UNIVERSITI PUTRA MALAYSIA

PATHOGENICITY STUDY OF EXTRACELLULAR PROTEINS (ECPs) AND CELLULAR MEMBRANE PROTEINS (CMPs) OF Streptococcus agalactiae AND ITS EFFECT AS IMMUNOMODULATOR IN AFRICAN CATFISH (Clariasgariepinus)

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PATHOGENICITY STUDY OF EXTRACELLULAR PROTEINS (ECPs) AND CELLULAR MEMBRANE PROTEINS (CMPs) OF *Streptococcus agalactiae* AND ITS EFFECT AS IMMUNOMODULATOR IN AFRICAN CATFISH (*Clariasgariepinus*)

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It is hereby certified that I have read this project paper entitled "Pathogenicity Study of Extracellular Proteins (ECPs) and Cellular Membrane Proteins (CMPs) of *Streptococcus agalactiae* and its effect as immunomodulator in African Catfish (*Clariasgariepinus)*", by Nor Aniskiha Mat Yunus 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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Specially dedicated to my beloved parents, family and friends

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LIST OF ABBREVIATIONS

%	**Percent**
µl	**Micro liter**
mL	**Milliliter**
^{o} C	**Degree Celcius**
i.p	**Intraperitoneal**
hpi	**Hour post inoculation**
dpi	**Day post inoculation**
SPSS	**Statistical Package for the Social Sciences**
CFU/ml	**Colony forming unit per ml**
ECPs	**Extracellular Proteins**
CMPs	**Cellular Membrane Proteins**
AGPT	**Agar Gel Precipitation Test**

ABSTRAK

Abstrakdaripadakertaskerjaprojek yang dikemukakankepadaFakultiPerubatanVeterinaruntukmemenuhisebahagiandaripadakeperluankursus VPD 4999- ProjekIlmiahTahunAkhirPelajar.

KAJIAN PATOGENISITIPROTEIN EKSTRASELULAR (PES) DAN PROTEIN MEMBRAN SEL (PMS) *Streptococcus agalactiae* DAN KESAN SEBAGAI IMUNOMODULATOR DALAM IKAN KELI AFRIKA (*Clarias gariepinus*)

Oleh

Nor Aniskiha Mat Yunus

2016

Penyelia: Prof. MadyaDr Hassan Hj. MohdDaud

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Protein Membran Sel (PMS) adalah protein permukaan bakteria yang boleh menjadi sumber immunogens manakala Protein Ekstraselular (PES) adalah produk yang dikeluarkan oleh bakteria yang dapat mengaktifkan tindak balas imun hos. Walau bagaimanapun, terdapat kekurangan dalam kajian sebelum ini untuk menilai tindak balas imun ikan keli terhadap PES dan PMS menggunakan AGPT. Kajian ini bertujuan untuk menilai kesan *in vivo*PES dan PMSbakteria *Streptococcus agalactiae* terhadap patogenisiti dan imuniti ikan keli Afrika (*Clarias gariepinus*).

PES dan PMS daripada *S.agalactiae* disuntik secara intraperitoneal (i.p) ke dalam ikan keli Afrika untuk menentukan sama ada bakteria produk dapat menyebabkan patogenisiti dan merangsang tindak balas imun ikan. Bakteria daripada kultur asal diambil menggunakan proses emparan pada 1800xg selama 15 minit untuk memisahkan PES dan PMS daripada larutan. Pencairan bersiri untuk PES dan PMS dilakukan pada 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, dan 10^{-5}. Morbiditi, mortaliti kumulatif dan peratusan kemandirian direkodkan setiap hari selama 7 hari. Sampel serum daripada ikan bagi setiap pencairanPES dan PMS serta kumpulan kawalan telah diambil melalui venakaudalpedunkel pada hari ke-8 selepas suntikan. Tanda-tanda klinikal seperti tiada selera makan, lesu, berenang tidak menentu, kelegapan kornea dan eksoptalmia diperhatikan dan sera diuji untuk melihat tahap kehadiran antibodi menggunakan Ujian Presipitasi Agar Gel (AGPT).

Hasil kajian menunjukkan bahawa PMSlebih virulen daripada PES berdasarkan kematian, bagaimanapun, secara statistik tidak ada perbezaan yang signifikan di antara ikan disuntik dengan PES dan PMS pada $p < 0.05$. Keputusan juga menunjukkan bahawa tidak terdapat perbezaan yang signifikan di antara pencairan inokula dan peratusan kemandirianuntuk kedua-dua PES dan PMS. Analisis serum melalui AGPT menunjukkan negatif untuk semua PES dan PMS dan ia menunjukkan bahawa tidak ada kompleks antigen-antibodi yang terbentuk bagi setiap pencairan.

Kata kunci: *Streptococcus agalactiae*, protein ekstraselular (PES), protein membransel (PMS), ikankeliAfrika, imunomodulasi

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine as a partial fulfilment of requirement for the course VPD 4999- Final Year Project.

