UNIVERSITI PUTRA MALAYSIA

COMPARISON OF SERUM AND ORAL FLUID ANTIBODY RESPONSES AGAINST PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) BY USING ELISA

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FPV 2017 31

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LIEW HUI XIAN

A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfillment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan

MARCH 2017

It is hereby certified that we have read this project paper entitled "Comparison of Serum and Oral Fluid Antibody Responses against Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) by using ELISA", by Liew Hui Xian and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 – Final Year Project.

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DEDICATION

To my parents,

Dear brother and sister,

And

Friends

ACKNOWLEDGEMENTS

First of all, millions thanks to my project supervisor, Dr. Ooi Peck Toung for his great support, guidance, patience and care throughout my study. I am very grateful to have him as my supervisor that he has been very helpful willing to work on the ground together with his students. I would like to also thank you for giving me moral support and working together with me as peer.

Besides that, lot of thanks also to Dr Kuiek Ah Meng, Dr Lee Chee Yien and Dr Cheah Zi Herk for the guidance, teaching and help during sample collection. Without them, the process wouldn't have been this smooth.

I wish to express my sincere gratitude to master student, Vynter Low Suet Ee in the virology lab for her kindness, technical guidance and support during my laboratory work. The time that we spent together in the virology lab is indeed one of the most precious and memorable. Special thanks to Dr Michelle Fong Wai Cheng who has provided advice on my data interpretation and help in sample collection.

Many thanks go to my final year project teammate, Ng Zi Yao for all the help and support from sample collection, processing and interpretation, project presentation up to writing this thesis.

Lastly, I would like to thank my parent for their moral support and weekly phone call when I am far away from home. They are the reasons that keep me going when time is hard.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
CSFV	Classical Swine Fever Virus
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
FeLV	Feline Leukemia Virus
FIV	Feline Immunodeficiency Virus
FMD	Food and Mouth Disease
G	Gauge
h	Hour
HIV	Human Immunodeficiency Virus
HRPO	Horseredish peroxidase
IFA	Indirect Fluorescent Antibody
Ig A	Immunoglobulin A
Ig G	Immunoglobulin G
Ig M	Immunoglobulin M
min	Minutes
ml	Mililiter
µl	Microliter
nm	Nanometer
OD	Optical Density
OF	Oral Fluid
PCVD	Porcine Circovirus associated Disease
PRRS	Porcine Reproductive And Respiratory Syndrome
PRRSV	Porcine Reproductive And Respiratory Syndrome Virus
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
S/P ratio	Sample Mean to Positive Mean ratio
SVN	Serum Virus Neutralization
TMB	Tetramethylbenzidine
TGE	Transmissible Gastroenteritis
˚C	Degree Celcius

ABSTRAK

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

PERBANDINGAN ANTARA ANTIBODI TERHADAP SINDROM REPRODUKSI DAN PERNAFASAN PORSIN VIRUS (PRRSV) DALAM CECAIR ORAL DAN DARAH DENGAN MENGGUNAKAN ELISA

Oleh

Liew Hui Xian

2017

Penyelia: Dr. Ooi Peck Toung

Sindrom Reproduksi dan Pernafasan Porsin (PRRS) adalah penyakit endemik di Malaysia. Penyakit ini penting sebab ia akan menyebabkan kegagalan reproduksi antara babi betina, kematian anak babi, penyakit pernafasan antara babi dalam fasa pertumbuhan dan seterusnya menyebabkan kerugian ekonomi. Oleh itu, kaedah yang berkesan dan cekap diperlukan untuk memantau penyakit ini. Kajian ini dijalankan untuk membandingkan antibodi terhadap PRRS antara cecair oral dan darah dengan menggunakan ELISA. Kajian ini dijalankan di dua ladang babi. 32 ekor babi yang

berusia 6 minggu digunakan sebagai subjek di setiap ladang. 32 ekor babi ini dibahagikan kepada empat kandang. 8 sampel darah dan 3 sampel cecair oral berdasarkan kandang dikumpulkan dari setiap kandang. Sampel tersebut dikumpulkan lagi dari babi dan kandang yang sama 4 minggu kemudian. Sampel darah dan cecair oral yang dikumpulkan telah diuji dengan menggunakan IDEXX PRRS X3 Antibody Test Kit and IDEXX PRRS Oral Fluid Antibody Test Kit masing-masing. Terdapat hubungan yang bererti, kuat dan positif antara sampel untuk kedua-dua ladang A ($r = 0.962$, $p < 0.01$) dan ladang B ($r = 0.933$, $p < 0.01$). Kesimpulannya, sampel cecair adalah bahan diagnostic yang berguna untuk memantau PRRS di ladang dan ia dapat mengganti sample darah.

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

ABSTRACT

An abstract of a project paper presented to the Faculty of Veterinary Medicine, University Putra Malaysia in partial fulfillment of the requirement for the course VPD 4999 – Final Year Project

COMPARISON OF SERUM AND ORAL FLUID ANTIBODY RESPONSES AGAINST PORCINE REPRODUCTIVE AND PESPIRATORY SYNDROME VIRUS (PRRSV) BY USING ELISA

By

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2017

Supervisor: Dr. Ooi Peck Toung

Porcine Reproductive and Respiratory Syndrome (PRRS) is endemic in Malaysia. It is a disease of concern as it can cause great economic loss due to reproductive failure in sows, high pre-weaning mortality in piglets and respiratory disease in growing and finishing pigs. Therefore, it is necessary to have an efficient and effective surveillance method to detect the disease. In this study, antibody responses against PRRS in oral fluid and serum were compared using IDEXX ELISA test kit. This study was conducted in two commercial pig farms. 32 head of six weeks

old animals were used as subject from each farm. These 32 animals were divided into four pens. 8 serum samples and 3 pen based oral fluid sample were collected from each pen. The samples were collected again from the same individual and same pen four weeks later. The serum and oral fluid samples collected were tested using IDEXX PRRS X3 Antibody Test Kit and IDEXX PRRS Oral Fluid Antibody Test Kit respectively. Statistical analysis shows that there is a significant, very strong and positive correlation between serum and oral fluid samples for Farm A ($r = 0.962$, $p < 0.01$). There is also a significant, very strong and positive correlation between serum and oral fluid samples for Farm B ($r = 0.933$, $p < 0.01$). In conclusion, oral fluid is a useful tool for PRRS surveillance in the farm and a good option alternative to replace the traditional serology method.

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

1.0 INTRODUCTION

Porcine Reproductive and Respiratory syndrome (PRRS) was first found in United States in the late 1980s. Not long after that, a disease of similar clinical sign was found in Europe. By early 1990s, the disease was documented in Asia. PRRS is now endemic in Malaysia. It is a disease of concern of pig farmers and veterinarians as it can cause great economic loss due to reproduction failure in sows, high pre-weaning mortality in piglets and respiratory disease in growing and finishing pigs. One survey was done on the incidence and impact of PRRSV in United States. The survey stated that PRRRSV had caused an estimated total productivity loss of US $664 million annually in the US national breeding and growing pig herd in 2010, an increased from the US $ 560 million annual cost estimated in year 2005. In year 2010, 45% of the total cost of productivity loss caused by PRRSV was due to losses in breeding herd. (Holtkamp et al., 2013) Therefore, it is necessary to have an effective and efficient surveillance method to be used widely in detecting the disease.

PRRS virus (PRRSV) is a single stranded, non-segmented, enveloped RNA virus that classified under Arteriviridae family and Nidovirales order. An important characteristic of PRRSV is that it replicates in macrophages. Macrophages are important immune cells that play a key role in defending infectious agent.(OIE, 2010) Replication of PRRSV in macrophages causes infected pig succumbs to severe secondary infection. This characteristic also enables the virus to replicate in the host

for an extended time without affected by active immune response. Therefore, pigs infected with PRRSV are able to transmit the virus for several months.

