ผลของเชื้อไวรัสพีอาร์อาร์เอสต่อสมรรถภาพทางการสืบพันธุ์ของสุกรสาวและแม่สุกร ในฝูงสุกรที่ติดเชื้อพีอาร์อาร์เอส โดยเฉพาะผลจากการทำวัคซีนและการจัดการ

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EFFECT OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) ON REPRODUCTIVE PERFORMANCE OF GILTS AND SOWS IN PRRSV-POSITIVE HERDS WITH SPECIAL REFERENCE TO VACCINATION AND MANAGEMENT STRATEGIES

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เอมอร โอฬารรัตน์มณี : ผลของเชื้อไวรัสพีอาร์อาร์เอสต่อสมรรถภาพทางการสืบพันธุ์ของสุกรสาวและแม่สุกรในฝูงสุกรที่ติดเชื้อพีอาร์อาร์เอส โดยเฉพาะผลจากการทำวัคซีนและการจัดการ (EFFECT OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) ON REPRODUCTIVE PERFORMANCE OF GILTS AND SOWS IN PRRSV-POSITIVE HERDS WITH SPECIAL REFERENCE TO VACCINATION AND MANAGEMENT STRATEGIES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.น.สพ.ดร. เผด็จ ธรรมรักษ์, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : ศ.น.สพ.ดร. อรรณพ คุณาวงษ์กฤต, ศ.น.สพ.ดร. รุ่งโรจน์ ธนาวงษ์นุเวช, 102 หน้า

การศึกษาในวิทยานิพนธ์นี้มีวัตถุประสงค์เพื่อประเมินผลของเชื้อไวรัสพีอาร์อาร์เอส ต่อสมรรถภาพทางการสืบพันธุ์ของสุกรสาวและแม่สุกรในฝูงสุกรที่ติดเชื้อพีอาร์อาร์เอส โดยเฉพาะผลจากการทำวัคซีนและการจัดการ ในการศึกษาที่หนึ่ง วัคซีนพีอาร์อาร์เอสชนิดเชื้อเป็นถูกนำมาใช้แบบปูพรมในฟาร์มสุกรขนาด 1,200 แม่ภายหลังการระบาดของเชื้อไวรัสพีอาร์อาร์เอส ข้อมูลสมรรถภาพทางการสืบพันธุ์ในช่วง 3 ปีถูกนำมาวิเคราะห์ การทำวัคซีนมีความสัมพันธ์กับการลดลงของอัตราเข้าคลอด (FR) (83.8% และ 90.0% *P*<0.001) จำนวนลูกสุกรแรกคลอดต่อครอก (TB) (10.6 และ 11.4 *P*<0.001) จำนวนลูกสุกรแรกคลอดมีชีวิตต่อครอก (BA) (10.0 และ 10.3 *P*=0.012) เปอร์เซ็นต์ลูกสุกรตายแรกคลอดต่อครอก (SB) (4.6% และ 7.0% *P*<0.001) และเปอร์เซ็นต์ลูกสุกรมัมมี่ต่อครอก (MM) (0.7% และ 1.6% *P*<0.001) และการเพิ่มขึ้นของอัตราการกลับสัด (RR) (11.3% และ 5.9% *P*<0.001) เมื่อเปรียบเทียบกับช่วงเวลาก่อนการระบาดของเชื้อไวรัสพีอาร์อาร์เอส สุกรอุ้มท้องที่ได้รับวัคซีนในช่วงต้นของการอุ้มท้องพบว่า BA ลดลงและ MM เพิ่มขึ้น ในขณะที่สุกรที่ได้รับวัคซีนในช่วงท้ายของการอุ้มท้องมี FR ลดลง การทำวัคซีนพีอาร์อาร์เอสชนิดเชื้อเป็นแบบปูพรมในฟาร์มสุกรจึงให้ผลทั้งเป็นกลาง เป็นบวก และเป็นลบต่อสมรรถภาพทางการสืบพันธุ์ ในการศึกษาที่สองข้อมูลสมรรถภาพทางการสืบพันธุ์จำนวนการผสม 211,009 ครั้งและการคลอด 180,935 ครั้งจากแม่สุกรที่ได้รับวัคซีนพีอาร์อาร์เอสชนิดเชื้อเป็นจำนวน 27,042 ตัวและแม่สุกรที่ไม่ได้รับวัคซีนจำนวน 45,816 ตัวจากฟาร์มสุกรที่ติดเชื้อไวรัสพีอาร์อาร์เอสจำนวน 20 ฟาร์มถูกนำมาวิเคราะห์ FR RR และ AR ในแม่สุกรที่ไม่ได้รับและได้รับวัคซีนได้แก่ 85.0% และ 89.7% (*P*<0.001) 6.9% และ 3.7% (*P*<0.001) และ 1.6% และ 2.0% (*P*=0.964) ตามลำดับ TB BA SB MM และจำนวนลูกสุกรหย่านมต่อครอก (WP) มีความแตกต่างอย่างมีนัยสำคัญ (*P*<0.001) ในสุกรสาวที่ไม่ได้รับและได้รับวัคซีน (11.2 และ 11.5 TB 10.0 และ 10.6 BA 6.9% และ 5.1% SB 3.2% และ 2.2% MM และ 9.2 และ 9.6 WP ตามลำดับ) การศึกษานี้สรุปได้ว่าการทำวัคซีนพีอาร์อาร์เอสชนิดเชื้อเป็นช่วยทำให้สมรรถภาพทางการสืบพันธุ์ของแม่สุกรในฟาร์มที่ติดเชื้อไวรัสพีอาร์อาร์เอสดีขึ้น ในการศึกษาที่สาม เนื้อเยื่อมดลูกของสุกรสาวที่ถูกคัดทิ้งจำนวน 100 ตัว จาก 6 ฟาร์ม ถูกเก็บเพื่อนำมาตรวจหาเชื้อไวรัสพีอาร์อาร์เอสโดยวิธีอิมมูโนฮิสโตเคมี เชื้อไวรัสพีอาร์อาร์เอสถูกพบในไซโตพลาสซึมของเซลล์แมคโครฟาจในชั้นเนื้อเยื่อเกี่ยวพันที่อยู่ใต้ชั้นผิวเยื่อบุมดลูก 33.0% ของสุกรสาวที่ถูกคัดทิ้ง เปอร์เซ็นต์เนื้อเยื่อมดลูกของสุกรสาวที่ตรวจพบเชื้อไวรัสพีอาร์อาร์เอสไม่มีความแตกต่างกันระหว่างฟาร์มที่สุกรสาวได้รับวัคซีนพีอาร์อาร์เอสชนิดเชื้อเป็นสายพันธุ์ยุโรป (24.5%) และสายพันธุ์อเมริกา (24.1%) แต่มีแนวโน้มน้อยกว่าสุกรสาวที่ไม่ได้รับวัคซีน (50.0%) เชื้อไวรัสพีอาร์อาร์เอสถูกพบแม้ในสุกรสาวที่อายุมากกว่า 11 เดือน การศึกษานี้สรุปได้ว่า เชื้อไวรัสพีอาร์อาร์เอสคงอยู่ในเนื้อเยื่อมดลูกของสุกรสาวที่ติดเชื้อได้นานหลายเดือนทั้งในสุกรสาวที่ได้รับและไม่ได้รับวัคซีน ในการศึกษาที่สี่ ลูกสุกรแท้ง ลูกสุกรมัมมี่ และลูกสุกรตายแรกคลอดจากแม่สุกรจำนวน 89 ตัวจาก 10 ฟาร์มถูกเก็บเพื่อนำมาตรวจหาเชื้อไวรัสพีอาร์อาร์เอสเชิงปริมาณโดยวิธีปฏิกิริยาลูกโซ่โพลีเมอเรส ผลการศึกษาพบว่า 67.4% ของตัวอย่างตรวจพบเชื้อไวรัสพีอาร์อาร์เอส เชื้อไวรัสถูกพบในลูกสุกรแท้ง 65.6% ลูกสุกรมัมมี่ 63.3% และลูกสุกรตายแรกคลอด 74.1% (*P*=0.664) เชื้อไวรัสพีอาร์อาร์เอสจพบได้ทั้งในตัวอย่างจากฟาร์มที่ไม่ได้รับวัคซีนพีอาร์อาร์เอสชนิดเชื้อเป็น (68.2%) และฟาร์มที่ได้รับวัคซีน (65.2%) (*P*=0.794) การศึกษานี้สรุปได้ว่าเชื้อไวรัสพีอาร์อาร์เอสตรวจพบได้ในลูกสุกรที่ตายในฟาร์มสุกรอุตสาหกรรมในประเทศไทยโดยไม่เกี่ยวข้องกับการทำวัคซีน

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EM-ON OLANRATMANEE : EFFECT OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) ON REPRODUCTIVE PERFORMANCE OF GILTS AND SOWS IN PRRSV-POSITIVE HERDS WITH SPECIAL REFERENCE TO VACCINATION AND MANAGEMENT STRATEGIES. ADVISOR : ASSOC. PROF. PADET TUMMARUK, D.V.M., Ph.D., CO-ADVISOR : PROF. ANNOP KUNAVONGKRIT, D.V.M., Ph.D., PROF. ROONGROJE THANAWONGNUWECH, D.V.M., Ph.D., 102 pp.

The aims of this thesis were to determine the effect of porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) on reproductive performance of gilts and sows in PRRSV-positive herds with special reference to vaccination and management strategies. In the first study, whole-herd PRRS modified-live virus (MLV) vaccination was performed in a 1,200-sow herd following a PRRSV outbreak. Reproductive performance data over a three-year period was available for analysis. Vaccination was associated with a lower farrowing rate (FR, 83.8% vs. 90.0%, $P<0.001$), number of total piglets born per litter (TB, 10.6 vs. 11.4 TB, $P<0.001$), number of piglets born alive per litter (BA, 10.0 vs. 10.3 BA, $P=0.012$), percentage of stillborn piglets per litter (SB, 4.6% vs. 7.0%, $P<0.001$), and percentage of mummified fetuses per litter (MM, 0.7% vs. 1.6%, $P<0.001$), and a higher return rate (RR, 11.3% vs. 5.9%, $P<0.001$) when compared with the period before the PRRSV outbreak. Pregnant females vaccinated during early gestation farrowed fewer BA and more MM than the comparison groups, whereas females vaccinated during late gestation had a lower FR. In this herd, PRRS MLV whole-herd vaccination had neutral, positive, and negative effects on reproductive performance. In the second study, the reproductive performance data contained of 211,009 mating and 180,935 farrowing records from 27,042 PRRS-MLV-vaccinated sows and 45,816 non-vaccinated sows from 20 PRRSV sero-positive herds were analyzed. FR, RR, and AR in non-PRRS-MLV-vaccinated and vaccinated sows were 85.0% and 89.7% ($P<0.001$), 6.9% and 3.7% ($P<0.001$), and 1.6% and 2.0% ($P=0.964$), respectively. TB, BA, SB, MM, and number of piglets weaned per litter (WP) differed significantly ($P<0.001$) between non-vaccinated and vaccinated sows (11.2 and 11.5 TB, 10.0 and 10.6 BA, 6.9% and 5.1% SB, 3.2% and 2.2% MM, and 9.2 and 9.6 WP, respectively). It could be concluded that PRRS MLV vaccination improved some reproductive performances of sows in PRRSV sero-positive herds. In the third study, uterine tissues of 100 culled gilts from six herds were collected for PRRSV detection using immunohistochemistry. PRRSV was detected in the cytoplasm of the macrophages in the subepithelial connective tissue layers of the endometrium in 33.0% of the culled gilts. The percentage of the gilts' uterine tissues containing PRRSV did not differ between herds with the gilts vaccinated with the EU-strain (24.5%) and the US-strain (24.1%) MLV PRRS vaccines but tended to be lower than the non-vaccinated gilts (50.0%). PRRSV could be found even in the gilts older than 11 months of age. It can be concluded that PRRSV remains in the uterine tissue of the infected gilts for several months in both vaccinated and non-vaccinated gilts. In the fourth study, aborted fetuses, mummified fetuses, and stillborn piglets from 89 sows from 10 herds were collected for PRRSV detection using quantitative polymerase chain reaction. The results showed that 67.4% of the samples contained PRRSV. The virus was found in 65.6% of the aborted fetuses, 63.3% of the mummified fetuses, and 74.1% of stillborn piglets ($P=0.664$). PRRSV antigen was retrieved from both non-PRRS-MLV-vaccinated herds (68.2%) and the vaccinated herds (65.2%) ($P=0.794$). It could be concluded that PRRSV was frequently detected in dead fetuses in swine commercial herds in Thailand regardless of vaccination.

Department : Obstetrics Gynaecology and Reproduction	Student's Signature .....
Field of Study : Theriogenology	Advisor's Signature .....
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LIST OF ABBREVIATIONS

°C	degree Celsius
µl	microliter
µm	micrometer
µM	micromolar
ADG	average daily gain
ADV	Aujezky's disease virus
AR	abortion rate
BA	number of piglets born alive per litter
bp	base pair
cDNA	complementary deoxyribonucleic acid
CRL	crown-rump-length
CSFV	classical swine fever virus
Ct	cycle threshold
Cy5	cyanine 5
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
EU	European
FA	fluorescent antibody
FAM	6-carboxy-fluorescine
FR	farrowing rate
g	gram
GLIMMIX	generalized linear-mixed models
GLM	general linear models
GP	glycoprotein
h	hour
H_2O_2	hydrogen peroxide
IFN-γ	interferon gamma
Ig	immunoglobulin

IHC	immunohistochemistry
ISH	*in situ* hibridization
kb	kilobase pairs
kg	kilogram
LSD	least significant difference
m	meter
min	minute
MLV	modified-live virus
MM	number of mummified fetuses per litter
NA	North American
nm	nanometer
NPD	non-productive day
ORF	open reading frame
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PCV2	porcine circovirus type 2
pH	potential of hydrogen ion
Ph.D.	doctor of philosophy
PPV	porcine parvo virus
PROC	procedure
PRRS	porcine reproductive and respiratory syndrome
PRRSV	porcine reproductive and respiratory syndrome virus
qPCR	quantitative polymerase chain reaction
RGJ	Royal Golden Jubilee
RNA	ribonucleic acid
RR	return rate
RT-PCR	reverse transcription-polymerase chain reaction
S/P	sample/positive
SAS	Statistical Analysis System

SB	percentage of stillborn piglets per litter
SD	standard deviation
SEM	standard error of mean
SVN	serum virus neutralization
TB	number of total piglets born per litter
Tris-HCl	trisaminomethane hydrochloride
US	American
VI	virus isolation
WP	number of piglets weaned per litter

CHAPTER I

INTRODUCTION

1.1 Importance and rationale

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases having high economic impact in the pig industry worldwide. The causative agent of the disease, porcine reproductive and respiratory syndrome virus (PRRSV), is an enveloped RNA virus (Cavanagh, 1997). PRRSV was classified by genetic, antigenic, and pathogenic differences into two genotypes, i.e., genotype 1 (European, EU) and 2 (North American, NA) (Meng, 2000). In Thailand, PRRSV infection in swine herds has been reported since 1995 and has become one of the most common diseases causing either respiratory diseases complex in nursery and fattening pigs or reproductive failures in gilts and sows (Oraveerakul et al., 1995; Meng, 2000). A retrospective study based on serological testing indicates that the antibody against PRRSV is detected for the first time in Thailand in early 1989 (Damrongwatanapokin et al., 1996) and both genotypes have been isolated (Thanawongnuwech et al., 2004; Amonsin et al., 2009).

PRRSV infection in gilts and sows is characterized by poor reproductive performance, i.e., high abortions rate (AR), which are vary from 10-50% and occur in any stage of gestation especially in the late stage, high return to estrus after mating (return rate, RR), low farrowing rate (FR), high premature farrowing, low number of piglets born alive per litter (BA), high percentage of stillborn piglets per litter (SB), high percentage of mummified fetuses per litter (MM), and high pre-weaning mortality (Done et al., 1996; Chung et al., 1997; Cho and Dee, 2006; Zimmerman et al., 2006). The economic losses in affected herds due to decreasing of the number of piglet weaned per sow, long farrowing intervals and increasing of the replacement rate (Brouwer et al., 1994). Under field conditions, the mode of transmission of PRRSV consists of direct contact, needle share for vaccination or medical injection, insects bite, and artificial insemination (Cho and Dee, 2006; Pringprao et al., 2006).

Several management strategies were applied to control and prevent PRRSV infection in swine commercial herds, e.g., acclimatization, management of replacement gilts, monitoring the prevalence of infection by serological profiling, and vaccination with PRRS modified-live virus (MLV) and/or killed virus vaccines (Cho and Dee, 2006). The vaccination of gilts and sows against PRRSV with either MLV or killed virus vaccines has been practiced in Thailand for over a decade. Although the killed or inactivated virus vaccine safe to be used in sows because the vaccine do not cause reproductive failures, generally no side effects has been observed, and can increase the serum neutralizing antibody titer and the number of interferon gamma (IFN-γ) producing cells (Nilubol et al., 2004, Misinzo et al., 2006; Papatsiros et al., 2006; Zimmerman et al., 2007; Vanhee et al., 2009), but it frequently failed to improve reproductive performance and to prevent the clinical signs of PRRSV infection, viremia, and transplacental infection after experimental challenged with PRRSV (Nilubol et al., 2004; Scortti et al., 2007; Kimman et al., 2009). Thus, the efficacy of the killed virus vaccines is still questioned (Kimman et al., 2009).

Apart from the killed virus vaccines, it has been demonstrated that the attenuated or MLV vaccines provide protection against clinical signs induced by homologous genotype of PRRSV infection and reduce viremia post challenge, although it provide incomplete protection against heterologous genotype of PRRSV infection (Labarque et al., 2003; Alvarez et al., 2006). The MLV vaccines are warranted to use in non-pregnant pigs since many researchers have proved the safety of the vaccine (Alexopoulos et al., 2005; Scortti et al., 2006b). However, the efficacy of MLV vaccines is under investigation. It has been found that the vaccinated pigs showed clinical signs, viremia, and viral shedding by transplacental transmission and transmission within herd although the immune responses occurred (Osorio et al., 1998; Scortti et al., 2006a; Kimman et al., 2009). Under field conditions, the use of MLV vaccines in pregnant pigs, sometime, resulted in an unfavorable outcome, such as, low BA, high SB and MM, and high pre-weaning mortality (Dewey et al., 1999; Nielsen et al., 2002; Dewey et al., 2004). Moreover, PRRS MLV can revert to virulent, causes the PRRS-like symptoms, spreads

transplacentally, especially when vaccination was performed at around 90 days of pregnancy, and the transplacentally infected piglets are capable to shed the virus (Mengeling et al., 1996; Botner et al., 1997; Mengeling et al., 1998; Dewey et al., 1999; Scortti et al., 2006a). Thus, the use of MLV vaccines has to be considered about the safety and the efficacy of the vaccines especially in pregnant females, and additional researches have to be carried out on the safety and the efficacy of using PRRS MLV vaccines in pregnant gilts and sows under field conditions.

In Thailand, the use of PRRS MLV vaccine to control and prevent PRRSV infection is increasing, but several vaccination programs have been used. Many studies on the characterization and the host immune responses after PRRSV infection have been carried out in Thailand (e.g., Suradhat and Thanawongnuwech, 2003; Thanawongnuwech et al., 2004; Amonsin et al., 2009). However, no comprehensive study on the control of reproductive failure caused by PRRSV infection under field conditions in relation to the use of PRRS MLV vaccine in combination with different types of management have been done. Therefore, some additional studies on the effect of PRRSV on reproductive performance of gilts and sows with special reference to PRRSV vaccination and management strategies are required to understand the strategies to control PRRSV infection in Thailand pig farms.

1.2 Keywords

Porcine reproductive and respiratory syndrome (PRRS), Modified-live virus (MLV) vaccine, Reproduction, Swine

1.3 Research coherence

In Thailand, likewise in other pig producing countries, PRRS is one of the important diseases and causes high economic loss in commercial swine herds during the past decades. However, limited studies on reproductive performances in PRRSV-infected herds and effect of PRRS MLV vaccination on reproductive performance under field conditions were reported.

The present research was comprised of two parts. The first part (Chapter II and III) studied about the PRRSV immune responses, viremia, and reproductive performances in gilts and sows in PRRSV sero-positive herds in relation to PRRS MLV vaccination. The second part (Chapter IV and V) studied about the detection of PRRSV in gilts and sows which had reproductive problems.

