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Review Article

Control and Prevention of Streptococcosis in Cultured Tilapia in Malaysia: A Review

Zamri-Saad, M.[1], Amal, M. N. A.[2]*, Siti-Zahrah, A.[3] and Zulkafli, A. R.[4]

[1]Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Malaysia
[2]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Malaysia
[3]National Fish Health Research Centre, Fisheries Research Institute, 11960 Batu Maung, Penang, Malaysia
[4]Freshwater Fisheries Research Center, Fisheries Research Institute, 71600 Jelebu, Negeri Sembilan, Malaysia

ABSTRACT

Streptococcosis in cultured fishes has been reported to cause severe economic losses to the aquaculture industry worldwide. Lancefield group B *Streptococcus agalactiae* has been recognised as the main pathogen in cultured tilapia. This review discusses the current scenario and risk factors of streptococcosis in tilapia and suggests the control and prevention measures for this disease. The preventive measures focus on combined aspects of selecting farm location, applying good aquaculture farm practices, utilization of antibiotics and proper vaccination programme. A combination of all these measures will perhaps be the key to improve the health of cultured tilapia and prevent the infection by *S. agalactiae*, which in turn will increase the economic profit of tilapia farm operators.

Keywords: Control, prevention, streptococcosis, tilapia, aquaculture, Malaysia

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E-mail addresses:
mzamri@upm.edu.my (Zamri-Saad, M.),
mnamal@upm.edu.my (Amal, M. N. A.),
siti.zahrah.abd@gmail.com (Siti-Zahrah, A.),
zulkaflirashid@gmail.com (Zulkafli, A. R.)
* Corresponding author

INTRODUCTION

Streptococcosis is an infection by Gram-positive bacteria of the genus *Streptococcus*. In cultured fish industry, infections by *Streptococcus* sp. have been reported to cause outbreaks leading to considerable morbidity and mortality worldwide. Klesius *et al*. (2008) estimated that worldwide annual losses due to streptococcosis alone

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were USD150 million in 2000 and exceeded USD250 million in 2008. Currently, *Streptococcus agalactiae*, *S. iniae* and *S. dysgalactiae* have been identified as the main pathogens that cause diseases, leading to severe economic losses in the aquaculture and fisheries industry throughout the world (Evans *et al.,* 2006; Amal & Zamri-Saad, 2011; Costa *et al.,* 2013).

Disease outbreaks following infections by *S. agalactiae* have been reported in various species of marine and freshwater fishes such as silver pomfret (*Pampus argenteus*), golden pompano (*Trachinotus blochii*), seabream (*Sparus auratus*), wild mullet (*Liza klunzingeri*), Nile tilapia (*Oreochromis niloticus*), red tilapia (*Oreochromis* sp.), ya-fish (*Schizothorax prenanti*), wild giant Queensland grouper (*Epinephelus lanceolatus*), estuary ray (*Dasyatis fluviorum*), mangrove whipray (*Himantura granulata*) and eastern shovelnose ray (*Aptychotrema rostrata*) (Evans *et al.,* 2002; Duremdez *et al.,* 2004; Suanyuk *et al.,* 2005, 2008; Hernandez *et al.,* 2009; Mian *et al.,* 2009; Geng *et al.,* 2011; Amal *et al.,* 2012; Azad *et al.,* 2012; Bowater *et al.,* 2012). Recently, streptococcal disease in cultured tilapia has become an emergence problem and is among the leading disease that causes severe economical impact worldwide. Therefore, *S. agalactiae* has been identified as one of the important tilapia pathogens among the streptococcal species that affects various species of fishes in the world.

In Malaysia, *Streptococcus* outbreak was first recorded in the late 1990s. The first outbreak of *S. agalactiae* in red hybrid tilapia (*Oreochromis* sp.) was observed in 1997 in Pahang river, Pahang. The disease affected tilapia weighing between 300 and 400g causing 60% mortality. Subsequently in 2000, outbreaks of *S. agalactiae* infection were reported in Kenyir Lake, Terengganu and Pergau Lake, Kelantan, killing approximately 50% of the cultured tilapia population. The outbreaks were observed between March and June of the year (Siti Zahrah *et al.,* 2004, 2005). Lately, reported cases of *S. agalactiae* infection, which included the wild and cultured tilapia, are widespread, covering almost all over Peninsular Malaysia (see Fig.1) (Amal, 2007, 2011; Najiah *et al.,* 2009; Nur-Nazifah *et al.,* 2009; Siti-Zahrah *et al.,* 2009; Zulkafli *et al.,* 2009; Amal *et al.,* 2010ab, 2013abc; Zamri-Saad *et al.,* 2010).

Affected tilapias showed either red discolouration of the skin, erratic swimming, whirling, corneal opacity, eye haemorrhage, cataract, exopthalmia, occasional sunken body or acute inflammation along the base of the pectoral and ventral regions, skin haemorrhages around the anus or at the base of anus, congested visceral organs (particularly liver, spleen and kidney), while the brain appeared soft and occasionally oedematous (Najiah *et al.,* 2009; Ali *et al.,* 2010; Zamri-Saad *et al.,* 2010).

Introduction of contaminated water and/or fry into the farm, high stocking density, poor husbandry management, deterioration of water qualities such as slow flowing water, high water temperature, high ammonia, low dissolved oxygen and unsuitable pH

and salinity in the culture system were reported to be the risk factors and stressors that increased the susceptibility of fish to streptococcal infection (Bunch & Bejerano, 1997; Bowser *et al.,* 1998; Shoemaker *et al.,* 2000; Nguyen *et al.,* 2002; Yanong & Floyd, 2002; Mian *et al.,* 2009; Amal, 2011; Amal *et al.,* 2013a; Milud *et al.,* 2013).

