UNIVERSITI PUTRA MALAYSIA

APPLICATION OF ORAL FLUID TO DETECT PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) BY USING ELISA

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APPLICATION OF ORAL FLUID TO DETECT PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) BY USING ELISA

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A project paper submitted to the

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It is hereby certified that I have read this project paper entitled "Application of Oral Fluid to Detect Porcine Reproductive and Respiratory Syndrome (PRRS) by Using ELISA", by Kuiek Ah Meng 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 – Final Year Project.

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DEDICATION

To,

My dearest family,

friends,

and everyone who work in the swine industry.

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LIST OF ABBREVIATIONS

Ab	Antibody
CSF	Classical Swine Fever
DPI	Day post infection
ELISA	Enzyme-Linked Immunosorbent Assay
HIV	Human Immunodeficiency Virus
HRPO	Horseradish Peroxidase
Ig	Immunoglobulin
IHC	Immunohistochemistry
IFA	Indirect Fluorescent Antibody
IPMA	Immunoperoxidase Monolayer Assay
µl	Microliter
nm	Nanometer
OD	Optical density
OF	Oral fluid
PAM	Porcine alveolar macrophages
PCV	Porcine Circovirus
PPV	Porcine Parvovirus
PRRS	Porcine Reproductive and Respiratory Syndrome
PRRSV	Porcine Reproductive and Respiratory Syndrome Virus
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SVN	Serum Virus Neutralisation
S/P ratio	Sample mean to positive control mean ratio
TMB	Tetramethylbenzidine
^{o}C	Degree Celcius

ABSTRAK

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

APLIKASI CECAIR ORAL UNTUK MENGESAN SINDROM REPRODUKSI DAN PERNAFASAN PORSIN (PRRS) DENGAN MENGGUNAKAN ELISA

Oleh

Kuiek Ah Meng

2015

Penyelia: Dr. Ooi Peck Toung

Sindrom Reproduksi dan Pernafasan Porsin (PRRS) adalah penyakit yang amat mudah berjangkit dan memainkan peranan ekonomi yang penting di Malaysia. Oleh itu, kaedah-kaedah diagnosis yang lebih baik diperlukan untuk tujuan pemantauan penyakit. Kajian ini dijalankan untuk menilai penggunaan sampel cecair oral selain daripada sampel serum untuk mengesan PRRS dengan menggunakan alat ujian IDEXX ELISA. Kajian ini melibatkan dua ladang babi yang terletak di Perak dan Selangor, Malaysia. 35 haiwan digunakan sebagai subjek dari setiap ladang. 35 haiwan ini dibahagikan kepada 7 kategori: babi dara, ibu babi muda, ibu babi tua, dan babi pedaging. Sampel cecair oral dan serum dikumpul secara individu daripada semua kategori manakala sampel cecair oral

berdasarkan kandang dikumpul daripada babi pedaging sahaja. Sampel cecair oral dan serum masing-masing diuji dengan menggunakan *IDEXX PRRS Oral Fluid Antibody Test Kit* dan *IDEXX PRRS X3 Anitbody Test Kit*. Terdapat hubungan yang bererti, kuat, dan positif antara sampel untuk kedua-dua Ladang A (p=0.0001, r=0.681) dan Ladang B (p=0.0001, r=0.601). Kesimpulannya, selain daripada sampel serum, cecair oral juga boleh digunakan sebagai alat diagnostik untuk pemantauan PRRS.

Kata Kunci: *Cecair Oral, IDEXX PRRS Oral Fluid Antibody Test Kit, IDEXX PRRS X3 Antibody Test Kit, Sampel Serum, Sindrom Reproduksi dan Pernafasan Porsin (PRRS).*

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.

APPLICATION OF ORAL FLUID TO DETECT PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) BY USING ELISA

By

Kuiek Ah Meng

2015

Supervisor: Dr. Ooi Peck Toung

Porcine Reproductive and Respiratory Syndrome (PRRS) is disease that is highly contagious and of great economic importance in Malaysia. Therefore, reliable and improved diagnostic methods are needed to help disease surveillance. This study evaluates the use of oral fluid samples instead of serum samples to detect PRRS by using IDEXX ELISA test kit. The study involves two pig farms located at Perak and Selangor, Malaysia. 35 animals were used as subjects from each farm. These 35 animals were divided into 7 different categories: gilts, young sows (2^{nd} to 5^{th} parities), old sows (6^{th} parities and above), and weaners (10 weeks old, 15 weeks old, 20 weeks old, and 25 weeks old). Oral fluid and serum samples were collected from these animals individually

whereas pen oral fluid samples were collected from weaners only. The oral fluid and serum samples were tested with IDEXX PRRS Oral Fluid Antibody Test Kit and IDEXX PRRS X3 Antibody Test Kit respectively. Statistical analysis shows that there is significant, strong, and positive correlation between samples for Farm A ($p=0.0001$, $r=0.681$) and significant, moderate, and positive correlation between samples for Farm B ($p=0.0001$, $r=0.601$). In conclusion, other than serum samples, oral fluids can also be used as diagnostic tool for PRRS surveillance in the farm.

Keywords: *IDEXX PRRS Oral Fluid Test Kit, IDEXX PRRS X3 Test Kit, Oral Fluid Porcine Reproductive and Respiratory Syndrome (PRRS), Serum Samples*

1.0 INTRODUCTION

Porcine Reproductive and Respiratory Syndrome (PRRS) is one of the major threats to pig industry as it will cause great economic loss due to reproduction failure such as abortions in sows, delayed return to estrus, and low conception rates. Besides that, due to high pre-weaning mortality up to 60%, it will also cause significant economic loss to the pig farmers as they are not able to increase the production of the farm. Base on a recent survey, it estimated that losses due to PRRS problems in the United State is as high as $668.58 million annually (Zimmerman, Karriker, Ramirez, Schwartz, & Stevenson, 2012).

There are two major genetic lineages of PRRS virus (PRRSV), which are type 1 (European type) and type 2 (North American type) genotypes currently. . The presence of type 2 PRRSV in Asia appears to be due to introductions of pigs from North American which then cause local diversification of PRRSV type 2 virus and lead to new disease outbreaks and increase in virulence. Hence, due to geographical distribution and transboundary disease transmission, it cannot be denied that PRRS has already entered our country, Malaysia. From two individual surveillance studies done in 2008 and 2012, both results showed high seroprevalence of PRRS in Malaysia and this suggested that Malaysia is endemic for PRRS. Therefore, it is important to carry out proper surveillance program for PRRS in Malaysia and in order to achieve that, it is necessary to come out with efficient and effective diagnostic methods that can be used widely in the industry.

Generally, tentative diagnosis of PRRS can be made by looking at the clinical signs such as reproduction problem in breeding stocks or respiratory disease in pigs of any age.

However, since clinical syndromes of PRRSV are not consistent and does not cause specific lesion, differential tests are needed in order to achieve definitive diagnosis. The differential diagnosis includes porcine parvovirus infection (PPV), porcine circovirus type-2 infection (PCV2), and classical swine fever (CSF) based on the clinical signs related to reproduction and respiratory problems. Hence, when the clinical signs and post-mortem findings are suggestive of PRRS, detection of viral antigens, viral genomic material, or isolation of virus from clinical specimens is necessary to confirm the tentative diagnosis made. Besides that, rising serum antibodies against PRRSV can also be used to support the diagnosis, provided the time frame is compatible with the clinical episodes.

There are several laboratory diagnosis of PRRS, such as detection of serum antibodies by using commercial PRRS ELISA (Enzyme-Linked Immunosorbent Assay), reverse transcriptase polymerase chain reaction (RT-PCR), frozen tissue section fluorescent antibody (FA) test, and immunohistochemistry (IHC) test.

One of the commercially used laboratory diagnosis of PRRS in Malaysia currently is by using commercial IDEXX PRRS X3 Antibody Test Kit which is a serological test that will detect for antibodies in pig serum against PRRSV for immunological disease status surveillance. Compared to other laboratory methods such as RT-PCR or IHC, this method will enable large samples size to be analyzed at one time, which can reduce cost and labor involved. However, sampling of blood in pigs is rather laborious, time consuming, and invasive as it requires restraining and a lot of man power. Besides that, it is almost impossible to get the whole picture of PRRS immunological status in the farm by using

blood sample alone because only around 30% of animal will be sampled at one time in the farm. Therefore, a novel method of detecting PRRS among the pigs is needed.

Oral fluid is the fluid collected from the mouth by placing an absorptive device in the mouth such as cotton rope. Oral fluid collected will contain both serum transudate and saliva of the animal. Serum transudate from the animals enter the mouth from various capillaries within the oral mucosa, crevicular gap, and gingival tissues. Hence, it is possible to use oral fluid samples from animals for epidemiological studies as it contains antibodies as well such as IgA and IgM. For example, in human beings, the ease of collecting oral fluid samples has enabled the use of this approach for large epidemiological studies such as Human Immunodeficiency Virus (HIV) study. In livestock animals, oral fluid has not been used for testing widely, but veterinary literature do report on the presence of antibodies, pathogens, and acute phase proteins in the oral fluid. For example, in swine, infectious agents, cortisol, acute phase proteins, and progesterone have all been detected in oral fluid samples in both experimental and field conditions (A. Kittawornrat, 2010).

