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General Septicemia of Streptococcal Infection in Cage-cultured Tilapia, *Tilapia mossambica*, in Southern Taiwan

臺灣南部箱網養殖吳郭魚之鏈球菌感染

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Abstract

From September to October 1984, epizootics of streptococcal infection outbroke in cage-cultured tilapia in Tzeng-Wen dam, Tainan. Heavy losses with accumulative mortalities of 50% and 60% within one month period were recorded.

The major external lesions of the diseased fish were hemorrhage and exophthalmia with corneal opacity. Blackish discoloration of the body with nodular or abscess formation of trunk and/ or peduncle muscle were frequently recognized among the affected fish. Internally, enlargement of liver and spleen was prominant, epicarditis, peritnitis with excess fluid were noticed. Gram's positive cocci were found in damp smears of eye, muscular abscesses, spleen, liver, kidney and ascites. Multiple necrosis, some granulomatous lesions with bacterial dissemination were seen in liver, spleen, kidney, heart, skeletal muscle, meninges, eye and serosa of stomach and intestine.

The causative organism of the disease could not grow on MacConkey agar, but it grew well on blood agar contained 5% defibrinated goat or sheep RBC with β-hemolysis. It was identified as *Streptococcus* species according to the morphological and biochemical properties of the isolates. The bacterium was highly sensitive to erythromycin and doxycycline. The treatment of the epizooties is discussed in the text.

Streptococcal infection usually is a sporadic disease in mammals. It has been incriminated as the cause of endocarditis, arthritis and mastitis in many species of animals (GILLESPIE & TIMONEY, 1982). In fish, it also has been reported in several species under a number of different conditions in the United States and Japan(ROBINSON & MEYER, 1 966; PLUMB *et al.*, 1974; BARHAM *et al.*, 1979; BOOMKER *et al.*, 1979; KUSUDA *et al.*, 1976; KUSUDA *et al.*, 1978; MINAMI *et al.*, 1978; KITAO *et al.*, 1981; OHNISHI & JO, 1981; UGAJIN, 1981; JO, 1982; SHIOMITSU, 1982; MIYAZAKI, 1982; MIYAZAKI *et al.*, 1984; 7 KAIGE *et al.*, 1984). In Taiwan, recently the streptococcal infection has frequently occredur among the pond cultured

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bullfrog (*Rama catesbeiana*) (LIU, 1982) and has become an important disease of this particular species of the cultured amphibia. Present report describes the clinical, symptoms, pathology and bacteriology of *Streptococcus* infection in cage-cultured tilapia (*Tilapia mossambica*) in Tzeng Wen dam, Tainan.

Materials and MethodsRes

Epizootiology and clinical manifestation

In September of 1984, epizootics outbroke in the separate tilapia cage-culture units in Tzeng-Wen dam, located in Tainan Hsien. In these units, approximately 10,000 and 15,000 tilapia with the size of 15-20 cm in length were raised. The commercial ration were used to feed them. The water level of the dam during the epizootics was very low due to the dry season of the year. The affected fish showed apparent circular swimming movement. The accumulative mortality of the epizootics within one month period was 50% and 60% respectively.

Gross pathology

The diseased fish presented signs of exophthalmia with or without conjunctival hemorrhage and corneal opacity (Fig. 1.). Body blackening and patches of skin discoloration with scale desquamation on the lateral and/or dorsol of the trunk and peduncle were frequently observed together with nodule or abscess formation of the affected muscle. Frayed, congested fins and swollen abdomen were often encountered. Enlargement of liver and spleen which always engorged with blood was prominent. Ascites with yellowish exudative material covered the peritoneum and epicardium was also frequently observed (Fig. 2). Gram's positive cocci were detected in the smear preparations from ascites, spleen, liver, kidney, eye and muscular lesions.

Tissues were taken and fixed in 10% buffered formalin solution, and 4-5μ thick sections were stained with hematoxylin and eosin, Gram and Giemsa stains.

Bacterial isolation, identification, serology and drug sensitivity

The primary bacterial isolation was routinely carried out with Blood agar (BA) contained 5% defibrinated goat RBC, Trypticase soy (TS) agar, MacConkey (MC) agar plates. The morphological, cultural and biochemical characteristcs were tested subsequently for the identification of the isolates. The Oxoid[1] streptococcal grouping kit was used for the identification of streptococcal Group A, B, C, D,F and G. Drug sensitivity test utilizing 13 BBL[2] sensi-discs of different antimicrobial agents were also performed.