Several methods, e.g., acclimatization, replacement gilts management, herd closure, and vaccination with either inactivated or MLV vaccines, were used to control PRRSV circulation in the infected herds by reducing the presence of subpopulation of susceptible pigs (Cho and Dee, 2006; Thanawongnuwech and Suradhat, 2010). Although PRRS MLV vaccine has been authorized and been broadly used in swine breeding herds in Thailand since 2005, but the efficacy of the vaccine varied among herds (Alexopoulos et al., 2005; Martelli et al., 2007; Martelli et al., 2009). Many studies were performed to determine the safety and the efficacy of PRRS MLV vaccine, e.g., immunological responses, viral shedding, and clinical signs after vaccination. However, limited information is found on the effect of the vaccine on reproductive performance of breeding gilts and sows, especially under field conditions. It leads to a hard decision of farmers whether to use the PRRS MLV vaccine to control PRRS or not. The first study, therefore, was performed to monitor the effect of whole-herd PRRS MLV vaccination on reproductive performance in the herd level over time of a PRRSV positive breeding herd and the effect of the vaccine on reproductive outcome in each stage of gestation at the time of vaccination (Chapter II). Furthermore, in the second study, the overall reproductive performance of gilts and sows in breeding herds in Thailand, both PRRS-MLV-vaccinated and non-vaccinated herds, were investigated (Chapter III).

In general practice, sows in breeding herds are culled and replaced by gilts around 35-55% annually (D'Allaire and Drolet, 1999). It is well established that replacement gilts are a major source of introducing new strains of PRRSV into the breeding herds. It was found that 47% of culled gilts were culled due to reproductive disturbances, i.e., anestrus, repeat breeding, not being pregnant, abortion, and

abnormal vaginal discharge (Tummaruk and Tantilertcharoen, 2012). Furthermore, 73% of them were infected with PRRSV (Tummaruk and Tantilertcharoen, 2012). Although there are some studies reported the presence of PRRSV in several organs, e.g., lung, liver, spleen, tonsil, and lymph node, of infected pigs (Laohasittikul et al., 2004), but no study has investigated the presence of PRRSV in reproductive organs and its association with the age at culling, culling reason, and PRRSV vaccination. Thereafter, the third study was performed to assure the presence of PRRSV in uterine tissues and its localization using immunohistochemistry (IHC) technique, and also to determine the prevalence of PRRSV in uterine tissues of gilts culled due to reproductive disturbances associated with age at culling, culling reason, herds, and PRRSV vaccination in swine commercial herds in Thailand (Chapter IV).

Due to the fact that PRRSV causes reproductive failures, i.e., abortion, stillborns, and mummies, in infected females (Zimmerman et al., 2006) and it is well known that Thailand is an endemic area of PRRSV infection, therefore, PRRSV was assumed as the causative agent in most cases of reproductive failures. However, the reproductive failures are influenced by both non-infectious causes, e.g., toxin, heat stress, and management, and infectious causes, e.g., PRRSV, pseudorabies virus (Aujezky's disease virus, ADV), porcine parvovirus (PPV), porcine enterovirus, classical swine fever virus (CSFV), swine influenza virus, encephalomyocarditis virus, porcine circovirus type 2 (PCV2), *Leptospira* sp., *Brucella suis*, and *Toxoplasma gondii* (Dias et al., 2012; Tummaruk and Tantilertcharoen, 2012; Tummaruk et al., 2013). Thus, the last study was performed to assure the presence of PRRSV and to investigate the prevalence of PRRSV in cases of reproductive failures by detection the virus in aborted fetuses, mummified fetuses, and stillborn piglets using real-time polymerase chain reaction (PCR) (Chapter V).

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1.4 Literature review

PRRS is one of the most important diseases in swine. The disease was discovered for the first time in the United State (US) in 1987 (Keffaber, 1989). In 1990, PRRSV was first identified in Lelystad, the Netherlands (Wensvoort et al., 1991) and was classified into two genotypes, i.e., genotype 1 (European genotype) and 2 (North American genotype), in 1992 (Nelson et al., 1993; Meng, 2000). Nowaday, PRRSV has been found and become an endemic disease in most major pig-producing areas thoughout the world (Benfield et al., 1999; Carlsson et al., 2009).

In 1995, PRRSV infection in Thai swine herds was first reported (Oraveerakul et al., 1995) but, by retrospective study using serological testing, the antibody against PRRSV had been detected as early as 1989 (Damrongwatanapokin et al., 1996). In addition, both genotype 1 and 2 of the PRRSV have been isolated in Thailand (Thanawongnuwech et al., 2004; Amonsin et al., 2009).

1.4.1 Etiology

PRRS is caused by PRRSV which is a lipid-enveloped with a diameter of 50-65 nm and single-stranded RNA virus. The virus was classified in the order Nidovirales, family *Arteriviridae*, genus *Arterivirus* (Benfield et al., 1992; Cavanagh, 1997). The genome of the PRRSV is approximately 15 kb in length and contained of nine open reading frames (ORFs), i.e., ORF 1a, ORF 1b, ORF 2a, ORF 2b, and ORFs 3 to 7 (Cavanagh, 1997; Snijder and Meulenberg, 1998). ORF 1a and 1b comprise around 80% of the viral genome and encode the RNA replicase required for viral replication, whereas ORFs 2 to 7 encode the viral structural proteins (Zimmerman et al., 2006).

The virus was classified by genetic, antigenic, and pathogenic differences into genotype 1 and 2 (Nelson et al., 1993; Meng, 2000). The original virus of genotype 1 and 2 are Lelystad virus and VR-2332, respectively. Due to the fact that RNA viruses are considered to have high rates of mutation, a high variation of PRRSV genetic by the virus itself is frequently observed. The genetic similarity between these two genotypes is about 55-65% (Meng et al., 1995; Murtaugh et al., 1995; Gagnon and Dea, 1998; Dea et al., 2000; Amonsin et al., 2009). The co-circulation of both genotypes in the infected

herds has been reported (Ropp et al., 2004). Since PRRSV mutates quickly in pigs, the filed isolated virus in either genotypes also vary genetically both within and among herds (Rowland et al., 1999; Goldberg et al., 2000a). Moreover, the multiple isolates of the virus can co-exist in the individual infected pigs, called quasispecies (Chang et al., 2002; Goldberg et al., 2003). The glycoprotein (GP) 5, which is coded from ORF 5 and induces neutralizing antibody, is the most variable structural protein (Kapur et al., 1996). This resulted in inefficiently neutralization of the antibody against progeny virus. In addition, the genetic recombination among PRRSV isolates has been reported (Yuan et al., 1999).

PRRSV is fragile and quickly inactivated by heat and drying (Pirtle and Beran, 1996; Cutler et al., 2012). PRRSV is stable for months to years at temperatures of -70°C and -20°C. Although, the infectivity of the virus is lost within a week at 4°C, but low titers of the virus can be detected for at least 30 days. In addition, the infectivity of the virus persists for 1-6 days at temperatures of 20-21°C and 3-24 hours at 37°C (Benfield et al., 1999). The infectivity is lost at pH below 6 and above 7.5 (Benfield et al., 1992). Since the virus is a lipid-enveloped virus, PRRSV is inactivated by the lipid solvents, such as chloroform and ether. Moreover, the virus is highly unstable in solutions containing low concentrations of detergents (Benfield et al., 1992).

In general, PRRSV primarily infects pulmonary alveolar macrophages during acute infection (Sur et al., 1997). It is well established that the alveolar macrophages as well as macrophages from other tissues are the primary cell type that sustains the *in vivo* viral replication (Thanawongnuwech et al., 2000). Using IHC evaluation of formalin-fixed tissues, it was found that 100% and 66% of the lung tissues of piglets infected with either genotype 1 or 2 of Thai PRRSV, respectively (Laohasittikul et al., 2004). An earlier study based on PRRSV antigen detection by the IHC technique has demonstrated that 75.0%, 50.0%, 37.5%, 37.5%, 37.5%, and 25.0% of PRRSV was found in liver, spleen, tonsil, turbinate bone, pulmonary lymph node, and ileum, respectively, of the experimentally infected piglets (Laohasittikul et al., 2004). In addition, PRRSV antigen is found in microglia-like cells and mononuclear cells in the

brain sections by IHC associated with neurovascular lesions (Thanawongnuwech et al., 1997a). Using the *in situ* hybridization (ISH), PRRSV is also detected in the epithelial germ cells of the seminiferous tubules, primarily spermatids and spermatocytes, and macrophages of the testes (Sur et al., 1997; Shin and Molitor, 2002). Moreover, PRRSV produces a persistent infection in pigs and the infected pigs can carry the virus for several months (Wills et al., 1997; Benfield et al., 2000).

1.4.2 Clinical signs

The clinical signs of PRRSV infection vary between herds depending on the age of the infected pigs, the genotype and virulence of the virus, the herd's size, herd's immune status, host susceptibility, concurrent infections, and other management factors (Halbur et al., 1995; Thanawongnuwech et al., 1997b; Thanawongnuwech and Suradhat, 2010). The clinical signs of PRRSV infection in sows include anorexia, pyrexia (39.0-41.0°C), agalactia, lethargy, pneumonia, skin discoloration, cyanosis on extremities, and subcutaneous edema (Done et al., 1996; Rossow, 1998; Zimmerman et al., 2006). The reproductive performances of infected herds are characterized by a decreasing of FR and an increasing of AR, which are vary from 10-50% and occur at any stages of gestation especially at the late stage, premature farrowing, SB, MM, the number of weak born piglets, pre-weaning mortality, which may occur up to 60%, the number of sows which had an irregular return to estrus, and non-productive sow days (Done et al., 1996; Benfield et al., 1999; Cho and Dee, 2006; Zimmerman et al., 2006).

1.4.3 Immunological responses

In general, after the animals were infected with PRRSV, the protective immunity is developed. In the acute phase of infection, viremia and high viral load in tissues were found (Figure 1). The viremia may last up to a month and the persistence of virus in tissues is at least five months (Albina et al., 1998; Allende et al., 2000). The anti-PRRSV antibodies can be detected by using enzyme-linked immunosorbent assay (ELISA) at 7-9 days post infection (Yoon et al., 1995; Lopez and Osorio, 2004). An earlier study has demonstrated that the anti-PRRSV immunoglobulin (Ig) M antibodies are detected by

5-7 days and undetectable after 2-3 weeks post-infection, whereas anti-PRRSV IgG antibodies are detected by 7-10 days post-infection and remaining for several months before declining to undetectable level by 10 months post-infection (Murtaugh et al., 2002). However, the antibodies in the early period can not neutralize the virus. The antibodies which have a neutralizing activity are developed as early as four weeks post-infection (Yoon et al., 1994; Lopez and Osorio, 2004). After the neutralizing antibodies are developed, the viremia is reduced, but the virus in tissues is still persist (Figure 1).

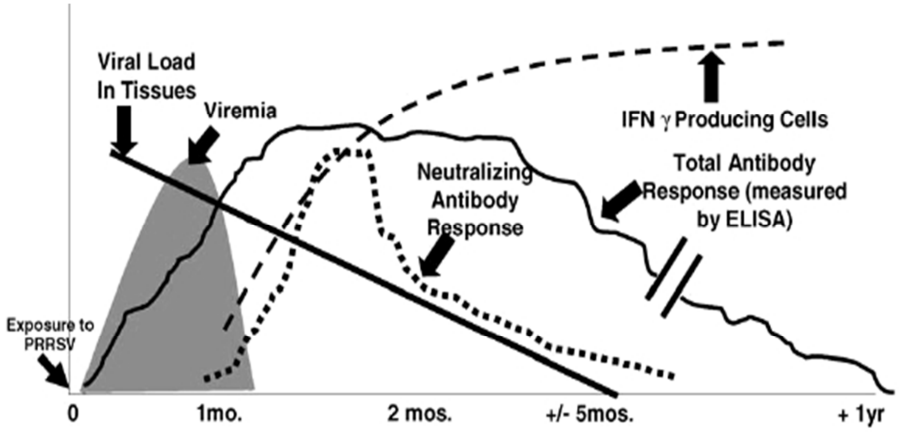

Figure 1 The immunological responses of porcine reproductive and respiratory syndrome virus infected pigs (Lopez and Osorio, 2004)

The cell-mediated immune responses against PRRSV are begin at four weeks post-infection, which are characterized by a developing of PRRSV-specific T cell responses (Bautista and Molitor, 1997). An increasing of specific IFN-γ, a cytokine which is produced by cytotoxic T cells, is indicated a cell-mediated immune responses to the virus in infected cells (Lopez Fuertes et al., 1999).

1.4.4 Diagnosis

There are many ways to diagnose the PRRSV infection in swine herds. The diagnosis of PRRSV infection included history taking, production record analysis, clinical signs, gross and microscopic lesions, serological test, and viral detection.

a) History taking, production record analysis, and clinical signs

From history taking and production record analysis, PRRSV infection was suggested in swine herds which had respiratory problems in pigs of all ages and reproductive problems in breeding herd. The reproductive problems are characterized by a decreasing of FR and an increasing of AR, which occur at any stages of gestation especially at the late stage, premature farrowing, SB, MM, the number of weak born piglets, pre-weaning mortality, the number of sows which had an irregular return to estrus, and non-productive sow days (Done et al., 1996; Benfield et al., 1999; Cho and Dee, 2006). The reproductive failures in sows may last up to four months. AR varies from 10 to 50% and pre-weaning mortality may be increased up to 60% in epidemic infected herds (Zimmerman et al., 2006).

b) Gross and microscopic lesions

There are no pathognomonic gross and microscopic lesions in PRRSV-infected pigs (Zimmerman et al., 2006). Interstitial pneumonia and lymph nodes enlargement can be observed (Stevenson et al., 1993; Lager and Halbur, 1996). Microscopically, moderate to severe multifocal interstitial pneumonia are observed. The alveolar septa are infiltrated with mononuclear cells (Benfield et al., 1999). In infected sows, mild multifocal perivascular lymphoplasmacytic and histiocytic myometritis and a moderate multifocal non-suppurative perivascular endometritis can be observed (Larger and Halbur, 1996). Endometrial edema is also common in PRRSV-infected sows (Benfield et al., 1999).

c) Serology

The serological diagnosis techniques, which are used to detect the PRRSV antibodies in the serum of the pig, include immunofluorescent antibody test, immunoperoxidase monolayer assay, ELISA, and serum virus neutralization (SVN) (Yoon et al., 1995; Christopher-Hennings et al., 2002). The antibodies can be detected by using ELISA as early as nine days post-infection, peak at 30-50 days post-infection, and

then decline to negative levels by 4-12 months post-infection (Zimmerman et al., 2006). The commercial ELISA (HerdChek®2XR PRRS ELISA, IDEXX Laboratories Inc., Westbrook, Maine) is generally recognized as the gold standard for detection of antibodies to PRRSV due to its high sensitivity (100%) and specificity (99.5%) (Zimmerman et al., 2006). However, the results cannot distinguish among natural infection, maternal immunity, and vaccinated immunity (Benfield et al., 1999). The antibodies detected by SVN test were the neutralizing antibodies (Christopher-Hennings et al., 2002). In addition, the antibodies can be detected over 1-2 months (Nelson et al., 1994) and persist up to a year after infection (Yoon et al., 1994).

d) Viral detection

The viral detections are including virus isolation (VI), fluorescent antibody (FA), IHC, ISH, PCR assay, and restriction fragment length polymorphism. The VI was used to detect viral antigen in the cells (Zimmerman et al., 2006). However, the VI cannot distinguish the vaccine and the field isolates (Nielsen et al., 1997). The FA and IHC were used to detect viral antigen in the infected tissues (Magar et al., 1993; Halbur et al., 1994; Rossow, 1998; Benfield et al., 1999), whereas the ISH was used to detect the viral nucleic acids (Shin and Molitor, 2002). These tests can be localized the virus within cells and tissues (Larochelle and Magar, 1997; Shin and Molitor, 2002). PCR technique, which is used to diagnose PRRSV, is a reverse transcription-polymerase chain reaction (RT-PCR). This method has high sensitivity and specificity for detection of viral RNA (Benfield et al., 1999; Benson et al., 2002).

1.4.5 Prevention and control

The Thai Swine Veterinarian Association (TSVA) has classified the pattern of PRRSV infection status in swine herds in Thailand, which are based on the serological profile of the herds using a commercial ELISA test, into four groups (I to IV, Table 1). In group I, acute PRRSV outbreak, the sows have high reproductive failure and the pigs in the herd have respiratory signs. In group II, chronic PRRSV infection, the sows have

low reproductive problems, but the pigs in the herd still have respiratory signs. In group III, PRRS stable sow herd, no clinical sign of PRRSV infection is found in the herd, and have low or no viral shedding in the sows herd. In group IV, the PRRS negative herd, no PRRSV is carried in the herd (Thanawongnuwech and Suradhat, 2010).

Table 1 the pattern of PRRSV infection status in swine herds in Thailand based on seroprofile of the herd using a commercial ELISA test (IDEXX ELISA)

Farm patterns	Sows	Weaners	Growers	Finisher >16 weeks
Acute PRRSV outbreak	+	+	+	+
Chronic PRRSV infection	+	+/-	+	+
PRRS stable sow herd	+	+/-	-	+/-
Negative herd	-	-	-	-

+ = individual or average S/P ratio ≥0.4, - = individual or average S/P ratio <0.4

The control and prevention of PRRSV in swine commercial herds include intensive acclimatization, management of replacement gilts, monitoring the prevalence of infection by serological profiling, and vaccination with PRRS MLV vaccine and/or killed vaccines (Cho and Dee, 2006).

The vaccine against PRRSV consists of two types, attenuated-live and inactivated PRRSV vaccines (Kimman et al., 2009). Inactivated or killed virus vaccine against PRRSV is safe to be used in sows because it does not induce the reproductive failures and vaccine side effects (Misinzo et al., 2006; Papatsiros et al., 2006), but it is questioned in the efficacy of the vaccine (Kimman et al., 2009). It has been reported that the vaccine failed to prevent the clinical signs of PRRSV infection in gilts. In addition, it failed to prevent viremia (Nilubol et al., 2004; Scortti et al., 2007) and transplacental infection of their piglets after challenged with PRRSV (Scortti et al., 2007). Although the pre-weaning mortality of the piglets born from the vaccinated gilts was lower than that of the piglets born from the non-vaccinated and infected gilts, but the others reproductive performances among vaccinated and non-vaccinated gilts was not differed (Scortti et

al., 2007). However, killed-virus vaccine can increase the serum neutralizing titer and the number of IFN-γ producing cells (Nilubol et al., 2004; Misinzo et al., 2006; Zimmerman et al., 2007; Vanhee et al., 2009).

Attenuated or MLV vaccine can protect against clinical signs induced by homologous genotype of PRRSV, but it cannot be able to completely prevent infection, transplacental transmission, and transmission within herds (Osorio et al., 1998; Scortti et al., 2006b; Kimman et al., 2009). The MLV has the ability to replicate *in vivo* and induces longer and more intense immune responses than inactivated virus vaccines (Alvarez et al., 2006). It has been reported that MLV vaccine provided the protection against homologous PRRSV infection, reduced viremia post challenge (Alvarez et al., 2006), and reduced the respiratory signs after infected with field isolate (Marteli et al., 2009), but it provided incomplete protection against heterologous PRRSV infection (Labarque et al., 2003). In addition, the experimentally vaccinated gilts that showed the neutralizing antibody could have the evidence of reproductive failure (Thanawongnuwech and Suradhat, 2010). The MLV can reverts to virulent and causes the PRRS-like symptoms which are characterized by an increasing of AR, SB, and mortality in the nursing period (Botner et al., 1997). Due to the fact that the MLV can replicates in pigs, persists for several weeks or months, has the ability to cross the placenta, can be shed to the non-vaccinated pigs both within and among herds, and can reverts to virulent that causes the PRRS-like symptoms (Mengeling et al., 1996; Botner et al., 1997; Scortti et al., 2006a), the use of MLV vaccine has to be considered about the safety and the efficacy of the vaccine especially in pregnant gilts and sows.

There were many studies reported the effects of MLV vaccine on reproductive performances. In non-pregnant gilts and sows, MLV vaccination improved the FR, BA, and number of piglets weaned per litter (WP) and lessened SB and MM (Alexopoulos et al., 2005; Scortti et al., 2006b). However, the congenital infection of piglets is also found (Alexpoulos et al., 2005). In pregnant gilts and sows, the vaccination with MLV vaccine had several negative effects. It was found a decreasing of the BA and WP and an increasing of the SB, MM, and mortality rate in nursery pigs (Dewey et al., 1999; Dewey

et al., 2004; Nielsen et al., 2002). Moreover, the largest association was seen in sows that were vaccinated in the last four weeks of gestation (Dewey et al., 1999).

