Multiple Streptococcal Species Infection in Cage-cultured Red Tilapia But Showing Similar Clinical Signs

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ABSTRACT

From year 2000, periodic mortality outbreaks of cage-cultured red tilapia in the reservoirs of Tasik Kenyir, Terengganu and Tasik Pergau, Kelantan were recorded. These incidents were associated with the onset of the dry season from March until June. Bacterial isolation of the sampled organs especially the eyes, brain and kidney conducted during the sampling study from September 2002 until December 2003, revealed small pinpoint or minute transparent colonies on Blood Agar. The colonies were characteristically presumptive of *Streptococcal* spp., Gram positive cocci, confirmed by API 20 STREP. *Streptococcus agalactiae* made up 70% of the total streptococcal species identified. The remaining 30% were other species of Streptococcus that included *Leuconostoc* spp. and *S. constellatus.* Although multiple streptococcal species infection was identified, clinical signs and gross morphological changes were similar. Typical signs observed included swimming in isolation prior to erratic swimming behavior and not feeding. Almost all of the sick tilapia indicated eye abnormalities such as corneal opacity or cloudiness; unilateral or bilateral exopthalmia or sunken eyes. Popeye was hemorrhagic at times and inflammation of the ventral region was more obvious in bigger tilapias. In Tasik Kenyir, streptococcal infection was more abundant in 150-250g compared with Tasik Pergau where the affected tilapias were between 300-450g.

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INTRODUCTION

Recent years have shown an increase in tilapia production in Malaysia (Table 1; Ann. Fisheries Stats., 2004). This appeared to be more pronounced after the introduction of red tilapia (*Oreochromis niloticus* hybrid) to Malaysia in the mid-1980s (Figure 1;http://agrolink.moa.my).

Table 1. Production of two main freshwater fish species culture in Malaysia from 1990-2004, including wholesale values.

Year	Species	Total of fish (ton)	Wholesale (RM)
1990	Red Tilapia	314.14	1,523,800
	Cat fish	133.33	1,110,000
1995	Red Tilapia	4827.03	26,646,350
	Cat fish	1752.26	8,195,940
2000	Red Tilapia	15636.02	76,782,370
	Cat fish	12115.68	48,001,910
2004	Red Tilapia	21458.88	67,990,380
	Catfish	20848.92	53,825,360

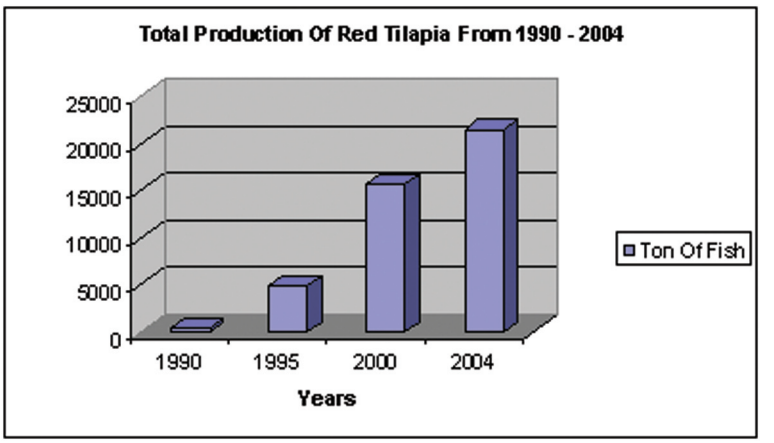

Figure 1. Total red tilapia production recorded from 1990-2004. (Source http://agrolink.mao.my).

The red tilapia is mostly cultured in ponds but now there is an increasing number of floating net-cages located in natural or man-made inland water bodies (Annual Fish. Stats. 2000). These include reservoirs or ex-mining pools. Cage-cultured red tilapia is also being produced along the river banks in Sungai Pahang, Temerloh, Pahang and Sungai Terengganu, Terengganu (Siti-Zahrah *et al.,* 2004. With the rapid growth in the Malaysian red tilapia industry, intensification of culture systems has resulted. It is anticipated that with this intensification there may be an increase in the incidence and severity of microbial

pathogens. Even though tilapia has been otherwise reported to be hardy (Roberts and Sommerville, 1982), tilapia continue to succumb to bacterial pathogens after a period of culture in cages.

The increased susceptibility was reported in 1997 at Temerloh, Pahang in cage-cultured fish at Sg. Pahang (Siti-Zahrah *et al.,* 2004). The case study conducted revealed that a high mortality rate was closely related to increased water temperature of 30-32^{0}C during the dry season. Laboratory diagnosis showed only the presence of *Aeromonas hydrophila* from kidney of affected animals, thus it was suggested that the mortality was possibly due to `heat–stress related syndrome'. This syndrome affected tilapia of more than 300g causing erratic swimming behavior and/or eye abnormalities such as exophthalmia, (Figure 2).

