UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF PIG IMMUNITY STATUS AFTER PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) MODIFIED LIVE VIRUS (MLV) VACCINATION WITH ELISA AND PCR TECHNIQUE

CHUA VI VIAN

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CHUA VI VIAN

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It is hereby certified that we have read this project paper entitled "Determination of Pig Immunity Status after Porcine Reproductive And Respiratory Syndrome (PRRS) Modified Live Virus (MLV) Vaccination with ELISA and PCR Technique", by Chua Vi Vianand in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

DR. OOI PECK TOUNG
DVM (UPM), PHD (GLASGOW),
Lecturer,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

DR. CHEAH ZI HERK
DVM (UPM),
Animal Health Division,
BoehringerIngelheim Malaysia
(Co-Supervisor)

DEDICATION

To

Love

Friendship

Passion

Life

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

PRRS	Porcine Reproductive and Respiratory Syndrome
HP-PRRS	Highly pathogenicPorcine Reproductive and Respiratory Syndrome
PRRSV	Porcine Reproductive and Respiratory Syndrome Virus
RNA	ribonucleic acid
DNA	deoxyribonucleic acid
bp	Base pair
ORF	Open Reading Frame
PCR	polymerase chain reaction
RT-PCR	reverse transcriptase-polymerase chain reaction
nPCR	nested-PCR
ELISA	enzyme-linked immunosorbent assays
IFA	Indirect Fluorescent Antibody test
IPMA	Immunoperoxidase monolayer assay
mRNA	messenger RNA
GP5	Glycoprotein 5
kDa	kilo Dalton
min	minute or minutes
mg	milligram
ml	mililiter
mM	mili Molar
g	gravity
µl	microliter
s	second
cDNA	complementary DNA
dNTP	Deoxyribonucleotide triphosphate
TAE	Tris-acetate-EDTA
UV	Ultra-violet
SP ratio	sample to positive ratio
M	Marker
KV	Killed Virus
MLV	Modified-Live Virus
VN	Virus neutralizing
AASV	American Association of Swine Veterinarians

ABSTRAK

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

PENENTUAN IMMUNITI STATUS

SELEPAS VAKSINASI PORCINE REPRODUCTIVE AND RESPIRATORY

SYNDROME (PRRS) VIRUS HIDUP DIUBAH SUAI

DENGAN ELISA DAN PCR

Chua Vi Vian

2016

Supervisor: Dr.Ooi Peck Toung

Porcine Reproductive and Respiratory Syndrome (PRRS) adalah satu penyakit yang mempunyai ciri-ciri seperti masalah keguguran di peringkat akhir kehamilan dalam khinzir betina dan khinzir betina dara, serta masalah pernafasan dalam khinzir kecil dan yang sedang membesar.Virus PRRS boleh dibahagikan kepada dua jenis berdasarkan antigen dan genetik yang berbeza iaitu Jenis I (Eropah) dan Jenis II (Amerika Utara).Dalam kajian ini, 240 sera telah dikumpul dari 4 ladang yang menggunakan program vaksinasi yang berbeza selama satu tahun dan vaksinasi telah dijalankan dua bulan sebelum sampling.15 sera daripada empat kumpulan umur: khinzir betina, khinzir yang sedang membesar, khinzir cerai susu dan anak khinzir telah diuji dengan IDEXX PRRS X3 ELISA yang mampu mengesan antibodi PRRS. Sera juga dikumpul untuk mengasingkan PRRSV bagi pengelasan menggunakan “nested-

PCR‟ .Daripada 80 sera sampel yang dikumpul, tiada yang positif bagi PRRSV dan ini menunjukkan semua kumpulan umur tidak viremik selepas vaksin virus hidup.ELISA pula menunjukkan semua ladang yang diuji adalah seropositive. S/P ratio bagi anak khinzir, khinzir sedang membesar and ibu khinzir dari keempat-empat ladang adalah lebih tinggi daripada 0.4 iaitu nilai tanda positif ELISA tetapi tiada perbezaan yang didapati antara lading kecuali ladang B yang mempraktikkan US-vaksin virus hidup keseluruhan ladang. Ladang B menunjukkan keputusan S/P ratio signifikan ($p<0.05$) yang rendah dalam kumpulan anak khinzir, khinzir sedang membesar dan ibu khinzir dan ini mencadangkan circulasi virus yang rendah dalam ladang. Farm A yang mempraktikkan vaksinasi US- vaksin virus hidup adalah satu-satunya ladang yang terdapat sero negatif status dalam kumpulan cerai susu. Data ini membuktikan bahawa vaksin hidup PRRS tidakakan meyebabkan viremik dan vaksinasi keseluruhan lading dengan vaksin hidup boleh membantu megurangkan virus circulasi dalam ladang endemik PRRS.