1.5 Research Objectives

1) To monitor the antibody titer responses to PRRSV infection and viremia of PRRSV-infected gilts and sows and the reproductive performance of the herd following whole-herd PRRS MVL vaccination under field conditions
2) To investigate the reproductive performance of gilts and sows in PRRSV-positive herds in Thailand in relation to different types of management strategies and PRRS MLV vaccination
3) To determine the prevalence of PRRSV antigen in the reproductive organs of gilts in relation to period of acclimatization, age, PRRSV antibody titers, and PRRS MLV vaccination
4) To investigate the prevalence of PRRSV detection in cases of reproductive failure in gilts and sows in swine commercial breeding herds in relation to PRRS MLV vaccination

1.6 Research Outline

The present thesis was conducted in totally 28 commercial swine breeding herds, which were located in every part (central, eastern, western, northern, north-eastern, and southern parts) of Thailand, the Department of Obstetrics, Gynaecology and Reproduction, the Department of Pathology, the Department of Veterinary Public Health, and the Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University from June 2008 to October 2012. The research was focused on the effect of PRRSV on reproduction of gilts and sows in PRRSV positive swine herds with special reference to vaccination and management strategies. The dissertation consists of six chapters (chapter I-VI).

Chapter I was an introduction about the dissertation included the important and rational of the research, keywords, research coherence, literature review, research objectives, research outline, and research benefits.

Chapter II was the study of the immunological response, viremia, and reproductive performance of gilts and sows in a 1,200 sows-on-production PRRSV-positive breeding herd in Thailand subsequent to a PRRSV outbreak and whole-herd vaccination with PRRS MLV vaccine. The effect of PRRS MLV whole-herd vaccination on overall reproductive performance of the herd and on reproductive outcome of gilts and sows in each stage of gestation at the time of vaccination were determined in this study. Serum samples were obtained from 36 gilts and sows before and after whole-herd vaccination and then were tested for antibody response against PRRSV by using ELISA technique and viremia by using RT-PCR. Three-year period of reproductive performance of the herds from 2,337 gilts and sows were retrospectively collected and analyzed for fertility and litter traits.

Chapter III was the study of reproductive performance of gilts and sows in 20 PRRSV-positive, with and without PRRS MLV vaccination, breeding herds in Thailand. Reproductive performance, three-year period, from 72,386 sows, included FR, RR, AR, number of total piglets born per litter (TB), BA, SB, MM, and WP in each herd were collected, analyzed, and demonstrated in this chapter.

Chapter IV was the study of the presence and prevalence of PRRSV antigen-positive uterine tissues in gilts culled due to reproductive disturbance. This study was performed by using IHC to detect the PRRSV antigen in 100 formalin-fixed uterine tissues of gilts culled due to reproductive disturbances from six swine herds in Thailand, both PRRS-MLV-vaccinated and non-vaccinated herds. The historical data of gilts were obtained to investigate the relationship among the prevalence of PRRSV, age at culling, culling reason, herds, PRRSV vaccination, and PRRSV antibody tested by ELISA.

Chapter V was the study of the presence and prevalence of PRRSV from 89 cases of reproductive failures in gilts and sows by detection the virus in aborted fetuses, mummified fetuses, and stillborn piglets using real-time PCR. The study was carried out in 10 commercial swine herds in Thailand, both PRRS-MLV-vaccinated and non-vaccinated herds.

In the last chapter (Chapter VI), the summarized of all results performed in this dissertation, research limitations, and suggestions for further investigations were included.

1.7 Research Benefits

PRRS highly causes economic losses in pig producing industry in Thailand due to the effect of PRRSV on gilts, sows, and also piglets, resulted in an increasing of production cost. Nowadays, PRRS is still one of the most important diseases causing problems in swine breeding herds, although many management strategies to control the disease and the economic losses from this virus were carried out during past decades. PRRSV vaccination with MLV vaccine is one of the ways to control the disease. However, the efficacy of the vaccine still varies among herds and the safety of the vaccine using in pregnant females pigs is still questioned. This leads the farmer and herd veterinarians difficult to make a decision of using PRRS MLV vaccine. This dissertation investigated the effect of PRRSV and PRRS MLV vaccination on reproductive performance of breeding gilts and sows. The information from this dissertation, i.e., current situation (importance and prevalence) of PRRSV infection in breeding herds in Thailand, effect of PRRSV on reproductive performance, and effect of PRRS MLV vaccine on pregnant gilts and sows, might lead farmer and herd veterinarians to understand about PRRSV infection and advantages and disadvantages of using PRRS MLV vaccine in swine breeding herds in Thailand.

CHAPTER II

REPRODUCTIVE PARAMETERS FOLLOWING A PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) OUTBREAK WHERE A WHOLE-HERD PRRS MODIFIED-LIVE VIRUS VACCINATION STRATEGY WAS INSTITUTED POST-OUTBREAK

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Reproductive parameters following a porcine reproductive and respiratory syndrome (PRRS) outbreak where a whole-herd PRRS modified-live virus vaccination strategy was instituted post-outbreak

2.1 Abstract

This study assessed the effect of whole-herd porcine reproductive and respiratory syndrome (PRRS) modified-live virus (MLV) vaccination on herd-level reproductive performance, PRRS virus (PRRSV) viremia, and antibody in a subset of females in a 1,200-sow commercial herd in Thailand. Following a PRRSV outbreak, the entire herd was vaccinated with PRRS MLV twice at 3-week intervals and at 3-month intervals, thereafter. Reproductive performance data over a 3-year period were available for analysis. Serum samples were collected before and after vaccination and tested by PRRSV ELISA and reverse transcription-polymerase chain reaction. Vaccination was statistically associated with a lower abortion rate (AR, 1.4% vs. 1.6%), farrowing rate (FR, 83.8% vs. 90.0%), number of total piglets born per litter (10.6 vs. 11.4 piglets/litter), number of piglets born alive per litter (BA, 10.0 vs. 10.3 piglets/litter), percentage of stillborn piglets per litter (SB, 4.6% vs. 7.0%), percentage of mummified fetuses per litter (MM, 0.7% vs. 1.6%), and a higher return rate (11.3% vs. 5.9%) when compared with the period before the PRRSV outbreak. Pregnant females vaccinated during early gestation farrowed fewer BA and more MM than the comparison group, whereas females vaccinated during late gestation had a lower FR. In this herd, PRRS whole-herd vaccination had neutral, positive, and negative effects on reproductive performance. Thus, the decision to implement whole-herd vaccination should be balanced between the benefits derived from reproductive performance improvements, e.g., fewer AR, SB, and MM, and the effect of vaccination on pregnant females.

Keywords: PRRSV, Modified-live virus vaccine, Whole-herd vaccination, Reproductive performance, Gestation

2.2 Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by PRRS virus (PRRSV), a member of family *Arteriviridae*. In general, PRRSV infection in pregnant gilts and sows is characterized by late-term abortions and an increase in percentage of mummified fetuses per litter (MM), percentage of stillborn piglets per litter (SB), and low viability piglets at birth (Chung et al., 1997). The disease was reported for the first time in the USA in 1987, and the virus was identified for the first time in Lelystad, the Netherlands, in 1990 (Wensvoort et al., 1991). In 1992, PRRSV was divided into two genotypes, i.e., types 1 (European genotype) and 2 (North American genotype) on the basis of genetic, antigenic, and pathogenic differences (Meng, 2000). To date, PRRSV has been found in most major pig-producing areas throughout the world (Zimmerman et al., 2006). A retrospective serological study determined that PRRSV was present in Thailand since 1989 (Damrongwatanapokin et al., 1996) and in 1995, it was estimated that 64% of the commercial swine herds in Thailand were PRRSV-infected (Oraveerakul et al., 1995). Both types 1 and 2 PRRSV genotypes have been isolated in Thailand (Thanawongnuwech et al., 2004).

In the PRRSV-endemic herds, the presence of subpopulations of susceptible pigs may lead to the continual circulation of PRRSV. Herd closure, gilts acclimatization, and whole-herd exposure to wild-type virus or vaccines have been recommended to eliminate these subpopulations (Cano et al., 2007a, b). The types of PRRSV vaccine available in Thailand include both modified-live virus (MLV) and inactivated virus vaccines. The use of vaccination to immunize pigs has been evaluated, in most cases, at the individual pig level and in nursery populations (Martelli et al., 2009). It has been demonstrated that PRRS MLV vaccination can reduce lung lesions in the PRRSV-infected pig and decrease the level and duration of viremia after challenge with homologous virus (Foss et al., 2002; Mengeling et al., 2003). In addition, PRRS MLV vaccination of the entire herd (whole-herd vaccination) was shown to reduce the persistence and duration of the viral shedding, even though wild-type virus was not eliminated (Cano et al., 2007a, b). However, the effect of PRRSV vaccination varies

among herds (Alexopoulos et al., 2005; Martelli et al., 2007) and, furthermore, limited information is available on reproductive performance of pregnant gilts and sows following PRRS MLV vaccination. Therefore, the objective of the present study was to monitor the PRRSV status (antibody and viremia) of a subset of gilts and sows and the herd-level reproductive performance over time of a PRRSV-positive breeding herd following whole-herd PRRS MLV vaccination.

2.3 Materials and methods

2.3.1 Project design

Reproductive data were collected in a commercial breeding herd prior to, during, and after a PRRSV outbreak and mass vaccination of gilts and sows with a PRRS MLV vaccine (Ingelvac® PRRS MLV, Boehringer-Ingelheim Vetmedica, Inc., St. Joseph, Missouri). The data were analyzed for the effect of mass vaccination on (1) PRRSV enzyme-linked immunosorbent assay (ELISA) response and viremia, (2) fertility parameters (farrowing rate (FR), return rate (RR), and abortion rate (AR)), and (3) litter parameters (number of total piglets born per litter (TB), number of piglets born alive per litter (BA), SB, and MM).

2.3.2 Herd management and vaccination protocols

The study was conducted in a 1,200-sow commercial breeding herd in central Thailand in which in-herd replacement gilts were produced using grandparent stock. Replacement gilts were acclimatized at 22-30 weeks of age, before entering the breeding herd and were assumed to be PRRSV positive. Gilts and sows were housed in a conventional open housing system, i.e., slatted floors and open sides, and the herd health management program was under the supervision of a herd veterinarian. Gilts and sows had never been vaccinated against PRRSV but did receive vaccines against foot-and-mouth disease (two weeks before farrowing), classical swine fever (two weeks after farrowing), Aujeszky's disease (mass vaccination every four months), and porcine parvovirus (gilts prior to placement in breeding herd, then 2 weeks after farrowing every third parity).

2.3.3 PRRSV monitoring data

Gilts and sows (n=20-30) were tested biannually using a commercial PRRS ELISA assay (HerdChek® PRRSV antibody test kit 2XR®, IDEXX Laboratories, Inc., Westbrook, Maine) for the three years prior to the PRRSV outbreak. Based on monitoring results, the herd was considered PRRSV positive, but stable. At the beginning of January 2009, reproductive failure characterized by abortions in gilts and sows mated during October to December 2008, increased RR, and increased mortality in suckling and weaned piglets were noted. In January 2009, a type 2 PRRSV was detected by reverse transcription-polymerase chain reaction (RT-PCR) in serum samples from sows and piglets submitted for testing at the Veterinary Diagnostic Laboratory, Chulalongkorn University (Bangkok, Thailand).

2.3.4 PRRSV vaccination and blood collection.

On 15 May 2009, all gilts and sows in the herd were vaccinated with a PRRS MLV vaccine at 3-week intervals, i.e., weeks 0 and 3. Thereafter, all gilts and sows (both pregnant and non-pregnant) were vaccinated every three months. Concurrently with the first PRRS vaccination, six age groups composed of six animals each were selected for PRRSV monitoring: (1) 7- to 8-month-old replacement gilts, (2) 9- to 11-month-old breeding gilts, (3) parity one sows, (4) parity 2 sows, (5) parity 3-4 sows, and (6) parity 5-6 sows. Blood samples were collected from these 36 animals one day before PRRSV vaccination and then 2, 5, 9, 12, and 18 weeks after the first vaccination. Blood samples were allowed to clot at room temperature, after which serum was harvested and either tested immediately for PRRSV antibodies or stored at -20°C for later testing. Serum samples (n=6) were pooled by age group and tested immediately by PRRSV RT-PCR.

2.3.5 PRRSV antibody and RT-PCR assay

Individual serum was tested for PRRSV antibody using a commercial assay performed according to the manufacturer's protocol. Pooled serum samples were tested for PRRSV using a commercial RT-PCR assay (AccessQuick™ RT-PCR system,

Promega Corporation, Madison, Wisconsin) capable of amplifying open reading frame 7 of either type 1 or 2 PRRSV genotypes. The reaction consisted of upstream and downstream primers (Amonsin et al., 2009), avian myelobalastosis virus reverse transcriptase (Promega Corporation), and RNA template. The reverse transcription and PCR amplification conditions were performed according to kit instructions. The amplified products and standards (GeneRuler™ 100 bp DNA Ladder, Fermentas Inc., Glen Burnie, Maryland) were electrophoresed on 1.0% agarose gel and stained with ethidium bromide. PRRSV genotypes were differentiated on the basis of the size of the products, i.e., 390 bp for type 1 and 430 bp for type 2 genotypes.

2.3.6 Reproductive performance dataset

Reproductive performance data were collected for the period from July 2007 to June 2010 from breeding productivity records (PigCHAMP®, version 4.10, Minnesota). The data dictionary was based on conventional definitions of industry terms and formulas. A mating was defined as the insemination of a gilt/sow during a 10-day estrus period and a service included one or more mating events during estrus (Takai and Koketsu, 2009). Return-to-estrus, abortion, and farrowing were defined as binomial traits (0, 1). The FR, RR, and AR were calculated as the number of females that returned to estrus or aborted or farrowed divided by the number of mated females multiplied by 100. TB was defined as the sum of BA plus the number of stillborn piglets per litter plus the number of mummified fetuses per litter. SB and MM were calculated as the number of stillborn piglets per litter or number of mummified fetuses per litter divided by TB multiplied by 100. Pregnant females were classified in terms of PRRSV vaccination status relative to the blanket vaccination that occurred on 15 May 2009: (1) 0 to 30 days of gestation at the time of blanket vaccination; (2) 31 to 60 days of gestation; (3) 61 to 90 days of gestation; and (4) vaccination at >90 days of gestation. The raw data consisted of 8,162 matings and 6,975 farrowings records from 2,543 sows. Records with missing data were removed from the dataset, leaving a total of 7,914 matings and 6,793

farrowings from 2,337 sows for the analysis. Records included sow identity, parity number at service, mating date, number of inseminations, mating result, days until the sow returned to estrus after mating, farrowing date, TB, BA, SB, and MM.

2.3.7 Statistical analyses

Statistical analyses were performed using SAS statistical software (SAS® version 9.0, SAS® Institute, Inc., Cary, North Carolina). Initially, fertility parameters (RR, AR, and FR) and litter parameters (TB, BA, SB, and MM) were analyzed for differences over time, i.e., before PRRSV infection (July 2007 to June 2008), during PRRSV field infection (July 2008 to June 2009), and after vaccination (July 2009 to June 2010), PRRSV vaccination status, parity (0, 1, 2-4, and ≥5), parity by time, and parity by vaccination status using generalized linear-mixed models. Tukey-Kramer adjustments were used for multiple comparisons. $P<0.05$ was considered statistically significant. Quantitative serum ELISA responses (S/P ratios) were evaluated by week of collection (0, 2, 5, 9, 12, and 18) using paired *t* tests. The qualitative ELISA response (positive vs. negative) was analyzed by logistic regression using generalized linear-mixed models that included the week of sample collection (0, 2, 5, 9, 12, and 18) and female classification (replacement gilt, bred gilt, and sow parity numbers 1, 2, 3-4, and 5-6).

2.4 Results

2.4.1. Serum testing results

No viremic animals were detected by PRRSV RT-PCR either before or after PRRS vaccination. Among the 36 animals monitored over time, 88.9% (32/36) were PRRS ELISA antibody positive prior to vaccination (Table 2). After mass vaccination, the percentage of sero-positive animals in this group ranged from 85.3% to a high of 94.4% for the 18 weeks over which the animals were monitored. Mean ELISA S/P ratios varied from 1.61 prior to vaccination to 1.23 at week 18 post-vaccination.

Table 2 Serum testing results by week post-vaccination

Weeks	PRRS ELISA (mean S/P ratio)	ELISA Positive	PRRSV RT-PCR
0	1.61 ± 0.19 a, b	32/36 (88.9 %) a	Negative
2	1.88 ± 0.16 a	34/36 (94.4 %) a	Negative
5	1.47 ± 0.16 b	31/36 (86.1 %) a	Negative
9	1.32 ± 0.15 b	32/36 (88.9 %) a	Negative
12	1.46 ± 0.17 b	29/34 (85.3 %) a	Negative
18	1.23 ± 0.07 b	31/33 (93.9 %) a	Negative

Different lowercase letters (a and b) within columns indicate statistically significant differences ($P<0.05$)

2.4.2 Reproductive performance

Herd fertility parameters (FR, RR, and AR) and litter parameters (TB, BA, SB, and MM) over time are summarized in Figure 2a, b and Tables 3 and 4, respectively. Before the PRRSV outbreak, FR, AR, RR, SB, and MM were 90.0%, 1.6%, 5.9%, 7.0%, and 1.6% respectively, while TB and BA were 11.4 and 10.3 piglets/litter, respectively. During the outbreak, especially November 2008 to January 2009, a high AR (16.7%) and a low FR (71.2%) were observed.

The lowest TB and BA, 9.7 and 8.3 piglets/litter, respectively, and the highest MM (8.4%) were observed in gilts and sows that farrowed in April 2009 (mated in January 2009). During the PRRSV outbreak, reproductive parameters were significantly affected compared with pre-outbreak levels, i.e., FR (83.9% vs. 90.0%, $P<0.001$), AR (5.2% vs. 1.6%, $P<0.001$), RR (8.0% vs. 5.9%, $P=0.048$), TB (10.9 vs. 11.4 piglets/litter, $P<0.001$), BA (9.9 vs. 10.3 piglets/litter, $P<0.001$), and MM (2.2% vs. 1.6%, $P=0.004$).

Following vaccination against PRRSV, the AR decreased from the outbreak period (1.4% vs. 5.2%, $P<0.001$) and returned to pre-outbreak levels (1.4% vs. 1.6%, $P>0.05$), whereas RR remained higher than before the outbreak (11.3% vs. 5.9%,

P<0.001) or during outbreak (11.3% vs. 8.0%, *P*<0.001) (Table 3). The FR did not differ from the outbreak period (83.8% vs. 83.9%, *P*>0.05), but it remained lower than before the outbreak (83.8% vs. 90.0%, *P*<0.001) (Table 3). TB and BA were lower than before outbreak (10.6 vs. 11.4 piglets/litter, *P*<0.001 and 10.0 vs. 10.3 piglets/litter, *P*=0.012, respectively) (Table 4). However, while TB was lower than during the outbreak period (10.6 vs. 10.9 piglets/litter, *P*=0.015), BA was higher (10.0 vs. 9.9 piglets/litter, *P*=0.012) (Table 4). SB and MM were both lower than before the outbreak (4.6% vs. 7.0%, *P*<0.001 and 0.7% vs. 1.6%, *P*<0.001, respectively) and during outbreak (4.6% vs. 6.1%, *P*<0.001 and 0.7% vs. 2.2%, *P*<0.001, respectively) (Table 4). Pre-weaning mortality before the outbreak, during the outbreak, and following PRRS MLV vaccination was 4.7%, 8.5%, and 4.4%, respectively. These estimates are based on pre-outbreak piglet numbers of 24,302 (BA) and 23,254 (weaned), outbreak piglet numbers of 20,999 (BA) and 19,217 (weaned), and post-vaccination numbers of 23,228 (BA) and 22,196 (weaned).

After PRRS vaccination, FR, BA, and MM varied by the stage of gestation at the time of vaccination (Tables 5 and 6). Gilts and sows vaccinated at ≥90 days of gestation had a lower FR than those vaccinated at 0-30 (77.3% vs. 88.3%, *P*=0.008), 31-60 (77.3% vs. 85.1%, *P*=0.055), and 61-90 days of gestation (77.3% vs. 84.7%, *P*=0.176) (Table 5). RR and AR were not significantly different among PRRSV vaccination status, although numeric differences were observed. Likewise, FR, RR, and AR varied by parity, but were not statistical significant (Table 5). BA was lowest (9.2 piglets/litter) and MM was highest (5.3 piglets/litter) in females vaccinated at 0-30 days of gestation (Table 6). However, TB and SB did not differ by parity or stage of gestation at the time of vaccination.

