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DIPLOSTOMIASIS OR BLACK SPOT DISEASE IN CLARIAS LAZERA AT ASSIUT

(With 2 Tables & 4 Fig.)

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مرض الدبلوستوما أو البقع السوداء فى القراميط بمحافظة أسيوط

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على صديق

أجريت بعض الفحوص الخاصة بعدوى الدبلوستوما على ثلاثمائة عينة من أسماك القراميط التى جمعت من الترع المتفرعة من نهر النيل بمحافظة أسيوط . وكانت الأعراض الاكلينيكية السائدة هى وجود بقع سوداء بكثافة عالية على جلد الأسماك المصابة أكثر من الزعانف . هذا وقد صنفت الميناسركاريا المستولة عن حدوث المرض مورفولوجياً كدلاله لتصنيف طفيل الدبلوستوما . وكان معدل إصابة الأسماك بالدبلوستوما فى موسم الصيف أكثر منه فى الشتاء . وكانت مستويات الهيموجلوبين والهيماتوكريت والبروتين الكلى لمصل الدم فى الأسماك المصابة منخفضه معنوياً عن مثيلاتها فى الأسماك السليمة وكذلك مستويات الجلوبيولين الكلى والفوسفور الغير عضوى والكلوريد كانت أيضاً منخفضه معنوياً . على الرغم من أن الألبيومين والجلوكوز والبوتاسيوم واليوريا والكالسيوم لم تظهر أى اختلافات معنويه فى مستواها .

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SUMMARY

Three hundred of *Clarias lazera* were collected from River Nile tributaries at Assiut-Egypt. Some investigations on Diplostomiasis infection were carried out. The clinical signs of Diplostomiasis in *Clarias lazera* are characterised by predominant black spots, that are noticed intensively on the skin rather than the fins. The metacercaria, which are responsible for occurrence of the disease have been identified morphologically as *Diplostomum* spp. Histopathological examination revealed the presence of numerous cysts of metacercariae in the musculature. The incidence of infestation with *Diplostomum* spp. was higher in summer than in winter season. The levels of haemoglobin, haematocrit, blood serum total protein, total globulin, inorganic phosphorus and chlorides were observed to be significantly decreased in the infested fish, however, blood serum levels of albumin, glucose, potassium, urea and calcium showed non significant variation.

Keywords: Diplostomiasis, *clarias lazera*.

INTRODUCTION

Clarias species is one of the major fish species inhabiting the River Nile and Egyptian lakes. Also, these fish species are considered to be important fish, for supporting the fishing industry of inland & lakes water in Egypt (EL-BOLCOK, 1972).

Diplostomiasis or black spot disease is a condition in fish caused by the resting metacercarial stage of digenean trematodes in the epidermis of fish. These parasites belong to the family Diplostomatidae. The host fish responds by secretion of melanin pigments around the cyst. So the infection appeared as a scattered of black spots over the body and fins (BAMBER and HENDERSON, 1985). The disease has been recorded in a variety of fresh water and marine fish (DAVIS, 1961; BAUER *et al.*, (1973); VAN DUJN, (1973); AMLACHER, (1981); BAMBER *et al.*, (1983); POYNTON and HOFFMANN, (1993).

The available literatures have no adequate informations on black spot disease in *Clarias lazera*.

The objectives of the present study were designed to investigate the incidence of black spot disease, its clinical

signs, histopathological and some haematological and blood serum biochemical changes of the infested fish.

MATERIAL and METHODS

A. Fish:

Three hundred *Clarias lazera* were collected from River Nile tributaries at Assiut (150 samples in winter and 150 samples in summer). The average weight of each fish ranged from 200-300 gm. Fish were brought as quickly as possible to the laboratory and kept in full glass aquaria supplied with chlorine free tap water and examined immediately.

Clinical examination of fish:

Fish were examined clinically to detect the presence of black spots on body surface and fins. Also other gross abnormalities were noticed.

B. Blood samples:

The blood samples were taken from fish only in summer, by section of the caudal peduncle for haematological and biochemical studies. Blood samples were also taken from 10 clinically healthy fish as control samples.

