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Comparative Clinicopathological Changes Associated with Experimental *Streptococcus agalactiae* and *Streptococcus iniae* Cohabitation Infection in Red Hybrid Tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*)

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ABSTRACT

Streptococcus agalactiae and *Streptococcus iniae* are the two main pathogens causing streptococcosis in fish. This study compares the clinicopathological changes in red hybrid tilapia experimentally infected with *S. agalactiae* or *S. iniae*. A total of 180 tilapias were divided into six groups. Groups 1A, 2A, and 3A were inoculated intraperitoneally with sterile phosphate-buffered saline, *S. agalactiae*, and *S. iniae*. Fish of Groups 1A, 2A, and 3A were then immediately allowed to cohabitate with fish of Groups 1B, 2B, and 3B, respectively. All fish were observed at 6-hr intervals for 120 hr before surviving fish were euthanized. The spleen, liver, and brain samples were collected for bacterial isolation and histopathology. Clinical signs were developed at 72 hr in Groups 2A and 3A and 96 hr in Groups 2B and 3B. Group 2A showed the highest clinical score (P<0.05). Significantly (P<0.05), more cohabitating fish (Groups 2B) were infected by *S. agalactiae* compared to *S. iniae* (Group 3B) at 55.0±0.0 and 43.70±1.25%, respectively. The mortality rate was significantly (P<0.05) higher for Groups 2A and 2B than other groups. The gross lesions were significantly (P<0.05) more common in fish of Group 2A. Histopathologically, encephalitis was observed in fish infected

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with *S. iniae* of Groups 3A and 3B, while meningoencephalitis was observed in fish infected with *S. agalactiae* of Groups 2A and 2B. The findings suggest that *S. agalactiae* is more pathogenic than *S. iniae*, producing slightly different histopathological lesions in the brain.

Keywords: *Streptococcus agalactiae*, *Streptococcus iniae*, streptococcosis, tilapia

INTRODUCTION

Tilapia (*Oreochromis* sp.) is an important fish species in aquaculture (Amal et al., 2010). In 2018, the global production of farmed tilapia was 6.03 million tonnes (Miao & Wang, 2020). Tilapia grows rapidly, can survive in water of poor quality, is tolerable to environmental conditions, and is resistant to various diseases (Amal & Zamri-Saad, 2011; Ghozlan et al., 2018). However, tilapia is susceptible to streptococcosis, a disease caused by *S. agalactiae* and *S. iniae* (Evans et al., 2006).

Since the first occurrence of streptococcosis in rainbow trout (*Oncorhynchus mykiss*) in 1957 (Hoshina, 1958), this disease has continued to cause significant losses in the global aquaculture industry (Mishra et al., 2018). The economic losses are largely attributed to the high mortality and morbidity caused by streptococcosis. Mortality rates due to *S. agalactiae* infection can reach as high as 80% (Al-Harbi, 2016), while *S. iniae* infection can result in mortality rates up to 50% (Eldar et al., 1995). Streptococcosis has infected various species of freshwater, estuarine, and marine fish worldwide, including Africa, the Middle East, North and South America, Australia, as well as East, South, and Southeast Asia (Amal & Zamri-Saad, 2011). The estimated economic impact of *S. agalactiae* and *S. iniae* infections in tilapia was around USD 150 million annually in 2000 and further increased to USD 250 million annually in 2008, approximately 5.7 and 6.7% of the total global value of tilapia, respectively (Amal & Zamri-Saad, 2011). Later, it was estimated that losses due to streptococcosis in China in 2011 could be as high as USD400 million (M. Chen et al., 2012).

Both pathogens can result in similar clinical signs and lesions, including loss of orientation and erratic swimming, lethargy, dyspnea, exophthalmia, and congestion of visceral organs (C.-Y. Chen et al., 2007; Rahmatullah et al., 2017; Zamri-Saad et al., 2010). Field observations revealed that *S. agalactiae* infection usually resulted in high morbidity and acute mortality, while *S. iniae* infection resulted in chronic mortality (Chu et al., 2016; Jantrakajorn et al., 2014; Yuasa et al., 2008). This article compares the clinical signs, pattern and rate of mortality, and histopathological lesions in red hybrid tilapia (*Oreochromis niloticus* × *O. mossambicus*) following experimental infection by *S. agalactiae* and *S. iniae* using a cohabitation infection method.

