

## Fetal losses associated with PRRSV, PPV, and PCV2 detection

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### *Abstract*

This study aimed to investigate the prevalence of porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), and porcine circovirus type 2 (PCV2) infections associated with fetal losses in 10 commercial swine herds in Thailand. Samples were collected from 100 sows and included 25 aborted fetuses, 35 mummified fetuses, and 40 stillborn piglets. The average parity number of the sows was  $2.8 \pm 2.4$ , ranging from 0 to 11. Fetal age was determined based on either the day of abortion or estimated using crown-rump length, and categorized as <70 days or  $\geq 70$  days. Organs from each case were pooled, homogenized, and tested for PRRSV (n = 100) using reverse transcription PCR, and for PPV and PCV2 (n = 60 each) using conventional PCR. PRRSV, PPV, and PCV2 were detected in 66.0% (66/100), 0% (0/60), and 93.3% (56/60) of samples, respectively. Specifically, PRRSV was identified in 52.0% (13/25) of aborted fetuses, 62.9% (22/35) of mummified fetuses, and 77.5% (31/40) of stillborn piglets ( $P = 0.096$ ). PCV2 was detected in 94.1% (16/17), 96.7% (29/30), and 84.6% (11/13) of the same groups, respectively ( $P = 0.343$ ). Co-infection with PRRSV and PCV2 was observed in 60.0% (36/60) of cases, specifically in 13.3% of aborted (n = 8), 30.0% of mummified (n = 18), and 16.7% of stillborn (n = 10) fetuses. No significant differences in PRRSV or PCV2 detection were found based on sow parity or fetal age group ( $P > 0.05$ ). Among PRRSV-positive samples, genotypes 1, 2, and mixed types accounted for 30.3%, 37.9%, and 31.8%, respectively ( $P = 0.728$ ). Interestingly, 92.3% of stillborn piglets tested positive for either PRRS or PCV2, while only 7.7% were free from all the viruses investigated. Similarly, all mummified fetuses (100%) were infected with either PRRS or PCV2, with none testing negative for the examined viruses. Among aborted fetuses, 94.1% were positive for either PRRS or PCV2, whereas only 5.9% were virus-free. These results highlight a strong association between PRRSV and PCV2 infections and fetal losses, underscoring the importance of targeted surveillance and control measures in Thai swine herds.

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**Keywords:** abortion, mummy, pig, reproduction, stillbirth, virus

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## Introduction

The primary objective in commercial swine herds is to maximize the number of piglets weaned per sow per year (Tummaruk *et al.*, 2023). However, several factors, including low litter size at birth, high preweaning mortality, and prolonged non-productive days, can negatively impact productivity (Adi *et al.*, 2024). Fetal losses, including abortion, mummification, and stillbirth, are common in swine herds and significantly reduce the number of piglets born alive. These losses can result from both infectious and non-infectious causes in gilts and sows. Among the major infectious agents associated with reproductive failure and fetal loss in the global swine industry are porcine reproductive and respiratory syndrome virus (PRRSV), Aujeszky's disease virus, porcine parvovirus (PPV), classical swine fever virus, and porcine circovirus type 2 (PCV2) (Tummaruk and Tantilertcharoen, 2012; Tummaruk *et al.*, 2014; Olanratmanee *et al.*, 2015; Pearodwong *et al.*, 2015; Kedkovid *et al.*, 2020; Maghfira *et al.*, 2025). The prevalence of PRRSV, PPV, and PCV2 infections associated with reproductive disorders varies across countries, for instance, 10.0%, 0.0%, and 1.0% in Spain (Maldonado *et al.*, 2005), and 10.7%, 67.3%, and 30.7% in South Korea (Kim *et al.*, 2004), respectively. In Thailand, research on the prevalence and co-infection of these viruses in cases of reproductive failure and fetal loss remains limited (Tummaruk and Tantilertcharoen, 2012). However, it has been reported that replacement gilts in most commercial Thai herds were seropositive for PRRSV (87.5%) and PPV (99.0%), while gilts culled due to abortion showed seropositivity rates of 81.0% for PRRSV and 75.0% for PPV (Tummaruk and Tantilertcharoen, 2012). Furthermore, PRRSV and PCV2 were detected in the uterine tissues of gilts culled for reproductive disturbances at rates of 33.0% and 45.1%, respectively (Olanratmanee *et al.*, 2011; Pearodwong *et al.*, 2015). These findings highlight the high prevalence of these viruses in the reproductive tracts of sows under field conditions. Therefore, routine monitoring of these infections and identification of associated risk factors are essential to inform improved management practices and strengthen biosecurity measures in swine production systems.

