



UNIVERSITI PUTRA MALAYSIA

***HISTOPATHOLOGICAL COMPARISON BETWEEN
EXPERIMENTAL STREPTOCOCCUS AGALACTIAE AND
STREPTOCOCCUS INIAE INFECTIONS IN OREOCHROMIS SP.***

DZULKIFLI BIN JAMALLUDIN

FPV 2018 26

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**FACULTY OF VETERINARY MEDICINE
UNIVERSITY PUTRA MALAYSIA
SERDANG SELANGOR**

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DZULKIFLI BIN JAMALLUDIN

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
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Serdang, Selangor DarulEhsan

MARCH 2018

CERTIFICATION

It is hereby certified that we have read this project paper entitled “Histopathological comparison between experimental *Streptococcus agalactiae* and *Streptococcus iniae* infections in *Oreochromis* sp.”, by Dzulkipli Bin Jamalludin and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement of the course VPD 4901 – Project.

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DEDICATION

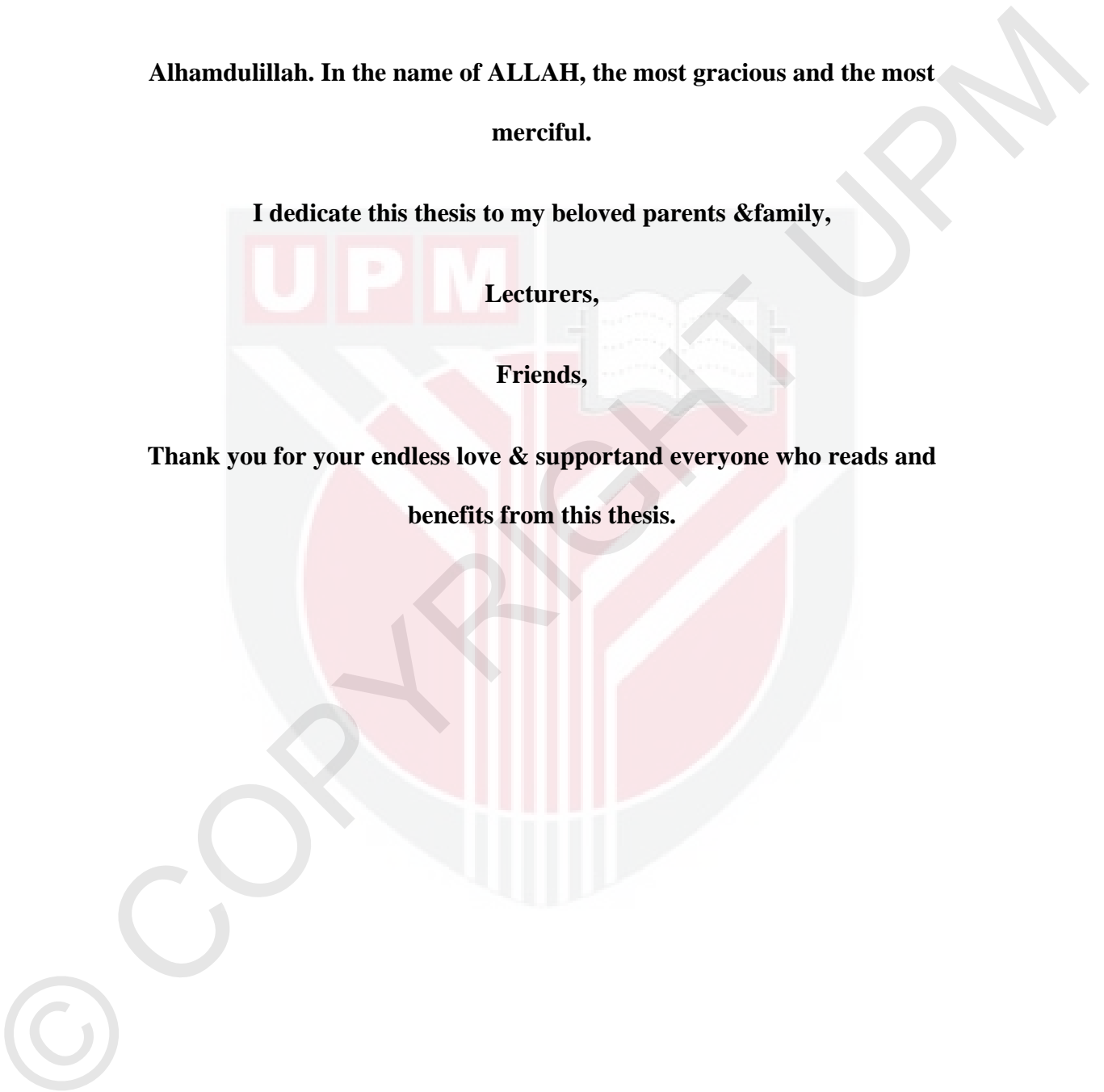
Alhamdulillah. In the name of ALLAH, the most gracious and the most merciful.

