## เอกสารอ้างอิง


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## ภาคผนวก

บทความทางวิชาการที่ตีพิมพ์ในวารสารทางวิชาการแล้ว (ถ้ามี)

**วารสารวิชาการระดับนานาชาติ**

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ORIGINAL RESEARCH

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

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

**Keywords** Pig · PRRSV detection · Reproductive failure · Uterus · Immunohistochemistry

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## Introduction

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

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

It has been suggested that the replacement gilts are a major source of introducing new strains of PRRSV into the herd. In practice, an intensive acclimatization of the replacement gilts with culled sows or infected nursery pigs is commonly practiced in most swine-breeding herds in Thailand. However, a high variability of the antibody titer against PRRSV of the gilts is observed both within and among herds (Tummaruk and Tantilertcharoen 2007). This problem causes difficulties for the farmer to mate the gilts. In our previous study, we have found that 73% (122/166) of the replacement gilts in Thailand culled due to reproductive disturbances were infected with PRRSV. A high proportion of PRRSV-seropositive gilts were found in the gilts culled due to abortion (81%) and repeat breeding (81%) (Tummaruk and Tantilertcharoen 2008).

In general, PRRSV primarily infects pulmonary alveolar macrophages during acute infection (Sur et al. 1997). It is well-established that the alveolar macrophages as well as macrophages from other tissues are the primary cell type that sustains the in vivo replication of the virus (Thanawongnuwech et al. 2000). Using immunohistochemical (IHC) evaluation of formalin-fixed tissues, we found that 66% and 100% of the lung tissue of piglets infected with either US or EU strain of Thai PRRSV, respectively, were observed (Laohasittikul et al. 2004). An earlier study based on PRRSV antigen detection by the IHC technique has demonstrated that 75.0%, 50.0%, 37.5%, 37.5%, 37.5%, and 25.0% of PRRSV was found in liver, spleen, tonsil, turbinate bone, pulmonary lymph node, and ileum, respectively, of the experimentally infected piglets (Laohasittikul et al. 2004). In addition, PRRSV antigen is found in microglia-like cells and mononuclear cells in the brain sections by IHC associated with neurovascular lesions (Thanawongnuwech et al. 1997). Using in situ hybridization (ISH), we found that PRRSV is also detected in the epithelial germ cells of the seminiferous tubules, primarily spermatids and spermatocytes and macrophages of the testis (Sur et al. 1997; Shin and Molitor 2002). However, to our knowledge, the presence of PRRSV in the uterine tissues of the gilts has not been demonstrated. Thus, the objective of this study is to determine the prevalence of PRRSV antigen in the uterine tissues of the gilts culled due to reproductive disturbances associated with age at culling, culling reason, herds, and PRRSV vaccination in selected swine commercial herds in Thailand.

## Materials and methods

### Animals and samples

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

### General management and vaccination

The herds in the present study are breeding herds located in the northeastern (A and B), middle (C), western (E), and eastern (D and F) parts of Thailand. The sows-on-production numbers were 900–3,500 sows per herd. Herds A and B produced replacement gilts within the herd using their own grandparent stock, while herds C, D, E, and F bought the replacement gilts from other breeders. The gilts in all herds were housed in a conventional open-housing system facilitated with a water sprinkler and fan for reducing heat stress. The health status of the herds was monitored routinely by the herd veterinarians. In general, the recommended gilt vaccination program consisted of foot-and-mouth disease, classical swine fever, Aujeszky's disease, and porcine parvovirus at between 22 and 30 weeks of age. Some herds were also given some extra vaccines against PRRSV, atrophic rhinitis, *Mycoplasma hyopneumo-*

*niae*, and *Actinobacillus pleuropneumoniae*. In herds B, E, and F, the replacement gilts were not vaccinated with PRRSV vaccine, while in herds A and D, they were vaccinated using the EU strain PRRS MLV vaccine (AMERVAC®, Laboratorios Hipra, Girona, Spain). Herd C, the replacement gilts, was vaccinated using the US strain PRRS MLV vaccine (Ingelvac® PRRS™ MLV, Boehringer-Ingelheim Vetmedica Inc., St. Joseph, MO, USA). The gilts were vaccinated against PRRSV twice during 22–30 weeks of age before being sent to the breeding house. Gilts were kept in each pen with a group size of 6–15 gilts per pen (depending on the herd) with a density of 1.5–2.0 $m^2$ per gilt. In general, the herds were recommended to breed the replacement gilts at about 32 weeks of age onwards with a body weight of at least 130 kg at the second or later observed estrus. The mating technique for all herds was performed by artificial insemination.

### Immunohistochemistry

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

### Serological test

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

### Statistical analyses

Statistical analyses were performed using Statistical Analysis System (SAS) version 9.0 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics (means, standard deviation, and range) and frequency tables were conducted for all reproductive parameters. The percentage of positive tissue was compared between groups of age at culling (6–8, 9–10, and 11–16 months), reason for culling (anestrus, vaginal discharge, repeat breeding, abortion, and not being pregnant), type of MLV vaccine against PRRSV (US and EU strain), and the detection of antibody titers against PRRSV by using ELISA (0.00–0.39, 0.40–0.99, 1.00–1.49, and 1.50–2.92) using $r \times k$ contingency table and *Fisher's exact* test. Logistic regression was performed to analyze the multiple effects of age at culling and the use of PRRSV vaccine on the incidence of PRRSV detection in the uterine tissues of the gilts. The analysis was carried out using the GLIMMIX macro of SAS. The statistical model included the effect of age at culling and PRRSV vaccination as independent variables. Least-square means of the *logit* scale were obtained and were compared by using the least significant different test. A value of $P<0.05$ was considered to be statistically significant.

## Results

### Reproductive data and culling reason

Reproductive data of the slaughtered gilts are presented in Table 1. On average, the gilts were culled at 303.3± 53.0 days of age and a body weight of 149.0±20.8 kg. They entered the herds at 218.9±53.1 days of age and were culled at 84.4±57.1 days after entering the herd. Of all the gilts, 52 gilts (52.0%) had been mated, and the interval from the first observed estrus to mating was 20.8±17.2 days (range 0 to 63 days). The reasons for culling of the gilts

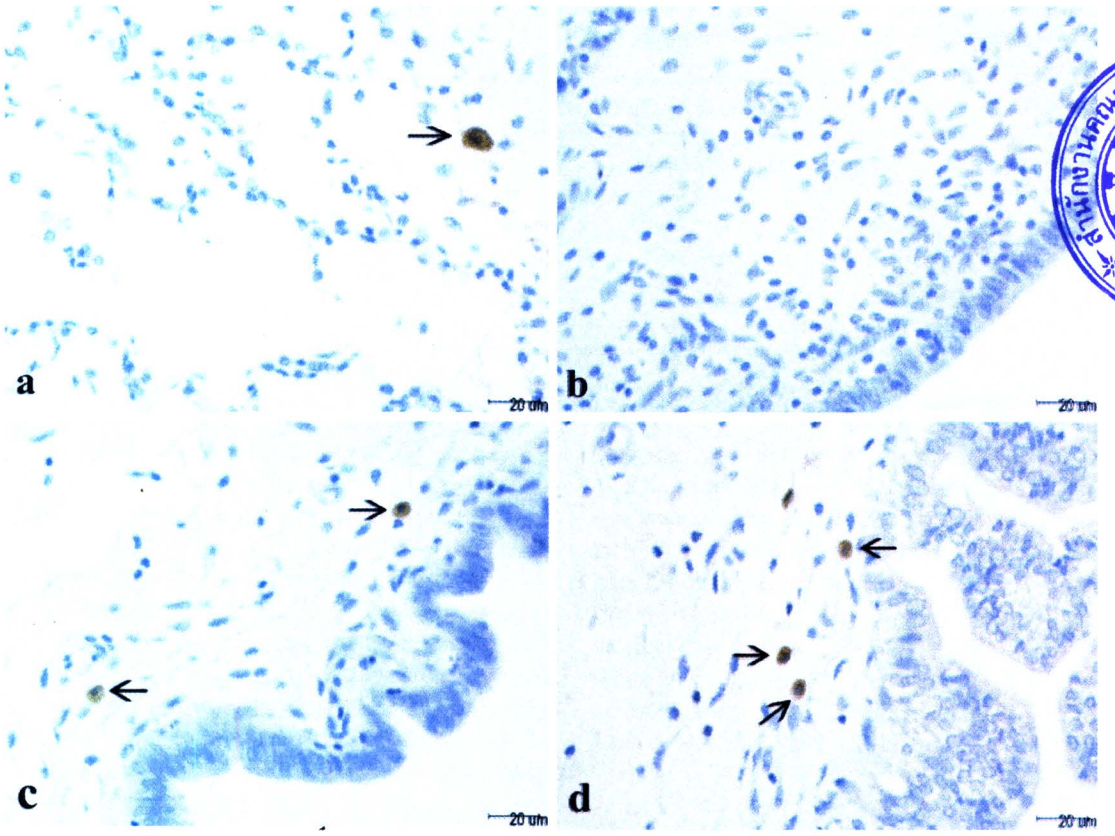


**Fig. 1** Expression of PRRSV antigen in the uterine tissue of gilts: **a** positive control (lung tissue); **b** negative control; **c**, **d** uterine tissue from gilts culled due to reproductive disturbance which expressed PRRSV antigen. *Black arrows* indicate positive staining cell

included anestrus, abnormal vaginal discharge, abortion, repeat breeding, and not being pregnant (Table 2). On average, the age at culling was 273.8, 298.0, 311.3, 342.9, and 368.9 days, and the interval from entry to culling was 73.0, 67.6, 68.3, 111.4, and 142.8 days for gilts culled due to anestrus, abnormal vaginal discharge, abortion, repeat breeding, and not being pregnant, respectively.

## Detection of PRRSV

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

**Table 1** Descriptive statistics for reproductive data of the replacement gilts culled due to reproductive failure

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

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

**Table 2** Number and percentage of gilts in relation to the presence of PRRSV antigen in the uterine tissue by IHC and the antibody titer against PRRSV by culling reason

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

Different letters within columns differ significantly ($P \leq 0.05$)

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

On average, gilts that had PRRSV in the uterine tissue were culled at 307.2±54.1 days of age (range 240 to 439 days), while those that had no PRRSV in the uterine tissue were culled at 301.3±52.8 days of age (range 209 to 489 days) ($P$=0.605). NPD of these gilts was 92.0±59.6 (range 0 to 225 days), and ADG was 488.1±80.6 g/day. The incidence of PRRSV manifestation in the uterine tissues of the gilts was 29.6%, 39.4%, and 40.9% in the gilts culled at 6–8, 9–10, and 11–16 months of age, respectively ($P$=0.698). PRRSV was found in 33.3%, 28.6%, 27.3%, 41.2%, and 33.3% of the uterine tissues of the gilts culled due to anestrus, abnormal vaginal discharge, abortion, repeat breeding, and not being pregnant, respectively ($P$=0.929) (Table 2). The detection of PRRSV in the uterine tissue of the gilts did not differ significantly between the virgin gilts (35.4%) and the gilts that were mated before culling (30.8%) ($P$=0.622).