Porcine reproductive and respiratory syndrome virus (PRRSV) is a single-stranded RNA virus classified into two genotypes based on genetic, antigenic, and pathogenic differences: genotype 1 (PRRSV1 or European strain) and genotype 2 (PRRSV2 or North American strain) (Kedkovid *et al.*, 2020). Infected herds commonly experience reproductive failures, including reduced farrowing rates, increased abortion rates (ranging from 10% to 50%), higher returns to estrus post-mating, and elevated incidences of mummified fetuses, stillborn piglets, weak-born piglets, and preweaning mortality, which can reach up to 60% (Olanratmanee *et al.*, 2013; Zimmerman *et al.*, 2019). PRRSV can be transmitted transplacentally, particularly during late gestation, and may induce apoptosis at fetal implantation sites, leading to abortion (Karniychuk *et al.*, 2011). Additionally, transplacental infection may cause fetal death,

contributing to increased rates of mummified fetuses and stillbirths (Zimmerman *et al.*, 2019). In field conditions, various management strategies are employed to control PRRSV clinical signs, including acclimatization, replacement gilt management, and vaccination with killed or modified-live virus (MLV) vaccines. However, despite MLV vaccination, reproductive failures associated with PRRSV infection may still persist in affected herds (Olanratmanee *et al.*, 2013).

Porcine parvovirus (PPV) is a single-stranded DNA virus, with reproductive failure being the primary, and often the only, clinical manifestation of infection (Streck and Truyen, 2020). Although the exact pathogenesis of PPV-induced reproductive failure remains incompletely understood, the virus is known to cross the placenta, spread progressively to the fetuses, and induce fetal death (Meszaros *et al.*, 2017). The clinical outcomes of PPV infection depend on the stage of gestation at the time of infection. During early gestation, infection typically leads to embryonic death followed by resorption. In mid-gestation, once fetal ossification has begun, fetal death results in mummification. In late gestation, PPV infection can cause stillbirth or the birth of weak piglets (Meszaros *et al.*, 2017; Streck and Truyen, 2020). Vaccination is the most widely used strategy to control PPV in commercial swine herds. However, despite vaccination, high antibody titers have been detected in some gilts and sows, suggesting that the immune response may not solely result from vaccination (Tummaruk and Tantilertcharoen, 2012). This implies that multiple factors may influence the effectiveness of PPV vaccination at the herd level (Oravainen *et al.*, 2005).