I dedicate this thesis to my beloved parents & family,

Lecturers,

Friends,

Thank you for your endless love & support and everyone who reads and benefits from this thesis.



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CONTENTS

	Page
TITLE.....	
CERTIFICATION.....	i
DEDICATION.....	iii
ACKNOWLEDGEMENTS.....	iv
CONTENTS.....	vi
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
ABSTRACT.....	x
ABSTRAK.....	xiii
1.0 INTRODUCTION.....	1
2.0 LITERATURE REVIEW.....	4
2.1 Streptococcus sp.....	4
2.1.1 <i>Streptococcus iniae</i>	
2.1.2 <i>Streptococcus agalactiae</i>	
2.2 Pathogenicity of Streptococcus sp.....	5
2.3 Streptococcosis disease.....	7
2.4 Diagnosis.....	7

2.4.1	Gross lesion of Streptococcosis	
2.4.2	Identification and isolation of <i>S. agalactiae</i> & <i>S. iniae</i>	
2.5	Treatment, Prevention and Control.....	11
3.0	MATERIALS AND METHODS.....	12
3.1	Experimental fish.....	12
3.2	Preparation of <i>S. agalactiae</i> & <i>S. iniae</i> inoculum.....	13
3.3	Pilot study.....	13
3.4	Bacterial concentration determination.....	14
3.5	Study design.....	14
3.6	Histological examination and evaluation.....	15
3.7	Statistical analysis.....	16
4.0	RESULTS AND ANALYSIS.....	17
4.1	Gross lesions.....	17
4.2	Histopathological lesions.....	18
5.0	DISCUSSION.....	23
6.0	CONCLUSION.....	25
7.0	REFERENCES.....	26

