many digeneans of land vertebrates used fish as a (secondary intermediate host) for their metacercarial stages. Encysted metacercariae are frequently the most common asymptomatic infections in many fish species especially of marine and estuarine zones. Such infections were recorded in different localities as heavy heterophyud muscle infections in grey mullets and juveniles of cichilids in lagoons in the eastern Mediterranean Sea **(Paperna, 1981 and 1991; Farstely, 1986 and Yossef et al., 1993)**. In addition, water bodies from the Jordan to the East African Great Lakes have common fishes (Cichlids, Clarias, and Barbus), which heavily infected with gill-heterophyids, centrocestus spp. and Haplorchis spp. occur in all young-of-the year Cichlids which inhabit shallow waters **(Paperna, 1980; Farstely, 1986 and Wittrock st al., 1991)**. Ocular diplostomiasis has been reported to occur in juvenile Cichlids and Barbus spp. from East and South Africa **(Thurston, 1965., Paperna, 1980 and Mashego, 1982)**.

Branchial Clinostomatids and renal Euclinostomatids are important and common encysted metacercariae recorded in O. niloticus in Egyptian and Sudan aquaculture **(Gado and El-Bahy, 1999)**.

At early time, Egyptian investigators studied and surveyed encysted metacercariae infestation among different wild or cultured Egyptian fish as Mugil cephalus, Tilapia, sp., Clarias lazera, Bagrus bayad and Synodontis schall at different localities as Edku, Cairo, Giza, Sharkia, Sohag, Manzala, Quaron and Wadi al-Raiyan Lake. They concluded that types of encysted metacercariae belonging to the families Heterophyidae, Diplostomatidae, Euclinostomatidae, Clinostomatidae, Cynodiplostomatidae.

Paperna, 1980 Concluded that hyperinfection may cause functional damage and death when the affected organs were displaced by encysted metacercariae.

The aim of the present study to figure out the different encysted metacercariae in Oreochro-

mis niloticus with trial of decreasing infestation load with Baylucid and Copper sulphate.

MATERIALS AND METHODS

Fish :

A total of 827 cultured fish samples (Oreochromis niloticus) were collected from different private fish farms at Dakahlia province, during a period extended from December 2001 to November 2002. Fish samples under investigations were transported alive as soon as possible to Fish Disease and Management at Faculty of Vet. Med. Mansoura Univ.

Clinical examination:

Fish were grossly examined for the presence of any visible cyst at branchial cavity, black spots on the skin and eye cataract according to **Schaperculaus (1996).**

Parasitological Examination :

Microscopical examination was carried out using compression technique according to **Lucky (1977).**

The branchial cavity was dissected and examined by naked eye. Gills were dissected and each gill arch was compressed between two slides and examined under a microscope.

Different internal organs such as liver, kidney, muscles and heart were examined macroscopically for the presence of white military nodules. A small piece of affected organ was squeezed between two slides and examined microscopically.

Examination of eyes macroscopically was done looking for opaqueness or cataract. Affected eyeball was removed from the orbit and cut opened on a slide to examine its fluid contents microscopically.

Histopathological Examinations

Freshly removed infested tissue specimens (liver, kidney, heart, gills, muscles and eyes) containing encysted metacercariae were fixed in 10% phosphate buffer formalin for 48 hours. The specimens then were dehydrated in ascending grades of ethyl alcohol, cleared twice-ascending grades of ethylalcohol, cleared twice in xylene one hour each and impeded in paraffin, sectioned at 5 - 7 microns thickness. Tissue sections on glass slides were stained with Haematoxylin and Eosin (H & E) according to **Carleton et al. (1967).**

Treatment Trials :

Treatment trials were carried out at a private fish farm in Gamasa, Dakahlia province.

An earthen pond 35 x 100 meter with a stocking density 7.000 Oreochromis niloticus fingerlings which heavily infested with black spots on their skin and marked snails at the pond bottom.

Chemical Molluscicides :

1- Baylucide. 73: (a product of Bayer).

It is the ethanolamine salt of niclosamid **(Gonnert, 1961)**, which is used as an anthelmintic for cestodes in mammals.

2- Copper sulphate: (a product of ADWIC):

It is used as fungicide, algicide and in eradication of snails.

