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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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 Vian 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 – Project.

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DEDICATION

To

Love

Friendship

Passion

Life

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LIST OF ABBREVIATIONS

PRRS	Porcine Reproductive and Respiratory Syndrome
HP-PRRS	Highly pathogenic Porcine 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 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 lading adalah lebih tinggi daripada 0.4 iaitu nilai tanda positif ELISA tetapi tiada perbezaan yang didapati antara ladang 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 seronegatif status dalam kumpulan cerai susu. Data ini membuktikan bahawa vaksin hidup PRRS tidak akan meyebabkan viremik dan vaksinasi keseluruhan ladang 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

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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 ELISA for 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 economically significant 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 first emerged in China, and infected pigs developed clinical signs including high fever ($\geqslant 41^{\circ}C$), anorexia, listlessness, red discoloration of skin, respiratory distress with very high morbidity and mortality rates (Tian et al, 2007), So far, HP-PRRSV strains have been discovered in most 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, genus Arterivirus. 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 of PRRSV from a production site or maintaining a PRRSV-free herd is attempted (Charerntantanakul, 2012), PRRSV is readily shed by infected swine and has an affinity for transmission via fomites and in aerosols, creating a persistent presence in the environment. Study had clearly support the fact that airborne transmission of PRRSV 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 generate stronger and more complete immune response, PRRSV neutralization, resulting in the ability to limit post-challenge viremia, transplacental infection and viral

shedding, protection against clinical disease following a single vaccine dose and some degree of cross-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 generally contraindicated in pregnant swine as a safety precaution and the main target populations are the growing and finishing pigs. The MLV vaccine virus replicates in the host, which allows shedding of attenuated PRRSV and creates the potential for exposure of immunologically 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, genus Arterivirus (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 suitable candidate for the detection of virus-specific antibodies and diagnosis of the disease (Dea *et al*., 2000).

Being an enveloped virus, PRRSV is very susceptible to adverse conditions and will die off within hours as conditions change from optimum. Its survivability outside of host is affected by temperature, pH and exposure to detergents. At normal environmental temperatures (25-27°C), PRRSV survives less than a day on pig-associated fomites but it can survive in water for up to 11 days. The PRRSV remains stable at pH ranging from 6.5

to 7.5 but with pH less than 6 or more than 7.65, the infectivity is reduced (Bloemraad *et al*, 1994). Thus, normal clean-up procedures with disinfectants such as chloroform and drying will kill the virus (Morrow, 2001).

2.2 Brief History of PRRS

In the late 1980s, PRRS was first documented as an unexplained reproductive mystery, so-called Mystery Swine Disease, in the mid-western United States (Wensvoort *et al*, 1991). Clinical signs included severe reproductive failure, post-weaning pneumonia, growth reduction, decreased performance, and increased mortality (Keffaber, 1989). About the same time, similar clinical outbreaks were reported in northwestern Germany and were rapidly spread throughout the swine-growing regions of North America and Europe (Wensvoort *et al*, 1991).

Type 1 PRRSV caused primarily reproductive disease in late gestation sows and appeared to change slowly, whereas type 2 PRRSV caused both reproductive disease in late gestation sows and respiratory disease in growing pigs and occasionally gave rise to more virulent forms (Murtaugh *et al*, 2010).

2.3 Epidemiology of PRRS in Malaysia and other countries

Within a few years, PRRS had become pandemic resulting in major economic loss to the swine industry (Neumann *et al*, 2005). In Malaysia, an article had reported syndrome very similar to PRRS in various pig farms where the farms typically showed

acute disease of one to three months, severe drop in conception and farrowing rate with increased stillbirth, and increased pre-weaning mortality. (Too, 1995). Respiratory distress and poor growth rate with some mortality were observed in weaners whereas secondary complications such as pneumonia were observed in growing pigs (Too, 1995). In Malaysia, a seroprevalence study were carried out where more than 90% of the farms involved were seropositive for PRRS and more than 80% of the pigs were seropositive (Jasbir *et al*, 2008). Later, a genetic characterization study done in 2012 showed a seroprevalence of 89.2% with only American PRRS virus strains detected in selected pig farms in Malaysia (Vania and Ooi, 2012). However, as opposed to the two previous studies, a study done in 2013 showed that both American and European strains are found in Malaysia (Seetha *et al*, 2013). To date, there had been no report of highly pathogenic PRRSV in Malaysia.

Highly pathogenic PRRS (HP-PRRS), which emerged in China in 2006, has spread to South-East Asian countries since 2007. The disease was characterized by high fever (40-42°C) in all age groups, abortions in sows and high mortality in suckling piglets, weaners and growers (Tian *et al*, 2007). The first case of HP-PRRS in Thailand was detected in Phitsanulok province in early 2010. The production system that has been affected is mainly the backyard sector with low farm biosecurity (Nguyen, 2013). The disease became established first in countries with a larger commercial production units and high animal densities (Vietnam, Thailand) and subsequently affected countries with a less developed commercial sector (Cambodia and Laos) due to the absence of disease surveillance at community level, weakness of the Veterinary Services in dealing with

outbreaks in a timely manner, lack of biosecurity in value chains and the absence of regulations and incentives to control pig diseases (Nguyen, 2013).

The virus is primarily transmitted directly via infected pigs and also by faeces, urine and semen (OIE, 2008). It can also be spread indirectly, presumably via aerosol routes, leading to chronic re-infection of herds in swine dense areas, and possibly by mechanical vectors (OIE, 2008). In PRRSV-infected boars and boars that have been vaccinated with live attenuated vaccine, PRRSV can be shed in semen, and changes in sperm morphology and function have been described (Christopher-Hennings *et al.,* 1997).

Many factors influence the effect PRRS virus has when it enters a herd. Clinical signs differ depending on management of herd, the strain of the virus, and the immune status of the herd. When the PRRS virus infects a totally naïve herd, 5-75% of pigs show acute illness with lethargy and anorexia and pigs will be off feed for 1-5 days. If pigs are well segregated by production stage, owners report a wave of off feed pigs moving through the farm as the virus spreads (Morrow, 2001). The intensity of the disease also appears to vary among isolates and variation in the pathogenicity of PRRSV virulence has been demonstrated in experimentally infected animals. Study had found that animals infected with mildly virulent isolates had transient pyrexia, dyspnea and tachypnea, whereas infection with highly virulent isolates induced labored breathing, pyrexia, lethargy, and anorexia (Halbur *et al*, 1996).

2.4 Different PRRS vaccination available in Asia

Vaccination is one of the main strategies for the prevention and control of economic losses caused by PRRSV. It is also the most economic strategy for all sizes of pig farms compared with other control strategies. There are two types of PRRSV commercial available vaccines which are Killed Virus (KV) or inactivated vaccines and Modified-Live Virus (MLV) or attenuated vaccines. Information about vaccination schedule of available commercial PRRSV vaccines in Asia are summarized in Table 1.

Inactivated PRRSV vaccines are used for the immunization of breeding herd. Their main advantage is safety, as the vaccine virus cannot transmit to other pigs and cannot revert to virulence (Papatsiros, 2012). Unfortunately, the efficacy of inactivated PRRS vaccines has been seriously questioned. Studies with commercial KV vaccines showed that the vaccination did not induce VN antibodies and did not sufficiently protect against viremia or prevent from the clinical signs of PRRS (Nilubol et al., 2004).

On the other hand, MLV vaccines are used for the prevention and control of PRRS infection both in breeding stock and young piglets. Those vaccine prepared from attenuated virus are the most likely to provide the highest level of clinical protection compare to other conventional vaccines available today. They have the potential to replicate extensively over a long period of time in the vaccinated pig and thus repeatedly expose the pig's immune system to the entire spectrum of viral antigens (Mengeling, 2005). This long interval of replication may be more important for PRRS immunity because neutralizing antibody against PRRSV is slow to develop. However, MLV vaccines usage remain debatable. The major concern is the safety and reversion of vaccine

virus to virulence result from genetic mutations or recombination with field strains (Murtaugh *et al*., 2010).

Table 2.4.1: Currently available commercial porcine reproductive and respiratory syndrome (PRRS) vaccines in Asia

Current name	Type	Virus strain	Manufacturer	Vaccination schedule
Amervac®PRRS	Attenuated	European	Hipra Laboratorios	Piglets: one dose (IM) at age of 4-5 wk
Ingelvac® PRRS MLV	Attenuated	North American	Boehringer Ingelheim	Gilts: 2 doses 30 days apart At least 30 days prior to entry Sows : All animals on site at the same time (Mass Vaccine) 1 dose , 4 times per year Piglets: one dose at day 10-14
Progressis®	Inactivated	European	Merial	Primary vaccination (gilts and sows): twice (IM), 3-4 wk interval at least 3 wk prior to mating Revaccination (booster) one dose (IM) at 60-70d of each gestation
Suipravac®PRRS	Inactivated	European	Hipra Laboratorios	Breeding stock Primary vaccination: entering the farm / Sows (pregnancy or lactation):

	Twice (IM), at 3-4 wk interval
	Revaccination (booster)

Note. Data adapted from Papatsiros (2012) in American Journal of Animal and Veterinary Sciences and Mengeling (2005) in Journal of Swine Health and Production.

