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Research article

# Distribution of *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*) after oral inoculation

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## Abstract

*Streptococcus agalactiae* is an important pathogen that causes severe mortality in Nile tilapia within a short period of time. The objective of this study was to determine the distribution of *S. agalactiae* in fish organs at various intervals of time after oral inoculation in order to understand the systemic dissemination of *S. agalactiae* after inducing the fish with the disease. Thirty Nile tilapia with an average weight of 14 g were used in this study. Nile tilapia (N=27) were each orally infected with 0.3 ml of bacterial concentration of $5.92x10^8$ CFU/ml and were maintained out of water for 5 min. Three fish were consecutively and randomly sampled within 30 min to 48 h. The control fish (N=3) were uninfected with *S. agalactiae* and sampled prior to *S. agalactiae* inoculation. All samples were investigated for *S. agalactiae* by bacteriological and polymerase chain reaction (PCR) techniques. *S. agalactiae* was recovered from the stomach, intestine, spleen, liver, kidneys and eyes after 30 min post inoculation but for the heart and brain, where were recovered *S. agalactiae* at 1 h post inoculation. All samples consistently recovered *S. agalactiae* by the end of the experiment, except the intestine and the eyes following 48 h of a period. PCR was performed on all sampled fish organs throughout the experiment. The results in this study demonstrated that the gastrointestinal tract successfully produced *S. agalactiae* infection that was then differently disseminated throughout important organs in the Nile tilapia.

**Keywords:** : Distribution, Nile tilapia, Oral inoculation, *Streptococcus agalactiae*

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## INTRODUCTION

Streptococcosis induced by *Streptococcus* sp. has become one of the most important fish diseases (Abuseliana et al., 2011) and caused some significant economic losses in the world aquaculture industry (Klesius et al., 2000). The main *Streptococcus* spp. that is affecting fish aquaculture are *S. iniae* (syn. *S. shiloi*) and *S. agalactiae* (syn. *S. difficili*) (Soto et al., 2016; Iregui et al., 2015). Both bacterial pathogens are affecting the Hybrid striped bass (*Morone chrysops* X *Morone saxalitis*) (Mcnulty et al., 2003; Evans et al., 2000), Barramundi (*Lates calcarifer*) (Bromage and Owens, 2002), Japanese flounder (*Paralichthys olivaceus*) (Nguyen et al., 2001), Golden shiners (*Notemigonus crysoleucas*) (Robinson and Meyer, 1966), Wild mullet (*Liza klunzingeri*), Seabream (*Sparus auratus*) (Evans et al., 2002), Ya-Fish (*Schizothorax prenanti*) (Geng et al., 2012), Queensland grouper (*Epinephelus lanceolatus*) (Delamare-Debouttevillle et al., 2015), Hybrid tilapia (*Oreochromis niloticus* X *Oreochromis aureus*) (Perera et al., 1997), Red tilapia (*Oreochromis* sp.) (Hernandez et al., 2009; Abuseliana et al., 2011; Abdullah et al., 2013) and Nile tilapia (*Oreochromis niloticus*) (Evans et al., 2000; Mian et al., 2009; Geng et al., 2012; Soto et al., 2016). The disease is characterised by an erratic swimming, anorexia, lethargy, exophthalmia, corneal opacity (Eldar et al., 1995; Austin and Austin, 2007). In the past decade, *S. agalactiae* has emerged as a major Nile tilapia pathogen and was frequently isolated from natural outbreaks (Suanyuk et al., 2008; Mian et al., 2009; Abuseliana et al., 2010; Geng et al., 2012; Li et al., 2014; Soto et al., 2016; Laith et al., 2017), which caused mortality within 3 days (Azad et al., 2012).

Experimental infection with *S. agalactiae* has been achieved using different exposure routes, including intraperitoneal (IP) injection in the Nile tilapia (Geng et al., 2012; Soto et al., 2016), Red tilapia (Abdullah et al., 2013), Queensland grouper (Delamare-Debouttevillle et al., 2015) and the Ya-Fish (Geng et al., 2012), intramuscular (IM) injection in the Nile tilapia (Soto et al., 2016), immersion exposure in the Nile tilapia (Geng et al., 2012; Soto et al., 2016), the Red tilapia (Abdullah et al., 2013) and Queensland grouper (Delamare-Debouttevillle et al., 2015),gills exposure in Nile tilapia (Mian et al., 2009) and oral inoculation in the Nile tilapia (Geng et al., 2012; Soto et al., 2016), the Red tilapia (Abdullah et al., 2013) and the Queensland grouper (Delamare-Debouttevillle et al., 2015). Naturally, *S. agalactiae* infection by intragastric route is probably the most important way of infecting fish culture conditions (Delamare-Debouttevillle et al., 2015; Iregui et al., 2015).

