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Case report

Case report of reproductive failure and dermatitis occurred by PCV3 and *Mycoplasma suis* in sows at a breeding farm

Dung Thi Thuy Do[1], Tram Thi Ngoc Ngo [1], Nam Minh Nguyen[2], Nga Thi To Bui[3] and Duy Tien Do[1,*]

[1]Faculty of Animal Science and Veterinary Medicine, Nong Lam University HCMC, Thu Duc City, Ho Chi Minh City 713000, Vietnam
[2]Center for Genetics and Reproductive Health (CGRH), School of Medicine, National University HCMC, Thu Duc City, Ho Chi Minh City 713000, Vietnam
[3]College of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi 12400, Vietnam

Abstract

Reproductive disorders in sows pose significant economic challenges to the swine industry worldwide. In this study, we investigated the presence of porcine circovirus type 3 (PCV3) and *Mycoplasma suis (M. suis)* in cases of reproductive failure at a breeding sow farm in Vietnam. PCR and RT-PCR assays were performed to detect related pathogens on samples collected from sows with reproductive disorders, and phylogenetic analysis was conducted to characterize the PCV3 strains. Additionally, the incidence and shedding patterns of PCV3 were assessed. Results revealed a high frequency of PCV3 and *M. suis* infections in sows with reproductive disorders (54.5% and 45.4% respectively), while other pathogens were not detected. Notably, 10 out of the 16 affected sows (62.5%) also showed signs of dermatitis lesions. Phylogenetic analysis showed that the PCV3 strains belonged to the PCV3b subtype. These findings suggest a significant role of PCV3 in causing reproductive issues in sows, including dermatitis, emphasizing the potential impact of early PCV3 infection on piglets during subsequent production stages.

Keywords: Case report, *Mycoplasma suis*, Porcine Circovirus type 3, reproductive failure, pigs

***Corresponding author:** Duy Tien Do, Faculty of Animal Science and Veterinary Medicine, Nong Lam University HCMC, Thu Duc City, Ho Chi Minh City 713000, Vietnam Tel. (+84) 918 356 744 E-mail: duy.dotien@hcmuaf.edu.vn.

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INTRODUCTION

Reproductive failure in sows poses a significant challenge for the pig production sector, leading to substantial economic losses in the global swine industry (Christianson et al., 1992; Althouse et al., 2019). Late-term gestation reproductive diseases can result in abortions, stillborn and/or weak-born piglets, or premature farrowing (Saporiti et al., 2021). Several viruses have been associated with reproductive problems in pigs, including porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus 1 (PPV1), porcine circovirus 2 (PCV-2), and Aujeszky's disease virus (ADV) (Althouse et al., 2019). Currently, the emergence of PCV3 has garnered considerable attention from scientists worldwide. PCV-3 has been detected in pigs with diverse clinical/pathological conditions, such as respiratory, reproductive, gastrointestinal, and neurological disorders (Sirisereewan et al., 2022). Interestingly, the virus has also been found in seemingly healthy animals (Palinski et al., 2017; Zheng et al., 2017; Franzo et al., 2018). Previous studies have reported high PCV3 loads within lesions of aborted fetuses and weak-born piglets, as well as cases of PDNS, pneumonia, periarteritis, myocarditis, or encephalitis using in situ hybridization methods (Phan et al., 2016; Arruda et al., 2019; Alomar et al., 2021; Saporiti et al., 2021; Vargas-Bermúdez et al., 2021; Molossi et al., 2022;). Recently, two major disease outcomes are proposed to standardize diagnostic criteria for PCV3-associated disorders: PCV3 reproductive disease in sows (PCV3-RD) and PCV3 systemic disease in pre- and post-weaning pigs (PCV3-SD) (Igriczi et al., 2022). In Vietnam, the first detection of PCV3 in pigs was recorded in the North (Quan et al., 2017), and later it was detected in the South (Duy et al., 2019).

