Advance Publication

The Journal of Veterinary Medical Science

Accepted Date: 30 Mar 2015
J-STAGE Advance Published Date: 12 Apr 2015
Prevalence of Porcine Reproductive and Respiratory Syndrome Virus Detection in Aborted Fetuses, Mummified Fetuses and Stillborn Piglets Using Quantitative Polymerase Chain Reaction

Em-on OLANRATMANEE1) Piya WONGYANIN2) Roongroje THANAWONGNUWECH3) and Padet TUMMARUK4)*

1) Faculty of Veterinary Medicine, Rajamangala University of Technology Tawan-ok, Chonburi, 20110, Thailand
2) Department of Medical Technology, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok, 10600, Thailand
3) Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand
4) Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand

Correspondence to: P. Tummaruk
Department of Obstetrics, Gynaecology and Reproduction, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand Tel: +662-2189651 Fax: +662-2520738 E-mail address: Padet.T@chula.ac.th

Running head: DETECTION OF PRRSV BY qPCR
ABSTRACT. The objective of the present study was to investigate the prevalence of porcine reproductive and respiratory syndrome (PRRS) virus detection in aborted fetuses (n=32), mummified fetuses (n=30) and stillborn piglets (n=27) from 10 swine herds in Thailand using quantitative polymerase chain reaction (qPCR). Pooled organs and umbilical cord from each fetus/piglet were homogenized and subjected to RNA extraction and cDNA synthesis. The qPCR was carried out on the ORF7 of the PRRS viral genome using fluorogenic probes for amplified product detection. The results revealed that 67.4% (60/89) of the specimens contained PRRS virus. The virus was found in 65.6% (21/32) of aborted fetuses, 63.3% (19/30) of mummified fetuses and 74.1% (20/27) of stillborn piglets (P=0.664). Genotype 1, genotype 2 and mixed genotypes of PRRS virus were detected in 19.1% (17/89), 25.8% (23/89) and 22.5% (20/89) of the specimens, respectively (P=0.316). PRRS virus antigen was retrieved from both non-PRRS-vaccinated herds (68.2%, 45/66) and PRRS-vaccinated herds (65.2%, 15/23) (P=0.794). These findings indicated that these specimens are important sources of the PRRS viral load and the viral shedding within the herd. Thus, intensive care on the routine management of dead fetuses and stillborn piglets in PRRS virus-positive herds should be emphasized.

KEY WORDS: mummy, qPCR, pigs, PRRS, reproduction
Reproductive failure in gilts and sows is influenced by both infectious and non-infectious causes. Major infectious agents associated with reproductive disturbances in gilts and sows commonly detected in swine commercial herds worldwide include porcine reproductive and respiratory syndrome (PRRS) virus, Aujeszky’s disease virus, porcine parvovirus, classical swine fever virus and porcine circovirus type 2 [8,25]. Recently, a serological survey on the evidence of these viruses in swine commercial herds in Thailand found that the sero-prevalence of PRRS virus, Aujeszky’s disease virus and porcine parvovirus in replacement gilts were 87.5%, 4.0% and 99.0%, respectively [25]. Furthermore, 81.0%, 50.0% and 75.0% of gilts culled due to abortion were seropositive for PRRS virus, Aujeszky’s disease virus and porcine parvovirus, respectively [25]. PRRS virus remains one of the most common viruses associated with reproductive failure in gilts and sows in the Thai swine industry. In most commercial swine herds in Thailand, replacement gilts and sows are routinely vaccinated against Aujeszky’s disease virus and porcine parvovirus, while PRRS virus vaccination has been applied only in some herds [18].

The reproductive failure caused by PRRS virus is characterised by a decrease in farrowing rate and an increase in abortion rate, the number of stillborn piglets, mummified fetuses, weak born piglets and pre-weaning mortality [4,29]. PRRS virus is a single-stranded RNA virus and is classified into two genotypes by its genetic, antigenic and pathogenic differences, i.e., genotype 1 (European genotype) and 2 (North American genotype) [14]. The genome of PRRS virus consists of nine open reading frames (ORFs). ORF1a and ORF1b encode the viral RNA polymerase, whereas ORFs 2a, 2b and 3-7 encode the viral structural proteins [15,24]. The complete nucleotide sequence of PRRS virus isolates in Thailand revealed that the percentage of homology between the Thai genotypes 1 and 2 is only 59.1% [1]. Additionally, homology between the Thai genotype 1 and the genotype 1 prototype (Lelystad virus) is 99.2%, and the homology between the Thai genotype 2 and the genotype 2 prototype (VR-2332) is 99.5% [1].