Although *S. agalactiae* could distribute and lead to the lesions in Red tilapia tissues in intraperitoneal injection, immersion cut and immersion exposures at 4 h pi (Abdullah et al., 2013) and in immersion exposure and oral inoculation at 30 min and 6 h pi, respectively (Iregui et al., 2015), the information of the early phases of *S. agalactiae* distribution in Nile tilapia has not been reported. In this study, we have investigated the distribution of *S. agalactiae* in the Nile tilapia organs at different times following oral inoculation in order to understand the early phases of the infection and the disease.

# MATERIALS AND METHODS

## Fish

Nile tilapia (*Oreochromis niloticus*) fingerling with an average weight of 14 g were obtained from a commercial production farm in the Chiang Mai province. For verification, the fish were checked for clinical signs of streptococcosis and were sampled for bacterial culture to ensure that they were free of *S. agalactiae*. The fish were maintained in a 1000-litre-fibreglass tank for at least 2 weeks before conducting the experiment and fed daily a commercial feed at a 2% volume of the fish body weight. The animal experiments in this study were approved and conducted in accordance with the guidelines of the Faculty of Veterinary Medicine, Chiang Mai University Animal Care and Use Committee (FVM-ACUC), approval no. S19/2017.

## Bacteria

*S. agalactiae* serotype III was isolated from naturally infected Tilapia (*Oreochromis* sp.) in some floating cage in the Chiang Mai province. The bacteria were identified using the standard conventional methods, biochemical characteristics by API 20 STREP system (BioMerieux, France), dividing the Streptococcal grouping base on Lancefield serogrouping by Streptococcal grouping kits (Oxoid, UK) and identified by the amplification and sequencing of the16S rRNA gene. The bacteria were grown in a Brain Heart Infusion (BHI) broth supplemented with 15% glycerol and stored at -80°C until use. The bacteria used in this study was approved by and complied with the guideline of the Chiang Mai University Institutional Biosafety Committee (CMU-IBC), approval no. CMUIBC A–0560003.

## Preliminary of *S. agalactiae* infection

Nile tilapia was injected by intraperitoneal (IP) with 0.1 ml of *S. agalactiae* concentration of $10^8$ CFU/ml, which was counted by total plate count technique, using a 1-ml syringe connected to a needle twice before the experiment started. For the initial preliminary trials of oral *S. agalactiae* infection in the Nile tilapia, results had showed that the fish maintained under normal conditions (life friendly environment) would rarely die as a result of the infection, in other words that the mortality rate was very low. Based on those results, the conditions were adjusted as well as some factors, including an increase of 0.3 ml in the volume of *S. agalactiae* concentration containing $10^8$ CFU/ml and an increase of 5 min in the stress time in order to accelerate the mortality from 15% to 20% at which the fish started to die from day 4 to day 1 post-infection (pi), respectively. Finally, the sampling time designed to measure the duration before death occurred as well as the adjusted conditions above were used for further study.

## Inoculation of *S. agalactiae* by oral route

Twenty-seven Nile tilapia were inoculated intragastrically with 0.3 ml of bacterial concentration containing $5.92 \times 10^8$ CFU/ml, using a 1-ml syringe connected to 24 gauges of a plastic catheter (BD medical system, USA). The

tube was slowly slipped along the mouth of the anaesthetised fish through the oesophagus until it reached the stomach and were maintained the fish out of the water for 5 min. The fish were returned to their respective tank. Three control fish were not inoculated.

## Tissue sampling

Three orally infected fish were randomly sampled at different times, including 30 min, 1, 3, 6, 12, 18, 24, 36 and 48 h pi. Samples of stomach, intestine, spleen, anterior kidneys, liver, heart, eyes and brain of infected fish were investigated for *S. agalactiae* by bacteriological examination and PCR analysis. Three control fish were sampled before the experimental infection and were investigated using the same methods.

