DETERMINATION OF LD50 FOR *STREPTOCOCCUS AGALACTIAE* INFECTIONS IN RED TILAPIA AND GIFT

AMAL A. M. N. [1]***, M. NUR-NAZIFAH*** [1]***, A. SITI-ZAHRAH*** [1]***, M.Y. SABRI*** [2*]***, M. ZAMRI-SAAD*** [2]

1. *National Fish Health Research Centre, 11960 Batu Maung, Penang, Malaysia*
2. *Department of Veterinary Pathology & Microbiology*
Faculty of Veterinary Medicine, Universiti Putra Malaysia
43400 Serdang, Selangor, Malaysia

Abstract

Red tilapia and GIFT were used in this study to see their susceptibility to *Streptoccocus agalactiae* using five different concentrations of the bacteria (10^8, 10^7, 10^6, 10^5, and 10^4 cfu/ml). For each species of red tilapia and GIFT, a total of one hundred and ten pieces of healthy, 4-5 inch size of average weight 85-100 g were used. Prior to experiment, they were conditioned and screened for any bacterial pathogens at the Fisheries Research Institute (FRI), Department of Fisheries Malaysia, Batu Maung, Penang. The water source was from dechlorinated water filter storage tank and water parameters were recorded before the trial start. All groups were exposed to the different concentrations of *S. agalactiae* by intraperitoneal injection at the rate of 0.1 ml of the inoculums and observed for any signs of abnormalities due to infections. Throughout the experiment eye abnormalities, erratic swimming behavior and body lesions were observed in most of the experimental fish, and mortality were recorded daily. From the experiment, both tilapias were observed to be susceptible to *S. agalactiae* concentration of 3×10^6 cfu/ml causing death. However, the mortality of red tilapia was recorded within 24 h, while GIFT was recorder after 24 h injection. From the plotted graph using recorded data, the LD50 of red tilapia and GIFT were 3.0×10^{10} and 3.0×10^7 cfu/ml, respectively. The result thus, showed that GIFT was comparatively more susceptible to *S. agalactiae* than red tilapia as cumulative mortality was also high at the end of experiment. All survival fish were humanely killed at the end of the experiment for re-isolation of *S. agalactiae*.

INTRODUCTION

Tilapia (*Oreochromis niloticus*) farming is one of the most important aquaculture industries in Malaysia since it has potential economic value, of highly international demand (Azeli, 2007). However, several diseases have been identified in tilapia farming. Streptococcal disease is considered to be the most devastating disease because it can cause enormous kills of large size of fish and responsible for huge economic losses (Pasnik *et al.*, 2005). Hence, most Asian countries including Malaysia rank Streptococcosis as one of the most economically important disease.

Red tilapia (*Oreochromis niloticus*) in Malaysia was first introduced in the mid 1980's. By the year 2000, almost 18,300 mt were harvested accounting for 36% of fresh water aquaculture production, where 10% were from the floating net cages. Although tilapia was first considered hardy and resistant to disease, mortality of tilapia weighing 300-400 g was first observed and reported in 1997-1998 in floating net cages of Sungai Pahang. This problem was subsequently observed in cages of Kenyir and Pergau Lake (Siti-Zahrah *et al.*, 2004, Siti-Zahrah *et al.*, 2005). Laboratory test revealed the presence of Gram-positive *Streptococcus agalactiae* as the most common isolate cultured from infected organs.

Experimental study conducted by Ferguson *et al.* (1994), has shown *S. agalactiae* to be more aggressive than any other environmental bacteria, where mortality may reach 100%. At present, Malaysia has brought stocks of GIFT tilapia from Philippine by the World Fish Centre (WFC) and kept in Jitra, Kedah, Malaysia, Aquaculture Extension Centre (AEC). The GIFT (Genetic Improved Farmed Tilapia) project involved collaboration between World Fish Centre, previously known as ICLARM then, Institute of Aquaculture Research of Norway (AKVAFORSK), and three Philippine Institutions; the Freshwater Aquaculture Centre of Central Luzon State University, the Marine Science Institute of the University of the Philippines, and the Bureau of Fisheries and Aquatic Resources. Our aim in this study is to determine the susceptibility to each of red tilapia and GIFT to *S. agalactiae* infection.

