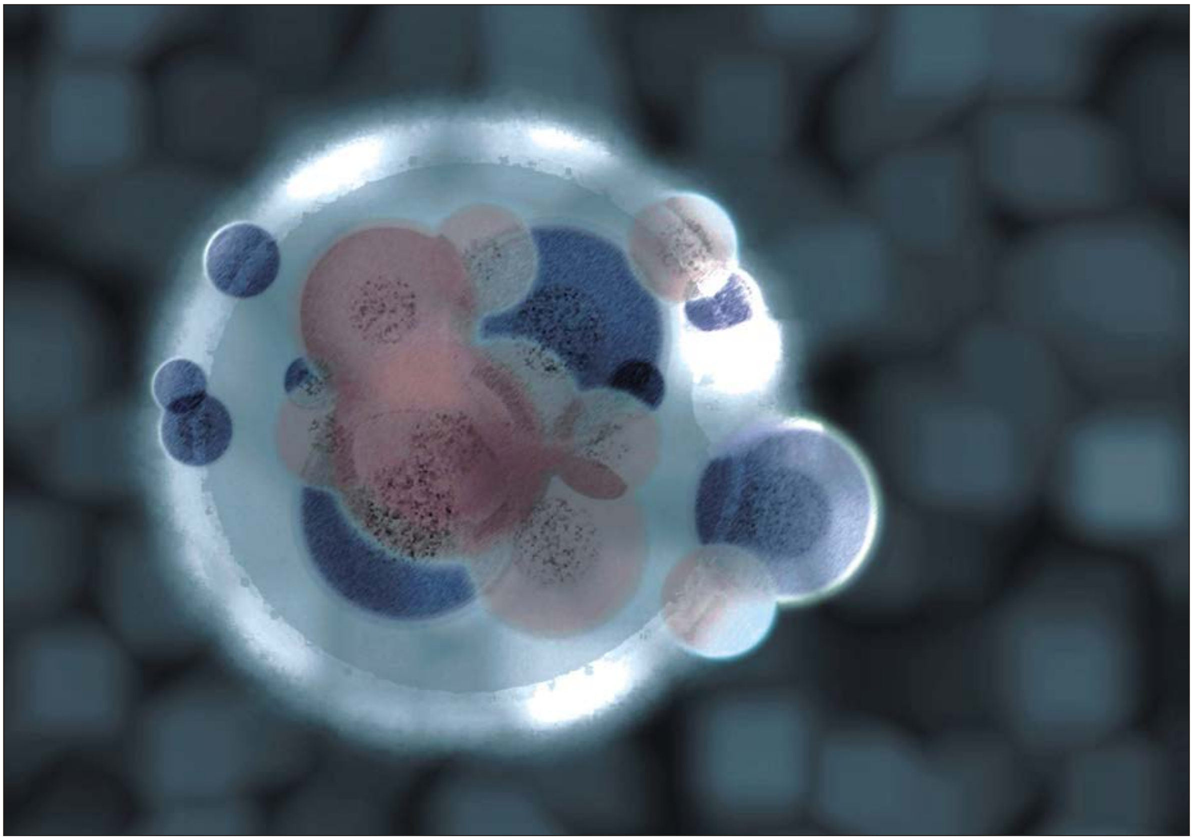

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Massive Mortality Associated with *Streptococcus agalactiae* Infection in Cage-cultured Red Hybrid Tilapia *Oreochromis niloticus* in Como River, Kenyir Lake, Malaysia