## Bacteriological examination

Samples of each organ from individually infected fish at each time were streaked on Tryptic Soy Agar (TSA) (Merck, USA) and incubated at 28°C for 48 h. The presence of colonies was subsequently identified by Gram staining, catalase test, oxidase test, CAMP test and lancefield serogrouping test (Oxoid, UK) for confirming the *S. agalactiae* infection.

## Polymerase Chain Reaction (PCR) analysis

Samples of each organ from three orally infected fish at each time were combined into the micro-centrifuge and stored at -20°C until use. DNA was subsequently extracted from the samples using a DNA extraction kit (Wacherey-Nagel, Germany) according to the manufacturer's protocol and it was then further evaluated by PCR. The universal primers of V1 (5′-TTTGGTGTTTACACTAGACTG-3′) and V2 (5′-TGTGTTAATTACT CTTATGCG-3′) were used to amplify the 16S rRNA gene as previously described by Meiri-Bendek et al. (2002). The reaction conditions for PCR were as follow: the initial hold at 94°C for 4 min, 30 cycles of denaturation step of 94°C for 45 s, an annealing step of 50°C for 45 s, an extension step of 72°C for 45 s and an indefinite final hold at 4°C. The PCR products were detected by electrophoresis through a 1.5% (w/v) agarose gel in 1X Tris Borate EDTA (TBE). The gel was stained with RedSafe nucleic acid staining (iNtRon Biotechnology, Korea). The DNA of *S. agalactiae* originally infected in fish was used as positive control while the Rnase/Dnase-free water was used as negative control. The amplification products were expected to yield approximately 120 bp for assuming the presence of *S. agalactiae*.

## Water quality evaluation

The water temperature, dissolved oxygen (DO) and pH were measured daily using a thermometer (SK SATO, Japan), a DO meter version YSI 550A (YSI, USA) and a pH meter (Eutech Instruments, Singapore), respectively. The ammonia and nitrite were determined daily following the Ammonia and Nitrite-measurement's protocols as described by Clesceri et al. (1998). The average temperature was 28°C, the average dissolved oxygen (DO) was 4.35 mg/l, the average pH was 8.17, the average ammonia was 0.03 mg/l and the average nitrite was 0.02 mg/l.

# RESULTS

## Bacteriological examination

*S. agalactiae* could be recovered from the stomach, intestine, spleen, anterior kidneys, liver and eyes of the infected fish after 30 min pi; while from the heart and brain at 1 h pi (Table 1). At the subsequent sampling time, the presence of *S. agalactiae* was evident in all sampled organs throughout the experimental period, except for the intestine and the eyes at 48 h pi. The fish of control group were not investigated for *S. agalactiae*.

**Table 1** Bacteriological examination of *Streptococcus agalactiae* in Nile tilapia infected by oral route

| Sampling time | N | Sampled organs | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | St | I | Sp | K | L | H | E | B |
| **30 min** | 3 | + | + | + | + | + | - | + | - |
| **1 h** | 3 | + | + | + | + | + | + | + | + |
| **3 h** | 3 | + | + | + | + | + | + | + | + |
| **6 h** | 3 | + | + | + | + | + | + | + | + |
| **12 h** | 3 | + | + | + | + | + | + | + | + |
| **18 h** | 3 | + | + | + | + | + | + | + | + |
| **24 h** | 3 | + | + | + | + | + | + | + | + |
| **36 h** | 3 | + | + | + | + | + | + | + | + |
| **48 h** | 3 | + | - | + | + | + | + | - | + |
| **Control fish** | 3 | - | - | - | - | - | - | - | - |

B=brain, E=eye, H=heart, I=intestine, K=anterior kidney, L=liver, St=stomach, Sp=spleen

Presence of *S. agalactiae*: +, presence; -, no presence

Data are from three fish at each sampling time.

The colonies of bacteria isolated from sampled fish organs were grown on TSA media after incubation at 28°C for 48 h. These colonies were white in color, translucent to slight opaque, round, convex and 1-2 mm in diameter. Basic physiological characterization of the bacteria indicated some characteristics of *Streptococcus* spp.: they were Gram-positive cocci cells in pairs or chains, catalase and oxidase negative. The bacteria were characterized further by a CAMP test and a Lancefield serogrouping test. The CAMP test results were shown the positive results. In order to use a commercial Streptococcal grouping kit base on the Lancefield serogrouping, the bacteria were reacted serologically with a group B antiserum. Base on the results of all these tests, the bacterial characteristics were found to be presumptive as *S. agalactiae*.

