農委會漁業特刊第二十四號、魚病研究專集(十)
COA Fisheries Series No. 24, Fish Disease Research (X), 40-45, 1990, 6

人工感染鏈球菌與氣單胞菌引致養殖吳郭魚之細菌性敗血症

The Experimental Infection of *Streptococcus* and/or *Aeromonas* to Induce Bacterial Septicemia in Cultured Tilapia

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摘　　要

以從養殖吳郭魚爆發細菌性敗血症之罹病魚分離菌株 *Streptococcus* sp. 及 *Aeromonas hydrophila* 行不同徑路之人工接種試驗。其結果顯示兩種細菌單獨或混合細菌浴、皮膚割傷後菌浴及經口接種等感染方式，皆能使吳郭魚致病死亡；尤其以兩種混合接種組病情最劇，死亡率最高，皮膚割傷後菌浴組次之，其他單純菌浴或口服各組則需觀察至第5天或第6天始能於各肢器中測出 10^{4-5} cells/g 之菌量，並顯現出非特異性之細菌性敗血症病徵。病（死）魚外觀見有體表出血斑點、突眼、眼角膜混濁、肛門月中突等臨床症狀。病灶常可見肝、脾及腎等實質器官之壞死灶、腸炎、心包炎及化膿性腦膜腦炎等組織病理變化。本試驗證實自然感染病例所分離之鏈球菌與氣單胞菌係引致細菌性敗血症之病原。

Introduction

Bacterial septicemia has long been recognized as the principal problem in the cultured tilapia in Taiwan. (Tung *et al.*, 1985) On survey, a total of 27 outbreaks of bacterial septicemia in cultured tilapia were diagnosed by the Livestock Disease Control Center(s) during 1984 to 1986. (Huang, 1987) Of these, streptococcosis, aeromonad septicemia and edwardsiellosis were presented as 18, 8 and 1 in cases, respectively.

Attempt was made in this study by using various ways of artificial inoculation to prove if *Streptococcus* spp. and *Aeromonas hydrophila*, isolated from the disease outbreaks, were pathogenic to the tilapia. Lesions and distribution and concentration of the inoculated bacteria in the viseral organs and blood were also measured in order to obtain the pathogenesis of the disease.

Material and Method

All the fish were sanitized in 10 ppm of Nitrofuran for 24 hours. Subsequently,

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some fish were ramdonly selected for rapid plate agglutination test by Gibbs' method (Gibbs, 1969) to confirm that the tested fish were not infected with the strains of *Strep.* and *A. hydrophila* previously. The fish were held in separated aquaria under controlling the water temperature within 25-28°C. The fish were fed with commercial feed during the tested period.

The inocula, *Strep.* (PST-3) and *A. hydrophila* (PAH-2), isolated from the field cases were inoculated intraperitoneally into tilapia six times to improve their toxicity. The LD_{50} of *Strep.* and *A. hydrophila* were 1.4×10^6 cells/ml and 7.5×10^5 cells/ml, respectively. Both strains were cultured in BHI broth at 28°C for 24 hours before used. The experimental design of this study was listed as Table 1.

所藏書 水產試驗

Table 1. Inoculation and Sampling Schedule

Group	Subgroup	No. of Tilapia	Sampling
Strep. sp.	A-a	35(35)*	(1) Two tilapias (or tiltpias died during test) were sampled for bacterial and pathological studies at 1/2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 17 days postinoculation. (2) Ten-folding dilution of blood and emulsified tissues as kidney, liver, spleen, G-I, and gill were cultured in blood agar and MacConkey agar for counting No. of bacteria (CFU/g).
	A-b	35(35)	
	B	35(35)	
A. hydrophia	A-a	35(35)	
	A-b	35(35)	
	B	35(35)	
Strep+*A. hydrophila*	A-a	35(35)	

A-a: Fish immersed with 10^6 cells/ml of *Strep.* or 10^5 cells/ml of *A. hydrophila.*
A-b: Fish incised before immersed with the same concentration of bacteriae as A-a.
B: Fish orally inoculated (2 ml) with 10^6 cells/ml of *Strep.* or 10^5 cells/ml of *A. hydrophila.*
*: Number of control. The body weight of fish ranged from 200 to 300 g.

