ภาคผนวก

บทความทางวิชาการที่ตีพิมพ์ในวารสารทางวิชาการแล้ว (ถ้ามี)

วารสารวิชาการระดับนานาชาติ

1. Tummaruk, P., Surapat, P., Sriariyakun, S., Seemakram, O., Olanratmanee, E., Tantilertcharoen, R. and Thanawongnuwech, R., 2013. Porcine reproductive and respiratory syndrome virus detection in Thailand during 2005-2010 in relation to clinical problems, pig types, regions, and seasons. **Tropical Animal Health and Production. 45: 771-779.** Impact factor (2011) 1.115
2. Olanratmanee, E., Nuntawan Na Ayudhya, S., Thanawongnuwech, R., Kunavongkrit, A. and Tummaruk, P., 2013. Reproductive parameters following a PRRS outbreak where a whole-herd PRRS MLV vaccination strategy was instituted post-outbreak. **Tropical Animal Health and Production. 45: 1099-1106.** Impact factor (2011) 1.115

การประชุมวิชาการระดับนานาชาติ

1. Tummaruk, P., Olanratmanee, E., Tantilertcharoen, R., Thanawongnuwech, R., 2012. Seasonal influence on the prevalence of porcine reproductive and respiratory syndrome virus in Thailand. Proc. 22nd International Pig Veterinary Society Congress, 10-13 June, 2012, Jeju, South-Korea. P. 1032.
2. Phoophitphong, D., Olanratmanee, E., Srisuwatanasagul, S., Wangnaitham, S., Thanawongnuwech, R., Tummaruk, P., 2012. Follicle development and number of ovulation in the ovarian tissue of gilts infected by porcine reproductive and respiratory syndrome virus. Proc. 22nd International Pig Veterinary Society Congress, 10-13 June, 2012, Jeju, South-Korea. P. 277.
3. Olanratmanee, E., Wongyanin, P., Thanawongnuwech, R., Tummaruk, P., 2012. Detection of porcine reproductive and respiratory syndrome virus in aborted fetuses, mummified fetuses and stillborn piglets in Thailand using real-time PCR. Proc. 22nd International Pig Veterinary Society Congress, 10-13 June, 2012, Jeju, South-Korea. P. 1034.
4. Olanratmanee, E., Thanawongnuwech, R., Kunavongkrit, A., Tummaruk, P., 2013. Preweaning mortality in porcine reproductive and respiratory syndrome virus (PRRSV) sero-positive herds in Thailand. Proc. 38th International Conference on Veterinary Science 2013 FAO Joint Symposium. 16-18 January 2013, Grand Diamond Ballroom, IMPACT Forum, Muang Thong Thani, Thailand. P. 316-318.
5. Phoophitphong, D., Olanratmanee, E., Srisuwatanasagul, S., Tummaruk, P., 2013. Effect of PRRS virus infection in the ovarian tissue on follicle growth in prepubertal and pubertal gilts. Proc. 38th International Conference on Veterinary Science 2013

FAO Joint Symposium. 16-18 January 2013, Grand Diamond Ballroom, IMPACT Forum, Muang Thong Thani, Thailand. P. 311-313.

6. Tienthai, P., Borhirunrat, W., Chalemchaikit, C., Kootpetch, P., Laprom, W., Tummaruk, P., 2013. Porcine reproductive and respiratory syndrome virus causes histological changes in endometrium of culling gilts. Proc. 38th International Conference on Veterinary Science 2013 FAO Joint Symposium. 16-18 January 2013, Grand Diamond Ballroom, IMPACT Forum, Muang Thong Thani, Thailand. P. 319-321.

การประชุมวิชาการระดับชาติ

1. Phoophitphong, D., Olanratmanee, E., Srisuwatanasagul, S., Tummaruk, P., 2013. Impact of PRRS virus detection in the ovarian tissue of replacement gilts on granulosa cells proliferation in the developing follicles. Proc. 51th Kasetsart University Annual Conference, 5-7 February 2013, Bangkok, Thailand, 7 pages.

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REGULAR ARTICLES

Porcine reproductive and respiratory syndrome virus detection in Thailand during 2005–2010 in relation to clinical problems, pig types, regions, and seasons

Padet Tummaruk · Pannin Surapat · Sutharat Sriariyakun · Oraphan Seemakram · Em-on Olanratmanee · Rachod Tantilertcharoen · Roongroj Thanawongnuwech

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Abstract The objectives of the present study were to determine the prevalence of porcine reproductive and respiratory syndrome virus (PRRSV) in Thailand between 2005 and 2010. The study was conducted by retrospectively investigating the detection of PRRSV from different pig types including boars, sows, piglets, nursery pigs, and fattening pigs from six regions of Thailand, i.e., the northern, eastern, northeastern, central, western, and southern parts. The data were obtained from cases submitted to the Chulalongkorn University Veterinary Diagnostic Laboratory for PRRSV detection between 2005 and 2010. Frequency analyses and generalized linear models were used to evaluate the prevalence of PRRSV in relation to various factors. In total, 2,273 tissues (n=636), semen (n=210) and serum (n=1,427) samples were included. PRRSV was detected in 32.6 % (740/2,273) of the pigs. The virus was found in 43.1 %, 15.7 %, and 30.3 % in the tissues, semen, and serum samples, respectively (P<0.001). The prevalence of PRRSV was highest in 2005 (43.6 %) and lowest in 2009 (23.6 %) (P<0.001). The prevalence of PRRSV was highest in nursery pigs (43.7 %) and lowest in boars (15.4 %) (P<0.001). The prevalence of PRRSV in the hot season (34.9 %) was higher than that found in the cool season (28.1 %, P=0.018) but did not differ significantly compared to rainy season (34.0 %, P=0.486). The strain of PRRSV isolated in the present study was genotype 2 (54.5 %), genotype 1 (31.0 %), and mixed genotypes (14.5 %). It can be concluded that PRRSV was detected in the tissue samples more frequently than the semen and serum samples. The prevalence of PRRSV was high in the nursery pigs. A high prevalence of PRRSV was found in the hot season, indicating that climatic factors may also contribute to the prevalence of PRRSV in Thailand. Of all the PRRSV detected, 31.0 %, 54.5 %, and 14.5 % belonged to genotype 1, genotype 2, and mixed genotypes, respectively.

Keywords Pig · Reproduction · Prevalence · PRRSV · RT-PCR

P. Tummaruk (✉) · P. Surapat · S. Sriariyakun · O. Seemakram · E. Olanratmanee
Faculty of Veterinary Science, Department of Obstetrics, Gynecology and Reproduction, Chulalongkorn University, Bangkok, Thailand 10330
e-mail: Padet.t@chula.ac.th

R. Tantilertcharoen
Faculty of Veterinary Science, Veterinary Diagnostic Laboratory, Chulalongkorn University, Bangkok, Thailand 10330

R. Thanawongnuwech
Faculty of Veterinary Science, Department of Pathology, Chulalongkorn University, Bangkok, Thailand 10330

Introduction

Late pregnancy loss (e.g., late term abortion and premature birth) in gestating gilts and sows is influenced by either infectious or noninfectious causes. Under field conditions, infectious agents directly cause reproductive disturbance in adult female pigs accounting for 30–40 %, while noninfectious causes (e.g., toxin, environment, and stress) account for up to 60–70 % of the clinical observations (Maldonado et al. 2005). Over the past decades, major infectious agents causing reproductive disturbance in pigs include porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus (ADV), porcine parvovirus (PPV), enterovirus, classical swine fever virus, encephalomyocarditis and porcine circovirus type 2 (PCV2) (O'Connor et al. 2001; Maldonado et al.

2005; Tummaruk and Tantilertcharoen 2012). Infection with these viruses leads to transplacental infection and cause fetal mortality (Christianson 1992). In Spain, Maldonado et al. (2005) investigated the prevalence of PRRSV, ADV, PPV, and PCV2 infections in 293 tissue samples from 100 clinical cases of sows with aborted fetuses and stillborn piglets and could detect only 9 % of PRRSV and 1 % of PRRSV in combination with PCV2, while neither ADV nor PPV was detected. The authors stated that, under field conditions, PRRSV remains the most common virus in aborted fetuses and stillborn piglets in Spain.

In general, PRRSV is classified into two genotypes, i.e., European (EU, genotype 1) and North American (NA, genotype 2) genotypes (Meng 2000). PRRSV develops and replicates in the macrophage in the lung and other visceral organs including the uterus of gilts (Karniychuk et al. 2011; Olanratmanee et al. 2011b). The common reproductive clinical symptoms of PRRSV infection include abortion, premature birth, stillborn piglets, weak-born piglets and a high pre-weaning mortality rate in suckling piglets due to secondary infection (Baysinger et al. 1997; Lager et al. 2003; Scortti et al. 2006). PRRSV vaccination has been introduced to many commercial pig farms around the world. Nevertheless, the efficacy of PRRSV vaccine has not yet been guaranteed in many countries and is still under investigation (Lager et al. 2003; Scortti et al. 2006; Martelli et al. 2009). Most of the modified live PRRSV vaccination trials have been conducted in nursery and fattening pigs, while limited number of the trials has been done in gestating gilts and sows (Dewey et al. 1999; Scortti et al. 2006). In Canada, it has been shown that the use of modified live PRRSV vaccination causes reproductive failure in pregnant sows if the vaccination is implemented during the last 4 weeks of the gestation period (Dewey et al. 1999). The reproductive failures induced by vaccination include a decrease in the number of piglets born alive per litter, a decrease in the number of weaning piglet and an increase in the proportion of stillborn and mummified fetuses per litter (Dewey et al. 1999). The efficiency of modified live PRRSV vaccination also depends on the genetic variation of the PRRSV field strains (Labarque et al. 2004). The antigenicity difference between genotype 1 (EU) and genotype 2 (NA) strains is one of the most important factors for unsuccessful vaccination in many commercial pig herds. In general, the EU strain is similar to the Lelystad virus in The Netherlands, while the NA strain is similar to VR2332 virus in the USA. Moreover, a high genetic variation within each strain is also found (Amonsin et al. 2009).

In Thailand, many commercial swine herds are infected with PRRSV and many strategies to control either respiratory or reproductive failures have been implemented. Nevertheless, clinical problems as well as reproductive failure caused by PRRSV are still frequently observed under field conditions, and it has become one of the most important diseases causing economic loss in the Thai swine industry over the last decade.

During 2011, 2,877,592 swine breeders (gilts, sows, and boars) are registered to the department of livestock development, ministry of agriculture and cooperative, Bangkok, Thailand. Of these pigs, 10.2 %, 16.7 %, 20.1 %, 21.6 %, 19.9 %, and 11.5 % are distributed in the central, eastern, northern, western, northern, and southern parts of Thailand, respectively. Most of the swine herds are medium–large independent farrowing-to-finishing producers. In general, all segments of the swine commercial herds can submit the samples to Chulalongkorn University Veterinary Diagnostic Laboratory (CU-VDL) in Bangkok. However, most of the cases are obtained from the central, eastern, and western regions of Thailand due to a short distance between the farm and the laboratory. In general, polymerase chain reaction (PCR) is a method routinely used for PRRSV detection at CU-VDL for many years. PCR is a method highly specific for PRRSV detection. A direct detection of PRRSV can be obtained via PCR based on reverse transcription of the viral RNA coupled to DNA amplification by PCR (Suarez et al. 1994). This method has been stabilized for PRRSV detection in Thailand since 2004 (Thanawongnuwech et al. 2004). The objective of the present study was to retrospectively investigate the prevalence of PRRSV in commercial swine herds in Thailand between 2005 and 2010 in relation to pig types, tissue sample, year, season, and herd locations.

Material and methods

Data

Data from 2,273 samples were collected from the CU-VDL, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand during the period from January 2005 to December 2010. The samples were sent to the CU-VDL for PRRSV detection. The types of sample were classified into three groups including tissue (i.e., lung, tonsil, spleen, liver, etc.) (n=636), semen (n=210), and serum (n=1,427). Data included date of sample submission to the laboratory, geographical location of the herds (northern, eastern, northeastern, central, western, and southern parts of Thailand), and pig types (boars, sows, piglets, nursery pigs, and fattening pigs). The data were manually collected from the laboratory registration book from 2005 to 2010; an individual case was manually recorded in a computer software program (Microsoft Excel 2007, WA, USA.). Types of sample and pig types were identified from the registration and the history book of the submitted cases. Season of the year was classified as hot (February-May), rainy (June-September) and cool (October–January).

Definition

Clinical cases were classified into two major groups, i.e., reproductive and respiratory problems. Semen samples from boars as well as serum and tissue samples from boars, sows, and suckling piglets were defined as "reproductive cases," and samples from nursery and fattening pigs were defined as "respiratory cases." Cases submitted as pooled samples were regarded as one single case. The results of PRRSV detection were regarded as a binomial trait, i.e., '0' was PRRSV negative and '1' was PRRSV positive samples. The identified genotype of the virus was classified into three categories, i.e., genotype 1, genotype 2, and mixed genotypes (i.e., both genotypes 1 and 2 were detected).

Reverse transcriptase–polymerase chain reaction

The detection of PRRSV was conducted by routine reverse transcriptase–polymerase chain reaction (RT-PCR) at the CU-VDL as described by Thanawongnuwech et al. (2004). Briefly, one-step RT-PCR (Qiagen, USA) was used to amplify the common genome of ORF 1b using the thermoregulator PTC-200 (MJ Research, USA). A total of 2 μL of the PCR product was utilized as a template in the nested multiplex PCR with the same conditions. The sizes of the expected multiplex PCR products (ORF 1b) were 186 and 107 bp for genotypes 1 and 2, respectively. Positive control of both genotypes 1 and 2 were included in each analysis. The Lelystad and SVI275 viruses were used as the positive controls for genotype 1 and 2, respectively. The viral detection result of each case was also identified as genotype 1, genotype 2, and mixed genotypes. A phylogenetic tree of PRRSV isolated in Thailand and published in NCBI database during 2003–2011 (62 Thai isolates nucleotide sequence of ORF5 gene and 13 isolates from other countries) was analyzed by MEGA 5.5 and was constructed by neighbor joining method and proved by bootstrap method.

