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EFFECTS OF DIFFERENT ROUTES OF VACCINATION AGAINST Streptococcus agalactiae IN RED HYBRID TILAPIA FINGERLINGS (Oreochromissp.)

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Effects of different routes of vaccination against *Streptococcus agalactiae* in Red hybrid tilapia fingerlings(*Oreochromis*sp.)", by 'AisyahBintiAminuddin and in my opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course of VPD 4999 – Project.

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DEDICATIONS

To my dear and lovely parents, Mr Aminuddin Ahmad

And

Mrs.Ruhani Mohamed

For making me who I am today

To my dearest siblings,

Ahmad SyahiqAminuddin

'AfifahAminuddin

Ahmad SyamimAminuddin

Ahmad Syazwan Aziz Aminuddin

For being there whenever I needed you

"You don't choose your family

They are God's gift to you, as you are to them."

-Desmond Tutu

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LIST OF ABBREVIATION

S. agakactiae	*Streptococcus agalactiae*
FKB	Formalin-Killed Bacteria
FCV	Formalin Crude Vaccine
PBS	Phosphate Buffered Saline
BHI	Brain Heart Infusion
ELISA	Enzyme-linked immunosorbent assay
PCR	Polymerase Chain Reaction
CFU/mL	Colony forming unit per mililiter
˚C	Degree celcius
rpm	Revolutions per minute
PBST	Phosphate Buffered Saline + Tween-20
BSA	Bovine serum albumin
IgM	Immunoglobulin M
DNA	Deoxyribonucleic acid
UV	Ultraviolet
ANOVA	Analysis of variance
mL	Mililiter
mm	Milimeter
µL	Microliter
<	Less than
Bp	Base pair

ABSTRAK

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

KESAN LALUAN VAKSINASI YANG BERBEZA TERHADAP Streptococcus agalactiae PADA ANAK TILAPIA HIBRID MERAH (Oreochromis sp.)

Oleh

'Aisyah Binti Aminuddin

2016

Penyelia: Prof. Madya Dr. Md Sabri Mohd Yusoff

Streptokokosis merupakan penyakit yang disebabkan oleh jangkitan Streptococcus sp. Penyakit ini merupakan masalah global bagi sector pengeluaran ikan sedunia dan saling berkaitan dengan kerugian yang tinggi dalam sector ekonomi.Kajian ini dilakukan bertujuan untuk mengenalpasti kesan terhadap penghasilan antibody bagi laluan vaksinasi yang berbeza terhadap Streptococcus agalactiae dalam anak ikan tilapia merah.Sembilan puluh anak ikan tilapia merah dibahagikan secara rawak kepada tiga kumpulan iaitu kumpulan 1, 2 dan 3 yakni setiap kumpulan mempunyai tiga puluh ekor anak ikan.Dua formulasi bagi formalin vaksin tidak aktif telah dihasilkan iaitu vaksin berasaskan makanan dan semburan.Kumpulan 1 menggunakan vaksin secara semburan selama 3 hari berturut-turut pada minggu pertama dan pada minggu ketiga sebagai dos ransangan jugas elama tiga hari berturut-turut.Bagi kumpulan 2, anak ikan divaksin secara semburan hanya sekali pada minggu pertama dan

diberi vaksin berasaskan makanan pada minggu ketiga sebagai dos penggalak manakala kumpulan 3 kekal sebagai kelopok kawalan tanpa vaksinasi.Kesemua kumpulan dijangkitkan dengan *S. agalactiae,* 109 CFU/mL pada kadar100 Ml melalui intraperitoneum. Setelah dijangkiti, anak ikan berada di bawah pemerhatian bagi sebarang petanda klinikal atau pun kadar kematian. Sampel mucus bagi lima ekor anak ikan daripada setiap kumpulan telah diambil dengan menggunakan steril calitan pada permukaan kulit ikan setiap ming. Cecair lavaj usus juga telah diambil dan kedua-dua sampel diproses menggunakan asaiimunoserap terangkai ensim (ELISA) tak langsung bagi mengenalpasti penghasilan antibody IgM terhadap *S.* agalactiae.Keputusan ELISA menunjukkan penghasilan antibody bagi sampel mucus dan cecair lava jusus bagi anak ikan tilapia yang telah divaksin tidak mempunyai perbezaan bererti walaupun berlaian kaedah vaksinasi telah digunakan.

Kata kunci:*Streptococcus agalactiae,* Tilapia hybrid merah, vaksin semburan, vaksin berasaskan makanan, asaiimunoserap terangkai ensim(ELISA)

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicines in partial for the course VPD 4999- Final year project.

EFFECTS OF DIFFERENT ROUTES OF VACCINATION AGAINST *Streptococcus agalactiae* IN RED HYBRID TILAPIA FINGERLINGS (*Oreochromis* sp.)