Clinical signs of PRRS are presented in two distinct forms, reproductive and respiratory. Gilts and sows infected with PRRSV might show inappetence and fever, while some might show no clinical sign. Most infected sow will undergo abortion or farrow prematurely in acute stage. Abortion usually occurs in sow at last trimester of pregnancy. Premature farrowing will result in birth of weak or dead pigs in various stages of mummification. Other clinical signs include delayed return to estrus, low conception rate and low farrowing rates. Boars generally show fewer clinical signs; fever and inappetence might be observed. Decline in semen quality and decreased libido had been reported in some cases. As for sucking, growing and finishing pigs, the common clinical sign observed are fever, dyspnea and blue discoloration of the skin on ears and hindquarters. (OIE, 2010)

There are several diagnostic assay used in the laboratory to diagnose PRRS. Assays for detecting PRRSV serum antibody include enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody (IFA) test and serum virus neutralization (SVN) test while assays for detecting PRRSV include reverse transcriptase polymerase chain reaction (RT-PCR). (Christopher-Hennings et al., 2002)

In Malaysia, IDEXX PRRS X3 antibody test kit is one of the commercially used laboratory diagnostic tools. It is a serological test that will detect pig serum antibody against PRRSV. IDEXX PRRS X3 antibody test kit is the most often cited

test and is generally reckoned to be the gold standard of ELISA to detect antibody against PRRSV. (Sattler, Wodak, Revilla-Fernández, & Schmoll, 2014) However, blood sample collection in pigs is very laborious as it require restraining and special technique, time consuming as only single sample can be collected at one time, invasive and stressful to the pigs. Oral fluid has now been highlighted as a promising, non-invasive alternative to blood serum for diagnostics. (Dawson, 2015)

Oral fluid had been use to assess health and diagnose disease in human and animal since long time ago. As early as 1909, oral fluid from patient diagnosed with Malta Fever was found to be able to agglutinate *Brucella melitensis.* This indirectly shows that antibody is actually present in saliva. However, finding in oral fluid diagnostics were then overshadowed by technical improvement in the detection of analytes in blood. Oral fluid was then getting attention again in last two decades following a report that found antibodies against human immunodeficiency virus (HIV) in oral fluid from patient suffer with acquired immunodeficiency syndrome (AIDS). (John Rodger Prickett, 2009) Detection of antibodies in oral fluid from pigs was first reported in 1976 when antibody against classical swine fever virus (CSFV) was detected in oral fluid.(Corthier, 1976)

Oral fluid consists of saliva and serum transudate. In pigs, saliva is produced by parotid, mandibular and sublingual glands. Saliva consist mostly water and some other molecules with biological function such as mucin, amylase, lysozyme, lipase and glycoprotein. Serum transudate originates from capillaries located in the buccal

mucosa and gingival tissues. Serum transudate carries antibodies which include Immunoglobulin G (IgG), Immunoglobulin M (IgM) and Immunoglobulin A (IgA) from serum into the oral cavity. IgA was also locally produced by serum derived plasma cell in salivary gland and duct associated lymphoid tissue. IgG and IgM were also found to be locally produced but at lower amount than IgA. (John R Prickett & Zimmerman, 2010)

Oral fluid collections from pigs are possible and relatively easy as compared to other domestic animal because it is compatible with their normal behavior. They are naturally curious and love to explore their environment and surrounding by biting, chewing and tasting. Object that are chewable, flexible and destructible, for examples ropes are their favorite object. (Zimmerman et al., 2014) So, to collect oral fluid sample from pigs, we can hang cotton ropes up to their shoulder level inside the pen and let them bite on it.

The purpose of this study is to compare the antibody responses towards PRRSV in serum and oral fluid. The study will be carry out in a commercial farm setting with continuous sampling from the same animals. The results obtained will provide information about the continuous usage of oral fluid as sampling mechanism compare with serology.

2.0 LITERATURE REVIEW

2.1 Porcine Reproductive and Respiratory Syndrome (PRRS)

PRRS was first detected in United States in year 1987 and it became pandemic within a few year times. (Bilodeau *et al*., 1991) PRRSV was discovered in Netherlands in year 1991 and is classified under order Nidovirales, family Arteriviridae and genus Arterivirus. (Brinton *et al*., 2000) PRRSV is a single stranded, non-segmented, enveloped RNA virus. PRRSV are divided into two antigenically difference genotype which are Type 1 (European Type) and Type 2 (North American Type). (Larochelle *et al*., 1997)

Clinical signs of PRRS are presented in two distinct syndrome, the reproductive syndrome and respiratory syndrome. The reproductive syndrome is characterized by abortion at third trimester of pregnancy and early or delayed farrowing which result in dead or mummified fetuses, stillborn pigs and week newborns. At acute phase of epizootic, increase in repeat breeder, low conception rate and low farrowing rate are commonly reported. Transient fever and inappetance may be observed in boars, gilts and sows. The respiratory syndrome is characterized by pneumonia, dyspnea, fever, inappetance and listlessness. Pneumonia can be observed from 3 to 28 days after infection and is most severe on day 10 post infection. Younger pig are more commonly having respiratory syndrome as compare to boars and sows. Some pigs might have subclinical infection and show no clinical sign. An increase in secondary infection is common and mortality is usually caused by severe secondary

infection. (Assembly, 2010) Lesions of PRRS virus commonly occur in lung and lymphoid tissue. At post mortem, the lung of infected pig will appeared slightly firm, non-collapsing like a wet sponge and is mottled, grey in colour. Lesions are usually seen at cranio-ventral part of the lung but can also be seen at the entire lung. Secondary bacterial infection might occur. Lymph node of infected pig will appeared enlarged, moist, firm and white in colour on cut surface. (OIE, 2010)

2.2 Porcine Reproductive and Respiratory Syndrome Serological Test

Serological tests to detect serum antibodies against PRRSV include enzyme-linked immunosorbent assay (ELISA), indirect fluorescent antibody (IFA) test and serum virus neutralization (SVN) test.

ELISA detects serum antibodies against PRRSV antigen indirectly. It has a few advantages, include easily perform with simple kit instruction, give rapid result, able to detect antibody against both European and North American PRRSV strain but cannot differentiate them and the result is reproducible because of the standardized format. Results of ELISA are analyses based on sample to positive ratio (S/P ratio). However, result of ELISA is very much of technician dependent. High precision is required in dilution of serum sample and mixing of solution.

IFA test is conducted by adding collected pig serum sample to the virus infected cells. Then, antigen-antibody reaction is detected by adding fluorescein labeled anti-porcine antibodies. An advantage of IFA is that it can estimate the magnitude of the antibody titer in specific serum sample. IFA has good specificity but

sensitivity of the test relies on several factors. Factors that can affect IFA test sensitivity are protocol used, incubation time, technician skill, cell type used in the assay. Interpretation of the result can be very subjective as it depends on the technician that read the result.

SVN test is also a cell culture based assay like IFA test which various dilution of serum to be tested are incubated with a known level of virus. After incubation, titer of antibody that was able to neutralize the PRRSV is determined. There is no standardized protocol or set of reagent used with this assays and most laboratories have their own independent system in carrying out this test. SVN test is less sensitive as compare to IFA test and ELISA because neutralizing antibodies against PRRSV will only developed 21 days post infection and some pigs produced relatively low neutralizing antibody titers. The SVN test is more expensive, more time consuming and more difficult to perform technically.