Statistical analyses

The statistical analyses were carried out using SAS (SAS version 9.0, Cary, NC, USA.). Frequency analysis was conducted for the presence or absence of PRRSV and the frequency of each genotype (genotypes 1, 2, and mixed genotypes) using PROC FREQ of SAS. The prevalence of PRRSV detection in each category was expressed as a percentage. The generalized linear models were conducted to evaluate the effect of various factors that influence the prevalence of PRRSV using the generalized linear model procedure (PROC GENMOD) of SAS. The generalized linear models allowed the mean of a population to depend on a linear predictor through a nonlinear link function. The statistical models included three logistic models for binary data. Generalized estimating equations were used to analyze the data. Three statistical models were conducted to analyze the influence of multiple factors influencing the proportion of PRRSV detection. Model 1 included the effect of sample types (tissue, semen, and serum), the pig types (boars, sows, piglets, nursery pigs, fatteners, and unidentified group) nested within samples types, the year of the sample collection (2005–2010), the month of the sample collection (January to December) and the herd location (northern, eastern, northeastern, central, western, and southern parts). Model 2 included the effect of clinical cases (reproductive and respiratory cases), year, month, and herd location. Model 3 included the effect of sample types (tissue, semen, and serum), the pig types (boars, sows, piglets, nursery pigs, fattening pigs, and unidentified group) nested within sample types, year, season (hot, rainy, and cool), and herd location. The binomial data were transformed to logit number, and least square means were estimated based on binomial distribution analyses. Maximum likelihood fitting method was used to estimate the scale parameter; the scaled deviance was used to assess the statistical models. The least square means were compared using least significant different test. $P<0.05$ were regarded to be statistically significant.

Results

PRRSV detection

Of all the samples (n=2,273), PRRSV was found in 740 samples (32.6 %). The percentage of PRRSV detection differed among types of samples (Table 1). A higher percentage of PRRSV was found in the tissue sample (43.1 %) than the semen (15.7 %, $P<0.001$) and the serum samples (30.3 %, $P=0.002$). The percentage of PRRSV detection in semen was lower than in the serum samples ($P=0.02$) (Table 1). PRRSV was found in 15.4 % (35/227) of the boars, 37.5 % (54/144) of the gilts and sows, 35.9 % (61/170) of the piglets, 43.7 % (160/366) of the nursery pigs,

Table 1 Percentage of positive samples and percentage of PRRSV strains by type of sample

Type of sample	Number of samples	Number positive (%)	Percent of positive samples by strain		
			EU	NA	Mixed
Tissue	636	43.1a	23.7	62.6	13.7
Semen	210	15.7b	29.0	64.5	6.5
Serum	1,427	30.3c	35.5	48.8	15.7
All	2,273	32.6	31.0	54.5	14.6

Different letters within a column differ significantly ($P<0.05$)

and 26.6 % (54/203) of the fattening pigs (Table 2). The percentage of PRRSV-positive samples obtained from respiratory cases (37.6 %) was higher than those from reproductive cases (27.7 %) (Table 3). The percentages of PRRSV-positive samples by strain for both reproductive and respiratory cases are present in Table 3.

Effect of herd location

Herd location significantly influenced the prevalence of PRRSV (Fig. 1). PRRSV was detected in 35.1 % (66/188), 32.7 % (222/680), 30.5 % (170/558) of the samples collected from the eastern, central, and western parts of Thailand, respectively. Of the positive samples, the highest proportion of genotype 1 (53.3 %) was found in the northern part, while the highest proportion of genotype 2 (57.8 %) was found in the central part of Thailand. The proportions of genotype 2 were 46.9 %, 55.6 %, 57.5 %, and 0 % in the eastern, northern, northeastern, western, and southern parts of Thailand, respectively.

Effect of season, month, and year

The prevalence of PRRSV in the hot season (34.9 %) was higher than that found in the cool season (28.1 %, P=0.018) but did not differ significantly compared to rainy seasons (34.0 %, P=0.486). The prevalence of PRRSV in the rainy season tended to be higher than that found in the cool season (P=0.062) (Table 4). The number of PRRSV detections by month is demonstrated in Fig. 2. As shown in the figure, the detection of PRRSV dramatically increased from April to July and then slowly declined from July to December (Fig. 2). The prevalence of PRRSV also differed among years. The prevalence of PRRSV in 2005 and 2007 was significantly higher than other years (Table 5). Number of PRRSV isolations by years is presented in Fig. 3. It was found that the highest number of PRRSV isolations was observed in 2005 and then continuously declined until 2009. In 2010, a slight increase in the number of PRRSV isolations was observed (Fig. 3).

Table 2 Percentage of positive samples and percentage of PRRSV strains by pig types

Pig types	Number of samples	Number positive (%)	Percent of positive samples by strain		
			EU	NA	Mixed
Boar	227	15.4a	33.3	60.6	6.1
Sow	144	37.5bc	18.9	41.5	39.6
Piglet	170	35.9bc	22.9	65.6	11.5
Nursery	366	43.7c	39.9	46.2	13.9
Fattening pig	203	26.6b	37.0	53.7	9.3

Different letters within a column differ significantly (P<0.05)

Table 3 Percentage of positive samples and percentage of PRRSV strains by clinical problems (reproductive and respiratory problems)

Clinical problems	Number of samples	Number positive (%)	Percent of positive samples by strain		
			EU	NA	Mixed
Reproductive	541	27.7a	23.8	55.8	20.4
Respiratory	569	37.6b	39.2	48.1	12.7

Different letters within a column differ significantly (P<0.05)

PRRSV genotypes

The genotypes of PRRSV isolated in the present study are demonstrated in Table 1. Of all the isolations (n=740), genotype 1, genotype 2, and mixed genotypes accounted for 31.0 %, 54.5 %, and 14.6 %, respectively. However, the proportion of PRRSV genotypes varied according to type of the sample (Table 1), the age group of the pigs (Table 2), clinical symptoms (Table 3), regions, seasons (Table 4), and years (Table 5). For instance, 64.5 % of the PRRSV that were isolated from semen was classified as genotype 2, while only 48.8 % of the virus isolated from serum was classified as genotype 2. PRRSV genotype 2 was found in 55.8 % of the cases associated with reproductive problems, while it was found in 48.1 % of the cases involving respiratory problems (Table 3). The detection of mixed genotypes was found in 20.4 % of the samples collected from reproductive problems cases (boars, sows, and piglets) and in 12.7 % of the samples collected from respiratory problems cases (nursery pigs and fattening pigs). Furthermore, the percentage of PRRSV genotypes varied among years. In 2005, PRRSV genotype 2 was detected in 39.8 % of the samples, while in 2010, PRRSV genotype 2 was detected in up to 95.5 % of the samples (Table 5). Interestingly, the proportion of PRRSV genotype 2 dramatically increased from 2008 onwards (Table 5). A phylogenetic tree of PRRSV isolated in Thailand is presented in Fig. 4.

Discussion

PRRSV has been serologically detected in Thai commercial swine herds since 1989 (Oraveerakul et al. 1995). Nowadays, both genotypes 1 (EU) and 2 (NA) have been isolated and have been comprehensively investigated in Thailand over the last decade (Thanawongnuwech et al. 2004; Amonsin et al. 2009; Thanawongnuwech and Suradhat 2010). During 2000–2003, a study based on 137 PRRSV isolates in Thailand found that 66.4 % of the

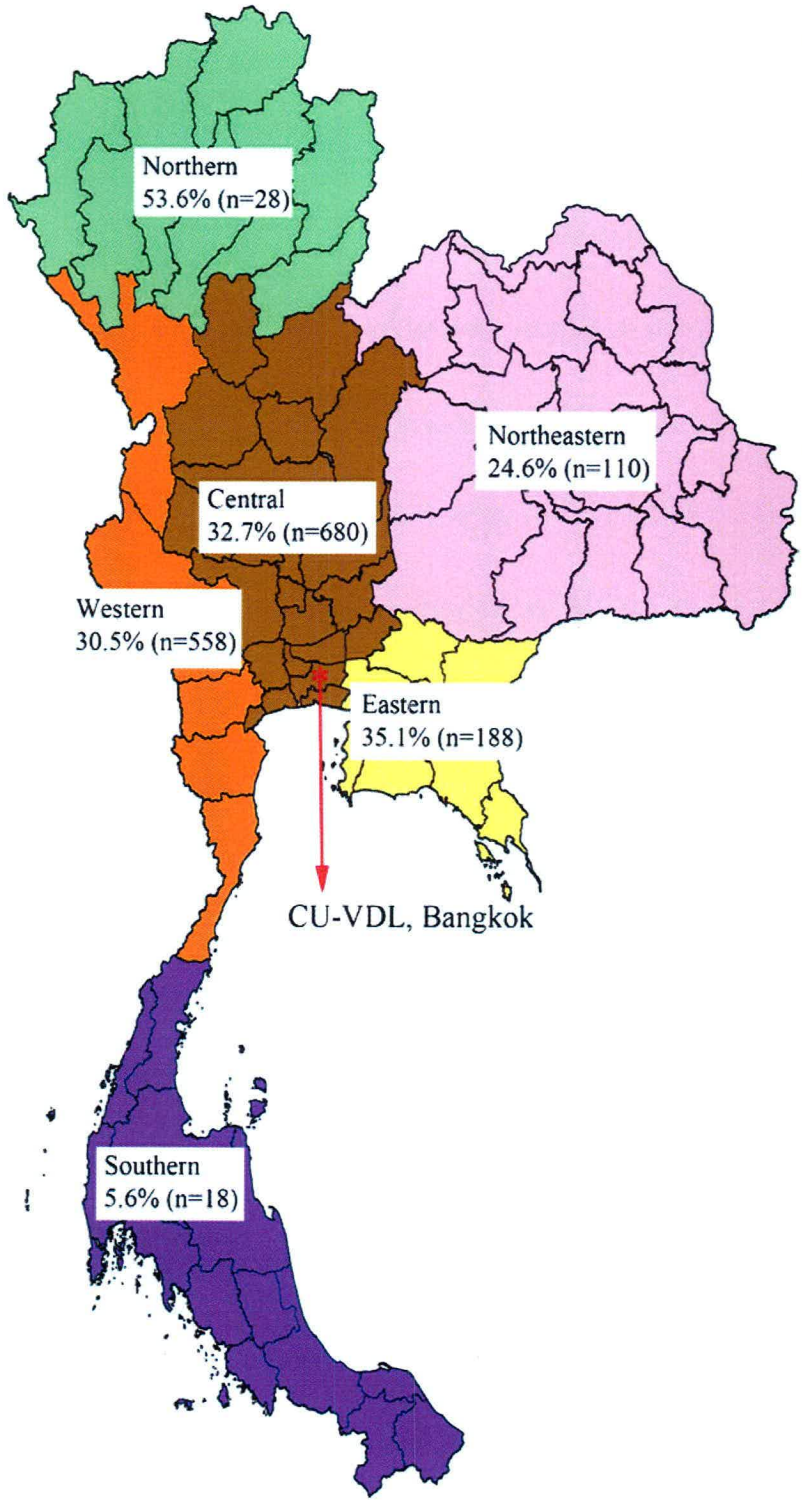

Fig. 1 Number of samples and percentage of positive samples of PRRSV by region in Thailand during 2005–2010

PRRSV isolated belong to genotype 1 and 33.6 % belong to genotype 2 (Thanawongnuwech et al. 2004). In contrast, the present study revealed that genotype 2 has become dominantly seen during 2005–2010, especially from 2008 onwards. In general, most of the great grandparent stock of pigs in Thailand was imported from Europe, especially from Denmark. Therefore, the PRRSV genotype 1 might have been introduced from European countries. However, in 2007, the PRRSV modified live virus vaccine belonging to genotype 2 was registered and became commonly practiced in the Thai commercial swine herds. PRRSV genotype 2, therefore, might have been quickly distributed due to the

Table 4 Percentage of positive samples and percentage of PRRSV strains by season

Season	Number of samples	Number positive (%)	Percent of positive samples by strain		
			EU	NA	Mixed
Cool	665	28.1a	21.3	70.5	8.2
Hot	667	34.9b	33.1	50.8	16.1
Rainy	941	34.0ab	34.9	47.9	17.1

Different letters within a column differ significantly ($P<0.05$)

Table 5 Number of samples, percentage of positive samples and percentage of PRRSV strains in Thailand from 2005 to 2010

Year	Number of samples	Number positive (%)	Percent of positive samples by strain		
			EU	NA	Mixed
2005	530	43.6a	38.5	39.8	21.7
2006	630	26.5b	41.5	43.8	14.8
2007	357	43.4a	31.8	48.0	20.1
2008	307	25.1c	11.8	86.8	1.3
2009	182	23.6c	19.0	78.6	2.4
2010	267	25.1bc	4.6	95.5	0.0

Different letters within a column differ significantly ($P<0.05$)

implementation of the PRRSV modified live virus vaccine in many commercial swine herds in Thailand since then. Additionally, PRRSV genotype 2 was introduced to Denmark after 1996 due to the use of PRRSV modified live virus vaccination based on genotype 2 of virus (Mortensen et al. 2002). Since then, both genotypes 1 and 2 of PRRSV have been also widely distributed among the Danish swine herds and also in some neighboring European countries (Beilage et al. 2009; Noremark et al. 2009). It is well documented that viral shedding from vaccinated pigs is commonly observed during the first few weeks after PRRS modified live virus vaccination (Alexopoulos et al. 2005; Scortti et al. 2006; Kim et al. 2009; Thanawongnuwech and Suradhat 2010; Olanratmanee et al. 2011a). Hence, the PRRSV isolates in the present study might, at least in part, belong to the vaccine strain. The isolation of the vaccine strain of PRRSV under field conditions has been shown by a genetic analysis on ORF5 of PRRSV in Korea (Kim et al. 2009). Furthermore, the high frequency of PRRSV detection during this period indicates that PRRS is a common disease in the commercial swine herds of Thailand.

