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CLINICAL, MICROBIOLOGICAL EXAMINATIONS AND PREVENTION OF SAPROLEGNIASIS INFECTION IN MORMYRUS KANNUME

(With 4 Tables & 1 Figure)

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الفحص الاكلينيكي والميكروبولوجي وطرق الوقاية من العدوى بمـــرض السابروليجنييس في أسماك البوبز

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شمل البحث ٤٠ عينة من أسماك البوبز المصاب بمرض السابروليجنييس في فصـل الشتاء عام ١٩٩٠م . هذا وقد تمثلت أعراض هذا المرض في نمو قطني على سطح الجلد والزعانف ومنطقة الرأس بالاضافة لوجود بقع نزفية على الجسم نتيجة العدوى بالبكتريـا. تم عزل فطري سابروليجينيا بارانتيكا وسابروليجنيا فيركس من الأسماك المصابـة بالاضافة إلى بكتريا الابرروموناس هيدوفيلا والسيدوموناس فلورسنس . أجري اختبـار الحساسية للفطريات المعزولة وكذلك البكتريا واتضح أن المالاكيت الأخضر أكثـر تأثيرا على فطر السابروليجنيا بينما النيوميسين والستربتميـن أكثر تأثيرا علـى البكتريا المعزولة . مما سبق يتضح أن علاج الأسماك المصابة بهذا المرض بعمـل حمام في المالاكيت الأخضر بالاضافة إلى تغذية الأسماك على طعام يحتوي على إحـدى هذه المضادات الحيوية .

SUMMARY

A total of 40 Mormyrus kannume naturally diseased with saprolegniasis were collected in winter season of the year 1990. Fish were investigated clinically, post-mortem and microbiological findings were also recorded. The disease was characterized by cotton like growth on the whole body, fins, and head region accompanied by septicemic picture due to bacterial infection. Saprolegnia parasitica and Saprolegnia ferax were isolated from infected fish, beside Aeromonas hydrophila and Pseudomonas fluorescens. Some selected antifungal agents and antibiotics sensitivity test were applied on isolated fungal and bacterial species. Bathing of infected fish in malachite green in addition to feeding of fish on medicated food containing neomycin or streptomycin which were the drugs of choice for treatment of saprolegnia infection.

INTRODUCTION

Saprolegnia is an important genus of family saprolegniaceae. (Oomycetes) with about 22 species known from various parts of the world (DICK, 1973).

Several species of this genus, especially Saprolegnia parasitica and Saprolegnia ferax were known to be pathogenic to fish and fish eggs (NOLARD, TINTIGNER, 1974; RICHARADS & PICKERING, 1978; WILLOUGHBY, 1978; ROBERTS, 1989 and AHMED & KHALLIL, 1991). Saprolegnia infection in fish were considered to be secondary to bacterial or viral infection (WOLKE, 1975 and RICHARDS, 1977) or the consequence of physical damage to the surface of fish (ROBERTS and SHAPHERD, 1974) but in certain conditions, saprolegnia species may act as primary pathogens (HOSHINA et al., 1960 and NEISH, 1977).

In Egypt, no satisfactory knowledges could be traced in the available literatures concerning saprolegniasis infection in Mormyrus kannume. Therefore, this work describes the clinical findings and post-mortem changes of saprolegnia infection in Mormyrus kannume. Furthermore, the study included isolation, identification of the causative agent and in vitro, the effect of some antifungal agents on isolated saprolegnia species.

MATERIAL and METHODS

From clinical examination of 40 Mormyrus kannume (50-100 gm) were suspect to be infected with Saprolegniasis, such infection was noticed in winter season, in which water level in canals of Assiut Governorate was decreased. The clinically diseased fish were brought to the laboratory in sterile plastic bags with enought amounts of water canal. The following examinations were carried out:

I- Clinical and Post-Mortem Findings:

Naturally infected fish were thoroughly examined for clinical and post-mortem findings according to the method described by AUSTIN and AUSTIN (1987).

II- Microbiological Examinations:

a) Mycology:

Pieces of tissue from skin lesions, fins, gills, liver, spleen and kidney were washed by sterile distilled water. Saprolegnia fungi were isolated by using baiting technique with hempseeds (ISMAIL et al., 1979). The seeded plates were incubated at 22°C for two weeks. Growing colonies were identified according to SEYMOUR (1970) and ISMAIL et al. (1979).

b) Bacteriology:

Specimens from gills, skin lesions, liver, spleen and kidneys were plated on brain heart infusion agar and blood agar. The plates were incubated at 22°C for 48 h. Isolates were subseguently purified by repeated subculture and identified biochemically according to COWAN and STEELS (1974) and ALLEN et al. (1983).

II- Sensitivity Tests:

a) For isolated saprolegnia species:

The isolated saprolegnia species were cultured on cytophaga (ANACKER & ORDAL, 1959 and BÖHM & FUHRAMANN, 1984) for three days at 22°C.