The two chemicals used at dose of 0.25 mg/liter of remaining water ponds after drainage and they were left for 3 days. Water irrigation in treated ponds to flush out the chemicals used and new fingerlings (10 - 15 g) Oreochromis niloticus were cultured in the breeding ponds for 3 months. Fish samples were collected after 3 months. (30 fish / pond) and total number of black spots on the skin, were counted and the average number was calculated in each previously treated pond.

RESULTS AND DISCUSSION

The clinical examination of 827cultured Nile tilapia (Oreochromis niloticus) revealed the presence of black spots on the skin and fins particularly among fish collected during summer season (fig. 3). Eye opacity was also reported in some infected fish (fig. 2). General emaciation (loss of body weight and thinning of the body muscles on both sides) as well as some infected fish showed dark discoloration of their skin.

Such observation of clinical signs and postmortem findings, which associated with encysted metacercariae infestation in fish, is in agreement with **(Britz et al., 1985; El-Naffar and El-Shahawy, 1986; Makhlouf et al., 1987; Mansour et al., 1987; El-Bouhy, et al., 1988 and Abd El-Rahim, 1998)**.

Regarding to the parasitological investigations in examined fish it was revealed the presence of black spots on the skin and fins (fig. 3), which proved parasitologically to belong to Heterophids (Table 3).

Internal organs of naturally infected fish had a multiple orange-yellowish cysts (2-8 cyst / fish) in the branchial cavity (fig. 1) (yellow grub disease), which identified as Clinostomum tilapiae as described by **Eissa and Hala (1993) and Eissa (2000)** (Table 3).

Grey whitish large nodules were also detected in kidney of infected fish (fig. 5), which identified parasitologically as Euclinostomum sp. as described in (Table 3).

Uni or bilateral eye cataract were also noticed in some infected fish as shown in (fig. 2), which identified as Diplostomum sp. as shown in (Table 3).

The present study showed that the total prevalence of encysted metacercariae in the examined Nile tilapia was 33.85%. The highest prevalence was in May and June (61.4% and 60% respectively) and the lowest one was in December (17.46%). Such results were nearly agree with Shaapan, (1997) who found that the infestation rate of encysted metacercariae was 77.37% in tilapia species, whereas **Abou-Zakham et al., (1990)** recorded higher prevalence and intensity of metacercariae in mugil and tilapia species collected from Lake Manzala in summer than in winter. **Mohamed (1996)** recorded 59.4% infestation rate in Tilapia zilli and 89.7% in Tilapia niloticus.

The infestation rate increases with the increase of water temperature, which coincide with increase infestation rate of snails with the increase snail size during summer: June, July and August **(Paperna, 1991 & 1996)**.

Dealing with the seasonal dynamics, the present study was disagreed with that recovered by **Zeng and Liao (2000)** who reported that the prevalence was not obviously correlated with the seasonal changes of water temperature.

Regarding to natural infestation of female O. niloticus compared to male fish infestation with different types of EMC, it is clear as shown in table (1) that female fish had a higher infestation rate than males. Such results were recorded by **El-Safiey et al., (1997)** who concluded that female fish had a higher infestation rate with nematodes, cestodes and digenetic trematodes than males. The highest total prevalence of EMC was recorded in muscular Heterophild of naturally infected fish with 52.14%, followed by infestation with branchial Clinostomatid with 23.39%, ocular Diplostomatid 21.78% and the lowest infestation rate was recorded in kidney Euclinostomatid 2.14% (Table, 2).

Many Egyptian investigations extensively studied the prevalence of different encysted metacercariae in internal organs of wild and cultured freshwater fish and their results were nearly agree with the present study results **(Eissa et al., 1988; El-Bohy et al., 1988; Shalaby, 1988; Abu-Zakham et al., 1990; Mohamed,1996; Shaapan, 1997 and Tantawy et al., 1998)**.

Dealing with the pathological alterations associated with encysted metacercariae of Heterophild in different internal organs of O. niloticus, firstly gill arch, head encysted metacercaria surrounded by inflammatory cells (Fig. 4) and the base of gill filaments showed encysted metacer-

carla (Fig 4') which may cause damage to gill epithelium resulting in respiratory distress which coincide with that of **(Lee and Cheng, 1970; Madhavi and Rukmini, 1991; Aly, 1994; Mahdy et al., 1995 and Mohammed, 1996)**.