Serological information from a serum sample cannot be used to diagnose clinical PRRS in an individual animal in endemic country like Malaysia. Current serological assays can only be used to measure PRRSV exposure and to confirm that the pigs were vaccinated but cannot differentiate antibodies produce by vaccine virus and antibodies produce from field virus. (Christopher-Hennings et al., 2002)

2.3 Porcine Reproductive and Respiratory Syndrome Molecular Test

Reverse transcriptase polymerase chain reaction (RT-PCR) is used to detect the present of genetic material of PRRSV in body tissue, serum, semen, oropharyngeal scrapings and lung lavage samples. In RT-PCR, RNA of the virus is first extracted and then converted to DNA by reverse transcriptase steps. Then, the DNA is amplified by PCR. The advantages of using RT-PCR are rapid turnaround time as a test can be completed within 1 to 3 days after receiving sample to be tested, high sensitivity and specificity. However, sensitivity and specificity of the test can be affected by a few factors such as technician skill level, type of specimen used, specimen condition and specimen volume. A disadvantage of RT-PCR is that viral genome might not be detected if a primer used in the assay is genetically difference with PRRSV. (Christopher-Hennings et al., 2002)

2.4 History of Oral Fluid Usage in Health Assessment and Disease Diagnosis

The use of oral fluid for health assessment and disease diagnosis has a long history in humans and animals surprisingly. In early days, investigators attempted to evaluate metabolic disease in human by using oral fluid, this early work however, had led to a finding that the principals present in serum were also present in oral fluid. As early as 1909, Pollaci and Ceraulo reported agglutination of *Brucella melitensis* by oral fluid from Malta Fever patient. But the development in oral fluid usage in diagnosis of disease had been overshadowed by technical improvement in the detection of analytes in blood. This began to change following detection of antibodies

against human immunodeficiency virus (HIV) in oral fluid of acquired immunodeficiency syndrome (AIDS) patient in year 1986.(Archibald, 1986) Since then, oral fluid based assays was widely developed and implemented.

2.5 Basic concepts of oral fluid

Oral fluid is a mixture of saliva and serum transudate. In pigs, saliva is produces by 3 major salivary glands which are parotid gland, mandibular gland and sublingual gland. (Sisson, 1975) Serum transudate passively diffuse through the oral mucosa and gingiva from the capillaries located in the oral mucosa and the gingival tissues into oral cavity. Saliva consist mostly water and some molecules with important biological function for examples, mucin, amylase, lysozyme, lipase and proline rich glycoprotein (Llena-Puy, 2006) while serum transudate consist of serum antibody like IgG, IgM and IgA. (Challacombe, 1978) This makes oral fluid a useful biological specimen for immunoassays.

2.6 Detection of PRRS Antibodies in Oral Fluid using ELISA

A study was done to evaluate the diagnostic performance of a commercial serum antibody ELISA to detect anti-PRRSV antibodies in pen based oral fluid specimen. The oral fluid specimen was collected from 2 different groups of pigs, 1 group is experimental pigs where PRRS infection was synchronized among all individual while the other group is field pigs that PRRS infection has not been synchronized. Anti-PRRSV IgM, IgA and Ig G were evaluated in pen based oral fluid samples collected in both group of pigs using isotype specific ELISA. The result was

IgM, IgA and IgG were readily detected in experimental pigs while only IgG was detected in field pigs. The reason explained why only IgG was detected is probably due to IgA and IgM from pigs at early stage of infection would have been diluted by oral fluid from animals not secreting those antibody isotypes. Result of the study suggests IgG oral fluid ELISA can provide efficient, cost effective PRRSV surveillance program. (Kittawornrat et al., 2012)

Another study was done to compare PRRSV antibody responses in oral fluid and serum samples following modified live PRRSV vaccination by using ELISA. The result shows that there are significant and positive correlation between antibody responses of both the oral fluid and serum sample. (Kuiek, 2015) This means that oral fluid can become an alternative to serum in PRRS monitoring program.

Other than that, another study was done to determine whether anti-PRRSV antibody were present in oral fluid at diagnostic level in pigs with different age group by using ELISA. The finding was low level of anti-PRRSV antibody were detected in oral fluid samples. This result also suggests that pen based oral fluid sampling could be efficient, cost effective approach to PRRSV surveillance program. (Prickett et al., 2008)

3.0 MATERIALS AND METHODS

3.1 Animals

This study was conducted at 2 commercial pig farms setting in Tanjung Sepat, Selangor. Farm A is an intensive, farrow to finish, open house with 1200 sows population while Farm B have same setting with 800 sows population.

In this study, 4 pens of 6 weeks old pigs were selected from each farm. Then, in each pen, 8 pigs were identified, tagged and their serum sample was collected. A total of 32 animals from each farm were used as subjects. Then, pen based oral fluid samples were collected from the 4 pens with tagged animal and their pen-mate. Each pen contain of 40 pigs. Four weeks later, when the pigs were at 10 weeks old, serum and oral fluid samples were collected again from same animals.

3.2 Sampling and Sample Handling

Serum sample was collected by the pig restrained at upside down position. Blood was taken from jugular vein by using 5ml syringe and 21G needle. The collected blood was then transferred to plain blood tube and the blood tube was labeled and stored immediately in an ice box at 4˚C. Then, serum was extracted from each blood sample by using pipette and then transferred into 1.5ml microcentrifuge tubes which was then labeled and stored in Acson® horizontal chest freezer at a temperature of -20˚C until serology test was to be conducted.

Pen based oral fluid was collected by using three strand twisted undyed cotton ropes. 3 cotton ropes were hanged at 3 different areas in a pen. The cotton ropes were hanged up to the pigs shoulder level for the ease of chewing by the pigs. All pigs in the pen were allowed to chew on the ropes for 30 min. After 30 min, wet end of the rope will be inserted into a clean plastic bag and the oral fluid is collected by pulling and squeezing of the rope. The collected oral fluid will be poured into a clean microcentrifuge tube. The microcentrifuge tube were labeled and stored immediately in an ice box at 4˚C. The oral fluid samples were kept in Acson® horizontal chest freezer at a temperature of -20˚C until serology test were to be conducted.

3.3 Serological Tests

The serum and oral fluid samples collected were tested using indirect ELISA. 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.

3.3.1 IDEXX PRRS X3 Antibody Test Kit

Serum samples which stored at a temperature of -20˚C were thawed at room temperature before they were diluted 40 folds by mixing 5 µl of sample with 195 µl of sample diluent. Firstly, 100 µl of undiluted positive and negative controls were added into duplicated wells of the antigen coated plate by using pipette. 100 µl of diluted samples were then dispensed into appropriate well of the antigen coated plate. After that, the antigen coated plate that filled with the solutions was incubated for 30 min at room temperature. After incubation, solutions were removed and each well was

washed with 300 µl wash solution for 3 to 5 times. Then, the plate was tapped onto paper towel after final wash to remove any residual wash fluid. After that, 100 µl of Anti-Porcine: Horseredish peroxidase (HRPO) conjugate were added into each well and incubate for 30 min. Each well was then washed again with wash solution and 100 µl of Tetramethylbenzidine (TMB) substrate solution were added into each well. The plate was incubated for another 15 min before 100 µl of stop solution was dispensed into each well to halt the reaction. Lastly, absorbance reading at 650 nm for the positive, negative controls and samples were obtained by using ELISA microplate reader.

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

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

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.