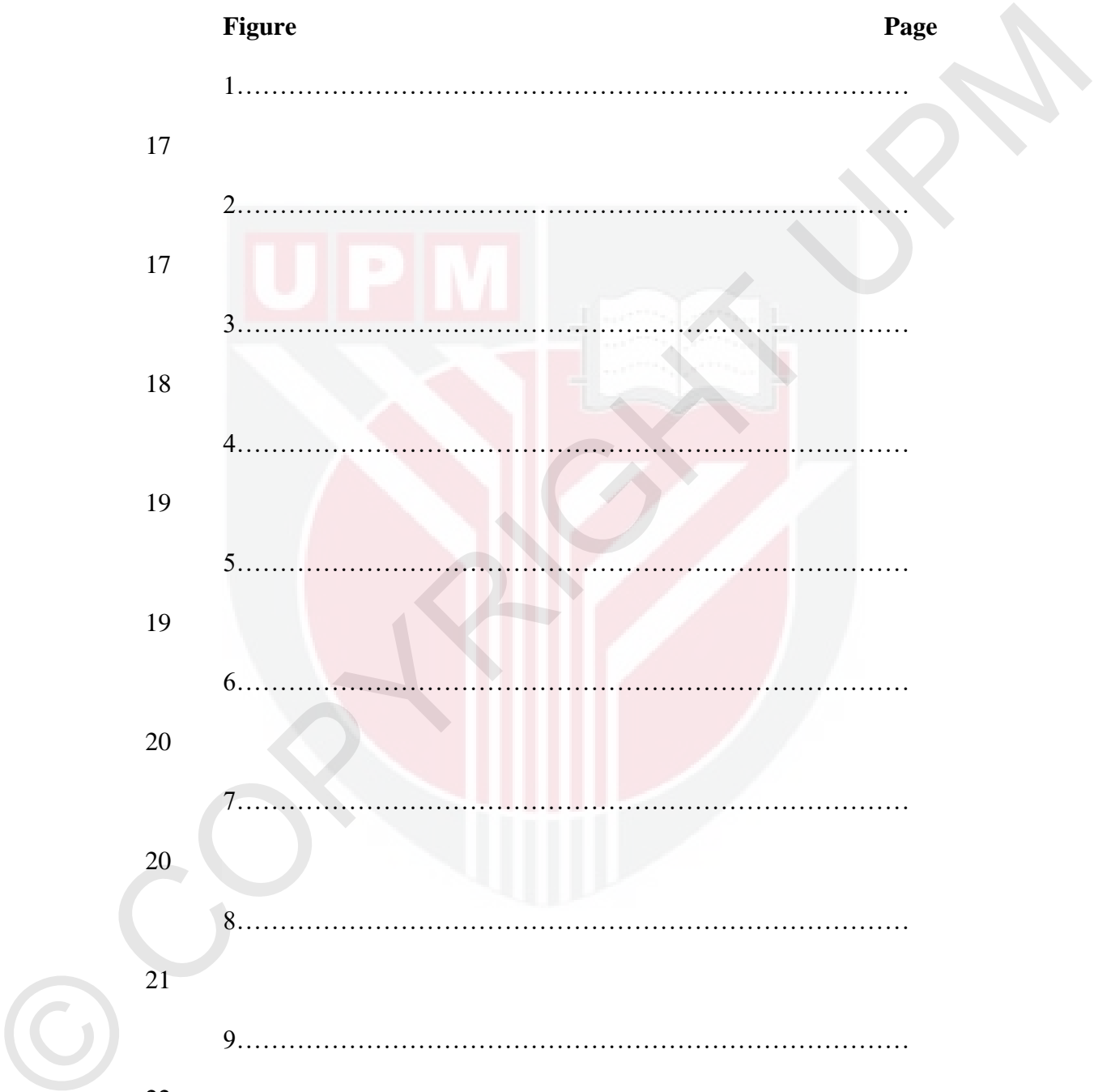
LIST OF TABLES

Table	Page
Table 1: Example of scoring table.....	16



LIST OF FIGURES

Figure	Page
1.....	17
2.....	17
3.....	18
4.....	19
5.....	19
6.....	20
7.....	20
8.....	21
9.....	22



ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine, UPM in partial requirement to fulfil the course of VPD 4999-Final Year Project.

**HISTOPATHOLOGICAL COMPARISON BETWEEN
EXPERIMENTAL STREPTOCOCCUS
AGALACTIAE AND STREPTOCOCCUS INIAE INFECTIONS IN
OREOCHROMIS SP.**

By

Dzulkifli Jamalludin

2018

Supervisor: Dr AnnasSalleh

Streptococcus agalactiae and *streptococcus iniae* are two main pathogens contributing to streptococcosis in fish. Streptococcosis is an important disease, leading to economic losses in aquaculture industry. The aim of this study was to compare the histopathological evaluations between *S. agalactiae* and *S. iniae* infections in *Oreochromis* sp.. Two experiments were conducted. In the first experiment, 60 healthy fish with the size of less than 4" were divided equally into three (3) groups; one control (G1) (n=20) and two treatment groups (G2, G3) (n=20) each. Each group was further divided equally into two groups, consisting of 10 inoculated fish and 10 fish were not inoculated but served as commingling fish. The former were inoculated with sterile PBS, *S. agalactiae*, and *S. iniae*, intraperitoneally at

the rate of 0.03 ml/g of 1×10^7 cfu/ml of bacteria. In the second experiment, the same experimental design was used, with the exception of the size of fish. Fish with more than 4" were used. G4 was inoculated with sterile PBS, G5 was inoculated with *S. agalactiae*, and G6 was inoculated with *S. iniae* at the aforementioned rate. All fish were observed every 6 hours for a period of 5 days. During the observation period, any dead fish was subjected to necropsy. After the observation period, any remaining fish were euthanized for necropsy. Samples of spleen, liver, and brain were collected and fixed in 10% neutral buffered formalin, and subjected to routine histopathology process. Gross lesions observed included cerebral oedema, ascites, integumentary haemorrhage, presence of pustules, and intestinal congestion. Tissue section of each organ was examined under microscope at 40x magnification and lesions were described and scored. In all infected groups, infiltration of inflammatory cells and haemorrhage were the most frequently observed lesion. All groups infected by *S. agalactiae* showed significantly ($p < 0.05$) more severe lesions compared to the groups infected by *S. iniae*, regardless of the size. Significant difference ($p < 0.05$) was also observed in comparison between the lesion score in fish of different sizes. Fish of more than 4" showed higher lesion severity score compared to fish of less than 4". In both streptococcosis, *S. agalactiae* is more pathogenic than *S. iniae* in *Oreochromis* sp., and fish more than 4" is more susceptible towards both streptococcosis.