Presence of Heterophild encysted metacercaria in the pancreatic tissue of the liver (Fig. 6) and embedded in hepatic tissue surrounded by fibrous capsule beside mild inflammatory cells (Fig. 6') in agreement with those of **(Lee and Cheng, 1970; Aly, 1994; Mahdy et al., 1995 and Mohammed, 1996)**.

The kidneys showed Heterophild encysted metacercariae surrounded by fibrous capsule (Fig. 7), affected kidney showed also hemorrhages and tubular necrosis (Fig. 7') beside coagulative necrosis of renal epithelium (Fig. 8). These kidney alterations may lead to a hemopoietic and renal failure which coincide with that of **(Gharib and Hamdy, 1969; Lee and Cheng, 1970; Aly, 1994 and Mahdy et al., 1995)**.

Finally, heart of fish showed Heterophild encysted metacercaria surrounded by fibrous capsule (Fig. 9) as described by **Lee and Cheng, (1970); Mahdy et al., (1995) and Mohammed, (1998)**.

Branchial cavity (Oropharynx) showed Clinostomum tilapiae encysted metacercaria surrounded by fibrous capsule (Fig. 8'), such observation recorded by authors **(Kabunda and Sommerville, 1984; Britz et al., 1985; El-Bouhy et al., 1988; Eissa, and Hala, 1993; Mahmoud and Sahlab, 1993 and Mahdy et al., 1995)**.

Concerning to the presence of Diplostomum spathceum in vitrous humor close to retina (Fig. 9'), others investigators recorded that the cyst embedded in the outer fibrous connective tissue and oculomotor muscles. The surrounded muscle bundles suffered from pressure atrophy and degenerative changes **(Lester and Huizinga, 1977; Shariff et al., 1980 and Aly, 1994)**.

Regarding to the presence of Euclinostomatid cyst in the kidney of fish, the large sized grayish cyst which can be detected by naked eye (Fig.5) which surrounded by thick fibrous capsule such results confirmed by **(Britz et al., 1985 and Gado and El-Bahy, 1999)**.

It is known that both of Baylucide and Copper sulphate are used in aquaculture as a molluscicides not to be used as a chemotherapeutics in living infested fish with different EMC, but they can be used in ponds bottom free from fish **(Gonnert, 1961 and Marzouk and Bakeer, 1991)**.

The present study results dealing with the use of Baylucide 73 for controlling snails at the earthen pond bottoms revealed that the product had the ability to reduce the average numbers of black spots on fish skin from 73 ± 8 to 31 ± 10 After treatment at a dose of 0.25 mg/liter. It is known that Baylucide is a molluscicide used in Egypt and many countries to eradicate the snails

which are the intermediate host for digenetic trematodes. However, it was proved that Baylucide has the same lethal effect on other aquatic organisms including fish (**Marzouk and Bakeer, 1991**). With low or sublethal concentrations, fishes may apparently survive and keep living. The molluscicide which doesn't kill individual fish with a specific concentration in a specific time, may still have perminant adverse effects which interfere with the health or the ability to survive and reproduce (**Koeman and Strik, 1975**). Such explanations are in agreement with our data results.

Regarding to the use of Copper sulphate as a molluscicide in the pond bottom at a dose of 0.25 mg/liter. It was cleared that Copper sulphate reduced the average number of black spots on fish skin from 78 ± 5 to 59 ± 9 However, some authors recommended Copper sulphate at a dose of 3 ppm as an effective molluscicide but they also recorded that such dose may be toxic to fish (**Sarig, 1970 and Paperna, 1991**). Other investigators suggested a continuous application of low concentrations of Copper sulphate (0.12 - 0.300 ppm) (**Noga, 1995**).

Table (1) : Monthly incidence of encysted metacercariae in *Oreochromis niloticus*.