A recent study has demonstrated that the PRRS virus can migrate cross the placenta of the pregnant female pigs, particularly during the last trimester of gestation [12]. The transplacental migration of the PRRS virus-induced apoptosis of the placental cells and caused late term abortion. Furthermore, transplacental infection of the virus also resulted in fetal mortality and an increase in the proportion of stillborn piglets per litter [29]. Investigation of the prevalence of PRRS virus from 100
clinical cases of sows with aborted fetuses and stillborn piglets in Spain found that PRRS virus could be detected in only 9.0% of the samples [13]. In practice, many types of management strategies including acclimatisation, gilt pool management and vaccination with killed virus vaccine and/or modified-live virus (MLV) vaccine, have been used to control the clinical signs of PRRS virus infection. However, PRRS virus still causes many types of reproductive failure in the infected herds, even though the herds have had PRRS MLV vaccination [18]. In addition, PRRS virus was detected in the uterine tissue in up to 33.0% of the replacement gilts culled due to reproductive disturbances [18]. However, the prevalence of PRRS virus in relation to fetal loss (i.e., abortion, mummification and stillborn) in swine herds has not been investigated. The objective of the present study was to investigate the prevalence of PRRS virus detection in aborted fetuses, mummified fetuses and stillborn piglets in swine commercial herds in Thailand.

MATERIALS AND METHODS

Specimens: The present study was conducted between February 2010 and August 2011 in 10 swine commercial herds in the high density pig raising areas in Thailand (i.e., herds A to J). Aborted fetuses (n = 32), mummified fetuses (n = 30) and stillborn piglets (n = 27) were collected from 89 Landrace x Yorkshire crossbred gilts/sows. The specimens were collected from the herd, placed on ice and transported to the laboratory within 24 hr.

Herd location and general management: The herds were located in the eastern (A and G), middle (B, D, E, I and J), northeastern (C and H) and southern (F) parts of Thailand. All herds were breeding herds with 900 to 5,000 sows per herd and were defined as PRRS virus-positive herds according to the results of a commercial ELISA test (HerdChek® PRRSV antibody test kit 2XR®, IDEXX Laboratories, Inc., Westbrook, ME, USA) and a reverse transcription polymerase chain reaction (RT-PCR) of the herd’s monitoring data [26]. Gilts and sows were housed in a conventional open housing system with equipment, e.g., water sprinklers, fans and roofs with heat reflecting material, to reduce the impact of high temperatures. On average, the outdoor 24-hr average temperature and humidity in these areas in the hot (15 February to 14 June), rainy (15 June to 14 October) and cool (15 October to 14 February) seasons
were 29.4°C/71.7%, 28.5°C/78.1% and 26.4°C/68.1%, respectively. The average minimum-maximum daily temperatures were 24.6 - 34.9°C, 24.8 - 33.0°C and 21.4 - 32.1°C in the hot, rainy and cool seasons, respectively. In general, gilts entered the gilt pool at a body weight of 80 - 100 kg. Water was provided up to ad libitum via water nipples. Feeding was provided twice a day at about 3 kg of feed/animal/day.

In general, the feed (corn-soybean-fish based) contained 16 - 18% crude protein, 3,000 - 3,250 kcal/kg metabolisable energy and 0.85 - 1.10% lysine. The herd management recommended breeding replacement gilts from 32 weeks of age onwards at the second or a later oestrus and at a body weight of at least 130 kg. All herds used conventional artificial insemination. Gilts and sows were routinely vaccinated against foot-and-mouth disease virus, classical swine fever virus, Aujeszky’s disease virus and porcine parovirus. Herds A, B, C, D, E and F did not vaccinate gilts and sows against PRRS MLV vaccine (n = 66), while herds G, H, I and J (n = 23) vaccinated all gilts and sows with PRRS MLV vaccine (Ingelvac® PRRSTM MLV, Boehringer-Ingelheim Vetmedica Inc., St. Joseph, MO, U.S.A. [herd G, n = 9] or AMERVAC®, Laboratories Hipra, Girona, Spain [herds H, I and J, n=14]). Among the PRRS-MLV-vaccinated herds, the gilts and sows were routinely vaccinated against PRRS MLV vaccine every 3 - 4 months.