There are various ways of collecting oral fluid samples from swine. One of the methods is by hanging cotton rope inside the pens for the pigs to chew on it and oral fluid will be collected from the cotton rope by squeezing the fluid into a clean tube. The usage of cotton rope to collect oral fluid sample from pigs has been done successfully under experimental and field condition (Prickett *et al.*, 2008).

Therefore, the purpose of this study is to evaluate the usage and efficacy of oral fluid samples for PRRS detection in Malaysia and hence, replace the conventional method of using blood serum to obtain the information about PRRS immunological status in the country. The immunological status of the farms will be determined by using IDEXX PRRS Oral Fluid Antibody Test Kit and IDEXX PRRS X3 Antibody Test Kit. By comparing the results of these 2 different test kits, we will be able to decide whether oral fluid can be used to replace the conventional method of using blood serum as PRRS surveillance in Malaysia.

2.0 LITERATURE REVIEW

2.1 Porcine Reproductive and Respiratory Syndrome

Porcine Reproductive and Respiratory Syndrome (PRRS) is a viral disease that will cause reproductive failure in sows, respiratory disease in growing and finishing pigs. PRRS is caused by Arterivirus which is a small single-stranded, non-segmented RNA virus (Gómez-Laguna, Salguero, Pallarés, & Carrasco, 2013). This virus is classified as a member of the order Nidovirales and family Arteriviridae (Dietze, Pinto, Wainwright, & Hamilton, 2011).

There are two major genetic lineages of PRRS virus (PRRSV), which are PRRSV type 1 and PRRSV type 2. PRRSV type 1 is known as European type because this type predominantly found in European region while type 2 is known as North American type because it is predominantly found in North American region. In addition, type 2 PRRSV is also found in Asian region which can be due to introduction of pigs from North American that cause local diversification of PRRSV type 2 virus that leads to disease outbreaks in the region. However, both genotypes of PRRSV are now distributed worldwide due to animals' transportation (Zimmerman *et al.*, 2012).

PRRSV impairs local pulmonary immune responses by injuring the mucociliary transport system, disrupting the function of porcine alveolar macrophages (PAMs), and inducing apoptosis of immune cells. The primary target cells of PRRSV for replication are PAMs, which are responsible for the phagocytosis of microorganisms within the alveoli. Hence, the replication of PRRSV within PAMs will directly affect their functions such as

phagocytosis, antigen presentation, and production of cytokines by the cells. Most importantly, PRRSV also induces apoptosis of PAMS and other leukocytes such as lymphocytes within the lung and hence, weaken the host immune response (Gómez-Laguna et al., 2013). Replication of PRRSV will take place within the lymphoid organs such as spleen, thymus, tonsils, lymph nodes, as well as Peyer's patches and cause viremia. Besides that, PRRSV also has the ability to cross the placenta during the late gestation period and affect the fetuses. The affected fetuses may die due to the infection. Hence, abortions are common in sows infected with PRRSV due to the effects of acute disease that result in fever in sows or due to infection and death of fetuses themselves (Vetmed.iastate.edu, 2015).

Clinical signs of PRRS are not specific and vary depend on the strain of virus, the immune status of the herd, and management. Clinical diseases of PRRS is the result of acute viremia in individuals and transplacental transmission from sows to their fetuses. Besides that, it can also occur concurrent with other pathogens such as *Mycoplasma hyopneumoniae* and *Haemophilus parasuis*. In sows, the clinical signs are lethargy, reduced in appetite, fever, respiratory distress, and abortions. Meanwhile, in growing and finishing pigs, the clinical signs include loss of appetite, lethargy, labored or rapid breathing, reddening of the skin, and rough hair coats. Since the clinical signs are not specific, other diseases such as Classical Swine Fever (CSF), Porcine Parvovirus (PPV), and Swine Influenza (SI) are possible differential diagnosis.

PRRS can be controlled by the use of vaccine, management of replacement gilts, and implementation of good biosecurity protocols in order to reduce the risk of PRRSV transmission within and between herds. Introduction of pigs into the farm must be carried out properly and all the pigs enter the farm must be quarantined. This is because the disease is able to spread quickly within the herd once it is introduced into the farm (OIE, 2008). Disease eradication is possible by whole herd depopulation and repopulation, test and removal of infected pigs, and herd closure. Up to now, countries that are free from PRRS are Argentina, Australia, Cuba, Finland, New Zealand, New Caledonia, Norway, Sweden, and Switzerland. This is because these countries have relatively small swine populations, use more traditional methods of swine production, and involve in less trade of live animals and by-products of swine origin (Extension.org, 2012).

2.2 Diagnosis of Porcine Reproductive and Respiratory Syndrome

Diagnosis of PRRS can be made by looking at few factors such as history of the farm itself, for example, vaccination status of the pigs in the farm. There are several vaccines available in the global market such as modified live-vaccine and killed vaccine. In Malaysia, most commonly used vaccine against PRRS is Ingelvac® PRRS MLV which is a type 2 modified live vaccine. Clinical signs which are associated with reproductive problems such as increase in abortion among sows and respiratory problems among any ages of pigs can also be used to diagnose PRRS in the farm. Furthermore, post mortem examination of affected pigs will show lesions such as enlarged lymph nodes, edema

eyelids, and firm with non-collapsing lungs. Meanwhile, further histopathology examination of the lungs will show interstitial pneumonia due to virus infiltrations.

However, since all these clinical signs and lesions are not definitive and can be caused by different type of agents as well, laboratory tests are necessary to make a definitive diagnosis of PRRS (Yoon & Nelson, 2003). Both molecular and serology tests are available to diagnose PRRS. Molecular tests include the use of Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) to demonstrate the involvement of the antigens. Virus isolation in swine pulmonary alveolar macrophages can also be done to isolate the virus and cytopathic effect will be observed in 1 to 4 days. Samples such as lung, lymph nodes, spleen, tonsils, serum, and buffy coats can be taken to make the diagnosis (OIE, 2008).

There are several serological tests can be done to diagnose PRRS such as Enzyme-Linked Immunosorbent Assays (ELISA), blocking ELISA, Immunoperoxidase Monolayer Assay (IPMA), serum virus neutralization (SVN), and Indirect Fluorescent Antibody (IFA) techniques. This is because there has been documentation about the rising in serum antibodies during the occurrence of clinical episodes of PRRS. Hence, serological tests can be useful to diagnose PRRS provided the time frame when clinical episodes take place is compatible.

2.3 Porcine Reproductive and Respiratory Syndrome Molecular Test

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) is used to detect the genetic material or genome of PRRSV in swine tissue homogenates as well as in clinical

specimens such as semen, serum, oral fluid, oropharyngeal scrapings, and lung lavage samples. Reverse transcriptase step is necessary to convert RNA to DNA, which enable it to be amplified by PCR and hence, enable detection of viral RNA (Christopher-Hennings *et al.*, 2002).

Advantages of using RT-PCR include rapid turnover time and high sensitivity, specificity. However, even though this method has high sensitivity and specificity, viral genome may not be detected if there are significant genomic differences between the PRRSV in samples and the primers being used in the assay. For example, some PCR primers detect type 2 PRRSV strains, but not European or European-like strains. Therefore, it is important to identify which type of PRRSV strains we are suspecting or going to test because correct primers need to be used (Christopher-Hennings *et al.*, 2002).

In addition, positive RT-PCR results may not necessarily indicate the presence of replicating virus in the sample. However, there is high correlation between RT-PCR results and detection of replicating virus. Next, we also cannot evaluate the severity of infection caused by PRRSV based on RT-PCR results alone (Han *et al.*, 2012).

2.4 Porcine Reproductive and Respiratory Syndrome Serological Test

Serology diagnosis of PRRS is more preferred by many swine practitioners because serum is easy to be collected and can be used for multiple tests as well as can be stored for future references. In addition to that, serological tests are also easier to be conducted with good sensitivity and specificity (OIE, 2010).

2.4.1 Enzyme-Linked Immunosorbent Assays

ELISA is the most common method used to diagnose PRRS because it can provide rapid results and more samples can be run at one time. Previously, ELISA only can work based on serum samples, however recently, oral fluid samples can also be used to detect antibodies against PRRS instead of using blood serum. This is because oral fluid contains both saliva and serum transudate (contains local and serum-derived antibodies, pathogens). Therefore, diagnosing of diseases become easier with the new oral fluid sampling methods although it is not widely used in the livestock industry yet.

IDEXX PRRS X3 Antibody Test Kits

Commercial ELISA is one of the more commonly used methods to diagnose PRRS serologically because it is affordable, easy to perform, and accurate. There are several ELISA test kits commercially available such as *HerdCheck® PRRS X3* by *IDEXX Laboratories*, *CIVTEST Suis PRRS®* by *Laboratories Hipra*, *Bio-Vet PRRS-Blocking®* by *Biovet Laboratories*, *LSIVet™ Porcine PRRS/EU Confirmation Serum ELISA* by *Life Technology* and *Ingezim PRRS DR* by *Ingenasa, Anigen Ab ELISA 4.0* by *Bionote*. All these test kits use blood serum to run the diagnosis. Commercial ELISA test kit *HerdCheck® PRRS X3* by *IDEXX Laboratories* is most commonly used in Malaysia and it is sensitive, specific, standardized, and rapid. This test kit gives 99.9% diagnostic specificity by using 1445 negative sera (Iowa State University, 2010).

