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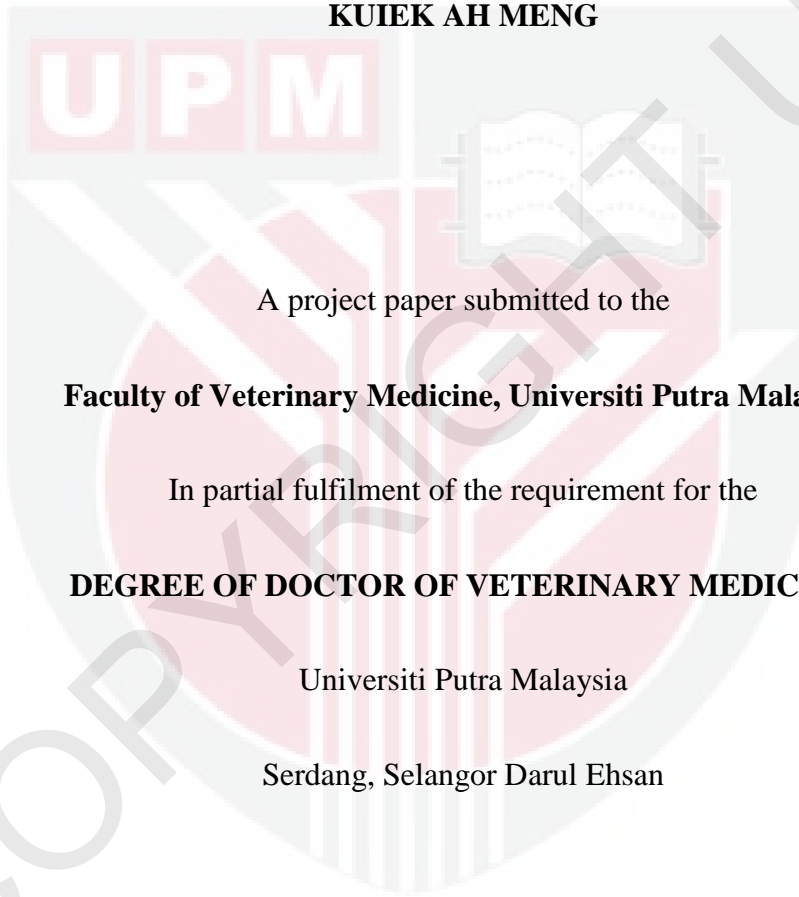
***APPLICATION OF ORAL FLUID TO DETECT PORCINE
REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) BY
USING ELISA***

KUIEK AH MENG

FPV 2015 59

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

KUIEK AH MENG



A project paper submitted to the

Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfilment of the requirement for the

DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan

MARCH 2015

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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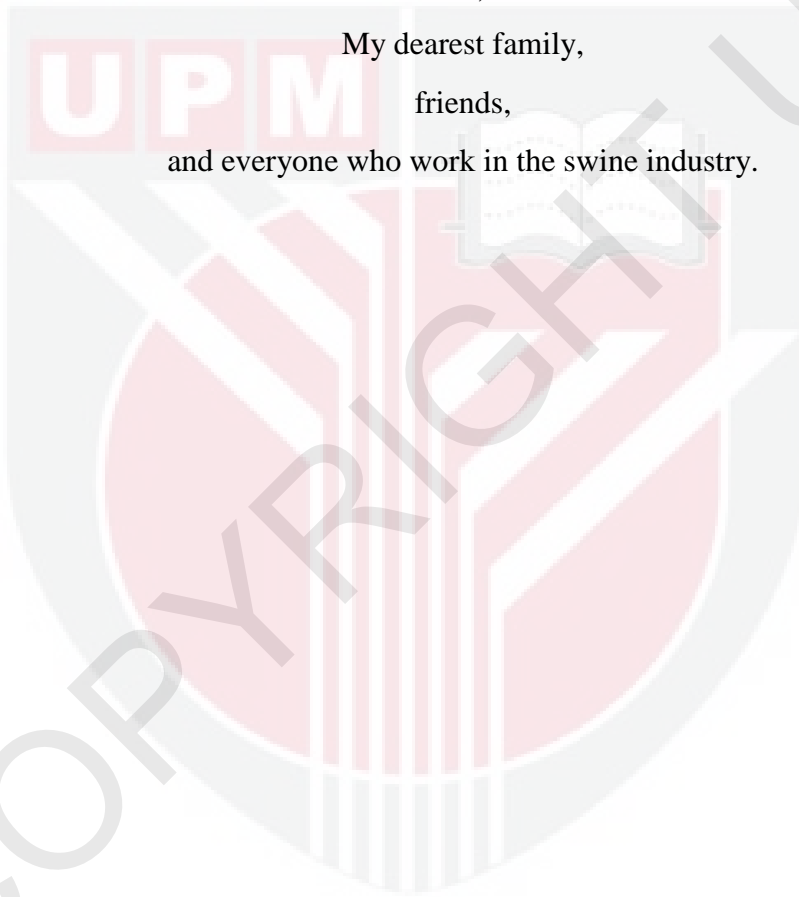
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DEDICATION

To,
My dearest family,
friends,
and everyone who work in the swine industry.



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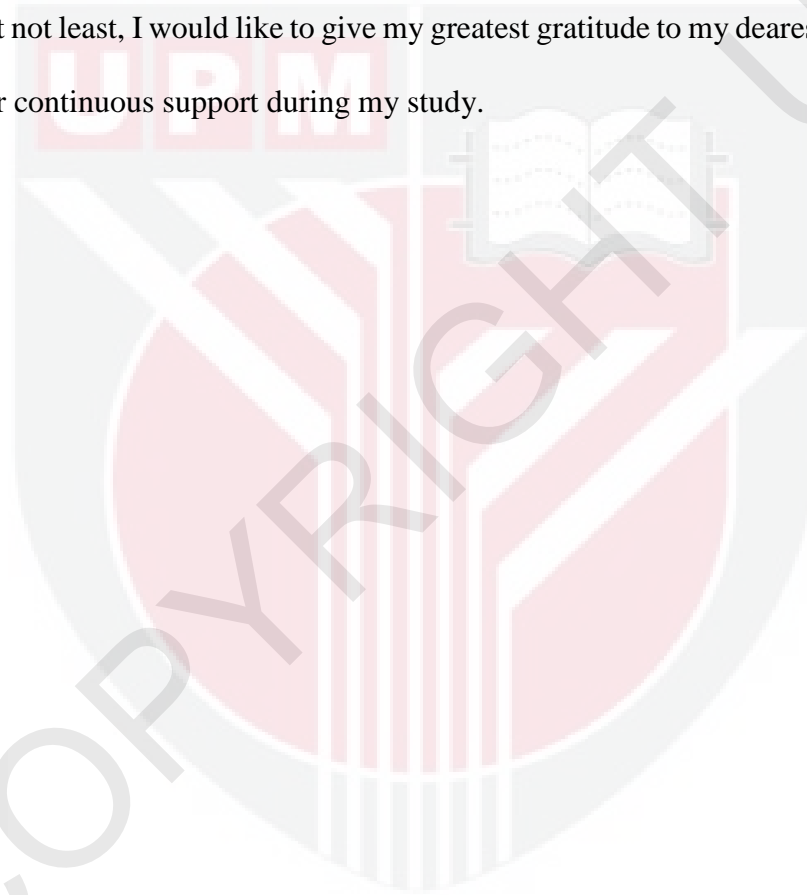