Porcine circovirus type 2 (PCV2) is a single-stranded DNA virus responsible for a group of conditions collectively known as porcine circovirus-associated diseases (PCVAD) (Opriessnig *et al.*, 2007). These include post-weaning multisystemic wasting syndrome, porcine dermatitis and nephropathy syndrome, porcine respiratory disease complex, necrotic enterocolitis, and reproductive failure, manifested as abortion, fetal mummification, stillbirth, preweaning mortality, and premature farrowing (Opriessnig *et al.*, 2007; Segales, 2012). Although the pathogenesis of PCV2-induced reproductive failure is not fully understood, fetuses are known to be susceptible to PCV2 infection (Opriessnig *et al.*, 2007; Segales, 2012). Various herd-level strategies, including vaccination, have been employed to control PCV2 (Opriessnig *et al.*, 2007; Paphavasit *et al.*, 2009). Vaccinating sows has been shown to improve farrowing rates, increase the number of piglets born alive, and enhance piglet survival to weaning (Pejsak *et al.*, 2012; Oliver-Ferrando *et al.*, 2018). However, pigs with high PCV2 antibody levels can still become infected, and antibody responses in sows vary across vaccinated herds (Sarli *et al.*, 2012; Oliver-Ferrando *et al.*, 2018). This variability may result in inconsistent maternal antibody transfer to piglets, complicating PCV2 control efforts (Sarli *et al.*, 2012). Given the significant impact of PRRSV, PPV, and PCV2 on reproductive failure and fetal loss in swine herds, including those in Thailand, this study aimed to

investigate the prevalence of these viruses using PCR and RT-PCR techniques.

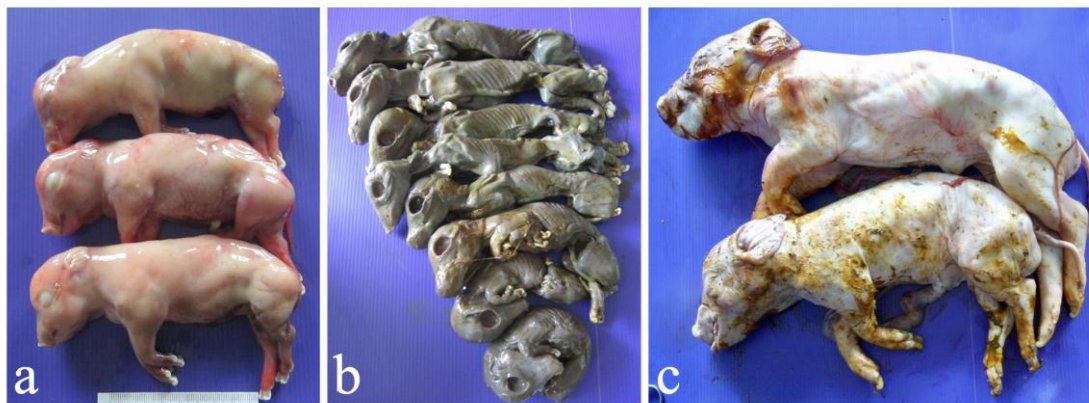
### Materials and Methods

This study complied with the Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes issued by the National Research Council of Thailand (NRCT). Ethical approval was granted by the Institutional Animal Care and Use Committee (IACUC) of Chulalongkorn University, in accordance with the university's regulations and policies on the care and use of experimental animals (Approval No. 11310033).

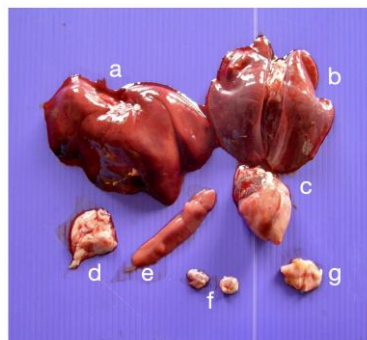
**Herd location and general management:** The study was conducted between 2014 and 2019 in 10 commercial swine herds across Thailand. These herds were located in the central (n = 1), western (n = 2), eastern (n = 2), northeastern (n = 3), and southern (n = 2) regions of the country, with each herd comprising 800 to 5000 sows. Based on routine monitoring data, all herds were seropositive for PRRSV, PPV, and PCV2. Gilts and sows were housed in conventional open systems equipped with water sprinklers and fans to mitigate heat stress. Animals were typically fed 3.0–3.5 kg of a corn-soybean-fish-based diet per day, containing 16–18% crude protein, 3000–3250 kcal/kg metabolizable energy, and 0.85–1.10% lysine. Water was available *ad libitum* via nipple drinkers. Herd health was managed under the supervision of a designated herd veterinarian. Artificial insemination was routinely practiced in all herds. Prior to breeding, gilts

underwent acclimatization and were vaccinated. Core vaccination programs for gilts and sows included immunization against foot-and-mouth disease virus, classical swine fever virus, pseudorabies virus, and PPV. In two herds, a commercial modified-live PRRSV vaccine was also used. The study included 100 fetal loss cases (i.e., abortion, mummification, or stillbirth) from 100 litters.