In the present study, detection of a single genotype of PRRSV (either genotype 1 or genotype 2) was more common than mixed genotypes. The detection of mixed genotypes was found in the samples collected from reproductive problem cases (boars, sows, and piglets) more than those collected from respiratory problem cases (nursery pigs and fattening pigs). The reasons might be that sows and boars, in most cases, stay in the herd for a longer period than nursery pigs and fattening pigs. Thus, they might have been exposed to several more PRRSV genotypes than nursery pigs or fattening pigs. Recently, Tummaruk and Tantilertcharoen (2012) demonstrated that replacement gilts have been exposed to PRRSV rather early in their life, either before, during, or after acclimatization as indicated by the seroconversion against PRRSV both in non-vaccinated and vaccinated herds. Furthermore, the replacement gilts are an important source of introducing a new strain of PRRSV into the breeding herds. Olanratmanee et al. (2011b) demonstrated that the virus could be found in the uterine tissue of gilts with either high or low antibody titer up to 11 months of age. Furthermore, in some cases, coinfection of PRRSV and PPV and/or ADV might possibly occur in the replacement gilts (Tummaruk and Tantilertcharoen 2012). This may cause a more complicated situation and lead to inferior subsequent reproductive performance in the gilts because PRRSV has been regarded as an immune suppressive pathogen (Thanawongnuwech and Suradhat 2010). These studies imply that understanding the factors that influence the

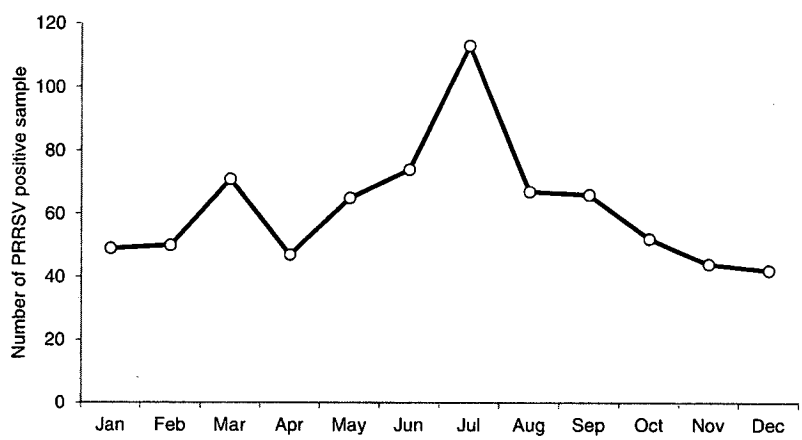

Fig. 2 Number of PRRSV detection by month

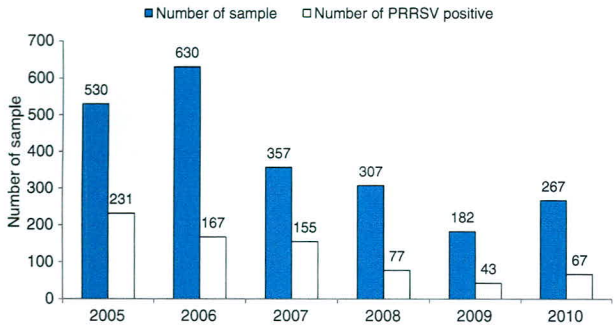

Fig. 3 Number of tested samples and number of PRRSV-positive samples from 2005 to 2010

prevalence of PRRSV is an important issue to be addressed in order to modify the management strategy of PRRSV control in Thailand.

In the present study, an increase in the number of PRRSV detections was found from April to July. This synchronized with an increase of infertility problems in gilts and sows mated during this period of the year (Tummaruk et al. 2004; Tummaruk et al. 2010a,b). Tummaruk et al. (2010a) has demonstrated that gilts and sows that were mated during the hot season and farrowed during late autumn and the cool season had a significantly lower number of total piglets born per litter than gilts and sows that were mated in cool season. These findings indicate that an increment in the viral detection during hot season may possibly contribute to a poor reproductive performance of gilts and sows mated during hot season in Thailand. In addition, during hot seasons, the temperature–humidity index is relatively high (Tummaruk et al. 2010a), and this could possibly induce moderate to severe heat stress in the pigs. Viremia as well as viral infections might be easily obtained from an animal under

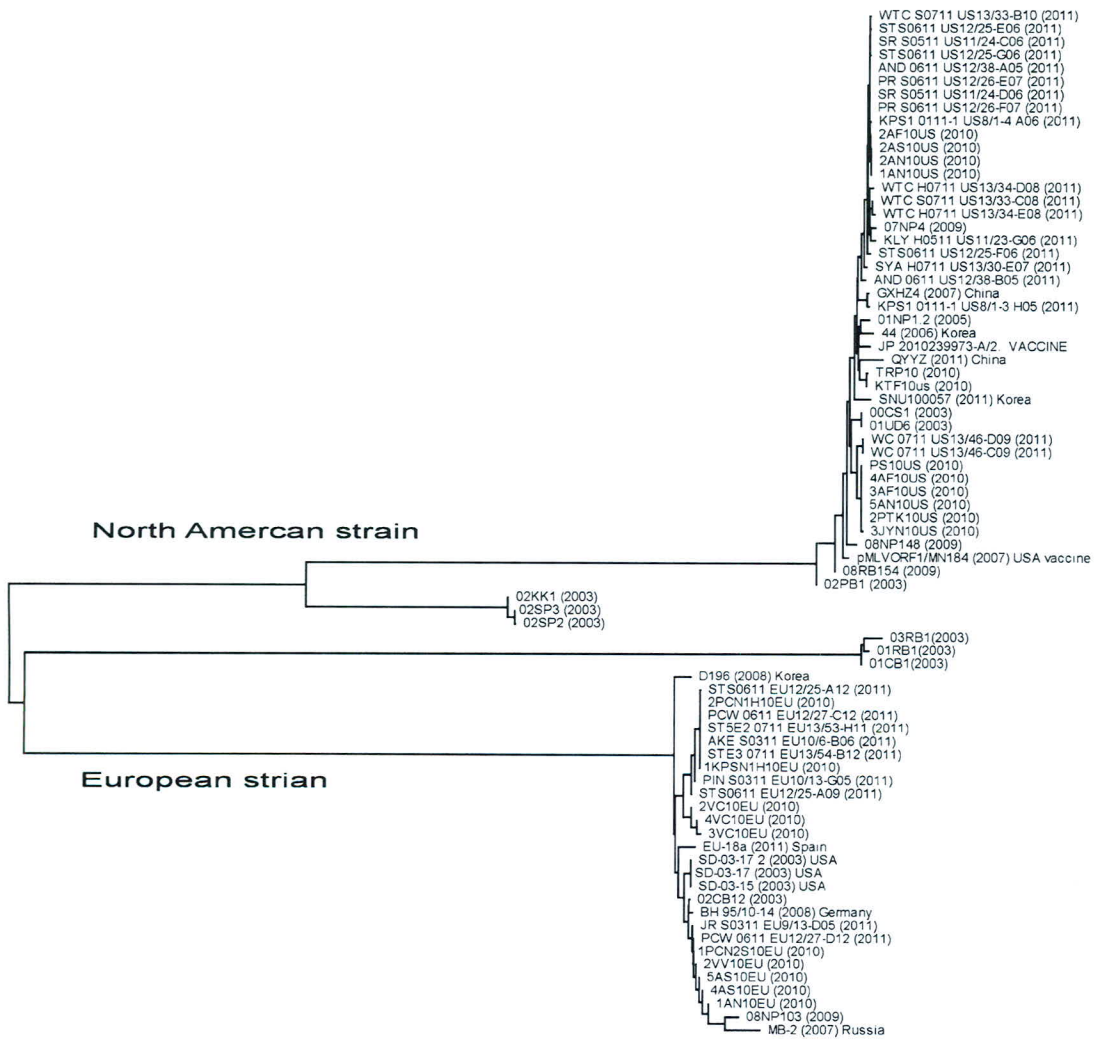

Fig. 4 Phylogenetic tree of PRRSV isolated in Thailand and published in NCBI database during 2003–2011 (62 Thai isolates nucleotide sequence of ORF5 gene and 13 isolates from other countries). The phylogenetic tree was analyzed by MEGA 5.5 and was constructed by neighbor joining method and proved by bootstrap method

stress due to its immunosuppressive conditions (Suradhat 2006). Furthermore, both high temperature and high humidity could be the favorable climatic condition for airborne transmission of the virus.

In Thailand, both genotype 1 and genotype 2 of PRRSV exist among Thai swine herds. The proportion of genotype 1 and 2 PRRSV also varies among regions. In general, the breed of pigs and management are similar among regions. However, the distribution of swine herds in northern and southern regions is not as dense as those in the western, eastern, and central regions. The climates are also different between the northern and southern regions. The relative humidity as well as the rain is more common in the southern than in the northern regions. In the northern and southern part of Thailand, genotype 1 can be isolated more frequently than genotype 2. On the other hand, genotype 2 is more dominant than genotype 1 in the rest of the country. This is in agreement with earlier studies in Thailand (Thanawongnuwech et al. 2004; Amonsin et al. 2009). However, genotype 2 of PRRSV is the dominant strain of PRRSV in other Asian countries, e.g., China (Li et al. 2010), Japan (Yoshii et al. 2005) and Korea (Cha et al. 2006; Kim et al. 2009). Li et al. (2010) analyzed the genetic diversity of 66 Chinese PRRSV field strains isolated from 1996 to 2009 and found that all of the tested samples belonged to genotype 2. Furthermore, the highly pathogenic PRRSV strains causing a high mortality rate in pigs in China in 2006 and in Southeast Asia in 2009–2010 also belonged to genotype 2 (Zhou and Yang 2010). In many countries in Europe, both genotypes 1 and 2 of PRRSV have also been detected (Mortensen et al. 2002; Beilage et al. 2009). But the presence of NA strains among European countries is, in most cases, associated with the introduction of North American type modified live virus vaccine (Mortensen et al. 2002; Beilage et al. 2009). In Germany, a study of postmortem examinations in 902 pigs found that 18.5 % of the samples were positive for wild-type EU strains, while EU and NA genotype vaccine viruses were also detected in 1.3 % and 8.9 % of the tissue samples, respectively (Beilage et al. 2009). These findings indicate that PRRSV genotypes in Thailand are closely related to European countries rather than other Asian countries and North America. A recent study on a complete nucleotide sequence of the Thai PRRSV isolates has suggested that the Thai EU isolate is likely to have evolved from the EU prototype, whereas the Thai NA isolate may originate from the vaccine virus or its derivatives (Amonsin et al. 2009). In addition, a recent phylogenetic study on PRRSV isolated in Thailand indicated that at least four independent introductions of PRRSV genotype 1 and three independent introductions of PRRSV genotype 2 had been observed (Tun et al. 2011). These findings indicate that PRRSV has been introduced to Thailand many times from both Europe and North America. The difference of PRRSV strains among regions might also be due to the fact that the Thai swine producers regularly import live pigs and/or semen from European countries for genetic improvement purposes. In addition, the North American type of the modified live virus vaccine are also continuously imported and have become nowadays commercially available in Thailand. Since the vaccine was registered in Thailand in 2007, the genotype 2 of PRRSV has become the most common genotype of the PRRSV isolates. The present study revealed that 78–95 % of the PRRSV isolated from 2008 to 2010 belong to genotype 2 (NA).

The limitations of the present study included the use of data from diagnostic laboratory to describe disease prevalence. The samples were mostly obtained from cases with suspicious PRRSV problems. Hence, the prevalence might be relatively high. Furthermore, the present data set was only based on samples collected from producers and veterinarians that choose to submit specimens to CU-VDL. Data from other laboratories were not included. Nevertheless, the CU-VDL is situated in the central part of Bangkok, and it is independent from private companies and producers. Thus, the analyses can be accomplished without any conflict of interest.

It can be concluded that the prevalence of PRRSV in Thailand between 2005 and 2010 accounted for 32.6 % of the cases submitted for viral detection. The prevalence of PRRSV varied according to the type of sample, the age group of the pigs, season, year, and herd location. PRRSV was detected in the tissue samples more frequently than the semen and serum samples. A high prevalence of PRRSV detection was found in the nursery pigs. A high prevalence of PRRSV was found in the hot season, indicating that climatic factors may also contribute to the prevalence of PRRSV in Thailand. Of all the PRRSV detected, 31.0 %, 54.5 %, and 14.5 % belonged to genotype 1, genotype 2, and mixed genotypes, respectively.

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References

Alexopoulos, C., Kritas, S.K., Kyriakis, C.S., Tzika E. and Kyriakis, S.C., 2005. Sow performance in an epidemically porcine reproductive and respiratory syndrome (PRRS)-infected farm after sow vaccination with an attenuated PRRS vaccine, Veterinary Microbiology, 111, 151–157.

Amonsin, A., Kedkovid, R., Puranaveja, S., Wongyanin, P., Suradhat, S. and Thanawongnuwech, R., 2009. Comparative analysis of complete nucleotide sequence of porcine reproductive and respiratory syndrome virus (PRRSV) isolates in Thailand (US and EU genotypes), Virology Journal, 6, 143.

Beilage, E.G., Nathues, H., Meemken, D., Harder, T.C., Doherr, M.G., Grotha, I. and Greiser-Wilke, I., 2009. Frequency of PRRS live vaccine virus (European and North American genotype) in vaccinated and non-vaccinated pigs submitted for respiratory tract diagnostics in North-Western Germany, Preventive Veterinary Medicine 92, 31–37.

Cha, S-H., Choi, E-J., Park, J-H., Yoon, S-R., Song, J-Y., Kwon, J-H., Song, H-J. and Yoon, K-J., 2006. Molecular characterization of recent Korean porcine reproductive and respiratory syndrome (PRRS) viruses and comparison to other Asian PRRS viruses, Veterinary Microbiology 117, 248–257.

Christianson, W.T., 1992. Stillbirth, mummies, abortions and early embryonic death, Veterinary Clinical of North America: Food Animal Practice 8, 623–639.

Dewey, C.E., Wilson, S., Buck, P. and Leyenaar, J.K., 1999. The reproductive performance of sows after PRRS vaccination depends on stage of gestation, Preventive Veterinary Medicine, 40, 233–241.

Karniychuk, U.U., Saha, D., Geldhof, M., Vanhee, M., Cornillie, P., van den Broeck, W. and Nauwynck, H.J., 2011. Porcine reproductive and respiratory syndrome virus (PRRSV) causes apoptosis during its replication in fetal implantation sites, Microbial Pathogenesis, 51, 194–202.

Kim, H.K., Yang, J.S., Moon, H.J., Park, S.J., Luo, Y., Lee, C.S., Song, D.S., Kang, B.K., Ann, S.K., Jun, C.H. and Park, B.K., 2009. Genetic analysis of ORF5 of recent Korean porcine reproductive and respiratory syndrome viruses (PRRSVs) in viremic sera collected from MLV-vaccinating or non-vaccinating farms, Journal of Veterinary Science, 10, 121–130.

Labarque, G., van Reeth, K., Nauwynck, H., Drexler, C., Van Gucht, S. and Pensaert, M., 2004. Impact of genetic diversity of European-type porcine reproductive and respiratory syndrome virus strains on vaccine efficacy, Vaccine 22, 4183–4190.

Lager, K.M., Mengeling, W.L. and Wesley, R.D., 2003. Strain predominance following exposure of vaccinated and naïve pregnant gilts to multiple strains of porcine reproductive and respiratory syndrome virus, Canadian Journal of Veterinary Research, 67, 121–127.

Li, B., Fang, L., Liu, S., Zhao, F., Jiang, Y., He, K., Chen, H. and Xiao, S., 2010. The genomic diversity of Chinese porcine reproductive and respiratory syndrome virus isolates from 1996 to 2009, Veterinary Microbiology, 146, 226–237.

Maldonado, J., Segalés, J., Martinez-Puig, D., Calsamiglia, M., Domingo, M. and Artigas, C., 2005. Identification of viral pathogens in aborted fetuses and stillborn piglets from cases of swine reproductive failure in Spain, The Veterinary Journal, 169, 454–456.

Martelli, P., Goziob, S., Ferraria, L., Rosinaa, S., Angelisa, E.D., Quintavallaa, C., Bottarellia, E. and Borghettia, P., 2009. Efficacy of a modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in pigs naturally exposed to a heterologous European (Italian cluster) field strain: Clinical protection and cell-mediated immunity, Vaccine 27, 3788–3799.