MATERIALS AND METHODS

Animals

A total of 190 red hybrid tilapias of total length of 8.0±1.5 cm were bought from a commercial fish farm in Serdang, Selangor,

Malaysia. These fish were maintained in 12 aquaria (75 cm length × 45 cm height × 37 cm width) with a closed water system, where approximately 20% of the water was replaced every 24 hr. Throughout the study period, the recorded water dissolved oxygen was 6.48±0.90 mg/L, pH was 7.4±0.5, temperature was 27.80±1.50°C, and ammonia-nitrogen was 0.02±0.01 mg/L, as recorded by a handheld YSI meter (YSI, USA) and a handheld colorimeter (DR900, Hach Company, USA). All fish were fed twice daily with commercial feed at the rate of 2% body weight. They were acclimatized for 10 days prior to the experiment, during which time 10 fish were randomly subjected to necropsy to ensure that they were free from external parasites, as well as *Streptococcus* spp. by isolation and identification of *Streptococcus* spp. using polymerase chain reaction (PCR) (Rahmatullah et al., 2017). The Institutional approved the experimental procedure for Animal Care and Use Committee, Universiti Putra Malaysia (UPM/IACUC/AUP-U035/2018).

Preparation of Inoculums

Streptococcus agalactiae strain UPM1357 (accession number AF151357) and *S. iniae* strain UPM17 (accession number KT722586) that were previously isolated from outbreaks of streptococcosis were obtained from stock cultures (Rahmatullah et al., 2017; Syuhada et al., 2020). The isolates were cultured in brain heart infusion (BHI) broth (Merck, Germany) and incubated at 37°C for 18 hr. The inoculums were adjusted using serial dilutions and plate counts to achieve an infective dose of 1×10^7 cfu/ml.

Experimental Design and Clinical Evaluation

The remaining 180 fish were divided into six groups of equal size, each being replicated thrice. Groups 1A, 2A, and 3A received intraperitoneal injections of 0.03 ml of sterile phosphate-buffered saline (PBS) (Merck, USA), 3×10^5 cfu/ml of *S. agalactiae*, and 3×10^5 cfu/ml of *S. iniae*, respectively. All fish that were inoculated were tagged with tail sutures for identification purposes. Immediately after inoculation, fish in Groups 1B, 2B, and 3B were introduced to their respective aquaria to cohabit (Table 1). All fish were observed for clinical signs at 6-hr intervals for 120 hr. During the observation period, dead fish were collected for necropsy. At the end of the study, surviving fish were anesthetized using MS222 prior to euthanasia by pithing. The fish were then necropsied. Samples of the brain, liver, and spleen were collected to isolate and identify *S. agalactiae*

Table 1
Summary of the groups and the respective treatment

Groups	Treatments	Cohabitating groups
1A	0.03 ml of sterile PBS	1B
2A	0.03 ml of 3×10^5 cfu/ml of live *Streptococcus agalactiae*	2B
3A	0.03 ml of 3×10^5 cfu/ml of live *Streptococcus iniae*	3B

Note. PBS = Phosphate buffered saline

and *S. iniae*. Similar samples were also fixed in 10% neutral-buffered formalin and processed for histopathology.

During observation, the following clinical signs were noted: lethargy, anorexia, exophthalmia, corneal opacity, dyspnoea, abnormal swimming, and death. At each observation point, a clinical score was given to each group, where observation of each clinical sign was scored 1, while death was scored 4. The cumulative mortality rates were calculated and plotted over time.