**Historical data and specimen collection:** A total of 100 litters were examined, comprising 25 aborted fetuses, 35 mummified fetuses, and 40 stillborn piglets (Figure 1). All specimens were stored on ice and transported to the laboratory within 24 hours of collection. For virus detection, tissue samples were collected and stored at  $-80^{\circ}\text{C}$ . Specifically, lung, liver, spleen, heart, thymus, tonsil, and lymph node tissues were collected from aborted fetuses and stillborn piglets (Figure 2), while only lung, liver, spleen, and heart tissues were collected from mummified fetuses. Historical data were retrieved for each case, including herd identification, animal ID, breed, parity, mating date, farrowing or abortion date, total number of piglets born (TB), number born alive (BA), number of mummified fetuses, and number of stillborn piglets per litter. The gestational age of aborted fetuses and stillborn piglets was calculated based on the interval between mating and either farrowing or abortion. For mummified fetuses, fetal age at death was estimated using crown-rump length (CRL), applying the formula: fetal age =  $21.07 + (3.11 \times \text{CRL})$ , as described by Ullery *et al.* (1965).



**Figure 1** Gross morphological features of different specimen types, including aborted fetuses (a), mummified fetuses (b), and stillborn piglets (c).



**Figure 2** Characteristics of the visceral organs in stillborn piglets, including the liver (a), lung (b), heart (c), thymus (d), spleen (e), lymph nodes (f), and tonsil (g).

**Viral detection using RT-PCR and PCR techniques:** Pooled organ samples from each case were homogenized and subjected to DNA and RNA extraction using a commercial kit (NucleoSpin® RNA Virus Kit, Macherey-Nagel Inc., Düren, Germany), following the manufacturer's protocol. The extracted nucleic acids were then analyzed for the presence of PRRSV ( $n = 100$ ) using reverse transcription polymerase chain reaction (RT-PCR), and for PPV ( $n = 60$ ) and PCV2 ( $n = 60$ ) using conventional PCR. PRRSV detection via RT-PCR followed the method described by Thanawongnuwech *et al.* (2004), while PCV2 detection followed the protocol outlined by Pearodwong *et al.* (2015). PPV detection was performed as part of routine diagnostic services at the Chulalongkorn University Veterinary Diagnostic Laboratory. Amplified products from both RT-PCR and PCR assays were visualized using gel electrophoresis.

**Statistical analyses:** Statistical analyses were conducted using the Statistical Analysis System (SAS), version 9.4 (SAS Institute Inc., Cary, NC, USA). Descriptive statistics, including the number of observations, mean, standard deviation, and range, were calculated for continuous variables such as fetal age, TB, BA, number of mummified fetuses, and number of stillborn piglets. Frequency analyses were used to determine the detection rates of PRRSV, PPV, and PCV2. These detection rates were compared across parity groups (0, 1, 2–4, 5–11, and unknown), specimen types (aborted fetuses, mummified fetuses, and stillborn piglets), and fetal age groups (<70 days and ≥70 days of gestation). All statistical comparisons were performed using chi-square tests, with a significance level set at  $P < 0.05$ .