Meng, X.J., 2000. Heterogeneity of porcine reproductive and respiratory syndrome virus: implications for current vaccine efficacy and future vaccine development, Veterinary Microbiology 74, 309–324.

Mortensen, S., Stryhn, H., Sogaard, R., Boklund, A., Stark, K.D.C., Christensen, J. and Willeberg, P., 2002. Risk factors for infection of sow herds with porcine reproductive and respiratory syndrome (PRRS) virus, Preventive Veterinary Medicine, 53, 83–101.

Noremark, M., Lindberg, A., Vagsholm, I. and Lewerin, S.S., 2009. Disease awareness, information retrieval and change in biosecurity routines among pig farmers in association with the first PRRS outbreak in Sweden, Preventive Veterinary Medicine, 90, 1–9.

O'Connor, B., Gauvreau, H., West, K., Bogdan, J., Ayroud, M., Clark, E.G., Konoby, C., Allan, G. and Ellis, J.A., 2001. Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit, Canadian Veterinary Journal, 42, 551–553.

Olanratmanee, E., Thanawongnuwech, R., Kunavongkrit, A. and Tummaruk, P., 2011a. Detection of porcine reproductive and respiratory syndrome virus (PRRSV) in the serum of gilts and sows after modified-lived PRRS vaccination, Proceeding of the 10th Chulalongkorn-Veterinary Annual Conference, page S40.

Olanratmanee, E., Wangnaitham, S., Thanawongnuwech, R., Kunavongkrit, A., Tummaruk, P., 2011b. Prevalence of porcine reproductive and respiratory syndrome virus (PRRSV) antigen positive uterine tissues in gilts culled due to reproductive disturbance in Thailand, Tropical Animal Health and Production, 43, 451–457.

Oraveerakul, K., Punarriwatana, D., Luengyosluechakul, S., Tantasuparuk W. and Kunavongkrit, A., 1995. The seroprevalence of porcine reproductive and respiratory syndrome (PRRS) virus among swine breeding farms in the central and north-eastern part of Thailand, The Thai Journal of Veterinary Medicine, 25, 233–240.

Scortti, M., Prieto, C., Simarro, I. and Castro, J.M., 2006. Reproductive performance of gilts following vaccination and subsequent heterologous challenge with European strains of porcine reproductive and respiratory syndrome vius, Theriogenology, 66, 1884–1893.

Suarez, P., Zardoya, R., Prieto, C., Solana, A., Tabares, E., Bautista, J.M. and Castro, J.M., 1994. Direct detection of the porcine reproductive and respiratory syndrome (PRRS) virus by reverse polymerase chain reaction (RT-PCR), Archives of Virology, 135, 89–99.

Suradhat, S., 2006. Relationships between the immune system and stress reactivity in pig: visualizing the immune-neuroendocrine framework in action, Thai Journal of Veterinary Medicine, 36, 9–18.

Thanawongnuwech, R. and Suradhat, S., 2010. Taming PRRSV: Revisiting the control strategies and vaccine design, Virus Research, 154, 133–140.

Thanawongnuwech, R., Amonsin, A., Tatsanakit, A. and Damrongwatanapokin, S., 2004. Genetics and geographical variation of porcine reproductive and respiratory syndrome virus (PRRSV) in Thailand, Veterinary Microbiology, 101, 9–21.

Tummaruk, P. and Tantilertcharoen, R., 2012. Seroprevalence of porcine reproductive and respiratory syndrome, Aujeszky's disease and porcine parvovirus in replacement gilts in Thailand, Tropical Animal Health and Production, 44, 983–989.

Tummaruk, P., Tantasuparuk, W., Techakumphu, M. and Kunavongkrit, A., 2004. Effect of season and outdoor climate on litter size at birth in purebred Landrace and Yorkshire sows in Thailand, Journal of Veterinary Medical Science, 66, 477–482.

Tummaruk, P., Tantasuparuk, W., Techakumphu, M. and Kunavongkrit, A., 2010a. Seasonal influence on the litter size at birth of pig are more pronounced in the gilt than sow litter, Journal of Agricultural Science, 148, 421–432.

Tummaruk, P., Tantasuparuk, W., Techakumphu, M. and Kunavongkrit, A., 2010b. Influence of repeat-service and weaning-to-first-service interval on farrowing proportion of gilts and sows, Preventive Veterinary Medicine 96, 194–200.

Tun, H.M., Shi, M., Wong, C.L.Y., Ayudhya, S.N.N., Amonsin, A., Thanawongnuwech, R. and Leung, F.C.C., 2011. Genetic diversity and multiple introductions of porcine reproductive and respiratory syndrome viruses in Thailand, Virology Journal, 8, 164.

Yoshii, M., Kaku, Y., Murakami, Y., Shimizu, M., Kato, K. and Ikeda, H., 2005. Genetic variation and geographic distribution of porcine reproductive and respiratory syndrome virus in Japan, Arch Virology, 150, 2313–2324.

Zhou, L. and Yang, H., 2010. Porcine reproductive and respiratory syndrome in China, Virus Research, 154, 31–37.

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REGULAR ARTICLES

Reproductive parameters following a PRRS outbreak where a whole-herd PRRS MLV vaccination strategy was instituted post-outbreak

Em-on Olanratmanee · Suparlark Nuntawan Na Ayudhya · Roongroje Thanawongnuwech · Annop Kunavongkrit · Padet Tummaruk

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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 (1.4 vs. 1.6 %), farrowing rate (83.8 vs. 90.0 %), total born (10.6 vs. 11.4 piglets/litter), liveborn (10.0 vs. 10.3 piglets/litter), stillbirths (4.6 vs. 7.0 %), mummies (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 liveborn and more mummies than the comparison group, whereas females vaccinated during late gestation had a lower farrowing rate. 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 abortions, stillborn piglets, and mummified fetuses, and the effect of vaccination on pregnant females.

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

E. Olanratmanee · P. Tummaruk (✉)
Department of Obstetrics, Gynecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330
e-mail: Padet.t@chula.ac.th

S. Nuntawan Na Ayudhya
Pfizer (Thailand) Limited, 323, Silom Rd,Bangrak, Bangkok, Thailand 10500

R. Thanawongnuwech
Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330

A. Kunavongkrit
The Office of the Commission on Agricultural Resource Education, Chulalongkorn University, Bangkok, Thailand 10330

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 mummified fetuses per litter, stillborn piglets per litter, 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 (Damrong watanapokin 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 in 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.

Materials and methods

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 PRRSV 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 ELISA response and viremia, (2) fertility parameters (farrowing rate, return rate, and abortion rate), and (3) litter parameters (total born, live born, stillbirths, and mummified fetuses).

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 (2 weeks before farrowing), classical swine fever (2 weeks after farrowing), Aujeszky's disease (mass vaccination every 4 months), and porcine parvovirus (gilts prior to placement in breeding herd, then 2 weeks after farrowing every 3rd parity).

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 3 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 return to estrus after mating, 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).

PRRSV vaccination and blood collection

On 15 May 2009, all gilts and sows in the herd were vaccinated with a PRRSV MLV vaccine at 3-week intervals, i.e., weeks 0 and 3. Thereafter, all gilts and sows (both pregnant and nonpregnant) were vaccinated every 3 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.

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.

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 farrowing rate (FR), the return rate (RR), and the abortion rate (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. Total born per litter (TB) was defined as the sum of born alive (BA) plus the number of stillborn piglets (SB) plus the number of mummified fetuses (MM). The percentage of SB and percentage of MM were calculated as the number of SB or MM 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 farrowing 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.

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).

Results

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 1). After mass vaccination, the percentage of seropositive 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.

Reproductive performance

Herd fertility parameters (FR, RR, and AR) and litter parameters (TB, BA, SB, and MM) over time are summarized in Fig. 1a, b and Tables 2 and 3, 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 per 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 %, $P=0.004$).

Table 1 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 ($P \leq 0.05$)

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 2). 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 2). 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 3). 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 3). 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 3). Preweaning 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 state of gestation at the time of vaccination (Tables 4 and 5). 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 4). 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 4). 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 5). However, TB and SB did not differ by parity or stage of gestation at the time of vaccination.

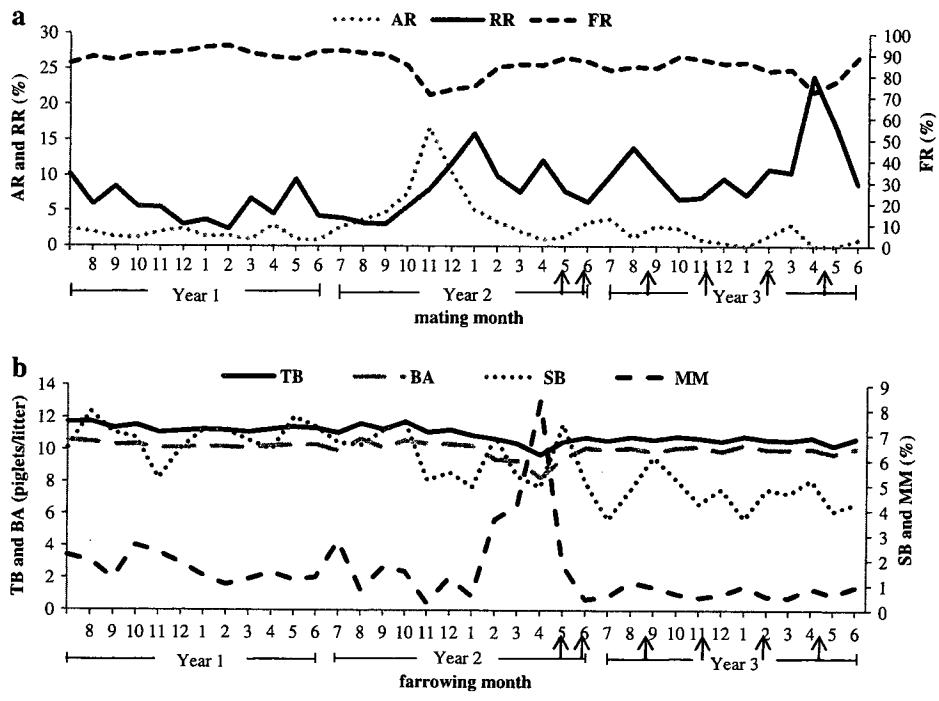

Fig. 1 **a** Farrowing rate (*FR*), abortion rate (*AR*), and return rate (*RR*); **b** the number of total piglets born per litter (*TB*), the number of piglets born alive per litter (*BA*), the percentage of stillborn piglets per litter (*SB*), and the percentage of mummified fetuses per litter (*MM*). *Arrow* indicates dates of PRRS MLV vaccination

Table 2 Comparison of fertility parameters 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
Farrowing rate (%)	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
Return rate (%)	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
Abortion rate (%)	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 ($P \leq 0.05$)

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 abortions and mummified fetuses, 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 PRRSV MLV vaccine, regardless of individual animals' stage in the reproductive cycle. In hindsight, taking this course of action 6 months earlier (at the peak of abortions) might have foreshortened overall reproductive losses (Fig. 1a).

Table 3 Fertility parameters 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
Farrowing rate (%)	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
Return rate (%)	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
Abortion rate (%)	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 ($P \leq 0.05$)

Table 4 Litter parameters (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
Total born	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
Born alive	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
Stillbirths (%)	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
Mummified fetuses (%)	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 ($P \leq 0.05$)

In agreement with previous reports, vaccination produced a measureable response both in terms of an increased proportion of seropositive 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 nonpregnant 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

Table 5 Litter parameters (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
Total born	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
Born alive	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
Stillborn (%)	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
Mummified fetuses (%)	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 ($P \leq 0.05$)

et al. 2005). Furthermore, vaccination against PRRSV has been shown to provide protection against reproductive losses. Scortti et al. (2006b) reported that inoculation of unvaccinated, seronegative gilts with PRRSV at 90 days of pregnancy resulted in 43.4 % stillborn piglets, 20 % 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 in 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 return rate 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 PRRSV 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.

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References

Alexopoulos, C., Kritas, S.K., Kyriakis, C.S., Tzika, E. and Kyriakis, S.C., 2005. Sow performance in an endemically porcine reproductive and respiratory syndrome (PRRS)-infected farm after sow vaccination with an attenuated PRRS vaccine, Veterinary Microbiology, 111, 151–157.

Amonsin, A., Kedkovid, R., Puranaveja, S., Wongyanin, P., Suradhat, S. and Thanawongnuwech, R., 2009. Comparative analysis of complete nucleotide sequence of porcine reproductive and respiratory syndrome virus (PRRSV) isolates in Thailand (US and EU genotypes), Virology Journal, 6, 143.

Cano, J.P., Dee, S.A., Murtaugh, M.P. and Pijoan, C., 2007. Impact of a modified-live porcine reproductive and respiratory syndrome virus vaccine intervention on a population of pigs infected with a heterologous isolate, Vaccine, 25, 4382–4391.

Cano, J.P., Dee, S.A., Murtaugh, M.P., Trincado, C.A. and Pijoan, C.B., 2007. Effect of vaccination with a modified-live porcine reproductive and respiratory syndrome virus vaccine on dynamics of homologous viral infection in pigs, American Journal of Veterinary Research, 68, 565–571.

Chung, W.B., Chang, W.F., Hsu, M. and Yang, P.C., 1997. Persistence of porcine reproductive and respiratory syndrome virus in intensive farrow-to-finish pig herds, Canadian Journal of Veterinary Research, 61, 292–298.

Damrongwatanapokin, S., Arsayuth, K., Kongkrong, C., Parchariyanon, S., Pinyochon, W. and Tantaswasdi, U., 1996. Serological studies and isolation of porcine reproductive and respiratory syndrome (PRRS) virus in Thailand, Journal of the Thai Veterinary Medical Association, 47, 19–31.

Dewey, C.E., Wilson, S., Buck, P. and Leyenaar, J.K., 2004. Effects of porcine reproductive and respiratory syndrome vaccination in breeding-age animals, Preventive Veterinary Medicine, 62, 299–307.

Foss, D.L., Zilliox, M.J., Meier, W., Zuckermann, F. and Murtaugh, M.P., 2002. Adjuvant danger signals increase the immune response to porcine reproductive and respiratory syndrome virus, Viral Immunology, 15, 557–566.

Martelli, P., Cordioli, P., Alborali, L.G., Gozio, S., Angelis, E.D., Ferrari, L., Lombardi, G. and Borghetti, P., 2007. Protection and immune response in pigs intradermally vaccinated against porcine reproductive and respiratory syndrome (PRRS) and subsequently exposed to a heterologous European (Italian cluster) field strain, Vaccine, 25, 3400–3408.

Martelli, P., Gozio, S., Ferrari, L., Rosina, S., De Angelis, E., Quintavalla, C., Bottarelli, E. and Borghetti, P., 2009. Efficacy of modified live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine in pigs naturally exposed to a heterologous European (Italian cluster) field strain: Clinical protection and cell-mediated immunity, Vaccine, 27, 3788–3799.