This report analyses and discusses the potential control and prevention measures for *S. agalactiae* infection in cultured tilapia (*Oreochromis* sp.), which can be used as a guide for the farmers and researchers based on previous reports and experiences of streptococcosis outbreaks in Malaysia.

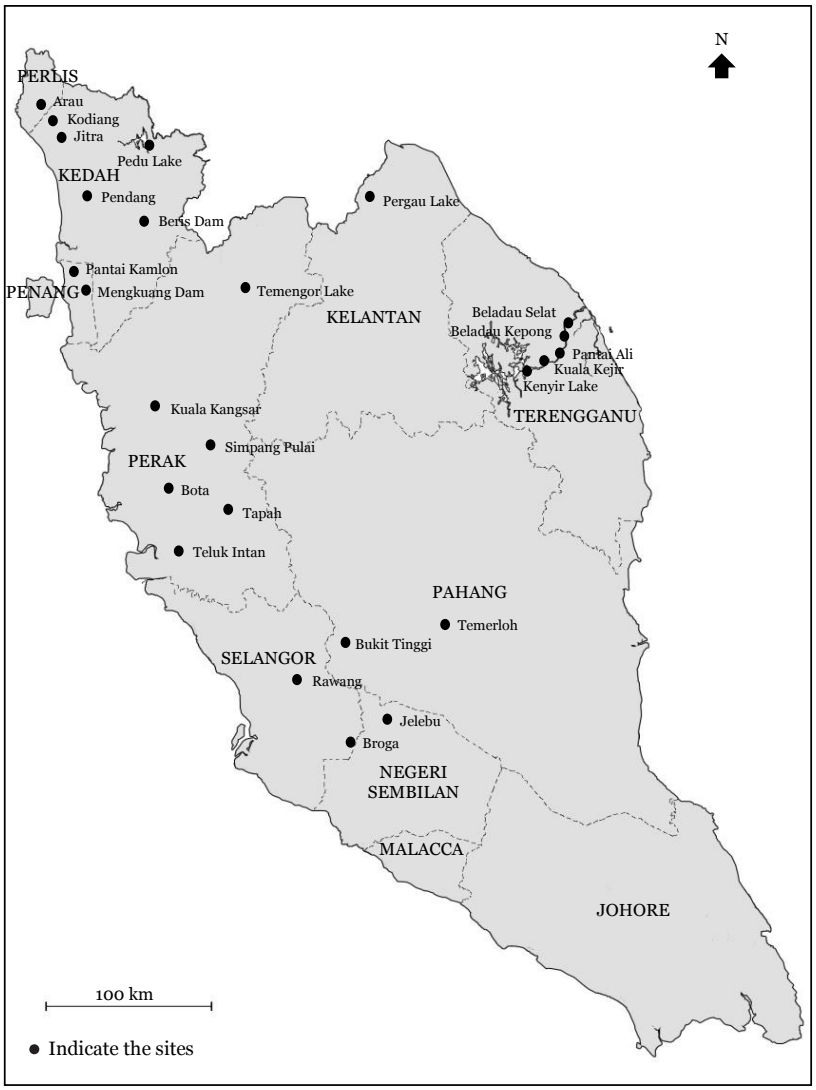

Fig.1: Sites with reported cases of *S. agalactiae* infection in wild and cultured tilapia in Peninsular Malaysia

POTENTIAL CONTROL AND PREVENTION MEASURES

Selection of Hatchery and Farm Sites

Siti-Zahrah *et al.* (2009) stated that cases of streptococcosis in tilapia were significantly higher in floating net cages kept in rivers, ponds and dams. *Streptococcus agalactiae* was also successfully isolated from the red tilapia cultured in commercial earthen pond farms (Najiah *et al.,* 2009; Nur-Nazifah *et al.,* 2009; Ali *et al.,* 2010). More recently, Amal (2011) reported that the mean prevalence of *S. agalactiae* infections in tilapia in Malaysia was significantly higher in floating net cage culture in huge-sized reservoirs (9.68±10.42%) and moderate-sized river (2.57±3.59%) compared to small-sized irrigation canal (0.28±0.9%), pond (0.69±2.77%) and ex-mining pool (0.17±0.82%). Moreover, infections by *S. agalactiae* have also been reported from various types of water bodies and culture methods such as raceway, hatchery, marine water floating net cage, freshwater floating net cage, shaded outdoor tank and open sea (Glibert *et al.,* 2002; Duremdez *et al.,* 2004; Mian *et al.,* 2009; Azad *et al.,* 2012). It seemed that certain types of water bodies and fish culture methods enhanced susceptibility of tilapia to *S. agalactiae* infections. However, no prohibition or preferable place was suggested as suitable culture site from previous studies.