IDEXX PRRS X3 Antibody Test Kit diagnoses ELISA results based on the sample to positive ratio (S/P ratio). The results are interpreted as positive when the S/P ratio is 0.4 or more. Negative results are when the S/P ratio are less than 0.4. The test targets antibodies to the nucleocapsid antigens for both North American and European PRRSV and it can detect antibodies as early as 9 days post infection (DPI), peak at 30 to 50 DPI, and then decline to negative 4 to 12 months post infection (Roberts, 2013).

Hence, proper analysis of serum antibodies level will enable the detection of ongoing active infection within the farm and determine whether the reproductive and respiratory problems in the farm are due to PRRSV or other antigens.

IDEXX PRRS Oral Fluid Antibody Test Kits

Oral fluid contains saliva and serum transudate. This mixture contains both local and serum derived pathogens and antibodies. Hence, it can be used as a very useful tool for disease investigation and epidemiology study. For example, in humans, lot of researches and epidemiology studies have been done by using oral fluid such as Malta Fever by Pollaci and Ceralo during 1909, and Human Immunodeficiency Virus (HIV) infection during 1986. Primary focus of using oral fluid is the detection of antibodies in the sample.

Testing oral fluids provide a more comprehensive picture of herd status if compared to conventional method of animal bleeding from a randomly selected herd. This is because oral fluids can be collected from different pens in the farm easily by just hanging the cotton rope in the pens and make sure the animals are chewing on it and contribute for the

oral fluids. Most importantly, this method is also less invasive and hence, can reduce stress implied to the animals.

IDEXX PRRS Oral Fluid Antibody (IDEXX PRRS OF Ab) test kit allows the detection of antibodies within 4 hours with specificity up to 98.7% and sensitivity up to 100% (with minimum specificity and sensitivity of 92.2% and 94.2% respectively, at 95% confidence interval). Therefore, it can be a very useful diagnostic tool for PRRS monitoring and surveillance within the farm.

Same as IDEXX PRRS X3 Antibody test kit, the results of IDEXX PRRS OF Ab test kit are also interpreted based on the S/P ratio. S/P ratio of 0.4 or more is considered as positive while any S/P ratio lower than 0.4 is considered as negative result.

Therefore, IDEXX PRRS OF Ab test kit can accurately detect PRRS antibodies in the oral fluid samples from a larger sample size within a short time, approximately 4 hours. It is also less invasive and can show a larger, more comprehensive picture of PRRS immunological status in the farm. Hence, it can be applied for the purpose of better disease monitoring and surveillance within the farm from time to time.

2.4.2 Serum Virus Neutralization

SVN test is also known as serum neutralization test (SNT) which is a cell culture based assay. A known level of virus is incubated in culture of sensitive cell line with various dilutions of test serum to determine the titer of antibody which is able to neutralize PRRSV. It is less sensitive than IFA or ELISA because neutralizing antibodies against

PRRSV develop slowly post infection, which is normally more than 21 days post infection (DPI). Besides that, some pigs also seem to develop low neutralizing antibody titers against PRRSV. Generally, SVN test is more expensive and time-consuming than other PRRS serological assays such as ELISA, and more difficult to perform as well (Christopher-Hennings *et al.*, 2002).

3.0 MATERIALS AND METHODS

3.1 Animals

This study was conducted at two commercial pig farms located in Tanjung Sepat, Selangor and Bercham Baru, Perak. Both farms were using the farrow-to-finish, open house, intensive farm system.

In this study, 35 pigs of different age groups and breeding stages were randomly selected as subjects from each farm. In general, the pigs from each farm were divided into 7 different groups based on their ages and breeding cycles, which were old sow (6^{th} parities and above) , young sow (2^{nd} to 5^{th} parities), gilt, 10 weeks old weaners, 15 weeks old weaners, 20 weeks old weaners, and 25 weeks old weaners.

Both farms practiced vaccination against Porcine Reproductive and Respiratory Syndrome (PRRS) at 3 weeks of age for piglets and this will provide duration of immunity for at least 4 months or throughout the period. Meanwhile, sows and gilts were vaccinated every 3 to 4 months or 3 to 4 weeks prior to breeding. This will provide duration of immunity at least 4 months as well.

3.2 Sampling and Sample Handling

Oral fluid samplings were done by using 3-strand twisted undyed cotton rope. Both individual and pen samplings were done for oral fluid. For individual oral fluid sampling, pigs were identified depending on their age groups and breeding cycles. After subjects were identified, untied and hanged the rope in a clean area in the individual pen and let

the subjects chewed on it for about 20 minutes. Individual oral fluid samplings were done in old sows, young sows, and weaners from different age groups which were 10 weeks old, 15 weeks old, 20 weeks old, and 25 weeks old. 5 subjects will be selected randomly from each category for the samplings. For individual oral fluid sampling, it is important to prevent other pigs from chewing the same rope to prevent cross contamination.

Next, for pen oral fluid sampling, the ropes will be hanged inside the pen for 20 to 30 min to allow all subjects to chew. It is important to ensure all the subjects within the pen contribute to the oral fluid so that a bigger picture of PRRS immunological status in the farm will be collected. Pen oral fluid samplings will be collected from 4 different age groups: 10 weeks old, 15 weeks old, 20 weeks old, and 25 weeks old. Pen oral fluid samples were collected up to three time as replicates.

After allowing the subjects to chew the rope, oral fluid collections will be done by using clean plastic bags attached to clean collection tubes. Gloves were wear while handling the samples to prevent contamination. Collection was done by inserting the wet end of ropes into a clean plastic bag. Next, squeezed the rope slowly and the fluid will slowly move into the collection tubes. Sealed and labelled the collection tubes properly for future references. A minimum of 2.5 ml of oral fluid sample is needed.

Next, blood samples were collected from pigs via jugular veins by using Vacutainer and Vacutainer Multi-Sample needles (18G x 1.5”) into plain tubes. All pigs were restrained in a standing position in their own pens by using a pig snare.

Blood and oral fluid samples collected were labelled and stored immediately in an ice box at 4^{o}C after each collection and left for about 4 hours. After that, serum was extracted from each blood sample carefully by using a pipette. The serums were transferred into individual 1.5ml microcentrifuge tubes, labelled accordingly, and stored in Acson® horizontal chest freezer at temperature -20^{o}C. Both samples were kept until they were ready to be used for serology testing.

3.3 Serological Tests

The serum and oral fluid samples were sent to private laboratory for *IDEXX ELISA* diagnostic test kit analysis. Serum samples were tested by using *IDEXX PRRS X3 Antibody Test Kit* whereas oral fluid samples were tested by using *IDEXX PRRS Oral Fluid Antibody Test Kit.* Both test kits used the concept of indirect ELISA.

IDEXX PRRS X3 Antibody Test Kit

Serum samples were thawed to suit the room temperature at 25^{o}C before they were diluted 40 folds with the sample diluent, 5 µl of serum sample was diluted with 195 µl of sample diluent. The mixture was mixed evenly by using *CappAero* 8-Multichannel Pipette. Next, 100 µl undiluted positive and negative controls were added into appropriate wells of the assay plate by using the same pipette. 100 µl of diluted samples were then added to the assay plate accordingly into each well. After that, all the samples were incubated for 30 minutes at 18^{o}C. After incubation, the plate was then washed 3 to 5 times with 300 µl of wash solution per well. Then, 100 µl of Anti-Porcine: Horseradish peroxidase (HRPO) conjugate were added into each well and incubated for another 30 minutes. After that,

each well was washed 3 to 5 times again before 100 µl of Tetramethylbenzidine (TMB) substrate solution were added into each well. The plate was then incubated for another 15 minutes before 100 µl of stop solution were dispensed into each well to halt the reaction. Finally, absorbance reading at 650 nm for the controls and samples were then obtained by using ELISA microplate reader (BioTek Instruments EL800). Color development was proportional to the amount of bound specific antibodies against PRRSV present in the sample.

For this test kit, the presence or absence of PRRSV antibody in the serum samples was determined by calculating the sample to positive control (S/P) ratio for each sample. To calculate the S/P ratio, optical density (OD) of the sample at 650 nm minus the mean OD of negative control. Then, this value was then divided with the difference between mean OD of positive control and negative control. The formula is as stated below:

$$Sample\ to\ Positive\ \left(\frac{S}{P}\right) Ratio = \frac{OD_{sample} - Mean\ OD_{negative\ control}}{Mean\ OD_{positive\ control} - Mean\ OD_{negative\ control}}$$

For the assay to be valid, the difference between mean OD of positive and negative control must be more than or equal to 0.150. The mean OD of negative control must also be less than or equal to 0.150.

Serum samples with S/P ratio more than or equal to 0.4 were interpreted as positive for PRRSV antibodies while serum samples with S/P ratio less than 0.4 were considered negative for PRRSV antibodies. The S/P ratios of all the serum samples were calculated and summarized by IDEXX XCheck® software.