Months	No. of examined	No. infected	%	♂	♀	M/F ratio
Dec	63	11	17.46	5	6	1:1.2
Jan	42	14	33.3	6	8	1:1.3
Feb	51	16	31.37	7	9	1:1.3
Mar	93	21	22.58	10	11	1:1.1
Apr	68	17	25	8	9	1:1.1
May	83	51	61.44	21	30	1:1.4
Jun	70	42	60.0	18	24	1:1.3
Jul	91	33	36.26	12	21	1:1.8
Aug	111	28	25.22	13	15	1:1.6
Sep	57	17	29.82	8	9	1:1.1
Oct	35	18	51.42	9	9	1:1
Nov	63	12	19.04	4	8	1:2
Total	827	280	33.85	121	159	1:1.4

Table (2) :Monthly dynamics of different types of encysted metacercariae in different organs of Oreochromis niloticus:

Months	No. of examined	No. infected	EMC in muscle	EMC in Branchial cavity	EMC in Kidney	EMC in Eye	Mixed Infestation
Dec	63	11	-	9	2	-	2
Jan	42	14	-	13	1	-	1
Feb	51	16	7	9	-	-	-
Mar	93	21	12	8	1	-	1
Apr	68	17	14	3	-	-	-
May	83	51	17	9	1	24	1
Jun	70	42	28	3	-	11	-
Jul	91	33	21	7	-	5	-
Aug	111	28	19	3	1	5	1
Sep	57	17	8	-	-	9	-
Oct	35	18	9	2	-	7	-
Nov	63	12	11	1	-	-	-
Total	827	280	146	67	6	61	6
% of infestation		33.85	52.14	23.93	2.14	21.78	2.14

Table (3): The main morphological differences between the collected species of encysted metacercariae (EMC):

Type of EMC Character	Clinostomadid	Diplostomatid	Euclinostomatid	Heterophyid
Host	Oreochromis niloticus			
Tissue	Branchial cavity	Eye	Kidney	Muscles, gills, liver and heart
Colour	Yellowish to orange	Light greyish	Greyish opaque	Black
Shape	Spherical	Oval	Spherical	Spherical to Oval
Size	4.0 mm in diam	0.8 – 0.6 mm	4.0 – 5.0 m	0.35 – 0.25 m
Cystwall	Thick	Thin	Very thick	Thick
Sukers	Well developed	Well developed	Well developed	Difficulty seen
Pseudo-sukers	-	Present	-	-
Balck pigments	-	-	-	Present
Light spots	-	-	-	-
Stalk like process	-	Present	-	-