Bacteriology

Swabs from the brain, liver, and spleen were placed on BHI agar (Merck, USA) containing 5% horse blood and incubated at 37°C for 18 hr. Initial identification of the cultured bacteria was based on the morphological characteristics of greyish-white, circular, and β-hemolysis of blood agar (Aisyah et al., 2015). Suspected colonies were subjected to identification using PCR. The bacterial DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA). The extracted DNA was subjected to PCR using forward primers 5′-ACG GAG TTA CAA AGG ACG AC-3′ and reverse primer 5′-AGC TCA GCC TTA ACG AGT AC-3′ for detection of *S. agalactiae*-specific 16S gene (Amal et al., 2012). For *S. iniae*, forward and reverse primers of 5'-CTAGAGTACACATGTAGCTAAG-3' and 5'-GGATTTTCCACTCCCATTAC-3', and forward and reverse primers of 5'-AAGGGGAAATCGCAAGTGCC-3' and 5'-ATATCTGATTGGGCCGTCTAA-3' were used for detection of 16S gene and lactate oxidase (*lctO*) gene, respectively (Rahmatullah et al., 2017).

Histopathology

The brain, liver, and spleen samples were fixed in 10% neutral-buffered formalin for 72 hr and processed using routine methods to isolate and identify bacteria. After embedding in paraffin, sections of 4 μm were obtained and stained with hematoxylin and eosin (R&M Chemicals, Malaysia) (Nurliyana et al., 2020). Histopathological lesions were evaluated by analyzing ten randomly selected fields at 200× magnification. Lesions for each sampled organ were identified before their severity was semi-quantitatively scored as previously described by Azzam-Sayuti et al. (2021) and Ulum et al. (2021), with minor modifications; score of 0 for normal tissue, a score 1 for tissue with ≤15% lesion, score 2 for tissue with 15-30% lesion, score 3 for tissue with 30-50% lesion, and score 4 for tissue with >50% lesion.

Data Analysis

Data on the clinical signs and cumulative mortality rates were analyzed using one-way analysis of variance (ANOVA), while the histopathology evaluations were analyzed using two-way ANOVA. The statistical analysis was done using the IBM® SPSS® Statistics (version 22).

RESULTS

Clinical Signs

After inoculation with *S. agalactiae* and *S. iniae*, the fish exposed to the pathogens

developed clinical signs observed from 72 hr until the end of the experiment. Similarly, the cohabitating fish in Groups 2B and 3B began exhibiting clinical signs starting from 96 hr until the end of the experiment. The clinical signs included lethargy, anorexia, exophthalmia, corneal opacity, dyspnoea, and abnormal swimming. Ten fish from Group 2A, five from Group 3A, and three from each Group 2B and 3B died without observable clinical signs. No clinical sign was observed in all control fish throughout the study period.

Group 2A, inoculated with *S. agalactiae*, had the highest clinical score of 8.9±1.7, significantly higher (P<0.05) than other groups. It was followed by the cohabitating Group 2B with a score of 7.5±0.5; Group 3A inoculated with *S. iniae* with a score of 6.6±0.7, and the cohabitating Group 3B with a score of 5.5±0.8. No significant difference (P>0.05) in the clinical score was observed between the cohabitating fish in Group 2B and those inoculated with *S. iniae* in Group 3A. Additionally, no significant difference (P>0.05) was observed between the fish inoculated with *S. iniae* in Group 3A and the cohabitating fish in Group 3B. A significant difference (P<0.05) was noted when 56.6±3.3% of the fish of Group 2B showed clinical signs compared to 43.3±3.3% of Group 3B, comparing the two cohabitating groups.

Fish in Group 2A inoculated with *S. agalactiae* experienced mortality starting from 78 hr at a rate of 23.3±3.3%. It was significantly higher (P<0.05) compared to other groups. On the other hand, mortality among the cohabitating Group 2B started at 90 hr at the rate of 6.7±3.3%. Similarly, fish of Group 3A that were inoculated with *S. iniae* showed mortality starting from 96 hr, while the cohabitating Group 3B started to show mortality from 102 hr. In all exposed Groups 2A, 2B, 3A, and 3B, the mortality rates consistently increased until the end of the experiment. At 120 hr, fish inoculated with *S. agalactiae* of Group 2A showed the highest cumulative mortality rate at 53.3±3.3%, followed by its cohabitating Group 2B at 36.7±6.7%, fish inoculated with *S. iniae* of Group 3A at 23.3±3.3%, and its comingling Group 3B at 20.0±0.0% (Figure 1). No mortality was observed in Groups 1A and 1B.