2.5 Enzyme-linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR)

PRRS can be diagnosed with various methods which include the typical clinical presentations, serological tests, characteristic light microscopic lesions and the demonstration of PRRSV by virus isolation and identification, detection of nucleic acids (PCR), and detection of viral proteins. However, the most widely used methods for PRRS diagnosis are ELISA and PCR. In herds with no clinical signs of PRRS, these diagnostic tests can be used to determine whether virus circulation is still present. The farmer can use this information to focus on the control or eradication of PRRSV (Duinhof *et al*, 2011).

Presence of antibodies to PRRSV indicates prior exposure to the virus and commercial ELISA tests are the preferred methods to detect antibodies. Antibody levels are detectable in serum within 14 days after infection and diminish over time to an undetectable level in individual animals (Molina *et al*, 2008). ELISA testing of serum is used to define exposure status in addition to confirm the seronegative status of negative breeding replacements. A positive ELISA test indicates previous exposure. However, a positive serologic test results may or may not mean that PRRSV caused clinical disease. In order to confirm the disease, demonstration of characteristic lesions and identification

of the agent are needed (Rossow, 1998). A study done in 2007 shown the Idexx HerdChek PRRS X3 test had highest specificity (99.9%) and sensitivity (98.8%) when compared to other commercial kit and had an earlier detection of experimental serum samples (Opriessnig, 2012). Other advantages cited for the new kit are a 90% reduction in false positive reactors and early detection around 8-14 days post infection of PRRS antibodies (HerdChek PRRS X3 information sheet: Idexx).

PRRS ELISA reactors can be sorted into six groups by S/P ratios in a format described by Dr Gene Erickson of Rollins Animal Disease Diagnostic Laboratory in Proceedings of the North Carolina Healthy Hogs Seminar (Erickson, 1996). Estimated endpoint titer ranges given for each grouping were listed in Table 1. The higher the S/P range, the higher the antibody end point titer range.

Table 2.5.1: Antibody Endpoint Titer Groups based on S/P Ranges of IDEXX PRRS ELISA

Titer Group	S/P Range	Antibody EP Titer Range
0	< 0.40	0-821
1	0.40-1.00	822-2290
2	1.01-2.00	2291-4877
3	2.01-2.50	4878-6220
4	2.51-3.00	6221-7587
5	> 3.01	> 7588

The best clinical specimens for virus detection by PCR in individual pigs are serum, lung, lymph node, tonsil or spleen sections. Viral RNA was detected by reverse transcription (RT)-PCR in serum and tonsil biopsy samples that had been collected through day 251 p.i., and the presence of infectious PRRSV was not detected by swine bioassay of tissue samples collected at necropsy. The results confirmed that RT-PCR is more sensitive than virus isolation in identifying PRRSV-infected pigs (Wills *et al*, 2003). The PCR assays should be able to detect and discriminate between the two genotypes of PRRSV, as they can co-infect individual pigs and pig populations. The ORF 7 is a strong target for diagnostic RT-PCR amplification. Primers from this region allowed the detection of the corresponding fragment in all isolates tested (Guarino *et al*, 1999). A nested PCR was developed to differentiate the European and North American type isolates of PRRS virus and was proved that detection of PRRS virus by the nested PCR was as sensitive as virus isolation in cultures of porcine alveolar macropahges from infected pigs at the acute stages (Kono *et al*, 1996).

Pooling is a testing strategy that has been used for a number of purposes including detecting disease and estimating the prevalence of disease in a population. It can result in major savings, predominantly of consumables and labor. However, pooling positive samples with negative samples will results in the dilution of the analyte. Study done had shown pooling samples at pool sizes of 3 and 5 resulted in a decrease in the sensitivity of RT-PCR. Sensitivity was reduced by 6% and 8%, respectively, when serum or blood swab

samples were run in pools of 5. The impact of pooling on the sensitivity of PCR was higher in samples taken during the beginning of the viremic period. (Rovira *et al*, 2007)

2.6 Herd PRRS Classification

According to Holtkamp *et al*, 2011, a herd classification system was developed for describing the PRRSV status of herds, based upon a set of definitions reflecting the biology and ecology of PRRSV. The herd classification system was established by a definitions committee formed by the American Association of Swine Veterinarians (AASV) and the United States Department of Agriculture PRRS-Coordinated Agricultural Project, and was approved by the AASV Board of Directors.

Breeding herds, with or without growing pigs on the same premises, are categorized as Positive Unstable (Category I), Positive Stable (Category II), Provisional Negative (Category III), or Negative (Category IV) on the basis of herd shedding and exposure status. Category II is further divided into two subcategories which are Positive Stable (II-A) and Positive Stable Undergoing Elimination (II-B). Growing-pig herds are categorized as Positive or Negative. Preferred testing methods to determine shedding include direct detection of the virus by PCR whereas exposure is determined by antibody testing: enzyme-linked immunosorbent assay (ELISA), immunofluorescent antibody (IFA), or immunoperoxidase monolayer assay (IPMA). ELISA is the preferred testing method.

Firstly, Positive Unstable (Category I) breeding herds have positive shedding and exposure statuses. Herds with clinical PRRS outbreak and those with persistently

shedding of virus will fall into Category I. It is also the default category when herds have not been tested and when herd shedding and exposure statuses have not been confirmed.

Next, Positive Stable (Category II) breeding herds have an uncertain shedding status and positive exposure status. Absence of clinical signs of PRRS in the breeding-herd population and confirmation of a constant lack of detectable viremia in sampled weaners and growers if present for at least three months are required. This classification requires a minimum of four consecutive negative PCR herd tests in weaners sampled every one month or more frequently. A distinction is made between Category II breeding herds that are not undergoing PRRSV elimination (Category II-A) and those that are (Category II-B). A breeding herd is undergoing elimination if it has began an elimination procedure. An elimination procedure is when the last seropositive breeding replacements are introduced or when the last intentional exposure to any vaccine occurs in the herd.

Besides, Provisional Negative (Category III) breeding herds have a negative shedding status. Continual introduction of negative breeding replacements without their seroconversion to the PRRSV is required. Lack of seroconversion in introduced animals is considered sufficient evidence to confirm that PRRSV is no longer being transmitted in the herd.

Lastly, Negative (Category IV) breeding herds have a negative shedding and exposure status. Confirmation of the negative exposure status of Category IV breeding herds varies depending on how the herd is established negative. Information from

production records may be used in conjunction with diagnostic information to confirm negative status.

3.0 MATERIAL AND METHODS

3.1 Sample Collection

A total of four farms were identified (Three from Perak and one from Selangor) and the farms chosen had been practicing different PRRS vaccination regime for more than one year. Farm A used PRRS US strain MLV on sows; Farm B practiced PRRS US strain MLV vaccine on both sows and piglets**;** Farm C vaccinated sow with PRRS killed vaccine and Farm D vaccinated sow and piglet with EU strain MLV. All farms were single site except for Farm B which had separated weaning-finishing unit and farrowing unit. Blood collection was done around one month after PRRS vaccination. In each farm, sera from

60 healthy pigs of five different age groups were collected (15 from five day old piglets, 15 from six weeks old weaners, 15 from 20 weeks old growers, 15 from sow). Thus, a total of 240 serum samples were collected. Pigs were sero-sampled with standard venepuncture using plain tube. Serum samples were kept cold with icepacks during transportation back to the diagnostic laboratory and then pipetted into 1.5 ml microcentrifuge tube for storage at -80°C. In order to control cost, three serum samples from pigs of same pen were pooled to become one PCR sample. Thus, a total of 80 PCR samples will be processed to evaluate the PRRS viremic status of piglets, weaners, growers and sows in each farm. Besides, the 240 sera samples were evaluated for their serological status of PRRS in each farm. The positive controls for nested-PCR were obtained from vaccines that are derived from type I and type II strains respectively.

3.2 Enzyme-linked Immunosorbent Assays (ELISA)

Serological test on the blood sera collected was carried out using commercial IDEXX PRRS X3 ELISA assay (HerdChek; IDEXX Laboratories Inc, Switzerland). Samples were considered positive if the calculated sample to positive (S/P) ratio was 0.4 or greater.