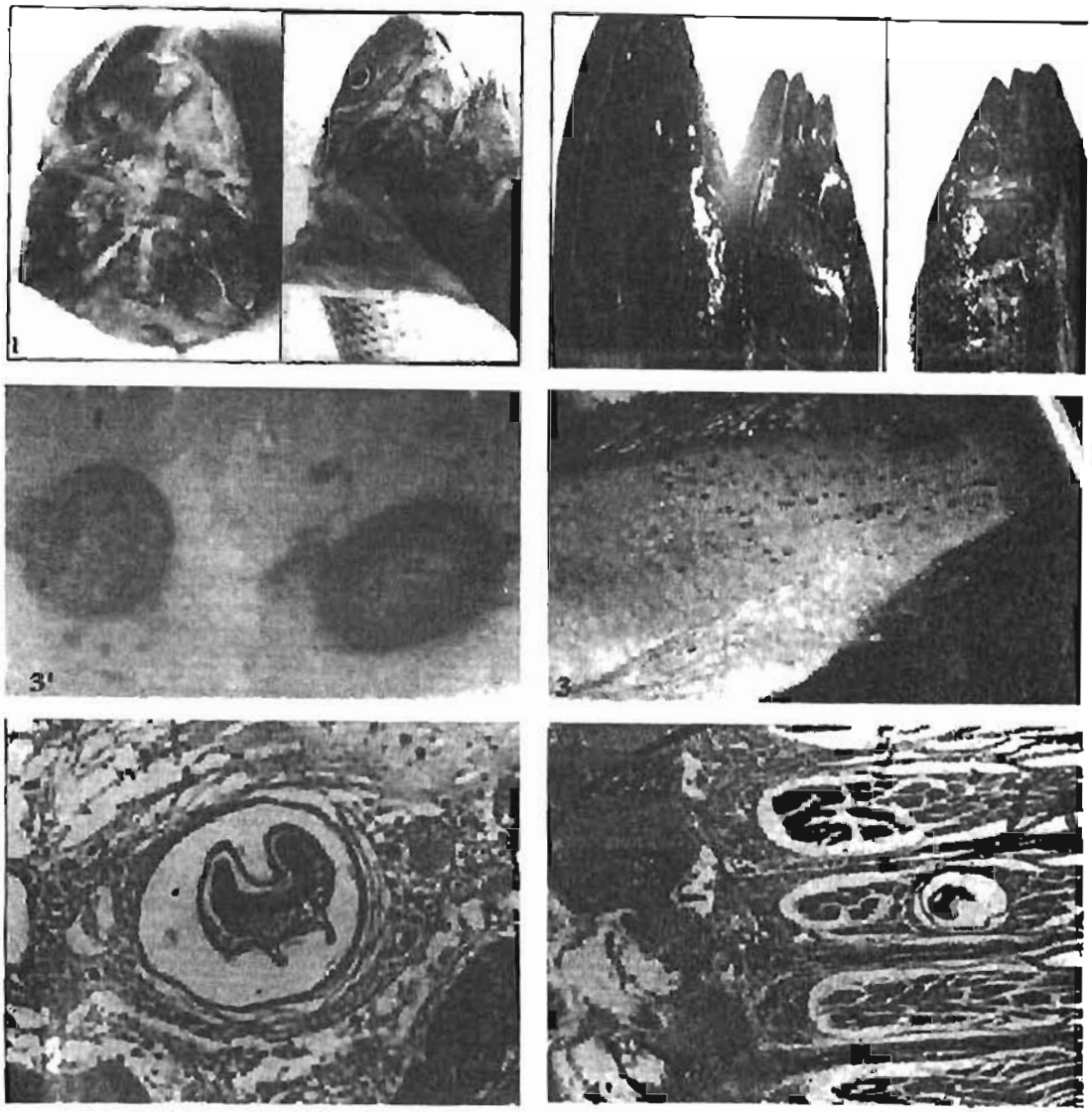

Fig. (1): Yellow grubs in branchial cavity Oreochromis niloticus(arrow).

Fig. (2): Eye cataract of O. niloticus (Diplostomum spathecum).

Fig. (3): Black spots in subcutaneous of O. niloticus.

Fig. (3'): Heterophid cyst in musculature tissue.

Fig. (4): Gill arch showing encysted metacercaria surrounded with inflammatory cells.

Fig. (4'): Gills of fish showing encysted metacercaria at the base of gill filaments.