MATERIALS AND METHODS

Fish

All the fish, red tilapia (*Oreochromis niloticus*) and GIFT were obtained from Aquaculture Extension Centre (AEC), Jitra, Kedah, Malaysia. They were then transferred and acclimatized to water conditioned in 3 tones fiberglass tanks at the National Fishes Health Research Centre (NaFisH), Fisheries Research Institute, Department of Fisheries Malaysia, Batu Maung, Penang, Malaysia. Prior to the experiment, all tanks were cleaned and disinfected. A week before the experiment, 5% of randomly selected fish were screened for any bacteria pathogen especially *S. agalactiae*. The fish were weighed and the water was aerated continuously throughout the study. The water source was from the dechlorinated water filter storage tank in FRI. Mean weight ranged between 85-100 ± 15g. Once they were found free from *S. agalactiae*, both types of two hundred and twenty tilapias were randomly stocked into twenty two 200-L tanks for each experiment. They were fed *ad-libitum* with a commercial feed.

Water Quality

The temperature, pH and dissolved oxygen were measured using YSI 556 (YSI, USA). The ammonia, sulfate and nitrites were determined using a DR 2800 Portable Spectrophotometer (Hach, USA). The water quality parameters were 4.97 ± 0.3 mg L^{-1} for dissolved oxygen, 29.6 ± 0.8°C for temperature, 7.47 ± 0.1 for pH, 0.01 mg L^{-1} for ammonia. These were recorded once, before the experiment.

Bacteria, bacterial culture and inoculums

S. agalactiae isolated from the outbreak cases was obtained from National Fish Health Research Centre (NaFisH), Batu Maung, Penang. Bacteria was cultured on the blood agar (Oxoid, UK) and further sub-cultured into the brain heart infusion broth (Oxoid, UK) in 30°C in incubator shaker for 18 hours. Following incubation the stock bacterial concentrations were determined by ten folds dilution of the Standard Plate Count using 'hotkey stick' technique. The final concentration of live *S. agalactiae* was calculated to be 3.0×10^9 cfu/ml. The inocula were used immediately.

LD_{50} challenge and sampling

This study was designed for six days after the inoculation. Experiment was divided into three groups with subgroup of five different concentrations (10^8, 10^7, 10^6, 10^5, and 10^4) with duplicate. Group A - red tilapia, Group B - GIFT, and Group C (non-infected control without duplicate). All groups of fish were exposed by intraperitoneal injection with 0.1 ml of live *S. agalactiae* according to their concentrations as described above. Mortality was recorded every 12 hours daily for the remaining days. All the dead fish were subjected for bacterial isolation. Samples of eyes, brain and kidney were collected for this purpose.

Statistical analysis

Differences in the mean mortality rate, dilution, day and group were analyzed using a one-way ANOVA by LSD all-pairwise comparisons tests. All data were analyzed using Statistix ver. 9 (Analytical software, USA) and tested at 5% level of significance.

RESULTS AND DISCUSSION

All infected fish in both red tilapia and GIFT showed multiple types of clinical signs either erratic, spiraling swimming, or some with curved body posture, or sometimes with haemorrhagic eyes, while some fish may also developed corneal eyes opacity or worst blinded eyes. In GIFT especially, clinical signs like decayed caudal fin, or some developed white spots ranging from 0.5 cm to 2 cm on the body surface were observed, or appearance of black pigmented body, swollen abdomen, and there were those that isolated themselves, which may finally died. The observations described above were similarly reported in experimental study conducted by Ferguson et al. (1994), which has shown that *S. agalactiae* to be more aggressive than any other environmental bacteria, where mortality may reach 100%.

From table 1 graph for LD_{50} was plotted accordingly. In the case of LD_{50}, in the red tilapia group ,LD_{50} was calculated to be 3.0×10^{10} and for the GIFT group, LD_{50} was much lower at 3.0×10^{7} cfu/ml. Though the LD_{50} of the red tilapia is higher, the results showed that within twelve hours post-injection of live *S. agalactiae*, mortality started earlier in red tilapia compared to GIFT, which shows mortality after 24 h exposure. However, cumulative mortality of fish was much higher in GIFT compared to red tilapia after the end of experiment, even though the lowest concentration causing mortality was similar (3×10^{6}).

Throughout the study, we observed that both tilapias were much more susceptible to bacterial concentration of 3×10^{8} cfu/ml (Fig 2 and 4). In addition to that both tilapia (red and GIFT) were susceptible due to mortality occurred for every single *S. agalactiae* concentration (Fig 3 and 4). As a conclusion to our study, we found that GIFT is highly susceptible due to high cumulative mortality occurred compared to red tilapia. However, there was no significant difference in mortality ($p>0.05$) between group, red tilapia and GIFT. There was only significantly difference in mortality ($p<0.05$) between day 2 to day 0 and day 5, but not in other days. It also indicated significant differences ($p<0.05$) between dilution of 10^{8} to dilution of 10^{6} and 10^{4} and control since high mortality occurred in both type of fish.

