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DEVELOPMENT OF POLYMERASE CHAIN REACTION (PCR) TECHNIQUE FOR DETECTION OF MYCOPLASMA HYOPNEUMONIAE AND PSEUDORABIES VIRUS IN PORCINE CLINICAL SAMPLES

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DEVELOPMENT OF POLYMERASE CHAIN REACTION (PCR) TECHNIQUE FOR DETECTION OF *MYCOPLASMA HYOPNEUMONIAE* AND PSEUDORABIES VIRUS IN PORCINE CLINICAL SAMPLES

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Development of Polymerase Chain Reaction (PCR) Technique for Detection of *Mycoplasma hyopneumoniae* And Pseudorabies Virus in Porcine Clinical Samples", by Tan Shin-Yi and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project

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DEDICATIONS

This project paper is dedicated to God, whom I was created and guided by Him

To my dearest family,

My late grandparents

Father

Mother

Sisters

& Kwang Yan

And to all my teachers and lecturers whom contributed to who am I now and who I will be in future.

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LIST OF ABBREAVIATIONS

%	Percent
µl	Microliter
µm	Micrometer
µM	Micromolar
ºC	Degree Celsius
AD	Aujeszky's Disease
bp	Base pairs
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxyribonucleotide triphosphate
g	Gram
kb	Kilobase
km	Kilometer
mA	Milliampere
MgCl2	Magnesium chloride
Mhyo	*Mycoplasma hyopneumoniae*
min	Minutes
ml	Milliliter
PCR	Polymerase Chain Reaction
PRV	Pseudorabies Virus
SEP	Swine Enzootic Pneumonia
PRDC	Porcine Respiratory Disease Complex
PRRSV	Porcine Reproductive and Respiratory Syndrome Virus
PCV2	Porcine Circovirus 2
RNA	Ribonucleic acid
rpm	Revolutions per minute

ABSTRAK

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

KEMAJUAN TEKNIK RANTAIAN REAKSI POLIMERASE DALAM PENGESANAN *MYCOPLASMA HYOPNEUMONIAE* DANPSEUDORABIES VIRUS DARIPADA SAMPEL KLINIKALPORSIN

Oleh

Tan Shin - Yi

2016

Penyelia: Dr Ooi Peck Toung

Penyelia bersama: Dr. Nor Yasmin Abd. Rahaman

Dengan kemajuan terkini dalam biologi molekul, pengesanan molekul telah menjadi antara satu teknik diagnosis alternatif. Oleh sebab *Aujeszky's Disease* dan *Enzootic Pneumonia* menyumbang kepada kerugian ekonomi yang besar dalam industri ternakan porsin, kajian ini bertujuan untuk menentukan set primer yang sesuai untuk pengesanan *Pseudorabies virus* (PRV) dan *Mycoplasma hyopneumoniae* (Mhyo) daripada sampel klinikal menggunakan PCR. 15 ekor porsin berumur lebih kurang 3 bulan yang mempunyai masalah sistem penafasan telah dikorbankan untuk memperolehi sampel tisu. Vaksin komersial dan sampel klinikal

positif telah digunakan sebagai kawalan positif. Setiap sampel tisu peparu dan tonsil telah diuji dengan kaedah PCR konvensional menggunakan 3 set primer yang berlainan yang direka untuk menyasar kawasan dipulihara DNA genomik untuk Mhyo dan PRV bermasing-masing. Berdasarkan keputusan PCR untuk sampel tisu peparu, antara 3 daripada 15 ekor porsin memperolehi keputusan positif untuk Mhyo dengan menggunakan ketiga-tiga primer tersebut manakala kesemua 15 ekor porsin memperolehi keputusan negative untuk PRV dalam sampel tisu tonsil. Untuk mengoptimumkan lagi protokol ini, penambahan sampel saiz dan perbezaan suhu ketara harus dilakukan. Kesimpulannya, semua set primers yang dipilih adalah sesuai untuk pengesanan Mhyo dan PRV menggunakan teknik PCR.

Kata kunci: *porsin, PCR, primer, Pseudorabies virus, Mycoplasma hyopneumoniae*

ABSTRACT

Abstract of a project paper submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia in partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

DEVELOPMENT OF POLYMERASE CHAIN REACTION TECHNIQUE FOR DETECTION OF *MYCOPLASMA HYOPNEUMONIAE* AND PSEUDORABIES VIRUS IN PORCINE CLINICAL SAMPLES

by

Tan Shin - Yi

2016

Supervisor: Dr. Ooi Peck Toung

Co-supervisor: Dr. Nor Yasmin Abd. Rahaman

With recent advancement in molecular biology, molecular detection has become an alternative diagnostic technique. As Aujeszky's disease and Enzootic Pneumonia caused great economic losses in swine livestock industry, this study aimed to determine suitable primers sets for Pseudorabies virus (PRV) and *Mycoplasma hyopneumoniae* (Mhyo) detection in clinical samples using PCR. 15 pigs aged approximately 3 months old showing clinical signs of respiratory distress were sampled. Commercial vaccine and positive clinical samples were used as positive control. Each lung and tonsil tissue samples were subjected to conventional

PCR assay using 3 different sets of primers designed to target conserved regions of genomic DNA for Mhyo and PRV respectively. Based on PCR assay for lung tissue samples, 3 out of 15 pigs were positive for Mhyo with all 3 primers sets whereas for tonsil tissue samples, all 15 pigs were negative for PRV. To further optimize the current protocol, more sample size and gradient PCR assay should be performed. In conclusion, all the primers sets chosen were suitable to be used for Mhyo and PRV detection using PCR.