IDEXX PRRS Oral Fluid Antibody Test Kit

Oral fluid samples were brought to room temperature at 18ºC before they were diluted 2 folds with the sample diluent, for example, 100 µl of oral fluid sample diluted with 100 µl of sample diluent. The mixture was then mixed evenly by using *CappAero* 8-Multichannel Pipette. Next, 100 µl undiluted positive and negative controls were added into appropriate wells of the assay plate by using the same pipette. 100 µl of diluted samples were then added to the assay plate accordingly. After that, all the samples were incubated for 2 hours at 18ºC. After incubation, the plate was then washed 3 to 5 times with 300 µl of wash solution per well. Then, 100 µl of Anti-Porcine: Horseradish peroxidase (HRPO) conjugate were added into each well and incubated for another 30 minutes. After that, each well was washed 3 to 5 times again before 100µl of Tetramethylbenzidine (TMB) substrate N.12 solution were added into each well. The plate was then incubated for another 15 minutes before 100 µl of stop solution N.3 were dispensed into each well to stop the reaction. Finally, absorbance reading at 450nm for the controls and samples were then obtained by using ELISA microplate reader (BioTek Instruments EL800). Color development was proportional to the amount of bound specific antibodies against PRRSV present in the sample.

For this test kit, the presence or absence of PRRSV antibody in the oral fluid samples was determined by calculating the S/P ratio for each sample. The formula to calculate S/P ratio is stated below:

$$Sample\ to\ Positive\ \left(\frac{S}{P}\right) Ratio = \frac{OD_{sample} - Mean\ OD_{negative\ control}}{Mean\ OD_{positive\ control} - Mean\ OD_{negative\ control}}$$

For the assay to be valid, the difference between mean OD of positive and negative control must be more than or equal to 0.150. The mean OD of negative control must also be less than or equal to 0.150.

Same as serum samples, oral fluid samples with S/P ratio more than or equal to 0.4 were interpreted as positive for PRRSV antibodies while oral fluid samples with S/P ratio less than 0.4 were considered negative for PRRSV antibodies. The S/P ratios of all the oral fluid samples were calculated and summarized by IDEXX XCheck® software.

3.4 Statistical Analysis

The data was analyzed by using IBM® Statistical Package for the Social Sciences (SPSS) software version 20. S/P ratios for both oral fluid and serum samples from both farms were used to run the analysis. Mann-Whitney U Test was used to run the analysis in order to determine whether there was any significant difference between the values of these two different type of samples taken from same individual. Next, Pearson's product-moment correlation test was used to determine the correlation between oral fluid and serum samples.

For pen oral fluid samples, paired samples t test was used to identify whether there is any difference between individual and pen oral fluid samples for the same age group.

4.0 RESULTS AND DISCUSSION

In general, the pig herds were divided into 7 categories (in each farm) : old sows (6^{th} parities and above), young sows (2^{nd} to 5^{th} parities), gilts, and weaners from different age groups (10 weeks old, 15 weeks old, 20 weeks old, and 25 weeks old). The subjects were divided based on age groups and breeding cycles because of the difference in immunological status due to previous vaccination history. Both blood and oral fluid samples were taken from all 7 categories. In addition, oral fluid samples were collected from 10 weeks, 15 weeks, 20 weeks, and 25 weeks old categories.

IDEXX PRRS X3 Ab Test Kit was used to test serum samples whereas IDEXX PRRS OF Ab Test Kit was used to test oral fluid samples in both farms. Results for Farm A sow herds and porkers IDEXX PRRS X3 Ab test are in Table 1 (Appendix I). Results for Farm A sow herds and porkers IDEXX PRRS OF Ab test are in Table 2 (Appendix II).

S/P Ratio	0.0-0.4	0.5-1.0	1.1-2.0	2.1-3.0	>3.0	S/P Ratio (Mean±SE)	Total Pigs
Gilt	0	0	0	1	4	4.278±0.578	5
Young Sows	0	2	2	1	0	1.430±0.382	5
Old Sows	0	1	2	1	1	2.314±0.873	5
10 Weeks	0	1	1	1	2	2.816±0.773	5
15 Weeks	0	0	0	2	3	3.012±0.234	5
20 Weeks	0	0	1	1	3	3.398±0.546	5
25 Weeks	0	0	1	3	1	2.416±0.272	5

Table 1: Summarized PRRS X3 Ab test results of Farm A sow herds and porkers.

S/P Ratio	0.0-0.4	0.5-1.0	1.1-2.0	2.1-3.0	>3.0	S/P Ratio (Mean±SE)	Total Pigs
Gilt	0	0	0	0	5	5.965±0.206	5
Young Sows	1	0	2	0	2	2.564±0.800	5
Old Sows	0	0	0	2	3	2.315±0.802	5
10 Weeks	0	0	0	2	3	3.969±0.727	5
15 Weeks	0	0	0	3	2	3.445±0.659	5
20 Weeks	0	0	0	0	5	5.925±0.117	5
25 Weeks	0	0	0	0	5	4.600±0.240	5

Table 2: Summarized PRRS OF Ab test results of Farm A sow herds and porkers.

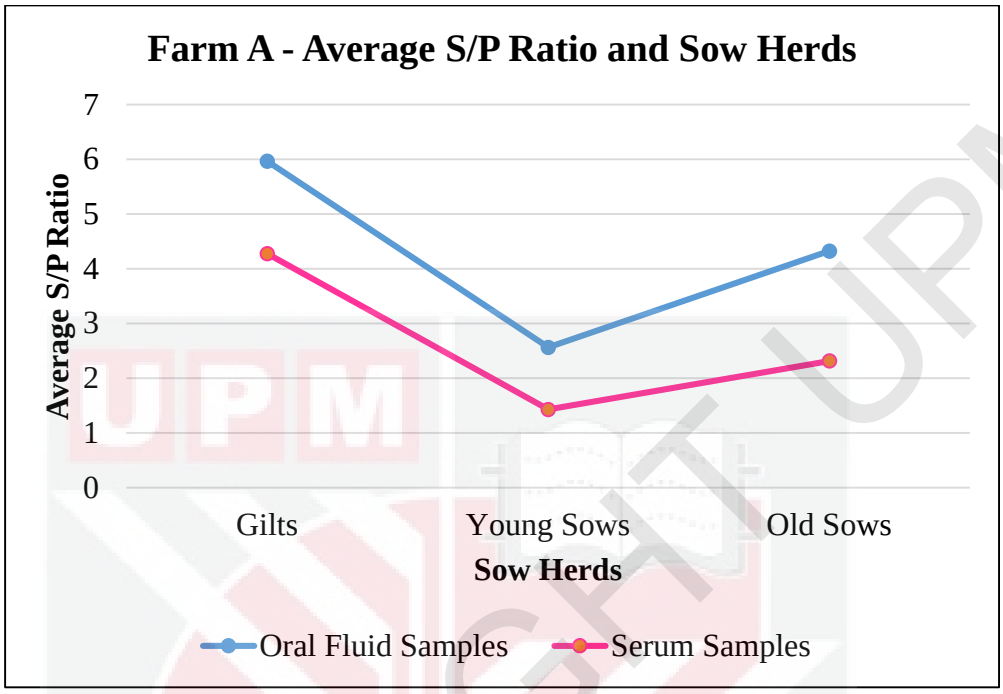

Figure 1.1: Average S/P ratio for oral fluid and serum samples of Farm A sow herds based on IDEXX PRRS OF Ab test and IDEXX PRRS X3 Ab test results respectively. The values of S/P ratio show same trend for both oral fluid and serum samples.

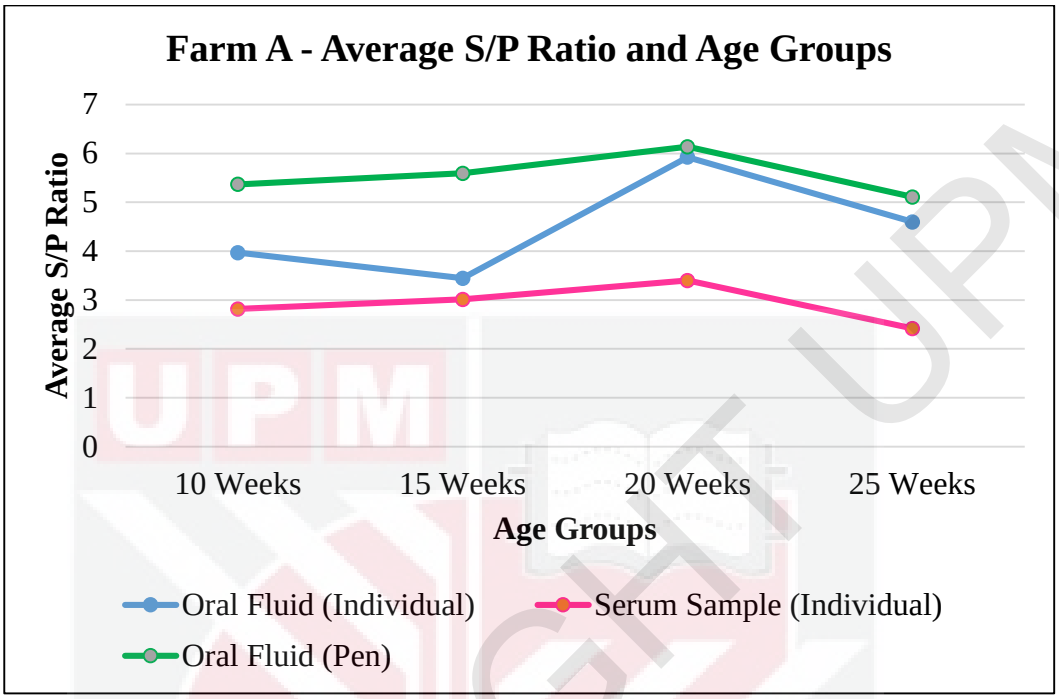

Figure 1.2: Average S/P ratio for oral fluid and serum samples of Farm A porkers based on IDEXX PRRS OF Ab test and IDEXX PRRS X3 Ab test results respectively. The values of S/P ratio show similar trend for both types of samples of all age groups in general except for porkers at 15 weeks which shows individual oral fluid samples has sharp increase trend compared to serum samples.

