National Institute of Oceanography and Fisheries

STREPTOCOCCUS FAECALIS AS A CAUSE OF MORTALITIES AMONG CULTURED MONOSEX-TILAPIA

(With One Table and 12 Figures)

By

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البكتريا السبحية من نوع ستربتكوكاس فيكالز كسبب للموت في أسماك البلطى وحيد الجنس المستزرع

صافيناز جمعة محمد

تم تسجيل وفيات عالية بنسبة ٢٠% في أحدى مزارع البلطى وحيد الجنس في مدينة الإسكندرية خلال الربيع الماضى. وتم عزل وتصنيف البكترية السبحية من نوع فيكالز كسبب للوفاه. تم عمل العدوى الصناعية بنجاح باستخدام البكتريا المعزولة في أسماك البلطى وحيد الجنس والمبروك الفضى عن طريق الحقن بالتجويف البريتونى. وكانت الأعراض الأكلينيكية عبارة عن خمول في حركة الأسماك وأعراض تنفسية وتحول لون الجلد للون الغامق مع تساقط القشور وحجوظ في أحدى العينين أو كلاهما ووجود أنزفة على الجلد وخاصة عند قواعد الزعانف واستسقاء بالبطن. وتمثلت الأعراض المرضية الداخلية في أحتقان بالأعضاء الداخلية. بينما كانت التغيرات الهستوباثولوجية تحلل وموت خلايا الكبد وأضمحلال بأنسجة تخليق الدم وتنقرز وموت خلايا الكلى. وقد وجد أن البكتريا السبحية من نوع فيكالز حساسة للعديد من المضادات الحيوية وأن كلاً من الأوكسى تتراسيكلين والأموكس سيللين كانا أكثر المضادات الحيوية تأثيراعلى البكتريا.

SUMMARY

During last spring (2006) severe mortalities among cultured monosex tilapia were recorded in a private fish farm at Alexandria governorate and caused up to 20% mortality. The isolated bacteria was identified as *Streptococcus faecelis*. Experimental infection was successful through intraperitoneal injection (i.p.). The clinical signs were sluggish movement, respiratory disorder, darkening, loss of scales, uni-or bilateral exophthalmia, haemorrhages of the skin especially in the base of fins and abdominal distention. The common postmortem lesions were congestion of internal organs. The histopathological changes were hydropic degeneration of hepatocytes, deplation of haemopoietic

elements and necrosis of kidney tissues. The isolated S. faecalis found to be sensitive to a wide range of antibiotic and oxytetracycline and amoxicillin were the drugs of choice.

Key words: *Fish, Tilapia, Strept faecalis.*

INTRODUCTION

Streptococcal infection of fish which were rearly reported before 1970, (Robinson and Meyer, 1966) become a major problem worldwide with the intensification of aquaculture (Baya *et al.*, 1990 and Carson *et al.*, 1993).

Now *streptococcus sp.* has recently created a major disease problem in cultured tilapia and considered of high importance in recent years due to increased reports of outbreaks and the high economic losses caused by gram-positive bacteria in both wild and culture fish (Domenech *et al.*, 1996).

Moreover, streptococcal disease has been documented in both wild and cultured fish with distribution being worldwide (Kitao 1993), specially in *Oreochromis niloticus* and common carp and caused heavy losses (Eldar and Ghittino (1999).

Also Zlotkin *et al.*, (2003) recorded that streptococcus iniae was capable of causing disease in human who had recently handled infected fish from farms.

In Egypt, massive mortalities from streptococcosis has been recorded in both wild and cultured freshwater fish (Badran, 1994, Khalil, 2002, Ebtesam, 2002 and Refaee, 2005).

Vaccination to streptococcosis has been largely disappointing but antimicrobial compound have met with considerable success (Kitao, 1982).