3.3.2 IDEXX PRRS Oral Fluid Antibody Test Kit

Oral fluid samples which stored at a temperature of -20˚C were thawed at room temperature before they were diluted 2 folds by mixing 100 µl of oral fluid sample with 100 µl of sample diluent. Next, 100 µl of undiluted positive and negative controls were added into duplicated wells of the antigen coated plate by using pipette. 100 µl of diluted samples were then dispensed into appropriate well of the antigen coated plate. After that, the antigen coated plate that filled with the solutions was incubated for 2 h at room temperature. After incubation, solutions were removed and each well was washed with 300 µl wash solution for 3 to 5 times. Then, the plate was tapped onto paper towel after final wash to remove any residual wash fluid. After that, 100 µl of Anti-Porcine: Horseredish peroxidase (HRPO) conjugate were added into each well and incubate for 30 min. Each well was then washed again with wash solution and 100 µl of Tetramethylbenzidine (TMB) substrate solution were added into each well. The plate was incubated for another 15 min before 100 µl of stop solution were dispensed into each well to halt the reaction. Lastly, absorbance reading at 450nm for the controls and samples were then obtained by using ELISA microplate reader. Colour development was proportional to the amount of 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 as below:

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

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.

As similar 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 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

ELISA results for both serum and oral fluid samples were analyzed by using IBM® Statistical Package for the Social Sciences (SPSS) software version 22. S/P ratio of both oral fluid and serum samples were tested using Pearson correlation coefficient test to determine the strength of association between those two samples.

4.0 RESULT AND DISCUSSION

In each farms, four pens were selected for the study. In Farm A, there are Pen A1, Pen A2, Pen A3 and Pen A4 while in Farm B there are Pen B1, Pen B2, Pen B3 and Pen B4. 32 pigs were selected and tagged; serum and oral fluid samples were collected from them at 2 different time points (6^{th} Week and 10^{th} Week).

IDEXX PRRS X3Ab 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 1^{st} time point are in Table 1 (Appendix I & V). Results for Farm A 2^{nd} time point are in Table 2 (Appendix II & VI). Results for Farm B 1^{st} time point and 2^{nd} time point are in Table 3 (Appendix III & VII) and Table 4 (Appendix IV & VIII) respectively.

Table 1: Summarized S/P ratio mean for serum and oral fluid samples of Farm A on 1st time point based on IDEXX PRRS X3 Ab test and IDEXX PRRS OF Ab test results respectively.

Pen	S/P Ratio (Mean ± Standard Error)	
	Serum	Oral Fluid
A1	0.968 ± 0.092	3.016 ± 0.269
A2	0.606 ± 0.085	2.866 ± 0.102
A3	0.908 ± 0.140	2.227 ± 0.189
A4	0.562 ± 0.139	1.693 ± 0.198

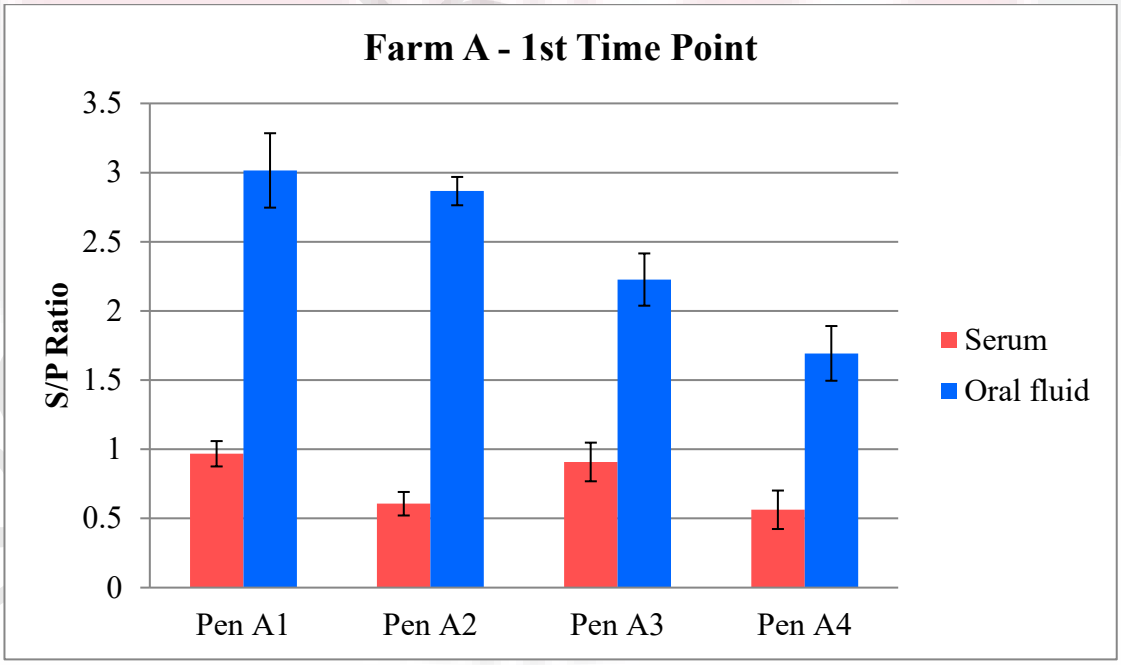

Figure 1: Average S/P ratio for serum and oral fluid samples of Farm A on 1st time point based on IDEXX PRRS X3 Ab test and IDEXX PRRS OF Ab test results respectively.

Table 2: Summarized S/P ratio mean for serum and oral fluid samples of Farm A on 2nd time point based on IDEXX PRRS X3 Ab test and IDEXX PRRS OF Ab test results respectively.

Pen	S/P Ratio (Mean ± Standard Error)	
	Serum	Oral Fluid
A1	1.769 ± 0.172	5.867 ± 0.054
A2	1.539 ± 0.180	5.755 ± 0.072
A3	1.794 ± 0.133	6.034 ± 0.039
A4	1.670 ± 0.180	5.845 ± 0.012

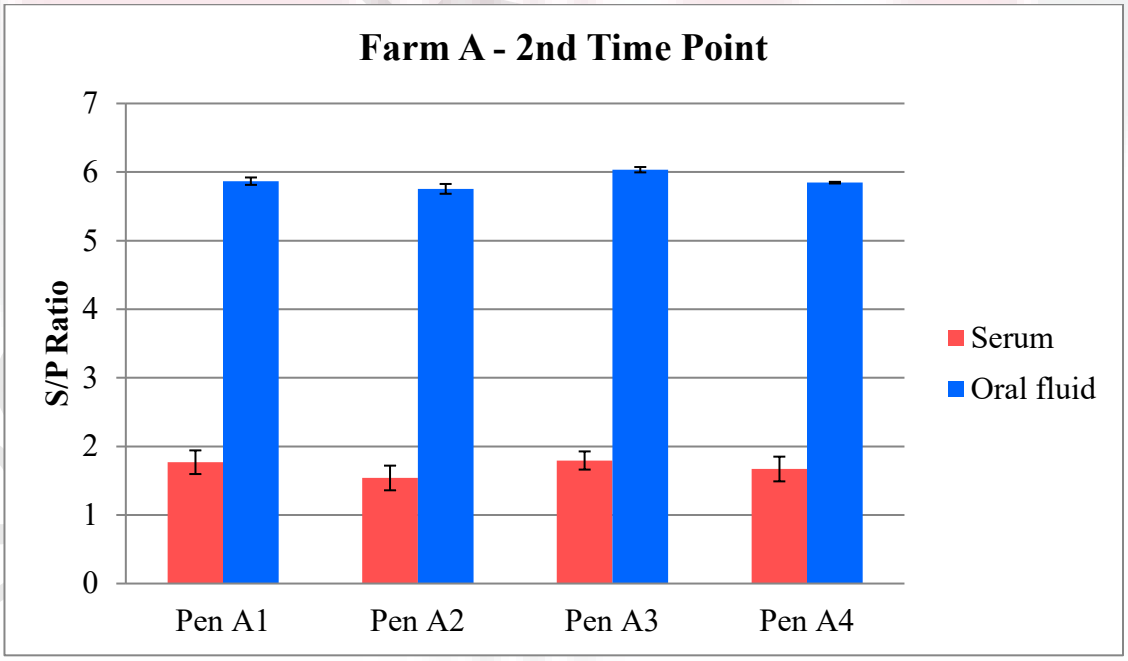

Figure 2: Average S/P ratio for serum and oral fluid samples of Farm A on 2nd time point based on IDEXX PRRS X3 Ab test and IDEXX PRRS OF Ab test results respectively.