Result and Discussion

The results indicated that mortality occurred in groups of fish which orally inoculated or immersed with either *Strep.* sp. or *A. hydrophila,* or both. The mortality in the group of mixed bacteria was higher than the group of infected single bacteria. (Table 2) The gill and G-I tract were the first place to show the bacteria within 12 hours postinoculation in each group. The presence of mortality coincided with the concentration of bacteria in blood. Four days after inoculations, the fish started dying and the bacteria concentrations between 10^{3-4} cells/ml in the blood. (Table 3) This phenomenon pointed out a bacteremia status and a wide distribution of the pathogens in these inoculated fish. More than five days was required for showing non-specific but significant lesions of bacterial septicemia with the bacteria

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Table 2. The Number of Tilapias Died during the Experimentally Infected period

Days postinoculation	*Streptococcus* sp.			*A. hydrophila*			*Strep.* sp. and *A. hydrophila*
	Immersion	Incision before immersion	Oral inoculation	Immersion	Incision before immersion	Oral inoculation	Incision before immersion
½	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	2	0	2
4	0	1	0	0	5	0	3
5	2	3	2	2	3	0	6
6	3	3	2	3	2	1	6
7	2	2	1	3	1	2	1
8	1	1	0	2	0	2	0
9	1	1	1	1	0	1	0
10	0	0	0	0	0	2	0
14	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0

Table 3. The Concentrations of *Strep.* sp. and/or *A. hydrophila* in the Blood of Tilapias Experimentally Infected with 10^6 cells/ml of *Strep.* sp. and/or 10^5 cells/ml of *A. hydrophila*

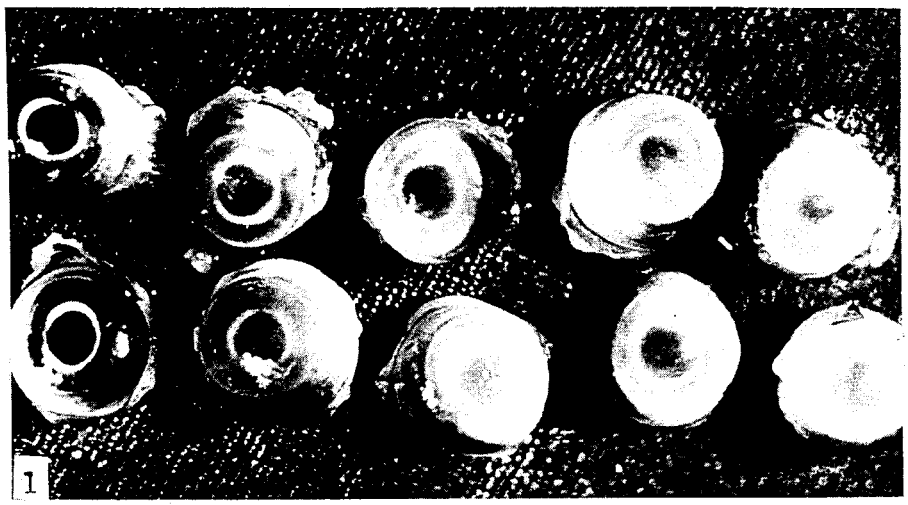
Days postinoculation	*Streptococcus* sp.			*A. hydrophila*			*Strep.* sp. and *A. hydrophila*
	Immersion	Incision before immersion	Oral inoculation	Immersion	Incision before immersion	Oral inoculation	Incision before immersion
½	—*	—	—	—	—	—	—/—
1	—	—	—	—	—	—	1.26/2.10#
2	—	2.12	—	—	—	—	2.00/3.43
3	2.12**	3.60	—	—	3.42	—	4.00/4.63
4	2.65	4.20	2.67	3.65	5.80	1.36	4.78/5.12
5	3.70	4.80	4.12	4.70	5.10	2.64	5.20/6.00
6	4.10	5.10	4.80	5.10	5.32	3.78	5.12/6.30
7	4.85	5.00	5.00	5.85	5.00	5.12	4.10/4.32
8	5.20	4.72	4.20	5.20	3.00	5.38	—
9	4.30	3.90	3.80	4.32	—	4.68	—
10	2.00	—	2.00	2.00	—	4.12	—
14	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—

* No Bacteria was isolated
** Presented in $\log_{10}$
\# Strep./Aeromonas

concentration up to 10^7 cells/g in the visceral organs.

No matter what inoculum or the route of inoculation, the typical signs and pathological lesions were similar. Initially, the affected fish appeared slow motion, dull and inappetence. The moribund and dead fish usually presented signs of exophthalmus with or without conjunctivitis and corneal opacity. (Fig. 1) Marked hyperemia and petechial hemorrhage were found on the body surface, base of fins and external site of the operculum. Redden anus and distended abdomen with ascite were also noticed. Marked external hemorrhage accompanied by erected scales was frequently seen in the tilapia inoculated with *A. hydrophila*. (Fig. 2) Internally the main features were peritonitis and pericarditis. (Fig. 3) Yellowish exudates rich in fibrin covered on the peritoneal and epicardial surfaces. The gall bladders were enlarged and engorged with excessive amount of bile. Most of the parenchymatous organs were usually hyperemic. The mucosa of G-I tracts was also redden. Histopathological examination revealed severe fibrinopurulent inflammation in the meninge of brain, pericardium and cornea. Large numbers of neutrophils and a few macrophages infiltrated in the leptomeninge. The cornea, conjunctivae and retina were also infiltrated by purulent exudate. The parenchymatous organs such as liver, spleen, testes and ovary were occasionally seen the lesion of focal necrosis. The submucosa of stomach was edematous and infiltrated with some inflammatory cells.