The average S/P ratios of oral fluid samples from Farm A show comparatively larger values if compared to that of serum samples for same age groups and sow herds. It is normal to have such results as oral fluid test results generally have higher S/P ratios if compared to serum samples. For example, S/P values which are considered normal for serum which is 0.5 to 1.5 would generally compare to S/P values of 3.0 to 6.0 for oral fluids (IDEXX, 2013).

Statistical analysis Mann-Whitney U Test also shows that there are significant difference between these two samples based on the S/P values (p=0.0001). However, despites the

significant differences in the values, these two samples show positive, significant, and strong correlation (p=0.0001, r=0.681) by using Pearson's correlation test. This strong correlation coefficient is further supported by the coefficient of determination, r^2 value which is 0.464. This means that about 46.4% of the total variation in S/P values of oral fluid samples can be explained by variation in S/P values of serum samples (Taylor, 1990).

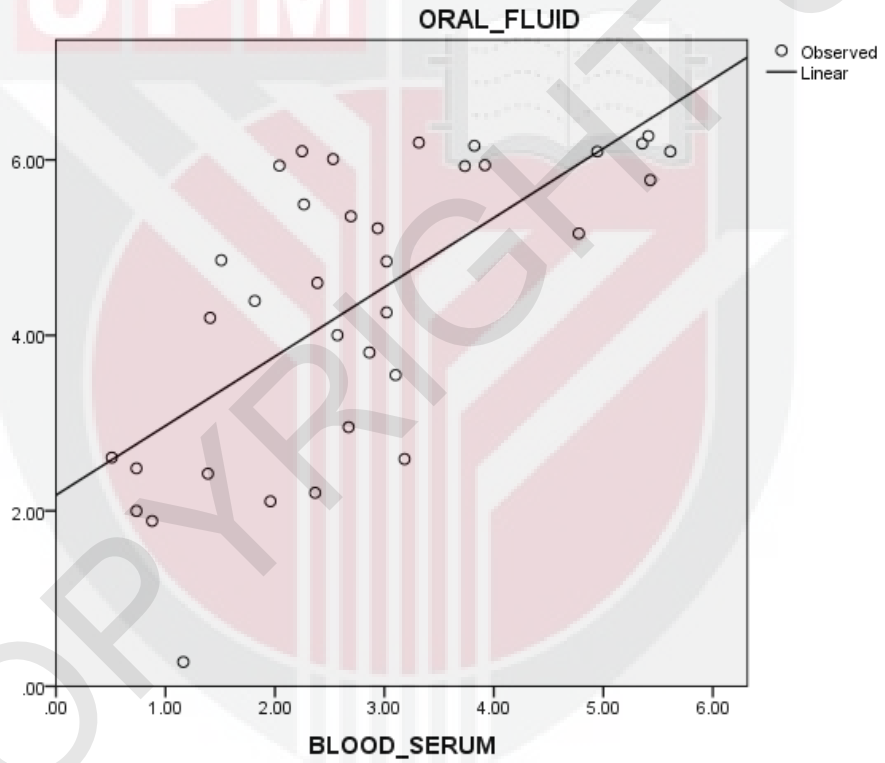

Figure 1.3: Correlation between S/P ratios for oral fluid and serum samples from individual subjects in Farm A as a summary statistic (Pearson's correlation coefficient, r=0.681).

On the other hand, the test results of Farm B also show similar pattern for both oral fluid and serum samples. Oral fluid samples consistently have higher S/P values if compared to serum samples. Results for Farm B sow herds and porkers IDEXX PRRS X3 Ab test

are in Table 3 (Appendix III) and the results for Farm B sow herds and porkers IDEXX PRRS OF Ab test are in Table 4 (Appendix IV). The trend of S/P ratio for sow herds in this farm is similar to Farm A while the trend for porkers is a bit varies. Sow immunity status tend to be more stable if compared to porkers. In porkers, the immunity challenge will be higher and therefore the immunity level is varies, which might cause S/P values fluctuation among age groups.

S/P Ratio	0.0-0.4	0.5-1.0	1.1-2.0	2.1-3.0	>3.0	S/P Ratio (Mean±SE)	Total Pigs
Gilt	1	0	0	0	4	3.749±1.006	5
Young Sows	1	1	3	0	0	0.931±0.245	5
Old Sows	1	0	0	0	4	3.062±0.726	5
10 Weeks	0	0	0	2	3	3.142±0.359	5
15 Weeks	0	0	0	1	4	3.902±0.482	5
20 Weeks	0	0	0	1	4	3.592±0.302	5
25 Weeks	0	1	1	0	3	2.500±0.597	5

Table 3: Summarized PRRS X3 Ab test results of Farm B sow herds and porkers.

S/P Ratio	0.0-0.4	0.5-1.0	1.1-2.0	2.1-3.0	>3.0	S/P Ratio (Mean±SE)	Total Pigs
Gilt	0	1	0	0	4	5.077±1.046	5
Young Sows	0	0	2	2	1	2.720±0.837	5
Old Sows	0	1	0	0	4	4.387±0.979	5
10 Weeks	0	0	0	3	2	4.263±0.684	5
15 Weeks	0	0	0	2	3	4.233±0.733	5
20 Weeks	0	0	0	1	4	4.244±0.659	5
25 Weeks	0	1	1	0	3	4.907±0.514	5

Table 4: Summarized PRRS OF Ab test results of Farm B sow herds and porkers.

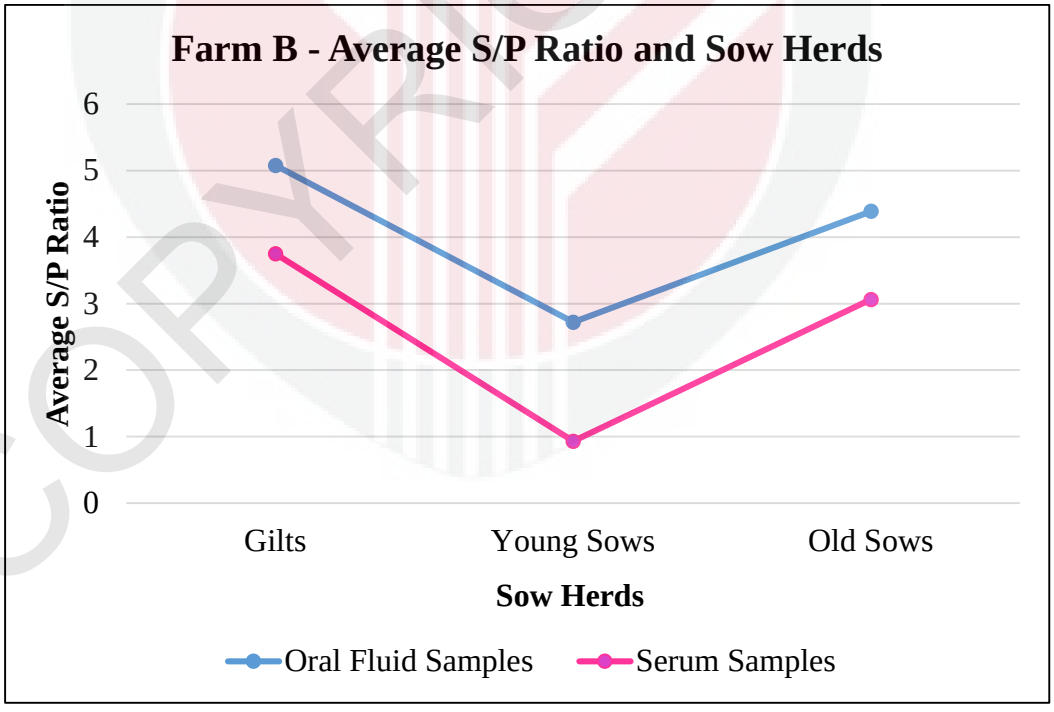

Figure 2.1: Average S/P ratio for oral fluid and serum samples of Farm B sow herds based on IDEXX PRRS OF Ab test and IDEXX PRRS X3 Ab test results respectively. The values of S/P ratio show same trend for both oral fluid and serum samples.