Figure 2. Bilateral exophthalmia in affected tilapia

The clinical signs observed for the previous disease outbreaks were again later observed in red tilapia reared in floating cages at Tasik Kenyir, Terengganu and Tasik Pergau, Kelantan of east coast, Peninsular Malaysia. The high tilapia mortality or outbreak was initially reported in June 2000, which was subsequently reported later every year during the dry season.

Thus, a study was initiated to examine the etiology of the disease affecting red tilapia causing very high mortality to the farmers. It was also observed that the outbreak was highly prevalent during the months of March to June which was associated with the coming of the dry season (Siti-Zahrah *et. al.,* 2004).

MATERIALS AND METHODS

Study sites

Two sites where floating cage-culture operated were selected. Both sites were in the east coast of Peninsular Malaysia, *i.e.* Tasik Kenyir in Terengganu and Tasik Pergau in Kelantan. Four operators were involved in the study, three of which were from Tasik Kenyir, Terengganu.

Tilapia sampling

Healthy and sick tilapia were randomly sampled from each operator, the number and size of fish sampled was dependent on the number of cages belonging to each operator. However, 25-30 fish samples were taken from Risda Sdn Bhd., who had the highest number of cages (>200) while the smallest number, about 10-12 fish were from NTL Sdn. Bhd. who operated about 20 cages. Tilapia samples taken monthly were observed and recorded for any clinical signs of disease and morphological changes exhibited.

Water sampling

Samples of water were taken monthly from a few sampling points selected from both inside and outside the cages. Water quality parameters (e.g. temperature, pH, DO, nitrite, ammonia and nitrate) were taken using YSI probe (Model 556), recorded, and analyzed to see any relationship with the prevalence of the outbreak.

Bacterial diagnosis

From the clinical signs observed in the tilapia, bacterial isolation was conducted by taking samples from the eye, brain and kidney, of affected fish using Blood agar (BA Oxoid) or Brain Heart Infusion (BHI Oxoid) agar. Secondary subculture was done to obtain pure colonies prior to Gram staining and presumptive biochemical tests including API 20 STREP (Biomereux).

RESULTS AND DISCUSSION

Water quality parameters

Periodic outbreaks of streptococcal infection with sudden high mortality of tilapia, were observed during the onset of dry season which lasted from March until June. This was associated with periodical changes and fluctuations in water quality parameters. The parameters (Table 2) were mainly temperature (30.5 to 32^0C), dissolved oxygen (3.2 to 10.2ppm), pH (6.3 to 8.7), nitrite (0 to 0.02ppm), iron (0.0 to 1.13ppm) and ammonia levels (0.0 to 0.55ppm) as recorded in Tasik Kenyir. The water temperature was well above 30^0C throughout the affected months in Tasik Kenyir Terengganu, almost similar to Tasik Pergau in Kelantan. The persistent high temperature with associated fluctuating water parameters throughout the dry season as above was reported by Siti-Zahrah *et al,* (2004), causing high mortality rate in cage-cultured tilapia.

Table 2. Mean water quality parameters recorded in cultured cages at Tasik Kenyir, Terengganu, and the associated percentage mortality and clinical symptoms observed in the affected tilapia.

Water quality parameters	Temp. (^{0}C)	DO (ppm)	pH	Nitrite (ppm)	Ammonia (ppm)	Fe^{2+} (ppm)
	30-32	3.2-10.2	6.3- 8.7	0- 0.02	0.0 - 0.55	(0.0-1.13
% mortality	60-70% in most of the reported cases					
Clinical symptoms	Isolation of tilapia at either corners of the floating cage prior to erratic swimming behavior, eye abnormalities either bilateral or unilateral exophthalmia, corneal opacity or cloudiness, hemorrhagic or sunken eyes and in some cases, blindness, inflammations along the ventral region from lower mandible to anus, and sometime pustule-like swellings at basal of dorsal fins.					

Bacterial diagnosis

A total number of more than 1000 tilapias were sampled during the study from both the reservoirs. Almost all the primary isolates from the sampled organs (eye, brain and kidney) showed uniquely uniform pinpoint or minute transparent colonies in the Blood or BHI agar. Some of the pure colonies showed distinct hemolytic properties (β- hemolytic) in the Blood Agar, while some showed otherwise. The colonies were minute (0.5-1.0mm), transparent, round and convex entire and some were occasionally very tiny pinpoint colonies (< 1mm) that can hardly be seen by the naked eye, after 24-48 hrs. They were Gram positive cocci (sometime ovoid) in chains of either single or in pairs. The catalase test was negative, and the biochemical profile of the isolates confirmed them to be *Streptococcus* spp. (data not presented).