## Polymerase Chain Reaction detection

The PCR was performed on all sampled organs as early as 30 min pi and remaining throughout the experiment (Table 2). The fish of control group were not detected for *S. agalactiae*. The amplification of extracted DNA from sampled organs of infected fish was positively present in 1.5% agarose gel as shown in Figure 1.

**Table 2** PCR detection of *Streptococcus agalactiae* in Nile tilapia infected by oral route.

| Sampling time | N | Sampled organs | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | St | I | Sp | K | L | H | E | B |
| **30 min** | 3 | + | + | + | + | + | + | + | + |
| **1 h** | 3 | + | + | + | + | + | + | + | + |
| **3 h** | 3 | + | + | + | + | + | + | + | + |
| **6 h** | 3 | + | + | + | + | + | + | + | + |
| **12 h** | 3 | + | + | + | + | + | + | + | + |
| **18 h** | 3 | + | + | + | + | + | + | + | + |
| **24 h** | 3 | + | + | + | + | + | + | + | + |
| **36 h** | 3 | + | + | + | + | + | + | + | + |
| **48 h** | 3 | + | + | + | + | + | + | + | + |
| **Control fish** | 3 | - | - | - | - | - | - | - | - |

B=brain, E=eye, H=heart, I=intestine, K=anterior kidney, L=liver, St=stomach, Sp=spleen

Presence of *S. agalactiae*: +, presence; -, no presence

Data are from three fish at each sampling time.

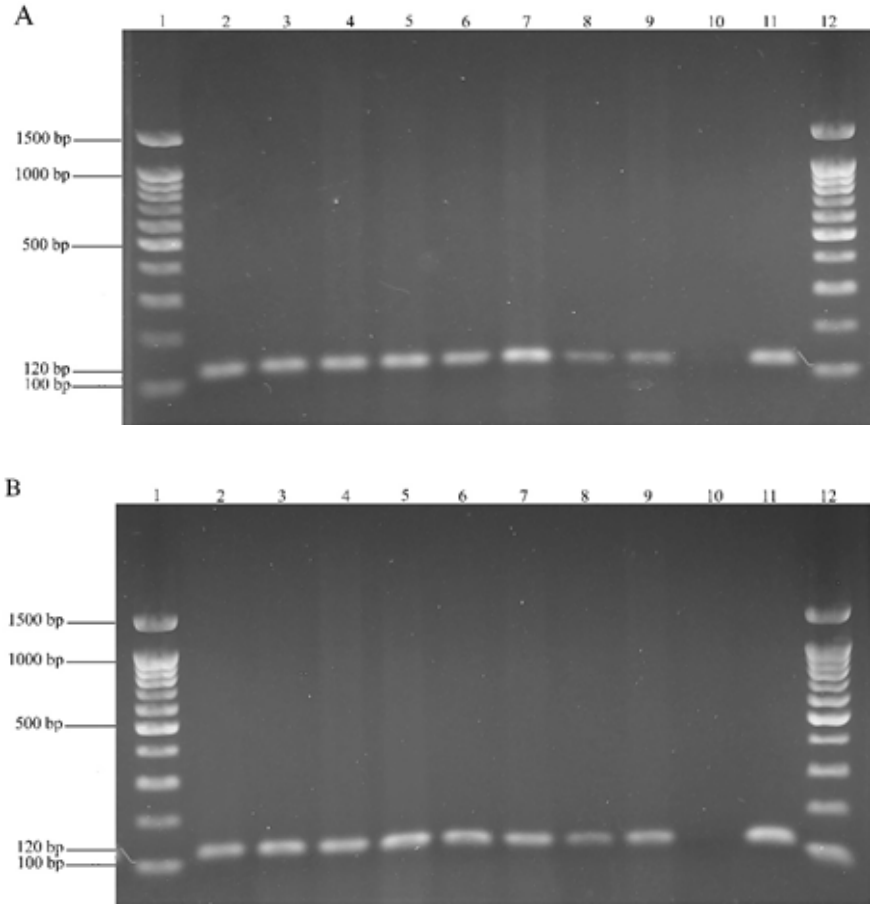


**Figure 1** The DNA amplification extracted from different organs of infected fish at 30 min pi (A) and 48 h pi (B): lane 1 and 12, DNA marker; lane 2, stomach; lane 3, intestine; lane 4, spleen; lane 5, kidney; lane 6, liver; lane 7, heart; lane 8, eyes; lane 9, brain; lane 10 positive control (*S. agalactiae*) and lane 11, negative control (no DNA).