## Results

Among the litters analyzed in this study, the mean ( $\pm$  SD) and range for each parameter were as follows: TB,  $13.5 \pm 3.4$  (range: 6–22); BA,  $8.0 \pm 5.2$  (range: 0–15); number of mummified fetuses,  $3.2 \pm 3.4$  (range: 0–10); and number of stillborn piglets,  $3.3 \pm 3.6$  (range: 0–13). The mean gestational age of aborted fetuses was  $69.1 \pm$

28.2 days (range: 31–107 days). Based on CRL estimations, mummified fetuses had an average fetal age of  $110.2 \pm 13.5$  days (range: 73–121 days). For stillborn piglets, the average gestation length was  $115.0 \pm 2.6$  days (range: 111–123 days).

Across all specimen types, PRRSV, PPV, and PCV2 were detected in 66.0% (66/100), 0.0% (0/60), and 93.3% (56/60) of cases, respectively. Table 1 presents the detection rates of PRRSV, PPV, and PCV2 by specimen type. Specifically, PRRSV was identified in 52.0% (13/25) of aborted fetuses, 62.9% (22/35) of mummified fetuses, and 77.5% (31/40) of stillborn piglets ( $P = 0.096$ ). PCV2 was detected in 94.1% (16/17), 96.7% (29/30), and 84.6% (11/13) of the same groups, respectively ( $P = 0.343$ ). Co-infection with both PRRSV and PCV2 was observed in 60.0% (36/60) of specimens, with rates of 13.3% (8/60) in aborted fetuses, 30.0% (18/60) in mummified fetuses, and 16.7% (10/60) in stillborn piglets.

PRRSV was detected in 13.0% (13/100) of samples from PRRSV-vaccinated herds and 53.0% (53/100) from non-vaccinated herds ( $P = 0.250$ ). The percentage of PRRSV detection by specimen type and viral genotype are presented in Table 2. Among the PRRSV-positive samples, genotype 1, genotype 2, and mixed genotypes were identified in 30.3% (20/66), 37.9% (25/66), and 31.8% (21/66) of cases, respectively ( $P = 0.728$ ). Detection rates of PRRSV and PCV2 by parity group are shown in Table 3, with no significant differences observed among parity groups. PRRSV was detected in 56.4% (22/39) of fetuses younger than 70 days and 72.1% (44/61) of those 70 days or older ( $P = 0.106$ ), while PCV2 was detected in 95.2% (20/21) and 92.3% (36/39) of these age groups, respectively ( $P = 0.664$ ). The mean fetal ages for PRRSV- and PCV2-positive samples were  $98.9 \pm 3.5$  days and  $98.0 \pm 3.8$  days, respectively.

Interestingly, 92.3% of stillborn piglets tested positive for either PRRS or PCV2, while only 7.7% were free from all the viruses investigated. Similarly, all mummified fetuses (100%) were infected with either PRRS or PCV2, with none testing negative for the examined viruses. Among aborted fetuses, 94.1% were positive for either PRRS or PCV2, whereas only 5.9% were virus-free.

**Table 1** Percentage detection of porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), and porcine circovirus type 2 (PCV2) across different specimen types.

| Types of specimens | Viral detection (%) |          |              |
|--------------------|---------------------|----------|--------------|
|                    | PRRSV               | PPV      | PCV2         |
| Aborted fetuses    | 52.0 (13/25)        | 0 (0/17) | 94.1 (16/17) |
| Mummified fetuses  | 62.9 (22/35)        | 0 (0/30) | 96.7 (29/30) |
| Stillborn piglets  | 77.5 (31/40)        | 0 (0/13) | 84.6 (11/13) |
| Total              | 66.0 (66/100)       | 0 (0/60) | 93.3 (56/60) |

**Table 2** Percentage of porcine reproductive and respiratory syndrome virus (PRRSV) detection by specimen type and viral genotype.