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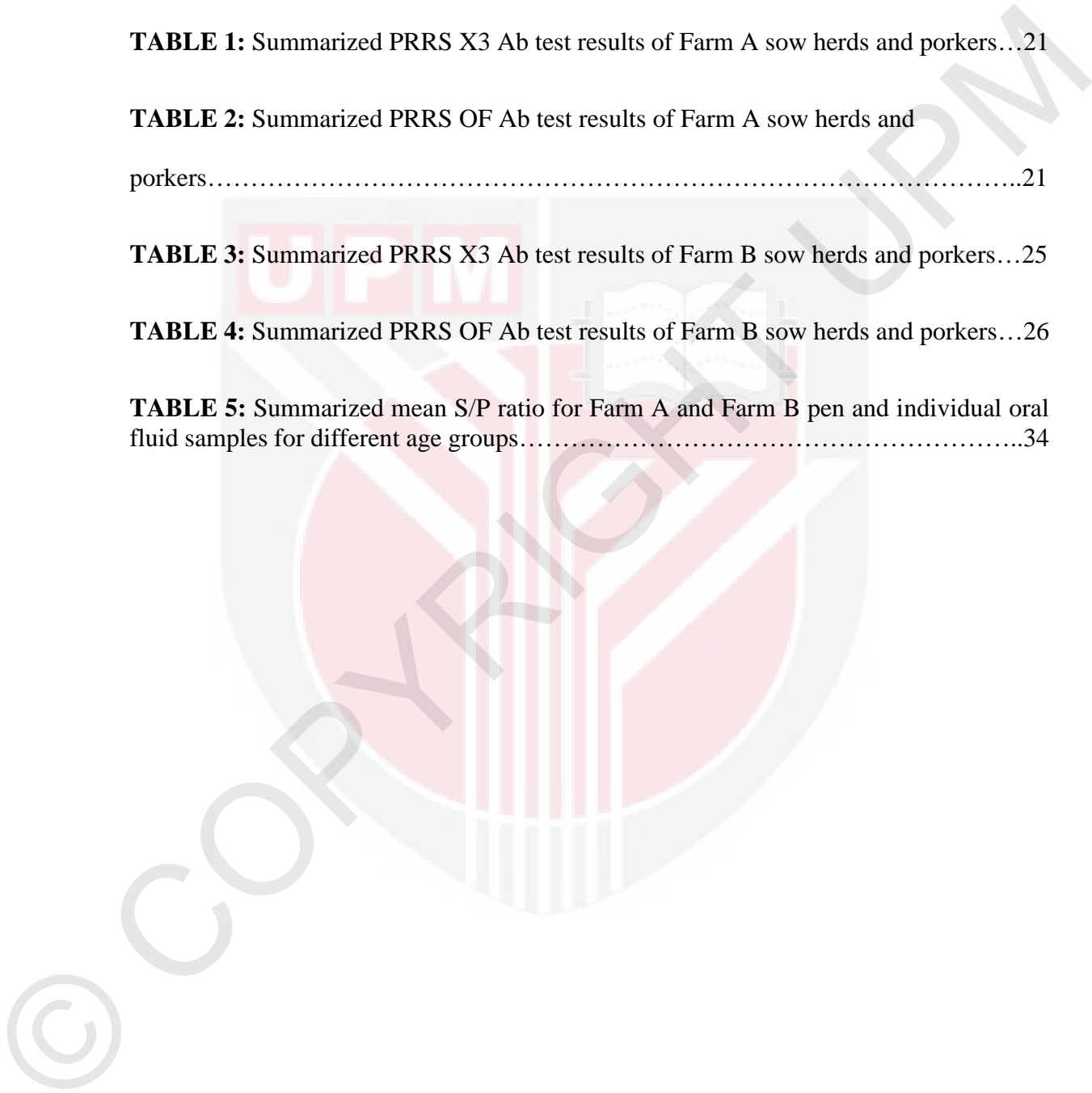
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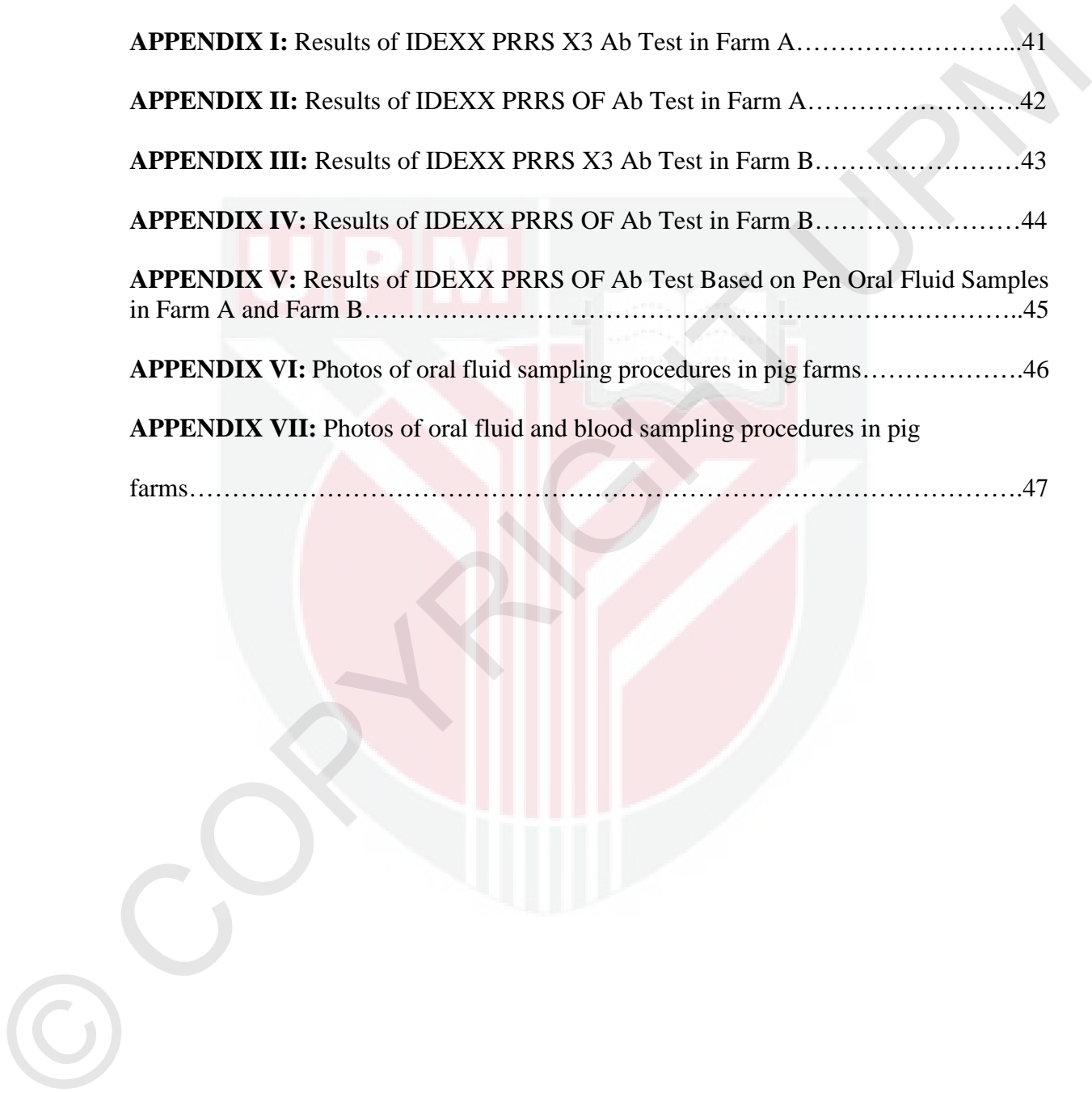
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LIST OF ABBREVIATIONS

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
°C	Degree Celcius

ABSTRAK

Abtrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan 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 Antibody 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 (2nd to 5th parities), old sows (6th 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.

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