## DISCUSSION

*Streptococcus agalactiae* infection is a serious disease in Tilapia (*Oreochromis* spp.) production and affects the mortality rate within a short time. The study of bacterial distribution has led to remarkable progress on the understanding of the early phases of the *S. agalactiae* infection in the Nile tilapia model. However, the information on the *S. agalactiae* infection in the Nile tilapia simulating a site of entry that most resembles a natural infection is still poorly understood. The results of this study demonstrated that the gastrointestinal (GI) tract could induce *S. agalactiae* infection in the Nile tilapia. In addition, the presence of *S. agalactiae* in different fish organs was confirmed after 30 min pi and remained strongly evident throughout the experiment, when using oral inoculation. Our results were very close to the distribution pattern of *S. agalactiae* infection in the Red tilapia (*Oreochromis* sp.), which also found the presence of *S. agalactiae* in different fish organs after oral inoculation with 0.3 ml of bacterial concentration of $10^7$ CFU/ml at 30 min (Iregui et al., 2015).

The infectivity and distribution of *S. agalactiae* in fish may be related to the closely fish species, the concentration and volume of bacteria as well as the route of infection. Moreover, Iregui et al. (2015) suggested that *S. agalactiae* does not only resist the acid pH of the stomach, but that it may also indicate that the bacteria are able to multiply in the mucus layer of both the apical surface and the cytoplasm of the epithelial cells of the GI tract.

In this results, *S. agalactiae* could not be recovered from the heart nor the brain of orally infected fish at the first sampling time (30 min pi) neither from the intestine nor the eyes at 48 h pi. This study suggests that the heart, which moves blood rapidly through its chambers, might have limited contact with the bacteria (Alonzo et al., 2011). For the brain, the bacteria may modify their pili in order to destabilize pilus fiber interactions, leading to the detachment of the bacteria from the original site to crossing the blood-brain barrier (Ribet & Cossart, 2015). Thus, the number of *S. agalactiae* in both the heart and the brain of infected fish at 30 min pi may have been too small to be recovered and grown as a colony on culture media. Besides, *S. agalactiae* in the intestine and the eyes may decrease the number after 48 h pi. The tight balance between the self-renewal of cells and the elimination as well as the enteric pathogen of the intestine may limit the infection and persistent colonization of the bacteria in their tissue (Ribet & Cossart, 2015). In addition, the eye may produce the soluble immune mediators and recruitment of phagocytic inflammatory cells to the site of infection (Callegan et al., 2002). However, the PCR enabled detection of *S. agalactiae* on all sampled organs of infected fish at 30 min pi and on all samples throughout the experiment; this confirmed the previous results of our study, where the PCR technique proved to be a more sensitive detection than the bacteriological technique in natural cases of Streptococcosis (Hernandez et al., 2009) and in the experimental infection of *S. agalactiae* in Queensland grouper (*Epinephelus lanceolatus*) (Delamare-Debouteville et al., 2015). The results of this study suggest that the PCR technique could be suitable and applied to detection of *S. agalactiae* from Streptococcosis in fish.

In natural cases of occurring Streptococcosis, the eyes and brain are common targets for tissue changes (Abuseliana et al., 2011). In this study, *S. agalactiae* was detected in the samples originating from the eyes and brain of orally infected fish after 30 min pi. Our results suggest that *S. agalactiae* is capable of invading the fish tissue and of penetrating the blood-brain barrier, resembling the invasion pattern of *S. iniae* in the Nile tilapia (Evans et al., 2001). Moreover, based on the detection of *S. agalactiae* from the eyes of infected fish in this study, we suggest that the bacteria are capable of tissue invasion, possibly leading to septicaemia. Similar to our study results, Evans et al. (2000) documentation on the *S. iniae* infection suggested that the bacteria are colonised hematogenously but that they do not play any role in the initial entry site. However, throughout the experiment, the presence of *S. agalactiae* in different fish organs as well as in the organs usually targeted by the disease, especially the eyes and the brain, demonstrated that *S. agalactiae* infection in the Nile tilapia does not develop any clinical signs of the disease until 48 h after the initial oral inoculation. Similarly, the distribution of *S. agalactiae* concentration of $10^7$ CFU/ml in Red tilapia could not be developed the clinical sings of disease either within 96 h after oral and immersion exposures (Iregui et al., 2015). On

the other hand, Red tilapia displayed an erratic swimming, c-shaped body curvature and hemorrhage around the eyes, operculum, fin and/or body at 2, 4 and 8 h after infected with $10^9$ CFU/ml by IP injection, immersion and immersion cut (0.5 cm) at the cuadal part (Abdullah et al., 2013). This study suggests that the differences of strain, concentration and volume of bacteria, fish species as well as the infection routs may be related to the infection processes leading to the starting presence of the clinical signs.