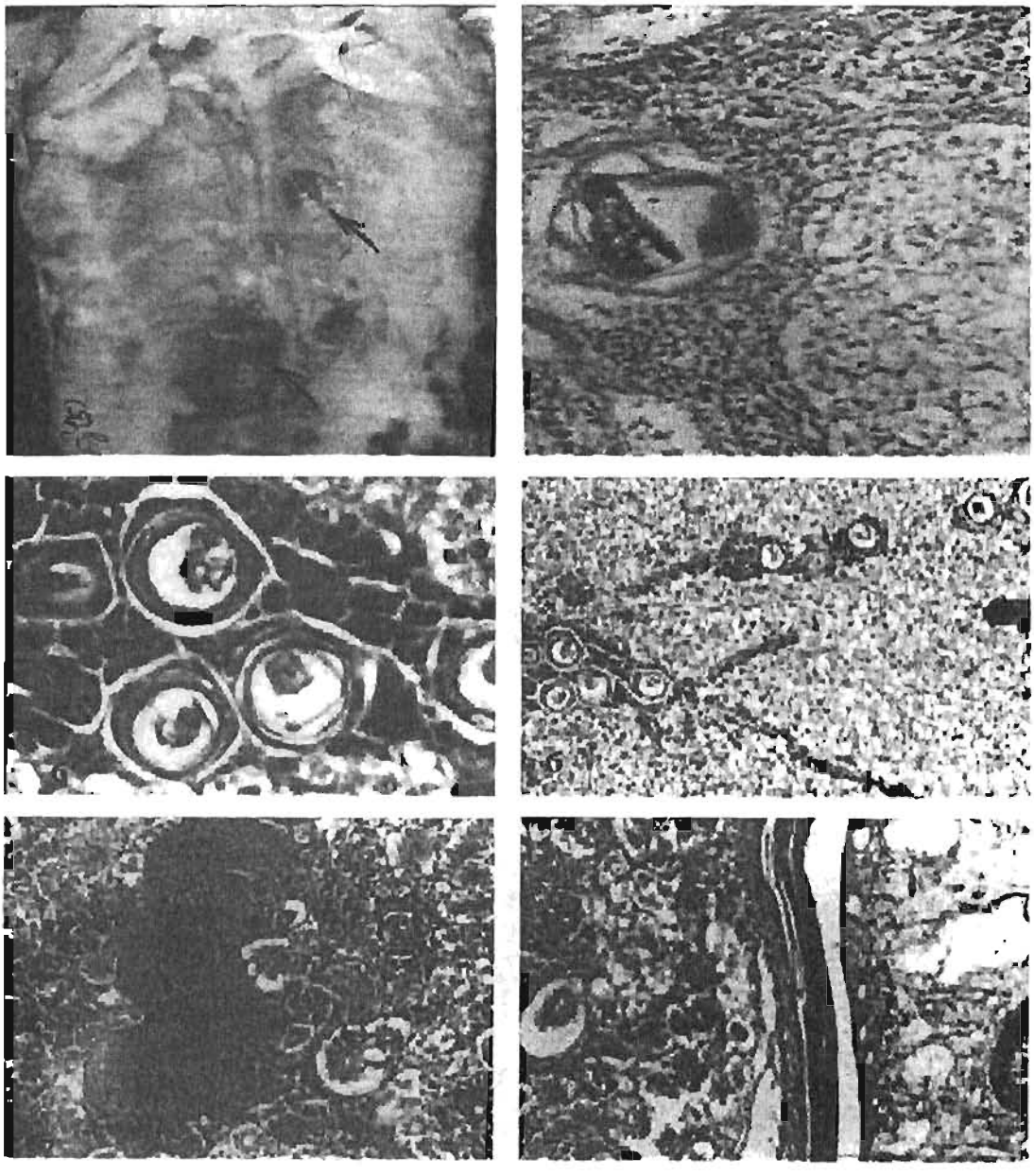

Fig. (5): Euclinotomatid cyst embedded in kidney of O. niloticus.

Fig. (6): Liver of fish showing encysted metacercariae in the pancreatic tissue of liver. (H & E) (X120).

Fig. (6'): High power of previous figure showing the encysted metacercariae surrounded by fibrous tissue capsule beside mild infiltration with inflammatory cells. (H & E) (X1200).

Fig. (7): Kidney of fish showing encysted metacercariae surrounded by fibrous tissue capsule. (H & E) (X300).

Fig. (7'): Kidney of fish showing hemorrahge and tubular necrosis. (H & E) (X120).