From the samples of tilapia examined, the average rate of bacterial infection was 39%. Where 29% of the isolates recovered from the affected fish were identified as *Streptococcus* spp. However, 70% of these were identified as *Streptococcus agalactiae* from the affected fish compared with any other *Streptococcus* spp. (*Leuconostoc sp., S. constellatus* and *Gemella hydrolysans)*.

The results from this study indicated that the affected tilapia displaying clinical signs of disease were mainly infected with *Streptococcus sp.*, the most common and dominant identified in this study was *S. agalactiae*. A similar finding was recently reported in Thailand and Indonesia (Yuasa *et al.,* 2005). These authors did not indicate periodic outbreaks which can be associated with dry seasons and fluctuating water quality changes (temperature, DO, etc. as reported by Siti-Zahrah *et al.* (2004). This was also true as reported by Bunch and Bejerano (1997) which indicated that certain water quality parameters affected tilapia susceptibility to streptococcal infection.

Furthermore in Kuwait Bay, which is a semi-enclosed embayment of the Arabian Gulf, *S. agalactiae,* caused massive kill to wild mullet and cultured sea bream (Gilbert *et al.,* 2002). Kitao (1993) also indicated that salmon, mullet, golden shiner, pinfish, sea trout and sturgeon were susceptible to streptococcal infection. *Streptococcus sp.* has also been isolated from a variety of ornamental fish including rainbow sharks, red-tailed black sharks, rosey barbs, danios, some cichlids including Venustus (*Nimbochromis ("Haplochromis*") *venustus*) and *Pelvicachromis sp*. and several species of tetras (Yanong and Francis-Floyd, 2002) demonstrating the global threat of *Streptococcus* spp. to both farmed and ornamental fish species.

The same typical clinical signs of streptococcal infections were observed in this study as reported by other authors (Evans *et. al.,* 2002,). Most striking or pronounced signs were isolation of tilapia at either corners of the floating cage, prior to erratic swimming behavior, with eye abnormalities either bilateral or unilateral exophthalmia, corneal opacity or cloudiness, hemorrhagic or sunken eyes and in some cases, blindness. Gross clinical signs of disease observed during this study were numerous and similar to previously reported Streptococcus infections in tilapia (Figure 3). All of these clinical signs support the morphological and behavioural changes observed in this study associated with streptococcus infections in tilapia.

A difference was found in the physical appearance of the fish depending on the size of the animals affected. As in this study the medium-sized tilapia, had a sunken body appearance, which was not observed in the larger fish. This appeared to be an additional clinical sign.

The study also showed that clear clinical signs associated with streptococcal infection prevailed more in tilapia of 150-250g in weight in Tasik Kenyir, whilst in Tasik Pergau, infected tilapia were of 300-450g in weight. The size or weight difference in affected tilapia at the respective sites of study was not fully understood but this may be due to the progression of the infection or route and duration of exposure to the pathogen.

The results of the study showed that, the high incidence of mortality in tilapia at both of the respective sites were due to a streptococcal infection. The dominant aetiological agent was identified as *S. agalactiae*, which has been reported to cause mortalities in other farmed tilapia species. The periodic disease outbreaks reported were also associated with the onset of the dry season. This resulted in increased and fluctuating water quality parameters, particularly water temperature which was 30-32^{0}C, during the dry season. Ferguson *et. al.* (1994), reported that populations of zebra danios and white cloud mountain minnows when exposed to high concentrations of *Streptococcus* spp. in the water experienced 100% mortality within 2-4 days of exposure. It is thus, important that streptococcal infections be quickly identified and managed to prevent major losses, as already experienced by operators in both reservoirs.

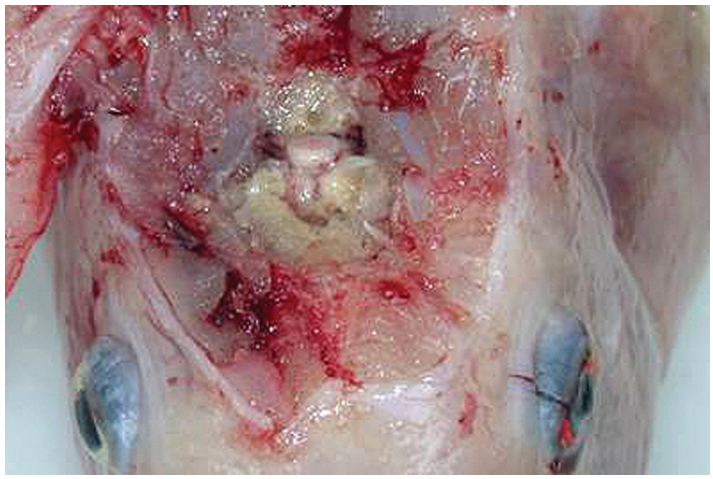

Figure 3. Haemorrhagic brain tissue observed grossly in affected tilapia

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