The mean S/P ratio of oral fluid samples from Farm A show larger value if compare to that of serum samples. It is normal as oral fluid test result generally have higher S/P ratio if compare to serum samples. High value of oral fluid test result is not surprising because of the sensitivity of the test. (IDEXX, 2013) Due to the low antibody concentration in the oral fluid, design of the test used to detect oral fluid antibody had to be more sensitive. For IDEXX PRRSV OF antibody test kit that used in our test, higher amount of sample, lesser sample dilution and longer incubation period had been applied to make the test more sensitive, therefore, its S/P ratio is higher compare to serum test kit.

In Figure 1 and Figure 2, Farm A 1st and 2nd time point, pattern of chart for S/P ratio of serum and oral fluid samples show high similarity. Pearson correlation coefficient test had been used to determine the strength of association in between serum and oral fluid samples of Farm A. Result shows that there is a very strong, significant and positive correlation between those two samples. ($r = 0.962$, $p < 0.001$). The coefficient of determination, r^2 value is 0.926. This means that for Farm A, about 92.6% of the total variation in S/P value of oral fluid samples can be explained by variation in S/P values of serum samples.

Table 3: Summarized S/P ratio mean for serum and oral fluid samples of Farm B on 1st time point based on IDEXX PRRS X3 Ab test and IDEXX PRRS OF Ab test results respectively.

Pen	S/P Ratio (Mean ± Standard Error)	
	Serum	Oral Fluid
B1	0.676 ± 0.198	2.538 ± 0.051
B2	0.601 ± 0.055	2.638 ± 0.180
B3	0.545 ± 0.083	2.054 ± 0.149
B4	0.696 ± 0.125	3.349 ± 0.116

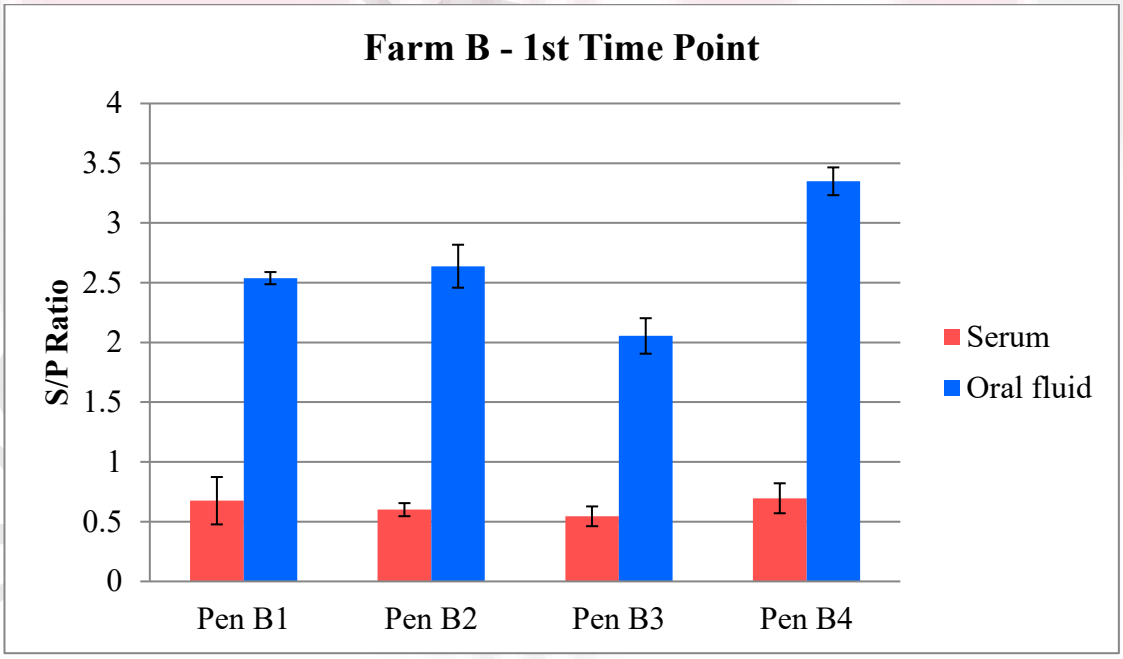

Figure 3: Average S/P ratio for serum and oral fluid samples of Farm B on 1st time point based on IDEXX PRRS X3 Ab test and IDEXX PRRS OF Ab test results respectively.

Table 4: Summarized S/P ratio mean for serum and oral fluid samples of Farm B on 2nd time point based on IDEXX PRRS X3 Ab test and IDEXX PRRS OF Ab test results respectively.

Pen	S/P Ratio (Mean ± Standard Error)	
	Serum	Oral Fluid
B1	1.614 ± 0.073	5.066 ± 0.212
B2	1.562 ± 0.071	5.781 ± 0.066
B3	1.196 ± 0.220	5.747 ± 0.125
B4	1.730 ± 0.095	6.094 ± 0.050

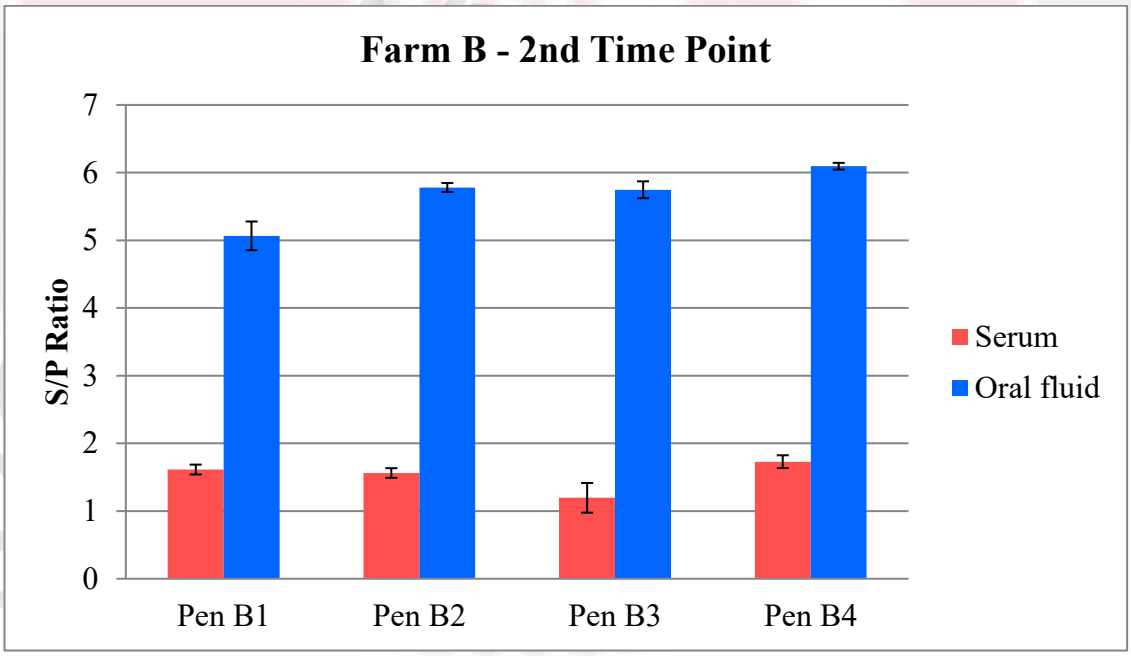

Figure 4: Average S/P ratio for serum and oral fluid samples of Farm B on 2nd time point based on IDEXX PRRS X3 Ab test and IDEXX PRRS OF Ab test results respectively.