**Historical data and post-mortem examination:** Historical data for all specimens including herd, animal identity, breed, vaccination protocol, parity number and date of mating, farrowing or abortion were collected. The age of the aborted fetuses and stillborn piglets were defined as the interval from mating to abortion or from mating to farrowing, respectively. Crown-rump-length (CRL) of the mummified fetuses was measured. The age of the mummified fetuses was estimated from CRL: age of fetus = 21.07 + (3.11 x CRL), where CRL was the fetal body length (from crown or frontal crest to anus in cm) [27]. Tissue samples, including lung, liver, spleen, thymus, tonsil, lymph node and umbilical cord, were collected from stillborn piglets and aborted fetuses and were kept at -80°C until RNA extraction. For mummified fetuses, only lung, liver and spleen were collected. The dead fetuses were classified into two age groups: < 70 days and ≥ 70 days. The prevalence of PRRS virus detection was compared between groups (see below).
**Viral RNA extraction and cDNA synthesis:** The pool organs (2.0 g) from each case were homogenized and suspended in 10% phosphate buffered saline (PBS) solution (10.0% w/v). The suspension was left at room temperature for sedimentation. The supernatant was collected and subjected to RNA extraction using a commercial kit (NucleoSpin® RNA virus test kit, Macherey-Nagel Inc., Duren, Germany). The extraction protocol was carried out according to the manufacturer’s instructions. Briefly, RNA virus was lysed, bound to the silica membrane and washed. The RNA was eluted from the silica membrane with 50 µl of RNase-free water. The concentration of the extracted RNA was measured by using Thermo Scientific Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE, U.S.A.). The RNA from each sample was diluted with RNase-free water to prepare 500 ng RNA per reaction and was subjected to cDNA synthesis using Omniscript® Reverse Transcriptase (QIAGEN, Hilden, Germany). The cDNA synthesis protocol was carried out according to the manufacturer’s instructions. Briefly, a 20 µl reaction (i.e., 0.5 µM dNTP, 1 µM random primer, 10 units of RNase inhibitor [Ribolock™ RNase Inhibitor, Fermentas Inc., Glen Burnie, Maryland, U.S.A.], 4 units of reverse transcriptase and RNA template from each sample in the kit’s buffer) was incubated at 37 °C for one hr. The synthesized cDNA was kept at -20°C until the quantitative polymerase chain reaction (qPCR) was performed.

**Quantitative polymerase chain reaction:** The qPCR was carried out on ORF7 of the PRRS viral genome using real-time polymerase chain reaction technique using a commercial kit (EXPRESS qPCR SuperMix Universal®, Invitrogen, Carlsbad, CA, U.S.A.). The primer sequences (US align EU forward and reverse primer, which yielded 96 bp for genotype 1 and 105 bp for genotype 2) and fluorogenic probe sequences used were taken from a previous study [10]. The fluorescent dyes labeling the probes for genotypes 1 and 2 detection were cyanine 5 (Cy5) and 6-carboxy-fluorescine (FAM), respectively. The reactions for genotypes 1 and 2 detection were performed separately. Each 20 µl reaction was composed of 10 µl of express qPCR Super Mix Universal, 1.25 µM of US align EU forward primer, 1.25 µM of US align EU reverse primer, 0.5 µM of genotypes 1 and 2 probe and 5 µl of cDNA template. The qPCR was carried out using Rotor-Gene RG-3000 (Corbett Research, Sydney, Australia) at 50°C for 2 min, 95°C for 2 min and 40 cycles of 95°C for 15 sec, 50°C for 20 sec and 60°C for 30 sec. The
known serial concentrations, i.e., $10^1$, $10^3$, $10^5$, $10^7$ and $10^9$ copies/µl of genotypes 1 and 2 PRRS viral cDNA were used as a positive control, and the cycle threshold (Ct) values were used to determine the standard curve. A negative control was performed using RNase-free water instead of cDNA template. The Ct values of each sample were plotted on the standard curve and were determined the amount of copy number (copies/µl) using Rotor-Gene Real-time Analysis Software 6.0 (Corbett Research). The amounts (i.e., copy number) of PRRS virus in the samples were transformed into logarithms ($\log_{10}$) for further analyses.

**Statistical analyses:** Statistical analyses were performed using Statistical Analysis System (SAS) version 9.0 (SAS Institute Inc., Cary, NC, U.S.A.). Descriptive statistics (means ± standard deviation) were conducted for the continuous data, i.e., gestation length and copy number of PRRS virus (log). Frequency analysis was conducted for the percentage of PRRS virus detection. The percentage of PRRS virus detection was compared among groups of types of specimen (aborted fetuses, mummified fetuses and stillborn piglets), PRRS MLV vaccination (yes, no) and parity number (0, 1, 2 – 4, 5 – 11 and unknown parity) by using $r \times k$ contingency table and Chi-square analyses. The copy number of PRRS virus was compared among types of specimen, PRRS MLV vaccination and sow’s parity number by using general linear model procedure (PROC GLM). Least-squares means were obtained from each class of the factors and were compared by using least significant difference (LSD) test. $P < 0.05$ was considered statistically significant.