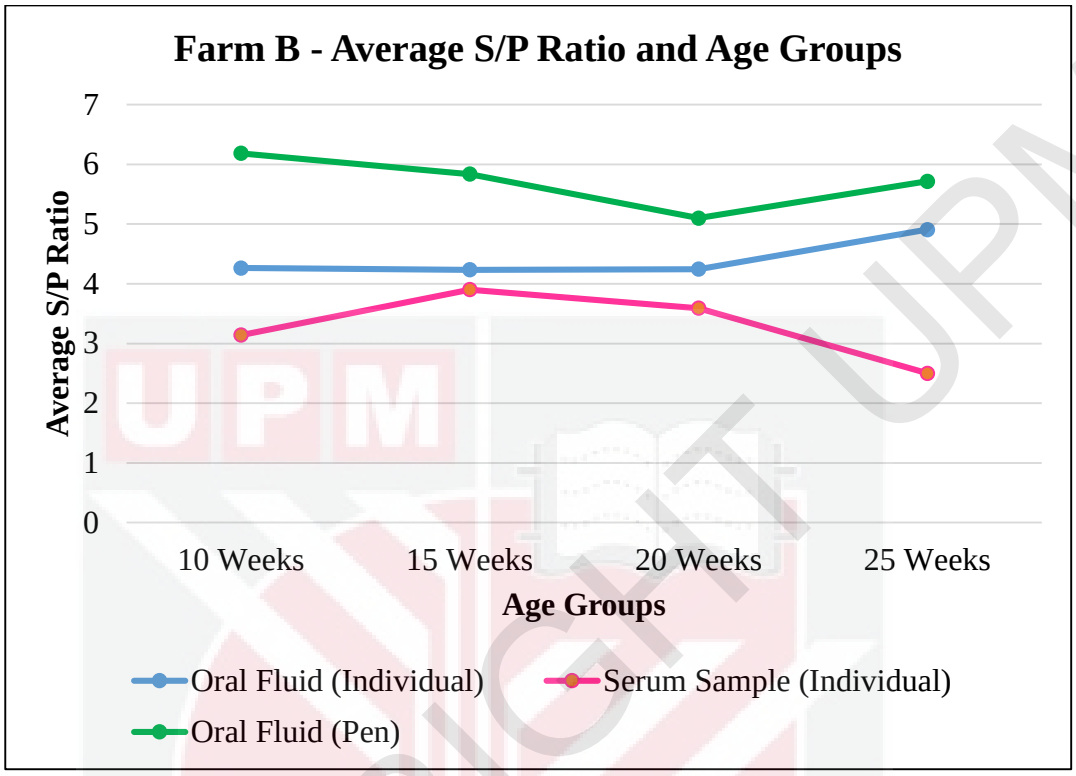

Figure 2.2: Average S/P ratio for oral fluid and serum samples of Farm A porkers based on IDEXX PRRS OF Ab test and IDEXX PRRS X3 Ab test results respectively. The values of S/P ratio show inconsistent trend regardless oral fluid or serum samples.

Statistical analysis Mann-Whitney U Test also shows that there are significant difference between these two samples based on the S/P values (p=0.003). Pearson's product-moment correlation test results also show positive, significant, and strong correlation between these two types of samples (p=0.0001, r=0.601). The coefficient of determination, r^2 value is 0.369. This means that for Farm B, about 36.9% of the total variation in S/P values of oral fluid samples can be explained by variation in S/P values of serum samples.

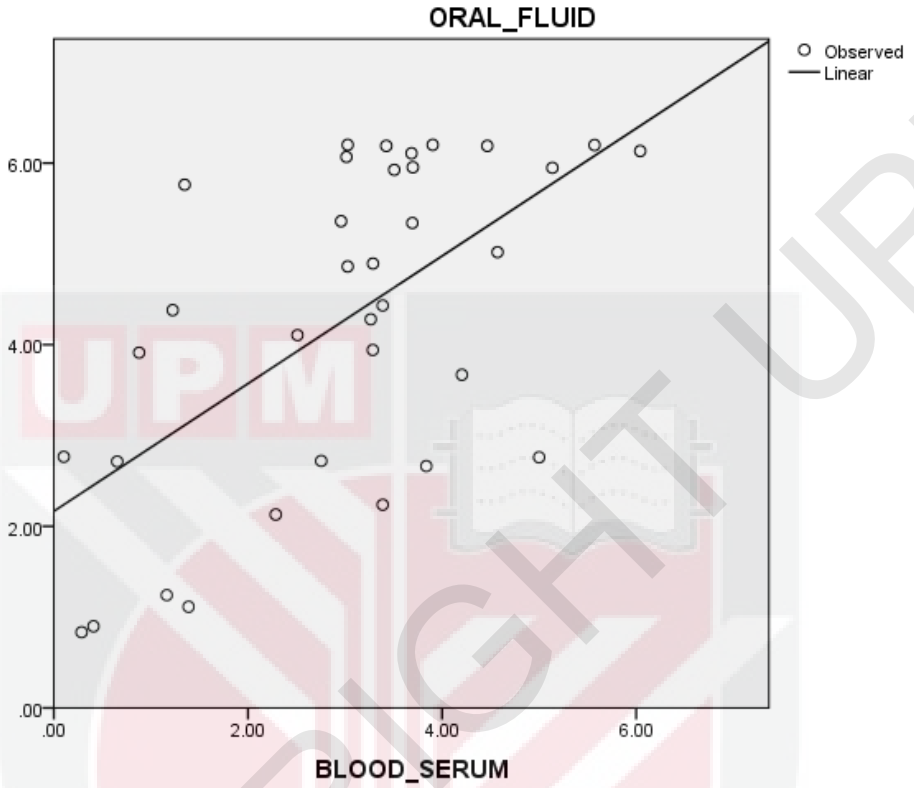

Figure 2.3: Correlation between S/P ratios for oral fluid and serum samples from individual subjects in Farm B as a summary statistic (Pearson's correlation coefficient, r=0.601).

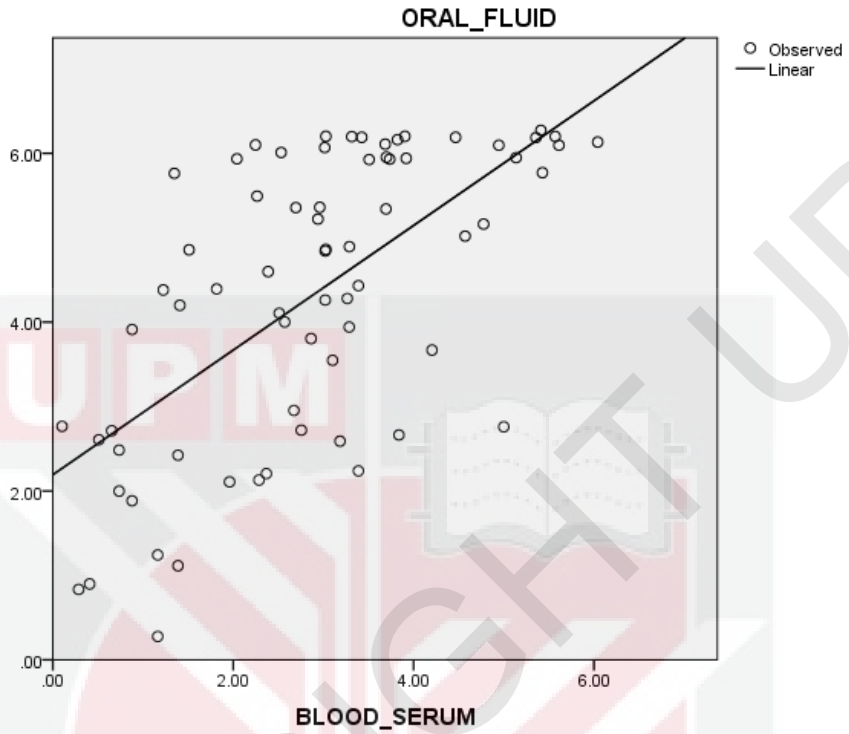

Figure 3.1: Correlation between S/P ratios for oral fluid and serum samples from individual subjects in both farms as a summary statistic (Pearson's correlation coefficient, r=0.638).

In addition, the oral fluid and serum sample results from both farms were also evaluated and they were statistically correlated with each other (p=0.0001, r=0.638). Besides that, based on Figure 3.2 and Figure 3.3, it can be seen that oral fluids and serum samples show similar pattern of results at different age groups and sow herds. This indicates that these two types of samples are closely correlated with each other.