In Figure 3 and Figure 4, Farm B 1st and 2nd time point, pattern of chart for S/P ratio of serum and oral fluid samples show high similarity. Pearson correlation coefficient test had been used to determine the strength of association in between serum and oral fluid samples of Farm B. As similar to Farm A, statistical result shows that there is a very strong, significant and positive correlation between those two samples. ($r = 0.933$, $p < 0.001$). The coefficient of determination, r^2 value is 0.870. This means that for Farm B, about 87.0% of the total variation in S/P value of oral fluid samples can be explained by variation in S/P values of serum samples.

From the results, there are strong and positive correlation between serum and oral fluid antibody responses in both Farm A and Farm B. This is due to oral fluid consist of serum transudate which come from capillaries in the oral mucosa, crevicular gaps and gingival tissue. Therefore, antibody content found in the serum can actually be found in the oral fluid (Kittawornrat et al., 2012).

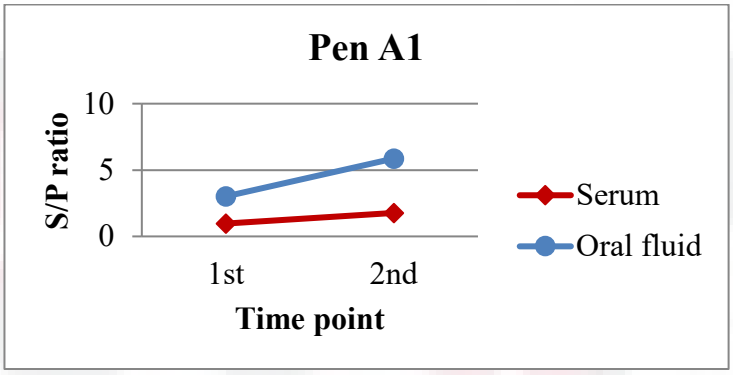

Figure 5.1: Average S/P ratio for serum and oral fluid samples of Pen A1 of Farm A at 2 different time points.

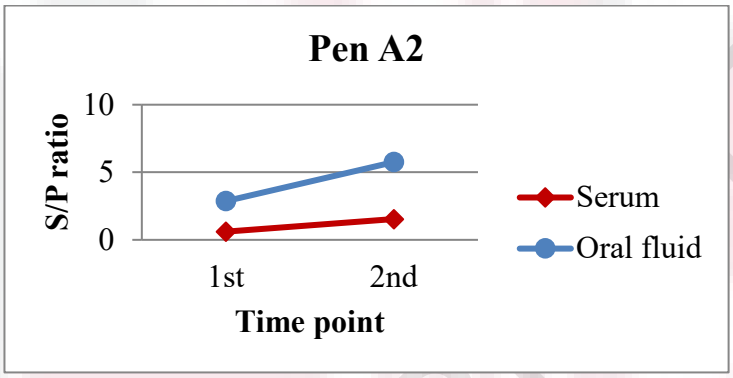

Figure 5.2: Average S/P ratio for serum and oral fluid samples of Pen A2 of Farm A at 2 different time points.

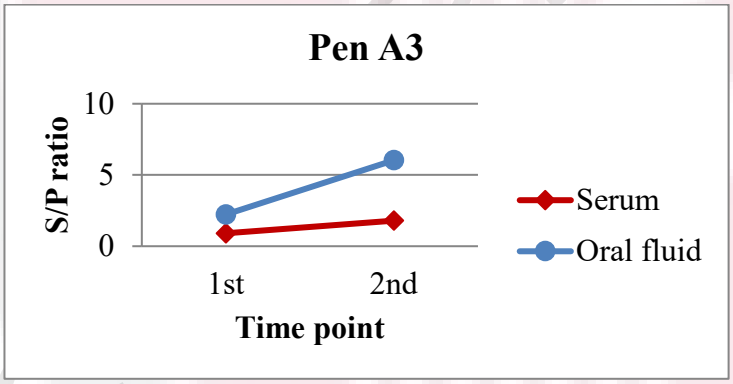

Figure 5.3: Average S/P ratio for serum and oral fluid samples of Pen A3 of Farm A at 2 different time points.

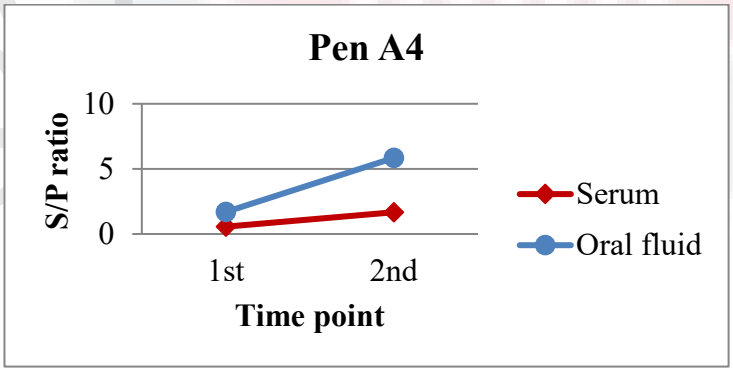

Figure 5.4: Average S/P ratio for serum and oral fluid samples of Pen A4 of Farm A at 2 different time points.

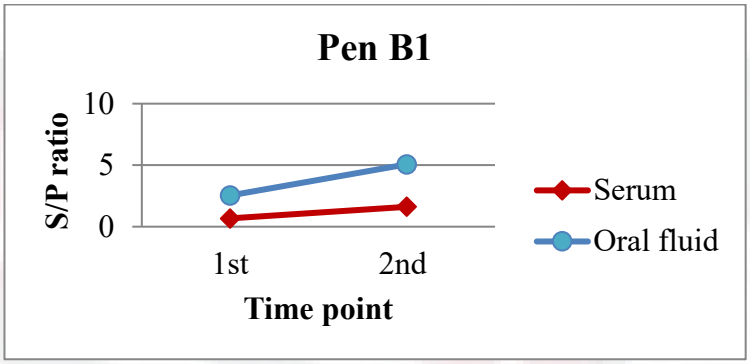

Figure 6.1: Average S/P ratio for serum and oral fluid samples of Pen B1 of Farm B at 2 different time points.

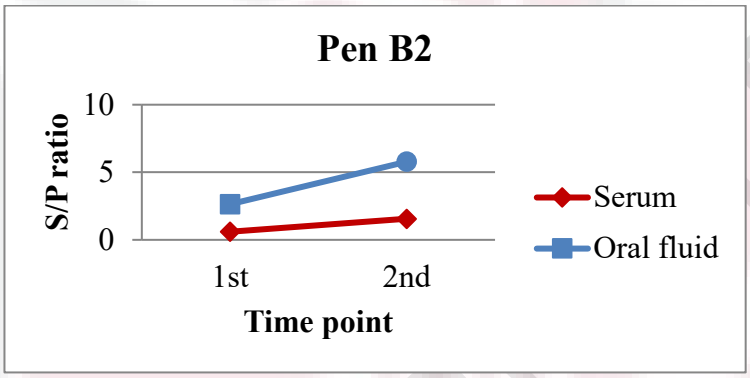

Figure 6.2: Average S/P ratio for serum and oral fluid samples of Pen B2 of Farm B at 2 different time points.

