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SOME INVESTIGATIONS ON SAPROLEGNIASIS IN TILAPIA SPECIES AT ASSIUT, EGYPT.

(With 3 Tables and 2 Figures)

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بعض الدراسات عن الإصابة بمرض السابروليجنيسيس
في أسماك البلطي بأسيوط

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في هذا البحث تم تجميع مائة عينة من أسماك البلطي من محافظة أسيوط. وجد أن عشرين عينة (خمس عشرة عينة من البلطي النيلي وخمس من البلطي الجاليلي) مصابة بمرض السابروليجنيسيس ، لقد تمثلت أعراض هذا المرض في نمو قطني على مناطق الرأس وغطاء الخياشيم والزعانف وسطح الجلد والعينان. هذا بالإضافة إلى وجود بعض التقرحات في منطقة الرأس لبعض الأسماك المصابة. لقد تم عزل فطر سابروليجنيس بارازيتيكا من جلد وزعانف وخياشيم الأسماك المصابة. باستخدام العدوى الصناعية معملها لوحظ أنه يمكن انتقال العدوى بفطر السابروليجنيس بارازيتيكا لأسماك البلطي النيلي والجاليلي عن طريق الحقن في العضل أو وضع الفطر على الجلد بعد تشريطه. كما أنه قد لوحظ أن أسماك البلطي النيلي أكثر قابلية للإصابة بهذا المرض عن البلطي الجاليلي.

SUMMARY

Out of 100 collected samples of Tilapia species, twenty fish (15 T.nilotica and 5 T.galilaea) were infected with Saprolegniasis. The most common clinical signs of the disease were cotton-like growth on the head region, operculum, fins, body surface and eyes. In addition, some ulcerations were observed on the head region of some infected fish. saprolegina parasitica coker was recovered from the skin lesions, fins and gills of infected fish.

Experimentally, the infection with Saprolegnia parasitica coker in Tilapia nilotica and T.galilaea was transmitted by intramuscular injection and by application of the fungal isolate on abraded skin. T.nilotica was more susceptible to the disease than T.galilaea.

INTRODUCTION

Saprolegniasis is a fungal disease of fish and fish eggs caused by the mold fungi which belong to the order Saprolegniales mainly of the genera Saprolegnia and Achlya (BAUER et al., 1973, NOLARD - TINTIGNER, 1974; POST, 1983; KABATA, 1985 and ROBERTS, 1989).

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The disease is characterized by the presence of cottonlike, white to gray or gray to gray-brown growth on the skin, fins, gills or eyes of fish (BAUER *et al.*, 1973; RICHARDS & PICKERING, 1978; WILLOUGHBY, 1978; NEISH & HUGHES, 1980; COPLAND & WILLOUGHBY, 1982; PICKERING & WILLOUGHBY, 1982; POST, 1983; BOHM & FUHRMANN, 1984 and SINGHAL *et al.*, 1987).

This investigation aimed to study each of the following:

- 1- The clinical signs of Saprolegniasis in natural and experimental infections in Tilapia nilotica and T.galilaea.
- 2- The mode of transmission of Saprolegnia parasitica coker in the same species.

MATERIAL and METHODS

A total No. of 100 tilapia samples (each about 60-75 grams) were collected from small canals at Assiut. Out of these collected fish, 20 fish (15 of T.nilotica and 5 of T.galilaea) were suspected to be infected with Saprolegniasis. The clinically diseased fish were brought to the laboratory in a sterile plastic bags containing a few amounts of the canal water.

A- Clinical and postmortem examination of fish:

Naturally infected fish were thoroughly investigated according to KABATA (1985) and AUSTIN & AUSTIN (1987).

Microbiological examination:

a- **Mycology:** Pieces of tissues from skin lesions, fins, gills and internal organs were washed by sterile distilled water. The zoospore fungi were recovered using baiting technique with sesame seeds as baits (KHALLIL, 1984). The seeded plates were incubated at 22 C for two weeks during which the growing colonies were identified according to SEYMOUR (1970).

b- **Bacteriology:** Specimens from the skin lesions, gills, liver and kidney were plated out on trypticase soy agar and blood agar. The plates were incubated at 22 C for 48 hours. Isolates were subsequently purified by repeated subculture and identified biochemically according to COWAN & STEELS (1974) and ALLEN *et al.* (1983).