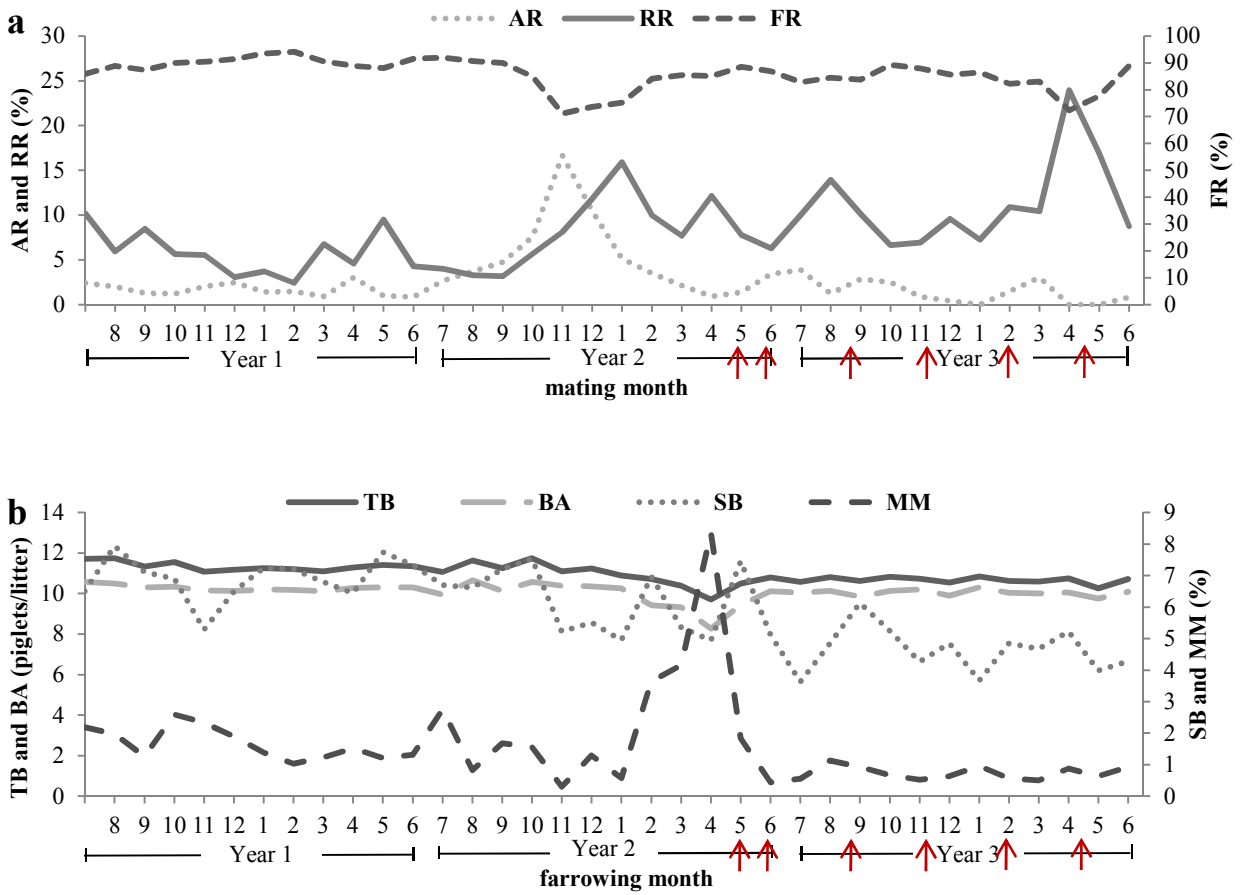

Figure 2 a Farrowing rate (FR), abortion rate (AR), and return rate (RR); **b** the number of total piglets born per litter (TB), the number of piglets born alive per litter (BA), the percentage of stillborn piglets per litter (SB), and the percentage of mummified fetuses per litter (MM); *Arrow* indicates dates of PRRS MLV vaccination

Table 3 Comparison of fertility parameters (farrowing rate (FR), abortion rate (AR), and return rate (RR)) by parity over time

Fertility parameters	Year 1 July 2007 - June 2008 (before outbreak)	Year 2 July 2008 - June 2009 (during outbreak)	Year 3 July 2009 - June 2010 (post vaccination)
Number of sows	1,332	1,253	1,452
Number of mating	2,582	2,540	2,792
FR (%)	90.0 a	83.9 b	83.8 b
Parity 0	86.6 a	87.0 a	87.2 a
Parity 1	91.2 a	84.4 a	85.8 a
Parity 2-4	91.5 a	82.1 b	84.3 b
Parity ≥5	88.0 a	84.6 a, b	78.3 b
RR (%)	5.9 a	8.0 a	11.3 b
Parity 0	7.5 a	8.4 a	10.1 a
Parity 1	5.4 a	8.6 a	10.9 a
Parity 2-4	5.4 a	8.9 b	10.0 b
Parity ≥5	6.0 a	5.5 a	14.8 b
AR (%)	1.6 a	5.2 b	1.4 a
Parity 0	1.8 a	2.8 a	1.0 a
Parity 1	1.3 a	4.4 a	1.0 a
Parity 2-4	1.4 a	5.9 b	1.7 a
Parity ≥5	2.9 a, b	6.5 b	1.6 a

Clinical signs suggestive of PRRS in late 2008, with virus detected in serum by RT-PCR in January 2009; PRRS MLV vaccination begun 15 May 2009; Different lowercase letters (a and b) across rows indicate statistically significant differences ($P<0.05$)

Table 4 Litter parameters (the number of total piglets born per litter (TB), the number of piglets born alive per litter (BA), the percentage of stillborn piglets per litter (SB), and the percentage of mummified fetuses per litter (MM), means±SEM) by parity over time

Litter parameters	Year 1 Jul 2007 - Jun 2008 (before outbreak)	Year 2 Jul 2008 - Jun 2009 (during outbreak)	Year 3 Jul 2009 - Jun 2010 (post vaccination)
Number of sows	1,233	1,120	1,365
Number of farrowing	2,362	2,116	2,315
TB (piglets/litter)	11.4 ± 0.1 a	10.9 ± 0.1 b	10.6 ± 0.1 c
Parity 1	10.3 ± 0.1 a	10.2 ± 0.1 a	10.2 ± 0.1 a
Parity 2-4	11.6 ± 0.1 a	11.0 ± 0.1 b	10.8 ± 0.1 b
Parity ≥5	11.6 ± 0.1 a	11.2 ± 0.1 a	10.7 ± 0.1 b
BA (piglets/litter)	10.3 ± 0.1 a	9.9 ± 0.1 b	10.0 ± 0.1 c
Parity 1	9.3 ± 0.1 a	9.0 ± 0.1 a	9.4 ± 0.1 a
Parity 2-4	10.6 ± 0.1 a	10.1 ± 0.1 b	10.3 ± 0.1 a, b
Parity ≥5	10.4 ± 0.1 a	10.1 ± 0.1 a	10.1 ± 0.1 a
SB (%)	7.0 ± 0.2 a	6.1 ± 0.2 b	4.6 ± 0.2 c
Parity 1	7.2 ± 0.5 a	6.9 ± 0.5 a	5.8 ± 0.4 a
Parity 2-4	6.3 ± 0.2 a	5.3 ± 0.3 a	4.1 ± 0.2 b
Parity ≥5	8.2 ± 0.4 a	6.9 ± 0.4 a	4.6 ± 0.3 b
MM (%)	1.6 ± 0.1 a	2.2 ± 0.2 b	0.7 ± 0.1 c
Parity 1	1.8 ± 0.3 a	3.7 ± 0.6 b	1.4 ± 0.3 a
Parity 2-4	1.6 ± 0.2 a	2.1 ± 0.3 a	0.6 ± 0.1 b
Parity ≥5	1.6 ± 0.2 a, b	1.8 ± 0.3 a	0.5 ± 0.1 b

Clinical signs suggestive of PRRS in late 2008, with virus detected in serum by RT-PCR in January 2009; PRRS MLV vaccination begun 15 May 2009; Different lowercase letters (a-c) across rows indicate statistically significant differences ($P<0.05$)

Table 5 Fertility parameters (farrowing rate (FR), abortion rate (AR), and return rate (RR)) by stage of gestation subsequent to blanket vaccination

Fertility parameter	Stage of gestation			
	0-30 days	31-60 days	61-90 days	>90 days
Number of animals	213	222	228	216
FR (%)	88.3 a	85.1 a, b	84.7 a, b	77.3 b
Parity 0	93.6 a	86.5 a	92.3 a	82.5 a
Parity 1	94.9 a	95.1 a	81.8 a	78.4 a
Parity 2-4	81.8 a	77.5 a	82.6 a	77.2 a
Parity ≥5	90.9 a	89.1 a	86.1 a	72.3 a
RR (%)	8.5 a	11.3 a	8.8 a	13.4 a
Parity 0	3.2 a	13.5 a	5.1 a	10.0 a
Parity 1	5.1 a	4.9 a	9.1 a	13.5 a
Parity 2-4	11.4 a	16.8 a	11.9 a	16.3 a
Parity ≥5	9.1 a	5.4 a	2.8 a	10.6 a
AR (%)	0.9 a	0.9 a	2.6 a	5.6 a
Parity 0	0.0 a	0.0 a	0.0 a	7.5 a
Parity 1	0.0 a	0.0 a	2.3 a	5.4 a
Parity 2-4	2.3 a	1.1 a	1.8 a	5.4 a
Parity ≥5	0.0 a	1.8 a	8.3 a	4.3 a

PRRS MLV vaccination on 15 May 2009; Different lowercase letters (a-c) across rows indicate statistically significant differences ($P<0.05$)

Table 6 Litter parameters (the number of total piglets born per litter (TB), the number of piglets born alive per litter (BA), the percentage of stillborn piglets per litter (SB), and the percentage of mummified fetuses per litter (MM), means±SEM) by stage of gestation subsequent to blanket vaccination

Litter parameter	Stage of gestation			
	0-30 days	31-60 days	61-90 days	>90 days
Number of farrowing	188	189	193	167
TB (piglets/litter)	10.5 ± 0.2 a	10.6 ± 0.2 a	11.0 ± 0.2 a	11.1 ± 0.2 a
Parity 1	9.8 ± 0.6 a	9.6 ± 0.6 a	10.0 ± 0.4 a	10.4 ± 0.5 a
Parity 2-4	10.1 ± 0.3 a	10.8 ± 0.3 a	11.3 ± 0.3 a	11.1 ± 0.3 a
Parity ≥5	11.2 ± 0.3 a	10.9 ± 0.4 a	11.0 ± 0.3 a	11.6 ± 0.4 a
BA (piglets/litter)	9.2 ± 0.2 a	9.4 ± 0.2 a	10.3 ± 0.2 b	10.3 ± 0.2 b
Parity 1	8.1 ± 0.6 a	8.4 ± 0.5 a	9.2 ± 0.4 a	9.5 ± 0.4 a
Parity 2-4	8.6 ± 0.3 a	9.9 ± 0.3 a, b	10.6 ± 0.2 b	10.4 ± 0.3 b
Parity ≥5	10.2 ± 0.3 a	9.1 ± 0.4 a	10.5 ± 0.3 a	10.4 ± 0.3 a
SB (%)	6.0 ± 0.6 a	6.5 ± 0.9 a	4.8 ± 0.6 a	5.6 ± 0.7 a
Parity 1	6.1 ± 1.7 a	4.8 ± 1.7 a	6.4 ± 1.4 a	7.1 ± 1.8 a
Parity 2-4	5.7 ± 1.0 a	5.2 ± 0.9 a	4.9 ± 1.0 a	3.9 ± 0.8 a
Parity ≥5	6.2 ± 0.9 a	9.0 ± 2.1 a	3.5 ± 0.9 a	7.4 ± 1.3 a
MM (%)	5.3 ± 1.3 a	4.2 ± 1.1 a, c	0.7 ± 0.3 b, c	1.6 ± 0.5 c
Parity 1	9.9 ± 4.1 a	5.3 ± 2.9 a	0.7 ± 0.5 a	1.4 ± 1.0 a
Parity 2-4	6.9 ± 2.2 a	2.7 ± 1.5 a	0.7 ± 0.4 b	1.5±0.8 a, b
Parity ≥5	1.5 ± 1.1 a	5.6 ± 2.0 a	0.7 ± 0.7 a	1.8 ± 0.9 a

PRRSV MLV vaccination on 15 May 2009; Different lowercase letters (a-c) across rows indicate statistically significant differences ($P<0.05$)

2.5 Discussion

In general, the reproductive performance of this herd was good relative to its peers in Thailand (Olanratmanee et al., 2010; Tummaruk et al., 2010). However, a decline in reproductive performance, i.e., an increase in AR and MM, was noted for several months before the use of the PRRSV vaccine. The decline in reproductive parameters was attributed to PRRSV based on the clinical experience of the herd veterinarians and the results of diagnostic testing, e.g., positive PRRSV RT-PCR testing. These data justified the decision to vaccinate the entire sow herd with PRRS MLV vaccine, regardless of individual animals' stage in the reproductive cycle. In hindsight, taking this course of action six months earlier (at the peak of abortions) might have foreshortened overall reproductive losses (Figure 2a).

In agreement with previous reports, vaccination produced a measureable response both in terms of an increased proportion of sero-positive animals and an increase in mean PRRSV ELISA S/P values (Murtaugh et al., 2002; Scortti et al., 2006b). Although the antibody ELISA does not measure neutralizing antibodies (Yoon et al., 1995; Foss et al., 2002), none of the monitored animals were viremic during the 2 to 18 week observation period post-vaccination. Vaccination against PRRSV in non-pregnant pigs has been shown to produce no negative reproductive consequences and improve some measures of reproductive performance, e.g., FR, BA, SB, and MM (Dewey et al., 2004; Alexopoulos et al., 2005). Furthermore, vaccination against PRRSV has been shown to provide protection against reproductive losses. Scortti et al. (2006b) reported that inoculation of non-vaccinated, sero-negative gilts with PRRSV at 90 days of pregnancy resulted in 43.4% stillborn piglets, 20.0% weak-born piglets, and 76.7% pre-weaning mortality. In contrast, vaccinated gilts challenged with PRRSV at 90 days of pregnancy farrowed 5.2% stillborn and reproductive performance otherwise indistinguishable from the negative control group (Scortti et al., 2006b). Overall, Scortti et al. (2006a) concluded that PRRS MLV vaccination did not cause clinical signs or affect reproductive performance of pregnant gilts. However, PRRS vaccination in pregnant pigs, especially during late gestation, has also been shown to have negative

consequences in terms of the number of BA, SB, MM, pigs weaned per litter, and an increase of the mortality rate in nursery pigs (Nielsen et al., 2002; Dewey et al., 2004).

Based on the data analyzed in this study, PRRS whole-herd vaccination had neutral, positive, and negative effects on reproductive performance. In particular, the stage of gestation at the time of vaccination affected the reproductive outcome. A lower FR was noted in gilts and sows vaccinated at >90 days of gestation; whereas, a lower BA and a higher proportion of MM was observed in animals vaccinated at 0-30 days of gestation. At the herd level, whole-herd vaccination reduced AR and SB and MM, but did not improve the FR over that observed during the outbreak period and was associated with an increased RR and a lower TB and BA.

A review of the literature showed that these data are compatible with previous reports that PRRS vaccination in PRRSV-infected herds reduced the duration of PRRSV shedding (Cano et al., 2007a, b) and improved some reproductive performance parameters, e.g., FR, BA, SB, and MM (Alexopoulos et al., 2005). Thus, it may be concluded that the decision to implement whole-herd vaccination using a PRRS MLV vaccine should be balanced between the benefits derived from reproductive performance improvements, e.g., fewer abortions, stillborn piglets, and mummified fetuses and the effect of vaccination on pregnant females.

CHAPTER III

Reproductive performances of gilts and sows with and without porcine reproductive and respiratory syndrome virus (PRRSV) vaccination in PRRSV sero-positive herds

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Reproductive performances of gilts and sows with and without porcine reproductive and respiratory syndrome virus (PRRSV) vaccination in PRRSV sero-positive herds

3.1 Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) infection causes reproductive failures including return to estrus, abortion, mummified fetuses, stillborn piglets, and weak-born piglets in gilts and sows. The objective of the present study was to investigate reproductive performances of gilts and sows in PRRSV sero-positive herds with and without vaccination against PRRSV. The study was conducted in 20 PRRSV sero-positive swine commercial herds in Thailand. PRRS modified-live virus (MLV) vaccine was used in 27,042 sows and not being used in 45,816 sows. Data were collected from the herd database for three-year period. In total, the data set contained records on 211,009 matings and 180,935 farrowings. Fertility traits including farrowing rate (FR), return rate (RR), and abortion rate (AR) in non-PRRS-MLV-vaccinated and vaccinated sows were 85.0% and 89.7% (P<0.001), 6.9% and 3.7% (P<0.001), and 1.6% and 2.0% (P=0.964), respectively. Number of total piglets born per litter (TB), number of piglets born alive per litter (BA), percentage of stillborn piglets per litter (SB), percentage of mummified fetuses per litter (MM), and number of piglets weaned per litter (WP) differed significantly between non-vaccinated and vaccinated sows (11.2 and 11.5 TB (P<0.001), 10.0 and 10.6 BA (P<0.001), 6.9% and 5.1% SB (P<0.001), 3.2% and 2.2% MM (P<0.001), and 9.2 and 9.6 WP (P<0.001), respectively). It could be concluded that PRRS MLV vaccination improved some reproductive performances of sows in PRRSV sero-positive herds. However, others management strategies including replacement gilts management, should be aware to minimize the reproductive losses from PRRSV infection.

Keywords: modified-live virus vaccine, reproductive performance, PRRSV, vaccination

3.2 Introduction

Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV), is one of the most important diseases in pig producing industry throughout the world. PRRSV is an enveloped RNA virus that is divided into two genotypes including genotype 1(European genotype) and 2 (North American genotype) (Meng, 2000). The virus persists in many visceral organs (i.e., lung, liver, spleen, tonsil, lymph node, and uterus) of the infected pigs for several months (Chapter IV; Benfield et al., 2000; Laohasittikul et al., 2004). The results from Chapter II and others previous studies found that PRRSV infection causes reproductive failures in gilts and sows i.e., an increase in the proportion irregular return to estrus and abortion and an increase in the number of mummified fetuses, stillborn piglets, weak-born piglets per litter, and also increase number of sow mortality annually (Done et al., 1996; Mengeling et al., 1996; Chung et al., 1997; Goldberg et al., 2000b; Zimmerman et al., 2006). The major economic losses in the PRRSV affected herds are mainly due to a decrease in the number of piglets weaned per sow per year and long farrowing intervals and an increase in the replacement rate (Brouwer et al., 1994). Several management strategies including intensive biosecurity, acclimatization of replacement gilts before being sent into the breeding herd, serological profiling to monitor the herd health status, all-in-all-out pigs flow system, segregated early weaning, and vaccination with inactivated and/or modified-live virus (MLV) vaccines have been used to control PRRS (Cho and Dee, 2006; Zimmerman et al., 2006; Thanawongnuwech and Suradhat, 2010). However, in practice, a high variation on gilts and sows reproductive performances among herds where a PRRSV vaccine has been implemented is still commonly found (Botner et al., 1997; Alexopoulos et al., 2005; Martelli et al., 2007; Martelli et al., 2009). To fulfill knowledge concerning the efficacy of PRRS MLV vaccination in herds with and without PRRSV vaccination, additional longitudinal study focusing on the important reproductive performances of gilts and sows, which can be directly linked to economic traits in the pig industry in Thailand, is needed to be performed.

It is well documented that Thailand is an endemic area of PRRSV (Oraveerakul et al., 1995; Thanawongnuwech et al., 2004; Amonsin et al., 2009; Tummaruk et al., 2013). PRRSV infection in Thailand has been reported for the first time in 1995 (Oraveerakul et al., 1995), while the antibody titer against PRRSV has been detected as early as 1989 (Damrongwatanapokin et al., 1996). Moreover, both genotype 1 and 2 have been clearly identified (Thanawongnuwech et al., 2004; Amonsin et al., 2009). Acute PRRSV-infected sows are characterized by reproductive failures and viral shedding (Chapter II; Thanawongnuwech and Suradhat, 2010). The common clinical signs of the acute PRRSV-infected sows included high abortion rate (AR), high returning to estrus after mating rate (return rate, RR), high percentage of stillborn piglets per litter (SB), high percentage of mummified fetuses per litter (MM), and high pre-weaning mortality rate (Chapter II; Mengeling et al., 2000). Moreover, the decrease in the number of total piglets born per litter (TB) and number of piglets born alive per litter (BA) are also observed (Chapter II). The objective of the present study was to investigate reproductive performances in gilts and sows in PRRSV sero-positive herds with and without vaccination against PRRSV.