Meng, X.J., 2000. Heterogeneity of porcine reproductive and respiratory syndrome virus: implications for current vaccine efficacy and future vaccine development, Veterinary Microbiology, 74, 309–324.

Mengeling, W.L., Lager, K.M., Vorwald, A.C. and Clouser, D.F., 2003. Comparative safety and efficacy of attenuated single-strain and multi-strain vaccines for porcine reproductive and respiratory syndrome, Veterinary Microbiology, 93, 25–38.

Murtaugh, M.P., Xiao, Z. and Zuckermann, F., 2002. Immunological responses of swine to porcine reproductive and respiratory syndrome virus infection, Viral Immunology, 15, 533–547.

Nielsen, J., Botner, A., Bille-Hansen, V., Oleksiewicz, M.B. and Storgaard, T., 2002. Experimental inoculation of late term pregnant sows with a field isolate of porcine reproductive and respiratory syndrome vaccine-derived virus, Veterinary Microbiology, 84, 1–13.

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, Animal Reproduction Science, 122, 42–51.

Oraveerakul, K., Punarriwatana, D., Luengyosluechakul, S., Tuntasuparak, W. and Kunavongkrit, A., 1995. The seroprevalence of porcine

reproductive and respiratory syndrome (PRRS) virus among swine breeding farms in the central and north-eastern part of Thailand, Thai Journal of Veterinary Medicine, 25, 233–240.

Scortti, M., Prieto, C., Martinez-Lobo, F.J., Simarro, I. and Castro, J.M., 2006a. Effects of two commercial European modified-live vaccines against porcine reproductive and respiratory syndrome viruses in pregnant gilts, The Veterinary Journal, 172, 506–514.

Scortti, M., Prieto, C., Simarro, I. and Castro, J.M., 2006b. Reproductive performance of gilts following vaccination and subsequent heterologous challenge with European strains of porcine reproductive and respiratory syndrome virus, Theriogenology, 66, 1884–1893.

Takai, Y. and Koketsu, Y., 2009. Double and triple matings associated with reproductive performance in first-serviced and reserviced female pigs in commercial herds, Journal of Veterinary Medical Science, 71, 635–639.

Thanawongnuwech, R., Amonsin, A., Tatsanakit, A. and Damrongwatanapokin, S., 2004. Genetics and geographical variation of porcine reproductive and respiratory syndrome virus (PRRSV) in Thailand, Veterinary Microbiology, 101, 9–21.

Tummaruk, P., Tantasuparuk, W., Techakumphu, M. and Kunavongkrit, A., 2010. Influence of repeat-service and weaning-to-first-service interval on farrowing proportion of gilts and sows, Preventive Veterinary Medicine, 96, 194–200.

Wensvoort, G., Terpstra, C., Pol, J.M.A., ter Laak, E.A., Bloemraad, M., de Kluyver, E.P., Kragten, C., van Buiten, L., Den Besten, A., Wagenaar, F., Broekhuijsen, J.M., Moonen, P.L.J.M., Zeststra, T., de Boer, E.A., Tibben, H.J., de Jong, M.F., van't Veld, P., Groenland, G.J.R., van Gennep, J.A., Voets, M., Verheijden, J.H.M. and Bramskamp, J., 1991. Mystery swine disease in the Netherlands: the isolation of Lelystad virus, Veterinary Quarterly, 13, 121–130.

Yoon, K.-J., Zimmerman, J.J., Swenson, S.L., McGinley, M.J., Eernisse, K.A., Brevik, A., Rhinehart, L.L., Frey, M.L., Hill, H.T. and Platt, K.B., 1995. Characterization of the humoral immune response to porcine reproductive and respiratory syndrome (PRRS) virus infection, Journal of Veterinary Diagnostic Investigation, 7, 305–312.

Zimmerman, J., Benfield, D.A., Murtaugh, M.P., Osorio, F., Stevenson, G.W. and Torremorell, M., 2006. Porcine reproductive and respiratory syndrome virus (Porcine Arterivirus), In: Straw, B.E., Zimmerman, J., D'Allaire, S., Taylor, D.J. (Eds.), Disease of swine, 9th edn. Blackwell, Ames, pp. 387–417.

VP-67-3

Seasonal influence on the prevalence of porcine reproductive and respiratory syndrome virus in Thailand

P. Tummaruk[1], E. Olanratmanee[1], R. Tantilertcharoen[2], R. Thanawongnuwech[3]
[1]*Department of Obstetrics, Gynaecology and Reproduction,* [2]*Veterinary Diagnostic Laboratory,* [3]*Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330,* Padet.t@chula.ac.th

Introduction

PRRSV has been serologically detected in Thai commercial swine herds since 1989[1]. Nowadays, both genotypes 1 (EU) and 2 (NA) have been isolated and have been being comprehensively investigated in Thailand over the last decade[2]. In Thailand, many commercial swine herds are infected with PRRSV and clinical problems as well as reproductive failure caused by PRRSV are frequently observed. PRRSV has become one of the most important diseases causing economic loss in the Thai swine industry. The objective of the present study was to retrospectively investigate the seasonal influence on the prevalence of PRRSV in swine herds in Thailand between 2005 and 2010.

Materials and Methods

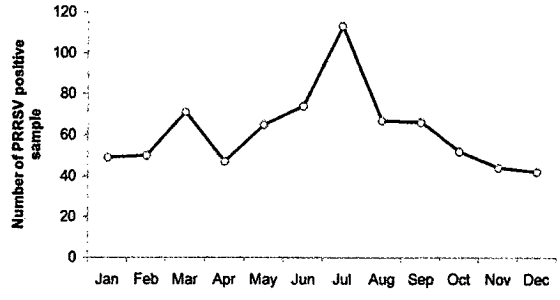
Data from a total of 2,273 samples were collected from the Chulalongkorn University-Veterinary Diagnostic Laboratory (CU-VDL), Bangkok, Thailand during January 2005 to December 2010. The types of sample included tissue (e.g., lung, tonsil, spleen, liver, etc.) (n=636), semen (n=210) and serum (n=1,427). Data included the date when the samples were collected, the location of the herds and the group of the pigs (boars, sows, piglets, nurseries and fattening pigs). Season of the year when the samples were collected was classified as hot (Feb-May), rainy (Jun-Sep) and cool (Oct-Jan) seasons. The data were collected from the registration book of the laboratory and each case was manually recorded. PRRSV was detected by routine RT-PCR at the CU-VDL[2]. The virus was classified into 3 genotypes, i.e., genotypes 1, 2 and mixed genotypes. Frequency analysis was conducted for the present or absence of PRRSV and the frequency of each genotype. Logistic regression was used to analyze the data.

Results

PRRSV was found in 740 out of 2,273 samples (32.6%). The PRRSV detection differed among types of samples. A higher percentage of PRRSV was found in the tissue sample (43.1%) than the semen (15.7%, $P<0.001$) and the serum samples (30.3%, $P=0.002$). Of all the isolations (n=740), genotypes 1, 2 and mixed genotypes accounted for 31.0%, 54.5% and 14.6%, respectively.

The prevalence of PRRSV in the hot season (34.9%) was higher than that found in the cool season (28.1%, $P=0.018$) but did not differ significantly compared to rainy seasons (34.0%, $P=0.486$). The prevalence of PRRSV in the rainy season tended to be higher than that found in the cool season ($P=0.062$). The number of PRRSV isolation by month is demonstrated in Figure 1. The detection of PRRSV dramatically increased from April to July and then slowly declined from July to December.

Figure 1. Number of porcine reproductive and respiratory syndrome virus (PRRSV) detection by months



Conclusions and Discussion

In the present study, an increase in the number of PRRSV detections was found from April to July. This synchronized with an increase of infertility problems in gilts and sows mated during this period of the year[3]. Tummaruk et al.[3] has demonstrated that gilts and sows that were mated during the hot season and farrowed during late autumn and the cool season had a significantly lower number of total piglets born per litter than gilts and sows that were mated in cool season. These findings indicate that an increment in the viral isolation during hot season may possibly contribute to a poor reproductive performance of gilts and sows mated during hot season in Thailand. In addition, during hot seasons, the temperature-humidity index is relatively high[3] and this could possibly induce moderate to severe heat stress in the pigs. Viremia as well as viral infections might be easily obtained from an animal under stress due to its immuno-suppressive conditions. It can be concluded that the prevalence of PRRSV in Thailand between 2005 and 2010 accounted for 32.6% of the cases submitted for viral detection. A high prevalence of PRRSV was found in the hot and rainy seasons in Thailand. Of all the isolates, 31.0%, 54.5% and 14.5% belonged to EU, NA and mixed genotypes, respectively.

References

1. Tummaruk, P. and Tantilertcharoen, R. 2011. Trop. Anim. Health Prod. (in press)
2. Thanawongnuwech, R., et al., 2004. Vet. Microbiol. 101: 9–21.
3. Tummaruk, P. et al., 2010. J. Agri. Sci.148:421-432.

VP-675

Detection of porcine reproductive and respiratory syndrome virus in aborted fetuses, mummified fetuses and stillborn piglets in Thailand using real-time PCR

E. Olanratmanee[1], P. Wongyanin[2], R. Thanawongnuwech[3], P. Tummaruk[1]
[1]*Department of Obstetrics, Gynaecology and Reproduction,* [2]*Department of Microbiology,* [3]*Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330,* Padet.t@chula.ac.th

Introduction

The infection of porcine reproductive and respiratory syndrome virus (PRRSV) in gilts and sows cause reproductive failure including abortion, high mummified fetuses, high stillborn piglets, low viability piglets at birth, infertility and an increasing of sow mortality rate[1]. In Thailand, the seroprevalence of PRRSV in gilts and sows during 2004-2007 was 79.3%[2]. The objective of the present study was to investigate the prevalence of PRRSV detection in aborted fetuses, mummified fetuses and stillborn piglets in Thailand using real-time polymerase chain reaction.

Materials and Methods

A total of 89 cases of aborted fetuses (n=22), mummified fetuses (n=28) and stillborn piglets (n=39) from gilts and sows which had reproductive failure from 10 swine commercial herds in Thailand were included. The tissues samples were obtained from either modified live PRRSV vaccinated pigs (n=23) or non-vaccinated pigs (n=66). Pooled organs of lung, liver, spleen, thymus, tonsil, lymph node and umbilical cord were homogenized and the RNA was extracted using NucleoSpin® RNA virus test kit (Macherey-Nagel Inc., Germany). The extracted RNA was subjected for cDNA synthesis using Omniscript® (QIAGEN, Germany). Quantitative real-time polymerase chain reaction (qPCR) was carried out on ORF7 of the viral genome using EXPRESS qPCR SuperMix Universal® (Invitrogen, USA) and the fluorogenic probe was used to amplify the qPCR product. The primers, probes and real-time PCR condition for gene detection were carried out according to previous study with some modification [3].

Results

It was found that PRRSV was detected in 67.4% (60/89) of the samples (i.e., 54.6%, 64.3% and 76.9% in aborted fetuses, mummified fetuses and stillborn piglets, respectively) (Table 1). The prevalence of North American (NA) and European (EU) strains were 48.3% and 41.6%, respectively (P>0.05) (Table 1). Moreover, the prevalence of PRRSV in PRRS modified live virus vaccinated pigs did not significantly differ compared to the non-vaccinated pigs (65.2% vs. 68.2%, respectively, P>0.05).

Table 1. Percentage of PRRSV detection by type of samples

Sample	PRRSV detection			
	Negative	EU strain	NA strain	Both strains
AF	45.5	0.0	40.9	13.6
MF	35.7	25.0	17.9	21.4
SP	23.1	25.6	23.1	28.2
total	32.6	19.1	25.8	22.5

AF=aborted fetuses, MF=mummified fetuses, SP=stillborn piglets

Conclusions and Discussion

The present study revealed that PRRSV was commonly found among gilts and sows with reproductive failures in Thailand. Although, PRRSV is well recognized as a causative agent of reproductive failure, some other viral pathogens may play a role in the reproductive failure but not investigated in this study. However, it could be speculated that PRRSV involved at least 2/3 of reproductive failure in gilts and sows in Thailand. Furthermore, co-infection of both PRRSV genotypes was found up to 22.5% of the clinical cases. This current situation manifests difficulty in PRRSV control by using only a single strain of PRRSV vaccination due to no cross protection against heterologous strains. The control of viral spreading from these reproductive failure cases are important because it can reduce the viral load within the herd and reduce the risk of infection in PRRSV subpopulation pigs.

References

1. Zimmerman, J. et al., 2006. Disease of swine 9th edition: 387-417.
2. Tummaruk, P. and Tantilertcharoen, R. 2011. Trop. Anim. Health Prod. (in press)
3. Egli, C. et al., 2001. J. Virol. Methods 98: 63-75.

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RO-203

| Reproduction/Breeding/Genetics-BREEDING |

Follicle development and number of ovulation in the ovarian tissue of gilts infected by porcine reproductive and respiratory syndrome virus

D. Phoophitphong[1], E. Olanratmanee[1], S. Srisuwatanasagul[2], S. Wangnaitham[3], R. Thanawongnuwech[3], P. Tummaruk[1]
[1]*Department of Obstetrics, Gynaecology and Reproduction,* [2]*Department of Anatomy,* [3]*Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand,* little_goy@yahoo.com

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) has been isolated from the ovary of the female pigs and may be involve with the reproductive failure in gilts and sows[5]. PRRSV can induce apoptosis in germ cells from testes, alveolar macrophage and mononuclear cells from lymphoid tissues[3,4]. Nevertheless, little information about the pathogenesis of PRRSV infection in the gilts ovary has been done[5]. Furthermore, the influence of PRRSV infection in the ovary on follicle development has not been elucidated. The aim of the present study was to determine follicles development and number of ovulation in the ovarian tissue of gilts infected by PRRSV.

Materials and Methods

Ovarian tissue sections were obtained from 19 Landrace x Yorkshire crossbred gilts aged 267.8±19.2 days and weighted 145.7±11.8 kg. The genital organs were collected from slaughterhouses, placed on ice and transported to the laboratory within 24 h of culling. Ovulation rate was defined as the total number of corpora lutea (CL) from both ovaries. The ovaries were fixed in 10% neutral-buffered formalin for 24-48 h, processed by an automatic tissue processor and embedded in paraffin block. The paraffin embeddings were cut into 5 μm thick by using microtome. At each ovarian tissue, two sections were cut and each section was placed on a separate slide. One section was used to determine PRRSV infection using immunohistochemistry[2], while another was stained by PCNA immunohistochemistry. For the PCNA sections, the follicles were categorized as primordial, primary and growing follicles and were quantified under light microscope. The number of follicles was expressed as the total number of follicles per 100 μm^2 of the tissue section. The gilts were classified on the criterion of body weight (≥150 kg, n=7 versus <150 kg, n=12) and the present or absent of PRRSV in the ovarian tissue (positive, n=10 versus negative, n=9). Multiple analysis of variance was used to analyze the effect of body weight and PRRSV infection on the number of follicles. $P<0.05$ were regarded to be statistically significant.