The used antifungal agents (malachite green, copper sulfate and methylene blue) were prepared in concentration of 1 mg/L, 5 mg/L, 10 mg/L, 50 mg/L and 100 mg/L. by using sterile distilled water while sodium chloride was tested also as antifungal agent by different concentrations ranged from 1% to 6%.

Agar plugs (5 mm in diameter) containing 3 days old fungal hyphae were removed from the edges of actively growing colonies. The plugs were placed in antifungal solution for 30 and 60 minutes (maximum exposure of 60 minutes was used. This is because more than 85% of hatcheries require that effective contact time for the fungicide must not exceed than one hour (BAILEY, 1984).

After exposure, the plugs were rinsed with sterile distilled water and then placed at the center of trisections petri dishes containing cytophaga agar. The culture were incubated at 22°C. The plates were examined for mycelial growth daily for 6 days.

b) For isolated bacteria:

The antibiotic sensitivity test for isolated bacteria were applied by different types of antibiotics discs according to AMTSBERG et al. (1973). The interpretation of inhibition zone was estimated according to the limits given by FINEGOLD & MARTIN (1982).

RESULTS

Clinical and Post-Mortem Findings:

Clinical signs of saprolegniasis in the naturally infected Mormyrus Kannume are characterized by the presence of cotton like growth on skin surface, fins especially tail fin, head region, eyes and operculum. The infection was accompanied by hemorrhages distributed on skin surface especially at the bases of the fins (Fig. 1). Gills were covered with mucous and have anaemic patches. Three fish had congested gills

which appeared more red than normal color of gills. Liver was congested but in only four fish, it was pale to yellowish in color. Kidneys were congested and swollen. Ascitic fluid was found in five fish.

Mycological Examinations:

Results of mycological examination revealed that 17 isolates of saprolegnia species were recovered and identified as 12 Saprolegnia parasitica coker and 5 Saprolegnia ferax. The total number of isolated saprolegnia species from various parts of examined fish were showed in Table 1.

Bacteriological Examinations:

14 bacterial isolates were recovered from investigated fish. According to morphological and biochemical characters shown in Table 2, isolates were identified as 10 Aeromonas hydrophila and 4 Pseudomonas fluorescens. Distribution of these bacterial species in different parts of naturally infected fish are shown in Table 3.

Effect of Antifungal Agents:

The growth of saprolegnia species did not occur after exposure of the fungi for 30 and 60 minutes at tested concentration of malachite green. The other antifungal agents had no effect on saprolegnia species and the growth appeared within 48 h. after incubation.

Drugs Sensitivity Test:

Results of antibiotic sensitivity test proved that isolated Aeromonas hydrophila and Pseudomonas fluorescens are highly sensitive to neomycin and streptomycin Table 4.

DISCUSSION

The clinical signs of saprolegniasis in Mormyrus Kannume were characterized the by presence of white, cotton like growth on skin, fins, operculum, gills and eyes. These findings were supported by WILLOUGHBY (1978), RICHARDS & PICKERING (1978); COPLAND & WILLOUGHBY (1982); POST (1983) and ANDERWS et al. (1988) in various species of fishes.

Saprolegnia parasitica and Saprolegnia ferax were found as predominant causative agents of saprolegniasis in Mormyrus Kannume. Both fungi were isolated from many species of fish in fected with the disease (BAUER et al., 1973; NOLARD-TINTIGNER, 1973 and COPLAND & WILLOUGHBY, 1982).

Aeromonas hydrophila and Pseudomonas fluorescens were isolated from examined fish. This explained that septicemic picture in diseased fish was due to bacterial infection. Similar result were obtained by EGUSA & NISHIKAWA (1965) and RICHARDS & PICKERING (1978) during examination of Anquilla anguilla L. elvers, Salmo trutta (L) and char Salvelinus alpinus (L) infected with saprolegniasis.

From the present study it was found that, reduction of water temperature during winter season, decreased the water level than regular in fish farms and invasion of fish with bacteria enhance the saprolegnia infection. BAUER et al. (1973), ISMAIL et al. (1979) and POST (1983) recorded that reduced water temperature, high or low PH and salinity ofwater may be responsible for secondary invasion by saprolegnia.

Growth of the fungi were inhibited by using of malachite green in concentration of 1 mg/L within 30 minute. ANDREWS et al. (1988) recorded that fungi, external parasites and skin and gills flukes could be treated with malachite green at concentration of 0.1 - 0.2 mg/L by bathing the fish for several days.

Copper sulfate, methylene blue and sodium chloride had no effect on fungal growth. This point needs further investigations to define the dose and time allowed for treatment of infected fish. However, AHMED et al. (1991) treated saprolegnia infection by bathing the infected fish in sod. chloride (1.5%) and copper sulfate solution (100 ppm) for 20 minutes for 5 days.

In vitro, Aeromonas hydrophila and Pseudomonas fluorescens had been found strongly sensitive to neomycin and streptomycin. These results agreed with ALLEN et al. (1983).