3.3 Materials and methods

3.3.1 Project design

Reproductive data recorded in 20 commercial swine breeding herds in Thailand were collected and were analyzed. A retrospective cohort study was conducted in each herd for three-year period. Reproductive performance data analyzed included both fertility traits (i.e., RR, AR, and farrowing rate (FR)) and litter traits (i.e., TB, BA, SB, MM, and number of piglets weaned per litter (WP)). Reproductive performances were compared between PRRS-MLV-vaccinated and non-vaccinated sows. All available management factors including herds, sow's parity number, month, and interactions were also taken into accounted. Multiple ANOVA and least-squares means procedure were used to estimate the reproductive data.

3.3.2 Animals, herd location, and general management

The present study was conducted in 20 swine commercial herds in Thailand. The herds were located in the middle (herds A, C, F, G, and I), eastern (herd D), western (herds J to T), and northeastern (herds B, E, and H) parts of Thailand between latitude 13° and 17°N and longitude 99° and 103°E. The numbers of sow-on-production in each herd varied from 900 to 3,500 sows. The majority of the females were crossbred Landrace x Yorkshire (LY) and some purebred Landrace (L) and Yorkshire (Y) breeds. Gilts and sows were kept in conventional open house system facilitated with a water sprinklers and fans. The gilts and sows were kept in individual stalls during gestation and in farrowing pens during lactation. The gilts and sows received water ad libitum via water nipples. The feed, which was a rice-corn-soybean-fish base, was provided twice a day (1.5-3.5 kg/day during gestation and 5.0-7.0 kg/day during lactation). Generally, the feed for gilts and sows contained 16.0-18.0% crude protein, 3,000-3,250 kcal/kg metabolisable energy, and 0.8-1.1% lysine. The health of the animals within the herds was monitored by herd veterinarians. The recommended vaccination program for gilts and sows in all herds included foot-and-mouth disease virus (FMDV), classical swine fever virus (CSFV), Aujeszky's disease virus (ADV), and porcine parvovirus (PPV). In general, the gilts were vaccinated against all of these diseases between 22 and 30 weeks of age. The sows were vaccinated against FMDV during the late gestation and CSFV and PPV were vaccinated during lactation. In most herds, whole-herd vaccination against ADV was conducted every 4 months. Six herds (herds A, B, D, E, H, and I) produced replacement within the herds using their own grandparent stock and 14 herds (herds C, F, G, and J to T) bought replacement gilts from other breeders. In general, the gilts were mated at 32 weeks of age onwards with a body weight at least 135 kg at the second or later observed estrus. In general, the target of replacement rate of sows by gilts was 40% annually and the sows were planned to be culled after parity six. Conventional artificial insemination was performed in all herds.

3.3.3 Status of PRRSV infection and vaccination

The herds included in the present study were sero-positive to PRRSV for over five years (based on routinely monitored seroprofiles from commercial PRRS enzyme-linked immunosorbent assay tests). PRRS MLV vaccine was used in herd A, B, and C during the three-year period of the analyses. The vaccination program as well as vaccination producer differed among herds. In herd A, whole-herd vaccination of gilts and sows with Ingelvac® PRRS MLV (Boehringer-Ingelheim Vetmedica Inc., St. Joseph, MO, USA) was performed every three months. In herd B, PRRS MLV vaccination with AMERVAC® PRRS MLV (Laboratorios Hipra, Girona, Spain) was performed twice in replacement gilts during 22-30 weeks of age. In herd C, both types of the PRRS MLV vaccine was used as a whole-herd vaccination program every three months.

3.3.4 Data

The reproductive performances dataset were obtained from the computer recording system of the herds (Table 7). Data of 212,421 mating and 181,163 farrowing records from 72,858 sows were obtained from the database (Table 7). The collected data included sow's identity, parity number, mating date, number of insemination, mating result, mating to return to estrus interval, farrowing date, BA, number of stillborn piglets per litter, number of mummified fetuses per litter, weaning date, WP, and weaning-to-service interval. The data were scrutinized for accuracy and completeness. Records with missing data were removed from the data analyzed. The complete data analyzed were based on a total of 211,009 mating and 180,935 farrowing records from 72,386 sows (Table 7).

3.3.5 Definition

The TB was defined as the sum of BA, the number of stillborn piglets per litter, and the number of mummified fetuses per litter. The SB was defined as the number of stillborn piglets per litter divided by TB multiplied by 100. The MM was defined as the number of mummified fetuses per litter divided by TB multiplied by 100. Farrowing

female, return to estrus after mating, and abortion were binomial traits (0, 1). Farrowing was defined as '0' when the sows were mated resulting in abortion, culling, not being pregnant or repeat mating, and were defined as '1' when the mating resulted in farrowing. The mated female not return to estrus after mating was given a null value of '0'. A value of '1' was assigned if the gilts/sows were mated and with either a regular or irregular return to estrus. Abortion was defined as '0' when the sows were mated and no evidence of abortion occur within 110 days after mating, and defined as '1' when the sows were mated resulting in abortion. The FR, RR, and AR were calculated using frequency analysis.

3.3.6 Statistical analyses

The statistical analyses were carried out by Statistical Analysis System version 9.0 (SAS, 2002). Analysis of variance (general linear model, GLM) was used to analyze continuous variables, i.e., TB, BA, SB, MM, and WP. The statistical models included the effect of PRRS MLV vaccination (vaccinated and non-vaccinated sows), parity number, farrowing month, herd nested within vaccination, and interaction between vaccination and parity number, and farrowing month and parity number. Least-squares means were obtained and were compared using the Tukey-Kramer adjustment for multiple comparisons. Discrete data including FR, RR, and AR were analyzed using generalized linear-mixed models (GLIMMIX). The statistical models included the effect of PRRS MLV vaccination (vaccinated and non-vaccinated sows), parity number, mating month, herd nested within vaccination, and interaction between vaccination and parity number, and mating month and parity number. In the statistical models, parity number $\geq$6 were pooled. Five statistical models were constructed for TB, BA, SB, MM, and WP using GLM, and three statistical models were constructed for FR, RR, and AR using GLIMMIX. Least-squares means were obtained and were compared using the Tukey-Kramer adjustment for multiple comparisons. $P<0.05$ were considered to have statistical significance.

Table 7 Structure of the analyzed data

Data collection	Databank	Edited data	FR, RR, AR	Databank	Edited data	TB, BA, SB, MM	WP
Period of analysis	3 years	3 years	3 years	3 years	3 years	3 years	3 years
No. of observations	212,421	211,009	211,009	181,163	180,935	180,935	175,836
Non-vaccinated sows	141,365	140,880	140,880	123,819	123,668	123,668	120,831
MLV vaccinated sows	71,056	70,129	70,129	57,344	57,267	57,267	55,005
No. of sows	72,858	72,386	72,386	64,782	64,719	64,719	63,003
Non-vaccinated sows	45,816	45,750	45,750	41,813	41,778	41,778	40,846
MLV vaccinated sows	27,042	26,636	26,636	22,969	22,941	22,941	22,157
Mean parity number	2.4	2.1	2.1	3.4	3.0	3.0	3.0
Non-vaccinated sows	2.2	2.0	2.0	3.3	2.9	2.9	2.9
MLV vaccinated sows	2.7	2.4	2.4	3.6	3.2	3.2	3.2
Statistical method	-	Descriptive	GLIMMIX	-	Descriptive	GLM	GLM
Criteria of exclusion[1]	-	1, 2	1,2	-	1,3	1,3	1,3,4

[1] Criteria of exclusion: 1, parity >10; 2, no record of mating result; 3, TB=0 or >25; 4, no record of WP

3.4 Results

3.4.1 Descriptive statistics

The average of FR, RR, AR, TB, BA, SB, MM, and WP of all herds included in the present study were 86.6%, 5.8%, 1.7%, 11.3 piglets/litter, 10.2 piglets/litter, 6.3%, 2.9%, and 9.3 piglets/litter, respectively. The effect of various factors on reproductive performances was analyzed and is presented in Table 8. On average, the number of insemination of gilts and sows monthly was 5,861.4±345.9 matings (range 5,234-6,182) and the number of farrowing monthly was 5,026.0±311.7 farrowings (range 4,621-5,552). The mean gestational period in PRRS-MLV-vaccinated and non-vaccinated sows were 115.5±1.6 days (range 109-120 days) and 115.2±1.8 days (range 109-120 days), respectively (P<0.001).

3.4.2 Farrowing rate, return rate, and abortion rate

The fertility traits of PRRS-MLV-vaccinated and non-vaccinated gilts and sows are presented in Table 9. FR of PRRS-MLV-vaccinated females was higher than that of non-vaccinated females (89.7% vs. 85.0%, P<0.001). RR of PRRS-MLV-vaccinated females was lower than that of non-vaccinated females (3.7% vs. 6.9%, P<0.001). However, AR between PRRS-MLV-vaccinated and non-vaccinated females were not difference (2.0% vs. 1.6%, P=0.965).

FR, RR, and AR were affected by the parity number sows (Table 10). FR were 83.2%, 85.3%, 88.7%, 88.2%, and 87.2% in gilts and sows parity numbers 1, 2-3, 4-5, and ≥6, respectively (P<0.001). FR of gilts and sows in each parity number of PRRS-MLV-vaccinated females were significantly higher than that of non-vaccinated females (P<0.001) (Table 10). RR were 8.7%, 6.8%, 4.9%, 4.3%, and 3.0% in gilts and sows parity numbers 1, 2-3, 4-5, and ≥6, respectively (P<0.001). RR of gilts and sows in each parity number of PRRS-MLV-vaccinated females were significantly lower than that of non-vaccinated females (P<0.001) (Table 10). AR were 2.0%, 1.6%, 1.4%, 1.9%, and 1.9% in gilts and sows parity numbers 1, 2-3, 4-5, and ≥6, respectively (P<0.001). However, AR of gilts and sows in each parity number were not significantly different between PRRS-MLV-vaccinated and non-vaccinated females (P>0.05) (Table 10).

Table 8 Levels of significance for factors included in the models

Factors	Dependent variables							
	FR	RR	AR	TB	BA	SB	MM	WP
Individual								
Vaccination[1]	<0.001	<0.001	0.965	<0.001	<0.001	<0.001	<0.001	<0.001
Parity	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Month[2]	1.000	1.000	0.985	<0.001	<0.001	<0.001	<0.001	<0.001
Herd[3]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Interactions								
Vaccination*parity	<0.001	<0.001	<0.001	0.001	<0.001	0.047	0.111	<0.001
Month*parity	<0.001	<0.001	0.004	<0.001	<0.001	0.230	<0.001	<0.001

[1] Status of PRRS MLV vaccination; [2] Month = month of mating for FR, RR, and AR and month of farrowing for TB, BA, SB, MM, and WP; [3] Herd = herd nested within PRRS MLV vaccination status

Table 9 Reproductive performance of gilts and sows by vaccination status

Parameters	Non-vaccinated sow			PRRS MLV vaccinated sow		
	n	Mean ± SEM	Range	n	Mean ± SEM	Range
FR (%)	140,880	85.0 a	-	70,129	89.7 b	-
RR (%)	140,880	6.9 a	-	70,129	3.7 b	-
AR (%)	140,880	1.6 a	-	70,129	2.0 a	-
TB	123,668	11.2 ± 0.01 a	1 - 25	57,267	11.5 ± 0.01 b	1 - 25
BA	123,668	10.0 ± 0.01 a	0 - 23	57,267	10.6 ± 0.01 b	0 - 24
SB	123,668	6.9 ± 0.03 a	0 - 100	57,267	5.1 ± 0.04 b	0 - 100
MM	123,668	3.2 ± 0.03 a	0 - 100	57,267	2.2 ± 0.03 b	0 - 100
WP	120,831	9.2 ± 0.01 a	0 - 25	55,005	9.6 ± 0.01 b	0 - 32

Different lowercase letters (a and b) across rows indicate statistically significant differences ($P<0.05$)

Table 10 Fertility parameters of gilts and sows by parity

Parameters	Parity				
	0	1	2-3	4-5	≥6
FR (%)	83.2 a	85.3 b	88.7 c	88.2 d	87.2 b
Non-vaccinated sow	81.3 a, A	83.2 b, A	87.4 c, A	87.3 cd, A	87.0 d, A
Vaccinated sow	88.6 ad, B	89.9 ac, B	91.2 b, B	89.8 c, B	87.4 d, B
RR (%)	8.7 a	6.8 b	4.9 c	4.3 c	3.0 d
Non-vaccinated sow	10.4 a, A	7.8 b, A	5.7 c, A	4.8 d, A	3.0 e, A
Vaccinated sow	4.2 a, B	4.6 a, B	3.4 b, B	3.3 bc, B	3.0 c, B
AR (%)	2.0 a	1.6 b	1.4 b	1.9 a	1.9 a
Non-vaccinated sow	1.7 ab, A	1.5 a, A	1.4 a, A	1.8 bc, A	1.8 c, A
Vaccinated sow	2.8 a, A	1.8 bc, A	1.5 b, A	2.1 ac, A	2.1 ac, A

Different lowercase letters (a-e) across rows and different uppercase letters (A and B) within columns (each parameter) indicate statistically significant differences ($P<0.05$)

Table 11 Litter parameters of sows by parity

Parameters	Parity			
	1	2-3	4-5	≥6
TB	10.7 ± 0.01 a	11.4 ± 0.01 b	11.7 ± 0.01 c	11.2 ± 0.02 d
Non-vaccinated sow	10.5 ± 0.02 a,A	11.3 ± 0.01 b,A	11.6 ± 0.02 c,A	11.3 ± 0.02 d,A
Vaccinated sow	11.1 ± 0.03 a,B	11.6 ± 0.02 b,B	11.9 ± 0.02 c,B	11.1 ± 0.03 d,B
BA	9.6 ± 0.01 a	10.4 ± 0.01 b	10.6 ± 0.01 c	10.0 ± 0.02 d
Non-vaccinated sow	9.4 ± 0.02 a,A	10.2 ± 0.01 b,A	10.4 ± 0.02 c,A	10.0 ± 0.02 d,A
Vaccinated sow	10.2 ± 0.03 a,B	10.8 ± 0.02 b,B	11.0 ± 0.02 c,B	10.1 ± 0.03 d,B
SB	6.4 ± 0.06 a	5.5 ± 0.04 b	6.4 ± 0.05 a	7.8 ± 0.07 c
Non-vaccinated sow	7.0 ± 0.08 a,A	6.1 ± 0.05 b,A	7.0 ± 0.06 a,A	8.5 ± 0.09 c,A
Vaccinated sow	5.0 ± 0.10 a,B	4.2 ± 0.06 b,B	5.2 ± 0.07 a,B	6.6 ± 0.11 c,B
MM	3.8 ± 0.06 a	2.6 ± 0.04 b	2.7 ± 0.04 bc	2.5 ± 0.05 c
Non-vaccinated sow	4.1 ± 0.08 a,A	2.9 ± 0.05 b,A	3.0 ± 0.06 bc,A	2.7 ± 0.07 c,A
Vaccinated sow	3.0 ± 0.09 a,B	2.0 ± 0.04 b,B	2.1 ± 0.05 bc,B	2.1 ± 0.06 c,B
WP	8.7 ± 0.01 a	9.5 ± 0.01 b	9.5 ± 0.01 c	9.3 ± 0.01 d
Non-vaccinated sow	8.6 ± 0.02 a,A	9.3 ± 0.01 b,A	9.4 ± 0.01 b,A	9.3 ± 0.02 c,A
Vaccinated sow	9.2 ± 0.02 a,B	9.8 ± 0.02 b,B	9.6 ± 0.02 c,B	9.4 ± 0.02 d,B

Different lowercase letters (a-d) across rows and different uppercase letters (A and B) within columns (each parameter) indicate statistically significant differences ($P<0.05$)

3.4.3 Litter size

The litter traits of PRRS-MLV-vaccinated and non-vaccinated sows are presented in Table 9. TB, BA, SB, MM, and WP in PRRS-MLV-vaccinated and non-vaccinated sows were 11.5 and 11.2 piglet/litter ($P<0.001$), 10.6 and 10.0 piglet/litter ($P<0.001$), 5.1% and 6.9% ($P<0.001$), 2.2% and 3.2% ($P<0.001$), and 9.6 and 9.2 piglet/litter ($P<0.001$), respectively.

Litter traits included TB, BA, SB, MM, and WP were also affected by the parity of sows (Table 11). TB were 10.7, 11.4, 11.7, and 11.2 piglets/litter ($P<0.001$), BA were 9.6, 10.4, 10.6, and 10.0 piglets/litter ($P<0.001$), SB were 6.4%, 5.5%, 6.4%, and 7.8% ($P<0.001$), MM were 3.8%, 2.6%, 2.7%, and 2.5% ($P<0.001$), and WP were 8.7, 9.5, 9.5, and 9.3 piglets/litter ($P<0.001$) in sows parity 1, 2-3, 4-5, and ≥ 6, respectively. All of the litter traits in each parity number were significantly different between non-vaccinated and PRRS-MLV-vaccinated sows ($P<0.001$) (Table 11).

3.5 Discussion

It is well established that PRRSV causes reproductive disorders, e.g., high AR, RR, MM, SB, and pre-weaning mortality rate (Done et al., 1996; Mengeling et al., 1996; Chung et al., 1997; Goldberg et al., 2000b; Zimmerman et al., 2006; Chapter II). Therefore, the PRRSV infected sows might have poor reproductive performances, although the reproductive disorders were not visible. Several methods, included PRRS MLV vaccination, were carried out to improve the reproductive performances of gilts and sows in the PRRSV infected herds. The present study demonstrated the reproductive performances of gilts and sows in the selected PRRSV sero-positive, both PRRS-MLV-vaccinated and non-vaccinated, commercial breeding herds in Thailand. In the present study, the reproductive performances of PRRSV sero-positive both of non-PRRS-MLV-vaccinated and vaccinated sows in Thailand were in an acceptable levels and comparable to reproductive performance of gilts and sows in Thailand reported earlier (Tummaruk et al., 2007; Olanratmanee et al., 2010). This implied that several types of management strategies, either vaccination or non-vaccination, to

control PRRSV infection in the Thai swine commercial herds could be effective. It was found that the reproductive performances, i.e., FR, RR, TB, BA, SB, MM, and WP, of PRRS-MLV-vaccinated sows were better than those of non-vaccinated sows, although the AR was not significantly different. These results might due to PRRS MLV vaccine can reduce viral shedding and partially prevent transplacental transmission, subsequent to a reduction of reproductive losses caused by wild type of PRRSV (Scortti et al., 2006a, b; Kimman et al., 2009). Although many studies on the effect of PRRS MLV vaccination on reproductive performances of gilts and sows has been done (Dewey et al., 1999; Dewey et al., 2004; Alexopoulos et al., 2005; Scortti et al., 2006a, b), but results are still controversial since negative, neutral, and positive effects were found. From the previous studies, PRRS MLV vaccination in pregnant sows, especially in the late stage of gestation, resulted in a decrease of BA and WP and an increase of SB and MM (Dewey et al., 1999; Dewey et al., 2004). However, the study of Scortti et al. (2006a) found that vaccination with PRRS MLV vaccine in pregnant female pigs was not influence the reproductive performances of vaccinated pigs. On the other hand, PRRS MLV vaccination in non-pregnant pigs showed positive effect on reproductive performances post-vaccination since the vaccination helped to prevent the reproductive failures from PRRSV infection in vaccinated pigs (Scortti et al., 2006b) and improved some reproductive traits, i.e., FR, RR, BA, SB, MM, and WP (Alexopoulos et al., 2005). In our previous study (Chapter II), we found that whole-herd PRRS MLV vaccination following a PRRSV outbreak improve some reproductive performances at herd level, i.e., AR, SB, and MM. However, TB and BA were decreased and FR was not affected. Moreover, we found the effects of vaccination on reproductive outcome of pregnant sows that were vaccinated at different stage of gestation. Sows that were vaccinated at early stage of gestation had lowest BA and highest MM, while those that were vaccinated at last stage had lowest FR (Chapter II).

The reproductive performances in PRRSV sero-positive herds were affected by the parity of gilts and sows. The poorest reproductive performances including FR, RR, AR, TB, BA, MM, and WP, were observed in gilts and primiparous sows. In general, gilts

and primiparous sows had lower reproductive performances, i.e., FR, RR, TB, and BA, than multiparous sows (Tantasuparuk et al., 2000; Tummaruk et al., 2000). In addition, under field condition, the replacement gilts should be immunized against PRRSV by exposed to field isolate of PRRSV or vaccination with PRRS MLV vaccine before being sent to the breeding herds. If the replacement gilts were not well immunized, such as the gilts were not exposed to the virus or the virus was heterologous to the virus persisted in the breeding herds, or the gilts still shed the virus (not cooled down) when they were mated, the PRRSV infection and transplacental transmission might occur which resulted in low reproductive performances. Therefore, the replacement gilts management should be carefully implement to control the PRRSV infection in the herds.