## CONCLUSION

The results of this study demonstrated that the gastrointestinal (GI) tract could induce *S. agalactiae* infection in the Nile tilapia. We have found that the bacteria were distributed into different fish organs and had penetrated the blood-brain barrier, which is the target organ of Streptococcosis in fish although the infection process did not reveal any clinical signs of the disease.

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## REFERENCES

Abuseliana, A., Daud, H., Aziz, S. A., Bejo, S. K., Alsaid, M. 2010. *Streptococcus agalactiae* the etiological agent of mass mortality in farmed Red tilapia (*Oreochromis* sp.). J. Anim. Vet. Adv. 9, 2640-2646.

Abuseliana, A. F., Daud, H. H. M., Aziz, S. A., Bejo, S. K., Alsaid, M. 2011. Pathogenicity of *Streptococcus agalactiae* isolated from a fish farm in Selangor to juvenile Red tilapia (*Oreochromis* sp.). J. Anim. Vet. Adv. 10, 914-919.

Abdullah, S., Omar, N., Yusoff, S. M., Obukwho, E. B., Nwunuji, T. P., Hanan, L., Samad, J. 2013. Clinicopathological features and immunohistochemical detection of antigens in acute experimental *Streptococcus agalactiae* infection in red tilapia (*Oreochromis* spp.). Springerplus 2, 286.

Alonzo, F., Bobo, L. D., Skiest, D. J., Freitag, N. E. 2011. Evidence for subpopulations of *Listeria monocytogenes* with enhanced invasion of cardiac cells. J. Med. Microbiol. 60, 423-434.

Austin, B., Austin, D. A., 2007. Bacterial fish pathogens. Diseases of farmed and wild fish. 4th ed. Springer/Prazis Publishing, Chichester. pp. 17-18.

Azad, I. S., Al-Marzouk, A., James, C. M., Almatar, S., Al-Gharabally, H., Qasem, J. A. 2012. Outbreak of natural Streptococcosis in hatchery produced Silver pomfret (*Pampus argenteus Euphrasen*) larvae in Kuwait. Aquaculture. 330-333, 15-20.

Bromage, E. S., Owens, L., 2002. Infection of Barramundi *Lates calcarifer* with *Streptococcus iniae*: effects of different routes of exposure. Dis. Aquat. Org. 52, 199-205

Callegan, M. C., Engelbert, M., Parke, D. W., Jett, B. D., Gilmore, M. S. 2002. Bacterial endophthalmitis: epidemiology, therapeutics, and bacterium-host interactions. Clin. Microbiol. Rev. 15, 111-124.

Clesceri, L. S., Green, A. E., Eaton, A. D., 1998. Standard methods for the examination of water and wastewater. 20th ed. United Book Press, Maryland. pp. 103-114.

Delamare-Deboutteville, J., Bowater, R., Condon, K., Reynolds, A., Fisk, A., Aviles, F., Barnes, A. C., 2015. Infection and pathology in Queensland grouper, *Epinephelus lanceolatus*, (Bloch), caused by exposure to *Streptococcus agalactiae* via different routes. J. Fish Dis. 38, 1021-1035.

Eldar, A., Bejerano, Y., Livoff, A., Horovitcz, A., Bercovier, H., 1995. Experimental streptococcal meningo-encephalitis in cultured fish. Vet. Microbiol. 43, 33-40.

Evans, J. J., Shoemaker, C. A., Klesius, P. H., 2000. Experimental *Streptococcus iniae* infection of hybrid striped bass (*Morone chrysops* x *Morone saxatilis*) and tilapia (*Oreochromis niloticus*) by nares inoculation. Aquaculture. 189, 197-210.

Evans, J. J., Shoemaker, C. A., Klesius, P. H., 2001. Distribution of *Streptococcus iniae* in hybrid striped bass (*Morone chrysops* x *Morone saxatilis*) following nares inoculation. Aquaculture. 194, 233-243.