Kata kunci: Porcine Reproductive and Respiratory Syndrome (PRRS), seroprevalence, vaksinasi, ELISA, nested-PCR.

ABSTRACT

An abstract of the project paper was presented to the Faculty of Veterinary Medicine as partial fulfilment of the course VPD 4999 – Project.

DETERMINATION OF PIG IMMUNITY STATUS AFTER PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) MODIFIED LIVE VIRUS VACCINE WITH ELISA AND PCR TECHNIQUE

Chua ViVian

2016

Supervisor: Dr.Ooi Peck Toung

Porcine reproductive and respiratory syndrome (PRRS) is a disease characterised by late-term reproductive failure in sows and gilts, and respiratory problems in piglets and growing pigs. In this study, 240 sera were collected from four farms that had been practicing different PRRS vaccination regime for more a year and vaccinations were done at 2 months before sampling. 15 sera from four age groups: sows, growers, weaners and piglets were collected from each farm and analysed using IDEXX PRRS X3 ELISAfor PRRSV antibodies. Pooled serum samples were tested by using nested-PCR that enable the differentiation of Type I and Type II PRRSV. Out of 80 pooled

serum samples, none were positive for PRRSV indicating all age groups were not viraemic after vaccination. As for ELISA, results showed all the farms were seropositive for PRRS. Mean S/P ratios of piglets, growers and sows of all four farms were higher than 0.4 which was the cut off value of positive ELISA result but there were no significant difference between the farms except for Farm B which practiced whole herd US MLV vaccination. Farm B showed significantly lower ($p<0.05$) S/P ratio in their piglet, grower and sow groups which suggest there was low virus circulation in herd. Farm A which practiced US MLV on sow was the only farm found to have seronegative status in their weaners. Thus, these data indicate PRRS MLV vaccination will not cause viraemia and whole herd MLV vaccination may help to reduce virus circulation in PRRS endemic farm.

Keywords: Porcine Reproductive and Respiratory Syndrome (PRRS), seroprevalence, vaccination, ELISA, nested-PCR.

1.0 INTRODUCTION

Porcine reproductive and respiratory and syndrome(PRRS)continues to be clinically relevant and economicallysignificant since it was first described and the causative agent, PRRS virus (PRRSV) was identified more than two decades ago (Neumann et al, 2005).Classical clinical signs of the disease include late-term reproductive failure in sows and gilts, and respiratory problems in piglets and growing pigs. In 2006, highly pathogenic PRRSV (HP-PRRSV) strains firstemerged in China, and infected pigs developed clinical signs including high fever (≥41◦C), anorexia, listlessness, red discoloration ofskin, respiratory distress with very high morbidity and mortality rates (Tian et al, 2007), So far, HP-PRRSV strains have been discovered inmost countries in East Asia including Cambodia, Laos, Philippines,Bhutan, Myanmar, Thailand, South Korea, and Russia (Ni et al, 2012).

The PRRSV is an RNA virus of the order Nidovirales, family Arteriviridae, genusArterivirus. It is small (approximately 50-65nm in diameter), enveloped, positive-sensed and single-stranded. There are two antigenically and genetically different strains of PRRSV which are Type 1 virus in Europe and Type 2 in North American. Nowadays, both types share worldwide distribution with Type 2 predominant in North America and Asia (Zimmerman et al., 2012). The viral genome is around 15 kbp long and encodes nine open reading frames, namely ORF1a, 1b, 2a, 2b, 3, 4, 5, 6, and 7(Stadejek et al, 2002).Within the different ORFs, the more conserved ORF7 is recommended as a

potential target site for detection of PRRSV of different strains using RT-PCR (Guarino et al, 1999).

The most frequently used tests to diagnose PRRS include ELISA, RT-PCR and serological assays. However, study has shown that the serology alone may not be adequate to define the PRRSV status of a breeding herd (Dee, 1997). Molecular techniques can be useful in conjunction with serology, observations of clinical signs, and analysis of production data in determining the PRRSV status of a breeding herd. It can also be used to define and pinpoint the period in the life of the piglet during which infection seems to be occurring (Dee, 1997).

Although elimination ofPRRSV from a production site or maintaining a PRRSV-free herd is attempted (Charerntantanakul, 2012), PRRSV is readily shedby infected swine and has an affinity for transmission via fomitesand in aerosols, creating a persistent presence in the environment. Study hadclearly support the fact that airborne transmission ofPRRSV can occur over distances as great as 9.1km, with the virus still remaining infectious (Otake et al, 2010).

