Pathologicl Findings of Experimental *Streptococcus Agalactiae* Infection in Red Hyprid Tilapia (*Oreochromis* sp.)

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***Abstract*—** An experiment was conducted to investigate the histological effect of *Streptococcus agalactiae* infection to adult red hybrid tilapia (*Oreochromis* sp.). The experiment was comprised of two groups, the first fish group acted as a control and inoculated intraperitoneally (IP) with 1 mL sterile saline solution (0.85%), and the second fish group was IP inoculated with 1 mL of $1x10^4$ cfu/mL *S. agalactiae*. Experimental infection caused gross clinical abnormalities such as erratic swimming behavior, exophthalmia, lethargy and mucoid fecal casts. In histopathological examination, marked mononuclear cell infiltration, congestion and haemorrhages were seen in the spleen, liver, kidney, brain and heart tissue. Hyaline droplet degeneration and vacuolation in liver and tubular cells of kidney were also observed. The present study has established that the histopathological changes of fish could be helpful for the diagnosis of pathological and physiological status in tilapia culture.

***Keywords*—** *Streptococcus agalactiae*, tilapia, *Oreochromi*s sp., pathology, histology.

I. Introduction

RED hybrid tilapia (*Oreochromis* sp.) is becoming very important commercial venture in Malaysia as they are grow fast and have rapid weight gain. They cultured widely in different culture systems and became well accepted by people [1]. Currently, tilapia considered commonly aquacultured fish and important seafood source for human consumption in many countries.

Up to date, still little is known about how tilapia responses histologically and pathologically to diseases infection. However, histopathological and hematological techniques which are frequently used to diagnose clinical diseases in veterinary, still limited in aquatic animal health medicine [2].

One of the most significant emerging diseases in tilapia farming in Asia-Pacific region is streptococcosis which considered the most destructive bacterial disease. It is usually caused by *S. agalactiae* [8] leading to an acute or chronic, systemic disease with high morbidity and mortality. The bacteria infect animals and human causing meningoencephalitis in fish, mastitis in cows, and neonatal sepsis in human [3]. This bacterium affects most of marine and fresh water fish, including Gulf killifish (*Fundulus grandis*) [4], red hybrid tilapia (*O. niloticus*) [5], sea bream (*Sparus auratus*) [6] and silver pomfret (*Pampus argenteus*) [7].

Histopathology provides method to detect effects of irritants and pathogens in various organs [10]. Most bacteria that cause disease in fish are likely to induce a number of lesions in different organs [11] such as kidney [12], brain [5], and liver [13], and these organs considered suitable for histological examination in order to determine the effect of bacteria.

In May 2010, mass mortality occurred in red tilapia farm in Selangor state, Malaysia. In an earlier report the mortality was determined to be associated with *S. agalactiae* [9]. There have been only a few studies that have evaluated the histopathological changes in relation to streptococcal disease of fish. Therefore, this experiment studied the histopathological changes in red hybrid tilapia injected IP with 1×10^4 cfu/ mL of *S. agalactiae* originally isolated from naturally infected fish.

II. Material And Methods

A. Experimental Fish

One hundred fifty eight adult red hybrid tilapia (*Oreochromis* sp.) with 235g average bodyweight and 21cm average body length were obtained from a commercial tilapia farm, the fish maintained in two fiberglass tanks (13.0 tonnes each) at the Aquatic Animal Health Unit, Universiti Putra Malaysia for 2 weeks.

During the acclimatization period, the fish were fed twice daily with commercial fish feed at a rate of 2% of their body weight until 2 days prior to experiment. The fish were starved for 24 hours before infection.

The water was constantly monitored and renewed with 50% fresh water daily. Water quality parameters were measured using YSI 85 (Temperature 29±1.2 °C, DO 5.8 ± 1.2 mg/l, pH 7.2 ± 0.3, and ammonia 0.3±0.1 mg/L). These conditions were kept constant during the experiment. During acclimatization, the fish were observed daily for any clinical signs of diseases. Eight tilapias were randomly collected for microbiological examination to confirm that the fish were disease free.