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Abstract: Massive mortality of cage-cultured red hybrid tilapia *Oreochromis niloticus* in Como River, Kenyir Lake, Malaysia was investigated. Moribund fish showing erratic swimming, sluggishness and hemorrhagic eyes were collected for the study. Water quality parameters were measured on-site. The highest temperature was 32.70°C at the surface while lower temperature was 30.85°C at 6 m. Dissolved Oxygen (DO) was highest 7.05 mg L^{-1} at 4 m while lowest 6.15 mg L^{-1} at 10 m. The pH value was highest 8.28 at 6 m and lowest 7.52 at surface. The result has shown that un-ionized ammonia levels ranged from 0.00046-0.01 ppm within the optimum range. While Nitrite (0.003 ppm) and Nitrate values (0.1-0.7 ppm). The water quality parameters were showed their values within the accepted range of water quality for aquaculture. Bacteria were isolated from diseased fish using blood and nutrient agars, and identified using BBL Crystal™ Enteric/Non-fermenter ID and Gram Positive ID Systems and subjected to antibiotic susceptibility analysis. Results showed the presence of *Streptococcus agalactiae, Burkholderia cepacia* in the fish liver and *Staphylococcus aureus* in the fish eyes, suggesting a possible case of warm-water streptococcosis associated with *S. agalactiae* with multiple bacterial complications. Nevertheless, a continuous long term monitoring is essential for the sustainable development of cage aquaculture in Como river of Lake Kenyir Malaysia.

Key words: Red hybrid tilapia, Como river, Kenyir Lake, *Streptococcus agalactiae*, water quality

INTRODUCTION

The main activities in lakes, rivers and reservoirs in Malaysia depend on fishing. Since last decade tilapia fish has shown rapid increase in production in Malaysian lakes and reservoirs (Table 1) (Siti-Zahrah *et al*., 2008). An extensive study has been conducted on diversity of fish communities in Malaysian lakes and reservoirs (Ambak and Jalal, 2006). According to their study, 13 families were identified as commercial and recreational fishes in tropical Lake Kenyir. Kenyir reservoir popularly known in Malaysia as 'Tasik or Lake Kenyir' is the largest man-made lake in South-East Asia. The lake is located in the state of Terengganu east coast of Peninsular Malaysia. Sungai Como which is locally known as 'River Como' in Lake Kenyir has been given priority at the state of Kuala Terengganu for fish culture activities since 2005. The Como river flowing into Kenyir Lake is a large scale Aquaculture Industrial Zone (AIZ). Local authorities of the state Kuala Terengganu have identified this river as permanent fish production park due to its potentiality in cage aquaculture activities (Department of Fisheries, 2005). It is projected that the cage culture production in 2012 will be 10,000 tons in Kuala Terengganu. As such the aquaculturists and fisheries scientists are expected that

Table 1: Production of main fresh water fish species Tilapia in Malaysia from 1990-2004

Year	Species	Total of Fish (ton)	Wholesale (RM)
1990	Red Tilapia	314.14	1,523,800
1995	Red Tilapia	4827.03	26,646,350
2000	Red Tilapia	15636.02	76,782,370
2004	Red Tilapia	21458.88	67,990,380

Department of Fisheries (2004), RM: Malaysian Ringgit

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80% of this production will be achieved from cage culture activities in Kenyir Lake (Department of Fisheries, 2012).

Recent survey has shown that an increase trend of cage fish culture activities have been operated in 6,000 fish cages growing mostly red hybrid tilapia *Oreochromis niloticus*. Red hybrid tilapia is the main species farmed in Malaysian freshwater cage-culture system with a production of 5 , 664.42 tonnes valued at RM 36.38 million in 2010 (Department of Fisheries, 2010). Due to this massive cage aquaculture activities around the river, the Como AIZ has recently encountered mass mortality of cage-cultured red hybrid tilapia with cumulative mortality approximately 20,556 fish in February 2012 (Department of Fisheries, 2012). In fact, these incidents were observed in the lake Kenyir and lake Pergau, Kelantan during dry season (March-June). It is evident that this mortality was occurred due the presence of pathogenic bacteria *Streptococcal* spp. which was isolated from the sampled organs especially the eyes, brain and kidney of infected fishes (Siti-Zahrah *et al*., 2008). It is predictable that severe microbial and fungal diseases could be resulted due to these intensifications of culture system. In fact Tilapia is known as hardy fish but still tilapia continues to affect by pathogens after a certain period of culture in cages (Roberts and Sommerville, 1982). Similar incidents also occurred in Pahang river cage cultured fishes (Siti-Zahrah *et al*., 2004). Based on above perspectives a study was initiated to investigate the possible causes of the massive mortality in these fish cages of Kenyir lake.