3.3 PRRSV RNA Extraction From Serum Samples

Frozen serum samples were thawed on ice and were pooled before RNA extraction. The RNA was extracted from the serum samples using QIAamp® Viral RNA Kits

(QIAGEN®, Germany) according to the manufacture protocol. 560 µl of prepared Buffer AVL containing carrier RNA was added into a 1.5 ml microcentrifuge tube. 140 µl of serum was pipetted into the Buffer AVL-carrier RNA in the microcentrifuge tube. The microcentrifuge tube was then pulse-vortexed for 15 sec to promote efficient lysis and incubated at room temperature for 10 min. The tube was then briefly centrifuged to remove drops from the inside of the lid. 560 µl of 98% absolute ethanol was added to the sample and mixed thoroughly by pulse-vortexing to ensure efficient binding.

Next, 630 µl of the solution was then transferred into a QIAamp spin column attached to a 2 ml collection tube using pipette and centrifuged at 8000 rpm for 1 min. The filtrate was discarded together with the collection tube and QIAamp spin column was transferred into a new collection tube. This step was repeated with another 630 µl of the solution.

500 µl of Buffer AW1 was pipetted onto the QIAamp spin column and centrifuged at 8000 rpm for 1 min to wash off any residual contaminants. After centrifugation, the spin column was once again transferred to a new collection tube and 500 µl of Buffer AW2 was added onto the spin column. It was the centrifuged at 13,000 rpm for 4 min. The filtrate was discarded together and spin column was transferred into a new collection tube. It was centrifuged at full speed for 1 min to remove any residual Buffer AW2 which may cause problems in downstream applications. After that, QIAamp spin column was placed into a clean 1.5 ml microcentrifuge tube and 60µl of Buffer AVE which is RNase-free water that contains 0.04% sodium azide was added into the spin column. It was then

incubated for 1 min at room temperature and centrifuged at 8000 rpm for 1 min. The RNA extracted will then detach from the QIAamp membrane and flow through the spin column with the RNase-free water. Viral RNA is stable for up to one year when stored at -30°C to -15°C or -70°C.

3.4 Synthesis of cDNA

RNA collected was converted into cDNA using QuantiNova™ Reverse Transcription Kit (Qiagen®, Germany). Genomic DNA removal reaction components were prepared on ice by adding 5 µl of RNA template extracted previously into a PCR tube which contains 8 µl of RNase-free water and 2 µl of gDNA removal mix. The PCR tube was incubated for 2 min at 45°C then immediately placed on ice. Next, Reverse-Transcription Master Mix which contains components required for first-strand cDNA synthesis was prepared on ice. 5 µl of the Reverse-Transcription Master Mix was pipetted into each tube containing the 15 µl of template RNA from entire genomic DNA elimination reaction. cDNA synthesis reaction was performed using PCR Cyclers at 25 °C for 3 min for annealing, followed by 45 °C for 10 min for reverse transcription step and 85°C for 5 min to inactivate reaction. The cDNA was then diluted with nuclease free water at ratio of 1 to 10.

3.5 Primers

Primers suggested in published journals were used (Pesch, 2003) with nested-PCR to amplify PRRSV that were extracted from the serum. A total of three sets of primers were used. The first set of primers with sequence of PRRS-F: 5'-ATG GCC AGC CAG

TCA ATC-3'; PRRS-R: 5'-TCG CCC TAA TTG AAT AGG TG-3' (Mardassi *et al.*, 1994) amplified a common site in ORF7 of both virus strains. After that, *n*PCR primers specific for North American genotype: NA-F: 5'-AGT CCA GAG GCA AGG GAC CG-3'; NA-R: 5'-TCA ATC AGT GCC ATT CAC CAC-3' and European genotype: EU-F: 5'-ATG ATA AAG TCC CAG CGC CAG-3'; EU-R: 5'-CTG TAT GAG CAA CCG GCA GCA T-3' were used to distinguish the two different strains. These sets of primers were chosen as this nested-PCR had proven its high sensitivity compared to other primers in detecting the virus (Truyen *et al*., 2006). At the end of amplification, nPCR products of different sizes were produced for different strain.

3.6 Amplification of ORF7 using Nested-PCR

The presence of PRRSV in the serum samples were assessed using a nested-PCR assay that will amplify 241 nucleotide or 337 nucleotide fragment of the ORF7 gene for European and North American strains respectively.

For RT-PCR, HotStarTaq® Plus Master Mix Kit (Qiagen®, Germany) was used. 1 µL of cDNA synthesised previously was added into a PCR tube containing 1 µl forward primer, 1 µl reverse primer, 10 µl of HotStarTaq Plus MasterMix, 2x (1x PCR buffer, 200 µM of each dNTP, 1 unit HotStarTaq Plus DNA Polymerase) and 7 µl RNase-free water. The RT-PCR cycle conditions were as follows: 95°C for 5 min to activate DNA polymerase and 30 cycles of 94˚C for 30 seconds, 58˚C for 45 seconds and 68˚C for 45 seconds, followed by a final prolongation of 10 min at 72˚C.

1 µL of RT-PCR product was used as template for the nested-PCR. Nested-PCR was carried out in a total of 20 µl containing 1 µl of template, 1 µl forward primer each, 0.5 µl reverse primer each, 10 µl of HotStarTaq Plus MasterMix, 2x (1x PCR buffer, 200 µM of each dNTP, 1 unit HotStarTaq Plus DNA Polymerase) and 7 µl RNase-free water. PCR cycle conditions were as follows: 95˚C for 5 min and 30 cycles of 94˚C for 1 minute, 58˚C for 1 min and 72˚C for 1 min, plus a final prolongation of 10 min at 72°C

3.7 Agarose Gel Electrophoresis and Photography

A 2% agarose gel was prepared by mixing 1 g of agarose powder into 50 ml of 1× TAE buffer. The mixture was then heated using microwave to dissolve the powder completely and 2.5 µl of nucleic acid staining solution (RedSafe™, iNtRON Biotechnology) was added when the temperature cooled down. The mixture was poured into the mould and left at room temperature for 45 min.

5 µL of the PCR product was mixed with 1 µL of DNA loading dye (6X Loading Dye Solution (XO), iNtRON Biotechnology, Korea) and loaded into the wells of the agarose gel. 100bp DNA ladder (BenchTop DNA Markers, Promega, USA) was used to pinpoint the size of the PCR products. The electrophoresis was conducted at 80V for 40 min. The results of PCR products gel electrophoresis were viewed under UV transillumination and photographs of the results were taken.

3.8 Statistical Analysis

The IBM® SPSS Statistics 20 statistical software was used. Comparisons of mean S/P ratio of pigs among the four farms for each age group were done using one way

ANOVA with Tukey's post hoc test. Statistical significance is recorded at $p < 0.05$ and confidence interval of 95%.

4.0 Results and Discussion

4.1 Nested-PCR results

PCR amplification was carried out on 80 serum samples collected from four farms. The band sizes of the second products derived from the samples were compared with the positive controls. Positive control of the American strain had band size of 354 bp whereas the European strain was 259 bp in size. Bands that appeared at approximately 250 bp or 350 bp were considered European strain or American strain respectively.

For the first set of result (Figure 4.1), lane 1 represents negative control; lane 2 is positive control for American strain; lane 3 is positive control for the European strain; lane 4-8 are samples from Farm A 5 days old piglets; lane 9 to 13 were loaded with samples from Farm A 6 weeks old weaners. For the second set of results (Figure 4.2), lane 1 represents negative control; lane 2 is positive control for American strain; lane 3 is positive control for the European strain; lane 4-8 are samples from Farm A 20 weeks old growers; lane 9 to 13 were loaded with samples from Farm A sow. Results for viral detection according to age groups and farms were displayed in Table 4.1.

Out of the 80 serum samples collected, none of the samples showed positive bands for either American strain or European strains. The negative results were suggestive of no active infection in the pigs, hence no detectable viraemia status in the pigs. It had been demonstrated that viraemia from PRRS virus able to be detected up to 92 days (Stadejek et al, 2005). In this project, PCR and ELISA were used as diagnostic methods. The actual virus circulation early infection can be determined by using PCR technique (Van Maanen et al., 2006). Compared to serological tests, the advantage of using PCR methods is that this test method is not influenced by the presence of either maternal antibodies, or antibodies induced by vaccination (Duinhof *et al*, 2011).

It is also possible that the negative results were obtained due to the unsuitable primers used as genotypic variances do exist (Guarino et al., 1999). Thus, more primers that detect more conserved area can be included to increase the sensitivity of the test.