Therefore, a good tilapia hatchery or farm should have electricity supply, proper roads, continuous water supply and water reservoirs, feed store, treatment or quarantine tanks, proper water aeration system, good water filters, etc. (Klesius *et al.,* 2008). Nevertheless, based on the observation of streptococcosis outbreaks in this country, farmers are advised not to choose huge size water body with extremely slow water flow rate such as big reservoirs or downstream huge rivers as site for tilapia culture due to retention of the heat at the upper water column during hot and dry seasons (Amal, 2011). Preferable culture sites for irrigation canal, raceway, river and sea should have flowing water, while for hatchery, indoor tank, shaded outdoor tank and earthen pond, the site should have continuous sources of water or water reservoir for partial or total water exchange in order to maintain a good water quality of their culture site (El-Sayed, 2006). Moreover, floating net cage culture site in rivers and reservoirs also must be equipped with excellent facilities mentioned above as an early step to prevent and control this disease.

Treatment of Water Supply and Fish Fry

In hatchery, farmers who culture their fry or adult tilapia in small ponds, concrete cemented ponds or tanks should filter and treat the water supply before channeling it to the culture system. Moreover, the farmers should ensure that the water is not or less contaminated by pathogens by using commercially available filters such as ozone and ultraviolet. In addition, the treatment of water can also be conducted using commercial biological filters and several types of solid removal filtration. It is important for fry production in hatchery

and small-scale farm where the input and output of water at the culture system can be manually controlled (pond, fiber tank, etc). By separating and treating the water supply, farmers can minimize the transmission of pathogenic bacteria from the water or wild fishes into the hatchery or culture system (Evans *et al*., 2002).

Previous study revealed that the wild fishes collected nearby the fish culture farms were infected by the same *S. iniae* strain, indicating the transmission of the pathogen from the wild to the cultured fish (Colorni *et al.,* 2002). Similarly, Bromage and Owen (2002) also reported that fish cohabiting barramundi pens had the same *S. iniae* strain infection as those of the barramundi. Moreover, Glibert *et al.* (2002) suggested that the transmission of *S. agalactiae* to the cultured fish was believed to have occurred via several routes and *Streptococcus* might have already present for a long period of time within the ecosystem (water, wild or cultured fish). Xu *et al.* (2007) also demonstrated that concurrent infection of tilapia with *Gyrodactyus niloticus* and *S. iniae* resulted in a significant increase in susceptibility to *S. iniae* disease. The authors also showed that *G. niloticus* could harbor *S. iniae* and might be a vector of infection for tilapia. Concurrent infection with *Trichodina* spp. and *S. iniae* or *S. agalactiae* also increased the susceptibility of channel catfish to both these streptococci (Evans *et al.,* 2007). Their results demonstrated that external parasites might play a role in the susceptibility of fish to *S. iniae* and *S. agalactiae* infections.

In order to treat the newly arrival fish fry before introducing them into the farm from most common fish parasites, Siti-Zahrah and Rokiah (1996) suggested to quarantine the fish using formalin and salt in concentrations of 25ppm and 1500ppm, respectively. The treatment should be conducted for three consecutive days in a week and repeated (if necessary) for the following week.

Currently, Arechavala-Lopez *et al.* (2013) reported the possibilities of reared fish, farmed escapees and wild fish stocks, which could contribute to a triangle of pathogen transmission. Thus, farmers should prevent the diseased fry or fish cultured in their hatchery or farm to not escape and transmit the diseases to the wild population, whether for same or different wild species of fishes.

Recently in Malaysia, Amal *et al*. (2013c) reported a case where *S. agalactiae* was transmitted to a newly established red hybrid tilapia farm in a reservoir via carrier fish fry and water from hatchery. Moreover, preventing the introduction of the wild fish into the culture system by additional nets surrounding the farm or cages with regular monitoring and by using improved netting materials for sea-cages (Jensen *et al.,* 2010), quarantine and treatment of the new stocks of fish fry for bacterial and parasitic diseases, and purchasing fish fry from a disease-free hatchery are important practices in order to control and prevent the transmission of streptococcosis.

Frequent Monitoring of Water Quality

Water quality is an important part of any aquaculture system. Successful aquaculture depends on the water quality (Boyd & Tucker, 1998). Water quality plays a major role in fish health because deterioration in water quality causes stress to the fish and leads to disease outbreaks. As *Streptococcus* spp. are opportunistic pathogens that are widely spread in aquaculture environment, they depend on stress to assert pathogenicity (Bunch & Bejerano, 1997). Therefore, farmers should always monitor and maintain water quality during the culture period. It is therefore necessary to understand the major water quality parameters and their interrelationships (relationship within the water quality parameters) which affect fish growth and health, and to determine the failure or success of the overall culture practices (El-Sayed, 2006).

For tilapia culture, the recommended water quality parameters suggested by Department of Fisheries, Malaysia are 6.5 – 8.5 for pH, >5mg/l for dissolved oxygen, 25 – 32^{0}C for water temperature, <0.02mg/l for ammonia, <3mg/l for nitrate, <2mg/l for iron and <120mg/l for alkalinity (Zulkafli, 2013). However, the water quality parameters may vary depending on the species, strains, sizes, duration of exposure, other environmental factors, culture system, geographical system, etc. (El-Sayed, 2006).

Environmental conditions surrounding the culture sites may affect water quality, and this will in turn stress the cultured fish due to disturbance on their physiology (Amal *et al.,* 2013a). This eventually lowers immune response and triggers bacterial infection, which are already in the water or the carrier fish leading to disease outbreaks. Even with low concentration of *S. agalactiae* in the water of culture system, bacteria are able to infect fish and express their virulency. This happens when water quality parameters are not at the optimum condition (Le Morvan *et al.,* 1998; Langston *et al.,* 2002).