MATERIALS AND METHODS

Sampling area: Investigation was conducted at the farm located at N05°02’23.7” E102°50’36.7” at Como river, lake Kenyir, where cage culture of tilapia was operated at floating-cages.

Water quality parameters: Water quality parameters (water temperature, dissolved oxygen and pH) were measured on-site using Hydrolab (Quanta, Germany) at 0 to 6 m deep in the morning (10.00 a.m.) and afternoon (2.00 p.m.). Water transparency was measured using Secchi disc. Ammonia, nitrite and nitrate levels were measured using water quality test kit (Hach, USA). The water quality parameters were analyzed according to Boyd (1998). The un-ionized ammonia levels were calculated according to Emerson *et al.* (1975) based on the water temperature, pH and ammonia nitrogen measured.

Fish collection: Moribund fish (90-280 g) showing erratic swimming, sluggishness and hemorrhagic eyes were collected from the affected cages for bacteriological studies.

Bacterial isolation and identification: Liver and eyes were aseptically taken for bacterial isolation on blood and nutrient agars at 30°C incubation for 24 h. The bacterial colonies of different morphologies were sub cultured several times to obtain pure cultures. The bacteria were Gram-stained and subjected to oxidase and indole tests prior to identification using BBL Crystal™ Enteric/Non-fermenter ID System (for Gram negative bacteria) and BBL Crystal™ Gram Positive ID System (BBL Crystal, USA).

Antibiotic susceptibility tests: Antibiotic susceptibility tests were carried out on the isolates by disk-diffusion method (Bauer *et al*., 1966) against tetracycline (30 μg, TE30), ampicillin (10 μg, AMP10), nitrofurantoin (50 μg, F50), amoxicillin (25 μg, AML25), lincomycin (15 μg, MY15) and erythromycin (15 μg, E15) (Oxoid, England). After 24 h incubation, diameter of the inhibition zone was measured and interpreted as susceptible (S), intermediate (I), or resistant (R) (CLSI, 2006).

RESULTS AND DISCUSSION

The water temperature showed a slight decline with depth of water from 31.93°C (surface) to 30.85°C (6 m) at 10.00 a.m. and from 32.70°C (surface) to 31.03°C (6 m) at 2.00 p.m. The dissolved oxygen level displayed slight increase with depth from 6.15 mg L^{-1} (surface) to 6.74 mg L^{-1} at 10.00 a.m. and from 6.52 mg L^{-1} to 7.03 mg L^{-1} (6 m) at 2.00 p.m.. The water pH also slightly increased with depth from 7.52 (surface) to 7.86 (6 m) at 10.00 a.m. and from 7.91 (surface) to 8.28 (6 m) at 2.00 p.m. (Table 2). The water transparency was 3. 9 m at 10.00 a.m. and measured the same 3.9 m at 2.00 p.m. The ammonia-

Table 2: Water temperature, dissolved oxygen concentration and pH at different depths of water

	Water temperature (°C)		Dissolved oxygen (mg L^{-1})		pH	
Depth (m)	10: 00 a.m.	2:00 p.m.	10:00 a.m.	2:00 p.m.	10:00 a.m.	2:00 p.m.
0 (surface)	31.93	32.70	6.15	6.52	7.52	7.91
1	31.04	31.46	6.31	6.69	7.63	7.92
2	31.05	31.29	6.49	6.74	7.63	8.08
3	31.02	31.19	6.40	6.96	7.69	8.21
4	31.00	31.10	6.58	7.05	7.75	8.26
6	30.85	31.03	6.74	7.03	7.86	8.28