**RESULTS**

On average, the ages of aborted fetuses, mummified fetuses and stillborn piglets were $73.9 \pm 26.4$ (range 31 – 105 days), $101.0 \pm 18.9$ days (range 62 – 119 days) and $114.4 \pm 1.7$ days (range 111 – 116 days), respectively.

**PRRS virus detection:** PRRS virus was detected in 67.4% (60/89) of the specimens. The genotype of the detected PRRS virus included genotype 1 (EU) 19.1% (17/89), genotype 2 (NA) 25.8% (23/89) and mixed genotypes 22.5% (20/89) ($P = 0.316$). The percentage of PRRS virus detection in each type
of specimen is presented in Table 1. As can be seen from the table, PRRS virus was detected in all types of the specimens. The percentages of PRRS virus detection were 65.6% (21/32), 63.3% (19/30) and 74.1% (20/27) in aborted fetuses, mummified fetuses and stillborn piglets, respectively ($P = 0.664$). The percentage of PRRS virus detection in two different age groups of the fetuses (<70 and ≥70 days) is presented in Table 2. On average, the percentages of PRRS virus detection in the fetuses <70 days of age tended to be lower than that of the fetuses ≥70 days of age (55% [11/20] versus 71.0% [49/69], $P = 0.179$). The age of the fetuses with PRRS virus detection did not differ significantly compared to the fetuses without PRRS virus detection (95.9 ± 26.0 days versus 88.6 ± 25.7 days, $P = 0.336$). Nevertheless, the age of fetuses with PRRS virus detection varied from 31 to 119 days. The fetal age in relation to PRRS virus detection is displayed in Table 3. The percentage of PRRS virus detection varied among the herds from 0.0% to 100.0% ($P = 0.001$) (Table 4). Nevertheless, the PRRS virus could be detected in both PRRS virus non-vaccinated sows (45/66, 68.2%) and in PRRS-MLV-vaccinated sows (15/23, 65.2%) ($P = 0.794$) (Table 4). The percentage of PRRS virus detection did not differ among parity number of sows ($P = 0.983$). PRRS virus was detected by 66.7% (2/3), 68.4% (13/19), 62.5% (10/16), 64.7% (11/17) and 70.6% (24/34) of the fetuses collected from the gilts and the sows parity numbers one, 2–4, 5–11 and unknown parity, respectively.

**Quantitative PCR detection of PRRS virus:** The amount (i.e., copy number) of PRRS virus detected by qPCR is presented in Table 5. On average, the amount of genotype 1 PRRS virus did not differ significantly among aborted fetuses (12.3 ± 2.5 copies/µl), mummified fetuses (10.8 ± 2.4 copies/µl) and stillborn piglets (12.2 ± 3.1 copies/µl) ($P = 0.373$). However, aborted fetuses had a higher amount (12.0 ± 1.3 copies/µl) of genotype 2 PRRS virus than mummified fetuses (10.4 ± 1.5 copies/µl, $P = 0.004$), but did not differ significantly compared to stillborn piglets (11.2 ± 1.4 copies/µl, $P = 0.345$). The amount of PRRS virus in PRRS-MLV non-vaccinated and vaccinated sows was not different in both genotype 1 (11.7 ± 2.7 and 11.8 ± 2.9 copies/µl, $P = 0.689$) and genotype 2 (11.2 ± 1.6 and 11.7 ± 1.3 copies/µl, $P = 0.273$). The amount for both genotypes 1 and 2 of PRRS virus did not differ significantly among parity number of sows. In gilts and sows, parity numbers one, 2–4, 5–11 and unknown parity, the amount of PRRS virus genotype 1 was 10.2 ± 1.9, 10.9 ± 1.7, 12.1 ± 2.9, 11.8 ±
3.0 and 12.2 ± 3.0 copies/µl, respectively (P = 0.655). Likewise, the amount of PRRS virus genotype 2 in gilts and sows parity numbers one, 2 – 4, 5 – 11 and unknown parity was 11.0 ± 0.0, 11.0 ± 1.7, 11.7 ± 1.7, 11.4 ± 1.5 and 11.2 ± 1.4 copies/µl, respectively (P = 0.716).

