UNIVERSITI PUTRA MALAYSIA

THE CLINICOPATHOLOGICAL EVALUATION OF 24 HOURS UPON CHALLENGE OF Streptococcus iniae IN RED HYBRID TILAPIA (Oreochromis sp.)

MUHAMMAD AQMAL HAKIM BIN MAZLAN

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MUHAMMAD AQMAL HAKIM BIN MAZLAN

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CERTIFICATION

It is hereby certified that I have read this project paper entitled "THE CLINICOPATHOLOGICAL EVALUATION OF 24 HOURS UPON CHALLENGE OF *Streptococcus iniae* IN RED HYBRID TILAPIA (*Oreochromis* sp.)" by Muhammad Aqmal Hakim Bin Mazlan and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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DR MD SABRI MOHD YUSOFF

DVM, MVSc, PhD (UPM)

Assoc. Prof.,

Department of Veterinary Pathology and Microbiology,

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

DEDICATIONS

This project paper is dedicated to Allah SWT, who had made everything possible,

To my family,

Father

Mother

Brothers & Sisters

Nur Arina

To all the lecturers, staffs and my friends who were involved directly or indirectly in this project.

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LIST OF ABBREVIATIONS

%	Percent
Bp	Base pair
CFU	Colony forming unit
DAB	3,3'-diaminobenzidine
h	Hour
Hpc	Hours post challenge
IHC	Immunohistochemistry
min	Minute
ºC	Degree Celsius
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
Rpm	Revolutions per minute
µl	Microliter
µM	Micromolar

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

PENILAIAN KLINIKOPATOLOGI 24 JAM SELEPAS DIUJI DENGAN *Streptococcus iniae* DALAM TILAPIA HIBRID MERAH (*Oreochromis* sp.)

Oleh

MUHAMMAD AQMAL HAKIM BIN MAZLAN

2016

Penyelia: Prof. Madya Dr. Md Sabri b. Mohd Yusoff

Kajian ini bertujuan untuk menggambarkan keterukan dan immunolokalisasi antigen di dalam otak, mata dan buah pinggang dalam setiap 6 jam untuk 24 jam dengan kehadiran atau ketiadaan faktor tekanan sebelum jangkitan. Lima belas tilapia hibrid merah di dalam setiap akuarium telah disuntik secara intraperitoneal dengan 10^9 CFU/mL dicairkan dalam PBS dan satu set lagi, telah disimpan untuk kawalan negatif. Tanda-tanda klinikal telah direkodkan dan diperhatikan, dan sampel dari insang, otak, mata dan buah pinggang telah dikumpulkan. Setiap sampel yang diperoleh daripada organ telah diuji dengan

kaedah pengasingan dan mengenalpasti jenis bakteria, dan histopatologi. Immunohistokimia (IHC) dan PCR konvensional juga telah dilakukan untuk mengesan kehadiran antigen. Walau bagaimanapun, tidak ada tanda-tanda jelas dan tiada penemuan makroskopik yang boleh diperhatikan sepanjang 24 jam selepas jangkitan penyakit ini. IHC telah dikesan seawal PCR dan pengasingan dengan nodaan yang teramat jelas dalam saluran darah, lumen dan dinding, makrofaj dalam koroid, pendarahan yang tertumpu dalam celahan buah pinggang dan meninges terutama dalam kumpulan yang diuji dengan tekanan haba diikuti oleh tiada tekanan. Immunolokalisasi oleh antigen dijelaskan dalam patogenesis streptokokosis dalam tilapia hibrid merah. Kesimpulannya, ikan yang tertekan lebih cenderung untuk membangunkan penyakit dan menunjukkan tanda-tanda yang lebih teruk penyakit berbanding ikan yang diuji dengan sebarang tekanan.

Kata kunci: Tilapia merah hibrid, *Streptococcus iniae*, intraperitoneal, immunolokalisasi, IHC, PCR

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project.