Results

On average, the total number of follicles in negative and positive PRRSV ovarian tissue was 21.6±3.1 and 19.2±2.7, respectively ($P=0.56$). Number of primordial, primary and growing follicles and ovulation rate in PRRSV positive and negative ovarian tissues are presented in Table 1. The number of primary follicles in gilts with a body weight of ≥150 kg was higher than gilts with a body weight of <150 kg (8.9±1.1 *versus* 4.6±0.8, $P=0.007$).

Table 1. Number of primordial, primary and growing follicles in PRRSV positive and negative ovarian tissue

Follicle	PRRSV	
	Positive	Negative
Primordial	12.2±6.7[a]	14.0±7.4[a]
Primary	6.4±4.5[a]	6.0±1.3[a]
Growing	0.6±0.2[a]	0.5±0.2[a]
Ovulation rate	15.0±2.9[a]	16.7±5.3[a]

[a] The same superscript within a row do not differ significantly (P>0.05)

Conclusions and Discussion

PRRSV in the gilt ovary is mainly found in the macrophages in the ovarian tissue[5]. Follicular count using PCNA immunohistochemistry revealed that no differences between number of follicles in negative and positive PRRSV ovarian tissues. This indicated that PRRSV might not affect the number and type of follicles in the gilts ovarian tissue. This is in agreement with earlier studies[1, 5]. However, a high variation on the number of primary follicles in the PRRSV positive ovarian tissues was remarked (Table 1). This indicated that abnormal follicles development may occurred only in some gilts. Additional works will be carried to determine the occurrence of apoptosis and also some additional number of gilts will be added.

References

1. Benson, J.E. et al., 2001. Theriogenology 56:777-785.
2. Olanratmanee, E. et al., 2011. Trop. Anim. Health Prod. 43:451-457.
3. Sur, J.H. et al., 1997. J. Virol. 71:9170-9179.
4. Sur, J.H. et al., 1998. Vet. Pathol. 35:506-514.
5. Sur, J.H. et al., 2001. Vet. Pathol. 38:58-66.

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A053-SW015 Preweaning mortality in porcine reproductive and respiratory syndrome virus (PRRSV) sero-positive herds in Thailand

Em-on Olanratmanee[1]* Roongroje Thanawongnuwech[2] Annop Kunavongkrit[1] Padet Tummaruk[1]

[1]*Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand .*

[2]*Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.*

*Corresponding author e-mail address: em_on.o@hotmail.com

Keywords: Pig, Reproduction, PRRSV, Vaccine, Preweaning mortality

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) infection in gilts and sows cause reproductive disorders, i.e., infertility, abortion, mummified fetuses, stillborn piglets, weak-born piglets, and sow mortality (1). In general, preweaning mortality in swine herds is around 10% (2). Preweaning mortality in PRRSV infected piglets may be as high as 60%, which is mostly associated with listlessness, emaciation, splay-legged posture, hyperpnea, dyspnea, and chemosis (1). Under field condition, several strategies are used to control PRRSV infection in swine breeding herds including intensive acclimatization, replacement gilts management, serological monitoring, and PRRSV vaccination (3). Preweaning mortality in PRRSV positive herds in USA has been reported to be varied from 45.0% to 58.4% (4-5). To our knowledge, no study on the preweaning mortality in PRRSV positive herds in Thailand has been done. The aim of the present study was to investigate the pre-weaning mortality in selected PRRSV sero-positive herds in Thailand.

Materials and Methods

Data of 150,147 weaning records from 58,542 sows were collected during 2005-2009 from 8 swine commercial breeding herds (A, B, C, D, E, F, G, and H) in the central, eastern, and north-eastern parts of Thailand. All herds were PRRSV sero-positive for over 5 years. North American (NA) strain of PRRS modified live virus (MLV) vaccine (Ingelvac® PRRS™ MLV, Boehringer-Ingelheim Vetmedica Inc., St. Josept, Missouri, USA) was used in herd A every 3 months as mass vaccination. Both NA and European (EU) strain of PRRS MLV vaccine (AMERVAC®, Laboratories Hipra, Girona, Spain) was used in herd B irregularly. EU strain of PRRS MLV vaccine was used in replacement gilts in herd C. In herds D-H, the gilts and sows has never been vaccinated against PRRSV. Sows with lactation length <18 days or >28 days were excluded from the analyses. Preweaning mortality rate (PWM) calculated by: PMN (%) = [(Number of litter mates after cross fostering – number of piglets at weaning) / number of litter mate] x 100. Due to a skew distribution of PWM data, the PWM were log transformed and were analyzed by multiple ANOVA. Other variables, i.e., the number of piglets born alive per litter (BA), the percentage of stillborn piglets per litter (SB), the percentage of mummified fetuses per litter (MM), and the number of piglets weaned per litter (WP), were analyzed and compared among herds using general linear model. The statistical models included the effect of vaccination (yes, no), herds (A-H) nested within vaccination, parity number, and month. Least square means were obtained from each class of the factors and were compared by using Tukey-Kramer test. $P<0.05$ was regarded to be statistically significant.

Results and Discussion

Preweaning mortality was presented in Figure 1. On average, preweaning mortality was 12.9%, which was varied among herds from 6.4% to 18.9% ($P<0.001$). Preweaning mortality in PRRSV vaccinated herds (mean=16.7%, ranged 13.6% to 18.9%) was higher than non-vaccinated herds (mean=10.8%, ranged 6.4% to 17.0%) ($P<0.001$) (Figure 1).

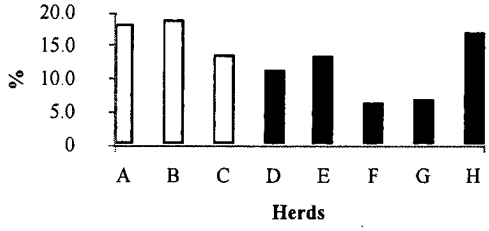

Figure 1. Preweaning mortality of sows by herd (A-C PRRSV vaccinated herds and D-H PRRSV non-vaccinated herds)

BA, SB, MM and WP in vaccinated and non-vaccinated groups are presented in Table 1. On average BA in PRRSV vaccinated herd was higher than non-vacinated herd. The litter traits of sows by herd are presented in Table 2.

Table 1. Means of the number of piglets born alive per litter (BA), stillborn piglets per litter (SB, %), mummified fetuses per litter (MM, %), and piglets weaned per litter (WP) by vaccination

Herds	BA	SB	MM	WP
Non-vaccinated	10.0^{a}	6.6^{a}	3.2^{a}	9.3^{a}
Vaccinated	10.5^{b}	5.0^{b}	2.1^{b}	9.3^{a}

^{a,b} Different superscripts within columns indicate statistically significant differences ($P<0.05$)

Table 2. Means of the number of piglets born alive per litter (BA), stillborn piglets per litter (SB, %), mummified fetuses per litter (MM, %), and piglets weaned per litter (WP) by herd

Herds	BA	SB	MM	WP
A	10.1	3.9	1.4	8.9
B	10.0	6.4	1.9	8.9
C	11.6	5.1	3.2	10.1
D	9.8	5.2	4.9	9.1
E	9.6	6.0	1.6	8.5
F	10.3	8.2	2.9	10.0
G	10.5	7.2	1.9	9.6
H	10.0	7.2	2.5	9.1
Total	10.2	5.9	2.8	9.3

The results indicated that the preweaning mortality of sows in PRRSV sero-positive herds in Thailand can be varied from 6.4% to 18.9% among herds. A previous study has demonstrated that PRRSV vaccination in gilts and sows could reduce the odds of preweaning mortality (5). However, in the present study, preweaning mortality in vaccinated herds was higher than in non-vaccinated herds.

Acknowledgments

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References

1. Zimmerman et al., 2006. Disease of swine 9th edition. p 387-417.
2. Shankar et al., 2009. Vet. World. 2(6): 236-239.
3. Cho and Dee, 2006. Theriogenology. 66: 655-662.
4. Goldberg et al., 2000. Prev. Vet. Med. 43: 293-302.
5. Young et al., 2010. Can. J. Vet. Res. 74(3): 170-177.

A051-SW013 Effect of PRRS virus infection in the ovarian tissue on follicle growth in prepubertal and pubertal gilts

Duangkamol Phoophitphong[1]* Em-on Olanratmanee[1] Sayamon Srisuwatanasagul[2] Padet Tummaruk[1]

[1]*Department of Obstetrics, Gynaecology and Reproduction,* [2]*Department of Veterinary Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand.*

*Corresponding author e-mail address: Phoophitphong.D@gmail.com

Keywords: gilts, granulosa cells, ovary, proliferating cell nuclear antigen, PRRS

Introduction

Porcine reproductive and respiratory syndrome (PRRS) virus has been isolated from ovary of the gilts (1). However, the involvement of PRRS virus in the follicle growth has not been clearly determined. Proliferating cell nuclear antigen (PCNA) is an auxiliary protein that necessary for DNA synthesis. It has been demonstrated that PCNA expression in the ovary involves with follicle growth in many species (2, 3). The purpose of the present study was to evaluate the effect of PRRS virus infection in the porcine ovarian tissue on follicle growth in prepubertal and pubertal gilts.

Materials and Methods

Ovarian tissue sections were obtained from 37 Landrace x Yorkshire crossbred gilts. For each gilt, two sections were cut and each section was placed on a separated slide. One section was used to determine PRRS virus infection by using immunohistochemistry and another was stained by PCNA immuno-histochemistry (3). The gilts were classified on the criterion of an immunohistochemical expression of PRRS virus in the ovarian tissues as positive (n=20) and negative (n=17) groups (Figure 1A and 1B). Stages of the reproductive cycle were classified according to the ovarian appearance and structures that is corpus luteum (CL) and follicles (4). Gilts with ovaries having only small follicles (<5 mm) were defined as a prepubertal (n=10) and those with ovaries having CL were defined as a pubertal (n=27) (Figure 1C and 1D). For the PCNA sections, a total of 273 follicles (197 pre-antral and 76 antral follicles) were determined for the proportion of PCNA expression under light microscope. The positive areas of granulosa cells with positive PCNA staining were calculated using Image-Pro® Plus software.

The statistical analyses were carried out using multiple ANOVA. The statistical model include effect of follicle type (preantral and antral), PRRS virus infection (positive and negative), ovarian status (prepubertal and pubertal), reason for culling (abortion, anestrus, repeat service, vaginal discharge and non-reproductive causes) and interaction between follicle type and PRRSV, follicle type and ovarian status and PRRSV and ovarian status. Least square means were obtained from each class of the factor and were compared by using least significant difference test. $P<0.05$ were regarded to be statistically significant.

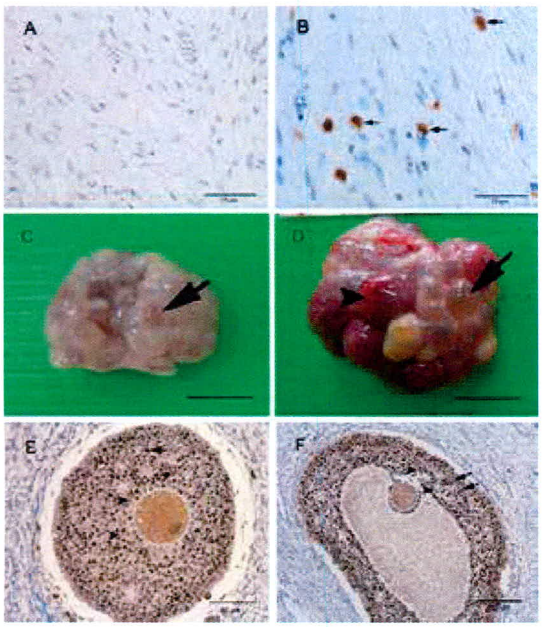

Figure 1. (A-B) Immunohistochemical expression of negative (A) and positive (B) PRRSV in macrophages of the gilt's ovarian tissue. (C-D) Small follicles (arrow) of ovary in prepubertal gilt and corpus luteum (arrow-head) in the ovary of pubertal gilt (bar = 1 cm). (E-F) PCNA immuno-staining of growing follicle demonstrate positively stained granulosa cells (arrow) and negatively stained granulosa cells (arrow-head) in pre-antral (E) and antral (F) follicles.

Result and Discussion

The follicle growth was demonstrated by the percentage of granulosa cells proliferation stained by PCNA (Figure 1E and 1F). The proportion of PCNA expression in the growing follicles of PRRSV negative and positive ovarian tissue is presented in Table 1. The present study demonstrated that the growth of the ovarian follicle of pubertal gilt was higher than pre-pubertal gilt in negative PRRS group but not in PRRS positive group (Table 1).

Table 1. Percentage of granulosa cells proliferation (least-squares means±SEM) in the ovarian tissue of gilts infected with porcine reproductive and respiratory syndrome virus.

Gilt status	PRRS virus infection	
	negative	positive
Prepubertal	$36.6\pm6.5^{a,A}$ (n=33)	$49.8\pm7.6^{a,A}$ (n=26)
Pubertal	$76.6\pm3.7^{a,B}$ (n=82)	$59.9\pm4.6^{b,A}$ (n=132)

n=number of follicle, ^{a,b,} Different letters within row differed significantly ($P<0.05$), ^{A,B} Different capital letters within column differed significantly ($P<0.05$).

This indicated that PRRS infection in the ovarian tissue of gilt influence the proliferation of granulosa cells and may lead to poor follicle growth as well as poor quality of oocyte. However, infection of PRRS virus in the ovarian tissue of prepubertal gilt did not influence the proliferation of granulosa cell of the follicle. This indicated that the acclimatization and/or vaccination should be introduced to the replacement gilt during prepubertal stage. The introduction of PRRS live virus to pubertal gilt is therefore not recommended. Additionally, the immunohistochemical expression of PCNA of granulosa cells in gilt can be applied to study the folliculogenesis in pig.

It could be conclude that PRRS virus infection effect the granulosa cell proliferation of pubertal gilts but not in pre-pubertal gilts.

Acknowledgement

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References

1. Sur et al., 2001. Vet. Pathol. 38: 58-66.
2. Picut et al., 2008. Toxicol. Pathol. 36: 674-679.
3. Phoophitphong et al., 2012. Livest. Sci. 150: 425-431.
4. Tummaruk et al., 2009. Theriogenology. 71: 369-385.