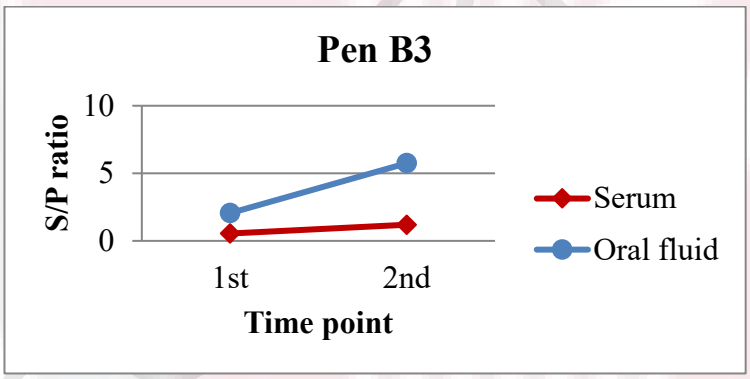

Figure 6.3: Average S/P ratio for serum and oral fluid samples of Pen B3 of Farm B at 2 different time points.

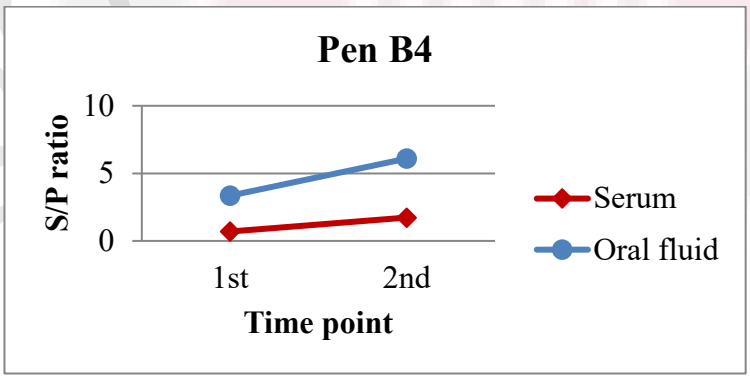

Figure 6.4: Average S/P ratio for serum and oral fluid samples of Pen B4 of Farm B at 2 different time points.

Figure 5 and 6 show pens in both Farm A and Farm B at 2 different time point, all of those figures show one similarity which are antibody responses on 2nd time point is always higher than antibody responses on 1st time point. The reason behind it is probably due to, at first time point when the pig was at 6 weeks old, maternal antibody had already wane off and then the pigs might be challenge by field strand PRRSV at the same time, this cause antibody responses to become high at 2nd time point.

This study involved only two time point, continuous time point assessment are recommended to observed the antibody trend of the pigs up to their market age. This antibody trend can then be used as baseline to monitor PRRSV infection on pigs of all age group in the farm. If the oral fluid antibody trend is higher than the established baseline, PRRS infection can be suspected and earlier control and preventive measure can be carry out.

Oral fluid collection has a few advantages as compare to serum collection. Oral fluid collection is not invasive and no risk to handlers because no sharp instrument for examples, needles is being used. Oral fluid collection is less laborious compare to serum collection. To collect serum sample, one person have to hold the pig at upside down position while one person holding the pig's forelimb to prevent the pig from struggling and one person take blood from the jugular vein. As for oral fluid collection, we only need to hang the rope inside the pen and let the pig chew on it. It is less time consuming and more samples can be collected at one time. (Prickett, 2009) Other than that, oral fluid collection is cheaper. Based on our estimation, it cost around RM12.50

to collect and process a single oral fluid sample, while RM25.00 is needed to collect and process a single serum sample.

However, oral fluid collection has its limitation. Oral fluid collection might not be applicable on newborn and young piglet as they are more interested in suckling and cuddling rather than exploring the environment. It was not advisable to be used on piglet that less than 18 days old as they might not interested in chewing the ropes. (IDEXX, 2013) Besides, environmental contamination is possible as the ropes have to be hanging up to the pigs shoulder level in the pen for the ease of the pig to chew on it. This provides a chance for the rope to be contaminated with the feces and urine on the pig's body. Other than that, oral fluid collection is only useful up to the level of disease monitoring, serum sample is still needed for individual diagnosis of disease. This is due to serum antibody detection test kit have higher sensitivity as compare to oral fluid antibody detection test kit.

Besides PRRS, other swine diseases that can be detected using oral fluid include Classical Swine Fever (CSF), Transmissible Gastroenteritis (TGE), Food and Mouth Disease (FMD), Porcine Circovirus associated disease (PCVD) and *Actinobacilus pleuropneumoniae* associated disease. Within those diseases, only PRRS and PCVD have commercial oral fluid test kit while for the other diseases, they have their own in house test kit because their demand are not as high as PRRS and PCVD.

Since oral fluid detection of diseases had been used widely in pig, it also had been experimented in few other species. Oral fluid detection of disease had been experimented in feline to test for Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV) and *Mycoplasma haemofelis*. In canine, rabies and *Bartonella sp.* were detected in oral fluid. Bovine oral fluid has been used to detect *E. coli, Salmonella sp.* and FMD. While in equine, oral fluid sample had been used to test for presence of performance altering chemical in race horse. (Prickett, 2009)

There are some factors that will affect amount of oral fluid production. Firstly, pigs kept in slatted floor pens will be more interested to the chew the rope as compare to pigs kept in straw bedding pens. This might be due to the pig had already enriched by the straws. Number of training was found to have significant effect on oral fluid yield for pig. There is progressive increase in oral fluid collected with increased number of training. Other than that, increased in rope number able to collect more oral fluid because number of pig engage with the rope also increase as this allow less dominant pig have chance to chew on the rope. On the other hand, study had showed adding flavor to the rope will not affect oral fluid production. Pig motivation to interact to the rope is independent of any flavored additives and presentation of the ropes itself is a sufficient stimulus to attract the pig to chew. (Dawson, 2015)

5.0 CONCLUSION

In early 1990s, PRRS was documented in Asia and this disease is now endemic in Malaysia. It is a disease of concern of pig farmers and veterinarians as it can cause great economic loss. Therefore, there is a need to develop better diagnostic methods of monitoring PRRSV in a herd. Serum based ELISA had been established in its application to diagnose PRRSV infection in a farm. However it is impractical to do blood samplings in all animals in the farm for PRRS monitoring.

Oral fluid had now been used widely in PRRS non-endemic country to detect the presence and absence of PRRS antibody. As for PRRS endemic country like Malaysia, the use of oral fluid will be different. As most of the oral fluid will be positive against PRRS we will be focusing on using the oral fluid to monitor the antibody trend. A baseline oral fluid antibody trend will first be established in the farm. Then, oral fluid collection can be done as frequent as biweekly to monitor the antibody level. If the antibody level increased from the baseline, then we can further confirmed by serology monitoring.

From our study it had been proved that there are strong and positive correlation between serum and oral fluid antibody responses tested by using ELISA test kit. Antibody content that found in the serum can also be found in the oral fluid. Other than that, oral fluid collection is not invasive, less laborious and cheaper compare to serum collection. This enable oral fluid sampling to be done frequently over a short time interval to facilitates ongoing disease monitoring.

In conclusion, oral fluid is a useful tool for PRRS monitoring in the farm and a good option alternative to replace traditional serology method.

6.0 RECOMMENDATIONS

Future studies may need to include more animals to reduce the variation of results and it is also recommended to take more samplings per pen.

In addition, we can try to collect the oral fluid at different frequency like once a week, biweekly and three times a week to determine the most suitable and practical sampling frequency to be used in the future and obtain the farm baseline.