Influence of antibody titer against PRRSV

Of the 100 replacement gilts, 83 serum samples were included in the present study. Of all the gilts, 61 of 83 gilts (73.5%) were positive to ELISA. The highest percentage of positive gilts (29/36 gilts, 80.6%) was observed in gilts culled due to anestrus. The percentage of positive gilts culled for abnormal vaginal discharge, abortion, repeat breeding, and not being pregnant, was 73.7%, 80.0%, 58.8%, and 0.0%, respectively (Table 2). Of the 61 gilts that were positive to ELISA, 22 gilts (36.1%) were positive to IHC. Of the 22 gilts that were negative to ELISA, five gilts (22.7%) were positive to IHC. According to all gilts that were positive to IHC, 81.5% (22/27 gilts) were positive to ELISA (Table 3). PRRSV was detected in the uterine tissue in 28.2%, 31.0%, 47.1%, and 33.3% of the gilts with antibody titers against PRRSV at 0.00–0.39, 0.40–0.99, 1.00–1.49, and 1.50–2.92, respectively ($P$=0.577).

## Discussion

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

**Table 3** Reproductive data of gilts culled due to reproductive disturbances in relation to percentage of ELISA-positive gilts to the results of IHC test

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

Different letters within columns differ significantly ($P \leq 0.05$)

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

remained in their uterine tissue. Therefore, the reproductive performance of these gilts might be compromised.

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

It has been demonstrated that PRRSV can be detected for at least 42 days post-infection in the lungs and in the tonsil by using IHC and ISH (Sur et al. 1996), at least 59 days post-infection in the brain stem by using ISH (Shin and Molitor 2002) and at least 15 days post-infection in the lung, liver, pulmonary lymph node, spleen, tonsil, turbinate bone, and heart by using IHC (Laohasittikul et al. 2004). In the reproductive organs, PRRSV can be detected by using ISH in the macrophages in the interstitium of the testis during 7–30 days post-infection and in the seminiferous tubules primarily in spermatocytes and round spermatids up to 25 days post-infection (Sur et al. 1997). Moreover, PRRSV has been found in the testis, epididymis, prostate gland, and bulbourethral gland at 7 days post-infection and in testis and epididymis at least 59 days post-infection (Shin and Molitor 2002). In this study, the exact timing of PRRSV infection in the replacement gilts is not known, but it is likely to be the period during PRRS MLV vaccination and acclimatization. These management practices are usually performed within a month after the gilts enter the herds. Most of these gilts are culled nearly 3 months after entering the herds. This indicates that PRRSV may remain in the uterine tissue of the infected gilts for several months, or re-infection might have occurred. In the boar, PRRSV infection causes viral shedding in semen for several months (Christopher-Hennings et al. 1995).

In this study, PRRSV is found in 6.0% of the uterine tissues of the gilts having no antibody titer against PRRSV. It has been demonstrated that PRRSV is widespread in the respiratory and lymphoid system of the pig by 1–2 days post-infection (Halbur et al. 1996) and in liver, ileum, kidney, and turbinate bone by 5 days post-infection (Laohasittikul et al. 2004). PRRSV antibodies can be detected early at 7–14 days post-infection using commercial ELISA; peak titers are seen by 30–50 days post-infection and undetectable titers by 4–6 months after infection (Benfield et al. 1999). Thus, the antigen of the virus can be detected while the antibody was undetected.

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

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ORIGINAL RESEARCH

# Seroprevalence of porcine reproductive and respiratory syndrome, Aujeszky's disease, and porcine parvovirus in replacement gilts in Thailand

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**Abstract** The present study investigated the seroprevalence of porcine reproductive and respiratory syndrome virus, Aujeszky's disease virus (ADV), and porcine parvovirus (PPV) in replacement gilts from selected five swine herds in Thailand. The study consisted of three parts. First, a retrospective data analysis on the seroprevalence of porcine reproductive and respiratory syndrome virus (PRRSV) and ADV glycoprotein I (gI) in gilts, sows, boars, nursery, and fattening pigs in five herds ($n$=7,030). Second, a cross-sectional study on seroprevalence of PRRSV, ADV, and PPV ($n$=200) in replacement gilts. Last, the seroprevalence of PRRSV, ADV, and PPV in gilts culled due to reproductive failure ($n$=166). Across the herds, the seroprevalence of PRRSV and ADV was 79.3% and 5.3%, respectively. The cross-sectional study revealed that 87.5%, 4.0%, and 99.0% of the replacement gilts were infected with PRRSV, ADV, and PPV, respectively. In the gilts culled due to reproductive failure, the seroprevalence of PRRSV, ADV, and PPV was 73.5%, 28.3%, and 86.0%, respectively. Of these culled gilts, 75.5% had been infected with at least two viruses and 18.9% had been infected with all three viruses. It could be concluded that most of the replacement gilts were exposed to PRRSV (84%), PPV (97%), and ADV (4%) before entering the breeding house. PPV was an enzootic disease among the selected herds. The prevalence of ADV was higher in gilts culled due to reproductive disturbance than in the healthy gilts.

**Keywords** Pig · Reproduction · Health · Disease · Acclimatization

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## Introduction

In practice, the replacement gilts have to be immunized against a number of pathogens via either acclimatization or vaccination before entering the herd. Generally, weaned sows selected for culling, nursery pigs, or fattening pigs are used for acclimatization. In most commercial herds in Thailand, replacement gilts are routinely vaccinated against Aujeszky's disease virus (ADV) and porcine parvovirus (PPV). But porcine reproductive and respiratory syndrome virus (PRRSV) vaccine is applied only in some herds. The viral pathogens causing a large impact to the swine industry in Thailand during the last decade include classical swine fever virus (CSFV), foot-and-mouth disease virus (FMDV), porcine circovirus type 2 (PCV-2), PRRSV, ADV, and PPV. Furthermore, the last three pathogens contribute to reproductive disorders in gilts and sows (Maldonado et al. 2005). Nowadays, co-infection of these pathogens is commonly observed in the modern swine industry (López-Soria et al. 2010). The co-infection in pigs may cause complicated clinical signs, such as porcine respiratory disease complex and post-weaning multisystemic wasting syndrome (Opriessnig et al. 2007). Although the influence of these complex diseases is well established in nursery and fattening pigs, information related to their influences on reproductive problems in gilts and sows are limited.

Based on serological examination, PRRSV has been first detected in Thailand in early 1989 (Oraveerakul et al.

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1995). Nowadays, both European (EU) and North American strains are isolated in Thailand (Thanawongnuwech et al. 2004). In 1995, a serological survey on glycoprotein I (gI) of ADV from 15 swine herds in Thailand indicated that 98% (597/608 samples) of the pig samples are positive (Wongwatcharadumrong and Platt 1995). It is known that the gI of ADV indicates natural infection (Mengeling et al. 1997). Therefore, monitoring of ADV-gI-positive pigs in the herd is an important key for ADV elimination program. At the present time, the prevalence of ADV in Thailand has declined because ADV vaccine is extensively used, together with the surveillance of ADV-gI is routinely performed. However, the prevalence of ADV causing different types of reproductive failure in gilts has never been investigated in Thailand. In general, PPV antigen can be detected in the sow's serum after infection up to 10 days (Miao et al. 2009). PPV antibody titer varies between 1:32 and 1:512 after vaccination. However, it may reach 1:40,960 within 19 days after challenging with field-strained PPV (Józwik et al. 2009). A high level of PPV antibody titer is commonly observed in both gilts and sows under field conditions. This is unlikely to be the result of PPV vaccination. Instead, it is associated with herd size, parity number, and reused of storage open vials vaccine (Oravainen et al. 2005). A study on the seroprevalence of high PPV antibody titer in replacement gilts in association with PRRSV and ADV may be important for investigation to understand the causes of reproductive failure in gilts raised in Thai swine herds (Tummaruk et al. 2009a). The objective of the present study was to investigate the seroprevalence of viruses causing reproductive disorders (PRRSV, ADV, and PPV) from different groups of pigs in commercial swine herds in Thailand with special emphasis on replacement gilts and gilts culled due to reproductive failures.

## Materials and methods

### Animals and blood samples

In the first part, herd monitoring data were collected from five commercial swine herds (A, B, C, D, and E) in Thailand between 2004 and 2007. The data totally included 7,030 pigs [764 boars, 3,364 gilts, 1,613 sows, 646 nursery pigs (4–9 weeks of age) and 643 fatteners (10–26 weeks of age)]. In general, the serological survey on PRRSV was done on a monthly basis in replacement gilts, and once or twice a year in the others. The serological survey for ADV was performed once a year in all pig groups. Both PRRSV and ADV-gI protein were examined in standardized laboratories in Thailand [most (except herd E) were done at the Faculty of Veterinary Science, Chulalongkorn University]. In the laboratories, PRRSV antibody was determined using HerdChek® PRRSV antibody test kit 2XR (IDEXX Laboratories, Inc., USA), while ADV-gI protein was examined using HerdChek® Anti-PRV gpI test kit (IDEXX Laboratories, Inc., USA). In herd E, PRRSV antibody was monitored using *HIPRA* PRRSV antibody test kit (*HIPRA* Laboratories, Inc., Spain; see below). In the second part, 200 blood samples (40 gilts per herd) were randomly collected from the jugular vein of healthy replacement gilts (244.7±5.8 days of age) at the same period (April to May 2005). The blood samples were collected from three groups of gilts, i.e., before acclimatization ($n$=50), after acclimatization ($n$=50) and mid-period of gestation (68.8±1.4 days, means±SEM) ($n$=100). In the last part, blood samples, genital organs, and reproductive data were collected from 166 slaughtered gilts from May 2005 to October 2008. The samples were kept on ice in the closed containers and transported to the laboratory within 24 h after culling. Data including gilt's identity, birth date, herd entry date, insemination date, culling date, body weight at culling, and reason for culling were collected from each herd. Age at first insemination and age at culling were calculated. Average daily gain (ADG) from birth to culling was calculated: ADG (grams per day)=(BW at culling−1.5/age at culling)×1,000 (Tummaruk et al. 2009b). The ADG was calculated to partially determine the health status of the gilts during their growing period. Culling reasons were classified into four groups, i.e., abortion, anestrus, repeated breeding, and abnormal vaginal discharge. Data relevant to gross morphological findings were presented in our previous study (Tummaruk et al. 2009a). In the second and the last parts, all serum samples were analyzed at the Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University (see below).