Table 4.1: Results of the nested-PCR according to farms, vaccination scheme and PRRSV strains.

Farm	Used vaccination: Type PRRSV-vaccine	Used vaccination: Used scheme	Piglets 5 days old	Weaners 6 weeks old	Growers 20 weeks old	Sow
A	US-MLV	S	Negative	Negative	Negative	Negative
B	US-MLV	S+P	Negative	Negative	Negative	Negative
C	EU-KV	S+P	Negative	Negative	Negative	Negative
D	EU-MLV	S+P	Negative	Negative	Negative	Negative

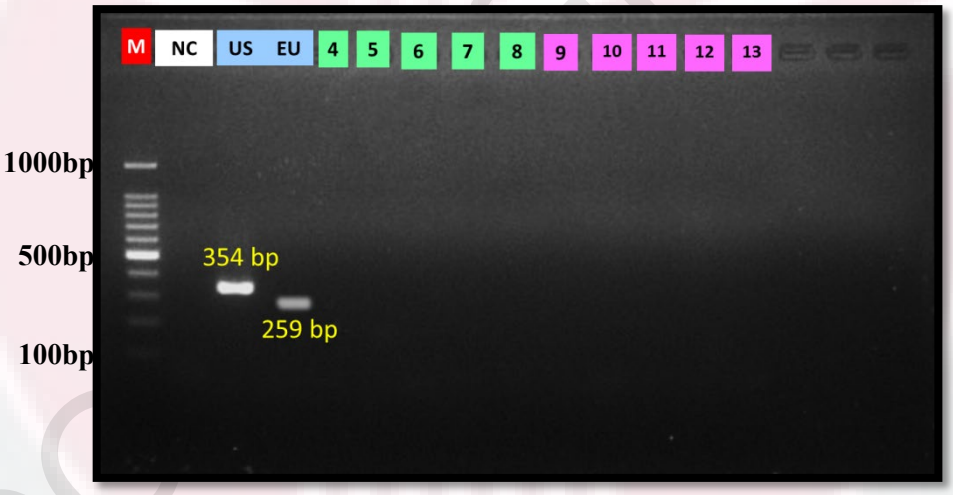

Figure 4.1.1: Nested-PCR amplification from Farm A. 5 days old piglets at lane 4-8; 6 weeks old weaners at lane 9-13. 'M' represents 100 bp DNA ladder markers. Positive controls were exhibited at Lane 'US' and Lane 'EU': American strain (354 bp) and lane 15: European strain (259 bp). Negative control was exhibited at lane 'NC'

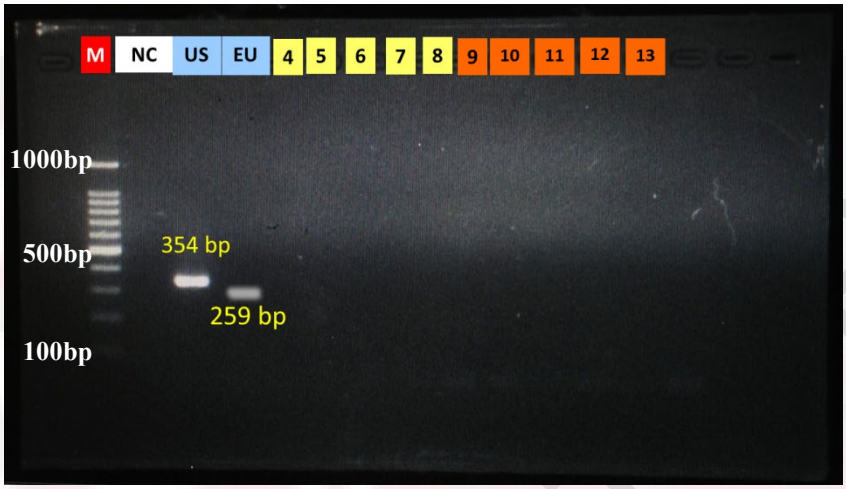

Figure 4.1.2: Nested-PCR amplification from Farm A. 20 weeks old growers at lane 4-8; sows at lane 9-13. 'M' represents 100 bp DNA ladder markers. Positive controls were exhibited at Lane 'US' and Lane 'EU': American strain (354 bp) and lane 15: European strain (259 bp). Negative control was exhibited at lane 'NC'

4.2 ELISA results

Used vaccination			Seroprevalence %			
Farm	Type PRRSV-vaccine	Used scheme	Piglets 5 days old	Weaners 6 weeks old	Growers 20 weeks old	Sow
A	US-MLV	S	93.3 (14/15)	13.3 (2/15)	93.3 (14/15)	93.3 (14/15)
B	US-MLV	S+P	46.7 (7/15)	46.7 (7/15)	86.7 (13/15)	53.3 (8/15)
C	EU-KV	S	100 (15/15)	86.7 (13/15)	100 (15/15)	100 (15/15)
D	EU-MLV	S+P	100 (15/15)	66.7 (10/15)	100 (15/15)	93.3 (14/15)
Mean			85	53.35	95	62.5

4.2.1 Seroprevalence rate in different farms

In total, 240 blood samples were screened for the presence of PRRSV antibodies. In all farms, antibodies against PRRSV were found and 191 (79.6%) of the pigs were seropositive for PRRS virus. The mean seroprevalence in Farm A, B, C and D on herd level were 73.3%, 58.3%, 96.7% and 90% respectively as shown in Figure 4.2.1a. When compared between the four age groups, the seroprevalence was the highest in growers of 20 weeks old where 95% of them were positive and lowest in weaners of 6 weeks old where only 53.3% of them were positive. Seroprevalence of each farm was shown in Table 4.2.1.

Table 4.2.1: Seroprevalence of the pigs according to different farms, vaccination scheme and age group.