A056-SW016 Porcine reproductive and respiratory syndrome virus causes histological changes in endometrium of culling gilts

Paisan Tienthai[1]* Weerapong Borhirunrat[2] Chakorn Chalermchaikit[2] Prakorn Kootpetch[2]
Watcharain Laprom[2] Padet Tummaruk[3]

[1]*Department of Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330 Thailand.*
[2]*6th year student, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330 Thailand.*
[3]*Department of Obstetrics, Gynaecology Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330 Thailand.*

*Corresponding author e-mail address: Paisan.T@chula.ac.th

Keywords: morphology, endometrium, pig, PRRSV

Introduction

At present, porcine reproductive and respiratory syndrome (PRRS) is considered to be the most important disease in the swine production and influences on the economic loss worldwide (1). It is known that PRRS virus (PRRSV) is capable to induce reproductive disorder in pregnant pigs and it combined with other bacteria, viruses or toxins causing respiratory malfunction in young pigs (2). The PRRSV-induced reproductive failure is often restricted to the last stage of gestation in sows and primarily characterized by late abortion, early farrowing, elevated mummified and dead fetuses as weak-born piglets (3). Interestingly, about 73% of replacement gilts in Thailand which were culled due to the reproductive failure had been infected with PRRSV (4). Although, most of these culling gilts exhibited the PRRSV antigen in the uterine tissue (5), the PRRSV mechanisms caused reproductive failures in gilts by the morphological changes in the uterus are not yet described. Therefore, the objective of the present study was to observe the histological changes by using light microscopy (LM) in the culling gilts infected with PRRSV.

Materials and Methods

Sixty four Landrace ´ Yorkshire crossbred culling gilts from commercial swine herds in Thailand were used in this study. The gilts were classified into the PRRSV infected groups (n=24) and control groups (n=40) by the serological test and the appearance of PRRSV antigens in the uterine tissue as already performed by previous study (5). The infected gilts were culled due to variable of reproductive disorders comprising of anestrus, repeat breeding, abortion or abnormal discharge vaginal whereas the control group was culled by non-reproductive problems. The uterine horns collected from each group were dissected, fixed in 10% buffered formalin, embedded in paraffin and cut into 5 mm thickness. The tissue slides were stained with hematoxylin and eosin (H&E) for further evaluation. The following parameters compose of 1) the general abnormalities and 2) the edema scores (0-3) of the endometrium were routinely observed under LM at 200´, 3) the height (mm) of uterine epitheliumwas measured by use of the digital camera (Micropublisher 5.0, Qimage, Surrey, Canada) via the program Image-Pro Plus version 6.0 (Media Cybernatics Inc., MD, USA) at 400´ and 4) the number of vessels nearby uterine epithelium was counted by the ocular micrometer with 25 squares corresponded to 125,000 mm^2 at 200´. All data was analyzed by using the GLM procedure (SAS Institute Inc., Cary, NC, USA) and expressed as mean ± SD. The mean values were compared by Student's *t*-test with $p<0.05$.

Results and Discussions

As shown, the infiltration of immune cells within the subepithelial layer and the uterine epithelial surface was more obviously seen in the PRRSV infected gilts (Fig. 1a). Remarkably, most of the infected group was found the sloughing of uterine epithelium (Fig 1b). Besides, the increase in the height of uterine epithelial surface (Fig. 2), the estimating edematous scores (Fig. 3), and the number of blood vessels underneath subepithelial layer (Fig. 4) were also significantly ($p<0.05$) detected in the PRRSV infected gilts. To be our knowledge, this study was the first research that attempted to investigate the morphological changes in the endometrium of the culling gilts infected with PRRSV in Thailand.

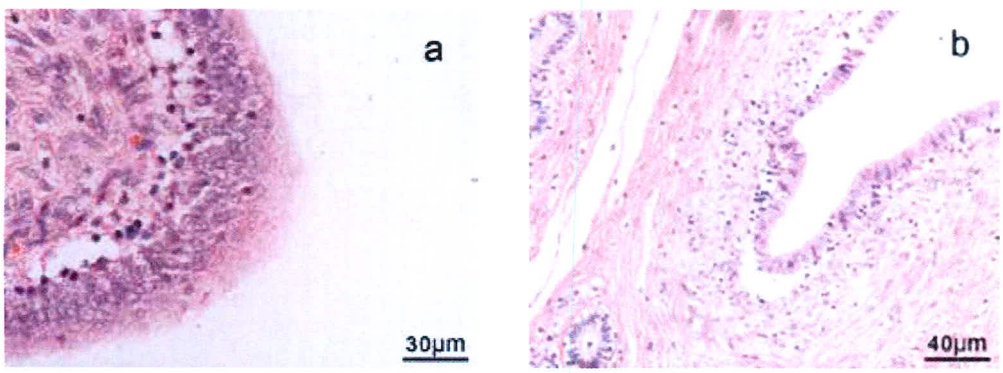

Figure 1. Characteristics of gilt endometrium infected with PRRSV by LM showed the infiltration of immune cells into uterine epithelium (a) and the detachment of epithelial surface from the subepithelial layer (b).

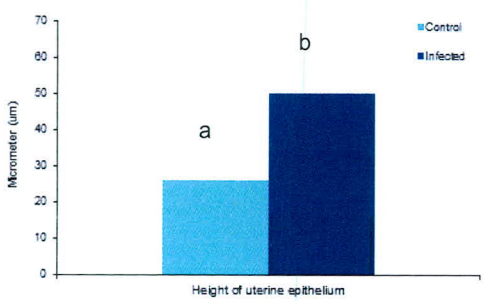

Figure 2. The height of uterine epithelial surface in the endometrium of control and infected PRRSV gilts. Different letters represent significantly difference.

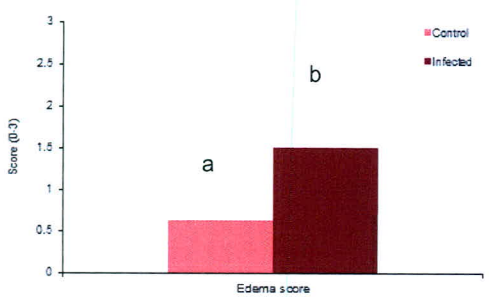

Figure 3. The estimating of edema scores observed in the subepithelial layer of gilt endometrium compared between control and infected PRRSV gilts. Different letters represent significantly difference.

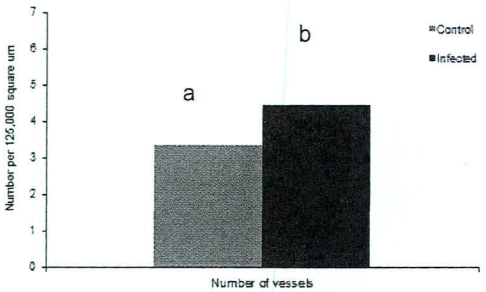

Figure 4. The number of blood vessels counted in the subepithelial layer nearby the uterine epithelium of gilt endometrium of control and infected PRRSV gilts. Different letters represent significantly difference.

In Thailand, more than 84% the replacement gilts were exposed to PRRSV and up to 73% of these were culled due to reproductive failure (4). Our results exhibited the deteriorated morphological changes occurred in the infectious endometrial gilts indicating the influence of PRRSV can cause the damage of endometrium. Simply explanation, several studies suggested that PRRSV induced the apoptosis and necrosis both in the cell culture (6) and the fetal implantation sites (7). Therefore, the lesions observed in this study might be the earlier evidence inducing the endometritis (8). Of course, this data might be enough to prove that PRRSV was the viral pathogen causing the reproductive disturbances in replacement gilts in Thailand. However, the apoptotic and necrotic detection need to be done in the near future.

Acknowledgement

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References

1. Neumann et al., 2005. J. Am. Vet. Med. Assoc. 227, 385-392.
2. Plana et al., 1992. Vet. Microbiol. 33, 203-211.
3. Rowland, 2010. Virus Res. 154, 114-122.
4. Tummaruk & Tantilertcharoen, 2012. Trop. Anim. Health Prod. 44, 983-989.
5. Olanratmanee et al., 2011. Trop. Anim. Health Prod. 43, 451-457.
6. Miller & Fox, 2004. Vet. Immunol. Immunopathol. 102, 131-142.
7. Karniychuk et al., 2011. Microb. Pathog. 51, 194-202.
8. Christianson et al., 1993. Can. J. Vet. Res. 57, 262-268.

ผลกระทบของการตรวจพบเชื้อไวรัสพีอาร์อาร์เอสในเนื้อเยื่อรังไข่ของสุกรสาวทดแทนต่อการงอกขยายของแกรนูโลซาเซลล์ในฟอลลิเคิลที่กำลังเจริญเติบโต

Impact of PRRS virus detection in the ovarian tissue of replacement gilts on granulosa cells proliferation in the developing follicles

Duangkamol Phoophitphong[1] Em-on Olanratmanee[1] Sayamon Srisuwatanasagul[2] Padet Tummaruk[1]

ดวงกมล ภูพิชญ์พงษ์[1] เอมอร โอฬารรัตน์มณี[1] ศยามณ ศรีสุวัฒนาสกุล[2] เผด็จ ธรรมรักษ์[1]

บทคัดย่อ

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อประเมินผลกระทบของการตรวจพบเชื้อไวรัสพีอาร์อาร์เอส (PRRS) ในเนื้อเยื่อรังไข่ของสุกรสาวทดแทนต่อการงอกขยายของแกรนูโลซาเซลล์ในฟอลลิเคิลที่กำลังเจริญเติบโต เก็บรังไข่จากสุกรสาวพันธุ์ผสมแลนด์เรซและยอร์กเชียร์ จำนวน 12 ตัว แบ่งเป็นกลุ่มที่ให้ผลบวก (n=6) และลบ (n=6) ต่อการตรวจพบเชื้อไวรัสพีอาร์อาร์เอส บนเนื้อเยื่อรังไข่จากการตรวจด้วยวิธีอิมมูโนฮิสโตเคมี นำเนื้อเยื่อรังไข่จากสุกรสาวทั้งหมดมาผ่านกระบวนการอิมมูนโนฮิสโตเคมี เพื่อตรวจหาการงอกขยายของกรานูโลซาเซลล์บนฟอลลิเคิลที่กำลังเจริญเติบโตจำนวน 56 ใบ โดยฟอลลิเคิลที่ทำการศึกษาประกอบด้วย 2 ระยะ ได้แก่ ระยะพรีแอนทรัม 41 ใบ และระยะแอนทรัม 15 ใบ ตรวจวัดสัดส่วนของกรานูโลซาเซลล์ที่พบโปรตีนพีซีเอ็นเอภายใต้กล้องจุลทรรศน์แสงสว่างแล้วทำการประเมินด้วยโปรแกรมวิเคราะห์ภาพ แล้วเปรียบเทียบสัดส่วนของกรานูโลซาเซลล์ที่กำลังมีการงอกขยาย (ติดสีพีซีเอ็นเอ) ในฟอลลิเคิลทั้ง 2 ระยะ บนรังไข่ที่ติดเชื้อไวรัสพีอาร์อาร์เอส เปรียบเทียบกับรังไข่ที่ไม่ติดเชื้อไวรัสพีอาร์อาร์เอส ผลการทดลองพบว่าในฟอลลิเคิลระยะพรีแอนทรัม กลุ่มเนื้อเยื่อรังไข่ที่ตรวจไม่พบเชื้อไวรัสพีอาร์อาร์เอส มีสัดส่วนของแกรนูโลซาเซลล์ที่กำลังมีการงอกขยายจำนวนสูงกว่ากลุ่มเนื้อเยื่อรังไข่ที่ตรวจพบเชื้อไวรัส พีอาร์อาร์เอส (57.4±5.8% และ 36.6±5.7% ตามลำดับ P=0.01) ส่วนในฟอลลิเคิลระยะแอนทรัมพบว่ากลุ่มเนื้อเยื่อรังไข่ที่ตรวจไม่พบเชื้อไวรัสพีอาร์อาร์เอส มีสัดส่วนของแกรนูโลซาเซลล์ที่กำลังมีการงอกขยายไม่แตกต่างจากกลุ่มที่เนื้อเยื่อรังไข่ที่ตรวจพบเชื้อไวรัสพีอาร์อาร์เอส (P=0.800) การศึกษานี้สรุปว่าการติดเชื้อไวรัสพีอาร์อาร์เอส ในเนื้อเยื่อรังไข่ของสุกรสาวทดแทนทำให้การเกิดการงอกขยายของแกรนูโลซาเซลล์ลดลงในฟอลลิเคิลระยะพรีแอนทรัม แต่ไม่มีผลต่อฟอลลิเคิลระยะแอนทรัม ผลกระทบนี้อาจทำให้ฟอลลิเคิลระยะพรีแอนทรัมมีการพัฒนาช้าลงและทำให้คุณภาพของโอโอไซต์ต่ำลง ซึ่งอาจเป็นสาเหตุของปัญหาการไม่เป็นสัดและความไม่สมบูรณ์พันธุ์ในสุกรสาวได้

Keywords: granulosa cell, PRRSV, Proliferating cell nuclear antigen, gilts

E-mail address: Phoophitphong.D@gmail.com

[1]ภาควิชาสูติศาสตร์ เธนุเวชวิทยาและการสืบพันธุ์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพ 10330
Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok
[2]ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพ 10330
Department of Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330

ABSTRACT

The purpose of this study was to evaluate the influence of PRRSV detection on the granulosa cells proliferation during follicle development in replacement gilts. The gilts were classified on the criterion of the PRRSV immunohistochemical expression in the ovarian tissues as positive (n=6) and negative (n=6) groups. Ovarian sections were evaluated for PCNA by use of immuno-histochemistry and categorized as pre-antral (n=41) and antral follicles (n=15). The proportion of PCNA expression in granulosa cells were determined under light microscope and calculated using Image-Pro® Plus software. The proportion of proliferating granulosa cells in either preantral and antral follicles were compared between PRRS positive and PRRS negative ovarian tissue. The results revealed that pre-antral follicle with negative PRRSV had a higher percentage of proliferative marker than those with positive PRRSV (57.4±5.8% and 36.6±5.7%, respectively, P=0.01). For antral follicles, the negative PRRSV had no difference in PCNA expression of granulosa cells compared with the positive PRRSV (P=0.800). It could be concluded that PRRSV infection in the gilt ovarian tissues reduced the proliferation of granulose cells in pre-antral follicles, but did not influence antral follicles. This impact may result in a delay follicle development and reduce the oocytes quality and subsequently caused abnormal estrus behavior and infertility problem in replacement gilts.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) has been isolated from the ovary of the gilts (Sur *et al*., 2001; Olanratmanee *et al*., 2011b). In boar, it has been clearly demonstrated that the virus is able to induces apoptosis of the testicular germ cells (Sur *et al*., 2001), while in the female's gonad, only limited information is known. To our knowledge, only one study has demonstrated that PRRS virus can penetrate the resident macrophages of the ovary, but its involvement in the follicle development, ovulation and corpus luteum formation has not been clearly determined (Sur *et al*., 2001).