Keywords: *porcine, PCR, primer, Pseudorabies virus, Mycoplasma hyopneumoniae*

1.0 INTRODUCTION

1.1 *Mycoplasma hyopneumoniae*

Mycoplasma hyopneumoniae is the aetiological agent of swine enzootic pneumonia (SEP) which is one of the important diseases in the swine production industry. Besides, Mhyo also contributes to Porcine Respiratory Disease Complex (PRDC) by interacting with respiratory viral pathogens such as PRRSV, PCV2 and PRV. Consequently, both SEP and PRDC cause significant economic losses in swine production industry by affecting feed conversion ratio (FCR) and average daily weight gain (ADG). It causes decreased growth rate, increased feed conversion ratio, increased treatment costs and increased mortality (Iris, 2010). Affected pigs with SEP will show a gradual onset of a chronic, dry, non-productive cough that is most evident when animals are aroused, particularly in pigs at the finishing stage of the production cycle (Thacker and Minion, 2012).

Although bacteriological culture is considered the gold standard among diagnostic technique to isolate Mhyo from clinical samples, it requires a special medium known as the Friis medium (Sibila *et al.*, 2009) and the isolation is very laborious, time-consuming as it needs at least 15 days for colony to grow and the culture can easily overgrown by other Mycoplasmas such as *M. hyorhinis* and *M. flocculare*. Serological tests such as ELISA can be performed especially for large amount of samples from pig herds but interpretation of serological results is difficult

and challenging (Erlandson *et al.*, 2005). Besides, in comparison with PCR, serological tests have lower sensitivity may be due to the organism normally colonizes the airways, resulting in minimal interaction with the systemic immune system and causing variable serological results.

With the recent advancement in molecular biology, PCR is an alternative of diagnostic method. It is more rapid, relatively inexpensive compared to bacteriological culture and is highly sensitive and specific. Hence, this study was undertaken to fulfil the following objectives:

i. To determine suitable primers set for detection of *Mycoplasma hyopneumoniae*.
ii. To detect *Mycoplasma hyopneumoniae* from clinically affected animals using the selected primers with PCR technique.

1.2 Pseudorabies Virus

Pseudorabies virus, also known as as Suid Herpesvirus type 1 (SuHV-1), belongs to the genus *Varicellovirus* in the subfamily *Alphaherpesviriae* of the family *Herpesviridae* (Kluge *et al.*, 1999). Pigs are the only natural host for PRV although the virus is capable of infecting a wide range of hosts. It is the aetiological agent of Aujeszky's disease that causes severe economical and production losses to the swine industry worldwide by fatal infection of piglets and abortions in pregnant sow. Fatal infections are predominant in younger pigs with the exhibition of neurological signs such as ataxia, convulsions, and sudden death whereas respiratory distress and

subclinical infection is primarily present in adult pigs. In pregnant sow, PRV results in infection of fetuses which lead to resorption, mummification, or abortion (Mettenleiter *et al.*, 2012). PRV is able to establish latent infection after primary replication and reactivation of the virus may take place following stressful events (Pomeranz *et al.*, 2005) which poses a threat to the health status of pigs in the herds.

Virus isolation (VA) is the commonly performed diagnostic technique to detect PRV from clinical samples followed by confirmation with immunofluorescence, immunoperoxidase or neutralization tests (OIE, 2004). However, with the recent advancement in molecular biology, PCR is an alternative to VA which can be used to detect PRV genomes with designed primers that is able to target conserved regions of PRV strains such as gB, gC, gD and gE. In comparison with VA, PCR can yield result in a shorter amount of time, is highly specific and sensitive and has the ability to detect dormant virus in latency (Ayala *et al.*, 2012). As a result, a better control and prevention of AD can be achieved.

Although there are several conventional PCRs targeting genes encoding gB, gC, gD and gE have been established (Yoon *et al.*, 2005; Perez and Arce, 2009; Huang *et al.*, 2004), but there is still no internationally agreed standard. Hence, this study was undertaken to fulfil the following objectives:

i. To determine suitable primers set for detection of Pseudorabies Virus.
ii. To detect Pseudorabies Virus from clinically affected animals using the selected primers with PCR technique.

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