SUMMARY

Thus, throughout this study it was revealed that the total cumulative mortality in GIFT was higher compared to red tilapia for every single concentration used (Table 1). The LD50 of 3.0×10^{7} in GIFT compared to LD50 of red tilapia (3.0×10^{10}) is much lower by IP injection, thus, GIFT is easily susceptible to *S. agalactiae* causing higher mortality compared to red tilapia as indicated in this study.

Table 1. Total cumulative mortality recorded for red tilapia and GIFT

Dilution	Total of mortality		% of Mortality	
	Red Tilapia	GIFT	Red Tilapia	GIFT
10^{8}	9	12	45%	60%
10^{7}	5	9	25%	45%
10^{6}	3	6	15%	30%
10^{5}	7	7	35%	35%
10^{4}	2	4	10%	20%

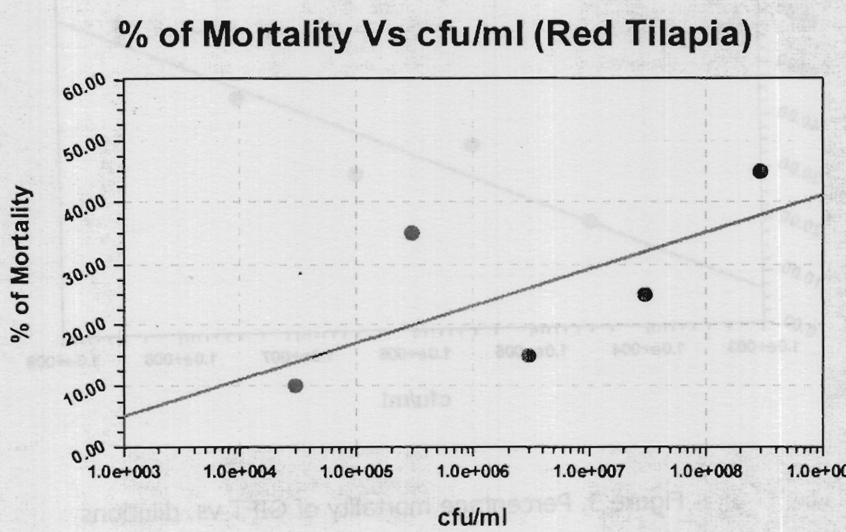

Figure 1. Percentage mortality of red tilapia vs. dilutions

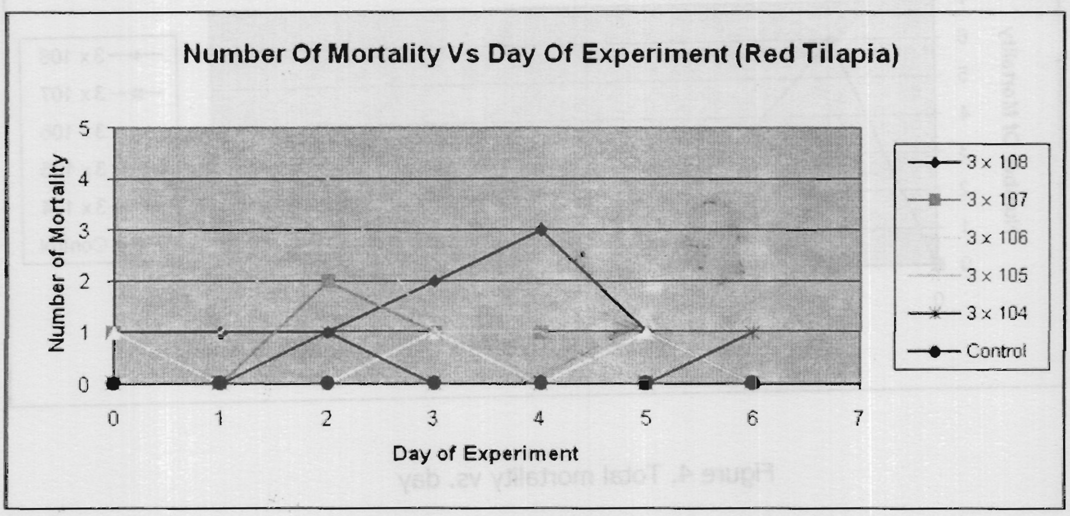

Figure 2. Total mortality vs. day

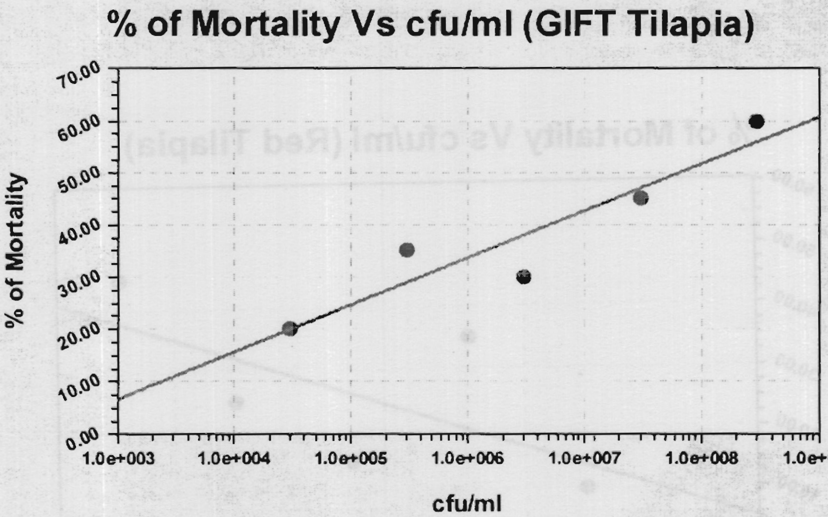

Figure 3. Percentage mortality of GIFT vs. dilutions

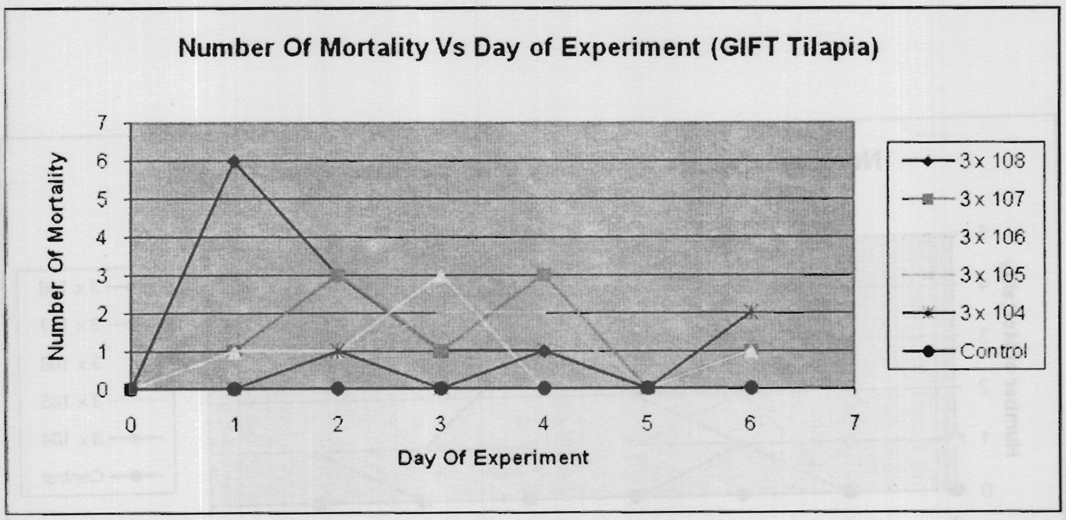

Figure 4. Total mortality vs. day

REFERENCES

1. Azeli, A. Potensi besar ternakan tilapia. KOSMO: 19th July 2007, p9.