Evans, J. J., Klesius, P. H., Glibert, P. M., Shoemaker, C. A., Al-Sarawi, M. A., Landsberg, J., Al-Zenki, S., 2002. Characterization of beta-haemolytic Group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* L., and wild mullet, *Liza klunzingeri* (Day), in Kuwait. J. Fish Dis. 25, 505-513.

Geng, Y., Wang, K. Y., Huang, X. L., Chen, D. F., Li, C. W., Ren, S. Y., Lai, W. M., 2012. *Streptococcus agalactiae*, an emerging pathogen for cultured Ya-Fish, *Schizothorax prenanti*, in China. Transbound. Emerg. Dis. 59, 369-375.

Hernandez, E., Figueroa, J., Iregui, C., 2009. Streptococcosis on a red tilapia, *Oreochromis* sp., farm: a case study. J. Fish Dis. 32, 247-252.

Iregui, C. A., Comas, J., Vasquez, G. M., Verjan, N., 2015. Experimental early pathogenesis of *Streptococcus agalactiae* infection in red tilapia (*Oreochromis* spp.) J. Fish Dis. 39, 205-215.

Klesius, P. H., Shoemaker, C. A., Evans, J. J., 2000. Efficacy of single and combined *Streptococcus iniae* isolate vaccine administered by intraperitoneal and intramuscular routes in tilapia. Aquaculture. 188, 237-246.

Laith, A. A., Ambak, M. A., Hassan, M., Sheriff, S. M., Nadirah, M., Draman, A. S., Najiah, M., 2017. Molecular identification and histopathological study of natural *Streptococcus agalactiae* infection in hybrid tilapia (*Oreochromis niloticus*). Veterinary World. 10, 101-111.

Li, Y. W., Liu, L., Huang, P. R., Fang, W., Luo, Z. P., Peng, H. L., Li, A. X., 2014. Chronic streptococcosis in Nile tilapia, *Oreochromis niloticus* (L.), caused by *Streptococcus agalactiae*. J. Fish Dis. 37, 757-763.

McNulty, S. T., Klesius, P. H., Shoemaker, C. A., Evans, J. J., 2003. *Streptococcus iniae* infection and tissue distribution in hybrid striped bass (*Morone chrysops* x *Morone saxatilis*) following inoculation of the gills. Aquaculture. 220, 165-173.

Meiri-Bendek, I., Lipkin, E., Friedmann, A., Leitner, G., Saran, A., Friedman, S., Kashi, Y., 2002. A PCR-based method for the detection of *Streptococcus agalactiae* in milk. J. Dairy Sci. 85, 1717-1723.

Mian, G. F., Godoy, D. T., Leal, C. A. G., Yuhara, T. Y., Costa, G. M., Figueiredo, H. C. P., 2009. Aspects of the natural history and virulence of *S. agalactiae* infection in Nile tilapia. Vet. Microbiol. 136, 180-183.

Nguyen, H. T., Kanai, K., Yoshikoshi, K., 2001. *Streptococcus iniae* infection in Japanese flounder *Paralichthys olivaceus*. Fish Pathol. 36, 40-41.

Perera, R. P., Johnson, S. K., Lewis, D. H., 1997. Epizootiological aspects of *Streptococcus iniae* affecting tilapia in Texas. Aquaculture. 152, 25-33.

Ribet, D., Cossart, P., 2015. How bacterial pathogens colonize their hosts and invade deeper tissues. Microbes Infect. 17, 173-183.

Robinson, J. A., Meyer, F. P., 1966. Streptococcal fish pathogen. J. Bacteriol. 92, 512.

Soto, E., Zayas, M., Tobar, J., Illanes, O., Yount, S., Francis, S., Dennis, M. M., 2016. Laboratory-controlled challenges of Nile tilapia (*Oreochromis niloticus*) with *Streptococcus agalactiae*: Comparisons between immersion, oral, intracoelomic and intramuscular routes of infection. J. Comp. Pathol. 155, 339- 345.

Suanyuk, N., Kong, F. R., Ko, D., Gilbert, G. L., Supamattaya, K. 2008. Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia (*Oreochromis* sp.) and Nile tilapia (*O. niloticus*) in Thailand-Relationship to human isolates. Aquaculture. 284, 35-40.

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