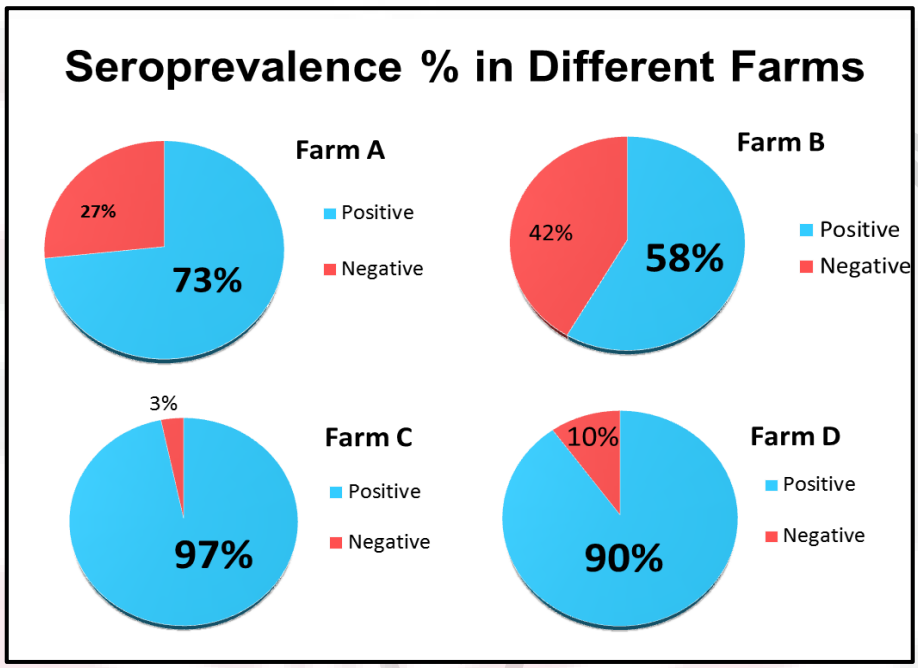

Figure 4.2.1a: Mean Seroprevalence % of different farms

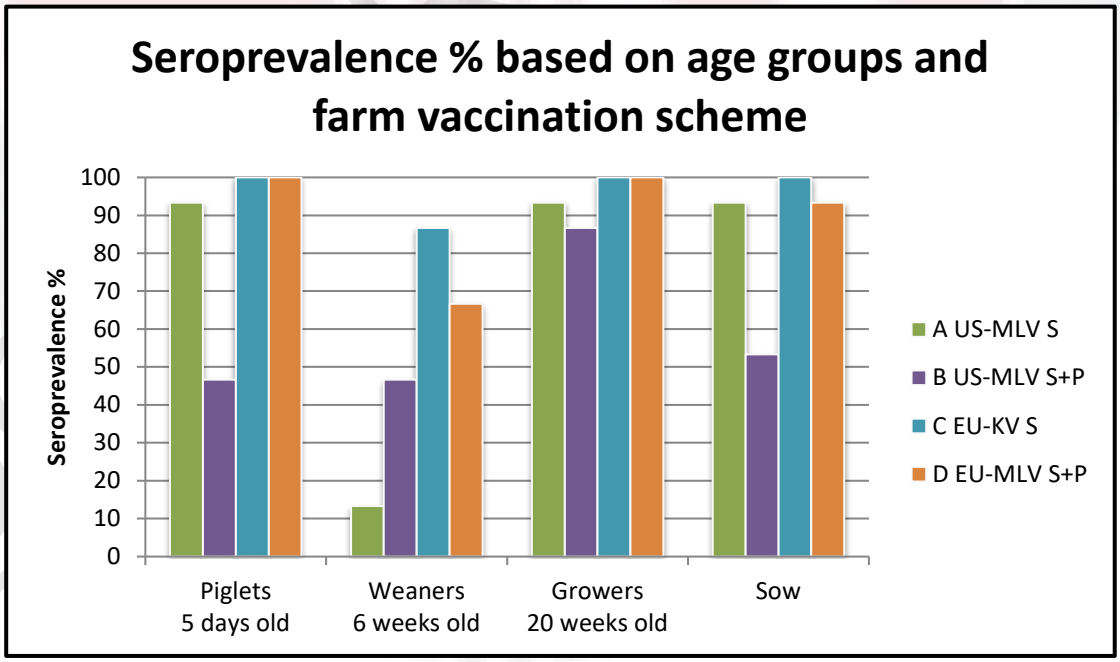

Figure 4.2.1b: Seroprevalence of the pigs according to different farms, vaccination scheme and age group.

Compared to other farm which had almost 100% seropositive of the sow sera samples tested, for Farm B, half of the sows were seronegative by ELISA. The seronegative immune state was most likely due to hyperimmunization of the cell mediated immune response, which cleared the vaccine virus from a vaccinated animal shortly after administration (Erickson, 1996). The sow would not need to mount an anamnestic humoral response. Moreover, antibody levels for positive animals in such herds were often low, S/P ratio usually less than 1.00, which in this case, the mean S/P ratio of the sow group of Farm B was only 0.44. This antibody profile which was obtained for a properly vaccinated sow herd under a four dose per year strategy suggested that the farm have excellent immunity.

As for Farm A, the seroprevalence of the weaners was only 13.3% where 13 out of 15 six weeks old weaners tested were seronegative for PRRS. This may be due to a low virus circulation load in the weaner pen and the pigs were not exposed or seroconverted for PRRS since their farm did not practice vaccination on piglets., vaccinated herds were more likely to be serologically stable than non-vaccinated herds. Pigs negative for PRRSV were produced repeatedly from these relatively small, closed, serologically stable herds (<700 sows) with good biosecurity (Rajic *et al*, 2001).

On the other hand, Farm C showed consistently high seroprevalence percentage across age groups which was suggestive of a high PRRSV load circulation in their farm. As the farm only practiced killed vaccination on the sow herd, the high seropositive

percentage in the growers and weaners group was highly due to seroconversion from field virus challenge.

4.2.2 Mean S/P ratio of the pigs according to different farms

A one-way ANOVA was conducted to determine if mean S/P ratio based on age group was different for farms with different vaccination scheme. Data was mean ± standard error as tabulated in Table 4.2.2. There were several important points that should be taken into account when interpreting PRRS ELISA results. First, it measured exposure, not protection which means a high antibody titer detected only suggested that the animal had been previously exposed to the virus and not the degree of protection from the disease (Christopher-Hennings *et al*, 2002). Secondly, PRRS antibodies presence was not correlated to protection. A variety of studies had shown that PRRSV viremia was often resolved before neutralizing antibodies were detected and PRRSV can be isolated from blood of pigs that have neutralizing antibodies (Murtaugh and Genzow, 2011). Thirdly, serological tests for PRRSV normally detect serum antibody response after 14–21 days post-infection, and do not allow the distinction between infected and vaccinated animals (Batista, 2005).

Table 4.2.2: Mean S/P ratio of the pigs according to different farms, vaccination scheme and age group

[a,b,c] = Means with different superscript within columns differed significantly at P<0.05

	Used Vaccination		Piglets 5 days old	Weaners 6 weeks old	Growers 20 weeks old	Sow
Farm	Type PRRSV-vaccine	Used scheme	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
A	US-MLV	S	$1.18^{a} \pm 0.13$	$0.15^{a} \pm 0.03$	$1.53^{a} \pm 0.10$	$1.00^{a} \pm 0.11$
B	US-MLV	S+P	$0.4^{b} \pm 0.07$	$0.59^{b} \pm 0.15$	$1.02^{b} \pm 0.16$	$0.44^{c} \pm 0.08$
C	EU-KV	S	$1.62^{a} \pm 0.19$	$1.62^{c} \pm 0.13$	$1.82^{a} \pm 0.10$	$1.10^{ab} \pm 0.14$
D	EU-MLV	S+P	$1.70^{a} \pm 0.10$	$0.47^{ab} \pm 0.05$	$1.55^{a} \pm 0.12$	$1.29^{b} \pm 0.09$

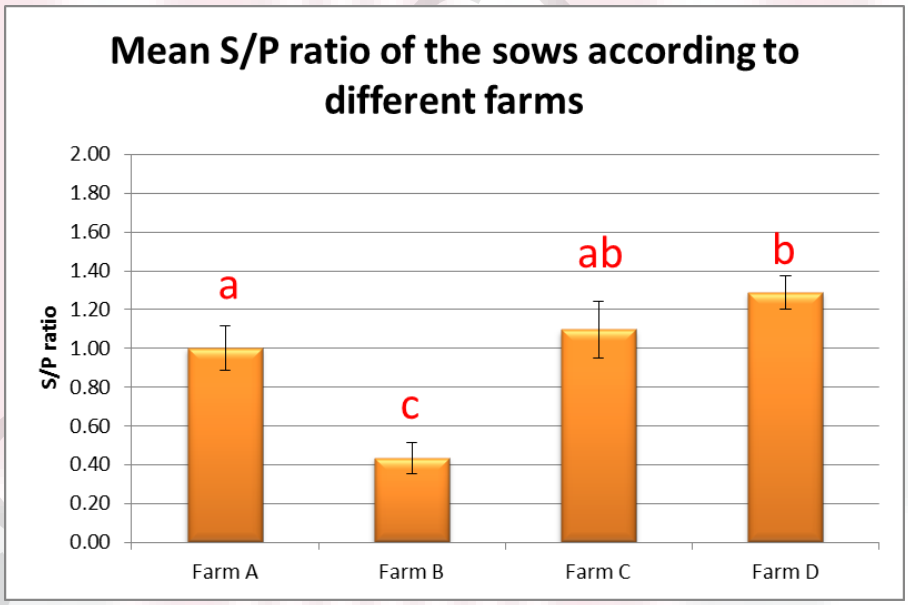

Figure 4.2.2a: Mean S/P ratio of the sows according to different farms. Error bars indicates standard error (SE) of mean. Means with [a, b,c] are significantly different (p<0.05)

For the sow group (Figure 4.2.2a), Farm B has significantly lower mean S/P ratio of 0.44 with p value less than 0.05 compared to Farm A, C and D. The research team believed

that farm B achieved the stabilization through mass vaccination, reduction of resident PRRSV circulation (Angulo, 2007). Thus, the low mean S/P ratio is likely due to lower exposure of pigs to virus.

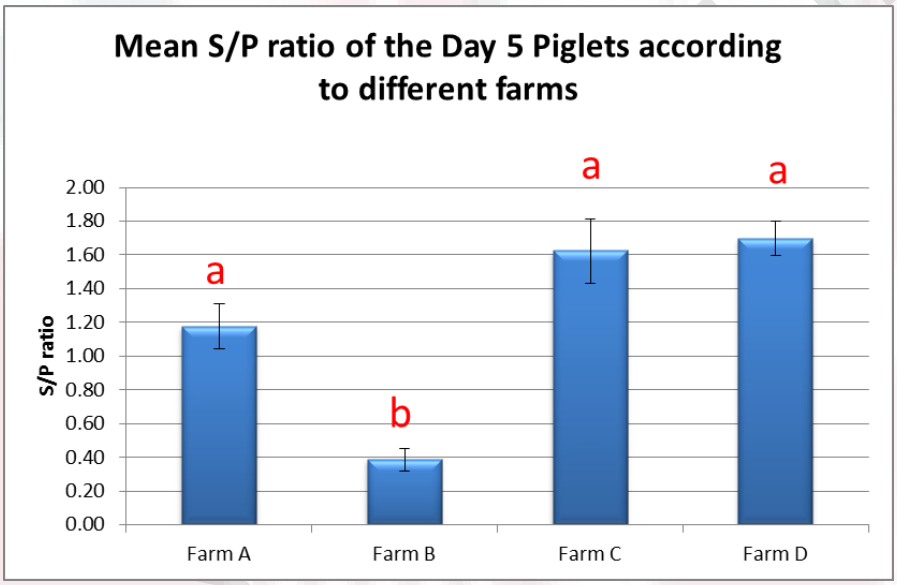

Figure 4.2.2b: Mean S/P ratio of the day 5 piglets according to different farms. Error bars indicates standard error (SE) of mean. Means with [a, b] are significantly different ($p<0.05$)

The mean S/P ratio of 0.4 for piglets in farm B was significantly different ($p<0.05$) from the rest of the farms (Figure 4.2.2b). The lower mean S/P ratio may be due to lower maternal antibody present in the piglets of Farm B. According to Albina et al., 1994, the maternal antibodies for PRRSV provided by colostrum were present in the serum until the third week of age, just before weaning. The results of piglets in farm B

correlates with the result obtained for the sow group in farm B as the mean S/P ratio was also low in the sow group and half of the sows were seronegative by ELISA.