It could be concluded that PRRS MLV vaccination improved some reproductive performances in PRRSV sero-positive herds. However, others management strategies, especially replacement gilts management, should be performed to reduce the reproductive losses from PRRSV infection.

CHAPTER IV

PREVALENCE OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDORME VIRUS (PRRSV) ANTIGEN-POSITIVE UTERINE TISSUES IN GILTS CULLED DUE TO REPRODUCITVE DISTURBANCE IN THAILAND

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Prevalence of porcine reproductive and respiratory syndrome virus (PRRSV) antigen-positive uterine tissues in gilts culled due to reproductive disturbance in Thailand

4.1 Abstract

The objective of the present study was to determine the prevalence of porcine reproductive and respiratory syndrome virus (PRRSV) antigen-positive uterine tissue in gilts culled due to reproductive disturbance in relation to age at culling, reasons for culling, herds, and PRRSV vaccination. Uterine tissues of 100 gilts from six swine herds in Thailand were collected. The immunohistochemistry was performed to detect the PRRSV antigen using a polymer-based non-avidin-biotin technique. PRRSV was detected in the cytoplasm of the macrophages in the subepithelial connective tissue layers of the endometrium in 33.0% of the culled gilts. The detection of PRRSV antigen varied among the herds from 14.3% to 80.0% (P=0.018). The detection of PRRSV in the uterine tissues at different ages was not statistically different (29.6%, 39.4%, and 40.9% in gilts culled at 6-8, 9-10, and 11-16 months of age, respectively, P=0.698), similar to the reasons for culling (P=0.929). PRRSV antigen was found in 24.5% of the gilts vaccinated against the genotype 1 modified-live PRRSV vaccine and in 23.1% of the gilts vaccinated against the genotype 2 modified-live PRRSV (P=0.941). The level of antibody titers against PRRSV had no impact on PRRSV antigen detection in the uterine tissues. Similarly, the detection of PRRSV antigen did not differ between the virgin gilts (35.4%) and the gilts mated before culling (30.8%) (P=0.622). It can be concluded that PRRSV remains in the uterine tissue of the infected gilts for several months even though vaccinations and acclimatization have been carried out.

Keywords: Pig, PRRSV detection, Reproductive failure, Uterus, Immunohistochemistry

4.2 Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by the PRRS virus (PRRSV), a member of Arterivirus, family Arteriviridae (Amonsin et al., 2009). The disease was discovered in the USA in 1987 (Keffaber, 1989). PRRSV was first identified in Lelystad, the Netherlands, in 1990 (Wensvoort et al., 1991). In 1992, PRRSV was classified by genetic, antigenic, and pathogenic differences into two genotypes, i.e., genotype 1 (European) and 2 (North American) (Meng, 2000). In Thailand, PRRSV infection in swine herds has been reported since 1995 and has become one of the most common diseases causing reproductive failure in gilts and sows (Oraveerakul et al., 1995). A retrospective study based on serological testing indicates that the antibody against PRRSV is detected for the first time in Thailand in early 1989 (Damrongwatanapokin et al., 1996). Both genotype 1 and 2 have been reported in Thailand (Thanawongnuwech et al., 2004). Presently, PRRSV has been found in most major pig-producing areas throughout the world (Benfield et al., 1999; Carlsson et al., 2009). The infection of PRRSV in gilts and sows is characterized by late-term abortion, mummified fetuses, stillborn piglets, and low-viability piglets at birth (Mengeling et al., 1996; Chung et al., 1997).

Under field conditions, the mode of transmission of PRRSV consists of direct contact, needle share for vaccination/medical injection, insects, and artificial insemination (Cho and Dee, 2006; Pringprao et al., 2006). The control and prevention of PRRSV in swine commercial herds include intensive acclimatization, management of replacement gilts, monitoring the prevalence of infection by serological profiling, and vaccination with PRRS modified-live virus (MLV) vaccine and/or killed vaccines (Cho and Dee, 2006). The vaccination of gilts and pregnant sows against PRRSV has been practiced in Thailand for over a decade. However, no comprehensive study has been carried out on whether the use of PRRS vaccination and/or different types of management programs is able to effectively control the transmission of the virus from the infected animals to the sero-negative pregnant gilts/sows.

It has been suggested that the replacement gilts are a major source of

introducing new strains of PRRSV into the herd. In practice, an intensive acclimatization of the replacement gilts with culled sows or infected nursery pigs is commonly practiced in most swine-breeding herds in Thailand. However, a high variability of the antibody titer against PRRSV of the gilts is observed both within and among herds (Tummaruk and Tantilertcharoen, 2007). This problem causes difficulties for the farmer to mate the gilts. In our previous study, we have found that 73.0% (122/166) of the replacement gilts in Thailand culled due to reproductive disturbances were infected with PRRSV. A high proportion of PRRSV sero-positive gilts were found in the gilts culled due to abortion (81.0%) and repeat breeding (81.0%) (Tummaruk and Tantilertcharoen, 2008).

In general, PRRSV primarily infects pulmonary alveolar macrophages during acute infection (Sur et al., 1997). It is well-established that the alveolar macrophages as well as macrophages from other tissues are the primary cell type that sustains the in vivo replication of the virus (Thanawongnuwech et al., 2000). Using immunohistochemical (IHC) evaluation of formalin-fixed tissues, we found that 100.0% and 66.0% of the lung tissue of piglets infected with either genotype 1 and 2 of Thai PRRSV, respectively, were observed (Laohasittikul et al., 2004). An earlier study based on PRRSV antigen detection by the IHC technique has demonstrated that 75.0%, 50.0%, 37.5%, 37.5%, 37.5%, and 25.0% of PRRSV was found in liver, spleen, tonsil, turbinate bone, pulmonary lymph node, and ileum, respectively, of the experimentally infected piglets (Laohasittikul et al., 2004). In addition, PRRSV antigen is found in microglia-like cells and mononuclear cells in the brain sections by IHC associated with neurovascular lesions (Thanawongnuwech et al., 1997a). Using *in situ* hybridization (ISH), it was found that PRRSV is also detected in the epithelial germ cells of the seminiferous tubules, primarily spermatids and spermatocytes, and macrophages of the testis (Sur et al., 1997; Shin and Molitor, 2002). However, to our knowledge, the presence of PRRSV in the uterine tissues of the gilts has not been demonstrated. Thus, the objective of this study is to determine the prevalence of PRRSV antigen in the uterine tissues of the gilts culled due to reproductive disturbances associated with age at culling, culling reason, herds, and PRRSV vaccination in selected swine commercial herds in Thailand.

4.3 Materials and Methods

4.3.1 Animals and samples

One hundred uterine tissues were obtained from gilts culled due to reproductive disturbance from six swine herds (A, B, C, D, E, and F) in Thailand. Blood samples were collected from the jugular vein prior to culling. After the swine were slaughtered, the ovary and uterus were collected, placed on ice, and transported to the laboratory within 24 h. Tissue samples were collected from the uterus of the gilts, fixed in 10% neutral buffered formalin, and embedded in paraffin blocks. Historical data for all culled gilts were also recorded, including the herd and gilt identity and breed. Also, the date of birth, entry into the herd, first observed estrus, insemination, and culling, as well as body weight at culling and reason for culling, were recorded. Ages at entry, at first observed estrus, at first insemination, and at culling were calculated. The average daily gain (ADG) from birth to culling was calculated: ADG (g/day) = (body weight at culling - 1.5 / age at culling) × 1,000. Non-productive days (NPD) of the culled gilts were defined as the interval from entry into the herd to culling.

4.3.2 General management and vaccination

The herds in the present study are breeding herds located in the northeastern (A and B), middle (C), western (E), and eastern (D and F) parts of Thailand. The sows-on-production numbers were 900-3,500 sows per herd. Herds A and B produced replacement gilts within the herd using their own grandparent stock, while herds C, D, E, and F bought the replacement gilts from other breeders. The gilts in all herds were housed in a conventional open-housing system facilitated with a water sprinkler and fan for reducing heat stress. The health status of the herds was monitored routinely by the herd veterinarians. In general, the recommended gilt vaccination program consisted of foot-and-mouth disease, classical swine fever, Aujeszky's disease, and porcine parvovirus at between 22 and 30 weeks of age. Some herds were also given some extra vaccines against PRRSV, atrophic rhinitis, *Mycoplasma hyopneumoniae*, and *Actinobacillus pleuropneumoniae*. In herds B, E, and F, the replacement gilts were not vaccinated with PRRSV vaccine, while in herds A and D, they were vaccinated using the

genotype 1 PRRS MLV vaccine (AMERVAC®, Laboratorios Hipra, Girona, Spain). Herd C, the replacement gilts, was vaccinated using the genotype 2 PRRS MLV vaccine (Ingelvac® PRRS™ MLV, Boehringer-Ingelheim Vetmedica Inc., St. Joseph, MO, USA). The gilts were vaccinated against PRRSV twice during 22-30 weeks of age before being sent to the breeding house. Gilts were kept in each pen with a group size of 6-15 gilts per pen (depending on the herd) with a density of 1.5-2.0 m^2 per gilt. In general, the herds were recommended to breed the replacement gilts at about 32 weeks of age onwards with a body weight of at least 130 kg at the second or later observed estrus. The mating technique for all herds was performed by artificial insemination.

4.3.3 Immunohistochemistry

Immunohistochemistry was carried out according to previous protocol in the lung tissue with some modification (Laohasittikul et al., 2004). Briefly, the samples were embedded in paraffin blocks, cut in 4-µm-thick sections, and placed on 3-aminopropyl-triethoxysilane-coated slides. The sections were deparaffinized in xylene and rehydrated in graded alcohol. A polymer-based non-avidin-biotin technique was applied in the present study. Briefly, the antigen retrieval technique was used in order to enhance the reaction between antigen and antibody by enzymatic treatment using 0.1% trypsin at 37°C for 30 min. After washing in phosphate-buffered saline (PBS), endogenous peroxidase activity was inhibited by immersing the sections in 0.3% hydrogen peroxide (H_2O_2) in absolute methanol for 30 min at room temperature. The sections were then blocked with 1.0% bovine serum albumin at 37°C for 30 min and incubated overnight (12-15 h) at 4°C with primary monoclonal antibody SDOW17 (Rural Technologies, Inc., USA) diluted 1:1,000. After washing in PBS, a dextran coupled with peroxidase molecules and goat secondary antibody (Dako REAL™ Envision™/HRP, Rabbit/Mouse®, Dako, Denmark) was applied on the sections and incubated at 37°C for 45 min. In the final step, the color of the bound enzyme (brown color) was obtained using 0.05% 3,3'-diaminobenzidine tetrahydrochloride (0.01 M Tris-HCl, pH 7.6) for 4-15 min. All sections were counterstained with Mayer's hematoxylin, dehydrated, and

mounted for investigation under a light microscope. Negative control procedures included an omission of primary antibody. Known PRRSV-infected lung and lymph node tissues served as positive controls. The sections were interpreted as positive if they contained at least one positive cell (brown intracytoplasmic staining, Figure 1).

4.3.4 Serological test

The blood samples were allowed to clot at room temperature, and the sera were obtained and were kept at -20°C for analyzing the antibody titers against PRRSV. The antibody against PRRSV was determined using a commercial enzyme-linked immunosorbent assay test kit (ELISA, HerdChek® PRRS virus antibody test kit 2XR, IDEXX Laboratories, Inc., USA). The protocol followed the kit's instructions. The serum sample/positive control (S/P) was calculated. The S/P ratio below 0.4 indicated that the sample had no antibody to PRRSV (negative), while the S/P ratio ≥0.4 indicated that the sample had antibody to PRRSV (positive).

4.3.5 Statistical analyses

Statistical analyses were performed using Statistical Analysis System (SAS) version 9.0 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics (means, standard deviation, and range) and frequency tables were conducted for all reproductive parameters. The percentage of positive tissue was compared between groups of age at culling (6-8, 9-10, and 11-16 months), reason for culling (anestrus, vaginal discharge, repeat breeding, abortion, and not being pregnant), type of MLV vaccine against PRRSV (genotype 1 and 2), and the detection of antibody titers against PRRSV by using ELISA (0.00-0.39, 0.40-0.99, 1.00-1.49, and 1.50-2.92) using $r \times k$ contingency table and *Fisher's exact* test. Logistic regression was performed to analyze the multiple effects of age at culling and the use of PRRSV vaccine on the incidence of PRRSV detection in the uterine tissues of the gilts. The analysis was carried out using the GLIMMIX macro of SAS. The statistical model included the effect of age at culling and PRRSV vaccination as independent variables. Least-square means of the *logit* scale were obtained and

were compared by using the least significant different test. A value of $P<0.05$ was considered to be statistically significant.

4.4 Results

4.4.1 Reproductive data and culling reason

Reproductive data of the slaughtered gilts are presented in Table 12. On average, the gilts were culled at 303.3±53.0 days of age and a body weight of 149.0±20.8 kg. They entered the herds at 218.9±53.1 days of age and were culled at 84.4±57.1 days after entering the herd. Of all the gilts, 52 gilts (52.0%) had been mated, and the interval from the first observed estrus to mating was 20.8±17.2 days (range 0 to 63 days). The reasons for culling of the gilts included anestrus, abnormal vaginal discharge, abortion, repeat breeding, and not being pregnant (Table 13). On average, the age at culling was 273.8, 298.0, 311.3, 342.9, and 368.9 days, and the interval from entry to culling was 73.0, 67.6, 68.3, 111.4, and 142.8 days for gilts culled due to anestrus, abnormal vaginal discharge, abortion, repeat breeding, and not being pregnant, respectively.

Table 12 Descriptive statistics for reproductive data of the replacement gilts culled due to reproductive failure

Parameters	Number of gilts	Mean ± SD	Range
Age at culling (day)	100	303.3 ± 53.0	209 - 489
Body weight at culling (kg)	96	149.0 ± 20.8	104.5 - 205.5
Age at entry (day)	98	218.9 ± 53.1	94 - 365
Age at first estrus (day)	69	229.3 ± 30.5	156 - 322
Age at first mating (day)	52	256.8 ± 24.4	211 - 322
ADG (g/day)	96	496.2 ± 78.2	245.6 - 674.5
NPD (day)	98	84.4 ± 57.1	0 - 250

ADG = average daily gain from birth to culling, *NPD* = non-productive day (the interval from entry into the herd to culling)

Table 13 Number and percentage of gilts in relation to the presence of PRRSV antigen in the uterine tissue by IHC and the antibody titer against PRRSV by culling reason

Culling reason	Number of gilts	Number of IHC positive gilts	Number of ELISA-positive gilts
Anestrus	42	14 (33.3%)	29 (80.6%) a
Abnormal vaginal discharge	21	6 (28.6%)	14 (73.7%) a
Abortion	11	3 (27.3%)	8 (80.0%) a
Repeat breeding	17	7 (41.2%)	10 (58.8%) a
Not being pregnant	9	3 (33.3%)	0 (0.0%) a
Total	100	33 (33.0%)	61 (73.5%)

Different lowercase letters (a and b) within columns indicate statistically significant differences ($P<0.05$)

4.4.2 Detection of PRRSV

The PRRSV-positive cells characterized by brown intracytoplasmic-stained macrophages in the subepithelial connective tissue layer of the endometrium were detected in the uterine tissue in 33.0% of gilts (33/100 gilts) (Figure 3). The detection of PRRSV in the uterine tissue of the gilts varied among the herds from 14.3% to 80.0% (*P*=0.018). PRRSV was found in 24.5% of the gilts vaccinated against genotype 1 PRRS MLV vaccine and in 23.1% of the gilts vaccinated against genotype 2 PRRS MLV vaccine (*P*=0.941). The detection of PRRSV in the uterine tissue of the gilts collected from non-vaccinated herds (17/34 gilts, 50.0%) was higher than the herds whose gilts were vaccinated against genotype 1 (13/53 gilts, 24.5%, *P*=0.023) and 2 (3/13 gilts, 23.1%, *P*=0.105) of PRRSV.

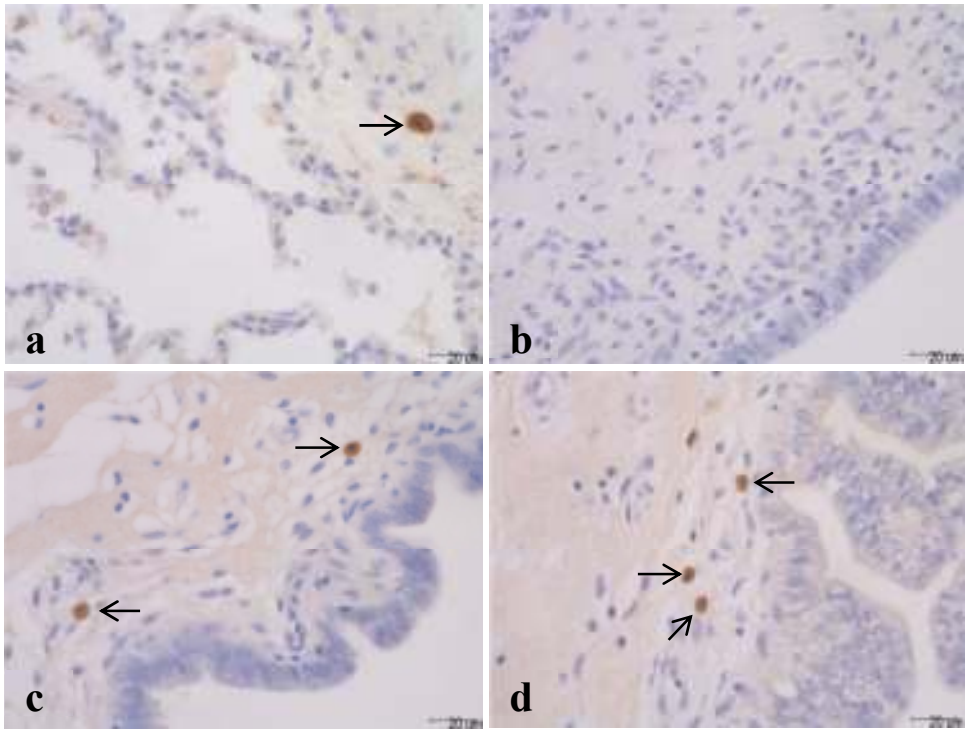

Figure 3 Expression of PRRSV antigen in the uterine tissue of gilts: **a** positive control (lung tissue); **b** negative control; **c**, **d** uterine tissue from gilts culled due to reproductive disturbance which expressed PRRSV antigen. *Black arrows* indicate positive staining cell

4.4.3 Influence of age at culling, reasons for culling, and mating

On average, gilts that had PRRSV in the uterine tissue were culled at 307.2±54.1 days of age (range 240 to 439 days), while those that had no PRRSV in the uterine tissue were culled at 301.3±52.8 days of age (range 209 to 489 days) (*P*=0.605). NPD of these gilts was 92.0±59.6 (range 0 to 225 days), and ADG was 488.1±80.6 g/day. The incidence of PRRSV manifestation in the uterine tissues of the gilts was 29.6%, 39.4%, and 40.9% in the gilts culled at 6-8, 9-10, and 11-16 months of age, respectively (*P*=0.698). PRRSV was found in 33.3%, 28.6%, 27.3%, 41.2%, and 33.3% of the uterine tissues of the gilts culled due to anestrus, abnormal vaginal discharge, abortion, repeat breeding, and not being pregnant, respectively (*P*=0.929) (Table 13). The detection of PRRSV in the uterine tissue of the gilts did not differ significantly between the virgin gilts (35.4%) and the gilts that were mated before culling (30.8%) (*P*=0.622).

4.4.4 Influence of antibody titer against PRRSV

Of the 100 replacement gilts, 83 serum samples were included in the present study. Of all the gilts, 61 of 83 gilts (73.5%) were positive to ELISA. The highest percentage of positive gilts (29/36 gilts, 80.6%) was observed in gilts culled due to anestrus. The percentage of positive gilts culled for abnormal vaginal discharge, abortion, repeat breeding, and not being pregnant, was 73.7%, 80.0%, 58.8%, and 0.0%, respectively (Table 13). Of the 61 gilts that were positive to ELISA, 22 gilts (36.1%) were positive to IHC. Of the 22 gilts that were negative to ELISA, five gilts (22.7%) were positive to IHC. According to all gilts that were positive to IHC, 81.5% (22/27 gilts) were positive to ELISA (Table 14). PRRSV was detected in the uterine tissue in 28.2%, 31.0%, 47.1%, and 33.3% of the gilts with antibody titers against PRRSV at 0.00-0.39, 0.40-0.99, 1.00-1.49, and 1.50-2.92, respectively (*P*=0.577).