1. Whole blood:

About 0.5-1.0 ml whole blood was collected in small plastic vials containing a suitable amounts of Di-potassium salt of Ethylene Diamine Tetra-Acetic acid (EDTA) as anticoagulant. These samples were used for the evaluation of blood Haemoglobin (Hb) and haematocrit using the methods of STOSKOPF (1993).

2- Blood serum:

About 3-4 ml whole blood were collected in plastic centrifuge tubes and left to clot for 3 hours at 4°C. The blood samples were centrifuged to separate the serum and kept in glass vials at -20°C until used.

Biochemical parameters determined in blood serum:

Total serum protein (g/L), albumin (g/L), urea (mmol/L), calcium (mmol/L) and inorganic phosphorus (mmol/L) were determined colorimetrically by means of commercial testkits supplied by Boehringer Mannheim Diagnostica-Germany. Total globulin and Albumin/globulin ratio were calculated mathematically. Blood serum potassium (K) was determined by Flame photometer (Corning 400). Serum chloride (Cl) was estimated by chloride analyzer model 925.

Parasitological examination:

The fish were examined by naked eye to show any macroscopic encysted metacercariae in different parts of fish

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muscles and other organs by dissection using forceps and scalpel.

The examination was carried out by taking small snips of the muscles from different parts of the body and internal organs. Each snip was compressed between two slides and examined microscopically for detection of metacercariae. Metacercariae were removed from the cyst and stained with haematoxylin & eosin to identify the encysted metacercariae morphologically according to HOFFMAN (1967) and BAMBER *et al.* (1983).

Histopathological examination:

Samples were taken immediately from muscles of trunk and tail regions of the infested fish. These samples were prepared for histopathological examination according to the method described by ROBERTS (1989) and stained with haematoxylin and eosin and examined by light microscope.

RESULTS

Clinical signs and postmortem findings:

Clinical examination of the infested *Clarias lazera* revealed the presence of black spots on the skin & fins of infested fish. The spots were concentrated on the tail region. The sizes of the spots ranged from 1-3 mm each. The cysts of metacercariae were embedded in the muscles of the infested fish and appeared as similar to granules of white sand (Fig., 1). The cysts were observed in the liver and gills of a few numbers of infested fish. No more clinical signs could be observed on the examined fish. The incidence of the infection and percentage of distribution of black spots on the skin and fins were recorded in table (1).

According to morphological characters of the encysted metacercariae, the parasites could be identified as species of *Diplostomum*.

Histopathological examination revealed the presence of numerous cysts of metacercariae observed in the musculature. Such cysts varied in shape and size and they were appeared as tubercles, each consisted of the metacercaria in the middle and surrounded by brown to black pigment cell and chromatophores (Fig. 2).

Mean values of haemoglobin, haematocrit and other biochemical parameters of blood serum are illustrated in table 2 and fig. (3&4).

DISCUSSION

The investigation revealed the presence of diplostomiasis or black spot disease in *Clarias lazera*. The disease is characterized by presence of black spots on the skin and fins. These spots were more concentrated on tail region. The clinical signs of the disease were previously described by VAN DUIJN, (1973), AMLACHER, (1981); POST, (1983); BAMBER, et al. (1983). and POYNTON and HOFFMANN, (1993) in different species of fishes. It was observed that, black spots were more predominant on the skin than fins (Tab, 1). An explanation to such picture can be offered on the view that the penetration of the skin by cercaria is more easier than penetration of the fins and at the same time *Clarias lazera* is scallless fish. BAMBER, et al., (1983). BAMBER and HENDERSON (1985) recorded that, the high incidence of metacercaria on the fins and tail of adult sand smelt in comparison to the body surface was attributed to inhibition of cercarial settlement on the body by the scales.

The incidence of infestation with black spot disease in *Clarias lazera* was higher in summer (80%) than in winter (10%). The authors suggest that, the increasing of the water temperature and presence of abundant snails which act as intermediate host in summer may be responsible for such increase in the rate of infestation. The obtained results were supported by POST (1983), who mentioned that, the water temperature and presence of aquatic snails are essential to the life cycle of the parasites, while BAMBER et al., (1983) recorded that, no seasonality changes in the levels of infestation were found.

