

## MICROBIOLOGICAL STUDIES ON CHARACTERISTICS OF *STREPTOCOCCUS INIAE* ISOLATED FROM DISEASED *TILAPIA NILOTICA* AND AQUATIC ENVIRONMENTS

J. A. RADWAN,

Bacteriology, Mycology and Immunology Department

Fac. Vet., Med., Cairo Univ., Beni-Suef

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### SUMMARY

Seventy-eight isolates of *Streptococcus iniae* were recovered from 200 diseased brackish water *Oreochromis niloticus* (Nile tilapia) fish with an incidence of 86.7% of the total number of isolated *Streptococcus* species. To investigate the effect of nitrite, alkaline pH, salinity and water temperature on the susceptibility of *Oreochromis niloticus* (Nile tilapia) to streptococcosis; ten water samples were collected and the results obtained were as follows: nitrite concentration was 3.2 ug/L water, pH was 8.2, salinity was 18 ppt (part per thousand) and water temperature was  $26 \pm 2^\circ\text{C}$ . environmentally stressed and experimentally infected fish showed mortality rate of 73.3% compared with a mortality rate of 46.6% in non-environmentally stressed fish. In the meantime, LD<sub>50</sub> values for *S. iniae* were  $1 \times 10^{5.1}$  cfu/ml for environmentally stressed fish compared with  $1 \times 10^{5.5}$  cfu/ml for non-stressed tilapia.

### INTRODUCTION

There are many reports on streptococcosis in marine, brackish and fresh water fishes. The first outbreak of streptococcosis was recorded by Hoshina et al. (1958) and the first report on the isolation of *Streptococcus iniae* (*S. iniae*) was recorded by Peir and Madin (1976) from fresh water dolphin. In the mean time, Kitao et al., (1981) isolated *S. iniae* for the first time from tilapia. Chang and Plumb (1996) found that exophthalmia, ocular opacity and ocular haemorrhage were the main signs of *S. iniae* in tilapia fish. Histopathological examination revealed the appearance of meningitis, polyserositis of heart, liver, spleen, ovaries and kidneys. Teska and Shotts (1994) isolated non-haemolytic group B *Streptococcus iniae* from aquatic animals and humans, which were phenotypically very similar and can cause mortality in catfish. Isolates consistently had a high affinity for brain tissues, with the recovery of bacteria

from moribund or dead fish being higher from brain tissues than kidneys (Sakata and Kawazu, 1990). *S. iniae* is a pathogen in fish capable of causing invasive disease and outbreaks in aquaculture farms and there was a cluster of four cases of invasive *S. iniae* infection in humans who had recently handled fresh, whole fish from such farms (Weinstein et. al., 1997).

#### The objective of this work was to:

- 1- Identify the most common *Streptococcus* species isolated from diseased tilapia fish.
- 2- Detect the effect of some environmental stress factors on the recovery rates of the isolates in naturally infected fish.
- 3- Investigate the effect of some factors on the pathogenicity of the most prevalent isolates on experimentally challenged tilapia fish.

## MATERIAL AND METHODS

### **Samples:**

Two hundreds diseased tilapia fish showing the signs of septicemia with twirling at the water surface, exophthalmia, petechiae on the inside of the opercula and congestion on the inside of the pectoral and caudal fins were collected from private fish farm at Fayoum Governorate during the period from January to November, 2001.

### Isolation and Identification:

Brain, kidney, liver and spleen samples were grown onto brain heart infusion agar (BHIA), blood agar and Todd-Hewitt agar plates. (Kusuda et al., 1991) and all inoculated plates were incubated at 25°C for 48 hs.

The suspected colonies were identified according to Kitao et al. (1981) and Sakata and Kawazu (1990)

### Effect of some environmental stress factors on the recovery rate in naturally infected fish:

Nitrite concentration, salinity and pH were detected in the natural environment by using sera kits (D52518, Heinsberg, Germany). Water temperature was also determined during the periods of sample collection.

### Pathogenicity of *Streptococcus iniae* in both environmentally stressed and non-stressed tilapia fish:

The experiment was carried out on a total of 45 apparently healthy tilapia fish with an average weight of 70.0 ± 10 grams.

The fish were divided into three equal groups in a separate glass aquaria (120 x 60 x 40 cm). The first group was infected with *S. iniae* in a dose of 0.5 ml of  $1 \times 10^7$  cfu/ml intraperitoneally (Perera

et. al. 1997).

To investigate the effect of high nitrite concentration, alkaline pH and salinity; potassium nitrite was dissolved in aquarium water at a concentration of 3.2 ug/L, also, water pH was adjusted to be 8.2 and sodium chloride was added to a final concentration of 18 ppt (part per thousand).

Aquarium water temperature was adjusted at 20°C (Perera et al., 1997).

The second group was infected with 0.5 ml of  $1 \times 10^7$  cfu of *S. iniae* per fish intraperitoneally at a thermostatically controlled aquarium water temperature of 25°C and pH 7.2 without any additives.