Keywords: Streptococcosis, *Streptococcus agalactiae*, *Streptococcus iniae*, histopathology, *Oreochromis* sp. (tilapia)



ABSTRAK

Abstrak daripada kertas project yang dikemukakan kepada Fakulti Perubatan Veterinar UPM untuk memenuhi sebahagian daripada keperluan kursus VPD 4999- Projek Tahun Akhir.

PERBANDINGAN HISTOPATOLOGIKAL DI ANTARA JANGKITAN EXPERIMENTAL *STREPTOCOCCUS AGALACTIAE* DAN *STREPTOCOCCUS INIAEDI* DALAM *OREOCHROMIS SP.*

Oleh

Dzulkifli Jamalludin

2018

Supervisor: Dr Annas Salleh

Streptococcus agalactiae dan *Streptococcus iniae* adalah dua pathogen utama yang menyebabkan streptococcosis di dalam ikan. Streptococcosis merupakan satu penyakit yang sangat penting di mana ia boleh membawa kepada kerugian ekonomi di dalam industry akuakultur. Tujuan penyelidikan ini adalah bagi membandingkan penilaian histopatologikal diantara jangkitan *S.agalactiae* dan *S.iniae* di dalam *Oreochromis sp.*. Dua eksperimen telah dijalankan. Dalam eksperimen yang pertama, 60 ekor ikan tilapia yang sihat berukuran kurang dari 4” telah dibahagikan sama rata kepada tiga (3) kumpulan; satu kawalan (G1) (n=20) dan 2 kumpulan rawatan (G2, G3) (n=20) setiap satu. Setiap kumpulan dibahagikan lagi secara sama rata kepada dua kumpulan yang terdiri daripada 10 ekor ikan diinokulasi dan 10 ekor ikan tidak diinokulasi tetapi bertindak sebagai ikan yang bergaul. Ikan yang diinokulasi telah diinokulasi dengan steril PBS, *S. agalactiae*, dan *S. iniae*, secara

intraperitoneal dengan kadar 0.03ml/g mengandungi 1×10^7 cfu/ml bakteria. Di dalam eksperimen kedua, kaedah eksperimen yang sama juga digunakan, dengan pengecualian saiz ikan yang digunakan iaitu ikan yang berukuran lebih dari 4". G4 telah diinokulasi dengan steril PBS, G5 diinokulasi dengan *S. agalactiae*, dan G6 telah diinokulasi dengan *S. iniae* menggunakan kadar yang telah disebutkan. Keseluruhan ikan telah diperhatikan setiap 6 jam dalam tempoh masa 5 hari. Ketika dalam tempoh pemerhatian, sebarang ikan yang mati akan terus melalui nekropsi. Selepas tempoh pemerhatian, mana-mana ikan yang masih hidup akan ditakai bagi tujuan nekropsi. Sampel; limpa, hati dan otak dipungut dan disimpan di dalam 10% neutral buffer formalin, dan diteruskan dengan proses rutin histopatologi. Lesi kasar yang dapat dilihat termasuklah permukaan berair di bahagian otak, air di dalam rongga abdomen, pendarahan integumentary, penampakan nanah dan penyesakan usus. Seksyen tisu setiap organ telah diteliti di bawah mikroskop pada kadar magnifikasi 40x dan setiap lesi dijelaskan dan dinilai. Di dalam semua kumpulan jangkitan, kehadiran selkera dangan dan pendarahan adalah merupakan lesi yang paling banyak dilihat. Kesemua kumpulan jangkitan dengan *S. agalactiae* menunjukkan secara signifikan ($p < 0.05$) lesi yang lebih teruk dibandingkan kepada kumpulan jangkitan dengan *S. iniae*, tidak kira sama ada saiz berbeza. Perbezaan signifikan ($p < 0.05$) juga dapat dilihat dalam perbandingan di antaras korlesi di dalam ikan yang berlainan saiz. Saiz ikan yang melebihi 4" menunjukkan kadar lesi yang lebih tinggi jika dibandingkan kepada saiz ikan yang kurang dari 4". Di dalam kedua-dua jangkitan streptococcosis, *S. agalactiae* adalah lebih patogenik daripada *S. iniae* di dalam *Oreochromis* sp. Dan ikan berukuran lebih dari 4" adalah lebih cenderung kepada jangkitan kedua-dua streptococcosis.