1.Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 OPW, UK.
2. BBL, Cockeysville, Maryland 21030, U.S.A.

Results

Histopathological changes

Systemic infection was evident with dissemination of bacteria and macrophages in the various internal organs.

Pericarditis was noticed in the tunica adventitia of bulbus arteriosus. Severe infiltration of bacteria-laden macrophages and accumulation of fibrinous exudates were seen in the outer layer of heart (Fig. 3.). The hepatic cells revealed various degree of fatty changes. Multiple necrosis distributed in the parenchyma which normal cells were replaced by the numerous bacteria-laden macrophages infiltrated in the necrotic area, even formed granuloma in some specimen (Fig. 4). Hyperplasia of reticuloendothelial cells was a main lesion found in the spleen. These splenocytes engulfed some bacterial agents. Few necrotic foci scattered here and there (Fig. 5 & 6). The renal tubules showed the changes of hyaline droplet degeneration and some reticuloendothelial cells phagocytosed bacterial cells in the interstitial tissues (Fig. 7). Catarrhal enteritis associated with desquamated necrotic cells was seen in the small intestine (Fig. 8). The same lesion was also found in the stomach. In spite of these changes, peritonitis was observed in the serosa of GI tracts and characterized by infiltration of bacteria-laden macrophages (Fig.9). Bacterial meningitis was noted by the infiltration of bacteria-laden macro phages accumlated in the lentomeninges of most cases (Fig. 10). Abscess formations were seen in the muscles which contained numerous necrotizing cells and macrophages (Fig. 11). In exophthalmic fish, severe invasion of bacteria and macrophages were observed in the conjunctiva, cornea, iris, orbital adipose tissue and oculo-motor musculature (Fig. 12).

Fig. 1. Tilapia showed exophthalmia, corneal opacity, hemorrhage and scale desquamation.
Fig. 2. Tilapia with ascites and peritonitis.