The third group of fish was kept as a control group at water temp. of about 25°C and injected intraperitoneally with sterile physiological saline (0.5 ml/fish). Prior to inoculation fish were anaesthetized with Ms 222 (100 mg + 50 mg sod. bicarbonate) (Black and Connor, 1964).

All inoculated fish were observed twice daily up to 3 weeks post infection. Clinical signs and P.M. lesions were recorded and trials for reisolation of the organism were done.

**Determination of the median lethal dose (LD50) of *S. iniae* in environmentally stressed and non-stressed condition:**

Sixty tilapia fish with an average weight of 70.0 grams were divided into three groups. First and second groups were divided into 5 equal subgroups each of 5 fish. The third group contained 10 fish and kept as a control group.

#### **Preparation of bacterial culture:**

*S. iniae* was grown in trypticase soya broth for 24 hrs at 25°C ten fold serial dilutions were done from the original broth; 1:10, 1:100, 1:1000, 1:10000 and 1:100000 using sterile saline.

#### **Bacterial inoculation (Lucky, 1977):**

Fish in the first group was injected intramuscularly with 0.2 ml of each dilution of *S. iniae* (original concentration containing  $1 \times 10^8$  cfu/ml) (Perera et. al., 1997) and kept in aquarium at water temp. of 25°C and pH 7.2.

Fish in the second group was injected intramuscularly with 0.2 ml of each dilution of *S. iniae* containing  $1 \times 10^8$  cfu/ml and kept in aquarium containing potassium nitrite at a concentration of 3.2 ug/L of water and sodium chloride at a concentration of 18ppt with pH 8.2 and water temp. of 20°C.

The third group was kept as control at 25°C and injected with sterile saline. Fish were kept under observation and the mortalities among all groups were recorded and LD50 was calculated according to Reed and Muench (1938).



## RESULTS AND DISCUSSION

The frequency of isolation of Gram positive and catalase negative chain cocci in two hundred brackish water diseased tilapia fish was 90 isolates with an incidence of 45.0 %.

Out of these isolates, 78 strains (86.7%) were non-haemolytic onto blood agar, ferment glucose, sucrose and salicin, hydrolyses casein, starch and chitin, does not grow at 10°C and 45°C, 6.5 % NaCl, pH 9.6 or onto 40 % bile agar or in 0.1 % methylene blue milk, also, they were Voges-Proskauer negative. similar results were obtained by Kitae et al. (1981); Sakata and Kawazu (1990) and Bunch and Bejerano (1997). These isolates were belonged to *S. iniae*. To investigate the effect of high nitrite concentration, high pH, salinity and water temp. on the susceptibility of tilapia niloticus to streptococcosis, ten water samples were collected and the results obtained were as follow: nitrite concentration was 3.2 ug/L water, pH was 8.2, salinity was 18 ppt and water temperature was  $26(C + 2^{\circ}C)$ .

Results obtained by Bunch and Bejerano (1997) showed that low dissolved oxygen levels and high nitrite concentrations increased the mortalities in tilapia hybrids experimentally infected with *S. iniae* and suggested that *Streptococcus* species are opportunistic pathogens because they are wide spread in aquaculture environment and because of their dependence on stress to assert pathogenicity.

The results of pathogenicity of *S. iniae* to both environmentally stressed and non stressed tilapia fish (Table 1) showed that environmentally stressed and experimentally infected tilapia fish started death 24 hours post inoculation (4 fishes) with clinical signs in the form of twirling and spiral and erratic movement. The naked eye pathological lesions were in the form of haemorrhaging of the opercula and at the base of the fins. Internally, the gut was filled with viscous fluid with haemorrhagic kidneys containing pinheaded nodules.

The mortality ratio was 73.3% with reisolation of the microorganism in pure culture from the brain and kidneys.

Perera et al., (1997) recorded a greater rate of mortalities in artificially infected tilapia with *S. iniae* and maintained at 20°C than those kept at 15, 25, 30 or 35°C; and in water at pH 9.0 compared with those maintained in highly acidic water.

As regards to non environmentally stressed tilapia that experimentally inoculated with *S. iniae*, the infected fish started its death at 48hs post inoculation (two fish) with clinical signs in the form of sluggish movement and loss of reflex. Congestion at the site of inoculation with darkness of the skin and exophthalmia were noticed. The intestine was swollen, containing yellow fluid with swollen kidneys containing white small nodules.