Proliferating cell nuclear antigen (PCNA) is an auxillary protein of DNA polymerase delta that necessary for DNA synthesis (Kurki *et al*., 1986; Bravo *et al*., 1987). The expression of PCNA increases during G1 phase, gets to the highest level in S-phase and decreases during G2/M phases of the cell cycle (Kurki *et al*., 1988). An earlier study has demonstrated that the expression of PCNA in the ovary involve with follicular development in many species, e.g, rat (Oktay *et al*., 1995), cow (Wandji *et al*., 1996), baboon (Wandji *et al*., 1995), pig (Tománek and Chronowska, 2006; Phoophitphong *et al*.,

2012) and human (Kelsey *et al.*, 2010). The purpose of the present study was to evaluate the granulosa cells proliferation during follicle development (pre-antral and antral stages) in the ovarian tissue of replacement gilts with and without PRRSV detection.

Material and methods

Animals and management

Ovarian tissue sections were obtained from 12 Landrace x Yorkshire crossbred gilts aged 275.2±34.2 days and weighted 142.7±19.1 kg. The gilts were obtained from two commercial swine herd in the middle and northern parts of Thailand. The replacement gilts were kept in the gilt pools for at least 60 days before sending to the breeding unit. All the replacement gilts were vaccinated against foot-and-mouth disease virus (FMDV), classical swine fever virus (CSFV), Aujeszky's disease virus (ADV), porcine parvovirus (PPV) and porcine reproductive and respiratory syndrome virus (PRRSV) between 154 and 210 days of age. Moreover, an acclimatization was conducted before service, by grouping the replacement gilts with the weaned sows selected for removal for about 28 days. The acclimatization process was initiated by introducing such weaned sows to the gilts' pen at 154–196 days of age with a ratio of one sow per 6-10 gilts. The sows used for acclimatization were rotated on a weekly basis and were removed from the herd after acclimatization. Using this acclimatization process, the gilts were exposed to many types of viral pathogens circulating within the herds (e.g., PRRSV, PPV and enterovirus) before sending to the breeding unit.

Sample collection and immunohistochemistry

The genital organs were collected from slaughterhouses, placed on ice and transported to the laboratory within 24 h of culling. Ovulation rate was defined as the total number of corpora lutea (CL) from both ovaries. The ovaries were fixed in 10% neutral-buffered formalin for 24-48 h, processed by an automatic tissue processor and embedded in paraffin block. At each ovarian tissue, two sections were cut and each section was placed on a separated slide. One section was used to determine PRRS virus infection using immunohistochemistry (Olanratmanee *et al.*, 2011b), while another was stained by PCNA immuno-histochemistry. PCNA immuno-staining technique has been modified after previous studies in the rat's ovarian tissue (Mushkelishvili *et al.*, 2005; Picut *et al.*, 2008). The slides were incubated with mouse monoclonal anti-PCNA (clone PC10, DAKO, Carpinteria, CA, USA) as a primary antibody at a dilution of 1:200 overnight at 4°C. After incubation with the primary antibody, the sections were incubated with DAKO EnVision™ reagent for 45 min at room temperature. The primary antibody was visualized by 3,3' -diaminobenzidine tetrahydrochloride hydrate (DAB, Sigma, Germany) for 3 min at room temperature. For negative control, PBS was used instead of the primary antibody.

Follicles categorization

The gilts were classified on the criterion of an immunohistochemical expression of PRRS virus in the ovarian tissues as positive (n=6) (Figure 1) and negative (n=6) groups. The follicles were classified into 2 categories as described earlier (Oktay *et al.*, 1995; Picut *et al.*, 2008). Pre-antral follicles were follicles having a central oocyte and visible zona pellucida surrounded by multiple layers of granulosa cells with no antral formation (Figure 2). Antral follicles were follicles having an oocyte and zona pellucida surrounded by multiple layers of granulosa cells with antral formation. For the PCNA sections, a total of 56 follicles were categorized as pre-antral (n=41) and antral follicles (n=15) and were determined for the proportion of PCNA expression under light microscope. The positive areas of granulosa cells with positive PCNA staining were calculated using Image-Pro® Plus software.

Statistical analysis

The statistical analyses were carried out using SAS version 9.0 (SAS institute Inc., Cary, NC, USA). The proportion of PCNA positive area was analyzed by using general linear model (GLM) procedure. The statistical model included follicle type (pre-antral and antral), PRRSV infection (positive and negative) and interaction between follicle type and PRRSV infection. Least squared means were obtained from the statistical models and were compared by using least significant different (LSD) test. $P<0.05$ were regarded to be statistically significant.

Result and Discussion

Figure 1 demonstrated PRRS virus detection in the ovarian tissue of replacement gilts and Figure 2 demonstrated the PCNA immnostaining of granulosa cells in a pre-antral and antral follicles. Regardless to the follicle types, follicles in the negative PRRS virus ovarian tissue tended to have a higher percentage of proliferative marker than those with positive PRRS virus ovarian tissue (57.0±5.2% versus 44.9±6.0%, $P=0.132$). For pre-antral follicles, negative PRRS virus ovarian tissue had 20.8% higher PCNA expression granulosa cells than the positive PRRS virus ovarian tissues (57.4±5.8% versus 36.6±5.7%, $P=0.01$). For antral follicles, the proliferation of granulosa cells in PRRS virus negative and PRRS virus positive ovarian tissue did not differ significantly (56.6±8.6% versus 53.2±10.5%, $P=0.800$) (Table 1). The proliferation of granulosa cells in antral and pre-antral follicles did not differ significantly (54.9±6.8% versus 47.0±4.1%, respectively, $P=0.324$).

In the present study, based on immunological detection of PRRSV in the ovarian tissue, 6 gilts were classified as positive and other 6 gilts were classified as negative. However, all of the reproductive organs were obtained from PRRSV sero-positive herds and PRRSV vaccination was

also performed in all replacement gilts. Thus, all of the gilts had been sero-positive gilts. Of these gilts, one group (n=6) detected PRRSV in the ovarian tissue, while another group (n=6) did not detected PRRSV in their ovarian tissue. Our hypothesis is that, if the gilts expose to PRRSV and the virus remains in their ovary until the time of insemination (i.e., at age >220 days). Would it be any significant impact on the follicles development and/or the oocyte qualities? It was found that the presence of PRRSV in the ovarian tissue of gilts significantly reduce granulosa cells proliferation of pre-antral follicles for 20% (36.6 versus 57.4%). This may reduce some of the ovarian growth factors (Sirotkin, 2011) and subsequently cause poor follicles development, reduced steroidogenesis and impair the oocyte's qualities. However, additional research should be carried to determine some more parameters indicating the ovarian function (e.g., apoptosis, expression of steroid receptor) as well as some ovarian growth factors.

Table 1 Granulosa cells proliferation (least-squares means±SEM) in the ovarian tissue of gilts infected with porcine reproductive and respiratory syndrome virus

	PRRS virus negative	PRRS virus positive
Pre-antral follicle	57.4±5.8%[a]	36.6±5.7%[b]
Antral follicle	56.6±8.6%[a]	53.2±10.5%[a]

[a,b] Different letters within row differed significantly ($P<0.05$)

Conclusions

It could be concluded that PRRS virus infection in the ovarian tissue of gilts significantly reduced proliferation of granulose cells of pre-antral follicles. This may subsequently resulted in an increase in the number of poor quality oocytes, poor steroidogenesis and may cause poor estrus behavior and/or infertility problems in replacement gilts.

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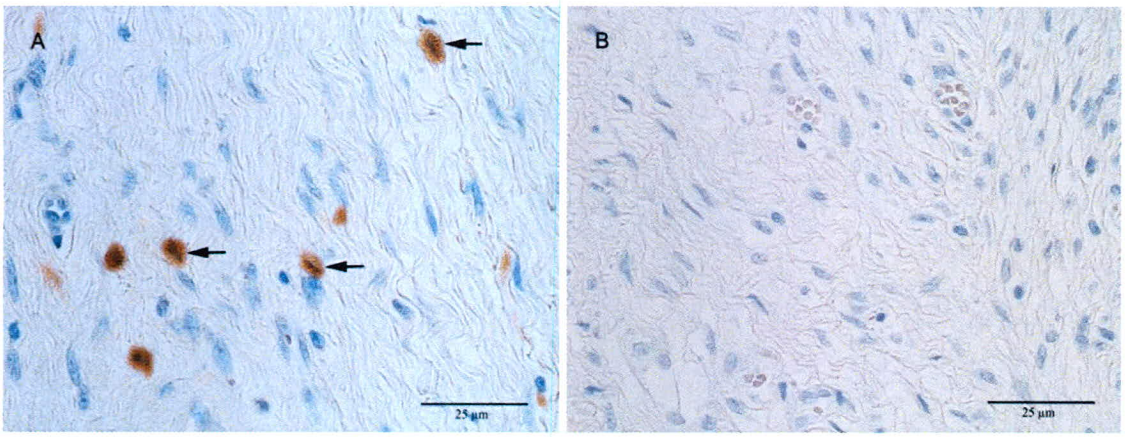

Figure 1 Porcine reproductive and respiratory syndrome virus (PRRSV) infection in macrophages of ovarian medulla (A) and negative PRRS infection (B) (magnification 400x).

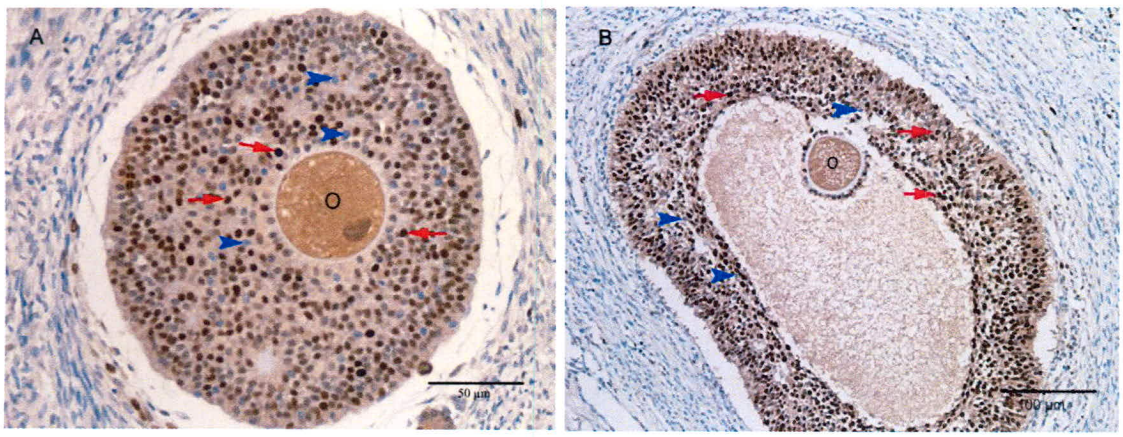

Figure 2 Proliferating cell nuclear antigen (PCNA) immuno-staining of growing follicles. Positive stained granulosa cells (arrow) and negative stained granulosa cells (arrow-head) in pre-antral (A) and antral (B) follicles. (magnification 200x and 100x).

References

Bravo, R. and McDonald-Bravo, H. 1987. Existence of two populations of cyclin proliferating cell nuclear antigen during the cell-cycle-association with DNA-replication sites. **J. Cell Biol.** 105: 1549-1554.

Kelsey, W.T., Caserta, B., Castillo, L., Wallace, B.H.W. and Gonzalvez, C.F. 2010. Proliferating cell nuclear antigen (PCNA) allows the automatic identification of follicles in microscopic images of human ovarian tissue. **J. Pathol. Lab. Med. Int.** 2: 99-105.

Kurki, P., Ogata, K. and Tan, E.M. 1988. Monoclonal-antibodies to proliferating cell nuclear antigen (PCNA) cyclin as probes for proliferating cells by immunofluorescence microscopy and flow-cytometry. **J. Immunol. Methods**. 109: 49-59

Kurkit, P., Vanderlaan, M., Dolbeard, F., Gray, J. and Tan, E.M. 1986. Expression of proliferating cell nuclear antigen (PCNA/cyclin) during the cell cycle. **Exp. Cell Res**. 166: 209-219.

Mushkelishvili, L., Wingard, K.S. and Latendress, R.J. 2005. Proliferating cell nuclear antigen-A marker for ovarian follicle count. **Toxicol. Pathol**. 33: 365-368.

Oktay, K., Schenken, S.R. and Nelson, F.J. 1995. Proliferating cell nuclear antigen marks the initiation of follicular growth in the rat. **Biol. Reprod**. 53: 295-301.

Olanratmanee, E., Wangnaitham, S., Thanawongnuwech, R., Kunavongkrit, A. and Tummaruk, P. 2011a. Prevalence of porcine reproductive and respiratory syndrome virus (PRRSV) antigen-positive uterus tissue in gilts culled due to reproductive disturbance in Thailand. **Trop. Anim. Health Pro**. 43: 451-457.

Olanratmanee, E., Wangnaitham, S., Thanawongnuwech, R., Tummaruk, P., 2011b. Detection of porcine reproductive and respiratory syndrome virus (PRRSV) antigen in ovarian tissue of gilts culled due to reproductive disturbances. **Thai J. Vet. Med. Suppl**. 41: 131-132.

Phoophitphong, D., Wangnaitham, S., Srisuwatanasagul, S. and Tummaruk, P. 2012. The use of proliferating cells nuclear antigen (PCNA) immuno-staining technique to determine the number and type of follicles in the gilt ovary. **Livest. Sci**. (inpress) doi: 10.1016/j.livsci.2012.10.008.

Picut, A.C., Swanson, L.C., Scully, L.K., Roseman, C.V., Parker, F.R. and Remick, K.A. 2008. Ovarian follicle counts using proliferating cell nuclear antigen (PCNA) and semi-automated image analysis in rats. **Toxicol. Pathol**. 36: 674-679.

Sirotkin, V.A. 2011. Growth factors controlling ovarian functions. **J. Cell. Physiol**. 226: 2222-2225.

Sur, J.H., Doster, R.A., Galeota, A.J. and Osorio, A.F. 2001. Evidence for the localization of porcine reproductive and respiratory syndrome virus (PRRSV) antigen and RNA in ovarian follicles in gilts. **Vet. Pathol**. 38: 58-66.

Tománek, M. and Chronowska, E. 2006. Immunohistochemical localization of proliferating cell nuclear antigen (PCNA) in the pig ovary. **Folia Histochem. Cyto**. 44: 269-274.

Tummaruk, P., Kesdangsakonwut, S. and Kunavongkrit, A. 2009. Relationships among specific reason for culling, reproductive data and gross-morphology of the genital tracts in gilts culled due to reproductive failure in Thailand. **Theriogenology** 71: 369-375.

Wandji, S.A., Sršeò, V., Nathanielsz, P.W., Eppig, J.J. and Fortune, J.E. 1997. Initiation of growth of baboon primordial follicles. **Hum. Reprod**. 12: 1993-2001.

Wandji S.A, Sršeò V, Voss AK, Eppig JJ, Fortune JE (1996) Initiation in vitro of growth of bovine primordial follicles. **Biol. Reprod**. 55: 942-948.