DISCUSSION

The present study revealed that PRRS virus was frequently detected in the dead fetuses. It was found that PRRS virus was detected in as high as 67.4% of the fetuses collected from gilts and sows with reproductive failure. The present study confirms that PRRS virus detection is strongly associated with reproductive failures in gilts and sows in the Thai swine commercial herds. This is in agreement with our previous clinical study on reproductive data in a commercial swine herd in Thailand [18]. In the previous study, although the homologous strain of PRRS MLV vaccine was implemented, some reproductive failures remained [6,7,18]. This can be explained by the finding of the present study that PRRS virus still exists and circulates within the herds, although vaccination has been undertaken. Interestingly, the prevalence of PRRS virus detection did not differ significantly between PRRS MLV vaccinated herds and non-vaccinated herds (i.e., 65.2% and 68.2%, respectively). This indicates that PRRS-MLV vaccination does not reduce the transplacental infection of the PRRS virus under field conditions. Therefore, although vaccination has been done, herd health monitoring, sanitation, biosecurity and general management are still important to minimize the viral circulation and the clinical signs of the PRRS virus infection.

In the present study, the age of the fetuses with PRRS virus detection varied from 31 to 119 days. This is in agreement with the previous studies that PRRS virus infection can occur at any stage of gestation [9,29]. Nevertheless, the sows with gestation length of ≥ 90 days are more sensitive to PRRS virus infection than sows with gestation length < 90 days [12,22]. In the present study, the prevalence of PRRS virus detection tended to be higher in fetuses aged ≥ 70 days compared to fetuses aged < 70 days. In general, the fetus ages > 70 days have had an immune competency. This, in fact, increases the potential of the viral clearance from the fetal tissue by the fetal immune system. However, it is known that PRRS virus infection and replication commonly occurred during the late state of gestation [11]. Thus, the viral detection is more common in the fetuses aged ≥ 70 days. The mechanism of reproductive
failure (i.e., abortion and fetal death) caused by PRRS virus infection is still unclear [3,12]. However, it was found that PRRS virus may induce apoptosis in PRRS virus infection site (i.e., endometrial connective tissues and fetal placenta) and subsequently caused reproductive disorders, e.g., abortion, premature farrowing, stillbirth and PRRS virus-infected live born piglets [12]. PRRS virus is able to replicate in the endometrium, cross the maternal epithelium, replicate in the fetal placenta and reach the fetal internal organs [12,20]. Additionally, sows experimentally infected with field strain of PRRS virus can farrow both non-infected and infected fetuses, and the infected fetuses are able to shed the virus [22]. Moreover, PRRS virus has been detected in many tissues of the infected fetus, i.e., umbilical cord, heart, lung, lymph node, spleen, tonsil and thymus [3,22]. Of these organs, the thymus is the primary site of the PRRS virus replication [3,22]. In the present study, thymus was not included in the mummified fetus samples. Thus, the amount of PRRS virus in mummified fetuses tended to be lower than that of the aborted fetuses and stillborn piglets. Furthermore, it has been demonstrated that PRRS virus can be diminished under dry or a low humidity conditions [5,21]. Therefore, the amount of virus might be low in the mummified fetus samples.

In the present study, the prevalence of PRRS virus from the dead fetuses did not differ significantly among parity groups of sows. This might be due to the fact that the specimens were collected only from the females that had reproductive failures. In the present study, 20% of the specimens had co-infection of genotypes 1 and 2 of PRRS virus. This finding is not surprising, because multiple isolates of the PRRS virus strains in infected piglets had been reported earlier, i.e., a so called “quasispecies” [2]. Interestingly, genetic combination among these multiple isolates has occurred and subsequently caused atypical clinical symptoms of PRRS virus infection under field conditions [17,22,28]. This may result in genetic diversity of the PRRS virus under field conditions, which may compromise immunological protection after PRRS–MLV vaccination.

In the present study, vaccination was conducted by two different commercial vaccines, one from European and another one from North American PRRS virus strain. Nowadays, both strains of the PRRS-MLV are used on most continents, because a strict geographical genotype barrier no longer exists [11]. In Thailand, both vaccine strains have been used in swine commercial herds for over 10 years. In fact, mixed genotypes of PRRS virus are commonly found in the herds using either European or North
American PRRS-MLV strains (Table 4). For instance, in a herd using North American PRRS-MLV strain, both genotypes of the PRRS virus are detected in 5 of 9 samples. Likewise, in a herd using European PRRS virus strain, both genotypes of the PRRS virus are detected in 2 of 2 samples (Table 4). These findings indicate that the PRRS viral shedding can be found in any vaccinated herds with regard to the vaccine strain used. Earlier studies have shown that PRRS-MLV is able to induce virus neutralizing antibodies, prevent PRRS virus replication in target cells, viremia and reduce clinical symptoms [11,23]. Furthermore, experimental inoculation with either European or North American strains of PRRS-MLV in gestating gilts and sows cause trans-placental infection to the fetus [16,23]. Therefore, the PRRS virus isolated from the fetus can be either vaccine or field strains. Nevertheless, in the present study, no significant difference on the prevalence of PRRS virus detection between vaccinated and non-vaccinated herds was found.

