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PRELIMINARY INVESTIGATION ON SRTEPTOCOCCOSIS AMONG FRESHWATER AND MARINE FISHES

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SUMMARY

Detection of Streptococcosis among fershwater fish, Nile tilapia *(O. niloticus)* and marine fish, grey mullet *(M. cephalus)* was confirmed. The disease of both fishes is characterized by numerous haemorrhagic areas on the body surface, particularly around the mouth and operculum. Corneal opacity with congested swelling around the eye ball was a common feature of the disease in *O. niloticus,* while, abdominal distention usually observed in *M. cephalus.* Internally, *O. niloticus* exhibited splenomegally while in *M. cephalus,* the abdominal cavity filled with yellowish fluid, the stomach and intestine contained yellow to yellowish green gelatinous material and pale coloured liver.

The bacteriological examinations revealed isolation of Streptococcal species linked with but not identical to *Streptococcus faecalis* and *Streptococcus faecium.*

The bacterial isolates able to produce the disease and cause 100% deathes when the fishes were injured prior to dipping in the bacetrial suspension. No deathes occurred among fishes dipped in or orally administered the bacterial suspension.

INTRODUCTION

Streptococcosis is of the most economically important diseases of fishes in Japanese mariculature (Kusuda et. al., 1978). There are few reports of streptococcal infections in freshwater fishes. It has been isolated from ayu (Ugajin, 1981), rainbow trout (Miyazaki, 1982) and tilapia (Kitao et. al., 1981).

This study was planned to declare the streptococcal infection among the freshwater Nile tilapia *(Oreochromis niloticus)* and the marine fish grey mullet *(Mcphalus)*, Moreover, studying the pathogenicity of isolated strpotococci to both fishes with different modes of infection were studied.

MATERIALS AND METHODS

1- Fish:-

A-Naturally infected fish:-

A total of 250 Nile tilapia *(O. niloticus)* and 190 grey mullet *(M. cephalus)* showing clinical abnormalities were collected from private freshwater fish farms and Lake Eltemsah respectivelly (during the period from Octobar 1992 through Septemper 1993). The collected fishes were subjected to full clinical and postmortem examinations according to the methods described by Lucky (1977).

B- Experimental fish :

Sixty of each apparently healthy live *O. niloticus* and *M. cephalus* with an average body weight 50 ± 5g were used in the pathogenicity test.

2- Water samples:

The surface water samples (200 ml/ each time) of both environments (freshwater farms and Lake Eltemsah) were collected by the method recommended by the American Public Health Association (1970).

3- Isolation and identifaction of Streptococcus species :

The primary isolation was made from fish external organs (skin, fins, gills and eyes), fish internal organs (liver, kidneys and spleen) and water deposite after centrifugation (16000 r. p. m. for 15 min.) on blood tryptose agar (BTA), macCnoky agar and tryptic soy agar plates (Foo et. al., 1985). The plates were incubated aerobically at room temperature (22°C) and at 10, 37 and 45°C for 24 hrs. One set of plates was incubated anaerobically. The bacterial isolates were identified by colony morphology, microscopic examination and biochemical tests (Cowan 1974, Bragg and Broere 1986 and Austin and Austin 1987). In addition, the bacteriall isolates were tested biochemically by AP120 strep (Foo et. al., 1985) and sulfamethoxazole sensitivity (American Society for Microbiology, 1981).

4- Pathogenicity test:

For pathogenicity test, the total number of each fish species (60) was divided into equal 6 groups of 10 fish each. The fishes were held in glass aquaria, supplied with declorinated tap water (for *O, niloticus*) and marine water (for *M. cephalus*), for 2 weeks prior to challenge. Each fish species was challenged with their corresponding bacterial isolate as descriped by Rasheed and Plumb (1984) as follow:

A- Dip challenge:

The fish were dipped in bacterial suspension contains approximately 5x1010 colony forming unit (CFU) ml for 1/2 hr. The fish of control groups were dipped in sterile saline solution for 1/2 hr.

B- Skin scrafication:

The fish were injured by skin scratching on eside of the abdomen with scalpel and dipped into the bacterial suspension (5x1010 CFU/ml) for 5min. The fishes of control groups were dipped in sterile saline solution for 5 mi after body scratching.