It concluded that:

a) The clinical signs of saprolegniasis in Mormyrus kannume were characterized by the presence of cotton like growth on the skin surface, fins and head region.

b) Saprolegnia parasitica and Saprolegnia ferax were the common cause of saprolegniasis. As well isolated fungi act as secondary infection to bacteria or enhanced by stress factors such as low water temperature and decrease the water level in fish farms.

c) Saprolegnia infection could be treated by bathing the infected fish through malachite green. It's preferred that food of fish must contains neomycin (20-100 mg/Kg. fish) or streptomycin (50-100 mg/Kg. fish) to prevent the bacterial infection.

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Tab. 1 : No. of the total isolates of S. parasitica coker and S. ferax recovered from naturally infected fish.

Saprolegnia species	No. of isolates			
	Skin	Fins	Gills	I.O(*)
S. parasitica coker	7	3	2	-
S. ferax	3	2	-	-

(*) = Internal Organs (Liver, spleen and kidney)

Tab. 2 : Morphological and biochemical characters of Aeromonas hydrophila and Pseudomonas fluorescens.

Tests	A. hydrophila (10 isolates)	Ps. fluorescens (4 isolates)
Gram stain	- ve short rods	- ve short rods
Haemolysis on		
5% sheep blood agar	+	-
Motility	+	+
Oxidase	+	+
O-F	+/+	+/-
Production of acid :		
Glucose	+	+
Trehalose	+	+
Salicin	+	+
Aesculin hydrolysis	+	-
Indole	+	-
H2S gas (TSI)	+	-
Gelatin lequfication	+	+
Methyl red	+	-
Fluoroescin pigment	-	+

Tab. 3 : Distribution of A. hydrophila and Ps. fluorescens among various tissues and organs of naturally infected fish.

Bacteria species	No. of isolates									
	Skin		Gills		Liver		Spleen		Kidney	
	No.	%	No.	%	No.	%	No.	%	No.	%
A. hydrophila	1	10	2	20	4	40	1	10	2	20
Ps. fluorescens	1	25	1	25	2	50	-	-	-	-

Tab. 4 : Sensitivity of Aeromonas hydrophila and psedomonas fluorescens to some antimicrobial drugs.

Drugs	Conc.	A.hydrophila	Ps. fluorescens
Ampicillin	25 µg	R	R
Chloramphenicol	30 µg	R	R
Erythromycin	15 µg	R	R
Neomycin	30 µg	SSS	SSS
Netilmicin	10 µg	SSS	R
Nitrofurantoin	300 µg	R	R
Oxytetracycline	30 µg	S	R
Penicillin	10 iu	R	R
Slufamethoxazole/ Trimethoprim	25 µg	R	SS
Streptomycin	10 µg	SSS	SSS

R = Resistant
S = Slight sensitive
SS = Moderate sensitive
SSS = Highly sensitive

SAPROLEGNIASIS, MORMYRUS KANNUME

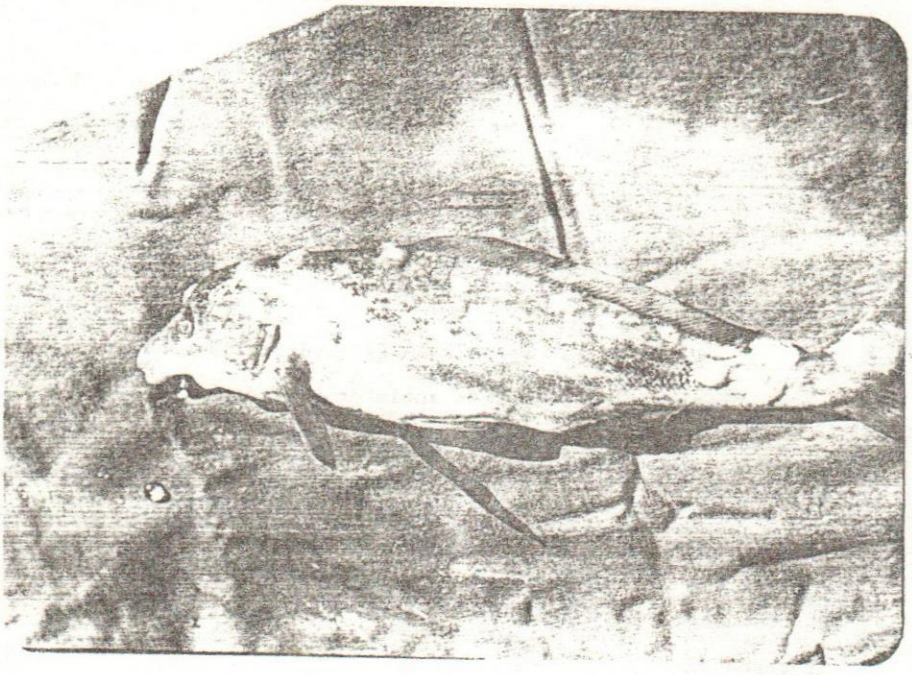

Fig. 1: Cotton like growth on the skin surface, fins and head region with hemorrhages on the body surface especially at the bases of the fins in Mormyrus kannume naturally in fected with saprolegniasis.