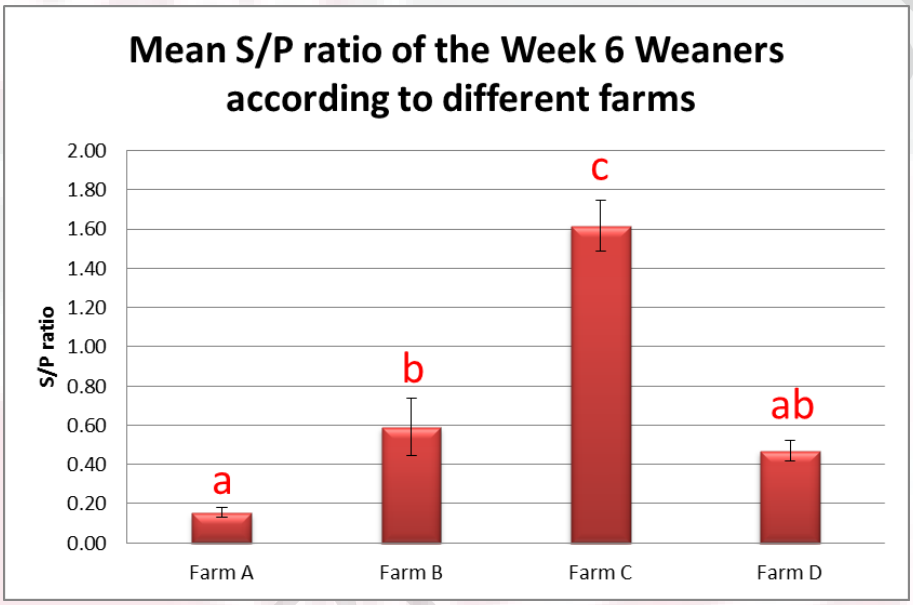

Figure 4.2.2c: Mean S/P ratio of the week 6 weaners according to different farms. Error bars indicates standard error (SE) of mean. Means with [a, b,c] are significantly different ($p<0.05$)

As for week 6 weaners (Figure 4.2.2c), there was no significant difference between Farm B and D. Both Farm B and D which practiced vaccination on piglets at day 10-14 had a higher mean S/P ratio of 0.59 and 0.47 respectively, which it suggested exposure to vaccine virus. Farm C which did not practice vaccination on piglet has the highest mean S/P ratio of 1.62 and it was significantly different ($p<0.05$) from Farm A, B and D. It was suggestive of exposure to field virus. As for Farm A which has low mean S/P ratio of 0.246, it may be because of there were no vaccination of piglets and lack of exposure of weaners to field virus. When there was little PRRS virus in circulation among

breeding adults in the herd, most piglets will be negative by six weeks of age as seen in Farm A where the S/P ratio of six weeks old weaners were generally lower than the cut off value of 0.4 in PRRS X3 ELISA test. (Rajik *et al*, 2001)

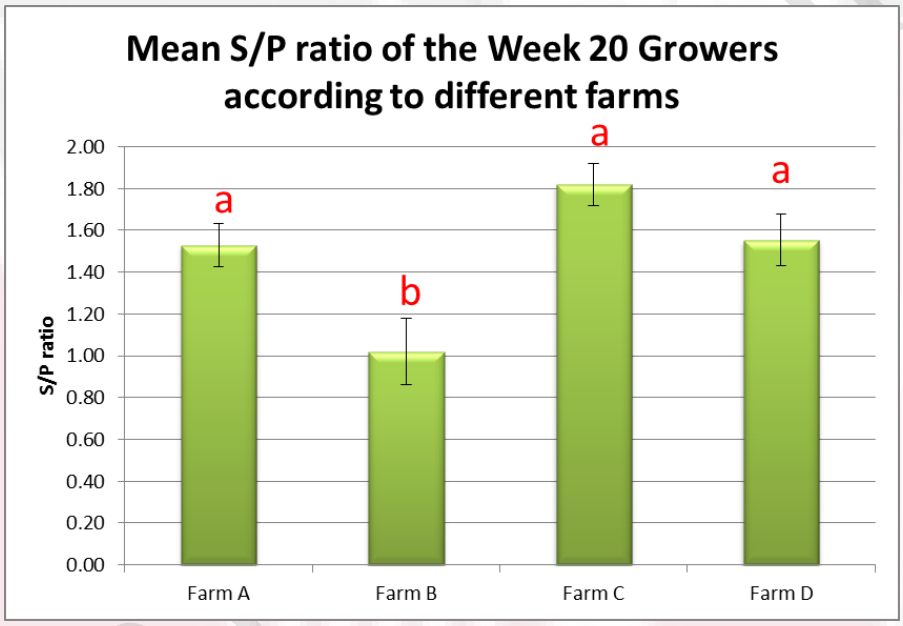

Figure 4.2.2d: Mean S/P ratio of the week 20 growers according to different farms. Error bars indicates standard error (SE) of mean. Means with [a,b] are significantly different ($p<0.05$)

Generally, all farms showed higher mean S/P ratio in grower group compared to other age group (Figure 4.2.2d). This may be because of when transferred to the finishing floor with other infected pigs, previously uninfected pigs will become infected and seroconvert. However, there is significantly lower mean S/P ratio of growers in Farm B ($p<0.05$). It suggests that there is a lower resident PRRSV circulation and thus a lower exposure to virus giving rise to a lower mean S/P ratio.

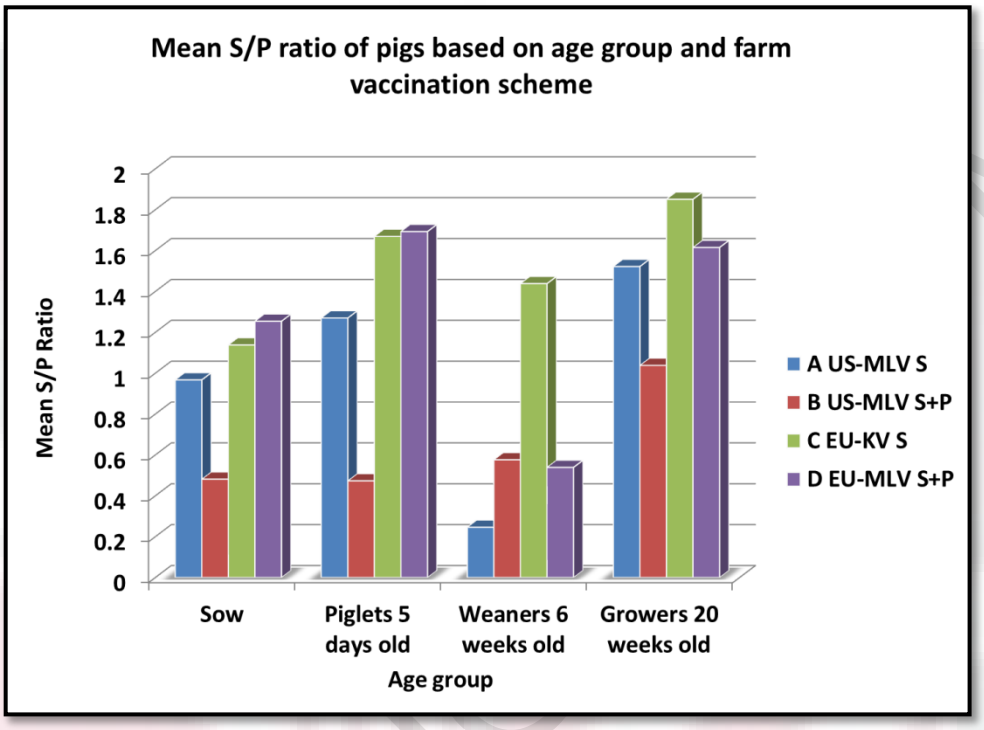

Figure 4.2.2e: Mean S/P ratio of the pigs according to different farms, vaccination scheme and age group.