Bacterial Isolation and Identification

Streptococcus agalactiae was isolated and identified by PCR from all dead fish of Groups 2A and 2B, while *S. iniae* was isolated from Groups 3A and 3B. All survivors from cohabitating Group 2B were positive for *S. agalactiae* compared to 43.75% of survivors from cohabitating Group 3B that were positive for *S. iniae*. Bacterial isolation from all Groups 1A and 1B fish yielded no growth.

Gross and Histopathologic Lesions

All of the exposed fish in Groups 2A, 2B, 3A, and 3B showed gross lesions, including corneal opacity, peritonitis, and abdominal distension containing serosanguineous ascitic fluid, as well as discoloration and hemorrhages at the base of the fin (Figure 2). However, no lesions were observed in the control fish of Groups 1A and 1B. The

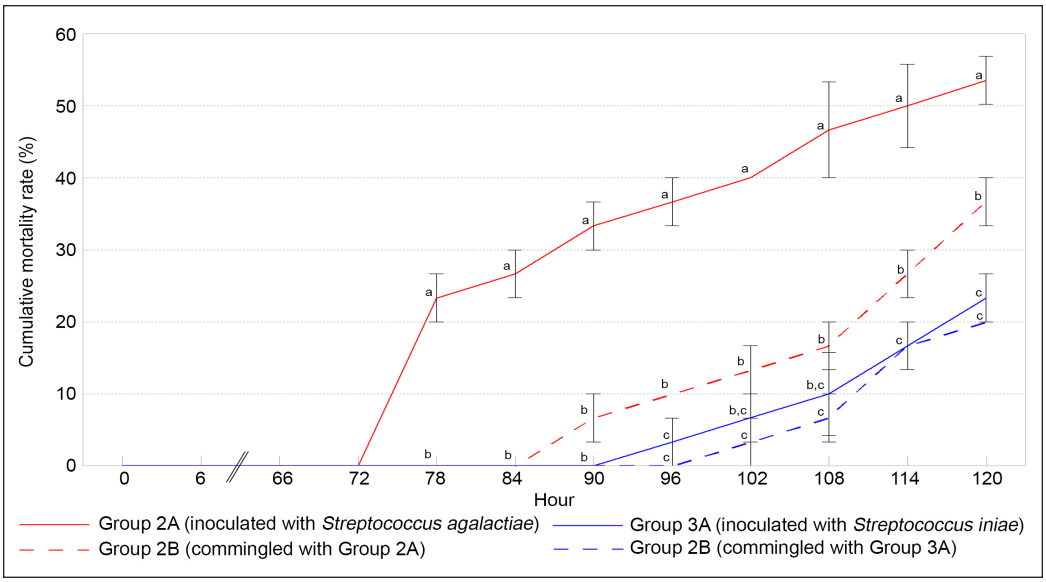
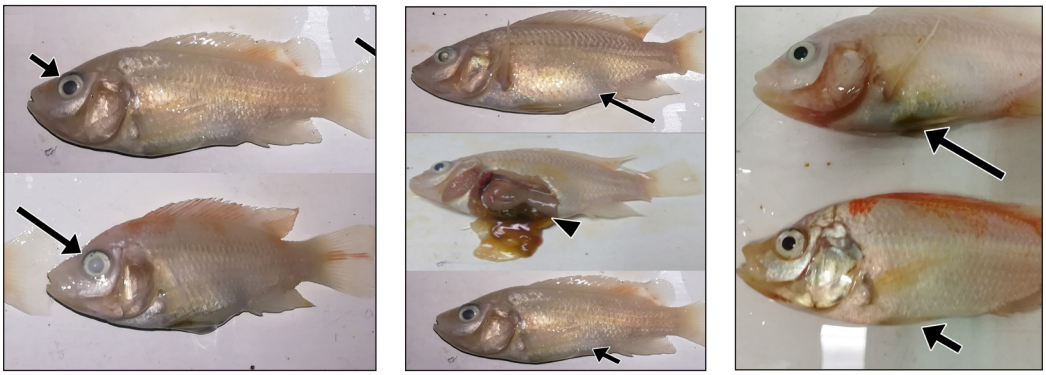