Experimental infection of streptococcus, which may or may not be representative a disease in the natural environment, have been achieved by injection (Cook and Lofton, 1975) and by exposure of fish to streptococcus species (Semino *et al.*, 1996). Monosex tilapia and carp species had been recived considerable attention during the last 10 years in Egyptian aquaculture and sharing in most as polyculture farms.

The aim of this study is to record the isolation and identification of streptococcus species during on outbreak in monosex tilapia farm in Alexandria – Cairo desert road at Alexandria Governorate during spring of 2005. Characterization and pathogenicity were carried out.

MATERIALS and METHODS

1. Fish:

During an out break in private fish farm heavy mortalities (up to 20%) was occurred in spring of 2005 among monosex-tilapia (average body weight 80 ± 5g).

Freshly dead and moribund fish were collected and subjected to clinical, bacteriological, parasitic and mycological examination according to Amlacher (1970). Bacterial isolation was done from blood, kidneys, liver, spleen and ascitic fluid of 50 naturally infected monosex-tilapia and striked on tripticase soya agar (TSA), brain heart infusion agar (BHIA), 5% sheep blood agar and Mackonky agar. The plates were incubated at 28C for 48 hours.

The isolated colonies were tested for morphological, culture and biochemical characterization according to Bergey *et al.*, (1994) and Elmer *et al.*, (1997). Also the isolated bacteria were tested biochemically by using APT-20 strep. System (Bio Merieux). The antibiogram of the recovered bacteria was done according to Cruickshank *et al.*, (1975) and Carter and Cole (1990).

Estimation of the medial lethal dose (LD_{50}):

The median to that dose (LD_{50}) was calculated for the isolated *streptococcus sp.* in monosex-tilapia and silver carp according to Reed and Muench (1938). Seventy fish of apparently healthy monosex tilapia (60 ± 5g) and 70 silver carp (80 ± 10g) were intraperitoneal (i.p) injected with serial ten fold dilutions (10^{-1} – 10^{-7}) of the isolated bacteria (10 fish/dilution). Ten fish from each fish species were injected (i.p) with 1ml steril saline and served as control. Mortalities were recorded for 8 days.

Experimental injection:

A total numbers of 60 apparently healthy (30 monosex tilapia and 30 silver carp) were used in experimental injection. Fish of each type were divided into 3 groups (10 fish/group). The first 2 groups of each species were injected (i.p) with 1ml of sublethal dose of *S. faecalis* (10^{-3} cfu/ml in case of monosex-tilapia and 10^{-5} cfu/ml in case of silver carp). The 3rd group of each fish species were injected with 1ml sterial saline and served as control. The fish were observed daily for 14 days for clinical signs and mortalities. Specimen from liver, kidney, spleen and gills were collected from injected fish fixed in 10% neutral buffered formalin for histopathological examination according to Roberts (1989).

Reisolation of injected bacteria was done from freshly dead fish for verification the specificity of death where the injected streptococcus isolate was rei-solated.

RESULTS

Clinical examination:

During the out breaks, mass mortalities (about 20%) was recorded. The naturally infected fish showed loss of appetite, sluggish movement, swimming close to the surface of the water, escape reflex (-ve), darkening of the skin, detached scales and haemorrhages of skin. Uni or-bilateral exophthalmia and eye turbidity with distented abdomen and congested vent were observed (Fig. 1).

Internally congestion of the liver, kidneys, presence of fluid in the abdomin and destended of gall bladder were recorded (Fig. 2).

Bacteriological examination:

The isolated bacteria was recovered from internal organs and blood of moribund fish during the outbreak. The bacteria was gram positive cocci, arranged in short chain, non motile, pen headed colony, white opaque colour, raised edges and gave α-haemolysis on 5% sheep blood agar. The biochemical characters of the isolated bacteria are illustrated in Table (1). From the morphological, cultures and biochemical characters by traditional method and the manufacturer cretaria of the API-20 streps, we abled to recovered one type of streptococcus, namely *streptococcus faecalis*.