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B. Bacterial preparation

The *S. agalactiae* strain used in the experiment was originally isolated from a naturally infected red tilapia [9]. The bacteria were subcultured on brain heart infusion agar (BHIA) at 30 ºC for 24 hours and identified as *S. agalactiae*, β-heamolytic, and group B using commercial identification kits (Streptococcal grouping Kit and BBL Crystal GP ID kit). Reference strain (*S. agalactiae*, β-haemolytic, ATCC 27956) was obtained from the American Type Culture Collection (ATCC) and used for identification.

The bacteria incubated in 10 ml BHI Broth for 24 h in an orbital shaker at 30 ºC. After incubation, the broth centrifuged at 15,000Xg for 15 minutes at 4 ºC and bacteria pellet obtained was washed with phosphate-buffered saline (PBS). The suspensions were diluted in a saline solution (0.85%) to reach the concentration of $1x10^4$ cfu/mL by a 10-fold serial dilution.

C. Experimental infection

The fish were divided into five groups; each group comprised of ten fish kept in 120-liter glass aquarium. Four groups were IP inoculated with 1 mL of 1× 104 cfu/mL of *S. agalactiae*. The fish in the fifth group were injected with 1mL of physiological saline and designated as the control. All fish were observed daily for any clinical signs, abnormal behavior or mortalities for a period of seven days post-infection.

D. Histopathological Examination

Fishes were euthanized on 0,1st, 3rd, 5th and 7th days post inoculation and dissected. Immediately after dissection kidney, liver, brine, heart and spleen were collected and fixed in 10% neutral buffered formalin then embedded in paraffin and processed. Fixed tissues were processed according to standard histological techniques and tissue sections were stained with haematoxylin and eosin (H&E).

III. Result

There were no significant observations in mortality, clinical or pathological changes in the control group. The other fish groups started to show signs of streptococcal disease on the 1st day post inoculation, such as staying motionless on the aquarium bottom and lethargic. On the 3rd dpi, the infected fish were still lethargic with rapid erratic swimming. One the 5th and 7th dpi fish showed exophthalmia, opaqueness of the eyes and fecal strings. On postmortem, the most significant pathological changes observed were exophthalmia, spleenic enlargement, hydroperitoneum and encephalomalacia as well as pale liver. The control fish showed no detectable gross and histopathological alterations, while all experimentally infected fish challenged with *S. agalactiae* revealed variable histopathological changes in the brain, kidney, spleen, heart and liver. The liver parenchyma showed acute hepatic cellular swelling, nuclear pyknosis, vacuolation of hepatocytes and increase in the numbers of melano-macrophages centre (MMC) In addition to that, hyaline droplet degeneration and congestion were also observed (Fig 1).

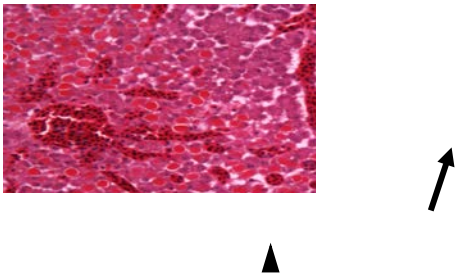

Fig 1. Liver of red hybrid tilapia infected with *S. agalactiae* showing severe congestion of liver blood capillaries (black arrow) and hyaline droplets formation (arrow heads) (H&E, 400x).

The spleen tissues showed red pulp degeneration, spleenic capillary congestion and focal haemorrhages. In addition, relatively large amount of yellowish brown areas of hemosiderin deposits and hypertrophy of melanomacrophage centers were also observed in spleen tissue (Fig 2).