Table 14 Reproductive data of gilts culled due to reproductive disturbances in relation to percentage of ELISA-positive gilts to the results of IHC test

Results of IHC	Number of gilts	Mean ± SD			Percentage of ELISA-positive gilts
		Age at culling (d)	NPD (d)	ADG (k/d)	
Positive	33	307.2 ± 54.1 a	92.0 ± 59.6 a	488.1 ± 80.6 a	81.5 a
Negative	67	301.3 ± 52.8 a	80.8 ± 56.0 a	500.4 ± 77.2 a	69.6 a

Different letters within columns differ significantly ($P<0.05$); *NPD* = non-productive day (the interval from entry into the herd to culling), *ADG* = average daily gain from birth to culling

4.5 Discussion

The presence of PRRSV antigen in the uterine tissues of the gilts culled due to reproductive failure was demonstrated. Apparently, the findings indicated that the replacement gilts remained at risk of introducing PRRSV into the breeding herd even though vaccinations and acclimatization have been carried out. Furthermore, the detection of PRRSV in the uterine tissue of the replacement gilts did not decrease when age at culling increased; PRRSV could be found even in the gilts older than 11 months of age. In Thailand, most of the gilts were usually mated between 8 and 9 months of age (Tummaruk et al., 2007). The detection of PRRSV in the uterine tissue varied considerably among the herds, from 14.3% to 80.0%. This indicated that, under field conditions, numerous gilts might be mated when the PRRSV antigen remained in their uterine tissue. Therefore, the reproductive performance of these gilts might be compromised.

Cells containing PRRSV are found in the subepithelial layer of the endometrium. This could be explained by the fact that PRRSV infection is a multisystemic disease characterized by viremia and, subsequently, viral distribution and replication in multiple organs (Thanawongnuwech et al., 1997a, b). Using IHC, PRRSV antigen has been detected at 56-100% in the lungs, 8-36% in the heart, 40-43% in the lymph node, 38-100% in the tonsil, 8-54% in the thymus, 4-50% in the spleen, 25-60% in the intestine, and 20-75% in the liver (Larochelle and Magar, 1997; Laohasittikul et al., 2004). Therefore, it is not surprising to detect the PRRSV antigen in 33.0% of the uterine tissues of the culled gilts since the infection of PRRSV results in the distribution of the virus via the blood system, and the virus is also detected in the macrophages of many organs. In the uterine tissue of the gilts, some macrophages have been observed in all tissue layers of the endometrium at all stages of the estrous cycle (Teamsuwan et al., 2010). Moreover, it is found that at least 73.5% of the culled gilts are infected with PRRSV as demonstrated by the serological response.

It has been demonstrated that PRRSV can be detected for at least 42 days post-infection in the lungs and in the tonsil by using IHC and ISH (Sur et al., 1996), at least 59 days post-infection in the brain stem by using ISH (Shin and Molitor, 2002) and at least 15 days post-infection in the lung, liver, pulmonary lymph node, spleen, tonsil, turbinate bone, and heart by using IHC (Laohasittikul et al., 2004). In the reproductive organs, PRRSV can be detected by using ISH in the macrophages in the interstitium of the testis during 7-30 days post-infection and in the seminiferous tubules primarily in spermatocytes and round spermatids up to 25 days post-infection (Sur et al., 1997). Moreover, PRRSV has been found in the testis, epididymis, prostate gland, and bulbourethral gland at seven days post-infection and in testis and epididymis at least 59 days post-infection (Shin and Molitor, 2002). In this study, the exact timing of PRRSV infection in the replacement gilts is not known, but it is likely to be the period during PRRS MLV vaccination and acclimatization. These management practices are usually performed within a month after the gilts enter the herds. Most of these gilts are culled nearly three months after entering the herds. This indicates that PRRSV may remain in the uterine tissue of the infected gilts for several months, or re-infection might have occurred. In the boar, PRRSV infection causes viral shedding in semen for several months (Christopher-Hennings et al., 1995).

In this study, PRRSV is found in 6.0% of the uterine tissues of the gilts having no antibody titer against PRRSV. It has been demonstrated that PRRSV is widespread in the respiratory and lymphoid system of the pig by 1-2 days post-infection (Halbur et al., 1996) and in liver, ileum, kidney, and turbinate bone by five days post-infection (Laohasittikul et al., 2004). PRRSV antibodies can be detected early at 7-14 days post-infection using commercial ELISA; peak titers are seen by 30-50 days post-infection and undetectable titers by 4-6 months after infection (Benfield et al., 1999). Thus, the antigen of the virus can be detected while the antibody was undetected.

It can be concluded that PRRSV antigen is detected in the uterine tissues in 33.0% of the gilts culled due to reproductive failure. The percentage of the gilts' uterine tissues containing PRRSV did not differ between herds with the gilts vaccinated with the genotype 1 and 2 of PRRS MLV vaccines but tended to be lower than the non-vaccinated gilts. The incidence of the gilts having uterine tissues containing PRRSV antigen varied among the herds from 14.3% to 80.0%.

CHAPTER V

Detection of porcine reproductive and respiratory syndrome virus in aborted fetuses, mummified fetuses and stillborn piglets using quantitative polymerase chain reaction

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Detection of porcine reproductive and respiratory syndrome virus in aborted fetuses, mummified fetuses, and stillborn piglets using quantitative polymerase chain reaction

5.1 Abstract

The objective of the present study was to investigate the prevalence of porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) detection in aborted fetuses (n=32), mummified fetuses (n=30), and stillborn piglets (n=27) from 89 gilts and sows using quantitative polymerase chain reaction (qPCR). The samples were collected from 10 swine herds in Thailand facing reproductive failure. Pooled organs of lung, liver, spleen, thymus, tonsil, lymph node, and umbilical cord from each fetus/piglet were homogenized, extracted for RNA, and subjected to cDNA synthesis. The qPCR was carried out on the ORF 7 of the PRRSV genome using fluorogenic probe for amplified product detection. The results showed that 67.4% of the samples (60/89) contained PRRSV. The virus was found in 65.6% (21/32) of the aborted fetuses, 63.3% (19/30) of the mummified fetuses, and 74.1% (20/27) of the stillborn piglets (*P*=0.664). Genotype 1, 2, and mixed (both genotype 1 and 2) of PRRSV were detected in 19.1% (17/89), 25.8% (23/89), and 22.5% (20/89) of the specimens, respectively (*P*=0.316). PRRSV was retrieved from both non-PRRS-modified-live-virus-vaccinated herds (68.2%, 45/66) and the vaccinated herds (65.2%, 15/23) (*P*=0.794). The virus was detected by 75.0% (3/4), 66.7% (12/18), 62.5% (10/16), 64.7% (11/17), and 70.6% (24/34) from gilts and sows parity numbers 1, 2-4, 5-11, and unknown parity, respectively (*P*=0.974). It could be concluded that PRRSV was frequently detected in aborted fetuses, mummified fetuses, and stillborn piglets in swine herds in Thailand regardless of vaccination. This indicated that the dead fetuses as well as stillborn piglets might be important sources of the PRRS viral load and transmission within the herd. Intensive care on the routine managements of the dead fetuses and stillborn piglets in PRRSV-positive herds should be emphasized in order to minimize the viral load and viral shedding within the herd.

Keywords: qPCR, pigs, PRRS, reproductive failure, Thailand

5.2 Introduction

Reproductive failure in gilts and sows is influenced by both infectious and non-infectious causes. Recently, major infectious agents associated with reproductive disturbances in gilts and sows commonly detected in swine commercial herds worldwide include porcine reproductive and respiratory syndrome virus (PRRS) virus (PRRSV), Aujeszky's disease virus, porcine parvovirus, classical swine fever virus, and porcine circovirus type 2 (Dias et al., 2012; Tummaruk and Tantilertcharoen, 2012; Tummaruk et al., 2013). Recently, a serological survey on the evidence of these viruses in swine commercial herds in Thailand found that the seroprevalences of PRRSV, Aujeszky's disease virus, and porcine parvovirus in replacement gilts were 87.5%, 4.0%, and 99.0%, respectively (Tummaruk and Tantilertcharoen, 2012). Furthermore, 81.0%, 50.0%, and 75.0% of gilts culled due to abortion were sero-positive against PRRSV, Aujeszky's disease virus, and porcine parvovirus, respectively (Tummaruk and Tantilertcharoen, 2012). PRRSV remains one of the most common viruses associated with reproductive failure in gilts and sows in the Thai swine industry. In most commercial swine herds in Thailand, replacement gilts and sows are routinely vaccinated against Aujeszky's disease virus and porcine parvovirus, while PRRSV vaccination has been applied only in some herds (Chapter II).

The reproductive failure caused by PRRSV is characterized by a decrease in farrowing rate and an increase in abortion rate, the number of stillborn piglets, mummified fetuses, weak born piglets, and pre-weaning mortality (Cho and Dee, 2006; Zimmerman et al., 2006; Chapter II). PRRSV is a single-stranded RNA virus and is classified into two genotypes by its genetic, antigenic, and pathogenic differences, i.e., genotype 1 (European genotype) and 2 (North American genotype) (Meng, 2000). The original prototypes of genotype 1 and 2 are Lelystad virus (Wensvoort et al., 1991) and VR-2332 virus (Benfield et al., 1992), respectively. The genome of PRRSV consists of nine open reading frames (ORFs). ORF 1a and ORF 1b encode the viral RNA polymerase, whereas ORFs 2a, 2b, and 3-7 encode the viral structural proteins (Meng et al., 1994; Snijder and Meulenberg, 1998). The complete nucleotide sequence of PRRSV

isolates in Thailand revealed that the percentage of homology between the Thai genotype 1 and 2 is only 59.1% (Amonsin et al., 2009). Additionally, the homology between the Thai genotype 1 and the genotype 1 prototype (Lelystad virus) is 99.2% and the homology between the Thai genotype 2 and the genotype 2 prototype (VR-2332) is 99.5% (Amonsin et al., 2009).

A recent study has demonstrated that PRRSV can migrate cross the placenta of the pregnant female pigs particularly during the late gestation (Karniychuk et al., 2011). The transplacental migration of the PRRSV induced apoptosis of the placental cells and caused late term abortion. Furthermore, transplacental infection of the virus also resulted in fetal mortality and an increase in the proportion of stillborn piglets per litter (Zimmerman et al., 2006). The investigation of the prevalence of PRRSV from 100 clinical cases of sows with aborted fetuses and stillborn piglets in Spain found that, PRRSV could be detected in only 9% of the samples (Maldonado et al., 2005). In practice, many types of management strategies including acclimatization, gilts pool management, and vaccination with killed virus vaccine and/or modified-live virus (MLV) vaccine, have been used to control the clinical signs of PRRSV infection. However, PRRSV still causes many types of reproductive failures in the infected herds even though the herds having PRRS MLV vaccination implementation (Chapter II). In addition, in Thailand, PRRSV was detected in the uterine tissue in up to 33.0% of the culled replacement gilts due to reproductive disturbances (Chapter IV). However, the prevalence of PRRSV in relation to fetal loss (i.e., abortion, mummification, and stillborn) in swine herds has not been investigated. The objective of the present study was to investigate the prevalence of PRRSV detection in aborted fetuses, mummified fetuses, and stillborn piglets in swine commercial herds in Thailand.

5.3 Materials and Methods

5.3.1 Animals and tissues

The study was conducted between February 2010 and August 2011. In total, dead fetuses and stillborn piglets collected from 89 Landrace x Yorkshire crossbred

sows from 10 swine herds in the high density pig raising areas in Thailand (i.e., Herd A to J) were included. The specimens including aborted fetuses (n=32), mummified fetuses (n=30), and stillborn piglets (n=27) were collected, placed on ice, and transported to the laboratory within 24 h.

5.3.2 Herd location and general management

The herds were located in the eastern (A and G), middle (B, D, E, I, and J), northeastern (C and H), and southern (F) parts of Thailand. All herds were breeding herds with 900 to 5,000 sows per herd and were defined as PRRSV-positive herds according to the results of a commercial ELISA test (HerdChek® PRRSV antibody test kit 2XR®, IDEXX Laboratories, Inc., Westbrook, Maine, USA) and a reverse transcription polymerase chain reaction (RT-PCR) of the herd's monitoring data (Tummaruk et al., 2013). Gilts and sows were housed in a conventional open housing system with equipment, e.g., water sprinklers, fans, and roofs with heat reflecting material, to reduce the impact of high temperatures. On average, the outdoor 24-h average temperature and humidity in these area in the hot (15 February to 14 June), rainy (15 June to 14 October), and cool (15 October to 14 February) seasons were 29.4°C/71.7%, 28.5°C/78.1%, and 26.4°C/68.1%, respectively. The average minimum-maximum daily temperatures were 24.6-34.9°C, 24.8-33.0°C, and 21.4-32.1°C in the hot, rainy, and cool seasons, respectively. In general, gilts entered the gilt pool at a body weight of 80-100 kg. Water was provided up to ad libitum via water nipples. Feeding was provided twice a day at about 3 kg of feed/gilt/day. In general, gilts and sows feed was a corn-soybean-fish based containing 16-18% crude protein, 3,000-3,250 kcal/kg metabolisable energy, and 0.85-1.10% lysine. The herd management recommended breeding replacement gilts from 32 weeks of age onwards at the second or a later estrus and at a body weight of at least 130 kg. All herds used conventional artificial insemination. Gilts and sows were routinely vaccinated against foot-and-mouth disease virus, classical swine fever virus, Aujeszky's disease virus, and porcine parvovirus. Herds A, B, C, D, E, and F did not vaccinate gilts and sows against PRRS MLV vaccine

(n=66), while herds G, H, I, and J (n=23) vaccinated all gilts and sows with PRRS MLV vaccine [Ingelvac® PRRS™ MLV, Boehringer-Ingelheim Vetmedica Inc., St. Joseph, MO, USA (herd G, n=9) or AMERVAC®, Laboratories Hipra, Girona, Spain (herd H, I, and J, n=14)]. Among the PRRS-MLV-vaccinated herds, the gilts and sows were routinely vaccinated against PRRS MLV vaccine every 3-4 months.

5.3.3 Historical data and post-mortem examination

Historical data for all specimens including herd, sows identity and breed, vaccination protocol, parity number of sows, and date of mating, farrowing or abortion was collected. The age of the aborted fetuses and stillborn piglets were defined as the interval from mating to abortion or from mating to farrowing, respectively. Crown-rump-length (CRL) of the mummified fetuses was measured. The age of the mummified fetuses was estimated from CRL: age of fetus = 21.07 + (3.11 x CRL), where CRL was the fetal body length (from crown or frontal crest to anus in centimeter) (Ullrey et al., 1965). Tissue samples including lung, liver, spleen, thymus, tonsil, lymph node, and umbilical cord were collected from stillborn piglets and aborted fetuses and were kept at -80°C until RNA extraction. For mummified fetuses, only lung, liver, and spleen were collected. The dead fetuses were classified into two age groups: <70 days and ≥70 days. The prevalence of PRRSV detection was compared among groups (see below).

5.3.4 Viral RNA extraction and cDNA synthesis

The collected pooled organs of each case were homogenized using a pestle and a mortar and was suspended to 10% organ suspensions with phosphate buffered saline (PBS) solution. Thereafter, the suspension was left at room temperature for sedimentation. The supernatant was collected and subjected to RNA extraction using a commercial kit (NucleoSpin® RNA virus test kit, Macherey-Nagel Inc., Germany). The protocol followed the kit's instruction. Briefly, RNA virus was lysed by a lysis buffer, and was bound to the silica membrane. Contaminations were removed by washing

many times. At the final step, the RNA was eluted from the silica membrane with 50 µl of RNase-free water. RNA concentration was measured by using Thermo Scientific Nanodrop 2000 (Thermo Fisher Scientific, USA). The RNA from each sample was diluted with RNase-free water to prepare RNA 500 ng per reaction and was subjected to cDNA synthesis using Omniscript® Reverse Transcriptase (QIAGEN, Germany). The protocol followed the kit's instruction. Briefly, the 20 µl reaction, which was composed of 0.5 µM each dNTP, 1 µM of random primer, 10 units of RNase inhibitor (Ribolock™RNase Inhibitor, Fermentas Inc., Glen Burnie, Maryland, USA), 4 units of reverse transcriptase, and RNA template from each sample in kit's buffer, was incubated at 37°C for one hour. The synthesized cDNA was kept at -20°C until quantitative polymerase chain reaction (qPCR) was performed.

5.3.5 Quantitative polymerase chain reaction

The qPCR was carried out on ORF 7 of the PRRSV genome using real-time polymerase chain reaction technique with a commercial kit (EXPRESS qPCR SuperMix Universal®, Invitrogen, USA). The primers sequence (US align EU forward and reverse primer, which yielded 96 bp for genotype 1 and 105 bp for genotype 2) and fluorogenic probes sequence were carried out according to previous study (Egli et al., 2001). The fluorescent dyes labeled to the probes for genotype 1 and 2 detection were cyanine 5 (Cy5) and 6-carboxy-fluorescine (FAM), respectively. The reaction for genotype 1 and 2 detection was performed separately. Each 20 µl reaction was composed of 10 µl of EXPRESS qPCR SuperMix Universal, 1.25 µM of US align EU forward primer, 1.25 µM of US align EU reverse primer, 0.5 µM of genotype 1 and 2 probe, and 5 µl of cDNA template. The qPCR was carried out using Rotor-Gene RG-3000 (Corbett Research, Australia) at 50°C for 2 minutes, 95°C for 2 minutes, and 40 cycles of 95°C for 15 seconds, 50°C for 20 seconds, and 60°C for 30 seconds. The known serial concentrations, i.e., 10^1, 10^3, 10^5, 10^7, and 10^9 copies/µl of genotype 1 and 2 PRRSV cDNA were used as a positive control and the cycle threshold (Ct) values were determined for standard curve. A negative control was performed using RNase-free

water instead of cDNA template. The Ct values of each sample were plotted on the standard curve and were determined the amount of copy number (copies/µl) using Rotor-Gene Real-time Analysis Software 6.0 (Corbett Research, Australia). The amounts of copy number of positive samples were transformed into logarithms (log_{10}) for further statistical analyses.

5.3.6 Statistical analyses

Statistical analyses were performed using Statistical Analysis System (SAS) version 9.0 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics (means±standard deviation) were conducted for the continuous data, i.e., gestation length and copy number of PRRSV (log), and frequency tables were conducted for the percentage of PRRSV detection. The percentage of PRRSV detection was compared among groups of types of specimen, PRRS MLV vaccination, and parity number (0, 1, 2-4, 5-11, and unknown parity) by using *r*×*k* contingency table and *chi*-square test. The copy number of PRRSV was compared among types of specimen, PRRS MLV vaccination, and sow's parity number by using general linear model procedure (PROC GLM). Least-squares means were obtained from each class of the factors and were compared by using least significant difference (LSD) test. $P<0.05$ was considered statistically significant.

5.4 Results

5.4.1 Reproductive data

On average, the age of aborted fetuses was 73.9±26.4 (range 31-105 days), the age of mummified fetuses was 101.0±18.9 days (range 62-119 days), and the age of stillborn piglets was 114.4±1.7 days (range 111-116 days).

5.4.2 PRRSV detection

PRRSV was detected in 67.4% (60/89) of the samples. The genotype of the PRRSV that were detected consisted of genotype 1 (EU) 19.1% (17/89), genotype 2 (NA) 25.8% (23/89), and mixed genotypes 22.5% (20/89) ($P=0.316$). The percentage of PRRSV detection in each type of specimens is presented in Table 15. As seen, PRRSV

was detected in aborted fetuses 65.6% (21/32), mummified fetuses 63.3% (19/30), and stillborn piglets 74.1% (20/27) (*P*=0.664). The percentage of PRRSV detection in two different age groups of fetuses (<70 and ≥70 days) is presented in Table 16. On average, percentages of PRRSV detection in the fetuses <70 days of age (55.0%, 11/20) tended to be lower than that of the fetuses ≥70 days of age (71.0%, 49/69, *P*=0.179). On average, the age of the fetuses with PRRSV detection was 95.9±26.0 days and fetuses without PRRSV detection was 88.6±25.7 days (*P*=0.336). Age of the fetuses with PRRSV detection varied from 31 days to 119 days. Age of the fetuses associated with PRRSV detection by genotypes is presented in Table 17.

The percentage of PRRSV detection varied among herds from 0.0% to 100.0% (*P*=0.001) (Table 18). Nevertheless, PRRSV was detected in both non-vaccinated sows (45/66, 68.2%) and PRRS-MLV-vaccinated sows (15/23, 65.2%) (*P*=0.794) (Table 18).

The percentage of PRRSV detection did not differ significantly among the fetuses obtained from different parity groups of sows. PRRSV was detected by 66.7% (2/3), 68.4% (13/19), 62.5% (10/16), 64.7% (11/17), and 70.6% (24/34) of the fetuses collected from the gilts and the sows in parity number 0, 1, 2-4, 5-11, and unknown parity, respectively (*P*=0.983).