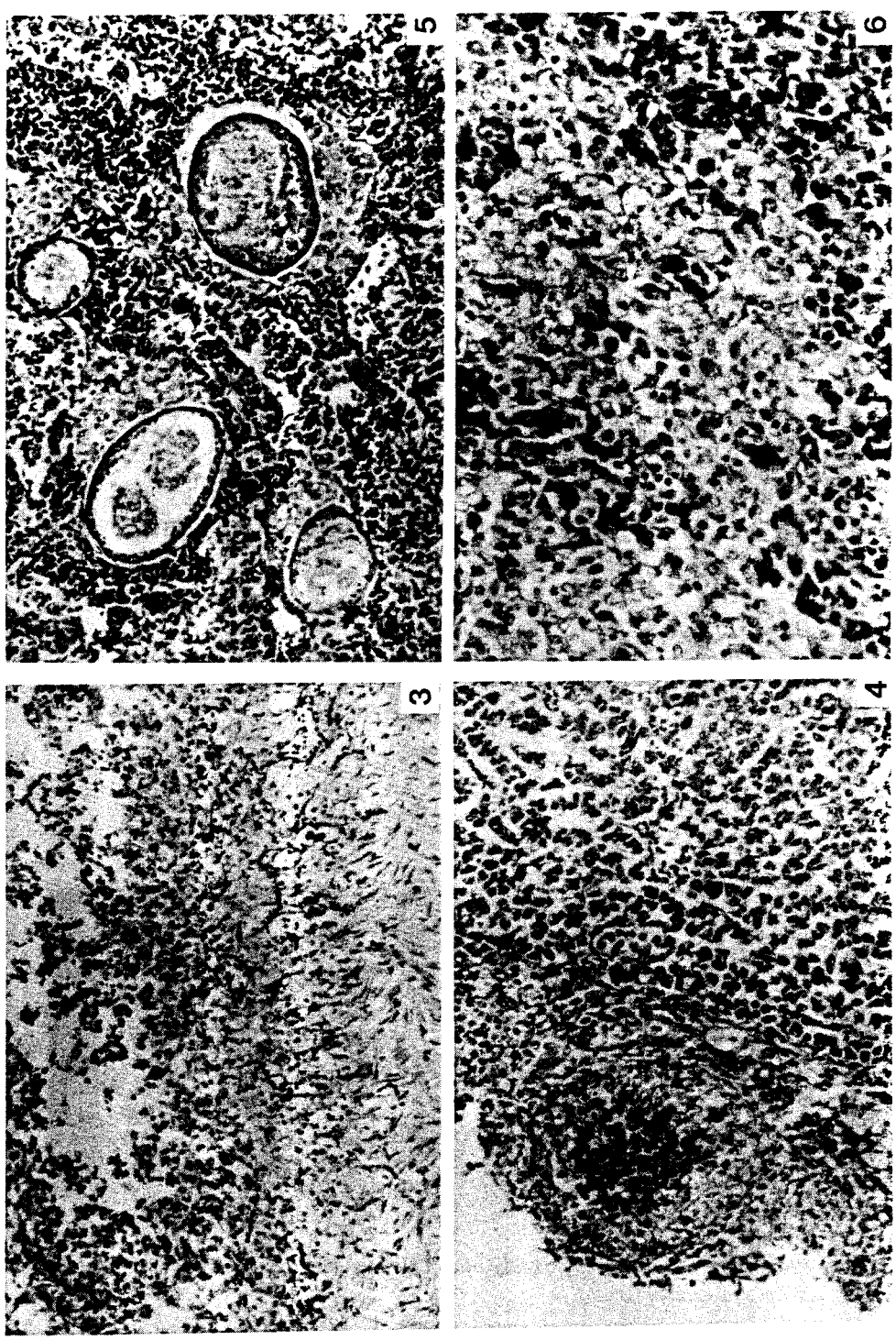
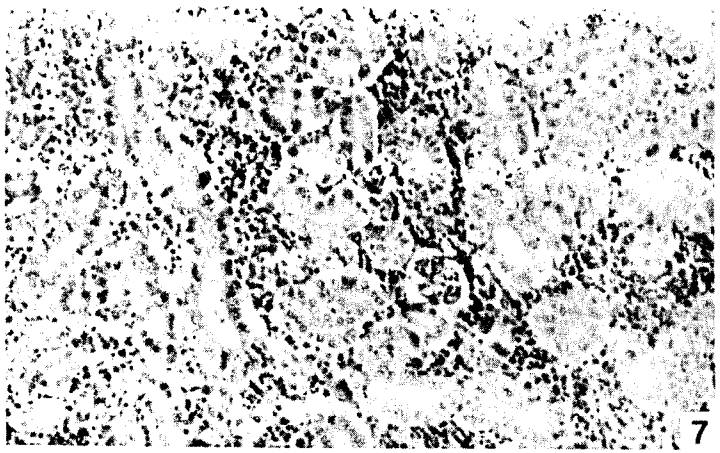


Fig. 3. Heart. Pericarditis was seen with evidence ol excess macrophages infiltrationin the bulbus of arteriosus H & E, x 200.

Fig. 4. Liver. A granuloma with necrotic center was found near the surface of liver. H &E x 200.

Fig. 5. Spleen. The melano-macrophage center showed necrotic alterations and had a tendency to form granulomatous changes. H & E. x 200.

Fig. 6. Spleen. Proliferation of reticuloendothelial cells was seen and some bacterial cells were engulfed in the cytoplasm. H & E. x 400.



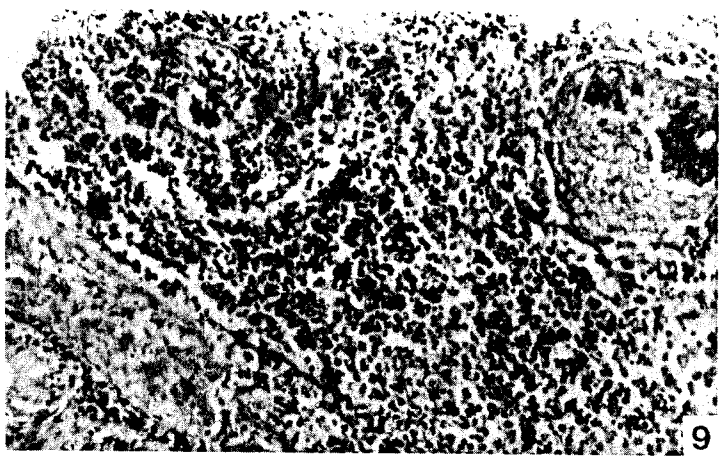


Fig. 7. Kidney. Some hyaline droplets were observed in the renal tubules. H & E. x 100.