Mycoplasma suis (*M. suis*) belongs to a highly specialized group of hemotrophic mycoplasmas (HM) and is uncultivable (Neimark et al., 2001; Hoelzle, 2008). It parasitizes the surface of erythrocytes in a variety of mammals, leading to erythrocytic deformity and damage (Zachary and Basgall, 1985). *M. suis* affects pigs of all age groups and exhibits two main clinical forms: an acute form with high fever and anemia, and a chronic form with multiple non-specific clinical signs that can vary in severity based on strain virulence and pig susceptibility (Stadler et al., 2014). The consequences of *M. suis* infection can be significant, particularly for the breeding herd during the period around farrowing (Biondo et al., 2009; Strait et al., 2012; Hoelzle et al., 2014; Stadler et al., 2014; Stadler et al., 2019), resulting in decreased birth weights (Zinn et al., 1983) and poor growth in post-weaning piglets (Hoelzle et al., 2014). Despite numerous studies on reproductive disorders in swine, a crucial knowledge gap persists in understanding the specific contributions of emerging pathogens, particularly porcine circovirus type 3 (PCV3) and *Mycoplasma suis* (*M. suis*), to cases of reproductive failure. This study aims to address this gap by investigating the prevalence and impact of PCV3 and *M. suis* in sows experiencing reproductive disorders at a breeding farm in Vietnam. Furthermore, we specifically focus on exploring the occurrence of dermatitis, a significant clinical manifestation observed in affected sows. By delineating the role of these pathogens and their association with dermatitis, we aim to contribute valuable insights to the understanding of reproductive health challenges in swine.

MATERIALS AND METHODS

Farm survey

In December 2022, a breeding sow farm in the South region of Vietnam, with 2400 sows (Landrace x Yorkshire) and utilizing a farrowing-to-finish two-site model with an open-window system, experienced a sudden increase in reproductive failure. One month later, the reproductive failure rapidly escalated. Notably, among the group of 300 sows transferred to the last stage of pregnancy for giving birth, 16 out of 300 pigs (5.3%) suffered from abortions, mummies, and stillbirths (Figure 1A, B). It was particularly striking that 10 out of the 16 affected sows (62.5%) also showed signs of dermatitis lesion (Figure 1C). Additionally, stunted and wasting piglets were observed in the affected litters at 1, 2, and 3 weeks of age, with the frequency of such occurrences gradually increasing from week 4 onwards.