Figure 1. Mean cumulative mortality rates over time between the different treatment groups
Note. Different superscripts (^{a,b,c,d}) at each point of time indicate significant (P<0.05) differences between the groups



(a) (b) (c)

Figure 2. Gross lesions of tilapia infected by *Streptococcus*. (a) Corneal opacity (long arrow) in infected fish, compared to non-infected fish with a normal eye (short arrow); (b) Infected fish showing abdominal distention (long arrow), and ascites (arrowhead), compared to non-infected fish with normal abdomen; (c) Intraperitoneally infected fish showing a discoloration of the injection site (long arrow), compared to non-infected fish with a normal gross appearance

percentage of fish exhibiting gross lesions was significantly higher (P<0.05) in Group 2A (46.7±0.7%) compared to all other groups (Table 2). It is followed by the fish of Group 3A (17.8±4.0%). The lesion scores for these two groups were significantly higher (P<0.05) than the remaining groups. Approximately 7.8±4.0% and 4.4±1.8% of fish from the cohabitating Groups 2B and 3B exhibited gross lesions, respectively.

Four types of histopathological lesions were frequently observed in the brain of

the infected fish. These were congestion (Figure 3a), hemorrhage, encephalitis, and meningitis. All exposed fish showed congestion and hemorrhages of various internal organs. The histopathology lesion scorings indicated no significant difference (P>0.05) among the four infected groups. Fish exposed to *S. agalactiae* (Groups 2A and 2B) exhibited microglial infiltration in the brain (Figure 3b), and 70.00±4.71% of fish exposed to *S. iniae* (Groups 3A and 3B) also showed the same lesion (P<0.05).

Table 2
Percentage (±SEM) of fish from different treatment groups showing the gross lesions following infection by Streptococcus agalactiae *or* S. iniae

Groups	Treatments	Corneal opacity	Peritonitis	Fin base hemorrhage	Overall
1A	Sterile PBS	0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{c}	0.0±0.0^{d}
1B	(cohabitated with 1A)	0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{c}	0.0±0.0^{d}
2A	*Streptococcus agalactiae*	63.3±3.3^{a}	53.3±3.3^{a}	20.0±0.0^{a}	46.7±0.7^{a}
2B	(cohabitated with 2A)	23.3±3.3^{b}	0.0±0.0^{d}	0.0±0.0^{c}	7.8±4.0^{c}
3A	*Streptococcus iniae*	10.0±0.0^{c}	33.3±3.3^{b}	10.0±0.0^{b}	17.8±4.0^{b}
3B	(cohabitated with 3A)	3.3±3.3^{c,d}	10.0±0.0^{c}	0.0±0.0^{c}	4.4±1.8^{c}

Note. Different superscripts (^{a,b,c}) indicate significant differences (P<0.05) of the same column; PBS = Phosphate buffered saline; SEM = Standard error of the mean