As a whole, Farm B had a lower mean S/P ratio across all age group. This may suggest the reduced circulation of PRRS virus in herd and therefore exposure status of pigs to virus was lower. Farm B which used MLV vaccination on sow and also piglet, it practice of multi-site farming system may help in reducing the circulating PRRSV resident in farm. As for farm C, the mean S/P ratio was always on the higher end, suggested there was still presence of challenge from field virus in farm. Farm C practiced killed vaccine on sows. Study had reported killed vaccine tested provided weak memory responses with sequential challenge without any obvious active immune responses in the vaccinated pigs (Kim *et al*, 2011). This lack of cell mediated immunity may cause a higher

risk of pigs being infected during weaning when maternal antibody had decayed. Thus, the shedding and circulation of virus in weaning and finishing unit remain high. The use of inactivated PRRSV vaccine should be administered on a regular basis for obtaining the maximum beneficial effect, as it has been observed that the higher the degree of immunization of sows, the better the improvement of their health status and reproductive performance (Papatsiros, 2012).

After revaccination, antibody response seems to be attenuated among vaccinated adults in a stable breeding herd; possibly greater than 20% of animals in a well maintained, vaccinated herd may be seronegative. An ideal herd average S/P ratio target would be 1.00 as seen in the ELISA result of sows from Farm B. Regular monitoring is needed to ensure that a new strain of PRRS virus has not entered the herd. If there is introduction of heterologous field virus which rapidly spreads through the vaccinated herd, typically 90% or more of the sows will become positive, with S/P ratio ranging up to 2.50 (Erickson, 1996).

Tentatively, all four farms participated in the study may be classified into Stage IIa (Positive stable breeding herds that are not undergoing PRRSV elimination) where there were an uncertain shedding status and positive exposure status. Farm B may be one step ahead compared to the other farms in terms of reducing virus shedding in farm. Steps had been taken to reduce virus shedding which are practice of whole-herd exposure to MLV vaccination and multisite farming system where the farrowing-weaning unit and weaning-finishing unit were separated. However, absence of clinical signs of PRRS in the

breeding-herd population 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 weaners sampled every 30 days or more frequently (Holtkamp *et al*, 2011).

Stage II is important for managing PRRSV in farm production setting. The absence of viremia is important for farm in management of pig flow, potentials about improved reproductive and grower performance. For breeding herds that are trying to control the virus, being Positive Stable (II) will be the goal of farm. In terms of national elimination efforts, subdivision of Stage II into II-A and II-B is crucial to differentiate the risk of existing or future shedding of virus by animals in the farm (Holtkamp *et al*, 2011).

5.0 CONCLUSION

As a conclusion, the present study showed that application of PRRS MLV vaccine will not cause viraemia post four weeks vaccination. Thus, the PRRS vaccines used in Malaysia are safe. In addition, MLV vaccination on sows and piglets may help to reduce

virus circulation in farm. Tentatively, all four farms participated in the study may be classified into Stage IIa (Positive stable breeding herds that are not undergoing PRRSV elimination) where there were an uncertain shedding status and positive exposure status.

6.0 RECOMMENDATION

A continuous time-point assessment of PRRSV shedding status & exposure status of weaners & breeding herd should be carried out using 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 weaners sampled every 30 days or more frequently. Thus, the study should be continued for at least 3 months with sampling once a month to 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 to integrate the information and evaluate interventions done in farm.

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APPENDIX I

Results for IDEXX PRRS X3 Antibody Test in Farm A

No.	Age	OD	S/P	Results
1.	A 5D	0.705	0.961	Pos
2.	A 5D	0.528	0.699	Pos
3.	A 5D	0.733	1.003	Pos
4.	A 5D	1.121	1.578	Pos
5.	A 5D	0.468	0.610	Pos
6.	A 5D	1.152	1.624	Pos
7.	A 5D	1.080	1.517	Pos
8.	A 5D	0.304	0.367	Neg
9.	A 5D	0.965	1.347	Pos
10.	A 5D	0.963	1.344	Pos
11.	A 5D	1.042	1.461	Pos
12.	A 5D	1.417	2.016	Pos
13.	A 5D	1.056	1.481	Pos
14.	A 5D	0.823	1.136	Pos
15.	A 5D	1.345	1.910	Pos
16.	A 6W	0.085	0.043	Neg
17.	A 6W	0.172	0.172	Neg
18.	A 6W	0.159	0.153	Neg
19.	A 6W	0.559	0.667	Pos
20.	A 6W	0.154	0.145	Neg
21.	A 6W	0.078	0.033	Neg
22.	A 6W	0.169	0.167	Neg
23.	A 6W	0.179	0.182	Neg
24.	A 6W	0.122	0.098	Neg
25.	A 6W	0.202	0.216	Neg
26.	A 6W	0.118	0.092	Neg
27.	A 6W	0.504	0.664	Pos
28.	A 6W	0.307	0.372	Neg
29.	A 6W	0.171	0.170	Neg
30.	A 6W	0.131	0.111	Neg

No.	Age	OD	S/P	Results
31.	A 20W	1.033	1.447	Pos
32.	A 20W	1.360	1.932	Pos
33.	A 20W	1.056	1.481	Pos
34.	A 20W	1.396	1.985	Pos
35.	A 20W	0.743	1.018	Pos
36.	A 20W	1.182	1.668	Pos
37.	A 20W	1.105	1.554	Pos
38.	A 20W	1.332	1.890	Pos
39.	A 20W	0.192	0.201	Neg
40.	A 20W	1.103	1.551	Pos
41.	A 20W	0.984	1.375	Pos
42.	A 20W	1.609	2.301	Pos
43.	A 20W	1.487	2.120	Pos
44.	A 20W	1.045	1.465	Pos
45.	A 20W	0.619	0.834	Pos
46.	A SOW	0.892	1.239	Pos
47.	A SOW	0.456	0.593	Pos
48.	A SOW	0.979	1.367	Pos
49.	A SOW	0.541	0.719	Pos
50.	A SOW	0.268	0.314	Neg
51.	A SOW	0.658	0.892	Pos
52.	A SOW	0.987	1.379	Pos
53.	A SOW	1.107	1.557	Pos
54.	A SOW	0.573	0.766	Pos
55.	A SOW	0.613	0.825	Pos
56.	A SOW	1.054	1.479	Pos
57.	A SOW	0.730	0.999	Pos
58.	A SOW	0.500	0.658	Pos
59.	A SOW	0.575	0.769	Pos
60.	A SOW	0.698	0.951	Pos

APPENDIX II

*6W: 6 weeks old weaners *20W: 20 weeks old growers

No.	Age	OD	S/P	Results
1.	B 5D	0.168	0.166	Neg
2.	B 5D	0.223	0.247	Neg
3.	B 5D	0.163	0.159	Neg
4.	B 5D	0.270	0.317	Neg
5.	B 5D	0.367	0.461	Pos
6.	B 5D	0.268	0.314	Neg
7.	B 5D	0.273	0.321	Neg
8.	B 5D	0.552	0.735	Pos
9.	B 5D	0.534	0.708	Pos
10.	B 5D	0.268	0.314	Neg
11.	B 5D	0.340	0.421	Pos
12.	B 5D	0.132	0.113	Neg
13.	B 5D	0.747	1.024	Pos
14.	B 5D	0.582	0.779	Pos
15.	B 5D	0.741	1.015	Pos
16.	B 6W	0.884	1.227	Pos
17.	B 6W	0.632	0.853	Pos
18.	B 6W	0.179	0.182	Neg
19.	B 6W	0.130	0.11	Neg
20.	B 6W	0.467	0.609	Pos
21.	B 6W	0.551	0.733	Pos
22.	B 6W	0.728	0.996	Pos
23.	B 6W	0.176	0.178	Neg
24.	B 6W	1.166	1.644	Pos
25.	B 6W	0.178	0.181	Neg
26.	B 6W	0.115	0.087	Neg
27.	B 6W	0.275	0.324	Neg
28.	B 6W	0.986	1.378	Pos
29.	B 6W	0.104	0.071	Neg
30.	B 6W	0.106	0.074	Neg