### Herd management

In the present study, the number of sows on production was 3,200, 1,700, 2,700, 3,500, and 900 sows in herd A, B, C, D, and E, respectively. The gilts entered the herd at a body weight of 92.2±1.1 kg (164.2±1.7 days of age) and they were sent to the breeding house at a body weight of 130.3± 2.0 kg (218.9±2.7 days of age). In the gilt pools, the gilts were kept in a pen with a group size of 6 to 15 gilts/pen with space allowance of 1.5 to 2 $m^2$/gilt. Water was ad libitum provided from water nipples. The feed (a corn–soybean–fish base, 16–18% CP, 3,000–3,400 kcal/kg ME, 0.85–1.00% lysine) was provided about 3 kg/day/head. In general, the gilts were vaccinated against FMDV, CSFV, ADV, and PPV between 22 and 30 weeks of age. Apart from these, vaccination in herd E also included PRRSV, atrophic rhinitis, mycoplasmosis, and *Actinobacillosis*

*pleuropneumoniae*. In most cases, the weaned sows selected for culling were taken to acclimatize the replacement gilts for about 4 weeks with a ratio of one sow per six to ten gilts; and were rotated on a weekly basis. After acclimatization, these sows were removed from the herds. The acclimatization program was applied in the replacement gilts at 22–28 weeks of age in order to naturally immunize the gilts via the weaned sows. Using this acclimatization process, the replacement gilts were exposed to many types of viral antigens circulating within the herds (e.g., field-strained PRRSV and enterovirus) before sending to the breeding houses. In general, it was recommended to breed the replacement gilts at 32 weeks of age onwards with a body weight of at least 130 kg at the second or later observed estrus. The mating technique for all herds was performed by artificial insemination.

### Serological analyses

The blood samples were left at room temperature to clot, then the sera were obtained and kept at −20°C for further serological analyses. Antibody against PRRSV in most herds (except herd E) was determined using HerdChek® PRRSV antibody test kit 2XR (IDEXX Laboratories, Inc., USA). The analysis was performed according to the manufacturer's instructions. Briefly, positive and negative controls were carried in the same plate as the sample. The serum sample/positive control (*S/P*) ratio was calculated. The *S/P* ratio below 0.4 indicated that the sample had no PRRSV antibody (negative), while *S/P* ratio ≥0.4 indicated that the sample was positive to PRRSV. In herd E, in the first part of the study, PRRSV antibody was examined using *HIPRA* PRRSV antibody test kit (*HIPRA* Laboratories, Inc., Spain). The cut-off value of the positive samples was set at >20 percentage relative index. Antibody against ADV-gI protein in all herds was determined using HerdChek® Anti-PRV gpI test kit (IDEXX Laboratories, Inc., USA). The procedures followed the kit's instructions. The antibody against PPV was determined by means of haemagglutination inhibition (HI) test. Briefly, 0.2 ml of serum was incubated at 56°C for 30 min and mixed with 0.6 ml of 25% Kaolin suspension in phosphate buffer solution (PBS). The samples were left at room temperature for 20 min, were centrifuged and were mixed with 0.1 ml of 50% guinea pig red blood cells in PBS, then were left at room temperature for 1 h. Diluents were added into microplate and mixed with 50 μl of serum samples. Serial dilutions (the samples were diluted for 1:2 each time) were carried out on the microplate using a multichannel micropipette for 12 dilutions (1:4 to 1:4,096). Positive and negative controls were also included in all plates. A 25 μl of PPV 8 hemagglutination unit was added to all channels with 0.6% guinea pig red blood cells and was left at room temperature for 60–90 min or until the precipitation was observed. A PPV antibody titer of <1:8 indicated no seroconversion, 1:16 to 1:512 indicated intermediate seroconversion and >1:512 indicated high level antibody (Oravainen et al. 2005). Since all the samples tested for PPV were positive, the antibody titer was divided into two groups, i.e., low (≤1:512) and high (>1:512). All serological examinations, in the second and last parts of the study, were carried out in the same laboratory (Veterinary Diagnostic Laboratory, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand).

### Statistical analyses

The statistical analysis was performed using SAS Version 9.0 (SAS Inst. Inc., Cary, NC., USA.). Continuous data were presented as means±SEM and categorical data were expressed as percentage. In the first study, the percentage of pigs which were seropositive to PRRSV, ADV-gI protein, and PPV (titer >1:512) were obtained by using frequency analysis ($r \times k$ contingency table). The proportional data were compared among herds (five herds), years (2004–2007), and age groups of pigs (nursery, fattener, gilt, sow, and boar) by logistic regression using GENMOD procedure of SAS. In the second study, the replacement gilts were classified into three groups (i.e., before acclimatization, after acclimatization, and mid-period of gestation). The percentage of pigs which were seropositive to PRRSV, ADV-gI protein, and PPV were compared among groups by Chi-squares test. In the last study, the gilts were classified according to the reasons for culling. The percentage of pigs which were seropositive to PRRSV, ADV-gI protein, and PPV were compared by Chi-squares test. Continuous data including age at culling (days), body weight at culling (kilogram), ADG (grams per day), age at first mating (day) and number of ovulation (number of corpora lutea) were analyzed by using general linear model procedure. Reasons for culling were included in the statistical models as an independent variable. Least-squares means were obtained from the statistical models and were compared using Tukey–Kramer adjustment. Values of $P<0.05$ were considered statistically significant.

## Results

### Herds monitoring data

Herds monitoring data revealed that all herds had been infected with PRRSV for more than four years. The overall percentage of PRRSV-positive pigs was 79.3% (4,492/5,664 pigs). The percentage of PRRSV-positive pigs differed significantly among years and herds. The percentage of PRRSV-seropositive pigs was 64.8%, 74.8%, 83.8%,

and 87.7% in 2004, 2005, 2006, and 2007, respectively. The prevalence of PRRSV was significantly increased year after year from 2004 to 2007 ($P<0.001$). The percentage of PRRSV-seropositive pigs was 81.7%, 67.9%, 60.6%, 80.9%, and 79.3% in herds A, B, C, D, and E, respectively. Herd A, D, and E had a higher percentage of PRRSV-positive pigs than herd B ($P<0.001$) and C ($P<0.001$). The prevalence of PRRSV was not significantly different among herds A, D, and E ($P>0.05$). The prevalence of PRRSV was higher in fatteners (84.1%), gilts (82.6%), sows (82.0%), and boars (79.4%) than in nursery pigs (48.4%; $P<0.001$; Table 1).

The percentage of ADV-seropositive pigs from all herds was 5.3% (70/1,332 pigs). The percentage of ADV-seropositive pigs was 17.8%, 5.9%, 0.8%, 0.0%, and 2.2% in herds A to E, respectively ($P<0.001$). Herd A had a higher prevalence of ADV than herd B ($P<0.001$), C ($P=0.001$), D ($P<0.001$), and E ($P<0.001$). Herd B had a higher prevalence of ADV than herd C ($P=0.052$), D ($P<0.001$), and E ($P=0.016$). The prevalence of ADV was higher in sows (11.9%), boars (4.6%), and nursery pigs (3.2%) than in gilts (0.0%) and fatteners (0.9%; $P<0.001$; Table 1). Across the herds, the prevalence of ADV also varied among years. The prevalence of ADV was 3.8%, 3.4%, 8.5%, and 2.3% from 2004 to 2007, respectively ($P<0.001$). The prevalence of ADV in 2006 was higher than in 2004 ($P=0.008$), 2005 ($P=0.005$), and 2007 ($P=0.009$).

### Replacement gilts

On average, the gilts were first mated at 242.7±2.5 days of age (range 212–348 days); the ADG from birth to first mating was 588.6±5.9 g/day (range 485.6–434.4 g/day). Across the herds, 87.5% (175/200) of the gilts had antibody titer against PRRSV. The *S/P* ratio of the PRRSV-seropositive gilts varied from 0.428 to 3.673. The percentage of PRRSV-seropositive gilts before and after acclimatization were 84.0% and 92.0%, respectively ($P=0.218$). The percentage of PRRSV-seropositive gilts was 85%, 95%, 100%, 55%, and 100% in herds A, B, C, D, and E, respectively. In the pregnant gilts, the *S/P* ratio of PRRSV varied from 0.03 to 3.7; 87.0% of them were PRRSV seropositive.

The antibody titer against ADV was found 4.0% from all gilts (8/200). Of the ADV-seropositive gilts, two out of eight were observed before acclimatization, while the rest was observed after acclimatization (during pregnancy). However, the ADV-positive gilts were found only in herd A.

All of the replacement and pregnant gilts were PPV seropositive with a titer of ≥1:128. Of these gilts, 99.0% had high PPV antibody titer (>1:512) and 97.0% had very high PPV titer (≥1:4,096).

**Table 1** Percentage of pigs which were seropositive to Aujeszky's disease virus (ADV) and porcine reproductive and respiratory syndrome (PRRSV) in different groups of pigs in commercial herds in Thailand during 2004–2007

| Group of pigs | Percentage of seropositive pigs | |
|---|---|---|
| | ADV | PRRSV |
| Nursery | 3.2 (5/158)[ab] | 48.4 (235/486)[a] |
| Fattener | 0.9 (3/341)[b] | 84.1 (254/302)[b] |
| Gilt | 0 (0/178)[b] | 82.6 (2,616/3,168)[b] |
| Sow | 11.9 (52/436)[c] | 82.0 (955/1,164)[b] |
| Boar | 4.6 (10/219)[a] | 79.4 (432/544)[b] |
| All | 5.3 (70/1,332) | 79.3 (4,492/5,664) |

The parenthesized figures are the number of tested positive/number of the tested sample

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

### Culled gilts

The gilts were culled at 313.1±3.6 days of age (range 211–504 days) at a body weight of 143.7±1.8 kg (range 92.0–205.5 kg). Reproductive data and reasons for culling of them are presented in Table 2. The ADG of the gilts from birth to culling was 461.3±7.0 g/day (range 197.0–689.0 g/day). The age at first mating was 265.5±3.6 days (range 204–347 days). The number of ovulations was 15.6±0.4 ova per gilt (range 2–25 ova).

Number and percentage of the gilts that were positive to PRRSV, ADV, and PPV are presented in Table 3. The *S/P* ratio of PRRSV-seropositive gilts ranged from 0.41 to 2.43. The number of PRRSV-seropositive gilts was lower in those culled due to abnormal vaginal discharge than those culled from anestrus ($P<0.05$). The incidence of ADV was higher in the gilts culled due to abortion and repeated breeding than those culled due to anestrus and abnormal vaginal discharge ($P<0.05$) (Table 3). High PPV titer was found in the gilts culled due to abnormal vaginal discharge more than the others ($P<0.05$; Table 3). PPV antibody titer ranged from 1:32 to 1:32,768. It was found that 86.0% of the gilts had PPV antibody titer of >1:512. Besides, 72% of them had PPV antibody titer of ≥1:4,096. Of all the culled gilts, 75.5% of them were exposed to at least two viruses, 18.9% of them were exposed to all the three viruses and 45.9% of them were exposed to both PRRSV and PPV (Table 4).