A survey on bacterial isolation from pond water and apparently healthy tilapia had been made by the authers recently. (Huang *et al.*, 1989) A total of 3,044 strains were isolated and grouped into 24 genera. Among them, *A. hydrophila* was one of the major isolates (21.8%) isolated from pond water and fish body. On the contrary, *Strep.* spp., the most popular cause of bacterial septicemia in tilapia, was only in small percentage of isolation rate (2.9%). This finding agrees with the popular point of view that *A. hydrophila* is one of the normal florae which might cause the disease problem in fish under stress circumstances. A number of freshwater and saltwater fish have been found to be affected by *Strep.* spp.. (Jo, 1982 and Miyazaki, 1982) In Taiwan, Tung suggested that tilapia was probably the most susceptible freshwater species to *Strep.* spp in our culture system. (Tung *et al.*, 1985) The strain of *Strep.* sp. used for this study and other pathogenic streptococci isolated from various fish species in our laboratory has not yet been classified into the suitable species and serogroup of the present category of classification.



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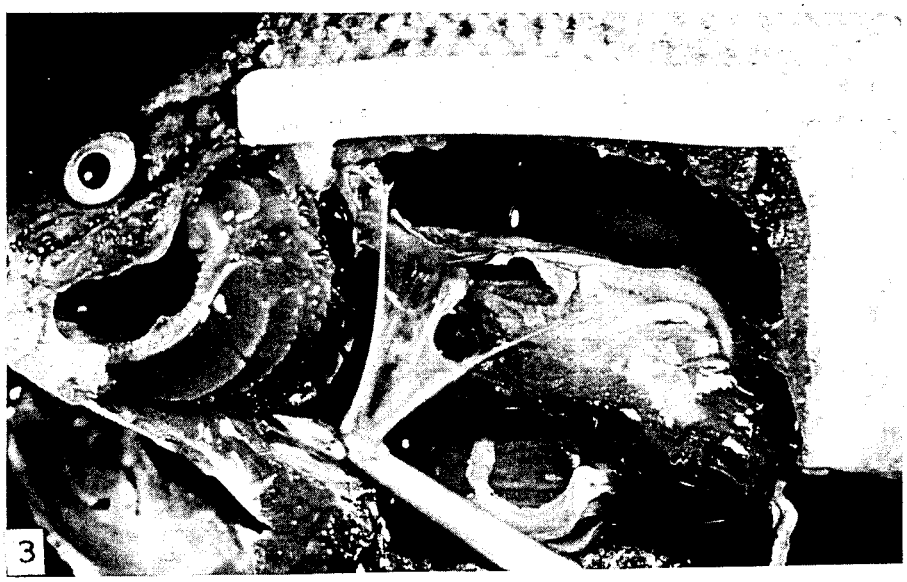

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Abstract

The pathogenesis of bacterial septicemia was studied in cultured tilapia experimentally infected with *Streptococcus* sp. and/or *Aeromonas hydrophila*, by means of immersion and oral inoculation. The results indicated that mortality occurred in groups of fish which orally inoculated or immersed with either *Streptococcus* sp. or *Aeromonas hydrophila*, or both. Mortality in the group of mixed bacteria was higher than the groups of single bacteria. More than five days was required for showing significant but non-specific lesions of septicemia with the bacteria concentration of 10^{4-5} cells/g in organs in groups of oral inoculation and single bacterial immersion. The moribund or dead fish showed typical lesions of petechia, exophthalmus and corneal opacity, redden anus and distended abdomen as found in field cases. Microscopically, focal necrosis was usually noted in liver, spleen and kidney and commonly associated with enteritis, pericarditis and suppurative meningoencephalitis. The experimental data prove that *Streptococcus* spp. and *Aeromonas hydrophila* are the causes of bacterial septicemia of cultured tilapia.

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Explanation for Figures

Fig. 1. Varying degrees of exophthalmia and corneal opacity of tilapias experimentally infected with *Strep.* sp or *A. hydrophila*. The left-hand eyes are clear and transparent as the normal control

Fig. 2. Marked external hemorrhage accompanied by erected scales in a tilapia immersed with 10^5 cells/g of *A. hydrophila* after 7 days.

Fig. 3. Tilapia orally inoculated with 2 ml, 10^6 cells/g of *Strep.* sp. 6 days P. I.. Showing marked fibrinopurulent exudates accumulation on the liver surface as the evidence of peritonitis.