The encysted metacercarial stage of infested fish could be identified morphologically as *Diplostomum* species. Histopathological examination cleared that the metacercaria situated in middle and surrounded by melanin pigment which considered as specific response of the host to penetration and development of the parasites. These findings were agreed with that reported by BAMBER, et al. (1983).

Blood haemoglobin, haematocrit, total protein and total globulin levels were significantly ($P < 0.05$) decreased in blood serum of infested fish when compared with healthy fish (Tab. 2 and Fig. 3 and 4). This indicated the lowering of the resistance of the *Clarias lazera* to pathogen and environment changes. MURAD and MUSTAFA (1988) recorded that haematocrit and haemoglobin were decreased in cat fish infested with *Diplostomum* species. Moreover significant decrease ($P < 0.05$) was observed in inorganic phosphorus level in the serum of infested

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sample which may be attributed to persistent of the infestation. KATO and WATANABE (1971) reported that a white wasting infectious disease lead to remarkable changes in phosphorus metabolism in the tissues of *Porphyra tenera*. Chloride level in serum of infested *Clarias lazera* was decreased as compared with healthy fish. These changes in chloride could be caused by stress from penetration and development of metacercaria in fish body. WEDEMEYER, et al. (1976) recorded that stresses on fish lead to some metabolic changes, one of them is decreased in chloride level in blood. The blood serum levels of glucose, calcium, urea, potassium showed non significant variations.

We could conclude that infestation of *Clarias lazera* with *Diplostomum* species is more prominent in summer than in winter and the clinical signs were in a form of black spots usually on the skin and sometime on both skin and fins. The disease was associated with a reduction in haemoglobin and haematocrit, blood serum total protein, globulin, inorganic phosphorus and chloride.

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Table 1: Incidence of black spots on the skin and fins of infested fish

No. of collect- ed fish	time of collect- ion	No. of infested fish	% of infest- ation	site(s) of lesion		
				skin only	fins only	skin & fins
150	winter	15	10	3 (20 %)	2 (13.3 %)	6 (40 %)
150	summar	120	80	40 (33.3%)	10 (8.5 %)	70 (58 %)

Table 2: Mean values of some blood biochemical parameters in both healthy and diseased fish.

Element No.	unit	healthy fish 10	Diseased fish 30	Level of significance
Haemoglobin	g/l	75.00 ± 13.00	57.00 ± 11.00	0.0200
Haematocrit	%	24.00 ± 4.6	19.3 ± 2.5	0.012
Total protein	g/l	38.29 ± 6.30	27.29 ± 6.42	0.0208
Albumin	g/l	9.27 ± 0.63	11.29 ± 3.26	0.37
Globulin	g/l	29.03 ± 6.74	16.07 ± 5.91	0.1293
A/G ratio	—	0.34 ± 0.10	0.76 ± 0.28	0.088
Glucose	mmol/l	4.45 ± 1.82	5.6 ± 2.2	0.64
Calcium	mmol/l	2.48 ± 0.22	2.62 ± 0.48	0.6164
Inorg.phosph.	mmol/l	5.52 ± 2.05	2.68 ± 1.65	0.02
Urea	mmol/l	1.52 ± 0.24	1.95 ± 0.5	0.17
Potassium	mmol/l	5.38 ± 0.37	5.4 ± 2.22	0.98
Chloride	mmol/l	98.66 ± 2.30	70.3 ± 18.7	0.019