## Discussion

The present study provided information concerning with antibody titers against the selected reproductive diseases in the pigs raised in Thailand with special emphasis on

**Table 2** Age at culling (days), body weight at culling (kilogram), average daily gain from birth to culling (ADG; grams per day), age at first mating (AFM; days) and number of ovulation in gilts by culling reasons

| Culling reason | *N* | Age at culling | Body weight | ADG | AFM | Ovulation |
|---|---|---|---|---|---|---|
| Abortion | 16 | 312.3±7.6[ab] | 153.2±5.2[ab] | 489.5±20.9[a] | 260.2±8.0[a] | 16.2±0.9[a] |
| | | (252–367) | (116–193) | (375–673) | (204–302) | (10–21) |
| Anestrus | 85 | 308.9±4.9[b] | 139.4±2.5[b] | 455.8±10.8[a] | – | 16.1±0.7[a] |
| | | (211–504) | (95–198) | (197–689) | | (5–25) |
| Repeated breeding | 26 | 341.6±11.6[a] | 160.0±4.0[a] | 470.9±17.5[a] | 264.5±6.5[a] | 15.2±1.0[a] |
| | | (274–479) | (117–205) | (283–661) | (224–347) | (2–22) |
| Abnormal vaginal discharge | 39 | 303.5±6.0[b] | 138.6±3.2[b] | 455.5±11.3[a] | 269.9±4.8[a] | 15.0±0.7[a] |
| | | (240–405) | (92–173) | (342–625) | (227–323) | (4–20) |
| Total | 166 | 313.1±3.6 | 143.7±1.8 | 461.3±7.0 | 265.5±3.6 | 15.6±0.4 |
| | | (211–504) | (92–205) | (197–689) | (204–347) | (2–25) |

Numbers in parenthesis are range of the data

[a,b] Different superscripts within column differed significantly ($P<0.05$)

replacement gilts. It was found that most of the replacement gilts were exposed to PRRSV (84%), PPV (97%), and ADV (4%) before entering the breeding houses. Furthermore, up to 75.5% of the culled gilts were exposed to at least two viruses, and almost 20% of them were exposed to all the three selected viruses. The data in the culled gilts indicated that they had a relatively delayed age at first mating (265.5 days) and low ADG (461.3 gram/day). The gilts with a poor growth performance, as well as those with a delayed age at first mating might have health problems and/or had exposed to extremely hot and humid climates during their growing periods. Tummaruk et al. (2009b) demonstrated that the replacement gilts reared under tropical climate attained puberty at approximately 200 days of age, which is about 2 weeks later than those in Europe and North America (Karlbom 1982; Patterson et al. 2010). Furthermore, it has been demonstrated that the gilts with a superior ADG attained puberty earlier than those with inferior ADG (Tummaruk et al. 2009b). These data indicated that the health status of the gilts might, partially, influence their reproductive functions and subsequent reproductive performance.

The gilts in all herds, in this study, had a high PPV antibody titer. Although PPV vaccination was applied in every herd, such high a level of the antibody titer is unlikely to be the result of the vaccination. It is well established that PPV caused embryonic and fetal mortality in pregnant gilts and sows. The antibody against PPV could be detected as early as 5 days after live-virus exposure and could be persistent for years (Mengeling et al. 2000; Józwik et al. 2009). PPV has been recognized as an enzootic disease in most swine herds (Oravainen et al. 2005). Likewise, the present results indicated that PPV was an enzootic disease in all of the selected herds. Furthermore, the present study indicated that replacement gilts were commonly exposed to PPV rather early in their lives. It has been shown that the pigs transmitted PPV for about 2 weeks after an exposure, and the pen in which they were kept remained infectious for at least 4 months (Mengeling et al.

**Table 3** Number and percentage of porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus (ADV), and porcine parvovirus (PPV) seropositive culled gilts

| Culling reason | *N* | PRRSV | ADV | PPV[a] |
|---|---|---|---|---|
| Abortion | 16 | 13 (81%)[bc] | 8 (50%)[bc] | 12 (75%)[b] |
| Anestrus | 85 | 65 (76%)[b] | 10 (12%)[d] | 70 (85%)[bc] |
| Repeated breeding | 26 | 21 (81%)[bc] | 16 (62%)[c] | 21 (81%)[b] |
| Abnormal vaginal discharge | 39 | 23 (59%)[c] | 13 (33%)[c] | 34 (97%)[c] |
| Total | 166 | 122 (73%) | 47 (28%) | 137 (86%)[c] |

[a] Number of gilts with titer >1:512

[b,c,d] Different superscripts within column differed significantly ($P<0.05$)

[c] Seven missing values

**Table 4** Number and percentage of porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus (ADV) gI and porcine parvovirus (PPV)-seropositive culled gilts ($n=159$)

| PPV[a] | PRRSV | ADV | Number | Percentage |
|---|---|---|---|---|
| Negative | Negative | Negative | 5 | 3.1 |
| Negative | Positive | Negative | 11 | 6.9 |
| Negative | Positive | Positive | 6 | 3.8 |
| Positive | Negative | Negative | 23 | 14.5 |
| Positive | Negative | Positive | 11 | 6.9 |
| Positive | Positive | Negative | 73 | 45.9 |
| Positive | Positive | Positive | 30 | 18.9 |

[a] Gilts are defined as high antibody titer (positive) when the titer >1:512

2000). Since passive immunity against PPV declined around 22 weeks of age (Too and Love 1985), the gilts might be exposed to the virus during the beginning of the acclimatization period, in most cases, before the first PPV vaccination. This indicated by the fact that, in the second part of the present study, most of the gilts already had high PPV antibody titer before acclimatization. In addition, the genetic variation of PPV was reported in many recent studies (Zimmermann et al. 2006; Józwik et al. 2009; Miao et al. 2009). The PPV field isolate in Germany was genetically different from the reference and vaccine strain (Zimmermann et al. 2006). The phylogenetic analyses of PPV indicated that at least two genotypes have been defined; their antigenicity was also different. The genetic variation of PPV caused a high titer against PPV in gilts and sows although vaccination had been performed (Józwik et al. 2009; Miao et al. 2009). To our knowledge, genetic variation on PPV has never been reported in Asia. However, most of the pigs utilized as the parentstock in Thai swine industry were regularly imported from European countries. Therefore, the genetic diversity of PPV in Thai swine herds might have occurred. In addition, if the immunity against PPV of gilts and sows could not be properly developed, reproductive failures, e.g., repeated breeding and abortion, could occur. Recent data in Thailand indicated that the percentage of mummified fetus in the gilts' litter was relatively high (3.1%) compared to what was reported in the literature (Tummaruk et al. 2010). We suggest that PPV should be properly controlled by vaccinating the replacement gilts twice before mating, and every 4–6 months in sows. Furthermore, careful herd monitoring on PPV should be performed regularly.

In the present study, the replacement gilts in all herds were exposed to PRRSV before, during, and after acclimatization as indicated by the seroconversion both in non-vaccinated and vaccinated herds. This signified that the replacement gilts were an important source of introducing PRRSV into the breeding herds. However, only antibody titer (*S/P* ratio) might not be a good indicator for the existence of PRRSV in tissues or blood circulation of the pigs (Thanawongnuwech and Suradhat 2010; Olanratmanee et al. 2011). Olanratmanee et al. (2011) demonstrated that the virus could be found in the uterine tissue of the gilts with either high or low antibody titer. In the present study PRRSV antibody titer differed considerably among the herds. The proportion of PRRSV-seronegative gilts in herd D was higher than the others. The reason might be due to genetic variation of PRRSV among the herds. Furthermore, PRRS modified live-virus vaccine has also been performed in herd E. Since the antibody formation of PRRSV was greatly affected by genetic variation and amino acid sequence of PRRSV (Kim et al. 2009); therefore, only antibody titers may not be enough to examine the PRRSV circulation within the herds. Nevertheless, the antibody titer of PRRSV, in many PRRSV non-vaccinating herds in Thailand, was intensively examined in replacement gilts for several times prior to being introduced to the breeding houses. In some breeding herds, PRRS modified live-virus vaccine was used in the replacement gilts to control PRRSV (Cho and Dee 2006). However, the use of PRRS modified live-virus vaccine should be carefully considered due to cross-protection among different strains of PRRSV still is controversial; the shedding of virus from vaccinated pigs was commonly observed during the first few weeks after vaccination (Alexopoulos et al. 2005; Scortti et al. 2006; Kim et al. 2009; Thanawongnuwech and Suradhat 2010). Furthermore, in some cases, co-infection of PRRSV and PPV and/or ADV might possibly occur in the replacement gilts. 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). Recently, Olanratmanee et al. (2011) demonstrated that the PRRSV antigen could remain in the female reproductive tract of the replacement gilts for several months (up to 11 months of age). In this case, the postponement of first mating in gilts should be considered. These findings indicated that the health status of the replacement gilts was an important issue which should be considered before first mating decision.

In the present study, herd C and D could have either negative or low incidence of ADV, while herd B and E obviously were ADV positive. However, no ADV-positive gilt was introduced into the herds during the study period. In herd A, the introduction of ADV-positive gilts was still observed. This might be due to the fact that the replacement gilts were produced within the ADV-positive herd. Therefore, the elimination program for ADV in this herd should be revised. Although the prevalence of ADV was relatively low, the circulation of the virus was still observed in three out of five herds. In addition, to our knowledge, ADV has never been found separately in the culled gilts in earlier studies. The present study has been the first report on the presence of ADV in the culled replacement gilts. In the last part of the present study, it was found that the prevalence of ADV-seropositive gilts was relatively high. This indicated that natural infection with ADV among gilts may partially result in reproductive failures and may lead to culling of the gilts. The reproductive disturbance that has previously connected with ADV infection included abortion and repeated breeding (Mengeling et al. 1997). However, in the present study, ADV was also found in the gilts culled due to anestrus and abnormal vaginal discharge.

In conclusion, most of the replacement gilts were exposed to PRRSV (84%), PPV (97%), and ADV (4%) before entering the breeding house. PPV was an enzootic disease in all of the selected herds; the replacement gilts

were commonly exposed to PPV rather early in their lives. Replacement gilts were an important source of introducing PRRSV into the breeding herds. The prevalence of ADV was higher in gilts culled due to reproductive disturbance than in healthy gilts. Immunization of replacement gilts against PRRSV and PPV, along with the elimination of ADV was the important issue which should be addressed in the swine breeding herds in Thailand.