Air temperature at 10:00 a.m. was 27.21°C and at 2:00 p.m. was 29.71°C

Table 3: Water quality parameters within 1 meter deep

Water quality parameters	10:00 a.m.	2.00 p.m.	Permissible/optimum level (Boyd, 1998)
Transparency	3.9 m	3.9 m	30-40 cm
Ammonia nitrogen (NH_3-N) (ppm)	0.01	0.07	-
Un-ionized ammonia (NH_3) (ppm)	0.00046	0.01	0-0.1
Nitrite (ppm)	0.003	0.003	0-0.5
Nitrate (ppm)	0.1	0.7	0.1-3

Table 4: Result of antibiotic susceptibility test

	TE30	AMP10	F50	AML25	MY15	E15
Streptococcus agalactiae	S	R	S	R	I	I
Staphylococcus aureus	S	R	S	R	S	S
Burkholderia cepacia	S	R	S	R	R	S

S: Susceptible, I: Intermediate, R: Resistant, TE30: Tetracycline 30 μg, AMP10: Ampicillin 10 μg, F50: Nitrofurantoin 50 μg, AML25: Amoxicillin 25 μg, MY15: Lincomycin 15 μg, E15: Erythromycin 15 μg

nitrogen (NH_3-N) was 0.01 ppm at 10.00 AM but increased to 0.07 ppm at 2.00 p.m. Un-ionized ammonia (NH_3) was 0.00046 ppm at 10.00 AM and increased to 0.01 ppm at 2.00 p.m. The nitrite levels were 0.003 ppm both at 10.00 AM and 2.00 PM, whereas the nitrate level were 0.1 ppm at 10.00 AM and 0.7 ppm at 2.00 PM (Table 3). Table 3 also compares the existing chemical parameters with the optimum level of un-ionized ammonia, Nitrite and Nitrate (Boyd, 1998). The levels of un-ionized ammonia in the sampling area was ranged from 0.00046-0.01 ppm, Nitrite (0.003 ppm) and Nitrate values (0.1-0.7 ppm) were showed their values within the accepted range of water quality for aquaculture (Boyd, 1998).

Streptococcus agalactiae and *Burkholderia cepacia* were isolated from the fish liver whereas *Staphylococcus aureus* was isolated from the fish eye. Table 4 reveals that all the bacterial isolates were showed their resistance to ampicillin (AMP10: ampicillin 10 μg) and amoxicillin (AML25: amoxicillin 25 μg) while *B. cepacia* isolate was also shown resistant to lincomycin (MY15: lincomycin 15 μg). Table 4 also showed that Tetracycline (TE30: tetracycline 30 μg), nitrofurantoin (F50: nitrofurantoin 50 μg) and erythromycin (E15: erythromycin 15 μg) were effective against all the tested bacteria.

In the present study, Gram positive bacteria, *S. agalactiae* and *S. aureus* as well as Gram negative bacterium, *B. cepacia* were isolated from the diseased fish. *S. aureus* and *B. cepacia* are considered as opportunistic fish pathogens that cause mortalities in stressed fish, whereas *S. agalactiae* is considered as true pathogen capable of causing diseases in healthy fish with normal immune defenses. A previous epidemiological investigation of *S. agalactiae* infection in Nile tilapia farming in Brazil has suggested close relationship between dynamic change in water temperature and susceptibility to the disease as outbreaks usually occurred when the water temperature is above 26°C (Mian *et al*., 2009). A study by Rodkhum *et al*. (2011) also indicated the susceptibility of Nile tilapia to *S. agalactiae* via water transmission at high water temperature 30°C. Siti-Zahrah *et al*. (2008) has suggested that the occurrence of high mortality of red tilapia in floating cages at Kenyir Lake could be due to the presence of high water temperature, which was recorded to be 32°C, although other chemical parameters were within permissible limits. According to Siti-Zahrah *et al*. (2004), the high mortality of tilapia fishes in the lakes of Malaysia generally occurs during high temperature with associated fluctuating water parameters throughout the dry season. A similar finding was also reported by Yuasa *et al*. (2005). These authors did not discuss in details periodic outbreaks associated with season and water quality changes. In the present study, water transparency (3.9 m) largely deviated from 30-49 cm optimum range (Boyd, 1998) and has likely contributed to the high water temperatures (31.00-31.10°C) at 4 m deep where the fish were located due to high sunlight penetration, which in turn stressed the fish and increased the fish's susceptibility to streptococcosis as well as other bacterial infections. The fish farmers perhaps could consider placing the fish cages deeper in water to reduce exposure of fish to direct sunlight and high water temperature, provided the dissolved oxygen levels remain permissible. This observation was also agreed with the findings as reported by Bunch and Bejerano (1997) which indicated that certain water quality parameters affected tilapia to streptococcal pathogens.