C- Oral challenge:

Each fish was administered with 0.1 ml of the bacterial suspension (5x1010 CFU/ml) using 1-cc syringe with 10-cm long polyethylene tubing. The fishes of control groups were orally administered with 0.1 ml sterile saline solution.

The fish of all groups were placed under daily observation for 2 weeks and the dead fishes were used for bacterial re-isolation.

RESULTS

The clinical examination of naturally infected Nile tilapia *(O. niloticus)* revealed corneal opacity with highly congested swelling around the eye ball and dermal haemorrhages in the lower jaw and operculum (Fig. 1). Internally, the spleen was enlarged (Fig. 2), while both stomach and intestine contained food.

The clinical examination of naturally infected grey mullet *(M. cephalus)* revealed numerous hameorrhagic areas particularly around the mouth and operculum with abdominal distension (Fig. 3). The abdominal cavity was filled with

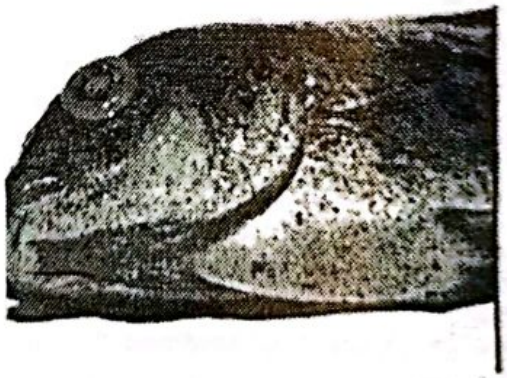

Fig. (1): Nile tilapia (*O. niloticus*) showing cornela opacity with highly congested swelling around the eye ball and dermal hameorrhages in the lower jaw and operculum.

yellowish fluid, the stomach and intestine contained yellow to yellowish-green gelatinous material and pale coloured liver (Fig. 4).

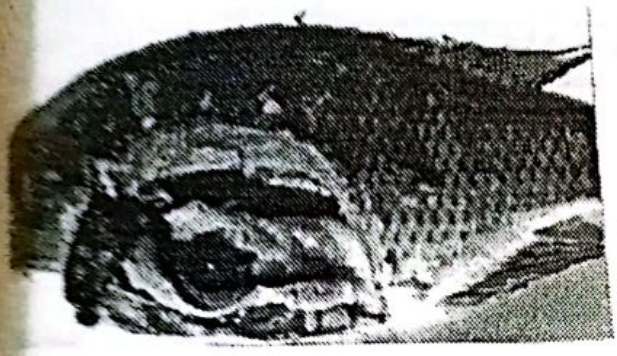

Fig. (2): Nile tilapia (*O. niloticus*) showing enlatgement of spleen.

The bacteriological examination revealed isolation of gram-positive cocci, almost in pure culture, from eyes, liver, kidneys and spleen. The colonies of isolated organisms on BTA had a diameter of about 1-2 mm, were dull greyish white colour and were surrounded by an area of α-haemolysis. The morphological, culture and biochemical characters of the isolates isolated from both environments (*O. niloticus*, freshwater and *M. cephalus*, marine water) linked with, but not identical to *Streptococcus faecalis* and *Streptococcus faecium* respectivelly (Table 1).

The bacterial isolates able to produce the disease in apparently healthy *O. niloticus* and *M. cephalus* only when the fish body was injured prior to dipping in the bacterial suspension.

The mortalities were 100% in both fish species after 10 days (Table 2) and the organism was re-isolated from eyes, liver, kidneys and spleen of all dead fishes. No deathes in any of the dip or oral challenges, nor the pathogen was isolated from the fish eyes, liver, kidneys and spleen.