C- Experimental infections:

a- **Fish:** A total number of 84 clinically normal Tilapia nilotica and T.galilaea (50-55 grams each) were collected from the river Nile at Assiut. These fish were acclimatized to water temperature 18 ± 2 C and kept in full glass aquaria, supplied with chlorine free tap water for 15 days prior to the experimental infection. Fish were observed daily for detection any lesion on the body surface. Four of clinically healthy fish were subjected thoroughly to microbiological examination to ensure that fish free from any pathogenic agent especially Saprolegnia infection.

b- **Preparation of inocula:** The inocula of Saprolegnia parasitica coker, which was isolated from naturally infected fish, were prepared according to the method of SINGHAL *et al.* (1987) using sterile distilled water:

i- **Intramuscular injection:** 10 fishes of each species (T.nilotica and T.galilaea) were injected intramuscular on each side of the body above the lateral line with

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experimental inoculum (0.5 ml to each fish). For comparison, 10 fishes related to each of the two species were used as control and injected in the same manner by the same volume of sterile distilled water (0.5 ml to each fish).

ii- **Scarification:** 3.5 mm patches of scales were removed from each side of each tested fish (20 T.nilotica and 20 T.galilaea) immediately anterior to the dorsal, caudal fins and on side of the body under the lateral line. Very slightly scarifications were made on this patches. 0.5 ml of prepared inoculum was applied directly on each of the patches of 10 T.nilotica and 10 T.galilaea. Also, for comparison, 0.5 ml of sterile distilled water was used to controlled fish (10 T.nilotica and 10 t.galilaea). Fish in both experiments were observed daily for 30 days during which the clinical signs of the saprolegniasis, morbidity and mortality rates were detected.

RESULTS

Clinical and postmortem findings:

The most prevalent clinical signs of Saprolegniasis in the natural infected Tilapia species were the presence of cotton like growth on the head region and the operculum including both eyes and lead to blind of the fish (Fig. 1).

In addition there are some ulcerations on the head region under the fungal growth. Also, the fungal growth was distributed on the dorsal fin, side of the body and on the tail fin. Septicaemic picture was not observed (Fig. 1). These clinical signs were noticed more prominent and intensive on Tilapia nilotica than T.galilaea. In few number of the collected samples, gills were swollen, hyperaemic and covered with large amount of mucous. There were no changes on the internal organs.

Mycological examination:

During this investigation 24 isolates of Saprolegnia species were isolated. These isolates were identified as Saprolegnia Parasitica coker (Fig. 2). The total number of Saprolegnia isolates, which were recovered from different parts of examined fishes were illustrated in Table (1).

Bacteriological examination:

Only one species of bacteria namely Aeromonas hydrophila was isolated from two samples of T.nilotica particularly from skin lesions and gills.

Experimental infections:

The clinical signs of Saprolegniasis began to appear after incubation periods of 5-7 and 7-10 days in case of T.nilotica and T.galilaea respectively after infection by intramuscular injection as well as scarification. The clinical signs and postmortem findings of experimented fish were as previously described in the case of natural infection. However, the ulceration on head region was not found. The morbidity, mortality rates and the time of death are illustrated in tables 2, 3. The fungal isolate reisolated from inoculated fish.

DISCUSSION

In this investigation, the clinical signs of Saprolegniasis in Tilapia species is characterized by the presence of cotton like growth on head region, operculum, fins, sides

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of the body and eyes. Such clinical symptoms were also described in Tilapia nilotica by EASA and AMIN (1987). The incidence of the disease was frequently occurred and the clinical signs were intensive and clear in T. nilotica than T. galilaea. Experimentally, the signs of the disease were observed earlier with high mortality in T. nilotica than T. galilaea. This can be explained that the T. nilotica is more susceptible to the infection with saprolegniasis than T. galilaea. This variation in susceptibility is probable due to the genetic characteristics. In this respect, SNIESZKO (1980) mentioned that the genetic make up of fish was the one of the most important factors that contribute to outbreaks of the diseases of fish.

Saprolegnia parasitica coker or Saprolegnia diclina Humphrey were the most common Saprolegnia Spp. isolated from fish infected with Saprolegniasis (WILLOUGHBY, 1986, 1970; NOLARD TINTGER, 1970; BAUER et al., 1973; POST, 1983 and EASA & AMIN, 1987).

Only one zoosporic fungal species namely Saprolegnia parasitica coker was isolated from skin lesions and gills of naturally infected fish (Table 1). SEYMOUR (1970) and ISMAIL et al. (1979) reported that S. parasitica coker comes very near to S. diclina Humphrey. NEISH & HUGHES (1980) mentioned that the S. parasitica was the synonym of S. diclina. Generally, the term S. parasitica diclina complex is widely used and accepted (WILLOUGHBY 1985 and SINGHAL et al., 1987).