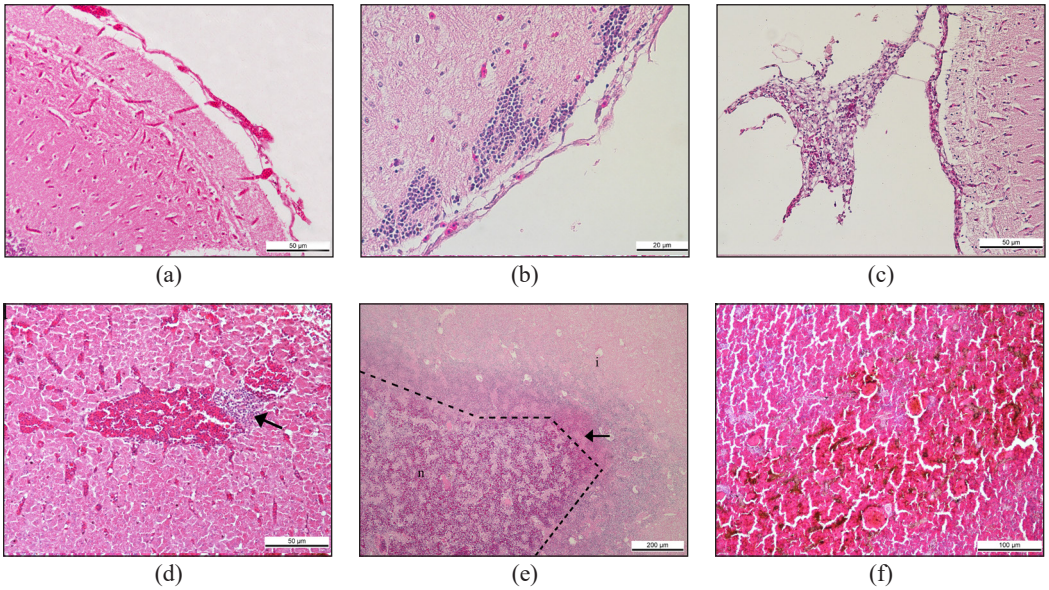


Figure 3. Histological lesions in organs of fish infected by *Streptococcus*. (a) Meningeal and cerebral congestion (bar = 50 µm, hematoxylin, and eosin [HE]); (b) Microglial cells in the brain (bar = 20 µm, HE); (c) Infiltration of inflammatory cells and congestion of meninges (bar = 50 µm, HE); (d) Congestion and infiltration of mononuclear cells (arrow) in the liver (bar = 50 µm, HE); (e) Spleen of fish showing normal (n) and infarcted (i) areas, accompanied by hemorrhage (arrows) (bar = 200 µm, HE); (f) Severe hemorrhage in the spleen (bar = 100 µm, HE)

Fish in Group 2A had the most severe encephalitis, significantly more severe (P<0.05) than Groups 3A and 3B, as revealed by encephalitis severity scores (Table 3). Meningitis (Figure 2c) was observed only in tilapia of Groups 2A and 2B infected with *S. agalactiae*, with moderate severity scores of 2.90±0.74 and 2.20±1.14, respectively. The overall histopathological lesion scores were significantly higher (P<0.05) in Groups 2A and 3A compared to Groups 2B and 3B.

The liver showed four types of lesions, namely congestion, hepatitis (Figure 2d), hemorrhage, and necrosis, which were observed in all infected fish, and there was no significant difference (P>0.05) in the number of fish affected by these lesions between the infected groups. Additionally, there was no significant difference (P>0.05) in the severity of histopathological lesions between Groups 2A, 2B, 3A, and 3B. The severity of all liver lesions was generally mild to moderate.

The spleen of the infected fish exhibited three types of lesions: congestion, infarction (Figure 3e), and hemorrhage (Figure 3f). Congestion and hemorrhages were found in all infected fish, whereas splenic infarction was observed in 85.00±3.33% of fish infected with *S. agalactiae* in Groups 2A and 2B and in all fish infected with *S. iniae* in Groups 3A and 3B. There was no significant difference (P>0.05) in the severity of all three types of spleen lesions among the infected groups.