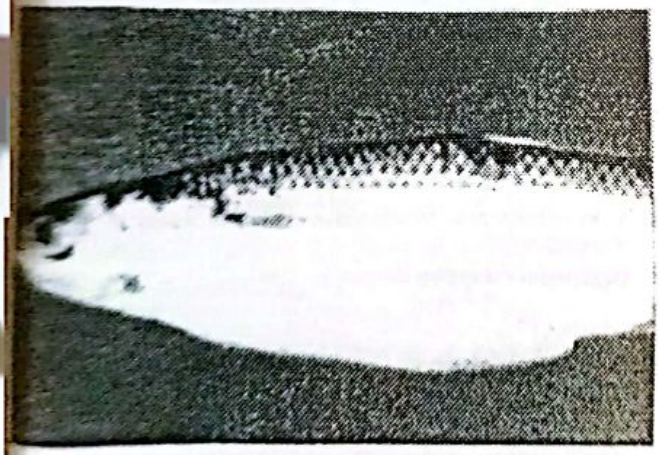

Fig. (3): Mullet (*M. cephalus*) showing haemorrhagic areas on the head region and slight abdominal distention.

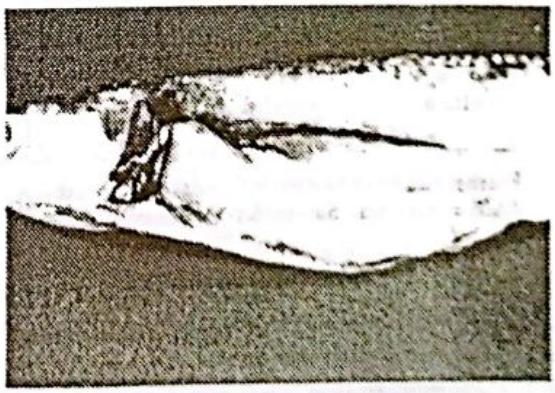

Fig. (4): Mullet (*M. cephhalus*) showing pale coloured liver and the stomach and intestine contained yellow to yellowish-green gelatinous material.

Table 1:Morphological, cultureal and biochemical characters of Streptococcal isolates.

Character	Isolates of fresh-water environ-ment	Isolates of ma-rine environ-ment	Cowan (1974) S. faecalis S. fae-cium		ASM (1981) S.faecalis S. fae-cium.	
Gram-reaction	gram-positive	gram-positive	gram-positive		gram-positive	
cell form	cocci in chain	cocci in chain	cocci in chain		cocci in chain	
Motility	--	--	--	v	--	--
Hemolysis	α	α	r/β	r/α	α/β /r	α/r
Catalase	+	+	+	-	+	--
Oxidase	--	--				
O/F glucose	fermentative	fermentative	fermentative		fermentative	
Indole test	--	--	v	--	--	--
Gelatine hydrolysis	--	--	+	+	+	+
Starch hydrolysis	--	+	+	--		
Aesculin hydrolysis	+	+	+	+	+	+
Nitrate reduction	--	--				
Citrate utilization	+	--				
Arginin decarboxylation	+	+			+	+
Lysine decarboxylation	--	--				
Ornithine decarboxylation	--	--				
H. S. production	--	--				
Growth at 10C°	+	+				
gowth at 22 C°	+	+				
Growth at 37C°	+	+				
Growth 45C°	+	--	+	+		
Growth at pH 9.6	+	+	+	+		
Growth at 6.5 % NaCl	+	+	+	+	+	+
Production of acid from:		--				
Glycerul	+	+	+	--		
Glucose	+	+	v		+*	+
Sucrose	+	--	+	+	+*	+
Lactose	+	+	+	+	+	+
Mannitol	+	+	--	+	--*	+*
Arabinose	--	--	--	--	--*	+*
Raffinose	--	+	+	+		
Salicin	+	+				
Maltose	+					

Freshwater environment = O. niloticus + Freshwater Marine environment = M. cephalus + Marine water.
ASM = American Society for Microbiology V : Variable
* : Occasional exception occurs

Table (2): The mortality rate in experimentally infected *O. niloticus* and *M. cephalus* with the corresponding Strep-tococcus species.