No.	Age	OD	S/P	Results
31.	B 20W	0.784	1.014	Pos
32.	B 20W	0.768	0.992	Pos
33.	B 20W	0.846	1.1	Pos
34.	B 20W	1.231	1.635	Pos
35.	B 20W	1.058	1.395	Pos
36.	B 20W	1.522	2.04	Pos
37.	B 20W	0.287	0.323	Neg
38.	B 20W	1.070	1.411	Pos
39.	B 20W	0.587	0.74	Pos
40.	B 20W	0.417	0.504	Pos
41.	B 20W	0.770	0.994	Pos
42.	B 20W	0.690	0.883	Pos
43.	B 20W	1.100	1.453	Pos
44.	B 20W	0.208	0.213	Neg
45.	B 20W	0.682	0.872	Pos
46.	B SOW	0.273	0.304	Neg
47.	B SOW	0.106	0.221	Neg
48.	B SOW	0.246	0.266	Neg
49.	B SOW	0.657	0.837	Pos
50.	B SOW	0.410	0.494	Pos
51.	B SOW	0.507	0.629	Pos
52.	B SOW	0.125	0.098	Neg
53.	B SOW	0.505	0.626	Pos
54.	B SOW	0.355	0.418	Pos
55.	B SOW	0.731	0.94	Pos
56.	B SOW	1.036	1.364	Pos

* 5D: 5 day old piglets *6W: 6 weeks old weaners *20W: 20 weeks old growers

Results interpretation:

S/P ratios ≥ 0.4: positive

Results for IDEXX PRRS X3 Antibody Test in Farm C

No.	Age	OD	S/P	Results
1.	C 5D	0.506	0.628	Pos
2.	C 5D	0.508	0.630	Pos
3.	C 5D	0.660	0.842	Pos
4.	C 5D	1.326	1.767	Pos
5.	C 5D	1.301	1.732	Pos
6.	C 5D	0.886	1.156	Pos
7.	C 5D	1.013	1.332	Pos
8.	C 5D	1.183	1.568	Pos
9.	C 5D	1.080	1.425	Pos
10.	C 5D	1.746	2.351	Pos
11.	C 5D	1.890	2.551	Pos
12.	C 5D	1.684	2.265	Pos
13.	C 5D	1.687	2.269	Pos
14.	C 5D	1.655	2.224	Pos
15.	C 5D	1.706	2.295	Pos
16.	C 6W	1.299	1.730	Pos
17.	C 6W	1.421	1.899	Pos
18.	C 6W	1.354	1.806	Pos
19.	C 6W	1.303	1.735	Pos
20.	C 6W	0.166	0.155	Neg
21.	C 6W	1.241	1.649	Pos
22.	C 6W	0.997	1.310	Pos
23.	C 6W	1.374	1.834	Pos
24.	C 6W	0.487	0.601	Pos
25.	C 6W	1.373	1.833	Pos
26.	C 6W	1.582	2.123	Pos
27.	C 6W	0.643	0.818	Pos
28.	C 6W	1.644	2.209	Pos
29.	C 6W	0.330	0.383	Neg
30.	C 6W	1.119	1.479	Pos

No.	Age	OD	S/P	Results
31.	C 20W	1.272	1.692	Pos
32.	C 20W	1.168	1.548	Pos
33.	C 20W	1.502	2.012	Pos
34.	C 20W	1.146	1.517	Pos
35.	C 20W	1.403	1.874	Pos
36.	C 20W	1.487	1.991	Pos
37.	C 20W	1.475	1.974	Pos
38.	C 20W	0.888	1.158	Pos
39.	C 20W	1.211	1.607	Pos
40.	C 20W	1.627	2.186	Pos
41.	C 20W	1.136	1.503	Pos
42.	C 20W	1.700	2.287	Pos
43.	C 20W	1.807	2.436	Pos
44.	C 20W	1.662	2.234	Pos
45.	C 20W	1.307	1.741	Pos
46.	C SOW	0.536	0.669	Pos
47.	C SOW	0.315	0.362	Neg
48.	C SOW	1.032	1.359	Pos
49.	C SOW	1.459	1.952	Pos
50.	C SOW	0.812	1.053	Pos
51.	C SOW	0.893	1.165	Pos
52.	C SOW	0.683	0.874	Pos
53.	C SOW	0.510	0.633	Pos
54.	C SOW	1.064	1.403	Pos
55.	C SOW	0.372	0.441	Pos
56.	C SOW	1.458	1.951	Pos
57.	C SOW	0.786	1.017	Pos
58.	C SOW	0.717	0.921	Pos
59.	C SOW	1.319	1.757	Pos
60.	C SOW	1.145	1.516	Pos

APPENDIX IV

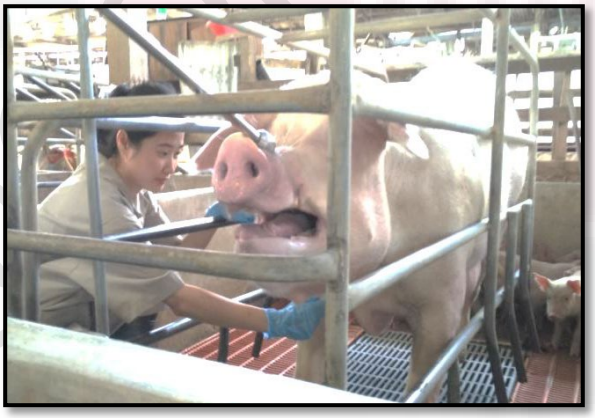
*6W: 6 weeks old weaners *20W: 20 weeks old growers

No.	Age	OD	S/P	Results
1.	D 5D	1.434	1.822	Pos
2.	D 5D	1.052	1.318	Pos
3.	D 5D	1.569	2.001	Pos
4.	D 5D	1.804	2.311	Pos
5.	D 5D	1.252	1.582	Pos
6.	D 5D	1.024	1.281	Pos
7.	D 5D	1.727	2.209	Pos
8.	D 5D	1.128	1.418	Pos
9.	D 5D	1.129	1.420	Pos
10.	D 5D	1.348	1.709	Pos
11.	D 5D	1.145	1.441	Pos
12.	D 5D	1.079	1.354	Pos
13.	D 5D	1.394	1.770	Pos
14.	D 5D	1.602	2.044	Pos
15.	D 5D	1.348	1.709	Pos
16.	D 6W	0.518	0.613	Pos
17.	D 6W	0.508	0.600	Pos
18.	D 6W	0.950	1.183	Pos
19.	D 6W	0.532	0.632	Pos
20.	D 6W	0.055	0.002	Neg
21.	D 6W	0.158	0.138	Neg
22.	D 6W	0.343	0.382	Neg
23.	D 6W	0.416	0.479	Pos
24.	D 6W	0.255	0.266	Neg
25.	D 6W	0.555	0.662	Pos
26.	D 6W	0.997	1.246	Pos
27.	D 6W	0.622	0.750	Pos
28.	D 6W	0.299	0.324	Neg
29.	D 6W	0.359	0.403	Pos
30.	D 6W	0.361	0.406	Pos

No.	Age	OD	S/P	Results
31.	D 20W	1.564	1.994	Pos
32.	D 20W	0.844	1.044	Pos
33.	D 20W	1.722	2.203	Pos
34.	D 20W	0.810	0.999	Pos
35.	D 20W	1.426	1.812	Pos
36.	D 20W	1.379	1.750	Pos
37.	D 20W	0.999	1.248	Pos
38.	D 20W	1.291	1.634	Pos
39.	D 20W	1.045	1.309	Pos
40.	D 20W	1.629	2.080	Pos
41.	D 20W	1.222	1.543	Pos
42.	D 20W	1.455	1.850	Pos
43.	D 20W	1.682	2.150	Pos
44.	D 20W	1.284	1.624	Pos
45.	D 20W	0.799	0.984	Pos
46.	D SOW	0.902	1.120	Pos
47.	D SOW	1.358	1.722	Pos
48.	D SOW	1.421	1.805	Pos
49.	D SOW	1.091	1.370	Pos
50.	D SOW	0.086	0.043	Neg
51.	D SOW	1.134	1.426	Pos
52.	D SOW	0.767	0.942	Pos
53.	D SOW	0.990	1.236	Pos
54.	D SOW	0.572	0.684	Pos
55.	D SOW	1.100	1.382	Pos
56.	D SOW	1.192	1.503	Pos
57.	D SOW	0.984	1.228	Pos
58.	D SOW	1.141	1.436	Pos
59.	D SOW	0.920	1.144	Pos
60.	D SOW	1.388	1.762	Pos

*6W: 6 weeks old weaners *20W: 20 weeks old growers

Blood Collection



Snare was used to restraint sow during blood collection.