**Table (1):** Pathogenicity of *Streptococcus iniae* to non-environmentally stressed and environmentally stressed tilapia fish:

Group of fish	No. of fish	Rate of death among inoculated fish after								No. of Dead fish	No. of survived fish	No. of dead No. of infected	Mortality percent
		24hs	36hs	48hs	72hs	96hs	1w	2w	3w				
-First group (non-environmentally stressed fish)	15	-	-	2	1	2	2	-	-	7	8	7/15	46.6
- Second group (environmentally stressed fish)	15	4	-	3	-	2	1	1	-	11	4	11/15	73.3
- Third group (control)	15	-	-	-	-	-	-	-	-	0	15	0/15	0.0

**Table (2):** Determination of the median lethal dose (LD<sub>50</sub>) of *Streptococcus iniae* in environmentally stressed tilapia fish:

Subgroup	No. of infected fish	Bacterial dilution	No. Dead	No. of survived	Survived			
					Dead	Survived	Mortality Values	Death percent
1	5	10-1	5	-	12	0	12/12	100
2	5	10-2	4	1	7	1	7/8	87.5
3	5	10-3	3	2	3	4	3/7	42.85
4	5	10-4	1	4	1	8	1/9	11.11
5	5	10-5	-	5	0	13	0/13	0

Original concentration:  $1 \times 10^8$  cfu/ml

Table (3):Determination of the median lethal dose (LD<sub>50</sub>) of *Streptococcus iniae* in environmentally stressed tilapia fish:

Subgroup	No. of infected fish	Bacterial dilution	No. Dead	No. of survived	Survived			
					Dead	Survived	Mortality Values	Death percent
1	5	10 <sup>-1</sup>	5	0	10	0	10/10	100.0
2	5	10 <sup>-2</sup>	4	1	5	2	5/7	71.42
3	5	10 <sup>-3</sup>	4	1	2	6	2/8	25.0
4	5	10 <sup>-4</sup>	2	3	1	10	1/11	9.09
5	5	10 <sup>-5</sup>	-	5	0	15	0/15	0.0

Original concentration: 1 x 10<sup>8</sup> cfu/ml

The mortality rate was 46.6 % with reisolation of the microorganism in pure culture. Similar results were obtained by Teska and Shotts (1994) and Chang and Plumb (1996).

LD50 value for *S. iniae* in environmentally stressed tilapia fish was 1 x 10<sup>5.1</sup> cfu/ml and for non environmentally stressed fish, it was 1x10<sup>5.5</sup> cfu/ml. Similar results were obtained by Perera et al., (1997) and Eldar et al., (1995).

## REFERENCES

- Black, E. C. and Conner, R. (1964): Effect of MS 222 as anaesthesia in Rainbow trout. Fish Res. Bd., 21 (6), 1539-1543.
- Bunch, E. C. and Bejerano, I. (1997): The effect of environmental factors on the susceptibility of hybrid tilapia to streptococcosis. J. Aquacul., 49 (2), 67-76.
- Chang, P. H. and Plumb, J. A. (1996): Histopathology of experimental *Streptococcus* spp. Infection in tilapia and channel catfish. J. Fish Dis., 19 (3), 235-241.
- Eldar, A.; Frelie, P. F.; Assenta, L.; Varner, P. W.; Lawhon, S. and Bercovier, H. (1995): *Streptococcus Shiloi*, the name for an agent causing septicemic infection in fish, is a junior synonym of *Streptococcus iniae*. Int. J. Syst. Bacteriol., 45 (4), 840-842.
- Hoshina, T.; Sano, T. and Morimoto, Y. (1958): A *Streptococcus* pathogenic to fish. J. Tokyo Univ. Fisheries., 44, 57-68.
- Kitao, T.; Aoki, T. and Sakoh, R. (1981): Epizootic caused by beta-haemolytic *Streptococcus* species in cultured fresh water fish. Fish Pathol., 15, 301-307.
- Kusuda, R.; Kawai, K.; Salati, F. and Fryer, J. L. (1991): *Enterococcus seriolicida* a fish pathogen. Int. J. Syst. Bacteriol., 41, 406-409.
- Luucky, S. (1977): Methods for the diagnosis of fish disease. Amerind publishing Co. Ltd., New Delhi, Bombay, Cal-

culta and New York.

per, G. B. and Madin, S. H. (1976): *Streptococcus iniae*, a beta hemolytic *Streptococcus* isolated from an Amazon fresh water dolphin. *Int. J. Syst. Bacteriol.*, 26, 545-553.

Perera, R. P.; Johnson, S. K. and Lewis, D. H. (1997): Epidemiological aspects of *Streptococcus iniae* affecting tilapia in Texas. *Aquacul.*, 152 (1/4), 25-33.

Reed, L. V. and Muench, H. (1938): A simple method of estimating fifty percent end points. *Amer. J. Hyg.*, 27, 493-497.

Sakata, T and Kawazu, T. (1990): Isolation of *Streptococcus* from fish and aquatic environments. *Mem. Fac. Fish. Kagoshima Univ. Kagoshima-Suisangakubu-K-iyo.*, 39, 151-157.

Teska, J. D. and Shotts, E. B. (1994): Non-haemolytic group B *Streptococci* from humans, fish and frogs. *Biomed. Lett.*, 199 (50), 195-201.

Weinstein, M. R.; Litt, M.; Keresz, D. A.; Wyper, P.; Rose, D.; Coutter, M. Willey, B. M. and Low, D. E. (1997): Invasive infections due to a fish pathogen, *Streptococcus iniae*. *N. Engl. J. Med.*, 337 (9), 589-594.