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# The Seroprevalence of Porcine Reproductive and Respiratory Syndrome Virus in Vaccinated and Non-vaccinated herds: a Retrospective Study

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***Keywords:*** *pig, PRRS, reproduction, seroprevalence*

## Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by an envelope, single-stranded positive-sense RNA virus known as PRRS virus (PRRSV) (1). The disease was observed first time in the United States of America (USA) since the late 1980s (2) and was found in Europe since 1990 (3). In Thailand, PRRSV sero-positive pig has been observed as early as 1989 (4). In 1995, the seroprevalence of PRRS was, on average, 64% with a variation among the herds from 20% to 90% (5). In general, PRRSV is classified according to their genotype as North American (US) and European (EU) strains (1, 4). In Thailand, both US and EU strains have been isolated (4). PRRSV causes many signs of reproductive failure in gilts and sow, such as infertility, abortion, death of sows and pre-weaning mortality (1, 6). Under field conditions, the infected sows develop a protective immunity and usually produce normal litters after rebreeding although the virus still circulate within the herds (7). The duration of the protective immunity is in fact unknown, but at least 604 days post infections have been proposed (7). Larger et al. (8) demonstrated that homologous PRRSV protective immunity was produced within 90 days post exposure and the virus-specific antibody was detected for at least 110 days post exposure in adult pig. Up to date, intensive acclimatization and/or vaccination in replacement gilts are commonly practiced in most breeding herds. However, high variability of the antibody titer against PRRS of the gilts is still observed both within and between herds. The objective of the present study was to retrospectively investigate the seroprevalence of PRRS antibody in pigs in 5 commercial herds in Thailand during 2004-2007. Furthermore, the seroprevalence of PRRSV between herds that vaccinated the gilts and sows with modified live virus (MLV) vaccine and those that performed intensive acclimatization in replacement gilts were compared.

## Materials and Methods

***Animals and data:*** The study was conducted in 5 commercial swine herds (A, B, C, D and E) in Thailand during January 2004 to December 2007. A total of 5,664 blood samples from 544 boars, 1,164 sows, 3,168 replacement gilts, 486 nursery pigs and 302 fattener pigs were collected and determined for PRRSV-specific antibody titer.

***Herd location, management and vaccination:*** The herds in the present study are located in the eastern (A, E), middle (B), western (C) and northeastern (D) of Thailand between latitude 13° and 17°N and between longitude 100° and 104°E. All herds included in the present study were breeding herd and the sows on production numbering about 900-3,500 sows/herd. Two herds (A and D) produced the replacement gilts within the herds, while 3 herds (B, C, E) bought the replacement gilts from other breeders. In general, the gilts entered the gilt pools at about 22-24 wk of age at 80-100 kg body weight (BW). Water was provided to *ad lib* from water nipples. The feed were provided twice a day (about 3 kg/day). The gilts were kept in a pen with a group size of between 6-15 gilts/pen with a space allowance of 1.5-2.0 m2/gilt and pregnant gilts and sows were kept in individual stall. Lactating sows were kept in individual pens. In most cases, the herds breed the replacement at ≥32 week of age with a BW of ≥130 kg at the second or later observed oestrus. Boar contact and estrous detection was applied to the gilts between 24-35 wk of age. The health of the herds was controlled by the herd veterinarian. In all herds, removal sows were taken to acclimatize the gilts for about 4 weeks period with a ratio of 1 sow per 6-10 gilts. The acclimatized sows were rotated weekly. Before breeding, the gilts were vaccinated against Foot-and-mouth disease (FMD), Swine fever (SF), Aujeszky's disease (AD) and Porcine Parvo virus (PPV) vaccine during 22-30 week of age. In herd B, the gilts, sows and nursery pigs were also vaccinated against US-strain of MLV vaccine (Ingelvac® PRRS™ MLV, Boehringer-Ingelheim Vetmedica Inc., Missouri, USA), and in herd C and E, the EU-strain of MLV vaccine (AMERVAC®, Lab. Hipra, Spain) were used.

***Serological test:*** Antibody of PRRS virus was tested by using HerdCheck PRRS virus antibody test kit 2XR® (IDEXX Lab., Inc., USA) (herd A, B, C and D). Briefly, the positive and negative control was also carried in the same plate as the sample. 100 µl of serum samples was added to the testing plated that coated with PRRS antigen and to the normal host cell (NHC) and incubated at room temperature for 30 min. Anti-Porcine: HRPO conjugate was added into the plate 100 µl for each sample and incubated. 100 µl of TMB substrate was added and incubated and then 100 µl of stop solution was added. OD was measured using ELISA reader at 650 nm. The serum sample/positive control (S/P) was calculated. The S/P ratio below 0.4 indicated that the sample had no antibody of PRRSV (negative), while S/P ratio ≥ 0.4 indicated that the sample had antibody of PRRSV (positive).

***Statistical analyses:*** The statistical analyzed was performed using SAS (SAS version 9.0, Cary NC, USA.). Frequency analysis was conducted using PROC FREQ of SAS. The proportional data were analyzed using Chi-squared test. $p<0.05$ were regarded to be statistical significance.

## Results and Discussion

Of all 5,664 tested samples, 4,492 pigs (79.3%) had antibody titer against PRRSV. The proportion of PRRS positive pigs were 79.4%, 82.0%, 82.6%, 84.1% and 48.4% in boars, sows, gilts, fattener and nursery pigs, respectively (Figure 1). The proportion of PRRS positive pigs were 81.7%, 67.9%, 60.6%, 80.9% and 79.3% in herds A, B, C, D and E, respectively ($p<0.001$). The S/P ratios were 1.5±1.1 (range 0-4.5), 1.3±1.2 (range 0-4.9), 1.0±0.9 (range 0-3.7), 1.4±0.9 (range 0-4.3) in herds A, B, C and D, respectively. Across the herds, the proportion of PRRS negative pigs varied among years from 36.6% in 2004 to 25.6%, 15.7% and 11.3% in 2005, 2006 and 2007, respectively ($p<0.001$). The proportion of PRRS positive boars varied among years from 69.2% in 2004 to 80.0%, 74.1% and 83.3% in 2005, 2006 and 2007, respectively ($p=0.03$). In the fattener, the proportion of PRRS positive pigs varied from 82.3% to 89.1% among years ($p=0.7$). The proportion of PRRS positive gilts were 62.1%, 78.6%, 91.5% and 94.4% in 2004, 2005, 2006 and 2007, respectively ($p<0.001$). The proportion of PRRS positive nursery pigs were 58.2%, 40.8%, 46.5% and 55.0% in 2004, 2005, 2006 and 2007, respectively ($p=0.03$). Proportion of PRRS positive pigs from 2004 to 2007 in the MLV PRRSV-vaccinated and non-vaccinated herds are demonstrated in Figure 2. Comparing between the PRRSV vaccinated and non-vaccinated herds, the proportion of PRRS positive pig was demonstrated in Figure 2. A higher proportion of PRRS-specific antibodies fattener pig was observed in the PRRSV-non-vaccinated herds than the PRRSV-vaccinated herds ($p<0.05$) (Fig. 3).

The present study provided descriptive data on the prevalence of PRRSV infection in 5 swine commercial herds in Thailand. The data indicated that the proportion of pigs infected with PRRS differed among herds, years and groups of pigs. The infection was found to be highest in the fattener (84.1%) and lowest in the nursery pigs (48.3%). High proportion of PRRS positive pigs were also observed in replacement gilts (82.6%) and sows (82.0%). Surprisingly, a relatively high prevalence of PRRS was found in the boars (79.4%). These indicate that the PRRSV circulation and re-infection remain relatively high either in vaccinated or in non-vaccinated herds. Boars, sows and replacement gilts seem to be the important reservoir of the virus. Interestingly, the exposure of PRRSV in the fattener pigs tended to be lower the vaccinated than the non-vaccinated herds.

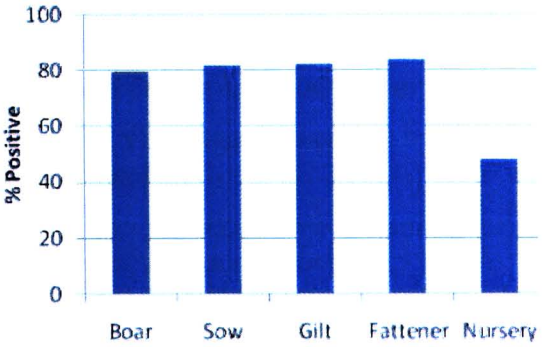


**Fig. 1** Percentage of PRRS positive pigs by groups

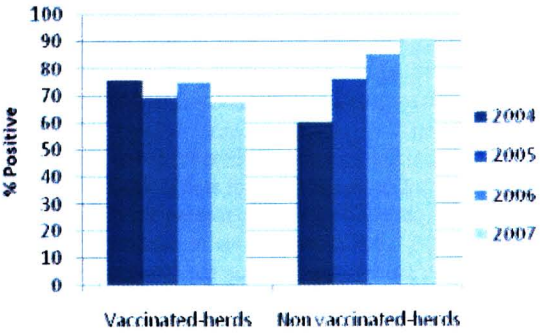


**Fig. 2** Proportion of PRRS positive pigs in PRRSV vaccinated and non vaccinated herds

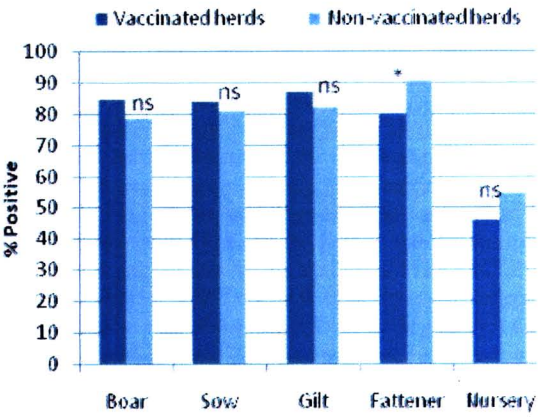


**Fig. 3** Proportion of PRRS positive pigs in PRRSV vaccinated and non-vaccinated herds by groups; $^{*}p<0.05$

## Acknowledgement

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# Porcine Reproductive and Respiratory Syndrome Virus Antigen Detection in the Uterine Tissue of Gilts Correlated to the Antibody Titer

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***Keywords:*** *immunohistochemistry, pig, PRRS, reproduction, uterus*



## Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by PRRS virus (PRRSV), a member of Arterivirus, family Arteriviridae (1). In general, the infection of PRRS in gilts and sows is characterized by late term abortion, mummified fetuses, stillborn piglets and low viability piglets at birth (2-4). The antibody titers against PRRSV infection are detected by 7-14 days after the animals are infected and remain for several months before declining (5). Under farm condition, intensive acclimatization and/or vaccination in replacement gilts are commonly practiced in most breeding herds. However, high variability of the antibody titer against PRRSV of the gilts is observed both within and between herds. This problem causes difficulties for the farmer to make decision to mate the gilts. Additional knowledge concerning the antibody titer of PRRS in the replacement gilts in different herds is needed to be investigated. It has been suggested that replacement management of gilts is a major source of introducing new strains of PRRSV into the herd. Our previous study has found that 73% (122/166) of the replacement gilts culled due to reproductive disturbance had been infected with PRRSV. In addition, a higher proportion of seropositive gilts was particularly found in those that were culled due to abortion (81%) and repeat breeding (81%) (6). It is well established that alveolar macrophages as well as macrophages from other tissues are the primary cell type sustaining the in vivo replication of the viruses (7). Using Immunohistochemistry (IHC) for evaluating formalin-fixed tissues, it was found that 66% and 100% of the lung tissue of piglets infected with US and EU stains of PRRS have been observed, respectively (7). An earlier study has demonstrated that 75.0%, 50.0%, 37.5%, 37.5%, 37.5% and 25.0% of IHC positive cells was observed in liver, spleen, tonsil, turbinate bone, pulmonary lymph node and ileum of the infected piglets, respectively (7). To our knowledge, the expression of PRRSV in the uterine tissue of gilts has not been dem onstrated. The objective of the present study was to determine the incidence of PRRSV in the uterine tissue of gilts in relation to the level of antibody titers.