5.4.3 Quantitative PCR detection of PRRSV

The copy number of PRRSV detected by qPCR is presented in Table 19. On average, the copy number of genotype 1 PRRSV did not differ significantly among aborted fetuses (12.3±2.5 copies/µl), mummified fetuses (10.8±2.4 copies/µl), and stillborn piglets (12.2±3.1 copies/µl) (*P*=0.373). However, the aborted fetuses had higher copy number (12.0±1.3 copies/µl) of genotype 2 PRRSV than that of the mummified fetuses (10.4±1.5 copies/µl, *P*=0.004) but did not differ significantly compared to the stillborn piglets (11.2±1.4 copies/µl, *P*=0.345).

The copy number of genotype 1 and genotype 2 PRRSV in non-PRRS-MLV-vaccinated and vaccinated sows were similar (11.7±2.7 and 11.8±2.9 copies/µl, *P*=0.689 and 11.2±1.6 and 11.7±1.3 copies/µl, *P*=0.273, respectively).

Table 15 The percentage of PRRSV detection in aborted fetuses, mummified fetuses, and stillborn piglets in Thailand

Specimen	n	PRRSV detection	PRRSV detection by genotypes		
			genotype 1	genotype 2	mixed
Aborted fetuses	32	21/32 (65.6%) a	5/32 (15.6%)	9/32 (28.1%)	7/32 (21.9%)
Mummified fetuses	30	19/30 (63.3%) a	7/30 (23.3%)	6/30 (20.0%)	6/30 (20.0%)
Stillborn piglets	27	20/27 (74.1%) a	5/27 (18.5%)	8/27 (29.6%)	7/27 (25.9%)
Total	89	60/89 (67.4%)	17/89 (19.1%)	23/89 (25.8%)	20/89 (22.5%)

Different lowercase letters (a and b) within columns indicate statistically significant differences ($P<0.05$)

Table 16 The percentage of PRRSV detection using a real time PCR based on age of fetuses

Age of fetuses	n	PRRSV detection	PRRSV detection by genotypes		
			genotype 1	genotype 2	mixed
<70 days	20	11/20 (55.0%) a	0/20 (0.0%)	7/20 (35.0%)	4/20 (20.0%)
≥70 days	69	49/69 (71.0%) a	17/69 (24.6%)	16/69 (23.2%)	16/69 (23.2%)
Total	89	60/89 (67.4%)	17/89 (19.1%)	23/89 (25.8%)	20/89 (22.5%)

Different lowercase letters (a and b) within columns indicate statistically significant differences ($P<0.05$)

Table 17 The means age of the fetuses in PRRSV positive and negative samples

Specimen	PRRSV negative		PRRSV positive	
	n	Age of fetuses/piglets	n	Age of fetuses/piglets
Aborted fetuses	11	71.4 ± 22.3 a, A	21	75.3 ± 29.0 a, A
Mummified fetuses	11	94.1 ± 23.5 a, B	19	104.2 ± 16.1 a, B
Stillborn piglets	7	113.2 ± 1.9 a, B	20	115.0 ± 1.3 a, B
Total	29	88.6 ± 25.7 a	60	95.9 ± 26.0 a

Different lowercase letters (a and b) across rows and different uppercase letters (A and B) within columns indicate statistically significant differences ($P<0.05$)

Table 18 The percentage of PRRSV detection in non-vaccinated and vaccinated herds

Herds	n	PRRSV detection	PRRSV detection by genotypes		
			genotype 1	genotype 2	mixed
Non-vaccinated herds	66	45/66 (68.2%)	12/66 (18.2%)	20/66 (30.3%)	13/66 (19.7%)
Herd A	43	32/43 (74.4%)	4/43 (9.3%)	17/43 (39.5%)	11/43 (25.6%)
Herd B	12	6/12 (50.0%)	3/12 (25.0%)	2/12 (16.7%)	1/12 (8.3%)
Herd C	5	5/5 (100.0%)	4/5 (80.0%)	0/5 (0.0%)	1/5 (20.0%)
Herd D	3	2/3 (66.7%)	1/3 (33.3%)	1/3 (33.3%)	0/3 (0.0%)
Herd E	2	0/2 (0.0%)	0/2 (0.0%)	0/2 (0.0%)	0/2 (0.0%)
Herd F	1	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)	0/1 (0.0%)
Vaccinated herds	23	15/23 (65.2%)	5/23 (21.7%)	3/23 (13.0%)	7/23 (30.4%)
Herd G	9	9/9 (100.0%)	4/9 (44.4%)	0/9 (0.0%)	5/9 (55.6%)
Herd H	7	4/7 (57.1%)	1/7 (14.3%)	3/7 (42.9%)	0/7 (0.0%)
Herd I	5	0/5 (0.0%)	0/5 (0.0%)	0/5 (0.0%)	0/5 (0.0%)
Herd J	2	2/2 (100.0%)	0/2 (0.0%)	0/2 (0.0%)	2/2 (100.0%)
Total	89	60/89 (67.4%)	17/89 (19.1%)	23/89 (25.8%)	20/89 (22.5%)

Table 19 The means of logarithms of copy numbers of PRRSV in PRRSV-positive aborted fetuses, mummified fetuses, and stillborn piglets in Thailand

Specimen	Number of PRRSV +ve samples	Genotype 1		Genotype 2	
		n	Log of copy number	n	Log of copy number
Aborted fetuses	21	12	12.3 ± 2.5 a	16	12.0 ± 1.3 a
Mummified fetuses	19	13	10.8 ± 2.4 a	12	10.4 ± 1.5 b
Stillborn piglets	20	12	12.2 ± 3.1 a	15	11.2 ± 1.4 a, b
Total	60	37	11.7 ± 2.7	43	11.3 ± 1.5

Different lowercase letters (a and b) within columns indicate statistically significant differences ($P<0.05$)

The copy number of both genotype 1 and 2 PRRSV did not differ significantly among parity number of sows. In gilts and sows parity numbers one, 2-4, 5-11, and unknown parity, the copy numbers of PRRSV genotype 1 were 10.2±1.9, 10.9±1.7, 12.1±2.9, 11.8±3.0, and 12.2±3.0 copies/µl, respectively ($P=0.655$). For genotype 2 PRRSV, the copy numbers of PRRSV in gilts and sows parity numbers one, 2-4, 5-11, and unknown parity were 11.0±0.0, 11.0±1.7, 11.7±1.7, 11.4±1.5, 11.2±1.4 copies/µl, respectively ($P=0.716$).

5.5 Discussion

The present study revealed that PRRSV was frequently detected in the dead fetuses. It was found that as high as 67.4% of gilts and sows having reproductive failure found PRRSV in their dead fetuses. This study confirms that PRRSV detection is strongly associated with reproductive failures in gilts and sows in the Thai swine commercial herds. This is in agreement with our previous clinical study on reproductive failure after PRRS outbreak (Chapter II). In the previous findings, although the homologous strain of PRRS MLV vaccine has been implemented, some percentages of reproductive failures are remains (Dewey et al., 1999; Dewey et al., 2004; Chapter II). This can be explained

by the finding of the present study that PRRSV still exists and circulated within the herds although vaccination has been done. It is also found that the prevalence of PRRSV was still similar between PRRS-MLV-vaccinated herds and non-vaccinated herds (i.e., 65.2% and 68.2%, respectively). This indicated that although vaccination has been done, herd health monitoring, sanitation, and biosecurity management are still the major keys to minimize the viral load and the clinical signs of PRRSV infection.

In the present study, the age of the fetuses with PRRSV detection varied from 31 days to 119 days. This is in agreement with previous study that PRRSV infection occurs at any stage of gestation (Done et al., 1996; Zimmerman et al., 2006). However, the prevalence of PRRSV detection tended to be higher in the fetuses age ≥70 days compared to the fetuses ages <70 days. Earlier studies have demonstrated that the sows with gestation length of ≥90 days are more sensitive to PRRSV infection than sows with gestation length <90 days (Rowland, 2010; Karniychuk et al., 2011). The mechanism of reproductive failure (i.e., abortion and fetal death) caused by PRRSV infection is still unclear (Cheon and Chae, 2001; Karniychuk et al., 2011). However, it was found that PRRSV may induce apoptosis in PRRSV infection site (i.e., endometrial connective tissues and fetal placenta) and subsequently caused reproductive disorders, e.g., abortion, premature farrowing, stillbirth, and PRRSV-infected live born piglets (Karniychuk et al., 2011). PRRSV is able to replicate in the endometrium, cross the maternal epithelium, replicate in the fetal placenta, and reach the fetal internal organs (Chapter IV; Karniychuk et al., 2011). Additionally, sows experimentally infected with the field strain of PRRSV can farrow both non-infected and infected fetuses and the infected fetuses are able to shed the virus (Rowland, 2010). Besides, PRRSV has been detected in many tissues of the infected fetus, i.e., umbilical cord, heart, lung, lymph node, spleen, tonsil, and thymus (Cheon and Chae, 2001; Rowland, 2010). Of these organs, thymus is the primary site of the PRRSV replication (Cheon and Chae, 2001; Rowland, 2010). In the present study, thymus was not included in the mummified fetus samples. Thus, the amount of PRRSV in mummified fetuses tended to be lower than that of the aborted fetuses and stillborn piglets. Furthermore, it has been demonstrated that PRRSV

can be diminished under a dry or a low humidity conditions (Pirtle and Beran, 1996; Cutler et al., 2012). Therefore, the amount of the virus might be low in the mummified fetus samples.

In the present study, the prevalence of PRRSV from the dead fetuses did not differ significantly among parity groups of sows. It might be due to the dead fetuses included in the present study were collected only from gilts and sows that had reproductive failure. In addition, in the present study, 20% of gilts and sows had co-infection of both genotype 1 and 2 of PRRSV. This is in accordance with previous studies that multiple isolates of the PRRSV can co-exist in the infected pigs, so called quasispecies (Chang et al., 2002; Goldberg et al., 2003). In these cases, genetic recombination among multiple isolates might occur (Yuan et al., 1999, Rowland, 2010). This may result in new isolates which might be heterologous to the isolates persisted in the herd, contributing to an incomplete immunological protection against heterologous isolates (Labarque et al., 2003; Cano et al., 2007a; Rowland, 2010).

It could be concluded that PRRSV was frequently detected in aborted fetuses, mummified fetuses, and stillborn piglets in swine commercial herds in Thailand both in PRRSV-vaccinated and non-vaccinated herds. This indicated that the dead fetuses as well as stillborn piglets are important sources of the PRRSV transmission within the herd. Intensive care on the routine managements of the dead fetuses and stillborn piglets in PRRSV-positive herds should be emphasized in order to minimize the viral load and viral shedding within the herd.

CHAPTER VI

DISCUSSION

6.1 General Discussion

This thesis demonstrated the effect of PRRSV on reproductive performances of gilts and sows in PRRSV-positive herds in Thailand with special reference to vaccination and management strategies.

6.1.1 Effect of PRRSV infection on reproduction

PRRSV infection influenced the reproductive performance of the infected females. Under field condition, high AR and RR, a low FR, and high MM were commonly found at the initial stage of the PRRSV infection in gilts and sows. In the present study (Chapter II), we clearly demonstrated typical clinical symptoms of PRRSV infection during the one year period of PRRSV outbreak, including a decrease in FR, TB, and BA and an increase in AR, RR, and MM. This was in agreement with a number of previous studies that PRRSV infection resulted in a decrease in FR and an increase in AR, premature farrowing, SB, MM, number of weak born piglets, pre-weaning mortality, RR, and non-productive sow days (Done et al., 1996; Benfield et al., 1999; Cho and Dee, 2006; Zimmerman et al., 2006).

We demonstrated that PRRSV strongly associated with reproductive failures in gilts and sows in the Thai swine commercial herds since 67.4% of the gilts and sows with reproductive failures, including abortion, SB, and MM, found PRRSV in their dead fetuses (Chapter V). The reproductive failure caused by PRRSV infection could be occurs at any stage of gestation. However, the percentage of fetuses loss in the late gestation (age ≥70 days) tended to be higher than the earlier stage (Chapter V). Although, the mechanism of reproductive failures caused by PRRSV infection is unclear but it might be associated with PRRSV infection in the gilt/sow's reproductive organs. PRRSV replicate in the endometrium, cross the maternal epithelium

to fetal placenta, replicate in the fetal placenta, reach the fetuses, and cause reproductive failure including abortion, mummified fetuses, stillborn piglets, and weak-born piglets (Karniychuk et al., 2011). In the present study, PRRSV antigen was detected in 33.0% by IHC technique in the uterine tissues of gilts culled due to reproductive failures (Chapter IV). The PRRSV antigen was detected in 41.2%, 33.3%, and 27.3% of the gilts culled due to repeat breeding, not being pregnant, and abortion, respectively (Chapter IV). It was found that PRRSV induce apoptosis in PRRSV infection site (Karniychuk et al., 2011). Therefore, the uterus of these PRRSV-antigen-positive gilts may not suitable for embryo implantation and may result in reproductive failures.

6.1.2 Effect of PRRS MLV vaccination on reproduction

PRRS MLV vaccination in gilts and sows affected reproductive performances. The first year of whole-herd vaccination with PRRS MLV vaccination in a PRRSV-infected herd resulted in positive (a reduction of 0.2% AR, 2.4% SB, and 0.9% MM), neutral (not improve FR), and negative effects (an increase of 5.4% RR and a reduction of 0.8 TB and 0.3 BA) (Chapter II). However, the females from the herds using PRRS MLV vaccine for over three years had a significant improvement in the gilts and sows reproductive performances (+4.7% FR, -3.2% RR, +0.3 TB, +0.6 BA, -1.8% SB, -1.0% MM, and +0.4 WP) compared to those from non-vaccinated herds (Chapter III). Previous studies found that using PRRS MLV vaccination in pregnant females resulted in many negative effects (Dewey et al., 1999; Dewey et al., 2004; Nielsen et al., 2002). Our results found that the stage of gestation at the time of PRRS MLV vaccination affected the reproductive outcome. Gilts and sows vaccinated at >90 days of gestation had the lowest FR, whereas those vaccinated at 0-30 days of gestation had the lowest BA and the highest MM (Chapter II).

Although the prevalence of PRRSV detection in the uterine tissues of gilts culled due to reproductive failures from PRRS-MLV-vaccinated herds was tended to be lower than those from non-vaccinated herds (Chapter IV), but the prevalence of PRRSV

detection in dead fetuses collected from gilts and sows having reproductive failure was not difference (Chapter V). This indicated that although vaccination have been implemented, other managements, e.g., biosecurity, sanitary, and herd health monitoring, are still important keys to control the clinical signs and reproductive failure caused by PRRSV infection.

6.1.3 PRRSV infection in relation to management strategies

It is well established that replacement gilts are the major source of introducing PRRSV into the breeding herd. In addition, in Thailand, the replacement gilts were usually sent into the breeding herd and were mated between eight or nine months of age. In the present study, it was found that the replacement gilts older than 11 months of age were still detected PRRSV in their uterine tissue (Chapter IV). This indicated that several gilts were sent into the breeding herd when they still carried PRRSV in their body and they were mated when the PRRSV remained in their uterine tissue. Since PRRSV infection in reproductive organs associated with reproductive failure, therefore, the reproductive performance of these gilts might be compromised. Moreover, as reviewed that PRRSV is able to replicate in the endometrium, transplacental transmission to the fetuses, caused the PRRSV infection in fetuses, and resulted in reproductive failure (Karniychuk et al., 2011), therefore, it was not surprising that high prevalence of PRRSV detection in dead fetuses collected from gilts and sows having reproductive failure (67.4%) were observed (Chapter V). In addition, in the present study, it was found that the antibody detected by using commercial ELISA test was not associated with PRRSV detection in the tissues of female pigs since PRRSV was found 6.0% of the uterine tissues collected from gilts that were sero-negative against PRRSV (Chapter IV). This indicated that although the antibody declined to the undetectable level, PRRSV might still remained in the uterine tissue. Thus, from the results of the present study, it was confirmed that the replacement gilts management should be carefully implement to control the PRRSV infection in the herds.

6.2 Conclusion

PRRSV is still one of the most major infectious agents associated with reproduction in gilts and sows in Thai swine commercial herds nowadays. The economic losses caused by PRRSV infection including extended farrowing interval due to a decrease in FR and an increase in AR and RR, and low piglets produced per sow per year due to a decrease in TB, BA, and WP and an increase of SB and MM. Under the field conditions, using PRRS MLV vaccine seemed to improve the reproductive performance of gilts and sows in PRRSV positive herds at herd level, although the reproductive performance might vary in the first year of vaccination. However, at the individual level, using PRRS MLV vaccine in pregnant females should be aware since the negative effects were observed. It could be concluded that PRRS MLV vaccination in gilts and sows had positive, neutral, and negative effects on reproduction, therefore, the decision to implement PRRS MLV vaccination in the swine breeding herds should be balanced between the benefits derived from positive effects and the negative effects of PRRS MLV vaccination, especially vaccination on pregnant female pigs. However, others management strategies, especially replacement gilts management, should be carefully performed to reduce the reproductive losses from PRRSV infection.

6.3 Research Limitations

The researches in this thesis focus on reproductive performances and reproductive failures of gilts and sows in PRRSV-positive herds in Thailand. However, PRRSV affected all the pigs in the herds including boars, nursery pigs, grower, and finisher pigs. Furthermore, the effects of PRRSV infection and PRRS MLV vaccination on reproductive performance and reproductive failures of gilts and sows in PRRS-negative herds might be different. Moreover, all of the researches in this thesis were carried out under field conditions. Therefore, several factors, e.g., herd managements, climate, and other diseases status might affect the results. However, these factors were adjusted in the models of statistical analyses to balance all the factors between the comparison groups. In Chapter IV and V, the samples were collected from gilts and sows having

reproductive problems. Thus, the prevalence of PRRSV detection might not refer to the whole-herd prevalence of PRRSV infection in gilts and sows in swine commercial herds in Thailand.

6.4 Suggestions for Further Investigations

Due to PRRS is one of the most importance diseases in pig industries in Thailand and also in pig producing countries throughout the world, limited information about the effect of PRRSV on reproductive performance of gilts and sows under field conditions were reported. Therefore, the effect of PRRSV infection on reproduction, especially under field conditions, should be performed.

In Chapter II, PRRS MLV vaccine was first applied in the herd and the reproductive data pre-vaccination and the first year post-vaccination were collected and analyzed. It was found that the effect of PRRS MLV vaccination on reproductive performance was varied (positive, neutral, and negative effects were observed). Therefore, it is interesting to continue the investigation of the reproductive performance after the first year post-vaccination since it was found that using PRRS MLV vaccine for over three years could improve reproductive performance of the herds.

In Chapter IV, it was found that gilts culled due to repeat breeding, not being pregnant, and abortion were detected PRRSV antigen in their uterine tissue 41.2%, 33.3%, and 27.3%, respectively. It is interesting to investigate the histology and pathology of uterine tissues of these gilts in relation to PRRSV infection. This might help to explain the mechanism of reproductive failure in PRRSV infected females. Moreover, other reproductive organs, especially the ovary, should be further investigate for the prevalence of PRRSV detection, the ovarian function, and ovarian histology in relation to PRRSV infection since it was found that 33.3% of gilts culled due to anestrus were detected PRRSV antigen in their uterine tissues.

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APPENDIX

List of international publications

Olanratmanee, E.-o., Kunavongkrit, A. and Tummaruk, P. 2010. Impact of porcine epidemic diarrhea virus infection at different periods of pregnancy on subsequent reproductive performance in gilts and sows. Anim Reprod Sci. 122: 42-51.

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Olanratmanee. E., Wangnaitham, S., Thanawongnuwech, R. and Tummaruk, P. 2010. Effect of age at culling on the existence of porcine reproductive and respiratory syndrome virus in the uterine tissue of replacement gilts. Proceeding of the RGJ Seminar Series LXXI, Bangkok, Thailand. 37.

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BIOGRAPHY

Miss Em-on Olanratmanee was born on June 4th, 1983 in Bangkok, Thailand. She graduated Bachelor's degree of Doctor of Veterinary Medicine (D.V.M.) (2nd Class Honors) from the Faculty of Veterinary Science, Chulalongkorn University, Thailand in 2007. After graduation, she worked as a sale representative at Philips International Co., Ltd. for a year. In 2008, she enrolled a Ph.D. program in Theriogenology at the Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University and received a Ph.D. program scholarship of the Royal Golden Jubilee (RGJ) Ph.D. Program, Thailand Research Fund and the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). Her field of interest was swine reproduction and diseases. During the Ph.D. study, she had 4 international publications and 20 proceedings.