There are a number of studies that evaluate the effects of water quality parameters on streptococcosis in tilapia. Some stressors that have been associated with *Streptococcus* outbreaks include high water temperature (>26°C) (Mian *et al.,* 2009; Amal *et al.,* 2013a; Milud *et al.,* 2013), high salinity (>15ppt) (Milud *et al.,* 2013) and pH (6-8) (Zhou *et al.,* 2008; Amal *et al.,* 2013a), low dissolved oxygen concentration (<3.4mg/l) (Bunch & Bejerano, 1997), high ammonia (>0.18mg/l) (Hurvitz *et al.,* 1997) or nitrite concentration (>10mg/l) (Bunch & Bejerano, 1997), slow flowing water (<0.25m/s) (Amal, 2011) and very high clarity of water (>334cm) (Amal *et al.,* 2013a).

An experimental study involving red hybrid tilapia (*Oreochromis* sp.) revealed that environmental temperature of 33°C, water salinity of 15ppt and water pH of 6 increased the susceptibility of the fish to *S. agalactiae* (Milud *et al.,* 2013). A field study conducted in Malaysia revealed that tilapia cultured in deep reservoirs were exposed to high water temperature (>29^{0}C) for up to 8m deep for a long period of time (during hot and dry season) due to extremely high water clarity (>425cm) and very slow water flow

(<0.01cm/s) increased their susceptibility to *S. agalactiae* infection (Amal *et al.,* 2010b). Moreover, Amal *et al.* (2013a) found that high water temperature (>27^{0}C), clarity (>334cm) and pH (7.37±0.53) of lake water, as well as high ammonia (>0.2mg/l), temperature (>29^{0}C) and low dissolved oxygen (<5mg/l) in down-stream river showed significant correlation with the presence of *S. agalactiae* in the cultured tilapia farms. Since it is not always possible to properly manage water temperature and dissolved oxygen levels, the conditions that favour disease outbreaks should always be kept minimal.

Controlled water bodies and culture systems such as earthern pond, tank, pool and hatchery, frequent partial or total water exchange are very important. According to Sipauba-Tavares *et al.* (2000), continuous water exchange increases the growth performance of Nile tilapia compared to no water exchange treatment when cultured in earthern pond. If this practice is not effective and practical in certain area due to water limitation and water cost, partial exchange and continuous water aeration are recommended. This practices are very important to remove and also lower the level of faeces and other total solids and dissolved metabolites such as ammonia and nitrite in the culture system, which negatively affect the culture performance.

For uncontrolled water bodies such as river and reservoir, lowering the fish culture density may help to reduce the stress of fish, which could lead to disease outbreaks. Sipauba-Tavares *et al.* (2000) revealed that pH, dissolved oxygen, carbon dioxide, nitrite, ammonia, phosphorus and chlorophyll a did not differ across the treatment of tilapia that received no water exchange, no water exchange but provided with aeration at night, or continuous aeration due to low fish stocking density (1.3 fish/m^3). The recommended stocking density is discussed later in this review.

Good Management Practice in Tilapia Hatchery and Farm

Farmers should minimize unnecessary handling or transportation of fish to reduce the stress of fish, which may contribute to disease outbreaks. It is also recommended to keep the hatcheries and farms clean all the time in order to reduce the risks of disease transmission and outbreaks. Furthermore, periodic cleaning and disinfection of all production units and equipment are also suggested as these will decrease the transmission of pathogens. In farm, for example, cleaning of the culture net cages, tanks and ponds should be conducted before the introduction of new fry by physical cleaning and drying, while drying and liming (calcite or dolomite) for the ponds. Equipment such as scoop nets, buckets, small tanks, containers, etc., should be regularly cleaned using the recommended commercial bleach (sodium hypochlorite at 200 - 500ppm) (Dvorak, 2009). Purchasing specific pathogen-free stocks and proper quarantine of newly arrived fish stocks are highly recommended while screening the new arrival fish stocks against *Streptococcus* is a compulsory (Dvorak, 2009). This is

because once the disease is introduced into the culture systems, it is difficult to eradicate them.

Maintenance and monitoring of fish production and health records can also help to detect disease problems and highlight their severity that often provides clues for disease diagnoses. In fact, maintaining accurate records of fish illnesses or deaths, while keeping records on fish production parameters such as water quality, growth and feed conversion ration also aids in detecting subclinical disease problems. Moreover, it is suggested that farmers record all new introductions or returning fish, their sources and movements on or off the hatcheries and farms. This practice can actually help identify potential disease entry points in the event of a disease outbreak (Dvorak, 2009).

High water temperature is a stress for fish but a preferential condition for the bacteria. Therefore, lowering the water temperature is extremely important and this can be achieved by recirculation systems. Small-size ponds and net cage farms often utilize sunscreens and water sprinklers to reduce water temperature. Activating paddle wheels during hot day is an additional method that can help reduce water temperature and increase the concentration of dissolved oxygen. Floating net-cage culture involving huge water bodies like reservoirs and dams, high-pressure multijet air under the cages is quite practical in reducing the water temperature and improving supply of the dissolved oxygen. However, these practices are costly and can cause upwelling in shallow water bodies and they can be deleterious. Thus, utilization of sunscreens is widely practiced in this country.

Reasonable Fish Stocking Density in the Culture System

Farmers should reduce fish stocking density to be reasonable according to the size of cages, size of fish or type of culture systems. High productivity in tilapia farming is achieved by balancing stocking density with a good survival rate. When mortality increases, lowering the stocking density helps to lower both stress level and pathogen load within the population.