PATHOGENICITY STUDY OF EXTRACELLULAR PROTEINS (ECPs) AND CELLULAR MEMBRANE PROTEINS (CMPs) OF *Streptococcus agalactiae* AND ITS EFFECT AS IMMUNOMODULATOR IN AFRICAN CATFISH (*Clariasgariepinus*)

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2016

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Cellular Membrane Proteins (CMPs) are bacterial surface proteinsthat could be a source of immunogens while the Extracellular protein (ECPs) are bacterial secretory products that able to activate host'simmune response. However, there is lack of previous studies done to evaluate the immune response of catfish against ECPs and CMPs using AGPT. This study aimed to evaluate the *in vivo* effect of the ECPs and CMPs of *Streptococcus agalactiae* on pathogenicity and immunity of the African catfish (*Clariasgariepinus*) fingerlings.

The ECPs and CMPs of *S.agalactiae* were intraperitoneally (i.p) injected into African catfish fingerlings to determine whether the bacteria products able to cause disease and also stimulate fish's immune response. Bacteria cells from pure culture were harvested by centrifugation at 1800xg for 15 minutes to separate ECPs and CMPs from bacteria suspension. Serial dilutions for ECPs and CMPs were done to give 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} diluents. The morbidity, cumulative mortality and percentage survivability of the fish were recorded within 7dpi. Pooled serum samples from fingerlings of each dilution of ECPs and CMPs injected fish as well as control group were taken via caudal peduncle vein at 8^{th} dpi. Clinical signs observed include anorexia, lethargy, erratic swimming, corneal opacity and exopthalmia. The sera were tested for antibody production by Agar Gel Precipitation Test (AGPT).

Results showed that CMPs was more virulence than ECPs based on mortality, however, statistically there was no significance difference between the fingerlings injected with ECPs and CMPs at $p<0.05$. Results also showed that there was no significant difference between the dilutions of the inocula and the percentage survivability of fingerlings for both ECPs and CMPs. Serum analysis via AGPT showed negativity for all ECPs and CMPs indicating that there was no antigen-antibody complex formed for every dilution.

Keywords: *Streptococcus agalactiae*, Extracellular Proteins (ECPs), Cellular Membrane Proteins (CMPs), African catfish, immunomodulation

1.0 INTRODUCTION

According to Department of Fisheries Malaysia, (2013) the total freshwater aquaculture in Malaysia reached about 132, 892.42 metric tonnes and about 38% from total production (50,533.79 metric tonnes) was contributed from freshwater catfish production from all culture system. Freshwater catfish production had the highest production compared to other fish species in Malaysia. Therefore, infectious diseases caused by pathogenic organisms such as *Streptococcus agalactiae* are an important issue that has caused a lot of financial loss in the aquaculture industry and it is increasingly being recognized as a potential constraint on aquaculture production and trade, and cause massive financial loss either through mortality or reduced meat quality, resulting in reduced profit margins (Smith *et al*., 2003).

Streptococcus agalactiae is a group B streptococcus, it was reported to cause neonatal pneumonia and meningitis in human (Brimil *et al*., 2006; Johri *et al*., 2006), mastitis in cows (Brochet*et al*., 2006; Yildirim *et al*., 2002) and streptococcal infection in fish (Toranzo *et al*., 2005).*Streptococcusagalactiae* also reported to cause such erratic swimming, loss appetite, exophthalmia and visceral cavity distension as main clinical signs in infected fish.According to Song *et al*. (2013), the development of vaccines is one of the solutions against the pathogen as a sustainable prevention method to control this emerging disease.Besides outer membrane proteins as a source of immune protective immunogens, recent increasing interests have been paid on extracellular secretory proteins The extracellular secretory proteins easily activate host'simmune response since they are secreted out of cells and are easily contacted with the host (Zhang *et al*., 2012). Thus, investigation on protective immunogens from extracellular

proteins may provide efficient candidates for development of vaccines (Song *et al*., 2013).

The objectives of this study were:

1) To identify the Extracellular Proteins (ECPs) and Cellular Membrane Proteins (CMPs) of *S.agalactiae*
2) To determine the clinical signs, mortality and Percentage Survivability in *Clariasgariepinus* fish when injected with ECPs and CMPs of *S.agalactiae*
3) To study the pathogenicity and immunity of *Clariasgariepinus* fish towards ECPs and CMPsof *S.agalactiae*.

Justification for this study:

Streptococcosis caused by *S.agalactiae* cause high morbidity and mortality in aquaculture industry primarily in cultured fish and cause great financial loss to the farmer. Therefore, the aim of the study is to evaluate the pathogenicity (clinical signs and mortality) and immune response of *Clariasgariepinus* fish when administered with Extracellular Proteins (ECPs) and Cellular Membrane Proteins (CMPs). Besides the host- agent immune response, the antigenic protein of the bacteria is important and potential for vaccine development. Other than that, there is also lack of study done on pathogenicity and immune response of African catfish fingerlings when challenged with ECPs and CMPs of *S. agalactiae*.

Hypothesis of this study were:

The hypothesis for this study is the Extracellular Proteins (ECPs) and Cellular Membrane Proteins (CMP) of *S. agalactiae*is able to produce pathogenicity (in terms of morbidity and mortality) and produce immunity in African catfish (*Clariasgariepinus*) fingerlings.

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