Lastly, this study should be conducted in more farms with different setting around Malaysia so that a baseline of oral fluid antibody trend against PRRSV in Malaysia can be established.

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APPENDIX I

Results of IDEXX PRRS X3 Ab Test in Farm A – 1st Time Point

Pen	Animal ID	S/P Ratio
A1	1609	1.259
	1611	0.802
	1617	0.620
	1876	1.045
	1878	1.005
	1879	1.074
A2	1601	0.767
	1602	0.492
	1603	0.565
	1604	0.949
	1605	0.674
	1606	0.541
	1607	0.134
	1608	0.729
A3	0085	1.598
	0086	0.630
	0087	0.448
	0088	0.699
	0089	1.484
	0090	0.884
	0091A	0.633
	0091B	0.592
	0092	1.206
A4	1612	1.226
	1613	0.401
	1614	0.087
	1615	0.451
	1616	0.185
	1618	0.440
	1619	1.011
	1620	0.700

APPENDIX II

Results of IDEXX PRRS X3 Ab Test in Farm A – 2nd Time Point

Pen	Animal ID	S/P Ratio
A1	1609	1.549
	1611	1.619
	1617	1.207
	1876	1.734
	1878	2.386
	1879	2.120
A2	1601	0.740
	1602	1.596
	1603	1.332
	1604	1.602
	1605	2.076
	1606	1.315
	1607	2.115
A3	0085	1.850
	0086	1.838
	0090	1.749
	0091	2.181
	0092	1.353
A4	1612	1.904
	1613	1.740
	1616	1.301
	1618	2.386
	1619	1.181
	1620	1.509

APPENDIX III

Results of IDEXX PRRS X3 Ab Test in Farm B – 1st Time Point

Pen	Animal ID	S/P Ratio
B1	1825	0.388
	1827	0.157
	1828	1.591
	1829	0.637
	1830	1.500
	1832	0.265
	1834	0.580
	1836	0.286
B2	1848	0.374
	1850	0.726
	1852	0.706
	1853	0.585
	1854	0.829
	1855	0.646
	1856	0.466
	2000	0.472
B3	1826	0.846
	1841	0.391
	1843	0.429
	1844	0.109
	1845	0.762
	1846	0.672
	1847	0.568
	1849	0.582
B4	1821	0.814
	1822	1.369
	1823	0.331
	1824	0.543
	1831	0.717
	1837	0.646
	1838	0.508
	1840	0.183

APPENDIX IV

Results of IDEXX PRRS X3 Ab Test in Farm B – 2nd Time Point

Pen	Animal ID	S/P Ratio
B1	1825	1.511
	1827	2.038
	1828	1.468
	1829	1.682
	1830	1.417
	1832	1.683
	1834	1.678
	1836	1.431
B2	1848	1.402
	1850	1.457
	1852	1.715
	1853	1.663
	1854	1.234
	1855	1.540
	1856	1.886
	2000	1.602
B3	1826	1.725
	1841	0.058
	1843	1.700
	1844	1.463
	1845	1.591
	1846	0.503
	1847	0.985
	1849	1.540
B4	1821	2.015
	1822	1.454
	1823	1.822
	1824	1.988
	1831	1.797
	1837	1.471
	1838	1.948
	1840	1.342

APPENDIX V

Results of IDEXX PRRS OF Ab Test in Farm A – 1st Time Point

Pen	Oral Fluid ID	S/P Ratio
A1	A1 - 1	2.279
	A1 - 2	2.952
	A1 - 3	3.443
	A1 - 4	3.388
A2	A2 - 1	2.737
	A2 - 2	3.068
	A2 - 3	2.792
A3	A3 - 1	1.849
	A3 - 2	2.407
	A3 - 3	2.424
A4	A4 - 1	1.546
	A4 - 2	1.449
	A4 - 3	2.085

APPENDIX VI

Results of IDEXX PRRS OF Ab Test in Farm A – 2nd Time Point

Pen	Oral Fluid ID	S/P Ratio
A1	A1 - 1	5.970
	A1 - 2	5.787
	A1 - 3	5.844
A2	A2 - 1	5.670
	A2 - 2	5.697
	A2 - 3	5.897
A3	A3 - 1	5.989
	A3 - 2	6.112
	A3 - 3	6.002
A4	A4 - 1	5.864
	A4 - 2	5.849
	A4 - 3	5.823

APPENDIX VII

Results of IDEXX PRRS OF Ab Test in Farm B – 1st Time Point

Pen	Oral Fluid ID	S/P Ratio
B1	B1 - 1	2.438
	B1 - 2	2.571
	B1 - 3	2.605
B2	B2 - 1	2.307
	B2 - 2	2.683
	B2 - 3	2.925
B3	B3 - 1	1.989
	B3 - 2	2.338
	B3 - 3	1.836
B4	B4 - 1	3.525
	B4 - 2	3.392
	B4 - 3	3.129

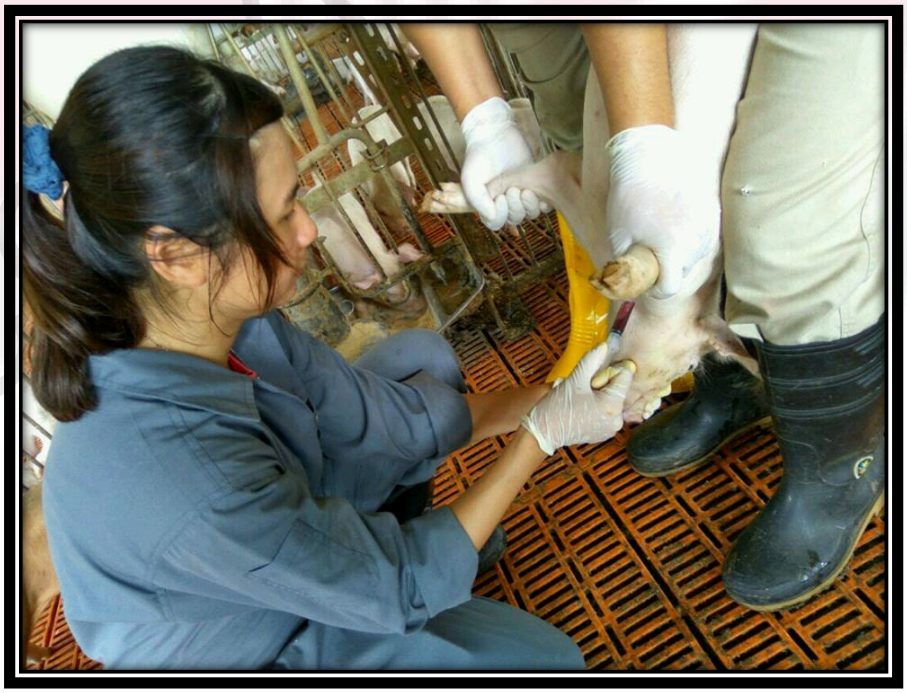
APPENDIX VIII

Results of IDEXX PRRS OF Ab Test in Farm B – 2nd Time Point

Pen	Oral Fluid ID	S/P Ratio
B1	B1 - 1	4.419
	B1 - 2	4.816
	B1 - 3	5.134
	B1 - 4	5.296
	B1 - 5	5.666
B2	B2 - 1	5.874
	B2 - 2	5.815
	B2 - 3	5.654
B3	B3 - 1	5.501
	B3 - 2	5.835
	B3 - 3	5.905
B4	B4 - 1	6.025
	B4 - 2	6.192
	B4 - 3	6.065

APPENDIX IX

Photos of blood sampling procedures in the farms



APPENDIX X

Photos of oral fluid sampling procedures in the farms