Under field condition, many strategies including PRRS-MLV vaccination have been used to control infertility problems caused by PRRS virus. In Thailand, Olanratmanee et al. [19] found that reproductive performance of sows in selected PRRS virus seropositive herds with PRRS-MLV vaccination were superior compared with those in PRRS-MLV non-vaccinated herds. The reason might be that PRRS-MLV vaccination is able to reduce viral shedding and partially prevent transplacental transmission [23], and thus, reduced infertility problems caused by the field strain PRRS virus infection. Nevertheless, although a number of comprehensive studies on the effect of PRRS-MLV vaccination on reproductive performance of sows have been conducted worldwide, the results are still controversial (i.e., negative, neutral and positive effects have been found) [11, 18, 19, 23]. In addition, Olanratmanee et al. [20] demonstrated that PRRS virus antigen was detected in the uterine tissues in gilts culled due to reproductive failure in both PRRS-MLV vaccinated and non-vaccinated herds. Furthermore, vertical transmission of PRRS virus has been clearly demonstrated [11,12,23]. Therefore, it is not surprising to detect the PRRS virus in aborted fetuses, mummified fetuses or stillborn piglets under field condition, although PRRS-MLV has been implemented.

It can be concluded that PRRS virus was frequently detected in aborted fetuses, mummified fetuses and stillborn piglets in swine commercial herds in Thailand, both in PRRS-MLV vaccinated herds and non-vaccinated herds. This indicated that the dead fetuses as well as stillborn piglets are
important sources of the PRRS virus within the herd. Therefore, the routine management of dead fetuses and stillborn piglets in PRRS virus-positive herds should be emphasized in order to minimize the viral load and the viral shedding within the herd.

ACKNOWLEDGEMENTS. Financial support of the present study was provided by Grant for International Research Integration: Chula Research Scholar, Ratchadaphiseksomphot Endowment Fund. E. Olanratmanee is a grantee of the Royal Golden Jubilee (RGJ) Ph.D. Program of the Thailand Research Fund.

REFERENCES

1. 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). Virol. J. 6: 143.

2. Chang, C.C., Yoon, K.J., Zimmerman, J.J., Harmon, K.M., Dixon, P.M., Dvorak, C.M. and Murtaugh, M.P. 2002. Evolution of porcine reproductive and respiratory syndrome virus during sequential passages in pigs. J. Virol. 76: 4750–4763.

3. Cheon, D.-S. and Chae, C. 2001. Distribution of porcine reproductive and respiratory syndrome virus in stillborn and liveborn piglets from experimentally infected sows. J. Comp. Pathol. 124: 231–237.

4. Cho, J.G. and Dee, S.A. 2006. Porcine reproductive and respiratory syndrome virus. Theriogenology 66: 655–662.

5. Cutler, T.D., Wang, C., Hoff, S.J. and Zimmerman, J.J. 2012. Effect of temperature and relative humidity on ultraviolet (UV254) inactivation of airborne porcine respiratory and reproductive syndrome virus. Vet. Microbiol. 159: 47–52.

6. 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. Prev. Vet. Med. 40: 233–241.
7. Dewey, C.E., Wilson, S., Buck, P. and Leyenaar, J.K. 2004. Effects of porcine reproductive and respiratory syndrome vaccination in breeding-age animals. *Prev. Vet. Med.* 62: 299–307.

8. Dias, A.S., Gerber, P.F., Araujo, A.S., Auler, P.A., Gallinari, G.C. and Lobato, Z.I.P. 2013. Lack of antibody protection against Porcine circovirus 2 and Porcine parvovirus in naturally infected dams and their offspring. *Res. Vet. Sci.* 94: 341–345.

9. Done, S.H., Paton, D.J. and White, M.E.C. 1996. Porcine reproductive and respiratory syndrome (PRRS): a review with emphasis on pathological, virological and diagnostic aspects. *British Vet. J.* 152: 153–174.

10. Egli, C., Thür, B., Liu, L. and Hofmann, M.A. 2001. Quantitative TagMan® RT-PCR for the detection and differentiation of European and North American strains of porcine reproductive and respiratory syndrome virus. *J. Virol. Methods* 98: 63–75.

11. Karniychuk, U.U. and Nauwynck, H.J. 2013. Pathogenesis and prevention of placental and transplacental porcine reproductive and respiratory syndrome virus infection. *Vet. Res.* 44: 95.

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

13. Maldonado, J., Segales, J., Martinez-Puig, D., Calsamiglia, M., Riera, P., Domingo, M. and Artigas, C. 2005. Identification of viral pathogens in aborted fetuses and stillborn piglets from cases of swine reproductive failure in Spain. *Vet. J.* 169: 454–456.