Fig. 8. Small intestine. Muco-necrotic enteritis was evident and characterized by excess mucus mixed with the necrotic debries mucosal layer accumlated in the lumen. H & E. x 200.

Fig. 9. Mesentery. Peritonitis accompanied by the formation of granuloma was observed in the mesentery near the serosa of small intestine. H & E. x 200.

Fig. 10. Brain. Meningitis was evident and characterized by infiltration of numerous macrophages in the meninges. H &E. x 200.

Fig. 11. Muscle. Muscle fibers revealed severe necrosis which was completely replaced by macrophages and hyperplastic fibers. H & E. x 200.
Fig. 12. Eye. Bacteria-laden macrophages infiltrated in the iris. H &E. x 100.

Bacterial isolation, identification serology and drug sensitivity

The causative microorganism was isolated in pure culture from kidney as well as from other tissues, including liver, spleen, nodular lesion of muscle and opaque, exophthalmic eye. The bacterium was primary isolated from BA with β-hemolysis. It grew well on BA with 5% sheep or goat RBC incorporated. It also grew on Brain Heart Infusion (BHI) and TS agar, whereas it would grow better when additional 0.5% NaCI was supplemented to BHI or TS agar. However, it could not grow on MC, EMB and SS agars. The bacterium was Gram-positive, spherical cells occurred in chains of various length.Thd morphological, cultural and biochemical properties of the isolate are listed in Table 1. It was not agglutinable to any group of A, B, C, D, F and G antiserum. Thus, it was identified as *Streptococcus* species.

The microorganism was shown susceptible to erythromycin, doxycycline and etc. but was resistant to ampicillin, kanamycin, neomycin, penicillin and etc. (Table 2).

Table 1. Biochemical cultural and morphological characteristics of the isolate.

Characteristics	*Reactions*	*Characteristics*	*Reaction*
Cell morphology	Cocci	Indol	—
Gram Stain	+	Nitrate reduction	—
Oxidase	—	Citrate	—
Catalase	—	Tartrate	—
OF test	Fermentive	Urease	—
Motility	—	Malonate	—
Growth at/in/on		Litmus milk	—
10°C	—	Voges-Proskauer test	—
45°C	—	Methyl red test	—
6.5% NaCl	—	Acid from:	
pH9.6	—	Adonitol	—
0.1% Methylene	—	Arabinose	—
blue milk	—	Cellobiose	+
40% bile	—	Dulcitol	—
EMB	—	Fructose	+
SS	—	Galactose	+
Heat tolerance		Glucose	+
(60°C, 30min)	—	Glycerin	—
Hydrolysis of:		Inositol	—
Hippurate	—	Inulin	—
Gelatin	—	Lactose	—
Starch	—	Maltose	+
Arginine	—	Mannose	+
Esculin	—	Mannitol	—
Hemolysis of:		Raffinose	—
Goat blood	β	Rhamnose	—
Sheep blood	β	Salicin	—
		Sorbitol	—
		Sucrose	+
		Trehalose	+
		Xylose	—

OF: Oxidation Fermentation, EMB: Eosin Methylene Blue agar
SS: Solmonella-Shigella agar

Table 2. Drug sensitivity of the isolate

Sensitive	Resistant
Chloramphenicol	Amikacin
Doxycycline	Ampicillin
Erythromycin	Kanamycin
Gentamycin	Methicillin
Nalidixic acid	Neomycin
Sulfamonomethoxine	Novobiocin
+trimethoprim	Penicillin