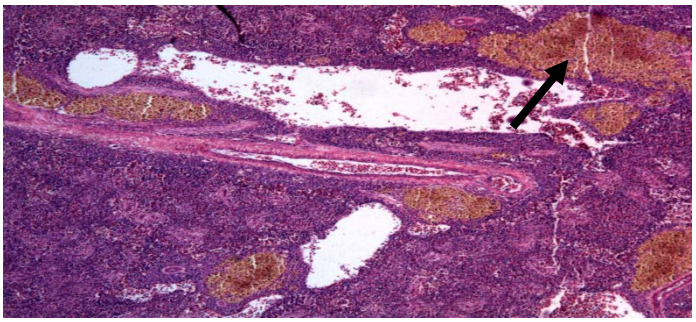

Fig 2. Spleen parenchyma from an infected fish showing multifocal hemosiderin deposition (white arrow) at 5dpi (H&E, 150x)

Histopathology of heart tissue showed thickened and congested blood vessel, accompanied with infiltration of mononuclear cellular infiltration in the epicardium (Fig 3)

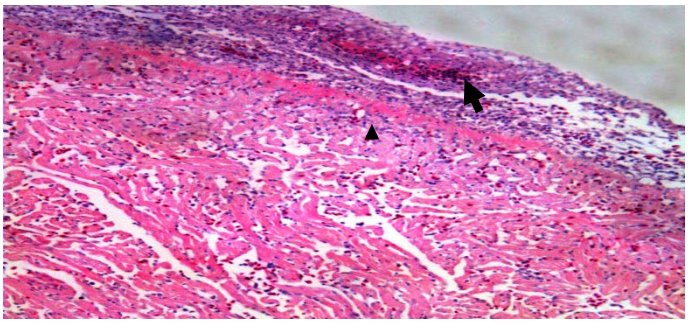

Fig 3. Heart of adult red hybrid tilapia experimentally infected with *Streptococcus agalactiae* showing increase thickness of pericardial epithelial layer (arrow head) and slight edema (arrow head) in epicardium. Seen at 7 dpi (H&E, 400x)

Within the myocardium, infiltrations of mononuclear cells were also present. Examination of kidney hematopoietic tissue showed cloudy swelling and deposition of hyaline droplets in the tubular epithelial cells and increases mononuclear inflammatory cells infiltration. In addition to numerous melanomacrophages centers (MMC), there were increase presences of haemosiderin deposits (Fig 4).

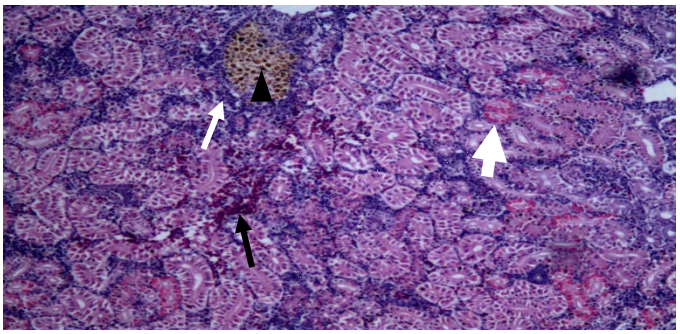

Fig. 4 Kidney of red hybrid tilapia infected with *S. agalactiae* showing mononuclear cell infiltration (white arrow), haemorrhage (black arrow), tubular hyaline degenerations (thick white arrow) and melanomacrophage centre (MMC) (head arrow) at 7 dpi (H&E, 200x)

The brain tissues revealed that there was severe congestion of meningeal blood vessels at the end of the experimental period. Mononuclear infiltrations in meninges and brain tissue were often seen in the late stages of experimental of *S. agalactiae* infection (Fig 5)

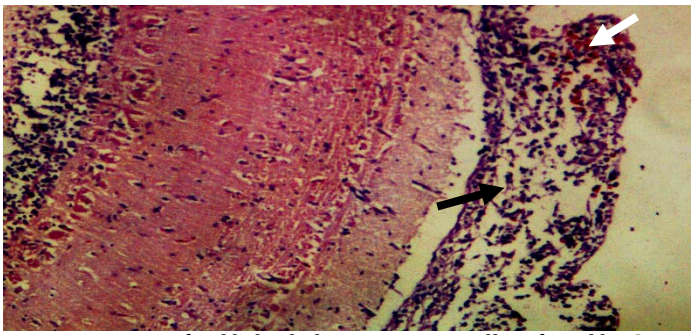

Fig. 5 Brain of red hybrid tilapia experimentally infected by *S. agalactiae* showing haemorrhages (white arrow) and mononuclear cell infiltration and increase intercellular space indicating oedema (black arrow) in the meninges at 7 dpi (H&E, 400x).