Kata kunci: Streptococcosis, *Streptococcus agalactiae*, *Streptococcus iniae*, histopatologi, *Oreochromis* sp (tilapia)

1.0 INTRODUCTION

Oreochromis sp., also known as Tilapia, is a prolific species of fish. This fish is reared in most parts of the world as it is assumed to be resistant to infection and environmental stressors. However, recently, it is found that *Oreochromis* sp. is prone to streptococcosis infection. Streptococcosis is an infection by Gram-positive bacteria of the genus *Streptococcus*. This bacteria is non-motile and non-spore forming. *Streptococcus* sp are facultatively anaerobic and they require nutritionally-rich media for growth. On blood agar supplemented with 5% blood, the organisms produces greenish discolouration (α -hemolysis) or complete clearing (β -hemolysis). Additionally, it is also fermentative in metabolism, releasing lactic acid, but without gas and catalase-negative. (Amal and Zamri-Saad, 2011). This disease has become a major problem in the aquaculture and fisheries industry throughout the world (Hernandez *et al.*, 2009).

Currently, *S. agalactiae* and *S. iniae* have been identified as the main pathogens causing this disease leading to severe economic losses in the aquaculture and fisheries industry throughout the world (Evans *et al.*, 2006; Amal and Zamri-Saad, 2011; Costa *et al.*, 2013). Amongst the *Streptococcus* sp., *S. agalactiae* has a broad host range, infecting both terrestrial and aquatic animals. This bacterium can causes neonatal meningitis and mastitis in humans and cattle, respectively. However, other animals such as mice, cats, dogs, hamsters, camels and frogs can also be

infected (Hernandez *et al.*, 2009). *S. agalactiae* has been isolated from numerous fish species in natural outbreaks of disease and has been shown to be pathogenic to several fish species in experimental trials using different routes of infection such as cohabitation, immersion, intraperitoneal and intra-muscular injections (Evans *et al.* 2002). According to Pretto Giordano and Scarpassa in 2015, *S. iniae* can be consider as an important pathogen in aquaculture and it is an emerging zoonotic pathogen. The first isolation of *S. iniae* occurred in the 1970s from skin lesions on dolphins (*Iniageoffrensis*). Then, it was subsequently identified in fish in North America, Middle East, Asia-Pacific region and Europe (Pretto-Giordano and Scarpassa, 2015).

The main clinical signs presented on the fishes are unilateral or bilateral exophthalmos, ocular haemorrhages, increase in corneal opacity, distended abdomen, curvature of the spinal cord, stiffness, erratic swimming, bleeding tendency at the base of the fins and difficulty in breathing. Some fish may not show clinical signs prior to death (Yanong and Floyd, 2002; Pulido *et al.*, 2004). Gross findings reveals haemorrhagic ascites, mucous content with reddish-brown colour in the intestine, pale and enlarged liver and a haemorrhagic brownish appearance of the retro-bulbar tissue and meninges (Pulido *et al.*, 2004; Zamri-Saadet *al.*, 2010).

At microscopic levels, most tilapias develop a primary inflammatory response of mononuclear cells with the subsequent formation of granulomatous nodules. Lesions include severe haemorrhagic or

granulomatous meningoencephalitis accompanied by large areas of encephalomalacia (Noraini et al., 2013). Similar tissue lesions can be found in the choroid, sclera and the eyeball. Histopathological changes may be found in organs such as spleen, brain, gut and liver. Findings are necrosis of epithelial gut, bacterial clumps in the blood vessel of liver and congestion and inflammation in the spleen. Severe lesions are haemorrhage of meningitis and erosion and necrosis of mucosal gut. Although both bacteria shows similar gross and histopathological lesion findings but currently, there were no studies conducted specifically to compare the gross lesions and severity of histopathological lesions between *Oreochromis* sp. infected with *S. agalactiae* and *S. iniae* infection via intra peritoneal route. Therefore, this study is proposed with the aim to compare the histopathological lesion in experimentally infected *Oreochromis* sp. following exposure to *Streptococcus agalactiae* or *Streptococcus iniae* via intraperitoneal injection.