## Materials and Methods

Uterine tissues from 50 replacement gilts were collected from three commercial swine herds (A, B and C) in Thailand. All of the gilts were culled due to reproductive disturbance. The culling reasons included anestrus (n=29), vaginal discharge (n=10), repeat breeding (n=5), abortion (n=5) and not being pregnant (n=1). Historical data for all gilts was collected. All herds included in the present study were breeding herds and the sows on production were between 900-3,500 sows/herd. Herd A produced replacement gilts within the herd using their own grand parent (GP) stock, while herds B and C bought the replacement gilts from other breeders. The gilts in all herds were housed in a conventional open housing system facilitated with a water sprinkler and fan. The health of the herds was monitored by the herd veterinarian. In general, the veterinarian gave the recommendation to vaccinate the gilts against foot-and-mouth disease, classical swine fever, Aujeszkyís disease and porcine parvovirus (PPV) at between 22-30 weeks of age. In addition, herd B vaccinated the replacement gilts using US-strain modified-live virus (MLV) vaccine (Ingelvac® PRRS™ MLV, Boehringer-Ingelheim Vetmedica Inc., St. Josept, Missouri, USA), while herd A and C vaccinated the gilts using EU-strain MLV vaccine (AMERVAC®, Lab. Hipra, Girona, Spain). Blood samples were collected from jugular vein of the gilts prior to culling. Serum were obtained and kept at -20°C for analyzing antibody titer of PRRSV. After slaughter, the ovary and uterus were collected, placed on ice and transported to the laboratory within 24 h of culling. Tissue samples were collected from the uterus of the gilts, fixed in 10% neutral buffered formalin for at least 24 h and embedded in paraffin blocks. Immunohistochemistry (IHC) was performed on the uterine tissues of the gilts using the protocol of the lung tissue with some modification (7). A polymer-based non-avidin-biotin technique was applied in the present study. Primary monoclonal antibody SDOW17 (Rural Tech., Inc., USA) diluted 1:1000 was used. Negative control procedures included omission of primary antibody. Known PRRSV-positive lung and lymph node tissues served as positive controls. The sections were interpreted as positive if contained at least 1 positive cell (brown intracytoplasmic staining, Fig. 1). PRRSV antibody was

determined using a commercial enzyme-linked immunosorbent assay test kit (ELISA, HerdChek® PRRS virus antibody test kit 2XR, IDEXX Lab., Inc., USA). The protocol followed the kit's instructions. The serum sample/positive control (S/P) was calculated. The S/P ratio below 0.4 indicated that the sample had no PRRSV antibody (negative), while S/P ratio ≥ 0.4 indicated that the sample had PRRSV antibody (positive). Statistical analyses were performed using SAS (SAS, 2002). The percentage of positive tissue was compared with the detection of antibody titers against PRRSV by using ELISA (positive and negative) using Fisherís exact test. $p<0.05$ was considered as statistically significant.

## Results and Discussion

PRRSV antigens were detected in the cytoplasm of macrophage-like cells in the sub-epithelial connective tissue layers of the endometrium in 28% (14/50) of the gilts. The PRRSV positive cells were observed in the cytoplasm of the macrophages in the endometrium (Figure 1). Of all the gilts, 77.6% (38/49 gilts) were positive to the ELISA test (Table 1). Of the seropositive gilts, 28.9% (11/38) had PRRSV antigen in the uterine tissues, while 18.2% (2/11) of the seronegative gilts had PRRSV antigen in the uterine tissues (p=0.70). Compared to the seronegative gilts, seropositive gilts had a 1.83 (95% confidence interval=0.34-9.89) higher odds for detecting PRRSV antigen in the uterine tissue. Among the seropositive gilts, high level of antibody titer (S/P ratio ≥1.2) was found in 47.4% of the gilts. The incidence of IHC-PRRSV positive staining cells was found in 33.3% of the high antibody titer gilts and in 25.0% of low antibody titer gilts ($p$=0.72).

The present study demonstrated the present of PRRSV in the uterine tissue of gilts. The site of positive cells was at the subepithelial layer of endometrium. PRRSV infection is a multisystemic disease which is characterized by viremia and subsequent virus distribution and replication in multiple organs (8). In the present study, it was found that gilts that had PRRSV antigen in the uterine tissue were culled at 287 days of age. Most of these gilts have been sent into the breeding herd and might shed the virus to the susceptible pigs in the herd. In the present study, the percentage of gilts culled due to reproductive disturbance that were detected the antibody against PRRSV was in agreement with the earlier study (6). It was not surprise to see both seropositive and seronegative gilts had PRRSV antigen presented in the uterine tissue of the culled gilts since PRRSV antibody titer cannot determine the persistent infection. Although the proportion of IHC-PRRS positive gilts tended to be higher in the gilts that had a high level of S/P ratio, a certain amount of the IHC-PRRSV positive uterine tissue were also observed in the gilts with low S/P ratio and even in the PRRSV seronegative gilts. This imply that the use of antibody titer as a criteria to introduce replacement gilts into the breeding house may not be good enough and remain a risk of introducing IHC-PRRS positive gilts into the herds. It has been demonstrated that the duration of protective immunity against homologous strain of PRRSV may persist for at least 604 days post experimental exposure to the field PRRSV, while the duration of detectable PRRSV-specific antibodies that develop in sows following natural infection is thought to be as short as 4-8 months (9). These findings suggested that replacement gilts must be allowed to expose homologous strain of PRRSV before entering the breeding herds.

**Table 1** the number of gilts that were positive to IHC test in relation to the results of ELISA test

| | IHC + | IHC - | Total |
|---|---|---|---|
| ELISA + | 11 | 27 | 38 |
| ELISA - | 2 | 9 | 11 |
| Total | 13 | 36 | 49 |

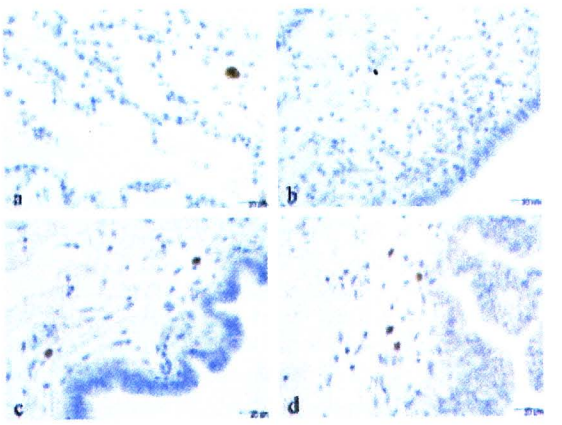


**Fig. 1** Demonstration of PRRSV antigen in (a) the positive control (lung tissue), (b) the negative control and (c-d) the uterine tissue of gilts

## Acknowledgements

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# Abortion rate in gilts and sows in porcine reproductive and respiratory syndrome virus (PRRSV) sero-positive herds in Thailand

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**Abstract**

PRRSV was discovered in USA since 1987 and currently, veterinarians control PRRSV in swine breeding herds via several types of strategies including acclimatization, housing management, monitoring and vaccination. The present study investigated abortion rate in gilts and sows in 8 selected PRRSV sero-positive swine herds in Thailand. Reproductive data of 192,765 mating records from 67,537 gilts and sows were collected during 2007-2009 from 8 swine herds (A, B, C, D, E, F, G and H) in Thailand. All herds had been infected with PRRSV for over 5 years. PRRSV vaccination was applied regularly (every 3 months) in herd A, irregularly in herd B, and only in replacement gilts in herd C, while the rest were not vaccinated. Abortion rate were compared among the herds using logistic regression. The statistical models included parity, mating month, mating year, herds and interaction between parity and herds. On average, abortion rate was 1.9%, which were varied among herds (2.4%, 0.7%, 2.2%, 1.2%, 2.8%, 0.5%, 1.3% and 2.6% in herds A to H, respectively ($P$=0.048)). Abortion rate was 2.3%, 1.8%, 1.9% and 2.0% in gilts and sows parity 1, 2-5 and >6, respectively ($P$<0.01). The results indicated that the impact of PRRSV on abortion rate among swine commercial herds in Thailand were relatively low. This implies that pregnancy failure in pig caused by PRRSV during the past 3 years in these herds could be effectively controlled by several types of management strategies including either vaccination or non-vaccination strategies.

**Keywords**: Pig, Reproduction, PRRSV

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# Sows mortality in porcine reproductive and respiratory syndrome virus (PRRSV) sero-positive herds in Thailand

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## Introduction

In general, the infection of porcine reproductive and respiratory syndrome virus (PRRSV) in gilts and sows cause reproductive signs including of abortion, mummified fetuses, stillborn piglets, low viability piglets at birth, infertility and an increase of sow mortality rate [2, 5, 8]. In practice, PRRSV in breeding herds is controlled by several types of strategies including acclimatization, housing management, monitoring and vaccination [1]. Under field conditions, most (73%) of the replacement gilts are infected with PRRSV [7] and PRRSV antigen in the uterine tissue has been found in 33% of the gilts culled due to reproductive disturbances [6]. In USA, the annual sow mortality account for 5.7% of the breeding females, where 42% of sows are culled annually [3]. The aim of the present study was to investigate the mortality of gilts and sows in selected PRRSV sero-positive herds in Thailand.

## Materials and methods

Data from 26,435 culled gilts and sows were collected from 7 swine herds (A, B, C, D, E, F and G) in Thailand during 2007-2009. All herds had been infected with PRRSV for over 5 years. PRRSV vaccination was applied regularly (every 3 months) in herd A, irregularly in herd B, and in replacement gilts in herd C, while the remaining herds were not vaccinated the gilts and sows against PRRSV. Sow mortality rate and culling rate were analyzed by using frequency analysis and logistic regression.