The results of mycological examination proved to be negative while, the parasitic one showed presence of slight infestation with ciliate protozoa (*Trichodina sp.*).

Antibiogram:

The isolated *Streptococcus faecalis* were proved to be sensilive to amoxicillin, chloramphenical, penicillin, oxytetracycline, Trimethoprim & sulfamethoxazole, nalidexic acid and colistin sulphate and resistant to ciprofloxacin, erythromycin, kanamycin, Tetracyclin and ampicillin.

Median Lethal dose (LD_{50}):

The results of LD_{50} were proved that the isolated S. faecalis was highly virulent to monosex tilapia than silver carp. LD_{50} value were 10^4 and 10^6 respectively.

Experimental infection:

The experimental infection was successfully induced by (i.p) with streptococcus fecalis with no difference in both fish species except that in silver carp, caudal fin erosion was observed. Reisolation of the injected bacteria was succeeded from all dead fish. The control group showed neither clinical signs nor post mortem (P.M.) changes.

The clinical signs and PM lesions were appeared on injected fish as no escape reflexes, restlessness, swim near the water surface and strong respiratory disorder. Death started after 4 and 6 days post-infection with a total mortalities of 30 and 40% incase of monosex tilapia and silver carp respectively.

The fish showed haemorrhage in the base of the fins, uni or bilateral exophthalmia, with haemorrhage of the eye. Slight ascites and detached scales. Congetion of the internal organs was observed and some fish from both species showed yellowish liver (Fig. 3, 4, 5, 6 and 7).

Histopathological alteration:

The histopathological lesions of naturally and experimentally infected fish were more or less similar.

The gills showed telangictasis beside edema at the base of secondary lamellae Fig. (8).

Hyperactivation of melanomacrophage centers and marked depletion of white pulp with multifocal hyrmphocytic cells depletion were observed in the spleen Fig. (9 and 10).

The liver showed severe hydropic degeneration and congestion of blood vessels Fig. (11).

Depletion of intertubular haemopiotic tissue, cloudy swelling of renal tubules and necrotic of convoluted tubules were the changes recognized in the kidney Fig. (12).

Table 1: Morphological and biochemical characters of isolated bacteria.

Test	Result	Test	Result
- Gram stain	+	- Citrate utilization	+
- Chain	Short	- α-galactosidase	-ve
- Motility	-ve	- Hydrolysis of:	
- Growth on:		* Argenin dihydrolase	+
* Triptic soya agar	pen head.	* Esculin hydrolysis	+
	ed white	* Hippurate hydrolysis	+
	Opque	- Acid produced from:	
	circular	* Ribose	+
	Colonies	* Arabinose	-ve
* Macconkey agar	Pink	* Mannitol	+
	Colour	* Sorbitol	+
- 5% sheep blood	α-hae-	* Lactose	+
Agar	moloysis	* Inulin	-ve
- At 6.5% NaCl	+	* Raffinose	-ve
- At 10 and 45C	+	* Sucrose	+
- Fermentation metab		* Glucose	+
olism	+	* Dulcitol	
- Production of:			
*Urease	-ve		
*Catalase	-ve		
* Oxidase	-ve		
* Indole	-ve		
* Voges Perskauer	+		