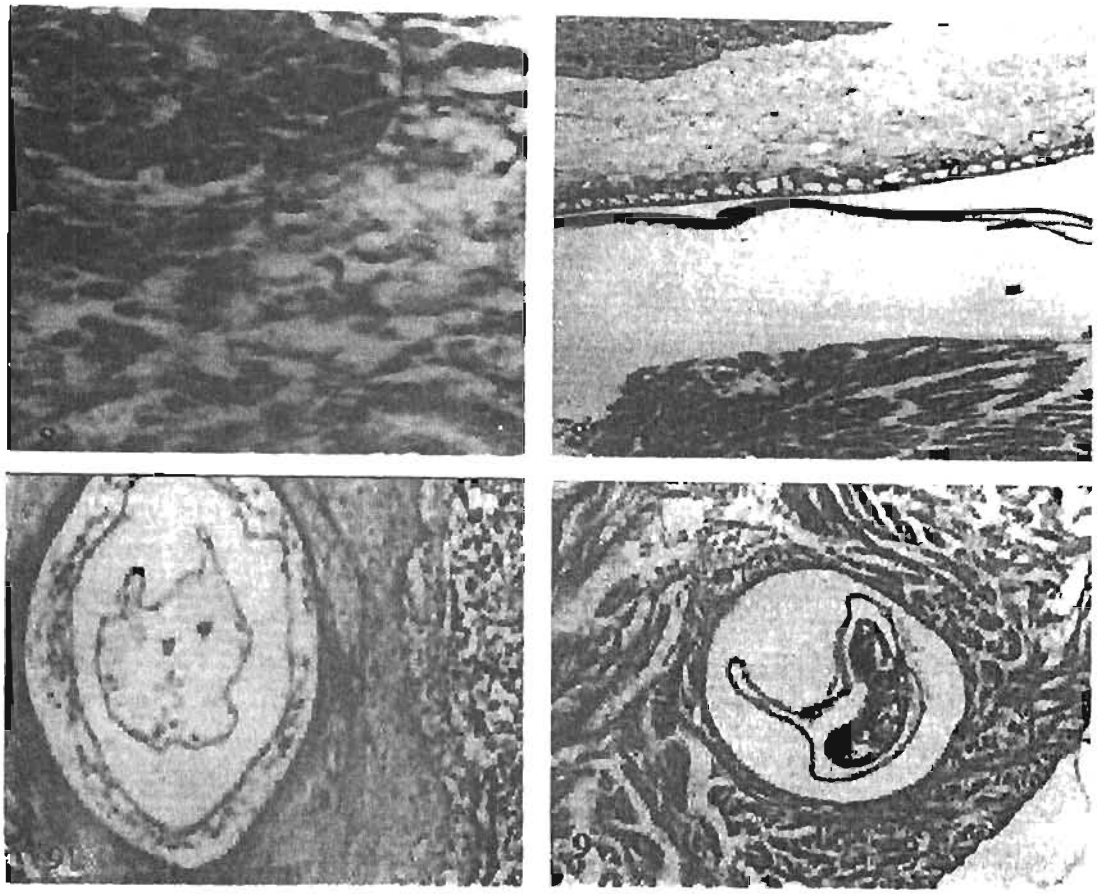

Fig. (8) : High power of previous figure to show congestion of glomerulus and intertubular blood vessels beside coagulative necrosis of renal epithelium. (H & E) (X1200).

Fig. (8') : Oropharynx showing encysted metacercaria surrounded by fibrous capsule. (H & E) (X120).

Fig. (9) : Heart of fish showing encysted metacercaria surrounded by fibrous tissue capsule (H & E) (X300).

Fig. (9') : Eye of fish showing encysted metacercaria in vitrous humour close to retina (H & E).

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الملخص العربى

معدل إنتشار اليرقات المتحوصلة البلطى النيلى بمحافظة الدقهلية مع إشارة خاصة للعلاج

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تمت الدراسة بفحص ٨٢٧ سمكة بلطى نيلى مجمعة من مزارع مختلفة بمحافظة الدقهلية خلال الفترة من ديسمبر عام ٢٠٠١ وحتى نوفمبر عام ٢٠٠٢ وكان أعلى معدل إنتشار اليرقات المتحوصلة بالأسماك فى شهر مايو بنسبة إصابة ٦١ر٤٤٪ وأقل معدل إصابة هو شهر ديسمبر بنسبة ١٧ر٤٦٪ والتى من الملاحظ إرتفاع نسبة الإصابة مع إرتفاع درجة حرارة الجو.

وقد صنفت اليرقات المتحوصلة المعزولة من أسماك البلطى النيلى إلى كلنيوستوماتيد فى التجويف الخيشومى (٢٣ر٩٪) وديبلوستوماتيد فى العين (٢١ر٨٪) وأبوكلينوستوماتيد فى النسيج الكلوى (٢ر١٪) وهبتيروفيد فى النسيج العضلى (٥٢ر١٪).

وكانت أهم العلامات المرضية التى ظهرت علي الأسماك المصابة فقد فى الأوزان ودكانة لون الجلد ووجود سحابة بيضاء على العين فى الأسماك المصابة بالديلوستوماتيد.

وقد سجلت التغيرات النسيجومرضية المصاحبة للإصابة فى أنسجة كل من الكبد والعين والقلب والكلية والخياشيم والتجويف الخيشومى للأسماك المصابة.

وقد تم عمل محاولات علاجية بكل من سلفات النحاس ومبيد البيلوسيد كمواد مؤثرة وقاتلة للقواقع والتى تعتبر العائل الوسيط للعدوى (وليس علاجاً للأسماك المصابة) فى أحواض ترابية لتربية البلطى النيلى المصابة بمرض البقع السوداء (الهتيروفيد). فقد لوحظ أن البيلوسيد أكثر فاعلية فى إبادة القواقع وكذلك تقليل عدد البقع على جلد الأسماك المصابة من (٧٣±٨) إلى (٣١±١٠) بينما كان تأثير سلفات النحاس على البقع السوداء من (٧٨±٥) إلى (٥٧±٩) بعد العلاج كما لوحظ أن للبيلوسيد تأثير سلبى على حيوية الأسماك وكذلك ردود فعلها مقارنة بالأسماك المعالجة بسلفات النحاس.