Because of the huge impact of PRRS in the swine industry, vaccination is a key component of PRRS disease control strategies. Various types of vaccines, including killed virus (KV) and modified-live virus (MLV) vaccines had been developed for the control of the disease in both grower and breeding sow (Martínez-Lobo et al, 2013). PRRS MLV vaccine is well known for its protective efficacy against PRRSV that are genetically homologous to the vaccine virus. The advantages it has over killed vaccine

are the ability to generatestronger and more complete immune response, PRRSV neutralization, resulting inthe ability to limit post-challenge viremia, transplacental infection and viral shedding, protection against clinical disease following a single vaccine dose and some degree ofcross-protection against heterologous strains of PRRSV (Schelkopfa et al, 2014).

However, there is concern for the immunogenicity and safety of MLV vaccine (Charerntantanakul, 2012). Vaccination with PRRS-MLV is generallycontraindicated in pregnant swine as a safety precaution and the main target populations are the growing and finishing pigs. TheMLV vaccine virus replicates in the host, which allows sheddingof attenuated PRRSV and creates the potential for exposure ofimmunologically naïve swine to the vaccine virus. Experimental and field studies reported that MLV strains can cause viremia, revert to virulence and spread transplacentally affecting the piglets born (Papatsiros, 2012). Piglets born to these MLV-infected sows can become carriers of PRRSV, shedding the MLV vaccine virus to other naive pigs (Rowland, 2010). Study has reported that after introduction of vaccination program using attenuated live PRRS vaccine in a previously unaffected Danish pig population, acute PRRS like disease was observed in non-vaccinated and in vaccinated herds. The same study has demonstrated that a field isolate of PRRS vaccine-derived virus (VDV) could cause disease in swine consistent with PRRS, thus confirming the etiological role of VDV (Nielsen, 2011).

Thus, this study aims to determine the viremic and serological status of piglets, weaners, growers and sows in farm after PRRS MLV vaccination with ELISA and PCR.

2.0 LITERATURE REVIEW

2.1 Aetiological Agent

The aetiological agent of PRRS is a small, enveloped, single-stranded positive-sense RNA virus of the order Nidovirales, family Arteriviridae, genusArterivirus (Kapur et al, 1996). Properties of these viruses include the ability to induce prolonged viremia, persistent infections, and replication in macrophages (Plagemann and Moennig, 1992). Generally, although PRRS is clinically similar in North America and Europe, the respective strains of virus differ in antigenic and genetic properties.These differences have led to the classification of PRRSV isolates into two genotypes which are type 1 that comprises viruses related to the European prototype Lelystad-virus and type 2 that includes viruses related to the American prototype strain VR-2332 (Wensvoort et al., 1991). The genome of PRRSV consists of 8 open reading frames (ORFs), 6 of which are expressed by the formation of subgenomic RNAs. ORFs 1a and 1b encode the viral RNA polymerase whereas the remaining 6 ORFs encode small polypeptides consisting of 128–265 amino acids each (Guarino *et al.*, 1999). The major structural proteins consist of an envelope glycoprotein (GP5), an unglycosylated membrane protein, and a nucleocapsid (N) protein, encoded by ORFs 5, 6 and 7, respectively. The N protein is the more abundant protein of the virion and is highly antigenic, which thus makes it a

6.0 RECOMMENDATION

A continuous time-point assessment of PRRSV shedding status & exposure status of weaners& breeding herd should be carried outusing ELISA and PCR for confirmation of PRRS herd classification. Absence of clinical signs of PRRS in the breeding-herd unit and a constant lack of detectable viremia in sampled weaners and growers for a minimum of 90 days is required for the confirmation of the PRRS herd staging. This classification also requires a minimum of four consecutive negative PCR herd tests in weanerssampled every 30 days or more frequently. Thus, the study should be continued for at least 3 months with sampling once a monthto collect the data for PCR and ELISA. This will help to ensure the vaccine is working well as well as to monitor the farm PRRS status.If possible, similar experiment should be carried out in an animal experimental house where environment condition and management can be controlled to monitor the status of pigs after vaccination.

Besides, by using ELISA IDEXX measured through Boxplot charts to determine variability of SP values can be used as another tool to measure sow herd stability in the farm. Less variability in ELISA serology SP values results can be demonstrated in boxplot chart when the farms achieve the stabilization through mass vaccination(Angulo, 2007).It is also important to monitor clinical signs and reproductive parameters data in order tointegrate the information and evaluate interventions done in farm.

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