Fig.1

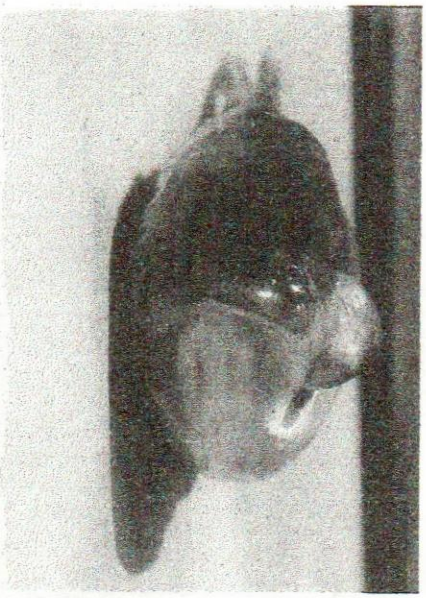

Fig.3

Fig.2

Fig. 4

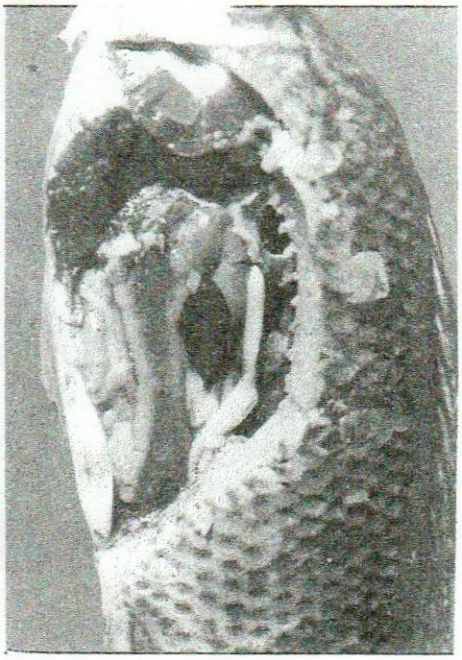

Fig.5

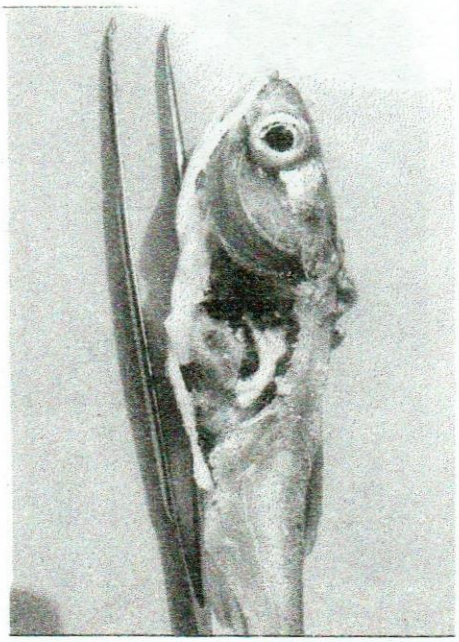

Fig. 7

Fig.6

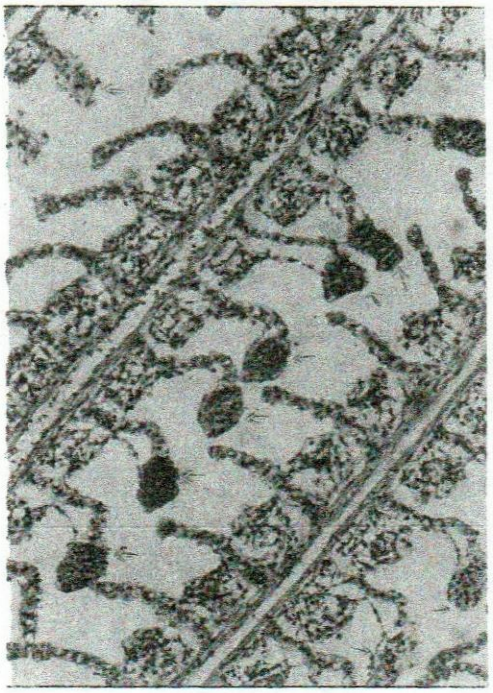

Fig.8

Fig. 9

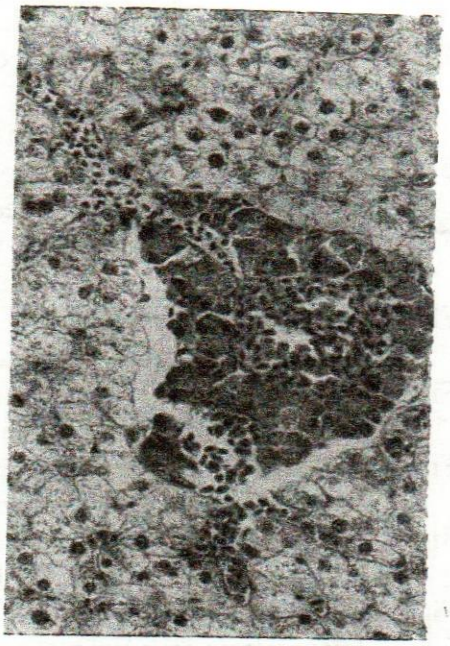

Fig. 11

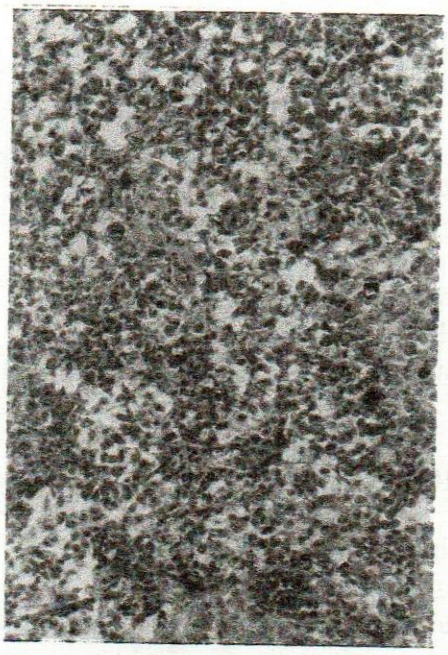

Fig. 10

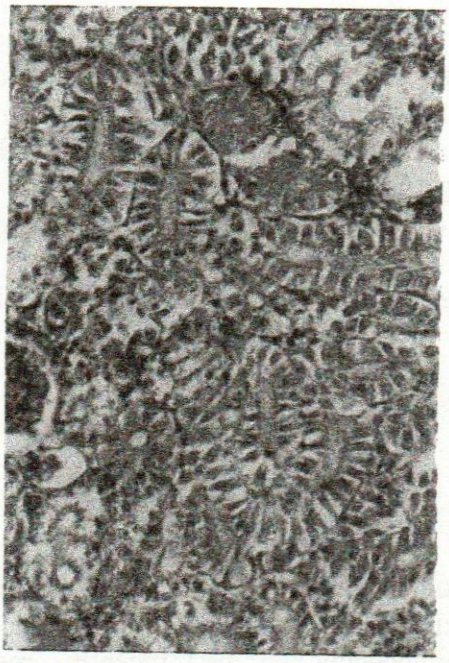

Fig. 12

LEGEND OF FIGURES

Fig. 1: Naturally infected monosex tilapia showing darking of the skin, congestion of base of fins, fin erosion and unilateral exophthalmia.

Fig. 2: Naturally infected monosex tilapia showing congestion of internal organs and distended gall bladder.

Fig. 3: Experimentally injected monosex-tilapia with *Streptococcus faecalis* showing sever exophthalmia.

Fig. 4: Experimentally injected monsex-tilapia with *Streptococcus faecalis* showing detached scales and fins errosion.

Fig. 5: Experimentally injected monosex-tilapia with *Streptococcus faecalis* showing congestion of internal organs.

Fig. 6: Experimentally injected silver carp showing darking, haemorrhagic eye exophthamia.

Fig. 7: Experimentally injected silver carp showing congestion of internal organs and yellowish liver.

Fig. 8: Gills of experimentally infected monosex tilapia showing telangictasis an edema of the base of secondary lamellae. H & E (X 250).

Fig. 9: Spleen of naturally infected monosex tilapia showing hyperactivation of melano macrophages centers H & E (X250).