CLINICOPATHOLOGICAL EVALUATION OF 24 HOURS AFTER CHALLENGE OF *Streptococcus iniae* IN RED HYBRID TILAPIA (*Oreochromis* sp.)

By

MUHAMMAD AQMAL HAKIM BIN MAZLAN

2016

Supervisor: Assoc. Professor Dr. Md Sabri Mohd Yusoff

This study was aimed to describe the severity and immunolocalisation of the antigen of lesions in the brain, eyes and kidney in every 6 hours for 24 hours by presence or absence of stress factors before infection. Fifteen Red hybrid tilapia in duplicates were inoculated intraperitoneally with 10^9 CFU/mL diluted in PBS while another set, was kept for negative control. Clinical signs were recorded and observed, and samples from gills, brain, eyes and kidney were collected. Each of the samples was subjected to bacterial culture and isolation and histopathology. Immunohistochemistry (IHC) and polymerase chain reaction (PCR) were also done to detect the presence of the antigen. However, there were no obvious signs,

and no macroscopic finding can be observed throughout 24 hours post challenge (hpc) of the disease. IHC were detected as early as PCR and isolation with intense staining in a blood vessel, lumen and wall, macrophages in the choroid, focal haemorrhages in the renal interstitium and meninges especially in heat stressed followed by no stressors. The immunolocalisation of the antigen is explained in the pathogenesis of streptococcosis in red tilapia. In conclusion, fish that is stressed are more likely to develop diseases and showing more severe signs of disease compared to fish that was not subjected to stress.

Keywords: Red hybrid tilapia, *Streptococcus iniae*, intraperitoneal, immunolicalisation, IHC, PCR

1.0 INTRODUCTION

1.1 Study background

Tilapia is one of the most important aquaculture products that is expanding in Malaysia. It has a high demand and potential locally and also from international (Azeli, 2007). Tilapia was introduced to Malaysia in 1970's and a decade after that a cross between *Oreochromis niloticus* and *Oreochromis mossambicus* was introduced, which is Red hybrid tilapia. Initially, tilapia were regarded to be more resistant to bacterial, fungal, viral and parasitic diseases compared to other cultured fish species (Amal et al., 2011). However, recently tilapia has been found to be susceptible to these diseases. Among the fish species that are infected by *Streptococcus iniae* also include tilapia. *Streptococcus iniae* was first discovered on freshwater dolphin in Amazon, *Inia geoffrensis* with caused the dolphin to develop a "golf ball" lesion on the skin layer due to its appearance resembling a golf ball (Pier and Madin, 1976). Hence, the name *Streptococcus iniae* was derived from the "Inia", which refers to the genus of the river dolphin in South America. Phylogenetically, *S. iniae* is closely related to *S. parauberis*, both being clustered with *S. agalactiae* and *S. dysgalactiae*, and the whole group clustering with *S. pyogenes* according to sequence similarity in their 16S rRNA gene (Facklam, 2002). Streptococcosis is a general name for a variety of diseases caused by a group of bacteria called *Streptococcus* (strep-TOE-coccus). Some "strep" organisms normally live on the body of humans or animals and do not cause disease. Others may cause disease (sometimes severe) in both people and animals.

Justification to do the study on the disease was that from literature there are no reports or comparative study of clinicopathological evaluation of 24 hours upon the challenge of *Streptococcus iniae* in red hybrid tilapia. So from this study, we hope that the finding will help to better understanding of the clinicopathology of *Streptococcus iniae* in Red hybrid tilapia.

This study was done to fulfil the following objectives:

1. to determine the severity of lesions in the brain, eyes and kidney in every 6 hours to 24 hours by presence or absence of stress factors before infection.
2. to determine the immunolocalisation of the antigen in the brain, eyes and kidney by immunohistochemical detection (IHC).

For this study, the following hypotheses were proposed:

1. There is a different level of severity of lesions in the brain, eyes and kidney observed in every 6 hours to 24 hours by presence and absence of stress factors before infection.
2. Presence of the immunolocalisation of the antigen in the brain, eyes and kidney by immunohistochemical detection (IHC).

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