The septicaemic lesions were not observed on naturally infected fish. However, Aeromonas hydrophila was isolated from the skin lesions and gills of two samples (out of 15) of T. nilotica. It can be said in this investigation that Saprolegnia parasitica coker acts as a primary pathogen. Moreover in nature, we can not discount the possibilities that the Saprolegnia infection was secondary to viral or parasitic or physical damage. This is in accordance with the results obtained by RICHERDS & PICKERING (1978) and COPLAND & WILLOUGHBY (1982).

It was proved that the intramuscular injection and scarification were effective in transmission of the Saprolegniasis. SINGHAL et al. (1987) reported that the Saprolegniasis was transmitted to Cyprinus carpio, Labeo rohita and Cirrhina mrigala by intramuscular injection or by application of the fungal isolates to skin lesions. In contrast, EASA and AMIN (1987) failed in transmission of the Saprolegnia parasitica to Tilapia nilotica by intramuscular or intraperitoneal injection.

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Table (1): No. of the total isolates of *Saprolegnia parasitica* Coker recovered from naturally infected fish.

| Fish species | No. of isolates from | | | |
|----------------------------|----------------------|------|-------|-----------------|
| | Skin lesions | Fins | Gills | Internal organs |
| <u>T. nilotica</u> (15) | 10 | 5 | 3 | 0 |
| <u>T. galilaea</u> (5) | 3 | 2 | 1 | 0 |

Table (2): Illustrate the I/M injection.

| Fish species | No. of | | | | Time of death/ day postinoculation |
|--------------------|--------------------|------------------|-------------------------------|-------------------------------|--|
| | inoculated fish | infected fish | dead fish after 30 days | survivors after 30 days | |
| <u>T. nilotica</u> | 10 | 10 | 8 | 2 | 17-23 |
| <u>T. galilaea</u> | 10 | 10 | 4 | 6 | 21-26 |
| Control | | | | | |
| <u>T. nilotica</u> | 10 | 0 | 0 | 10 | 0 |
| <u>T. galilaea</u> | 10 | 0 | 0 | 10 | 0 |

Table (3): Illustrate the experimental infection with scarification.

| Fish species | No. of | | | | Time of death/ day postinoculation |
|--------------------|--------------------|------------------|-------------------------------|-------------------------------|--|
| | inoculated fish | infected fish | dead fish after 30 days | survivors after 30 days | |
| <u>T. nilotica</u> | 10 | 10 | 10 | 0 | 15-21 |
| <u>T. galilaea</u> | 10 | 10 | 6 | 4 | 19-25 |
| Control | | | | | |
| <u>T. nilotica</u> | 10 | 0 | 0 | 10 | 0 |
| <u>T. galilaea</u> | 10 | 0 | 0 | 10 | 0 |

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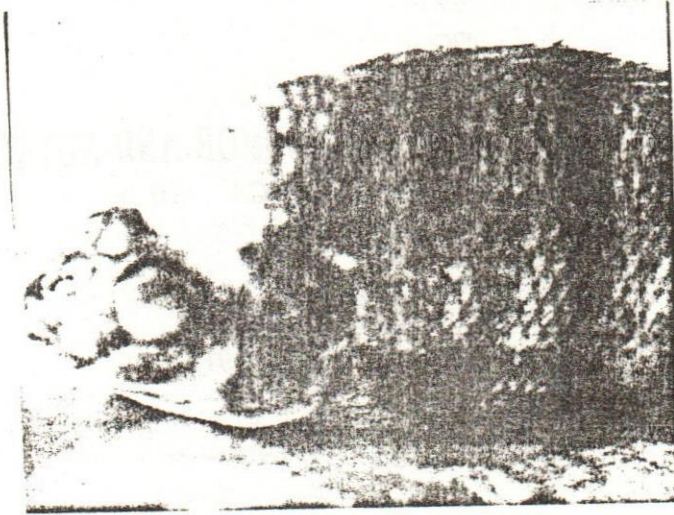
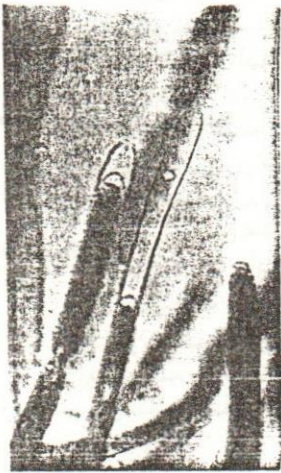


Fig.(1): Showing cotton-like growth on the head region, operculum eyes and on the body surface, with some ulcerations on the head region.

Fig.(2): Saprolegnia parasitica coker



A-Zoosporangial proliferation



B-Oogonium containing large number of Oospores and characteristics antheridium



C-Gemmae