Environmental parameters are important factors in aquatic habitat as it could influence the size and composition of any aquatic orgarisms. So, the study on diversity and distribution of the microbes with respect to physicochemical parameters is an essential need for the sustainable development of fisheries resources in any water body. As such this streptococcal infection also observed in mollusks and blood cockle in the coastal water Pahang (Jalal *et al*., 2009). Similarly, a massive mortality of wild mullet and cultured sea bream was observed in Kuwait Bay due to the presence of *S. agalactiae* (Gilbert *et al*., 2002).

The typical clinical signs of streptococcal infections on tilapia make isolated at either corners of floating cage, prior to erratic swimming behavior, with eye abnormalities either bilateral or unilateral exophthalmia, corneal opacity or cloudiness and hemorrhagic eyes as described by other authors (Evans *et al*., 2002; Siti-Zahrah *et al*., 2008).

Musa *et al*. (2009) has previously reported *S. agalactiae* from commercially farmed red hybrid tilapia in Malaysia to be resistant to spiramycin, oleandomycin, sulfamethoxazole, oxolinic acid, kanamycin and nalidixic acid. In comparison, the *S. agalactiae* isolate in the present study was found to be resistant to ampicillin and amoxicillin. In Malaysia, most of the fish farmers operate on a small scale basis with little technical support and apply treatment rather than prevention as antibiotics are largely available with little regulations on the use in aquaculture (Choo, 2000). This has probably contributed to the antibiotic susceptibility profiles of the bacteria isolated in this study. In the case of Streptococcosis, till now, there is still no effective commercial vaccine available that can be used to prevent Streptococcosis in tilapia.

Generally, aquaculture management strategies along with environmental factors depend on various types of fish culture techniques. Globally, most of the aquaculture activities have been conducted with high stocking densities per unit area (Teichert-Coddington and Green, 1997). *Streptococcus agalactiae* has been associated with numerous disease outbreaks in several fish species in both tropical and temperate countries (Baya *et al*., 1990; Plumb, 1994; Evans *et al*, 2002). It has been reported that intensive culture of susceptible fish species has resulted in severe losses due to streptococcal disease with reports of up to 50% in ponds (Eldar and Ghittino, 1999) and 75% mortality in closed culture systems (Perera *et al*., 1994; Stoffregen *et al*., 1996). However, the present bacteriological study provides important information in regards to fish health management particularly in terms of quarantine procedures which plays an important role in disease prevention and control. In the current practice, tilapia is only grown in Kenyir Lake with the fry sourced from various states in Malaysia, thus the risk of introducing various bacteria to the lake water body. This practice has been alerted to be a possible serious source of diseases (Sayuthi, 1993), and therefore proper quarantine measures should be imposed to minimize the introduction of pathogenic bacteria to the fish cultures in Kenyir Lake.

CONCLUSION

In conclusion, this study documented a possible case of warm-water streptococcosis associated with *S. agalactiae* with multiple bacterial complications in red hybrid tilapia in a Como River, Kenyir Lake. For a tropical country like Malaysia, fish's susceptibility to streptococcosis due to high water temperature condition is significantly important. Nevertheless, a long term research should be initiated for continuous monitoring of the environmental parameters along with the health of commercial and recreational fishes in Lake Kenyir which could boost economical returns and ecotourism as well.

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