14. Meng, X.J. 2000. Heterogeneity of porcine reproductive and respiratory syndrome virus: implications for current vaccine efficacy and future vaccine development. *Vet. Microbiol.* 74: 309–329.

15. Meng, X.J., Pual, P.S. and Halbur, P.G. 1994. Molecular cloning and nucleotide sequencing of the 3’-terminal genomic RNA of the porcine reproductive and respiratory syndrome virus. *J. Gen. Virol.* 75: 1795–1801.

16. 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. *Vet. Microbiol.* 84:1–13.
17. Nilubol, D., Tripipat, T., Hoonsuwan, T. and Piriyapongsa, J. 2013. Genetic diversity of the ORF5 gene of porcine reproductive and respiratory syndrome virus (PRRSV) genotypes I and II in Thailand. *Arch. Virol.* 158: 943–953.

18. 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. *Trop. Anim. Health Prod.* 45: 1099–1106.

19. Olanratmanee, E., Thanawongnuwech, R., Kunavongkrit, A. and Tummaruk, P. 2014. Reproductive performance of sows with and without PRRS modified live virus vaccination in PRRS virus seropositive herds. *Trop. Anim. Health Prod.* 46: 1001–1007.

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

21. Pirtle, E.C. and Beran, G.W. 1996. Stability of porcine reproductive and respiratory syndrome virus in the presence of fomites commonly found on farms. *J. Am. Vet. Med. Assoc.* 208: 390–392.

22. Rowland, R.R.R. 2010. The interaction between PRRSV and the late gestation pig fetus. *Virus Res.* 154: 114–122.

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

24. Snijder, E.J. and Meulenberg, J.J.M. 1998. The molecular biology of arteriviruses. *J. Gen. Virol.* 79: 961–979.

25. Tummaruk, P. and Tantilertcharoen, R. 2012. Seroprevalence of porcine reproductive and respiratory syndrome, Aujeszky’s disease and porcine parvovirus in replacement gilts in Thailand. *Trop. Anim. Health Prod.* 44: 983–989.
26. 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. *Trop. Anim. Health Prod.* 45: 771–779.

27. Ullrey, D.E., Sprague, J.I., Becker, D.E. and Miller, E.R. 1965. Growth of the swine fetus. *J. Anim. Sci.* 24: 711–717.

28. Yuan, S., Nelsen, C.J., Murtaugh, M.P., Schmitt, B.J. and Faaberg, K.S. 1999. Recombination between North American strains of porcine reproductive and respiratory syndrome virus. *Virus Res.* 61: 87–98.

29. Zimmerman, J., Benfield, D.A., Murtaugh, M.P., Osorio, F., Stevenson, G.W. and Torremorel, M. 2006. Porcine reproductive and respiratory syndrome virus (Porcine Arterivirus). pp. 387–417. *In: Disease of Swine*, 9th ed. (Straw, B.E., Zimmerman, J.J., D’Allaire, S. and Taylor, D.J. eds.) Blackwell Publishing Professional, Iowa.
Table 1 The percentage of porcine reproductive and respiratory syndrome (PRRS) virus detection in aborted fetuses, mummified fetuses and stillborn piglets

| Specimen           | N  | PRRS virus detection | Genotypes of PRRS virus | Genotype 1 | Genotype 2 | Mixed       |
|--------------------|----|----------------------|-------------------------|------------|------------|-------------|
| Aborted fetuses    | 32 | 21/32 (65.6%)        | 5/32 (15.6%)            | 9/32 (28.1%)| 7/32 (21.9%)|             |
| Mummified fetuses  | 30 | 19/30 (63.3%)        | 7/30 (23.3%)            | 6/30 (20.0%)| 6/30 (20.0%)|             |
| Stillborn piglets  | 27 | 20/27 (74.1%)        | 5/27 (18.5%)            | 8/27 (29.6%)| 7/27 (25.9%)|             |
| Total              | 89 | 60/89 (67.4%)        | 17/89 (19.1%)           | 23/89 (25.8%)| 20/89 (22.5%)|             |