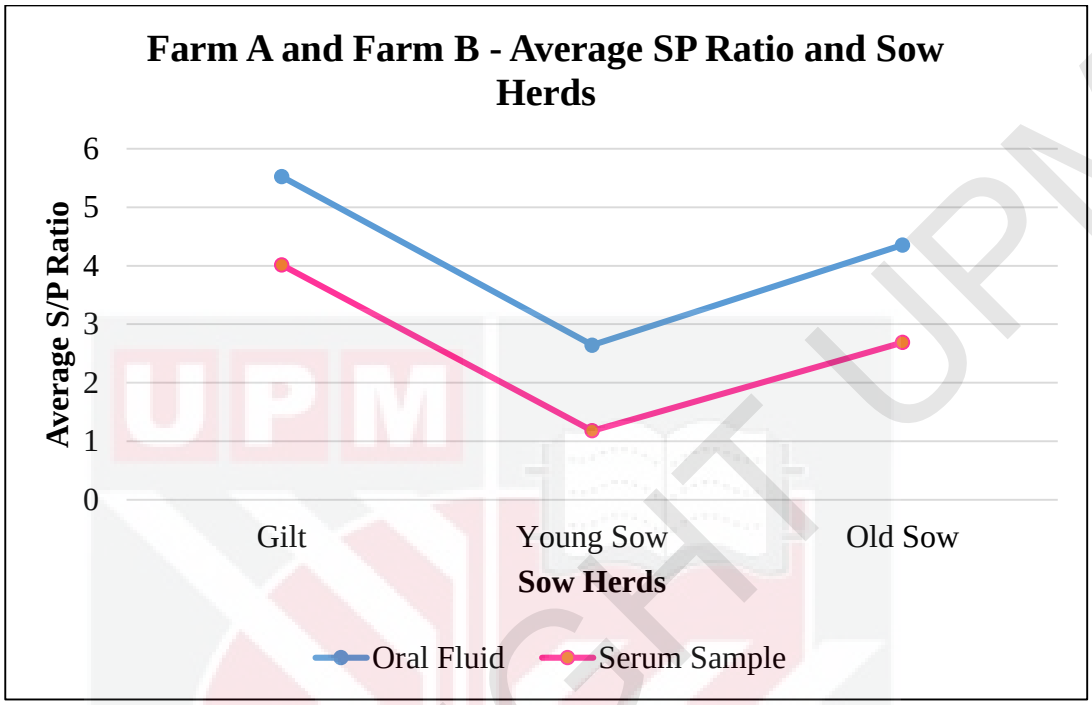

Figure 3.2: Average S/P ratio for oral fluid and serum samples of Farm A and Farm B sow herds based on IDEXX PRRS OF Ab test and IDEXX PRRS X3 Ab test results respectively. The values of S/P ratio show similar pattern for both samples.

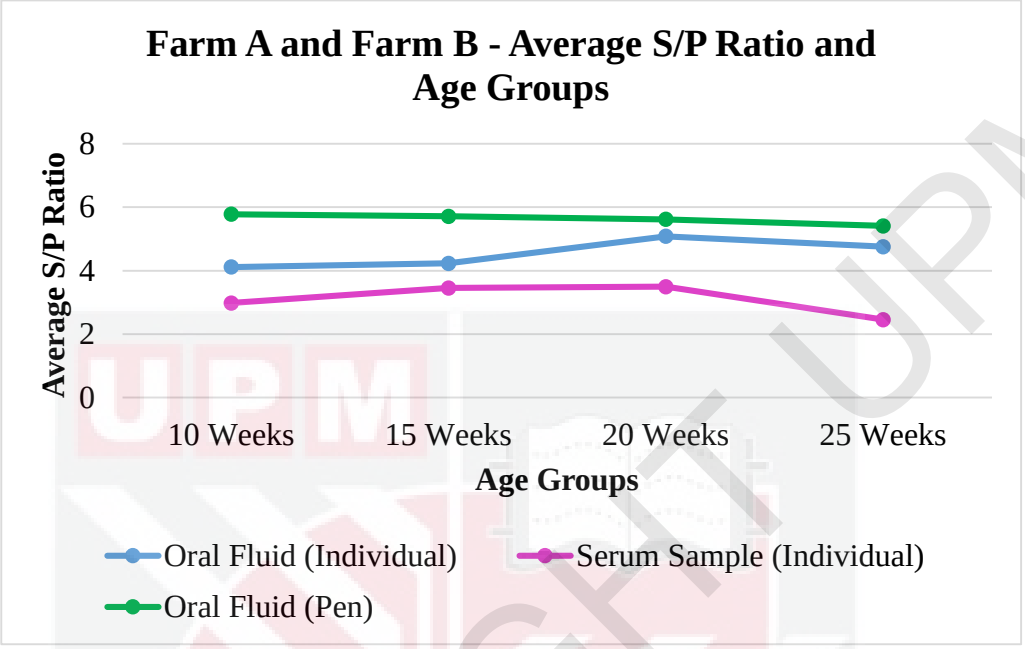

Figure 3.3: Average S/P ratio for oral fluid and serum samples of Farm A and Farm B at different age groups based on IDEXX PRRS OF Ab test and IDEXX PRRS X3 Ab test results respectively. The values of average S/P ratio of individual oral fluid and serum samples show similar pattern at different age groups. Meanwhile, average S/P ratios for pen oral fluid samples do not deviate much at different age groups.

Generally, based on the test results for both test kits, the S/P ratios for oral fluid samples are significantly higher than serum samples ($p<0.05$). As stated in this article before, normally PRRS vaccination is done at 3 weeks of age or older for piglets and every 3 to 4 months for sows and gilts. Hence, the most likely explanation for the higher S/P ratios for oral fluid samples will be due to the built up of immunity by the body 21 or more days post infection (DPI). This is because the value of S/P ratio for both test kits indirectly reflects the amount of antibody produced by the body. More antibodies produced will cause the S/P ratio to become higher. It has been reported that there will be higher concentration of virus in oral fluid than serum samples at 21 DPI (A. Kittawornrat, 2010).

Therefore, when there is more virus circulating in the bloodstream, this will cause more antibodies to be produced by the body post infection, and hence, increase the S/P ratio (Lopez & Osorio, 2004).

The most commonly method used to monitor PRRSV infection among swine population in Malaysia is by using IDEXX PRRS X3 Antibody Test Kit, which uses blood serum as specimens. This approach is laborious, time consuming, and may pose danger to the workers and practitioners if the subjects are not cooperative. Besides that, this approach is unable to achieve enough sampling requirements for a targeted level of disease detection because it is impossible to do blood sampling for all the animals in the farm (A. Kittawornrat, 2010). The animals are chosen randomly and based on few animals only it is not wise to represent the results as a whole.

Oral fluid is a good sample to be used as a diagnostic tool for PRRS surveillance in the farm because it contains both pathogens and antibodies produced by the body. Therefore, if the results of oral fluid samples show similar pattern as serum samples, then it will be a useful and reliable sample to be used to replace serum as diagnostic specimens in PRRS surveillance in the farm and the whole country. This is because oral fluid collection can be done easily by only one person on daily basis without posing danger to the personnel. Besides that, the natural behaviors of pigs that made them chew on anything surrounding them make it easier for the samples to be collected (A. Kittawornrat, 2010). Though collection of oral fluid from non-cooperative animals can be an issue, however, blood sampling from non-cooperative animal is also an issue and it can be dangerous to the

personnel during collection. Therefore, in both cases, it is wise to select more animals than minimum number of animals needed to run the experiment so that we will always have enough animals to be used as subjects.

From this experiment, although there are significant differences between two different samples used, however, there are significant, positive, and strong correlations between them for both Farm A and B. This positive and significant correlation indicates that increase in S/P ratios of serum samples will also cause increase in S/P ratios of oral fluid samples as well. This proves that oral fluid samples can also be used instead of serum samples as PRRS surveillance diagnostic tools because they are correlated to each other, even though approximately only 40% of the variability of S/P ratios for oral fluid samples can be explained by S/P ratios for serum samples at 95% confidence interval. A comparison has also been done before on the matched samples from individual boars and it revealed that oral fluid was equal to serum for the detection of PRRSV at DPI 7 and more likely to be more positive than serum on DPI 14 and 21 (A.Kittawornrat *et al*, 2013). Hence, this concludes that oral fluid was more superior than serum for over a 21 days observation periods and cumulatively, oral fluid offers more advantages over serum for the purpose of monitoring PRRSV infection. These advantages include easier sample collection, ability to collect samples more frequently, ability to select animals randomly, and the ability to cover more animals in the farm.

In addition to individual sampling of oral fluids, the sampling is also been done based on pen for different age groups: 10 weeks old, 15 weeks old, 20 weeks old, and 25 weeks old.

Oral fluid will be taken three times from each pen and the sample is tested by using same PRRS OF Ab Test Kit to compare whether the results for pen samples are the same or different from that of individual samples. The PRRS OF Ab test results based on pen oral fluid samples for both Farm A and Farm B are in Table 5 (Appendix V).

S/P Ratio				
Age	**Farm A**		**Farm B**	
	Pen Samples (Mean±SE)	**Individual Samples (Mean±SE)**	**Pen Samples (Mean±SE)**	**Individual Samples (Mean±SE)**
10 Weeks	5.369±0.279	3.969±0.727	6.185±0.022	4.263±0.684
15 Weeks	5.594±0.186	3.445±0.659	5.835±0.093	4.233±0.733
20 Weeks	6.136±0.030	5.925±0.117	5.098±0.174	4.244±0.659
25 Weeks	5.108±0.312	4.600±0.240	5.715±0.222	4.907±0.514

Table 5: Summarized mean S/P ratio for Farm A and Farm B pen and individual oral fluid samples for different age groups.

Statistical analysis of Farm A indicates that there is no difference between the results of pen and individual oral fluid samples for the same age group (p=0.094). This means that there is no difference between these two samples at 95% confidence interval. On the other hand, analysis of Farm B indicates that the results of pen and individual samples at same age group are the same except for week 20 (p=0.05).