Discussion

A number of freshwater and saltwater fishes has been found to be affected by *Streptococcus* spp. The earliest finding of *Streptococcus faecalis* infection in rainbow trout (*Salmo gairdneri*) was described by BAKER & HAGEN (1942). Later, HOSHINA *et al.*, (1958) also found the same infection and further proven that *Streptococcus faecalis* was the responsible agent of the infection in trout by the artificial inoculation. Recently, the streptococcus infection in fish received more attention from fish pathologists throughout the world. ROBINSON & MEYER (1966), PLUMB *et al.* (1974). WILKINSON *et al.* (1973) reported that non-hemolytic *Streptococcus* of the Lancefield group B was the causative agent of the epizootics in golden shiners (*Notemigonus crysoleucas*), menhaden (*Brevoortia patronus*). sea catfish (*Arius felis*), striped mullet (*Mugil cephalus*), pinfish (*Lagodon rhomboides*), Atlantic croaker (*Micropogon undulatus*), spot (*Leiostomus xanthurus*), Stringray (*Dasyatis* sp.) and silver trout (*Cynoscin nothus*). And the bacterium was found to be pathogenic to several fish hosts by the experimental inoculation, including bluegills (*Lepomis macrochirus*), green sunfish (*Lepomis cyanellus*) and American toads (*Bufo americanus*). Besides. BOOMKER *et al.* (1978) states the occurrence of group D *streptococcus* in rainbow trout. In Japan, hemolytic streptococcus similar to *S. faecalis and S. faecium* but not classified as Lancefield group D was associated with infection in eel (*Anguilla japonica*), yellowtail (*Seriola quinqueradiata*), striped beakperch (*Oplegnathus fasciatus*) and Japanese horse mackerel (*Trachurus japonica*) (KUSUDA *et al.*, 1978; KUSUDA *et al.*, 1976; KUSUDA & KOMATSU, 1978; KITAO, 1982; MIYAZAKI, 1982). In addition, another β-hemolytic *Steptocccccus* was isolated from ayu (*Plecoglossus altivelis*), amago (*Oncorhynchus rhodurus* var. *macrostomus*), tilapia (*Sarotherodon nilotica*), Japanese flounder (*Paralichthys olivaceus*) ane rainbow trout (OHNISHI & JO, 1981; KITAO *et al.*,1981; MIYAZAKI, 1962; MIYAZAKI *et al.*, 1984; UGAJIN, 1981; JO, 1982). Moveover, a β-hemolytic *Streptococcns* different from the above was isolated from cultured yellowtail and was identified as *S. equisimilis*

(MINAMI *et al.*, 1979). However, in Taiwan, streptococcal infections in cultured fish have not received adequate investigation, it is well known that streptococcal infection of bullfrog is one of the most widely spread and frequently encountered bacterial disease in cultured bullfrog (LIU, 1982; CHUNG, 1982). It has been suggested that tilapia is probably the most susceptible freshwater fish in the cultured system here, although some other fish species submitted to our Fish Disease Diagnostic Laboratory were found also to be infected with streptococci.

The drug sensitity test of our isolate is quite different to those streptococci reported from other investigators that the isolate was resistant to penicillin and synthetic penicillin such as ampicillin and methicillin, but was susceptible to erythromycin and doxycycline tested. Therefore, 100 mg/Kg erythromycin and 70 mg/Kg boody weight of doxycycline mixtare was prescribed for treatment. The oral administration of the medicated food was given for 6 days and the epizootics were effectively under control.

The results from this study and others reveals that except *S. faecalis* and *S. equisimilis* isolated from some infected rain bow trout and yellowtail, most fish pathogenic streptococci isolated from various fish species are diffcult to classify intothe present category of the Lancefield serological groups. Thus further comparative studies of these streptococci from fish origin are necessary in order to identify the wide variety of fish streptococci into the suitable species and serogroup.

中 文 摘 要

1984年9月至10月間，在臺南曾文水庫之箱網養殖吳郭魚發生嚴重之鏈球菌感染，該病一個月之累計死亡率達50%及60%。

罹病魚外觀上呈眼球突出、角膜混濁變白及出血，體表變黑、軀幹或尾柱有結節狀隆起或膿瘍，剖檢病變有肝、脾最著腫大、心外膜炎、腹膜炎及腹水。眼、脾、肝、腎、腹水及肌肉膿瘍抹片有格蘭氏陽性球菌，組織病理學上，可見多發性壞死、肉芽腫瘤及細菌散佈於肝、脾、腎、心、眼、腦膜、胃腸漿膜及骨骼肌等組織。

病原菌在 Mac Conkey agar 上不發育，在含5%山羊或綿羊血液之培養基發育良好，且有β溶血，經生化學性狀鑑定爲 *Streptococcus* sp. 該菌對 Erythromycin 及 Doxycycline 具有高度感受性。有關本病之治療在本文中討論之。

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