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

Table 2 The percentage of porcine reproductive and respiratory syndrome (PRRS) virus detection by age of fetuses

| Age of fetuses | N  | PRRS virus detection | Genotypes of PRRS virus | Genotype 1 | Genotype 2 | Mixed       |
|----------------|----|----------------------|-------------------------|------------|------------|-------------|
| < 70 days      | 20 | 11/20 (55.0%)        | 0/20 (0.0%)             | 7/20 (35.0%)| 4/20 (20.0%)|             |
| ≥ 70 days      | 69 | 49/69 (71.0%)        | 17/69 (24.6%)           | 16/69 (23.2%)| 16/69 (23.2%)|             |
| Total          | 89 | 60/89 (67.4%)        | 17/89 (19.1%)           | 23/89 (25.8%)| 20/89 (22.5%)|             |

a,b Different superscripts within columns indicate statistically significant differences ($P<0.05$)
Table 3 The means age of the fetuses in porcine reproductive and respiratory syndrome (PRRS) virus positive and negative specimens

| Specimen            | PRRS virus negative |                  | PRRS virus positive |                  |
|---------------------|---------------------|------------------|---------------------|------------------|
|                     | N       | age of fetuses  | N       | age of fetuses  |
| Aborted fetuses     | 11      | 71.4±22.3<sup>aA</sup> | 21      | 75.3±29.0<sup>A</sup> |
| Mummified fetuses   | 11      | 94.1±23.5<sup>abB</sup> | 19      | 104.2±16.1<sup>abB</sup> |
| Stillborn piglets   | 7       | 113.2±1.9<sup>aB</sup>  | 20      | 115.0±1.3<sup>aB</sup>  |
| Total               | 29      | 88.6±25.7<sup>a</sup>  | 60      | 95.9±26.0<sup>b</sup>  |

<sup>a,b</sup> Different superscripts within rows indicate statistically significant differences (<i>P</i>&lt;0.05)

<sup>A,B</sup> Different superscripts within columns indicate statistically significant differences (<i>P</i>&lt;0.05)
Table 4 The percentage of porcine reproductive and respiratory syndrome (PRRS) virus detection in PRRS virus non-vaccinated herds (n=66) and in PRRS-modified lived virus vaccinated herds (n=23)

| Herds | n  | PRRS virus detection | PRRS virus detection by genotypes |
|-------|----|----------------------|----------------------------------|
|       |    |                      | Genotype 1 | Genotype 2 | Mixed     |
| Non-vaccinated herds | 66 | 45/66 (68.2%) | 12/66 (18.2%) | 20/66 (30.3%) | 13/66 (19.7%) |
| Herd A | 43 | 32/43 (74.4%) | 4/43 (9.3%) | 17/43 (39.5%) | 11/43 (25.6%) |
| Herd B | 12 | 6/12 (50.0%)  | 3/12 (25.0%) | 2/12 (16.7%) | 1/12 (8.3%)  |
| Herd C | 5  | 5/5 (100.0%) | 4/5 (80.0%)  | 0/5 (0.0%)  | 1/5 (20.0%)  |
| Herd D | 3  | 2/3 (66.7%) | 1/3 (33.3%) | 1/3 (33.3%) | 0/3 (0.0%) |
| Herd E | 2  | 0/2 (0.0%)   | 0/2 (0.0%)  | 0/2 (0.0%)  | 0/2 (0.0%)  |
| Herd F | 1  | 0/1 (0.0%)   | 0/1 (0.0%)  | 0/1 (0.0%)  | 0/1 (0.0%)  |
| Vaccinated herds | 23 | 15/23 (65.2%) | 5/23 (21.7%) | 3/23 (13.0%) | 7/23 (30.4%) |
| Herd G | 9  | 9/9 (100.0%) | 4/9 (44.4%) | 0/9 (0.0%)  | 5/9 (55.6%) |
| Herd H | 7  | 4/7 (57.1%)  | 1/7 (14.3%) | 3/7 (42.9%)  | 0/7 (0.0%) |
| Herd I | 5  | 0/5 (0.0%)   | 0/5 (0.0%)  | 0/5 (0.0%)  | 0/5 (0.0%)  |
| Herd J | 2  | 2/2 (100.0%) | 0/2 (0.0%)  | 0/2 (0.0%)  | 2/2 (100.0%) |
| Total  | 89 | 60/89 (67.4%) | 17/89 (19.1%) | 23/89 (25.8%) | 20/89 (22.5%) |
**Table 5** The means of copy numbers (log_{10}) of porcine reproductive and respiratory syndrome (PPRS) virus in PPRS virus positive aborted fetuses, mummified fetuses and stillborn piglets

| Specimen             | Number of PRRS virus positive | Genotype 1 | Genotype 2 |
|----------------------|------------------------------|------------|------------|
|                      | N   | Copy number   | N   | Copy number |
| Aborted fetuses      | 21  | 12 12.3±2.5^a | 16  | 12.0±1.3^a |
| Mummified fetuses    | 19  | 13 10.8±2.4^a | 12  | 10.4±1.5^b |
| Stillborn piglets    | 20  | 12 12.2±3.1^a | 15  | 11.2±1.4^ab |
| Total                | 60  | 37 11.7±2.7   | 43  | 11.3±1.5   |

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