2. Ferguson, H. W., Morales, J. A. and V. E. Ostland. 1994. Streptococcosis in aquarium fish. Diseases of Aquatic Organisms 19: 1–6.
3. Pasnik, D. J., J. J. Evans, P. H. Klesius, R. A. Shelby and C. A. Shoemaker. 2005. Antigenicity of *Streptococcus agalactiae* extracellular product and vaccine efficacy. Journal of Clinical Microbiology 35: 2573-2579.
4. Siti-Zahrah, A., S. Misri, B. Padilah, R. Zulkafli, B. C. Kua, A. Azila and R. Rimatulhana. 2004. Pre-disposing factors associated with outbreak of Streptococcal infection in floating cage-cultured red tilapia in reservoirs. Abstracts of the 7th Asian Fisheries Forum 04, The Triennial Meeting of The Asian Fisheries Society 30th Nov-4th Dec 2004, Penang, Malaysia. pp129.
5. Siti-Zahrah, A., B. Padilah, A. Azila, R. Rimatulhana and H. Shahidan. 2005. Multiple Streptococcal species infection in cage-cultured red tilapia, but showing similar clinical signs. Proceedings of the Sixth Symposium on Diseases in Asian Aquaculture, 25-28th Oct 2005, Colombo, Sri Lanka. Editors: Melba G. Bondad-Reantaso, C.V. Mohan, Margaret Crumlish, & Rohana P. Subasinghe. pp332-339