Extensive research has been carried out on the effects of stocking density on tilapia production in different intensive culture systems (El-Sayed, 2002). However, the results revealed controversial outcomes and this is probably due to the differences in species, sexes, life stages, sizes, social hierarchies, nutrition, feeds, feeding regimes, culture systems and water qualities (Huang & Chiu, 1997; Muir *et al*., 2000). Nevertheless, El-Sayed (2006) concluded that tilapia are known to tolerate a high stocking density and can withstand extreme crowding condition. In contrast, Shoemaker *et al.* (2000) reported that high stocking density (≥11.2g/l) significantly affects the rate of mortality in tilapia exposed to *S. iniae*. In addition, Xu *et al.* (2007) revealed that the infection of this pathogen could involve direct infection through wounds and abrasions of the skin. This mechanism was reported to occur when the fish were

cultured in high densities. By reducing the stocking density, outbreaks of this disease could probably be controlled or at least minimized.

The relationship between stocking density and individual fish growth is generally negative (Sin & Chiu, 1983), while there is a positive correlation between stocking density and tilapia yield (i.e., high fish density leads to high yield) (Siddiqui *et al.,* 1997). According to El-Sayed (2006), farmers should therefore carefully define their target consumers and determine the appropriate stocking density that will produce the size of the fish preferred by the consumers. In addition, farmers should also adopt the stocking density that satisfies maximum production efficiency and the production of fish of a uniform size. Farmers should also determine whether they want to produce a high yield but small individual fish to be sold in rural areas at low prices, or produce larger individual fish but with a lower yield for restaurants or export purposes at higher prices.

In Malaysia, farmers usually stocked their fish based on the size of cage and fish. In theory, the bigger the size of the fish, the stocking density should be reduced accordingly. It is recommended to stock the cultured tilapia at 13 fish/m^3 of water (approximately 1500 fish in a cage of 6m length x 5m width x 4m depth) until the fish reached 100g and at the stocking rate should be 8 fish/m^3 of water (approximately 1000 fish in a cage of 6m length x 5m width x 4m depth).

Feed and Feeding

Partial reduction or completely stop feeding helps to control or reduce the mortality during streptococcosis outbreaks. This is because feeding facilitates proliferation of bacteria in the water. Furthermore, uneaten or excessive feed leads to deterioration of water quality. Besides, infected fish are low in appetite until they are recovered from the infection or in other words, the sick fish lose their appetite and will not eat. Therefore, feed reduction is one of the factors that can reduce and control the mortality rate of streptococcosis.

The use of contaminated thrash fish as feeds has also been implicated in the outbreaks of *Streptococcus* in Korea (Kim *et al.,* 2007), especially in the marine water fish aquaculture. Therefore in Malaysia, farmers are advised to feed tilapia with boiled visceral organ of chickens or trash fishes. This is to ensure that the feeds are free of pathogens which may contribute to disease outbreaks.

Susceptible Fish Size and Critical Period of Tilapia Farming

Study on streptococcosis in Malaysia revealed that tilapia weighing between 100 and 300g (10 - 30cm of total body length) were more susceptible to *S. agalactiae* infection (Zulkafli *et al.,* 2009; Amal, 2011; Amal *et al.,* 2013a). In addition, when tilapia of this size are cultured in huge water bodies (e.g., reservoirs and dams) with extremely slow water movement and high water temperature (≥30^0C) during the hot and dry months (April to September), the tilapia are

more susceptible to streptococcosis. Thus, avoiding fish of susceptible size during the critical months might be beneficial in reducing streptococcosis. For example, when a farmer starts culturing tilapia in early January by introducing fingerling of approximately ±20g, the harvesting size of ±500g can be reached in 5 to 6 months at the growth rate of approximately 3g/day. Therefore, fish should be weighing between 100 and 300g (susceptible size) in the months of February to April (non-critical period). This simple management practice avoids rearing susceptible tilapias size during the critical period of April to September and thus reduces chances of disease outbreaks. However, the success of this control measure depends on the growth rates, strains of tilapia, feeding regimes, nutrition, stocking densities, water qualities and other husbandry practices. Experienced farmers can manage their own strategies but the concept remains that they should avoid rearing susceptible size during critical period.

Practising "break cycle" Method

Another potential husbandry management that could be considered is breaking the disease cycle by not culturing tilapia during the critical period of hot months. By using this method, farmers can avoid the risks of economic losses due to streptococcosis.

Water temperature influences the presence of *S. agalactiae* in cultured red hybrid tilapia (Amal *et al.,* 2013a). Thus, by not culturing tilapia during hot seasons, the presence or population of *Streptococcus* in cultured fish, culture systems and farm water bodies could be reduced as well. Moreover, if abnormal mortality is detected, early precaution should be taken by harvesting the fish in order to reduce the economic losses and to break the cycle of fish culture. Indeed, they should avoid culturing the susceptible size of fish during the critical period. However, farmers are recommended to culture other fish species during this critical period.

Practising the "all-in-all-out" Method

Farmers may also control streptococcosis by practicing the all-in-all-out method. It is suggested that new tilapia fry (non-critical size) be introduced in November (non-critical period) before all fish are harvested in April (critical period). The fish reach susceptible size (critical size) in January or February (non-critical period). Following harvest, cages must be cleaned before another batch of fry (non-critical size) is introduced in May (critical period), reach susceptible size in July (critical period) and harvested in October (non-critical period). This method of culture can effectively reduce disease incidence. The newly introduced fish is the most important factor in introduction of *Streptococcus* into a fish farm while some fish survived an outbreak can harbour the bacteria and transmit them to susceptible tilapia (Nguyen *et al.,* 2002). However, this all-in-all-out method may have some difficulties in implementation particularly the consistent supply of the fry.