By

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2016

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Streptococcosis is a disease that develops following infection by *Streptococcus sp*. It is a major problem for fish production worldwide, and it is associated with high economic losses. This study was aimed at investigating the effects of different vaccination route against *Streptococcus agalatiae* in Red hybrid tilapia fingerlings. Ninety fingerlings were randomly divided into three groups, 1, 2 and 3 of 30 each. Two

formalin-killed vaccine formulations were developed, feed based and spray. Group 1 was vaccinated using spray vaccine for 3 consecutive days in the 1st week and a booster dose for 3 consecutive days in the 3rd week. Group 2 was vaccinated once by using spray vaccine followed by a booster dose using the feed-based vaccine. While Group 3 served as a control group without any vaccination. All groups were challenged with 100 µL of *S. agalactiae*(10^9 CFU/mL) intraperitoneally. Following challenge, the fingerlings were observed for any clinical signs and mortality. Mucus samples of five fish from each group at sampling time were collected by using sterile swab at the surface of the skin. Gut lavage fluid was also collected, and both samples were subjected to indirect enzyme-linked immunosorbent assay (ELISA) to determine the IgM antibody levels against *S. agalactiae*. The results showed that the IgM antibody response in mucus and gut lavage fluidsproduced by the tilapia immunized with vaccination were not significantly different with each other even though different routes of vaccination were used.

Keywords: *Streptococcus agalactiae*, Red hybrid tilapia, spray vaccine, feed-based vaccine, enzyme-linked immunosorbent assay (ELISA)

1.0 INTRODUCTION

1.1 Study Background

Streptococcosis can be acute or chronic. Acute streptococcosis usually occurs in the seasons where the water temperature is high resulting in peaks of mortality that last 2 - 3 weeks (Intervet, 2006). The chronic take place when the water temperature is lower and does not cause peaks of mortality. Amal and Zamri (2011) reported that streptococcosis occur by the infection of *Streptococcus* sp. The shape is spherical or ovoid and 0.5 - 2.0 μm in diameter, occur in pairs or chains when grown in liquid media, non-motile, non-spore-forming and appears purple or blue when stained using Gram stain.

Streptococcus bacteria are part of the normal flora on animal bodies, but infection and disease also can occur when the bacteria enter through the cuts, abrasions, wounds or when the immune system becomes weakened especially involving the stress. *Streptococcus*spp., which can cause diseases in fish, includes *S. agalactiae*(Suanyuk*et al*., 2005) *Streptococcus iniae*(Shoemaker *et al*., 2000) and *Streptococcus difficile*(Berridge*et al*., 2001). Evans *et al. (*2002) said that Group B *S. agalactiae* is another emerging fish pathogen of freshwater and saltwater fish species throughout the world.

Environmental stress is playing a major role in influencing the fish immunity for examples such as water temperature, high nitrate level and low dissolved oxygen. When the line of immunity in fish is declined, the fish are highly susceptible to streptococcosis. Siti-Zahrah*et al*. (2004) and Amal*et al*. (2008) reported that high

mortality of the fish infection is frequently recorded between April and July that depicts dry season in Malaysia.*When fish are infected with streptococcosis, the most common clinical signs shown include anorexia, exophthalmia, ascites and erratic swimming (Evans et al., 2002; Salvador et a., 2005)l*and meningoencephalitis in fish *(*Eldar*et al*., 1995). Mian*et al*. (2009) also said that streptococcosis caused by *S. agalactiae* is a major disease of many fish species, and it is characterized by septicaemia and meningoencephalitis.

Pathogenesis in infected fish involves septicaemia and colonization of several organs such as nares, brain, kidney and intestines (Pasnik*et al*., 2005). However, other than fish animals such as mice, cats, dogs, hamsters, camels and frogs can also be infected with streptococcosis (Evans *et al*., 2002).

The vaccine is an antigenic material that stimulates the immune system by developing the adaptive immunity to a pathogen. The main aim of vaccination against an infectious disease is to stimulate host adaptive immune responses to counteract the infection caused by a pathogen. Vaccination is the most effective method to combat disease (Karen and Scott, 2011) and it also an important disease prevention to maintain human and animal health worldwide. Craig *et al*. (2009) reported that vaccines developed for aquaculture have significantly reduced antibiotic use in fish production. Lombart*et al.* (2007) also stated that vaccination is the most effective and cost-effective method of preventing infectious diseases.

1.2 Justification

The study in determining the antibody response between spray vaccine and a feed-based vaccine against *S. agalactiae* was never done before. Thus, this study will reveal the information regarding the antibodyresponses production between these two different methods and at the same time giving the choices of vaccination methods that can be more practical to be practiced in future. Besides, *S. agalactiae* is one of emerging fish pathogen reported in tilapia fish throughout the world including Malaysia.

1.3 Objectives

i. To determine the antibody response in the mucus and gut-lavage in Red hybrid tilapia fingerlings.

ii. To isolate *S.agalactiae* post challenge in Red hybrid tilapia fingerlings.

1.4 Hypothesis

H_O: Presence of antibody responses in mucus and gut-lavage in different route in Red hybrid tilapia fingerlings

H_A: Absence of antibody responses in mucus and gut-lavage in different route in Red hybrid tilapia fingerlings

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