## Results

On average, culling rate and mortality rate for the breeding females were 48.5% and 3.4%, respectively. The mortality rate and culling rate for breeding females varied between 1.7-5.4% and 35.9-68.5% among herds (Fig. 1). Mortality of gilts and sows in the selected herds accounted for 6.8% (n=1,789) of the removal sows. Of the removal females (n=26,435), 9,539 females (36%) were removed at parity ≤2. Of these females, 879 females were found dead (9.2%). Of all the dead females, 49% died at parity number ≤2. The percentage of sow mortality by parity was demonstrated in Fig. 2.

## Discussion

The results indicated that the mortality of gilts and sows in PRRSV sero-positive herds in Thailand are varied in both PRRSV vaccinated (A-C) and non-vaccinated (D-G) herds. However, the mortality rate of gilts and sows based on the number of sows on production was in acceptable level. It has been demonstrated that at least three litters are required from sows before a positive cash flow could be obtained [4]. In the present study, a relatively high mortality was found in young sows (parity number ≤2). This might reduce the overall herd reproductive performances due to the decrease of sow's longevity. It has been reported that the occurrence of sow mortality in PRRSV positive herds varied between 1-4% and is, in most case, associated with respiratory sings [8].

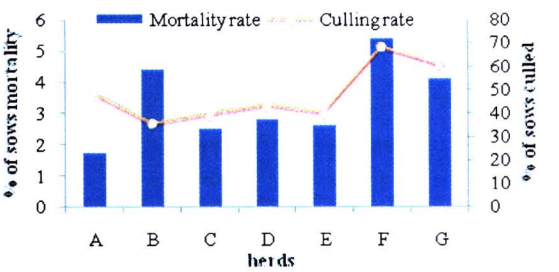


**Figure 1** Mortality rate and culling rate of gilts and sows by herds

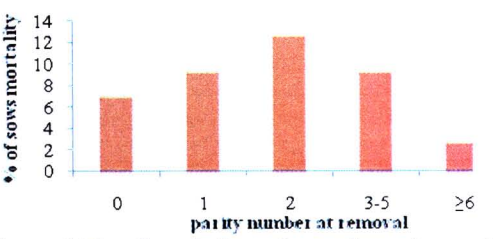


**Figure 2** Mortality of gilts and sows by parity number at removal

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# Antibody titer against porcine reproductive and respiratory syndrome virus (PRRSV) after modified live PRRSV vaccination

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## Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) can be detected in the lymphoid tissue of pig up to 8-9 months post infection (1). During this period, the virus can be replicated continuously at a low level and transmitted to susceptible animals via direct contact. In the PRRSV endemic herds, the present of PRRSV subpopulations (susceptible pigs) may lead to reoccurrence of the disease in the herds. Herd closure, gilts acclimatization and mass exposure have been recommended to eliminate the subpopulations (2). The use of vaccination to immunize the pigs has been evaluated, in most cases, at individual level. However, only few studies on the immune response of PRRSV in the infected herd have been evaluated. Earlier study has demonstrated that vaccination of the entire herd (mass vaccination) could reduce persistence and duration of shedding even though the wild type of virus was not eliminated from the pigs (3). However, the successful results are varied among the herds and a limited information on the immune response of PRRS modified live virus (MLV) vaccine is available in pregnant gilts and sows. The present study aims to evaluate the humoral immune response of gilts/sows after mass vaccination of PRRS MLV vaccine in a PRRSV infected herd in Thailand.

## Materials and Methods

The present study was conducted between May and September, 2009 in a 1,200-sow inventory swine herd in Thailand, which produced their own replacement gilts. The herd has never been vaccinated the gilts and sows with PRRSV vaccine. An occurrence of abortion in sows was observed in January 2009 and an American strain of PRRSV was detected in serum of aborted sows using reverse transcription polymerase chain reaction (RT-PCR). Gilts/sows were vaccinated against PRRS MLV vaccine (Ingelvac® PRRSTM MLV, Boehringer-Ingelheim, Vetmedica Inc., St. Josept, Missouri, USA) 2 doses 3 weeks apart and booster at 14 week and 3 months. Blood samples were collected from replacement gilts and pregnant gilt/sow (36 samples) one day prior to first vaccination (week 0) and subsequently at 2, 5, 9, 12 and 18 weeks after vaccinations in the same animals. The sera were obtained for viral detection using RT-PCR technique (pooled serum) and analyzing antibodies against PRRSV using ELISA (HerdChek® PRRS virus antibody test kit 2XR, IDEXX Laboratories, Inc., USA). The percentage of sero-positive gilt/sow was compared using logistic regression. The differences of means of the serum sample/positive control (S/P) ratio were compared using pair t-test. $P<0.05$ was considered as statistically significant.

## Results and Discussion

It was found that no viral shedding was detected during 2 to 18 weeks post vaccination (Table 1). Before vaccination, 11.1% (4/36) PRRSV susceptible pigs were observed. The percentage of PRRSV subpopulation was reduced to 6.1% within 18 week post vaccination ($P=0.401$). The S/P ratio was slightly increased two weeks post vaccination and significantly decreased at 5 weeks post vaccination (Table 1).

**Table 1.** *The S/P ratio (means±S.E.M.), the percentage of sero-positive gilts and sows and the viral detection by weeks post vaccination*

| weeks | S/P ratio | % positive | RT-PCR |
|---|---|---|---|
| 0 | $1.61\pm0.19^{ab}$ | $88.89^{a}$ | Negative |
| 2 | $1.88\pm0.16^{a}$ | $94.44^{a}$ | Negative |
| 5 | $1.47\pm0.16^{b}$ | $86.11^{a}$ | Negative |
| 9 | $1.32\pm0.15^{b}$ | $88.89^{a}$ | Negative |
| 12 | $1.46\pm0.17^{b}$ | $85.29^{a}$ | Negative |
| 18 | $1.23\pm0.07^{b}$ | $93.94^{a}$ | Negative |
| All | 1.50±0.06 | 89.57 | Negative |

$^{a,b}$ different letters differ significantly ($P<0.05$)

The present study demonstrated that mass vaccination of PRRSV MLV in pregnant gilts and sows did not cause viral shedding during 2-18 weeks post vaccination and might possibly minimize the number of PRRS subpopulations in the PRRSV infected herds. However, the shedding of PRRSV during 0-2 weeks post vaccination and reproductive performance of pregnant gilts/sows should be evaluated further.

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# Detection of porcine reproductive and respiratory syndrome virus (PRRSV) in the serum of gilts and sows after modified-live PRRSV vaccination

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**Keywords:** pigs, PRRSV, vaccination

## Introduction and objectives

PRRSV infection cause a number of reproductive failures in gilts and sows including abortion, high mummified fetuses, stillborn and weak-born piglets, and increase sow mortality rate [1-3]. The reoccurrence of PRRS in the sow herds mostly depends on the number of subpopulation pigs in the herds especially replacement gilts and old sows. To minimize the subpopulations, herd closure, gilts acclimatization and/or vaccination have been recommended [4]. Earlier studies have shown that the PRRSV modified-live virus (MLV) vaccination can reduce the duration of the viral shedding, although the virus still persist in the pigs [4-6]. However, under filed conditions, the duration of the viral shedding after vaccination varied among herds. The present study investigates the evidence of PRRSV detection in the serum of gilts and sows after PRRS MLV vaccination under field conditions.

## Materials and methods

The present study was conducted in a 2,700 sows on production swine herd in Thailand. The gilts and sows were vaccinated against PRRS MLV vaccine (Ingelvac PRRS™ MLV, Boehringer-Ingelheim Vetmedica Inc., St. Josept, Missouri, USA) at Day 0. Blood samples were collected from the jugular vein of gilts and sows at Day 0, 2, 4, 11, 13 and 15 after vaccination (n=6 per group). The serum were obtained and pooled in each group for PRRSV detection by reverse transcription-polymerase chain reaction. The strain of PRRSV was also identified. The percentages of positive sample were compared between first (Day 0, 2 and 4) and second weeks (Day 11, 13 and 15) after vaccination by Fisher's exact test.

## Results

PRRSV was detected in all groups (6/6) of the pig during the first week of vaccination (Day 0, 2, 4 after vaccination). In the second week of vaccination, PRRSV was detected in only 33% (2/6) of the pig (Day 13) ($P$=0.06). The strain of all the PRRSV isolates was identified as NA strain.

## Discussion

The present study demonstrated that the PRRSV MLV vaccination may caused viral shedding in both gilts and sows in the first week of vaccination (100%) and this proportion is reduced during the second week of vaccination (33%). This result is in agreement with an earlier study [4] and supports our previous study that viremia may not be observed in gilts and sows during 2-18 weeks post vaccination [6].

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# Litter size at birth of sows in a PRRSV-positive herd after modified-live PRRSV mass vaccination

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## Introduction and objective

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 increase of sow mortality rate [1]. The present study aims to evaluate the litter traits i.e., the number of piglets total born per litter (TB), the number of piglets born alive per litter (BA), the percentage of stillbirth piglets per litter (SB) and the percentage of mummified fetuses per litter (MM) of sows after PRRS modified-lived virus (MLV) vaccination in a PRRSV-positive herd in Thailand.

## Methods

The study was conducted in a 1,200-sow inventory PRRSV positive swine commercial herd during 2007-2010. Mass vaccination was done for the first time in May 2009 twice 3 weeks apart in all pigs and repeated every 3 months. Data of 6,793 litters from 2,468 sows were included. General linear models (GLM) of SAS were used. $P<0.05$ was considered as statistically significant.

## Results

The litter traits of sows before PRRSV outbreak (July 2007-June 2008), during outbreak (July 2008-June 2009), which were characterized by high abortion in gilts and sows and respiratory signs in nursery pigs, and after mass vaccination (July 2009-June 2010) were shown in Figure 1. After PRRSV vaccination, TB of sows parity≤2 was returned to normal, whereas TB of sow parity>2 were still lower than the period before outbreak ($P<0.001$).

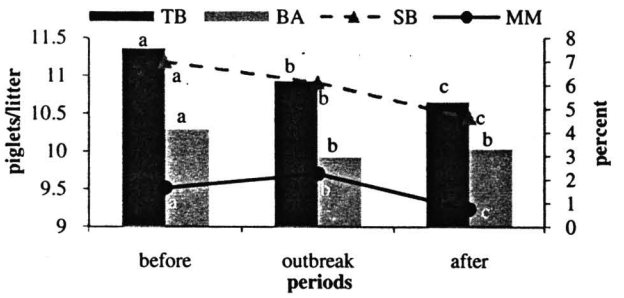


**Figure 1** Litter size at birth of sows before during and after PRRS-MLV mass vaccination

## Conclusion

Our study demonstrated that PRRS MLV vaccination resulted in an improvement of some reproductive performance, i.e., SB and MM [2]. However, the vaccination may cause some unfavourable outcome, such as, low litter size at birth [3]. Furthermore, the young sows had more response to the PRRS MLV vaccination than the older sows.