7.0 REFERENCES

- 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.*). *Journal of Animal and Veterinary Advances*, 10(7), 914–919. <https://doi.org/10.3923/javaa.2011.914.919>
- Against, L., Challenge, I., Rb, J., Tj, A., Roy, A., Singha, J., ... Pk, P. (2017). Histopathology and Wound Healing in Oxytetracycline Treated *Oreochromis*, 8(4), 4–9. <https://doi.org/10.4172/2155-9546.1000488>
- Alsaid Milud, Daud Hassan Hj Mohd, Mohamed Mustapha Noordin, B. S. K., Mohamed Abdelhadi Yasser, Abuseliana Ali Farag, and H. R. H., Milud Alsaid, Hassan Hj Mohd Daud, Noordin Mohamed Mustapha, Siti Khairani Bejo, Y., & Mohamed Abdelhadi, Ali Farag Abuseliana, and R. H. H. (2013). Pathological Findings of Experimental *Streptococcus Agalactiae* Infection in Red Hybrid Tilapia (*Oreochromis sp.*). *International Conference on Chemical, Agricultural and Medical Sciences (CAMS-2013)*, (January), 70–73. <https://doi.org/10.15242/IICBE.C1213075>

- Amal, M. N. A., & Zamri-Saad, M. (2011). Streptococcosis in Tilapia (*Oreochromis niloticus*): A review. *Pertanika Journal of Tropical Agricultural Science*, 34(2), 195–206. <https://doi.org/ISSN:1511-3701>
- Amal, M. N. A., Zamri-Saad, M., Iftikhar, A. R., Siti-Zahrah, A., Aziel, S., & Fahmi, S. (2012). An outbreak of *Streptococcus agalactiae* infection in cage-cultured golden pompano, *Trachinotus blochii* (Lacépède), in Malaysia. *Journal of Fish Diseases*, 35(11), 849–852. <https://doi.org/10.1111/j.1365-2761.2012.01443.x>
- Amal, M. N. A., Zamri-Saad, M., Siti-Zahrah, A., & Zulkafli, A. R. (2013). Transmission of *Streptococcus agalactiae* from a hatchery into a newly established red hybrid tilapia, *Oreochromis niloticus* (L.) × *Oreochromis mossambicus* (Peters), farm. *Journal of Fish Diseases*, 36(8), 735–739. <https://doi.org/10.1111/jfd.12056>
- Amal, M. N. A., Zamri-Saad, M., Siti-Zahrah, A., Zulkafli, R., Misri, S., Nur-Nazifah, M., & Shahidan, H. (2010). Prevalence of *Streptococcus agalactiae* in tilapia kept in different water bodies. *Online Journal of Veterinary Research*, 11(2), 153–162.

- Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., & Wahli, T. (1999). Histopathology in fish: Proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases*, 22(1), 25–34. <https://doi.org/10.1046/j.1365-2761.1999.00134.x>
- Chen, C. Y., Chao, C. B., & Bowser, R. R. (2007). Comparative histopathology of streptococcus iniae and streptococcus agalactiae-infected tilapia. *Bulletin of the European Association of Fish Pathologists*, 27(1), 2–9.
- Dewi, T. C., Amanu, S., & Widayati, R. (2015). Phylogeny and Histopathology of, 5, 135–140. <https://doi.org/10.17265/2161-6264/2015.02.008>
- Filho C I, Müller E E, Preto-Giordano L G, & BracarenseAna Paula F. R. L. (2009). Histological findings of experimental Streptococcus agalactiae infection in Nile tilapia (Oreochromis niloticus). *Brazilian Journal of Veterinary Pathology*, 2(1), 12–15.
- Hossain, M. M., Rahman, A., & Roy, H. S. (2016). Inactivation of Streptococcus iniae in substitution of chemicals and drugs to develop vaccine for mono-sex Nile tilapia, 4(4), 99–105.