IV. Discussion

Clinical and pathological changes have been studied in many fish species during bacterial infection to identify the variable parameters for determination of healthy and diseased fish. The present study describes details of lesions and changes in clinical induced by *S. agalactiae* infection in adult red hybrid tilapia. In the present study, there were marked signs of streptococcal disease in the experimentally infected red hybrid tilapia. Similar findings were recently reported with Japanese flounder (Paralichthys olivaceus) [14] Red porgy (Pagrus pagrus) [15], Sunshine bass (Morone chrysops x M. saxatilis) [16]. Interestingly, during current study whitish stringy faecal casts were observed among the infected fish. This was likely due to presence of sloughed intestinal wall cells mixed with mucus in the feces. This clinical sign of fecal string was in agreement with recent report [8].

A streptococcal infection of cultured tilapia is systemic infection in several internal organs [17]. The histopathogical examination revealed that pericarditis and meningitis were the distinctive indicator of infection by *S. agalactiae*. As demonstrated by [18], *Streptococcus parauberis* infection in Olive flounder (*Paralichthys olivaceus*) induced inflammation of the heart (pericarditis) and brain (meningitis). Our results were in agreement with these earlier findings. Similar pathological changes in tilapia also reported by [19], *O. niloticus* experimentally infected with *S. iniae* and *S. agalactiae*. Findings similar to current observation, including pericarditis, infiltration of macrophages and lymphocytes into internal organs, hyaline deposition in tubular cells in kidney, and meningitis were documented. In addition, our current results were in agreement with many recently published reports which have shown that naturally infected fish with streptococcal disease showed a variety of pathological conditions, including congestion of internal organs, particularly in the liver, spleen and kidney and brain [16],[20], [2]. The histopathological lesions in the liver observed in the present study were hyperemia, cloudy swelling, haemorrhages, eosinophilic granular inflammatory cells infiltration and hyaline droplet degeneration. Our results clearly showed that the lesions associated with the infection of tilapia by *S. agalactiae* were similar to those described [22] in experimentally infected Rainbow Trout, Onchorhyncus mykiss with Streptococcus sp. Moreover, these histopathological changes have been observed in naturally infected golden pompano, Trachinotus blochii in Malaysia [23].

In the spleen of infected tilapia, we found haemorrhages in intercellular spaces together with multiple foci of melanin deposits and melano-macrophage centers (MMC). These MMCs are macrophage aggregates containing pigments such as hemosiderin, melanin and lipofuscin[24]. Many studies have demonstrated that an increased size and number of MMCs were more apparent in livers and spleens of fish infected with bacteria [25]-[27]. As described by [28] the multifocal of hemosiderosis presence in color carp *Cyprinus carpio* spleens induced by exposure to A. hydrophila. Hemosiderosis is known to be caused by the increase in the rate of destruction of erythrocytes in the spleen; in which related to hemolysis condition. As described earlier, our *S. agalactiae* isolate showed beta-hemolytic activity.

The observed microscopic lesions in the kidney of adult red hybrid tilapia infected with *S. agalactiae* in the current study such as cloudy swelling, hyaline droplets in the tubular epithelial cells and vessels congestion, were also described in Nile tilapia, *O. niloticus*, infected with *Streptococcus* sp. [29]. It is well known that hyaline droplet degeneration occur from the absorption of excessive amounts of proteinaceous substances, such as bacterial toxins[30]. Clearly, there is considerable histopathological evidence that *S. agalactiae* is producing a toxin which may be important in the progress of the disease, and needs further investigation.

V. Conclusion

In conclusion, we have demonstrated, that adult red hybrid tilapia experimentally infected with *S. agalactiae* exhibited pathological signs including in liver, spleen kidney, brain and heart tissues. These findings will serve as guide for monitoring

the streptococcal disease infection and will be useful as a baseline data for early diagnosis of the disease in red hybrid tilapia

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