Farmers are also recommended to practice monosex culture for tilapia. According to El-Sayed (2006), monosex tilapia showed high growth rates and feed utilization efficiency, greater uniformity of size at harvest, high tolerance to severe environmental conditions or water qualities, high resistance to stress and diseases, etc., and all-male populations are more preferable. Moreover, in this country, farmers prefer practicing monospecies culture, namely the red hybrid tilapia (*Oreochromis* sp.) due to their higher price, demands and easily available fry compared to the black or Nile tilapia and GIFT (Genetically Improved Farmed Tilapia). Combination of monosex and monospecies culture practices could also be concurrently practiced with the all-in-all-out culture method to minimize disease outbreaks and increase farmers' economic profits.

Early Marketing

The suggested break cycle and the all-in-all-out methods of rearing will eventually result in rearing susceptible fish during the critical period. Therefore, early marketing may be considered where fish are sold when they reach <350g during critical period (Siti-Zahrah *et al.,* 2009). When farmers sell their fish of susceptible size upon entering the critical period, they could minimize the risk of disease outbreaks. Moreover, tilapia consumers in Malaysia prefer fish of the sizes between 250 and 350g (Siti-Zahrah *et al.,* 2009). This practice may reduce the cost of feeding through shortened rearing period.

Immediate Disposal of Dead Tilapia

Many researchers speculate on the mode of transmissions of *Streptococcus*. Transmissions of *Streptococcus* may be due to inhabitation of cage culture environment, carried over by contaminated fish fry or disseminated by reservoir adult fish (Nguyen *et al.,* 2002; Najiah *et al.,* 2009). Experimental cohabitation of dead infected fish with healthy fish resulted in infection of many healthy fish. Horizontal transmission of the pathogens between fish is another likely mechanism of dissemination (Xu *et al.,* 2007). Moreover, cannibalism of infected dead fish is an important issue in lateral disease transmission. The dead fish tissue in water is soft and sometimes attacked by the other fish will possibly disseminate the *Streptococcus*. Thus, in order to prevent outbreaks, it is important to remove dying and dead fish as promptly and frequently as possible. Farmers should bury or burn the dead infected tilapia to avoid transmission of the bacteria. The increasing pathogen load and deterioration of water quality could be easily observed in tilapia culture sites when farmers failed to remove the dead fish.

Antibiotics

Antibiotics are only effective in treating outbreaks of streptococcosis if the treatment is applied early. In most cases, oral antibiotic treatments are ineffective as the infected fish have a reduced appetite. Therefore, antibiotics are only able to partially control mortality rates during the period of

application. Once the course of antibiotic is over, mortality usually increases again. This phenomenon leads to non-sustainable behaviour; as mortality increases again after a normal antibiotic course, farmers are tempted to extend the duration of antibiotic application to longer periods or use higher doses. This in turn increases selection pressure toward resistant bacteria. The negative consequences of using antibiotics, such as emergence of antibiotic resistant bacteria and antibiotic residues in meat, must be carefully evaluated.

In Malaysia, farmers tend to use erythromycin and oxytetracycline to treat streptococcosis in tilapia as well as a prophylactic agent in healthy fish. These antibiotics are usually sprayed onto fish pellets and given orally to fish. Therefore, *S. agalactiae* isolated from tilapia in Malaysia were found to be resistant to spiramycin, oleandomycin, sulphamethoxazole, oxolinic acid, kanamycin and nalidixic acid, probably due to the previous overuse or misuse of these drugs to combat bacterial diseases in farms (Najiah *et al.,* 2009). Therefore, antibiotic treatment is generally ineffective and the need for proper vaccine has become necessary (Klesius *et al.,* 2000b).

Darwish and Griffin (2002) found that oxytetracycline is effective in controlling *S. iniae* in blue tilapia (*O. aureus*). Oxytetracycline was incorporated into the feed at 0, 25, 50, 75 and 100mg/kg fish body weight for 14 consecutive days. The 75 and 100mg doses significantly increased the survival rate of the fish from 7% in the infected non-medicated to 85% and 98%, respectively. Other reports concluded that erythromycin-incorporated feed is effective against streptococcal infections in cultured yellowtails (Shiomitsu *et al.,* 1980) and rainbow trout (Kitao *et al*., 1979) at the doses of 25-50mg/kg fish body weight for 4 to 7 consecutive days. Darwish and Hobbs also (2005) revealed that oral administration of amoxicillin-medicated feed for 12 consecutive days at a daily rate of 10, 30 and 80mg/kg fish body weight significantly increased the survival of *S. iniae* infected tilapia from 3.8% in the challenged, non-medicated positive control to 45, 75 and 93.8%, respectively. The survival rate was significantly higher in the 80mg treatment (93.75%) than the 10mg treatment (45%) but did not differ significantly between the 10mg (45%) and 30mg (75%) treatments. In conclusion, they stated that no carriers were detected in any challenged group receiving amoxicillin-medicated feed, whereas the bacterium was recovered from the non-medicated, challenged survivors of the infection.