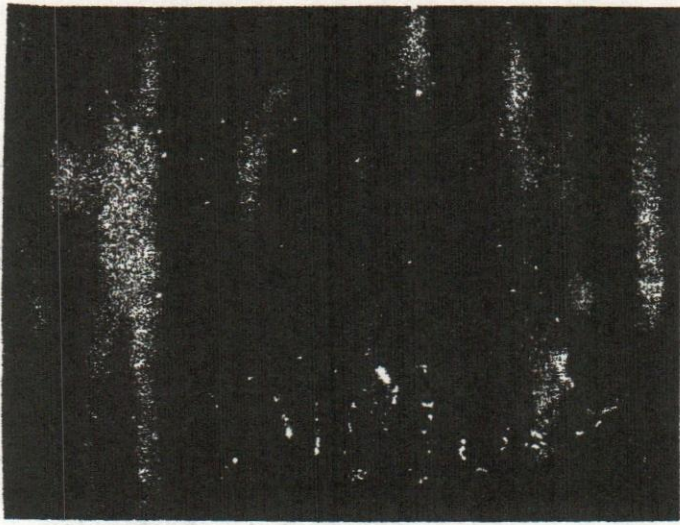


Fig. (1) Gross appearance of encysted metacercariae in the muscles of *Clarias Lazera*.



Fig. (2) Microscopic appearance of encysted metacercariae in the muscles of *Clarias Lazera* (the metacercaria is surrounded with melanin pigments)

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Fig. 3: mean values of t.prot., alb. & globulin in healthy and diseased fish

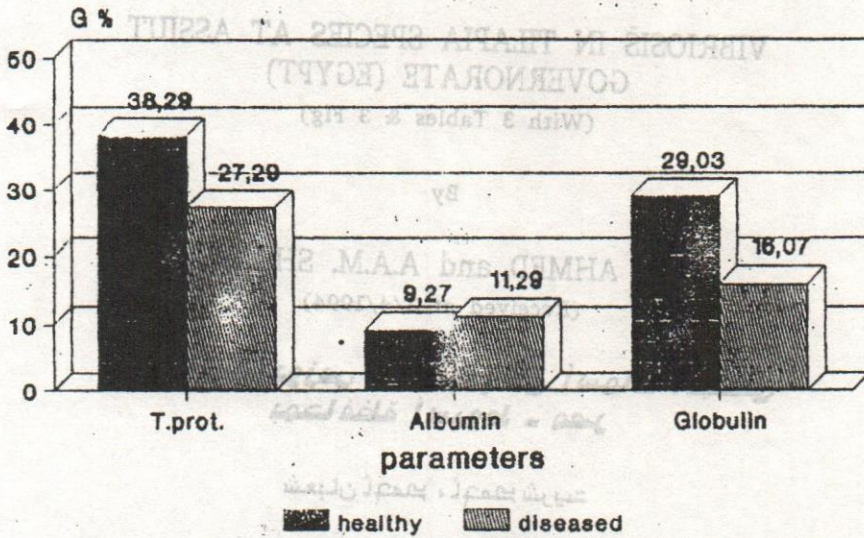
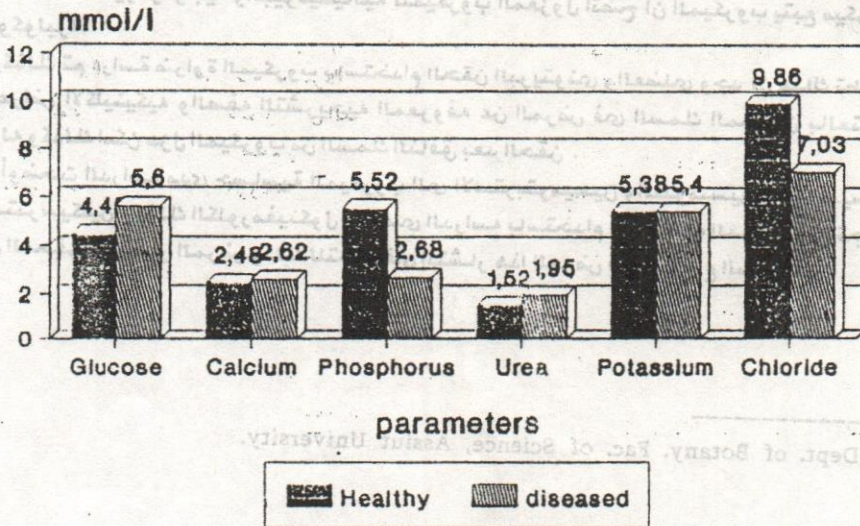


Fig. 4: mean values of blood serum glucose, Ca, in. Ph, urea, K and cl in healthy and diseased fish



Chloride value = Chloride X10