Nevertheless, based on Figure 1.2 and Figure 2.2, both pen and individual oral fluid samples show similar pattern of results. An increase in S/P values for individual oral fluid samples is coherent with an increase in S/P values for pen oral fluid samples of the same

age groups and vice versa. The only difference is that pen oral fluid samples generally have higher S/P ratios if compared to that of individual oral fluid samples of the same age group. It has also been reported before in previous paper that pen oral fluid samples generally have higher S/P values if compared to individual oral fluid samples (IDEXX, 2013). This is because more antibodies is detected by the test and causing significant difference between the value of pen and individual oral fluid samples. Therefore, pen oral fluid samples can also be used as diagnostic tool to monitor PRRS in the farm because the results do not show significant difference between individual and pen oral fluid samplings, it is easier to collect oral fluid by this method, and more animals can be covered at one single time. However, it is necessary to allow as much as animals to chew the rope during the pen oral fluid sampling so that more animals will be covered in the farm and PRRS status in the farm can be defined more accurately.

5.0 CONCLUSION

Since the discovery of PRRSV in the early 1900s, the virus has continued to spread throughout the world and cause a major economic losses to the swine industry. More understandings regarding the etiologic agent have been achieved through the efforts of many scientists and their researches. Although the use of modified live vaccines has been successful to reduce the economic losses caused by PRRSV, cross-protection against heterologous virus is still questionable. Therefore, there is a need to develop better diagnostic methods of monitoring PRRSV infection in the herd, especially in PRRS negative farm. Blood serum based ELISA has already been established in its application to diagnose PRRSV infection in farm. However, it is impractical to do blood samplings in all animals in the farm for PRRS monitoring.

Cumulatively, the application of oral fluid as a diagnostic based offers advantages over serum for the purpose of monitoring PRRSV infection in the farm by using ELISA. Advantages include the simple and non-invasive methodology needed. Oral fluid collection does not require restraining and can be done easily by trained personnel because of the natural behaviors of pigs to chew anything in their surroundings. This will greatly improve the welfare of the animals and will not affect their productivity due to stress during handling. In addition, oral fluid samplings can also be done more frequently over a short time interval that facilitates ongoing disease monitoring.

Therefore, it is highly beneficial if the application of oral fluid can be used more widely in the animals for disease monitoring, not only for PRRSV, but for other diseases as well

such as swine influenza, classical swine fever (CSF), and porcine circovirus type 2 (PCV2) infection which causes huge economic loss to the farmers because as stated before, oral fluid contains both pathogens and antibodies in the body.

As a conclusion, oral fluid is a powerful tool to be used for the purpose of disease monitoring and the data collected is crucial to improve health management in the herds. Therefore, based on my findings, oral fluids can be used as tool for the purpose of disease monitoring and surveillance in the farm.

6.0 RECOMMENDATIONS

The study presented is the first description of comparing oral fluid samples with serum samples in Malaysia. It is important to test the efficacy of oral fluid samples in detecting anti-PRRSV antibodies by using other ELISA test kits as well. If the other test kits show similar results and have high agreement among each other, then oral fluid samples are good to be used as tool in PRRS monitoring.

Besides that, more studies need to be done regarding the efficiency of oral fluid samples to detect both pathogens and antibodies by using other diagnostic tests such as RT-PCR and SVN.

Furthermore, future studies may need to include more animals to reduce the variation of results and it is also recommended to take more samplings per individual, for example, 3 oral fluid samples per individual so that the results are more accurate.

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APPENDIX I

Pigs		S/P Ratio
10 Weeks Old	**10W/A**	**2.939**
	10W/B	**3.020**
	10W/C	**5.429**
	10W/D	**1.959**
	10W/F	**0.735**
15 Weeks Old	**15W/B**	**3.184**
	15W/C	**3.735**
	15W/D	**3.102**
	15W/F	**2.673**
	15W/G	**2.367**
20 Weeks Old	**20W/A**	**3.822**
	20W/B	**2.265**
	20W/C	**2.041**
	20W/D	**4.945**
	20W/E	**3.918**
25 Weeks Old	**25W/A**	**3.020**
	25W/B	**1.408**
	25W/C	**2.694**
	25W/D	**2.388**
	25W/E	**2.571**
Gilts	**G1**	**5.356**
	G2	**2.531**
	G3	**3.315**
	G4	**5.411**
	G5	**4.776**
Young Sows	**S2/1679**	**1.163**
	S2/2020	**0.878**
	S4/319	**2.863**
	S4/2051	**0.735**
	S3/39	**1.510**
Old Sows	**S7/256**	**1.816**
	S6/545	**5.612**
	S6/1714	**0.510**
	S7/597	**2.247**
	S6/388	**1.388**

APPENDIX II

Pigs		S/P Ratio
10 Weeks Old	**10W/A**	**5.221**
	10W/B	**4.261**
	10W/C	**5.770**
	10W/D	**2.108**
	10W/F	**2.484**
15 Weeks Old	**15W/B**	**2.590**
	15W/C	**5.930**
	15W/D	**3.547**
	15W/F	**2.954**
	15W/G	**2.205**
20 Weeks Old	**20W/A**	**6.161**
	20W/B	**5.493**
	20W/C	**5.934**
	20W/D	**6.095**
	20W/E	**5.940**
25 Weeks Old	**25W/A**	**4.843**
	25W/B	**4.199**
	25W/C	**5.356**
	25W/D	**4.598**
	25W/E	**4.002**
Gilts	**G1**	**6.186**
	G2	**6.010**
	G3	**6.197**
	G4	**6.273**
	G5	**5.161**
Young Sows	**S2/1679**	**0.277**
	S2/2020	**1.884**
	S4/319	**3.805**
	S4/2051	**1.998**
	S3/39	**4.857**
Old Sows	**S7/256**	**4.393**
	S6/545	**6.095**
	S6/1714	**2.607**
	S7/597	**6.099**
	S6/388	**2.424**

APPENDIX III

Pigs		S/P Ratio
10 Weeks Old	**10W/A**	**3.014**
	10W/B	**4.204**
	10W/C	**2.286**
	10W/D	**3.694**
	10W/E	**2.510**
15 Weeks Old	**15W/A**	**3.027**
	15W/B	**5.000**
	15W/C	**5.137**
	15W/D	**2.959**
	15W/E	**3.388**
20 Weeks Old	**20W/A**	**4.571**
	20W/B	**3.288**
	20W/C	**3.837**
	20W/D	**2.755**
	20W/E	**3.507**
25 Weeks Old	**25W/A**	**1.224**
	25W/B	**3.286**
	25W/C	**0.878**
	25W/D	**3.425**
	25W/E	**3.685**
Gilts	**GA**	**5.571**
	GB	**3.699**
	GC	**6.041**
	GD	**0.408**
	GE	**3.027**
Young Sows	**S1/5616**	**0.102**
	S2/5412	**1.347**
	S4/5066	**1.388**
	S4/4896	**0.653**
	S4/4977	**1.163**
Old Sows	**S6/3264**	**4.466**
	S6/3453	**3.265**
	S7/3881	**3.388**
	S9/3596	**0.286**
	S12/2415	**3.904**

APPENDIX IV

Pigs		S/P Ratio
10 Weeks Old	**10W/A**	**6.068**
	10W/B	**3.669**
	10W/C	**2.130**
	10W/D	**5.340**
	10W/E	**4.106**
15 Weeks Old	**15W/A**	**4.861**
	15W/B	**2.760**
	15W/C	**5.948**
	15W/D	**5.360**
	15W/E	**2.238**
20 Weeks Old	**20W/A**	**5.019**
	20W/B	**4.894**
	20W/C	**2.663**
	20W/D	**2.720**
	20W/E	**5.926**
25 Weeks Old	**25W/A**	**4.381**
	25W/B	**3.942**
	25W/C	**3.913**
	25W/D	**6.190**
	25W/E	**6.108**
Gilts	**GA**	**6.199**
	GB	**5.954**
	GC	**6.133**
	GD	**0.899**
	GE	**6.201**
Young Sows	**S1/5616**	**2.766**
	S2/5412	**5.762**
	S4/5066	**1.114**
	S4/4896	**2.714**
	S4/4977	**1.242**
Old Sows	**S6/3264**	**6.190**
	S6/3453	**4.280**
	S7/3881	**4.431**
	S9/3596	**0.834**
	S12/2415	**6.201**

APPENDIX V

Farm A		Farm B	
Pigs	**S/P Ratio**	**Pigs**	**S/P Ratio**
10W/1	5.520	**10W/1**	6.219
10W/2	4.683	**10W/2**	6.209
10W/3	5.905	**10W/3**	6.128
15W/1	5.186	**15W/1**	5.979
15W/2	5.578	**15W/2**	5.595
15W/3	6.017	**15W/3**	5.932
20W/1	6.188	**20W/1**	4.669
20W/2	6.060	**20W/2**	5.195
20W/3	6.159	**20W/3**	5.429
25W/1	5.058	**25W/1**	6.132
25W/2	4.437	**25W/2**	5.849
25W/3	5.830	**25W/3**	5.164

APPENDIX VI

Photos of oral fluid sampling procedures in pig farms

APPENDIX VII

Photos of oral fluid and blood sampling procedures in pig farms