DISCUSSION

Streptococcosis is long-known to be an important disease in fish. Many studies

Table 3
Histopathological lesion scores (± SEM) of brain, liver, and spleen in fish following infection by Streptococcus agalactiae *or* S. iniae

Organs	Lesions	Inoculated groups			Cohabitating groups		
		1A	2A	3A	1B	2B	3B
Brain	Congestion	0.10±0.32^{a}	3.00±0.667^{b}	2.10±1.20^{b}	0.22±0.44^{a}	3.00±0.667^{b}	1.40±0.84^{c}
	Hemorrhage	0^{a}	1.8±1.14^{b}	1.0±0.67^{b}	0^{a}	1.7±1.16^{b}	0.7±0.82^{b}
	Encephalitis	0^{a}	2.90±0.74^{b}	1.60±0.84^{c}	0^{a}	2.60±0.97^{b,c}	1.70±0.95^{c}
	Meningitis	0^{a}	2.60±0.69^{b}	0^{a}	0^{a}	2.20±1.14^{b}	0^{a}
	Overall	0.03±0.16^{a}	2.48±0.99^{b}	1.10±1.13^{c}	0.06±0.23^{a}	2.33±1.10^{b}	1.025±0.98^{c}
Liver	Congestion	0.30±0.67^{a}	1.90±0.99^{b}	1.86±0.94^{b}	0.11±0.33^{a}	2.10±0.74^{b}	1.40±0.70^{b}
	Hemorrhage	0^{a}	2.1±1.29^{b}	1.50±1.08^{b}	0.33±0.71^{a}	2.00±1.05^{b}	1.50±0.85^{b}
	Hepatitis	0^{a}	1.90±0.57^{b}	2.1±0.57^{b}	0^{a}	1.80±1.23^{b}	2.30±0.95^{b}
	Necrosis	0.10±0.32^{a}	1.60±0.84^{b}	1.80±0.92^{b}	0.10±0.32^{a}	1.00±1.05^{b}	1.80±1.03^{b}
	Overall	0.10±0.38^{a}	1.88±0.94^{b}	1.83±0.90^{b}	0.06±0.24^{a}	1.73±1.09^{b}	1.75±0.93^{b}
Spleen	Congestion	0.10±0.32^{a}	2.00±0.94^{b}	1.60±0.70^{b}	0^{a}	2.00±0.94^{b}	1.80±0.92^{b}
	Hemorrhage	0^{a}	0.90±0.99^{b}	1.1±0.88^{b}	0^{a}	1.40±1.08^{b}	0.60±0.70^{b}
	Infarction	0^{a}	1.60±1.27^{b}	2.10±1.42^{b}	0^{a}	1.30±1.42^{b}	2.30±0.74^{b}
	Overall	0.03±0.18^{a}	1.50±1.14^{b}	1.60±0.86^{b}	0^{a}	1.57±1.17^{b}	1.57±1.04^{b}

Note. Different superscripts (^{a,b}) within the same row indicate significant differences (P<0.05) between groups; SEM = Standard error of the mean

were previously conducted to understand this disease. However, to date, there is a very limited number of studies on the various species of *Streptococcus* that cause streptococcosis. The findings from this study are believed to significantly contribute to understanding the disease. In this study, the clinical signs observed following *S. agalactiae* or *S. iniae* infections were generally similar, making it impossible to distinguish between the two bacterial infections based solely on these criteria. In streptococcosis, it is postulated that damage to the brain and nervous system leads to behavioral changes such as abnormal swimming and grouping at the bottom of the tank (Abuseliana et al., 2011), while death is largely incriminated to septicemia (Song et al., 2017). It is consistent with the findings in this study, where histopathological changes were noted in the brain samples of tilapia exposed to *S. agalactiae* and *S. iniae*.