Fish species	Total No.	Method of challenge	Daily mortality after challenge 1 2 3 4 5 6 7 8 9 10 11 12 13 14	Total dead fish	Mortality %
O. niloticus	10	Dip challenge	-- -- -- -- -- -- -- -- -- -- -- -- -- --	--	0.0
	10	Skin scrafication	-- -- -- -- 2 3 2 1 1 1 -- -- -- --	10	100
M. cephalus	10	Oral challenge	-- -- -- -- -- -- -- -- -- -- -- -- -- --	--	0.0
	10	Dip challenge	-- -- -- -- -- -- -- -- -- -- -- -- -- --	--	0.0
	10	Skin scrafication	-- -- -- -- 4 2 2 1 1 -- -- -- -- --	10	100
	10	Oral challenge	-- -- -- -- -- -- -- -- -- -- -- -- -- --	--	0.0

DISCUSSION

The local freshwater fish farms and Lake Eltemsah in Ismailia governorate are considered the most important sources for *O. niloticus* and *M. cephalus* respectivelly. Unfortunately, both environments were polluted with domestic wastewater. This pollution plays an important role in appearance and spreading of previously undescribed diseases as edwardsiellosis in Nile tilapia (Badran, 1993) and streptococosis in Nile tilapia (*O. niloticus*) and grey mullet (*M. cephalus*) in the present study.

The clinical pathology of streptococcus infection was varied acording to the water environment. In freshwater fishes, ayu (Ugajin, 1981), several species of tilapia (Kitao et. al., 1981 and Miyazaki et. al., 1984), rainbow trout (Miyazaki, 1982 and Bragg and Broere, 1986), Striped bass (Baya et. al., 1990) and Nile tilapia (*O. niloticus*), the disease was characterized by corneal opacity with haemorrhagic swelling around the eye ball (Fig. 1), dermal haemorrhages and slenomegally (Fig. 2). While in grey mullet (*M. cephalus*) the signs were as those described in marine fishes, yellowtail (Minami et. al., 1979), eels (Kusuda et. al., 1978), Siganus canaliculatus (Foo et. al., 1985) in the form of numerous haemorrhagic areas on the body surface, particularly around the mouth and operculum and abdominal distension (Fig. 3).

Moreover, the abdominal cavity was filled with yellowish fluid, the stomach and intestine contained yellow gelatinous material and pale coloured liver (Fig. 4).

The morphological, cultural and biochemical characters of Streptococcal species isolated from both environments were very similar to those of *S. faecalis* and *S. faecium* (Cowan, 1974 and American Society for Microbiology, 1981) (Table 2). Although the API20 strep results showed a close similarity of the isolated species to group A streptococci, the species were sensitive to sulfamethoxazole which does not affect group A Streptococcus (American Society for Microbiology, 1981). Moreover, the species are α-haemolytic Sterptococcus in contrast to the normal B-haemolytic Group A Streptococci. Furthermore, the relavent literature (Robinson and Meyer, 1966; Cowan, 1974; Minami et. al., 1979; Bullock, 1981; Ugajin, 1981; Kitao, 1982; Foo et. al., 1985 and Bragg and Broere, 1986) revealed that the fish pathogenic Streptococci have been linked wtih *S. equisimilis, S. faecalis, S. faecium, S. agalactiae, S. equimus, S. pyogenous* and *S. lactis*. Superficially this information could inter heterogeneity among these Streptococci or due to geographical differences (Austin and Austin, 1987).

Streptococcus species were detected in both fresh and marine water in which the fish were reared. Thus a possible source of infection was the water itself and the injured fish body was the route of bacterial invasion. This clarify whey gulf killifish (Rasheed and Plumb, 1984), rabbitfish (Foo et. al., 1985), Nile tilapia (*O. niloticus)* and grey mullet (*M. cephalus*) were infected artificially through injured fish body prior to dipping in the bacterial suspension and not orally or by depping. In contrast, the results of some studies (Minami, 1979 and Iwata, 1982) reported that the feed (trash fish) was the source of streptococcal infection in yellowtail by the oral rout. Moreover, the mortalities in rainbow trout farms were greatly reduced by feed boiling (Bragg and Broere, 1986). However, all authers attempted to isolate the Streptococcus species from the feed have been failed. Indeed, the bacteria can penetrate the wall of the intestine or stomach if it has previously invaded by other intestinal tract fauna in large numbers or has been chemically injured by pesticides or other pollutants in the water (Miyazaki, 1982).

Accordinglly, it could be concluded that streptococcal infection to freshwater and marine fishes may be one of the future problem in pisciculture under domestic wastewater pollution.

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