Several studies revealed the efficacy of florfenicol in controlling *S. iniae* infection in Nile tilapia, blue tilapia and sunshine bass (*Morone chrysops* x *M. saxatilis*) (Darwish, 2007; Bowser *et al.,* 2009; Bowker *et al.,* 2010; Darwish, 2010; Gaunt *et al.,* 2010; Gaikowski *et al.,* 2013). Gaunt *et al.* (2010), in a study to determine the dosage of florfenicol in feed to control *S. iniae*-associated mortality in Nile tilapia, found that cumulative mortality was 20.5±2.0% in the challenged, unmedicated group; 11.0±2.1% in the 10mg florfenicol/kg group;

and 5.5±2.4% in the 15mg florfenicol/kg group following intracoelomic injection with 0.1ml of 10^5 colony-forming units (CFU)/ml of *S. iniae* to the fish. The fish were given their treatment feed once per day at 2.5% body weight for 10 consecutive days after the infection. Moreover, the mortality was significantly less in the medicated groups than in the challenged, unmedicated control group (10mg/kg: p=0.0270; 15mg/kg: p=0.0007). Similarly, Darwish (2007) suggested the optimum therapeutic daily dose of florfenicol was between 10 and 15 mg/kg body weight for 10 consecutive days. Their studies also concluded that florfenicol was palatable, safe, and efficacious for control of Nile tilapia mortality due to *S. iniae* infection.

Recently, Lee and Park (2014) evaluated the combination of intramuscular amoxicillin-florfenicol in controlling *S. iniae* in olive flounder (*Paralichthys olivaceus*). They found that a single IM delivery at 10mg/kg body weight of the amoxicillin-florfenicol combination in olive flounder infected with 4.56 x 10^5 CFU/ml of *S. iniae* resulted in an increased rate of survival (77.5% after 5 days and 62.5% after 14 days) compared to the challenged but untreated control group.

The main issue in antibiotic utilization is the tissue withdrawal period of antibiotic. Bowser *et al*. (2009) showed a trend toward shorter half-lives of elimination of florfenicol in the smaller fish compared to those bigger sizes (100, 250 and 500g of experimental fish weight). The elimination times in muscle-skin and half-lives were 9.2 and 1.2 days (100g), 8.6 and 1.7 days (250g), and 12.7 and 2.2 days (500g), respectively. SEAFDEC (2000) and Serrano (2005) listed several antibiotics that are used in aquaculture and their withdrawal periods, ranging from five days (amoxicillin, ampicillin and florfenicol) to 30 days (erythromycin, spiramycin, oxytetracycline hydrochloride, sulfadimethoxine and oxolinic acid). However, in order to prevent development of antibiotic resistant bacteria and antibiotic residues in meat, farmers must practice prudent use of antibiotics, while referring to the local Fisheries Department Officer, veterinarians, fish health specialist and experienced aquaculturist is also recommended.

Vaccinations

Vaccination has been recognised as an important method of prevention of infectious diseases in farmed fish (Lombard *et al.,* 2007). The objective of vaccination is to provide a strong immune response against an administered antigen that is able to produce long-term protection against a pathogen (Klesius *et al.,* 2008). Killed and modified live vaccines have been developed for use in aquaculture. The type of immunity needed, antibody or cell mediated against a particular pathogens is among the deciding factor in the development of a vaccine.

The understanding on response of immune system of fish in relation to vaccination is very important. In general, vaccines are designed to protect fish from the consequences of infectious diseases. This is accomplished by exposing the

fish to inactivated, attenuated or other forms of the pathogen, giving rise to antibodies (including B cells, T cells, IgM, gut associated lymphoid tissue, and etc. in the serum, mucus and gut lavage fluid of the vaccinated fish) that protect the fish from the debilitating and often life-threatening consequences of infectious diseases. Vaccines are unique among modern medications in that they offer effective protection against the onset and progression of specific infectious diseases. Most other medications are therapeutic, i.e., they are used to treat the disease and/or its symptoms; few are preventative. Vaccination is also unique in harnessing the cells, tissues and molecules of fish immune system to mediate this protection through a variety of natural mechanisms and processes that are fundamental to fish biology (Anon, 2014).

Killed vaccines are usually administered by IM or intraperitoneal (IP) of individual fish. Injection is the least cost effective in terms of labour and time. Killed vaccines are considered safer than modified live vaccines, which may revert to virulence. Future trends in vaccination include oral and immersion delivery of killed vaccine, development of modified live vaccines and multivalent vaccines that require improved vaccine adjuvants and immunostimulant. Vaccine prevents disease and mortality but may not completely eliminate streptococci in surviving fish (Klesius *et al.,* 2008). To date, several vaccines have been developed against streptococcosis in fish (Eldar *et al.,* 1997; Klesius *et al.,* 2000a; Evans *et al.,* 2004; Shoemaker *et al.,* 2006).

There are several available commercial vaccines for *Streptococcus* disease in fish, such as:

- Vaccine from formalin killed *S. agalactiae* and administrated to fish by injection (United States Department of Agriculture).
- AQUAVAC® Strep Sa vaccine from attenuated *S. agalactiae* and addition of oil adjuvant against *S. agalactiae* Biotype 2. Administrated to fish by injection (Merck Animal Health Company, USA).
- Aquavac™ Garvetil™, vaccine against *Lactococcus* and *S. iniae* via immersion dip administration (Intervet/Schering-Plough Animal Health).
- Aquavac™ Garvetil™, an oral vaccine against *S. iniae* which was applied by mixing the vaccine with the feed (Intervet/Schering-Plough Animal Health).
- NORVAX® STREP Si vaccine, a monovalent vaccine containing an inactivated strain of *S. iniae* (Merck Animal Health Company, USA).