**Keywords:** pig, PRRSV, reproduction, vaccination

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# Fertility of gilts and sows after modified live PRRSV mass vaccination in a PRRSV-positive herd

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## Introduction and Objective

The infection of porcine reproductive and respiratory syndrome virus (PRRSV) in gilts and sows cause reproductive signs including of abortion, high mummified fetuses, high stillborn piglets, low viability piglets at birth, infertility and an increase of sow mortality rate [1]. The present study aims to evaluate return rate (RR), abortion rate (AR) and farrowing rate (FR) of gilts and sows after mass vaccination of PRRS modified lived virus vaccine in a PRRSV-positive herd in Thailand.

## Methods

The study was conducted in a 1,200-sow inventory swine commercial herd during 2007-2010. Mass vaccination was done for the first time in May 2009 twice 3 weeks apart in all pigs and repeated every 3 months. Data of 6,793 litters from 2,468 sows were included. Generalized linear-mixed models (GLIMMIX) of SAS were used. $P<0.05$ was considered as statistically significant.

## Results

On average, return rate (RR), abortion rate (AR) and farrowing rate (FR) before mass vaccination (July 2007-June 2008) were 5.2%, 1.7% and 90.7%. During the PRRSV outbreak (July 2008-June 2009) RR and AR were increased to 9.1% ($P<0.001$) and 5.4% ($P<0.001$), resp., and FR was decreased to 82.5% ($P<0.001$). After vaccination (July 2009-June 2010), AR was decreased to 1.1% ($P<0.001$), while RR (11.0%, $P=0.24$) and FR (84.3%, $P=0.08$) were not differed significantly compared to the outbreak period. After vaccination, RR was higher ($P<0.001$) and FR ($P<0.001$) was lower than the control period (July 2007-June 2008), while AR was returned to normal.

## Conclusion

Our study demonstrated that PRRS MLV vaccination might result in an improvement of some reproductive performance, i.e., AR [2, 3]. However, the vaccination may cause some unfavourable outcome, such as, low litter size at birth [2].

**Keywords:** Pigs, PRRSV, reproduction, vaccination

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Research field: swine, reproduction, disease, PRRSV

# Detection of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Antigen in Ovarian Tissue of Gilts Culled due to Reproductive Disturbances

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**Keywords:** immunohistochemistry, ovary, pig, PRRSV

## *Introduction*

Porcine reproductive and respiratory syndrome (PRRS) is caused by the PRRS virus (PRRSV). It is well established that macrophages are the primary cell type sustaining the *in vivo* replication of the viruses (1). Immunohistochemically (IHC), the PRRSV antigen is observed in several tissues of the infected piglets, e.g., lung, liver, spleen, tonsil, pulmonary lymph node and ileum (2). Replacement gilts are a major source of introducing new strains of PRRSV into the herd. Our previous study found that 73% of them were infected with PRRSV before entering the herd (3). Furthermore, PRRSV antigen is observed in the uterine tissue in 33% of the replacement gilts culled due to reproductive disturbance (4). The objective of the present study was to determine the incidence of PRRSV antigen positive ovarian tissue in gilts culled due to reproductive disturbance.

## *Materials and Methods*

Ovarian tissues from 100 replacement gilts culled due to reproductive disturbance were collected from 2 commercial swine herds in Thailand. After slaughter, the ovarian tissues were collected, fixed in 10% neutral buffered formalin, embedded in paraffin blocks and cut into 4-µm-thick sections. IHC, a polymer-based non-avidin-biotin technique, was applied to all ovarian tissues for PRRSV detection according to our previous protocol in the uterine tissue (4). Primary monoclonal antibody SDOW17 (Rural technologies, Inc., USA) diluted to 1:1000 was used. Negative control procedures included omission of primary antibody. Known PRRSV-positive lung tissues were served as positive controls. The sections would be interpreted as positive if they contained at least 1 positive cell (brown intracytoplasmic staining, Fig 1). Statistical analyses were performed using SAS (SAS, 2002). The percentage of positive tissue was compared with the reasons for culling using the Chi-square test. $P<0.05$ was considered statistically significant.

## *Results and Discussion*

PRRSV antigens were detected in the ovarian medulla (Fig 1) of 70% of the culled gilts. The percentage of positive tissue in different culling reasons is shown in Table 1. No significance of the percentage of positive tissue was found among culling reason groups ($p=0.496$).

**Table 1** The percentage of gilts with PRRSV antigen positive ovarian tissue by culling reasons

| Culling reason | Number of gilts | % IHC positive gilts |
|---|---|---|
| Anestrus | 50 | 78.0 |
| Vaginal discharge | 17 | 64.7 |
| Abortion | 10 | 60.0 |
| Repeated breeding | 9 | 66.7 |
| Miscellanies | 14 | 57.1 |
| Total | 100 | 70.0 |

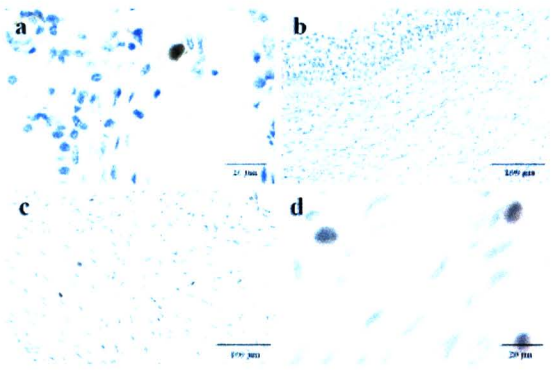


**Fig 1.** PRRSV antigen in (a) positive control (lung tissue), (b) negative control and (c-d) ovarian tissues

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# Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Strains Isolated from Sows and Boars in Thailand during 2005-2010

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**Keywords:** pig, PRRSV, reproduction, RT-PCR

## *Introduction*

Common infectious agents causing reproductive failures in pig include porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus (ADV), porcine Parvo-virus (PPV), Enterovirus (PEV), classical swine fever virus (CSFV), encephalomyocarditis virus (EMCV) and porcine circo virus type 2 (PCV-2) (1, 2). These viruses are able to infect the fetuses through placenta and result in fetal mortality (3). Maldonado et al. (2) found 9% of PRRSV and 1% of PRRSV combined with PCV2 positive samples from 100 clinical cases of aborted fetuses and stillborn piglet in Spain, while ADV and PPV were not detected in any tissue samples. This indicates that, under field conditions, PRRSV remains the most common virus in aborted fetuses or premature birth. It was suggested that this evidence could be a result of either PRRSV modified live virus vaccination commonly practiced in the pig industry and/or that PRRSV had becomes one of an enzootic disease in the commercial swine herds. PRRSV is classified into 2 genotypes, i.e. European (EU) and North American (NA) genotypes (4). PRRSV develops and replicates in macrophage in lung and other visceral organs including uterus of gilts (5). Common reproductive clinical symptoms of PRRSV in the sows include abortion, premature birth, stillborn piglet, weakborn piglet and high pre-weaning mortality rate in suckling piglets due to secondary infection. The purposes of the present study were to determine the prevalence of porcine reproductive and respiratory syndrome virus (PRRSV) in boars and sows in swine commercial herds in Thailand during 2005-2010.

## *Materials and Methods*

A total of 355 samples (27 tissues, 132 serum and 196 semen samples) from 213 boars and 142 sows submitted for PRRSV detection at the veterinary diagnostic laboratory, Chulalongkorn University was included in the analyses. PRRSV was detected using RT-PCR (6). The strain of the virus was classified into three categories, i.e. NA, EU and mixed strains (both EU and NA). Frequency analysis was conducted for the present or absence of PRRSV. Logistic regression analyses were conducted to evaluate the prevalence of PRRSV. The statistical model included effect of type of samples (serum, semen and tissue), year of the sample collection (2005-2010) and groups of the pig (boars and sows).

## *Results and Discussion*

PRRSV was detected in 23.4% (83/355) of the cases submitted for RT-PCR. PRRSV was found in 13.6% (29/213) of the boars and 38.0% (54/142) of the sows ($P$=0.023). The virus was also found in 48.2%, 13.4% and 32.6% of the tissue, semen and serum samples, respectively ($p$<0.05). The percentage of PRRSV positive sample was 39.7% (29/73), 14.0% (16/114), 38.8% (26/67), 23.1% (6/26), 13.2% (5/38) and 2.7% (1/37) in 2005, 2006, 2007, 2008, 2009

and 2010, respectively. The prevalence of PRRSV in 2005 and 2007 were higher than 2006, 2008, 2009 and 2010 ($p<0.05$). Across the year, the EU, NA and mixed strain of the PRRSV were 26.3%, 45.0% and 28.7%, respectively. However, the proportion of PRRSV strains differed among the years. The proportion of NA strain was 33.0%, 50.0%, 46.2%, 40.0%, 80.0% and 100.0% from 2005 to 2010, respectively (Table 1).

**Table 1** Strains of PRRSV detected in sows and boars during 2005-2010

| Year | N[1] | EU | NA | Mixed |
|---|---|---|---|---|
| 2005 | 27 | 9 | 9 | 9 |
| 2006 | 26 | 5 | 8 | 3 |
| 2007 | 26 | 4 | 12 | 10 |
| 2008 | 5 | 2 | 2 | 1 |
| 2009 | 5 | 1 | 4 | 0 |
| 2010 | 1 | 0 | 1 | 0 |
| All | 80 | 21 (26.3%) | 36 (45.0%) | 23 (28.7%) |

[1]N: number of PRRSV positive sample

A previous study from 137 PRRSV isolates obtained during 2000-2003 in Thailand found that 66.4% of PRRSV isolated belong to EU genotypes and 33.6% belonged to NA genotype (6). The present study demonstrated the PRRSV strains isolated from sows and boars from the Thai swine herds during 2005-2010. Our data revealed that 73.7% of the sample contained NA genotypes of PRRSV and 55.0% of the samples contained EU genotype of PRRSV. Furthermore, more than one forth (28.7%) of the samples had both NA and EU genotypes. These data represented the strain of virus involved with reproductive problems among the swine breeding herds in Thailand. It was found that during 2005-2010, NA strain was more common than EU strains in sows and boars. The reasons might be because PRRSV modified live virus vaccination has been registered and become commonly practiced in the Thai pig industry since 2007. The isolation of the vaccine strain of PRRSV under field conditions has been shown by a genetic analysis on ORF5 of PRRSV in Korea (7). Also, the high frequency of PRRSV isolation during this period indicates that PRRSV may become one of an enzootic disease in the commercial swine herds in Thailand.

***Acknowledgements***

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