Iregui, C., Barato, P., Rey, A., Vasquez, G., & Verjan, N. (2014). Epidemiology of *Streptococcus agalactiae* and Streptococcosis in Tilapia Fish. *Concept Press Ltd*, (January 2016), 18.

Jantrakajorn, S., Maisak, H., & Wongtavatchai, J. (2014). Comprehensive Investigation of Streptococcosis Outbreaks in Cultured Nile Tilapia, *Oreochromis niloticus*, and Red Tilapia, *Oreochromis sp.*, of Thailand. *Journal of the World Aquaculture Society*, 45(4), 392–402. <https://doi.org/10.1111/jwas.12131>

Noraini, O., Jahwarhar, N. A., Sabri, M. Y., Emikpe, B. O., Tanko, P. N., Latifah, M. H., & Jamil, S. (2013). The effect of heat stress on clinicopathological changes and immunolocalization of antigens in experimental streptococcus agalactiae infection in red hybrid tilapia (*Oreochromis spp.*). *Veterinary World*, 6(12), 997–1003. <https://doi.org/10.14202/vetworld.2013.997-1003>

Ortega, Y., Barreiro, F., Bueno, H., Huancaré, K., Ostos, H., & Manchego, A. (2016). First report of *Streptococcus agalactiae* isolated from *Oreochromis niloticus* in Piura , Peru : Molecular identification and histopathological lesions. *Aquaculture Reports*, 4, 74–79. <https://doi.org/10.1016/j.aqrep.2016.06.002>

Pretto-Giordano, L. G., Müller, E. E., de Freitas, J. C., & da Silva, V. G. (2010). Evaluation on the Pathogenesis of *Streptococcus agalactiae* in Nile tilapia (*Oreochromis niloticus*). *Brazilian Archives of Biology and Technology*, 53(1), 87–92. <https://doi.org/10.1590/S1516-89132010000100011>

Pretto Giordano, L. G., & Scarpassa, J. A. (2015). *Streptococcus iniae*: An Unusual Important Pathogen Fish in Brazil. *Journal of Aquaculture Research & Development*, 6(9), 9–11. <https://doi.org/10.4172/2155-9546.1000363>

Rahmatullah, M., Ariff, M., Kahieshesfandiari, M., Daud, H. M., Zamri-Saad, M., Sabri, M. Y., ... Ina-Salwany, M. Y. (2017). Isolation and pathogenicity of *Streptococcus iniae* in cultured red hybrid tilapia (*Oreochromis* sp.) in Malaysia. *Journal of Aquatic Animal Health*, 7659(August), 08997659.2017.1360411. <https://doi.org/10.1080/08997659.2017.1360411>

Suanyuk, N., Sukkasame, N., Tanmark, N., Yoshida, T., Itami, T., Thune, R. L., ... Supamattaya, K. (2010). *Streptococcus iniae* infection in cultured asian sea bass (*Lates calcarifer*) and red tilapia (*Oreochromis* sp.) in southern Thailand. *Songklanakarinn Journal of Science and Technology*, 32(4), 341–348.

Suwannasang, A., Dangwetngam, M., Issaro, A., Phromkunthong, W., & Suanyuk, N. (2014). Pathological manifestations and immune responses of serotypes Ia and III *Streptococcus agalactiae* infections in Nile tilapia (*Oreochromis niloticus*). *Songklanakarin Journal of Science and Technology*, 36(5), 499–506.

Wongsathein, D. (2012). Factors Affecting Experimental *Streptococcus Agalactiae* Infection in Tilapia , *Oreochromis Niloticus*, (September).

Zamri-Saad, M., Amal, M. N. A., & Siti-Zahrah, A. (2010). Pathological changes in red tilapias (*Oreochromis spp.*) naturally infected by *Streptococcus agalactiae*. *Journal of Comparative Pathology*, 143(2–3), 227–229. <https://doi.org/10.1016/j.jcpa.2010.01.020>

Zamri-Saad, M., Amal, M. N. A., Siti-Zahrah, A., & Zulkafli, A. R. (2014). Control and prevention of streptococcosis in cultured tilapia in malaysia: A review. *Pertanika Journal of Tropical Agricultural Science*, 37(4), 389–41