The behavioral changes mentioned earlier can be easily overlooked in real-world scenarios. Nonetheless, analyzing mortality patterns over time could offer significant clues to support the cause of infections. As observed in this study, earlier and higher mortality rates and higher percentages of isolation of pathogens in surviving fish suggested that streptococcosis caused by *S. agalactiae* develops rapidly. Furthermore, the transmission rate is faster for *S. agalactiae* than for *S. iniae*. Theoretically, in a septicaemic disease, rapid disease development and transmission are incriminated to rapid multiplication of the pathogens in the host tissues and circulation. It was previously observed that *S. agalactiae* usually results in severe septicaemic disease where it could be isolated from various organs, while *S. agalactiae* infection tends to be more localized, especially in the brain and the eye socket (Yuasa et al., 2008). Unfortunately, this study did not determine the concentrations of *S. agalactiae* and *S. iniae*. The mortality rate patterns between the four infected groups, as well as previous pathogenicity studies to determine the 50% lethal dose (LD_{50}) of these *S. agalactiae* and *S. iniae* isolated (Nur-Nazifah et al., 2011; Rahmatullah et al., 2017), proved that *S. agalactiae* is significantly more pathogenic compared to *S. iniae* in the tilapia. The findings from this and previous studies explain the previous field observation that *S. agalactiae* causes higher acute mortality in fish than *S. iniae* (Jantrakajorn et al., 2014; Yuasa et al., 2008). The field observations revealed that *S. agalactiae* results in cumulative mortalities that range from 40–60%. In contrast, mortality caused by *S. agalactiae* was low but consistent, accounting for only 0.1–0.2% per day (Yuasa et al., 2008).

Even though the gross lesions caused by both bacteria were similar, the histopathology evaluation showed some differences. These differences were primarily observed in the brain lesions, where *S. agalactiae* caused meningoencephalitis, while *S. iniae* caused only encephalitis. While the meningeal involvement in *S. agalactiae* infection in tilapia was recently explained (Eto et al., 2020), this comparative discovery has not been reported before. Furthermore,

these findings are actually in contrast with previous observations that both organisms cause similar histopathology findings (C.-Y. Chen et al., 2007). The different clinical and pathological features resulting from infection by *S. agalactiae* and *S. iniae* suggest different pathogenesis between these two pathogens.

The general pathogenesis of encephalitis or meningoencephalitis involves the early entry of the agent into the central nervous system (Quagliarello & Scheld, 1992). If the agent is equipped with certain virulence factors or due to the high concentration of the agent in the blood (Tunkel & Scheld, 1993), it could lead to meningeal invasion and breakdown of the blood-brain barrier causing the development of meningitis and cerebral edema (Quagliarello & Scheld, 1992). A recent study emphasized that a high concentration of pathogens in the bloodstream is crucial in invading the meninges and tropism of bacterial pathogens, such as Group B *Streptococcus* (Coureuil et al., 2017). On the other hand, without the meningeal invasion, inflammation occurs only in the brain. Studies to quantify the level of *S. agalactiae* and *S. iniae* in the circulation of infected tilapia, and their interactions with the blood-brain barrier should be conducted to elucidate the exact pathogenesis of infections of these two pathogens.

The variation in mortality rate and histopathological alterations observed in the two pathogens may be linked to the response of inflammatory cells to these pathogens. Previous research has demonstrated that the phagocytes of tilapia exhibit distinct reactions to *S. agalactiae* and *S. iniae*. When exposed to *S. agalactiae*, macrophages exhibit higher levels of chemotactic and chemokinetic activity than those exposed to *S. iniae*, as demonstrated by Klesius et al. (2007). Furthermore, macrophages exposed to *S. iniae* failed to show any chemokinetic activity. It is speculated that tilapia innate immunity could efficiently control *S. iniae* infection compared to *S. agalactiae* infection (C.-Y. Chen et al., 2007; Poyart et al., 2001), which possibly explains the meningeal invasion in the latter. Further investigations on the response and modulation of macrophages may offer valuable insights for the future prevention and treatment of streptococcosis.

CONCLUSION

This study showed that *S. agalactiae* is more pathogenic than *S. iniae*, leading to higher mortality rates and more severe clinical signs in red hybrid tilapia. Furthermore, the study also revealed that *S. agalactiae* caused meningoencephalitis, while *S. iniae* caused encephalitis in infected fish. These findings highlight the differences in pathogenicity and histopathological lesions between the two pathogens, possibly suggesting two different pathogenesis of infections of these two pathogens.

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