| Types of specimens | PRRSV detection (%) | Genotypes of PRRSV (%) |              |               |
|--------------------|---------------------|------------------------|--------------|---------------|
|                    |                     | Genotype 1             | Genotype 2   | Mixed         |
| Aborted fetuses    | 52.0% (13/25)       | 0% (0/25)              | 40% (10/25)  | 12% (3/25)    |
| Mummified fetuses  | 62.9% (22/35)       | 25.7% (9/35)           | 17.1% (6/35) | 20% (7/35)    |
| Stillborn piglets  | 77.5% (31/40)       | 27.5% (11/40)          | 22.5% (9/40) | 27.5% (11/40) |
| Total              | 66.0% (66/100)      | 20% (20/100)           | 25% (25/100) | 21% (21/100)  |

**Table 3** Detection rates of porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) according to parity number.

| Parity number  | Viral detection (%) |                |
|----------------|---------------------|----------------|
|                | PRRSV               | PCV2           |
| 0              | 60.0% (3/5)         | 100.0% (4/4)   |
| 1              | 71.4% (15/21)       | 100.0% (12/12) |
| 2-4            | 61.1% (11/18)       | 90.0% (9/10)   |
| 5-11           | 64.7% (11/17)       | 71.4% (5/7)    |
| Unknown parity | 66.7% (26/39)       | 96.3% (26/27)  |
| Total          | 66.0% (66/100)      | 93.3% (56/60)  |

### Discussion

In the present study, PRRSV and PCV2 were frequently detected in fetal samples associated with reproductive losses, whereas PPV was not detected in any case. PRRSV was identified in 66.0% of the samples, while PCV2 was found in 93.3%, indicating that these two viruses are likely key contributors to fetal mortality in the herds investigated. The absence of PPV detection suggests that this virus may no longer play a significant role in reproductive failures within the studied population, likely due to the widespread use of effective vaccination protocols against PPV in Thailand. Consistent with previous reports, PRRSV and PCV2 have been detected in various fetal tissues following in utero infection, including the heart, lungs, liver, spleen, tonsils, thymus, and lymph nodes (Rowland, 2010; Saha *et al.*, 2010). Supporting these findings, Olanratmanee *et al.* (2015) reported PRRSV detection in 67.4% of fetuses from gilts and sows with reproductive losses using quantitative PCR. Additionally, PRRSV was detected in 70.0% of ovarian tissues and 33.0% of uterine tissues of gilts culled for reproductive disturbances, as shown by immunohistochemistry (Olanratmanee *et al.*, 2011; Tummaruk *et al.*, 2015). Likewise, PCV2 was found in 30.0% of ovarian tissues and 45.1% of uterine tissues of culled gilts, based on PCR performed on paraffin-embedded samples (Pearodwong *et al.*, 2015; Limsaranrom *et al.*, 2016). These findings reinforce the significant involvement of both PRRSV and PCV2 in reproductive disorders and their widespread tissue distribution in affected animals.

Among PRRSV-positive cases, the distribution of genotypes was relatively balanced, with genotype 1, genotype 2, and mixed infections accounting for 30.3%, 37.9%, and 31.8% of cases, respectively. The absence of a statistically significant difference among these groups suggests that multiple PRRSV strains are co-circulating and contributing to fetal losses. This finding aligns with previous reports highlighting the genetic diversity of PRRSV strains in endemic regions (Tummaruk *et al.*, 2013; Olanratmanee *et al.*, 2015). Similarly, the current study confirmed the presence of both genotype 1 and genotype 2 in reproductive loss cases, as previously reported by Olanratmanee *et al.* (2015), indicating that both genotypes remain prevalent in Thai swine herds and continue to be associated with fetal losses. Interestingly, co-infection with both genotype 1 and genotype 2 strains of PRRSV is commonly observed. This raises concerns about the potential for genetic recombination between strains, potentially resulting in the emergence of novel variants that may evade existing herd immunity (Nilubol *et al.*,

2013). As a result, mixed-genotype infections may complicate disease control strategies and present additional challenges for effective PRRSV management at the herd level.