The administration of vaccine is another issue to be considered especially where a large number of fish is involved. Many possible routes of administration have been studied to facilitate vaccination, and these include immersion, IP injection, IM injection and oral. Immersion is one route that is suitable to be applied on fry in the sizes between 1 and 5g (Le Breton, 2009). Intraperitoneal and IM injections are

normally being used on large sized, valuable fish such as brood stocks and ornamental fishes. Oral administration fits to be used on all sizes of fish and fish that are cultured in large water bodies such as lakes and open sea cages (Le Breton, 2009). Oral feed vaccination is preferable route since it consumes less time, less labour work, low cost and easy to perform by farmers.

In Malaysia, several oral and spray vaccines against *S. agalactiae* infection in tilapia have been developed, even though at laboratory scale. The newly developed feed-based vaccines showed promising early results (Firdaus-Nawi *et al.,* 2011, 2012). According to Firdaus-Nawi *et al.* (2012), in their study to determine the systemic, mucosal immunity and protective capacity of the feed-based adjuvant vaccine (FAV) of *S. agalactiae* following oral vaccination against streptococcosis in tilapia, the FAV group had a significantly higher protection level compared to only feed-based vaccine and control group, which were 100%, 50% and 12.5% survival, respectively, after being challenged with 3.4 x 10^9 CFU/ml of live virulent *S. agalactiae*.

Moreover, in other study, Noraini *et al.* (2013) revealed that the formalin-killed cells of *S. agalactiae* vaccine with single spray exposure was able to induce IgM, giving moderate to high protection following the immersion (80% survival) and IP (70% survival) challenge with virulent strain of *S. agalactiae* compared to unvaccinated group (0% survival). They concluded that the spray administration of vaccines had a moderate to high protection level in tilapia by producing higher antibody responses in mucus and serum. Moreover, they also suggested that in order to further increase the length of protection against streptococcosis to a period longer than 2 months in cage-cultured tilapia (during the critical months), spray vaccination should be combined with oral vaccine (Firdaus-Nawi *et al.,* 2012).

Recently, Nur-Nazifah *et al.* (2014) successfully developed and evaluated the efficacy of feed-based recombinant vaccine encoding the cell wall surface anchoring the family protein of *S. agalactiae* against streptococcosis in *Oreochromis* sp. Their results showed that tilapia immunized with the feed-based recombinant vaccine developed a strong and significantly higher IgM antibody response in serum, mucus and gut lavage fluid samples compared to formalin killed of *S. agalactiae* feed-based vaccine and unvaccinated group. Following heat intervenes and IP challenge, the rate of survivors was 70% for the recombinant vaccinated group and 0% for the rest of the groups. Moreover, their study also showed that the newly developed vaccine significantly provided high protection against high dose challenge in heat stress environment and enhanced the production of the mucosal and humoral immunity.

Based on the study on the epidemiological pattern of *Streptococcus* infection among tilapia in Malaysia, vaccination is suggested to be conducted prior to the critical period on tilapia that have reached the susceptible size (100 - 300g) (Amal, 2011; Amal *et al*., 2013a). Immersion vaccination is recommended for fry and fingerling tilapia

of hatchery stage (Le Breton, 2009; Merck, 2013). After introducing fingerlings into cultured farms (floating net cage, pond, tank, etc.), the vaccine should be given when they enter the susceptible size (100±50g) before the hot seasons. Booster dose must be given on day 14 after the first vaccination (Firdaus-Nawi *et al.,* 2012). Interestingly, Noraini *et al.* (2013) and Nur-Nazifah *et al.* (2014) revealed that their newly developed vaccines could provide protection for about 8 weeks and thus protecting the fish during the critical hot season.

CONCLUSION

A combination of good aquaculture farm practices, selective utilization of antibiotics and proper vaccination programme are keys to improved fish health, reducing disease outbreaks and decreasing the devastating economic impact on tilapia farming in Malaysia.

Considering the prevalence and risk factors discussed earlier, the following combined control measures are suggested:

- Tilapia farm should be established at a site with moderate rate of water flow. The most suitable sites are the upstreams of river, irrigation canal and other small water bodies with moderate water flow and humanly controlled.
- The source of tilapia fry must be from disease-free hatcheries.
- Tilapia should be kept at reasonable stocking density at all time. As the size of fish increases, stocking density must also be modified accordingly.
- Always aware of the farm environment. This includes regular monitoring of water quality, proper disposal of dead fish and removal of debris.
- If possible, practice all-in-all-out management system. Otherwise, be aware of the critical fish size during critical months.
- Use proper antibiotics and vaccination regime.

Adopting biosecurity measures by all hatcheries and farms is recommended. Biosecurity involves practices, procedures and policies used to prevent the introduction of infectious diseases (Dvorak, 2009). Also, the spread of disease causing organisms (e.g., bacteria, viruses, fungi and parasites), as well as many aquatic invasive species, should be considered. Routine use of biosecurity measures can reduce the risks of introduction and reduce economic impacts of the diseases. Fish movement, water sources, fish health, equipment/vehicles and vectors (human and animal) should always be monitored and examined as they are the main risk factors for disease introduction and spread in aquaculture facilities. The use of biosecurity measures on the hatcheries and farms can help farmers to prevent the disease introduction and spread, and thus protect their fish and investment.

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