Fig. 10: Spleen experimentally infected silver carp showing marked deplation of white pulp and multifocal hymphocytic cells deplation (arrow) H & E (X400).

Fig. 11: Liver of experimentally infected monosex-tilapia showing congestion of hepatic blood vesels with severe hydropic degeneration of the most hepatic cells. H & E (X400).

Fig. 12: Kidneys of silver carp showing cloudy swelling of some convoluted tubules (arrows), multifocal tubular necrosis (arrows head) and depletion of inter tubular haemopiotic tissue H & E (X400).

DISCUSSION

Streptococcal infection of fish which were reported before 1970, (Robinson and Meyer, 1966) and become a major problem world wide with the intensification of aquaculture (Carson *et al.*, 1993). Now streptococcus sp. had recently created a major disease problem in

cultured tilapia and reported to cause high economic losses in both wild and cultured fish (Domench *et al.*, 1996).

The isolated bacteria from naturally infected monosex tilapia and comprised gram-positive cocci, non-motile, catalase, oxidase and indole negative, grew on blood agar and gave α-haemolysis. From the results of culture, morphological and biochemical tests, the isolated bacteria could be identified as *Streptococcus faecalis* as guided by Bergey *et al.*, (1994). Also the results agree with those reported by Baye *et al.*, (1990), El-Bouhy (2002) and Zeid (2004). Also Refaee (2005) isolated S. faecalis from naturally infected *Oreochromis niloticus*. The isolation of *S. faecalis* from kidney, spleen and liver may be attributed that, this organs more or less considered being tropism for this bacteria due to the nature of septicemia occurred by the microorganism and proved the pathogenicity of this bacteria to fish (Kimura and Kasuda, 1982). Moreover, presence of isolated bacteria in fish tissue revealed high concentration of these pathogen in the pond water.

The successful induction of the disease experimentally leaves no doubt about the potential pathogenicity of *Streptococcus faecalis* to monosex tilapia and silver carp. The fact that infection could occur following the presence in water contaminated with S. faecalis confirm the invasive character of the organism. These substantiate findings reported by Boomker *et al.*, (1979).

Variation in disease signs, including lethargic, darkening, deteched scales, exophthalmia and distended abdomin, while the common postmortem lesions were acute septicemia in nature as they revealed congestion of the internal organs. These results may be due to the haemolytic effect of the exotoxin produced by bacteria (Rasheed and Plumb, 1984).

The isolation of S. faecalis from diseased and dead fish during the epizootic in spring season indicate the pathogenicity of this organism and seasonal threat to fish industry posed by it. The differences in LD_{50} values and mortality rate in monosex tilapia and silver carp may be attributed to sensitivity of fish to infection and monosex tilapia proved to be sensitive to *S. faecalis* infection than silver carp. This differences among fish species had been reported by Khalil (2002) and Refaee (2005).

It is interesting to note that the disease was occurred during spring and among sexually mature fish. This may be due to the stress effect during this time of the year which initiates a series of physiological responses ending with immunosuppression (Peters, 1977).

The histopathological alterations were gill telangictasis, hydropic degeneration, activation of melanamacrophage centers and depletion of haemopiotic elements.

The possible explanation of these changes could be attributed to the strong action of α-haemolysin of S. faecalis (Minamia *et al.*, 1979 and Zeid, 2004).Some changes also reported by Khalil (2002) and Refaee (2005).

The results of antibiotic sensitivity of the isolated bacteria proved that they were sensitive to a wide range of antibiotics (Franks *et al.*,1998, Domenech *et al.*,1996 and Khalil, 2000).

The fact that streptococcus species are a common cause of human infections. The zoonotic importance of this organism specially *S. faecalis*. This work, raised some questions about the use of chicken manure infarms and dangerous of contamination of water used in farms with human sewage. However, it remain to be necessary to investigate how far is the role of man or fish in transmission of this bacteria.

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