Co-infection with PRRSV and PCV2 was identified in 60.0% of the cases, with the highest prevalence observed in mummified fetuses, followed by stillborn piglets and aborted fetuses. This high rate of co-infection underscores the potential synergistic interaction between PRRSV and PCV2 in exacerbating fetal pathology. Previous studies have suggested that concurrent infections can lead to more severe reproductive and systemic outcomes than single-virus infections (Sagrera *et al.*, 2024). Despite this, no significant associations were found between the detection rates of either virus and sow parity or fetal age group, indicating that these infections are widespread across reproductive stages and sow age categories. These findings highlight the urgent need for ongoing surveillance of PRRSV and PCV2 and support the implementation of integrated control strategies, such as effective vaccination programs (Paphavasit *et al.*, 2009; Jittimanee *et al.*, 2013; Olanratmanee *et al.*, 2013; Vu Anh Tuan Vo *et al.*, 2022) and stringent biosecurity measures (Poonsuk *et al.*, 2013; Zimmerman *et al.*, 2019), to mitigate reproductive losses in commercial Thai swine herds. It is well-established that both PRRSV and PCV2 primarily target the immune system, particularly mononuclear cells. Co-infection can lead to pronounced immunosuppression, increasing susceptibility to secondary infections and contributing to more severe clinical signs (Ouyang *et al.*, 2019). Previous research has shown that PRRSV-PCV2 co-infection extends the duration of PCV2 viremia and viral shedding compared to PCV2 infection alone (Sinha *et al.*, 2011). Additionally, pigs co-infected with these viruses exhibited greater genetic variation in PRRSV isolates, which may complicate disease control (Yin *et al.*, 2013). Given the limited cross-protection between heterologous PRRSV strains, managing infections under such conditions becomes increasingly difficult. While co-infections have been detected in aborted fetuses and stillborn piglets in herds experiencing elevated abortion rates (Mak *et al.*, 2018), the specific impact of PRRSV and PCV2 co-infection on reproductive failure and fetal loss has yet to be comprehensively investigated. Further studies are warranted to elucidate the pathogenesis and reproductive consequences of such co-infections.

The high detection rates of PRRSV and PCV2 observed in the present study are consistent with previous reports highlighting the significant role of these pathogens in reproductive failures in swine

herds worldwide. Several studies have demonstrated that PRRSV is a major cause of abortion, mummification, stillbirths, and weak-born piglets, particularly in endemic areas (Tummaruk *et al.*, 2014; Olanratmanee *et al.*, 2015; Zimmerman *et al.*, 2019). Similarly, PCV2 has been implicated in reproductive losses, often in association with porcine circovirus-associated diseases (PCVAD) (Opriessnig *et al.*, 2007; Segales, 2012). The co-infection rate of 60.0% between PRRSV and PCV2 found in the present study aligns with earlier findings, which reported that concurrent infections can exacerbate reproductive loss more severely than single infections (Mak *et al.*, 2018).

Interestingly, the absence of PPV detection in the present study contrasts with earlier reports in which PPV was traditionally recognized as a major cause of reproductive failure in swine (Mengeling *et al.*, 2000). This discrepancy is likely attributable to the widespread implementation and effectiveness of PPV vaccination programs in commercial herds, which have significantly reduced the virus's prevalence in recent years. These findings support the notion that vaccination against PPV can effectively prevent fetal death. In the present investigation, no cases of fetal loss were associated with PPV infection, which may reflect the routine and continuous vaccination of both gilts and sows within the studied herds. However, antibody titers against PPV were not assessed in this study. A previous investigation in similar Thai herds reported that 99.0% of replacement gilts were infected with PPV, and a survey of gilts culled due to reproductive failure found a seroprevalence of 86.0% (Tummaruk and Tantilertcharoen, 2012). In that study, all replacement and pregnant gilts were seropositive ( $\geq 1:128$ ), with 99.0% exhibiting high titers ( $> 1:512$ ) and 97.0% showing very high titers ( $\geq 1:4096$ ). Typically, PPV antibody titers range from 1:32 to 1:512 following vaccination, but they can rise to 1:40960 within 19 days after exposure to a field strain (Józwik *et al.*, 2009). These findings suggest that the high proportion of sows with elevated antibody titers may be due to either vaccination or natural infection. Consequently, the presence of PPV antigen is likely undetectable because most sows possess antibodies against the virus. Moreover, the exact timing of infection is unknown, which may have further limited PPV detection in this study. These results reinforce the recommendation that PPV vaccination should be administered consistently to all breeding females, including gilts and sows of all parities, at regular intervals (e.g., every 4–6 months) (Józwik *et al.*, 2009). Furthermore, ongoing monitoring of PPV circulation within the herd, along with periodic assessment of antibody titers, is essential to ensure adequate immunological protection and to prevent future reproductive losses associated with PPV.

Furthermore, the similar distribution of PRRSV genotypes and the lack of association between viral detection rates and sow parity or fetal age are comparable to previous observations that PRRSV and PCV2 infections can affect a broad range of reproductive stages and parities without clear predilection (Sanchez *et al.*, 2001; Johnson *et al.*, 2002; Karniychuk and Nauwynck, 2013). Overall, these comparisons reinforce the importance of ongoing monitoring and the adaptation of herd health

management practices to address the evolving epidemiology of reproductive pathogens in swine production systems.

The remarkably high detection rates of PRRSV or PCV2 in stillborn piglets (92.3%), mummified fetuses (100%), and aborted fetuses (94.1%) underscore the significant role these viruses play in reproductive failures in swine herds. The near-universal presence of at least one of these viruses among affected fetuses, particularly the complete absence of virus-negative mummified samples, suggests that vertical transmission of PRRSV and PCV2 is a major contributing factor to fetal compromise. These findings are consistent with previous reports indicating that both PRRSV and PCV2 can cross the placenta and lead to embryonic death, mummification, stillbirth, or abortion, depending on the stage of gestation and viral load (Sanchez *et al.*, 2001; Gerber *et al.*, 2012; Karniychuk and Nauwynck, 2013). The extremely low proportion of virus-free specimens further supports the need for improved control strategies, especially in herds with suboptimal vaccination or biosecurity protocols. Moreover, the high prevalence of these viruses, despite the use of vaccines in some herds, raises concerns about the adequacy of herd immunity and the potential for co-infections or viral evolution to undermine vaccine efficacy. These results emphasize the importance of comprehensive and continuous monitoring, the implementation of effective vaccination programs, and enhanced biosecurity measures to reduce the reproductive losses associated with PRRSV and PCV2 infections.

One limitation of this study is that the specimens analyzed were collected between 2014 and 2019, and the current prevalence of PRRSV types 1 and 2 in Thai swine herds may have since changed. Nonetheless, both genotypes remain prevalent and are still linked to fetal losses. Ongoing monitoring of the virus in relation to fetal loss may be beneficial for developing herd-specific PRRS control strategies. Furthermore, the present study assessed PRRSV prevalence across farms with differing vaccination protocols. Two farms employed PRRS vaccination, while eight did not. PRRSV was detected in 13.0% (13/100) of samples from vaccinated herds and 53.0% (53/100) from non-vaccinated herds. All herds were classified as PRRS-stable, with no apparent clinical signs during the sampling period. These findings suggest that viral load in specimens may not be solely influenced by vaccination status, highlighting the need for continued vigilance regarding PRRSV contamination regardless of vaccination practices.

In conclusion, the present study highlights the significant involvement of PRRSV and PCV2 infections in fetal losses in Thai swine herds, with a high rate of co-infection observed. The findings emphasize the need for integrated control measures, including effective vaccination programs and enhanced biosecurity practices, to mitigate reproductive failures and improve herd productivity.

**Conflicts of interest:** There were no conflicts of interest that may have biased the work reported in this study.

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