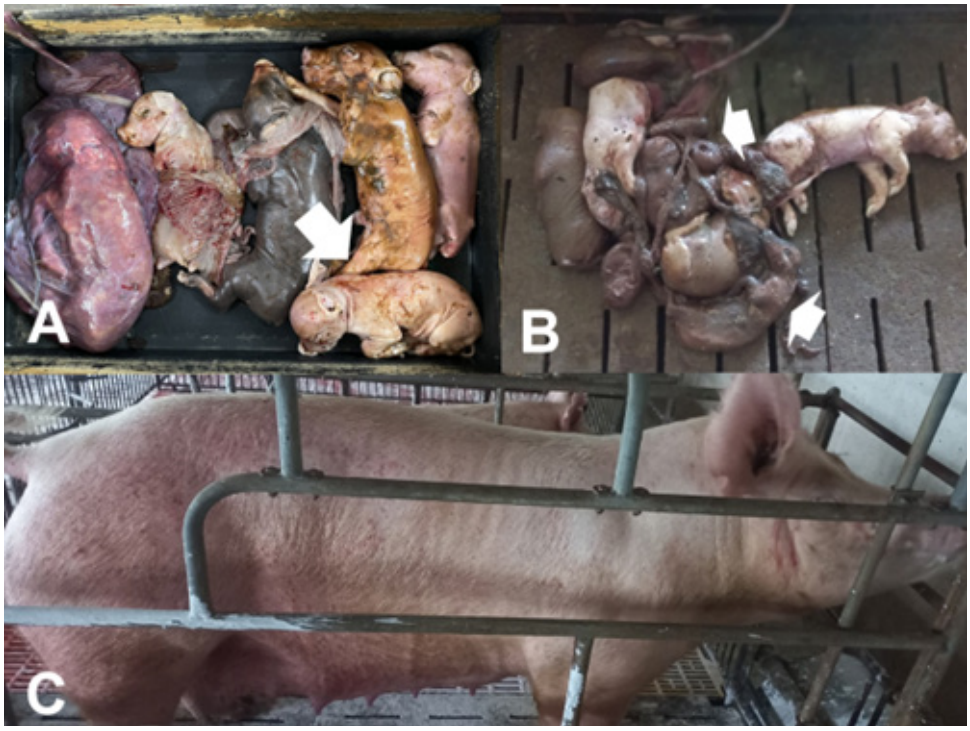

Figure 1 The reproductive abnormalities including stillbirth (A, arrow), mummies (B, arrow), and dermatitis lesion (C, sow).

The farm's vaccination scheme for PCV2 included vaccinating sows at 21 days of farrowing, female replacements at 34 weeks of age, and piglets at day 21 of age. Moreover, the vaccination program also covered CSFV, ADV, FMDV, PPV, PRRSV, and MH (Table 1).

Table 1 Vaccination program in sow farm

Pigs	Age	Type of vaccines						
		CSFV	**PRRSV***	**FMDV***	**PCV2**	**ADV**	**PPV**	**MH**
Piglets	14 days of age							x
	21 days of age				x			
	35 days of age	x						
	42 days of age			x				
Gilts	28 weeks of age		x					
	29 weeks of age						x	
	30 weeks of age			x				
	31 weeks of age	x						
	32 weeks of age					x		
	33 weeks of age			x				
	34 weeks of age				x			
Pregnant sows	11-week					x		
	12-week	x						
Boars	3 times/year (April, August, December)							
		x	x	x		x	x	

**PRRSV vaccine is massively vaccinated 3 times/year (February, June, October) and FMDV vaccine is massively vaccinated 3 times/year (April, August, December) in sow herd.*

Study design

At a breeding sow farm, an unusual birth defect was observed in a group of farrowing sows consisting of 300 individuals. These sows were closely monitored for clinical signs. 11 out of 16 clinical sows and 5 nonclinical sows from the same group were sampled to investigate the presence of pathogens associated with reproductive failure. The targeted pathogens for analysis included PCV3, PCV2, PCV4, PRV, PPV, PRRS, CSFV, APPV, JEV, ASFV and *M. suis*.

Various samples were collected from the sows, including serum, colostrum, vulva swabs, and oropharyngeal fluid within 2 hours after parturition. Colostrum was collected immediately before or at the beginning of the natural farrowing, without the use of oxytocin, by using hand pressure exerted approximately in the center of the mammary gland (Luise et al., 2020). Additionally, whole blood and visceral tissue samples were collected from their piglets at a rate of three pigs per sow. All collected samples were then transported to the research laboratory of the Faculty of Animal Science and Veterinary Medicine at Nong Lam University - HCMC for further analysis.

DNA extraction

DNA and RNA extraction was performed using the Thermo ScientificTM GeneJET Genomic DNA Purification and Thermo ScientificTM GeneJET Genomic RNA Purification kit (Thermo Fisher Scientific, USA). The extracted DNA and RNA were then subjected to PCR and RT-PCR analyses to evaluate the presence of the aforementioned pathogens. These molecular techniques allowed for the specific detection and identification of PCV3, PCV2, PCV4, PRV, PPV, PRRS, CSFV, APPV, JEV, ASFV and *M. suis* in the collected samples.

PCR/RT-PCR

After extracting DNA/RNA from the samples, the DNA/RNA extracts were used for PCR/RT-PCR reactions. The extracted products were then tested using PCR to detect PCV2, PCV3, PCV4, PRV, PPV, ASFV and *M. suis*. Additionally, remaining pathogens were tested using RT-PCR, including PRRSV, CSFV, JEV, and APPV. The PCR/RT-PCR reactions and thermal cycling protocols used in this case study were referenced from previous studies, specifically for detection of PRRSV (Do et al., 2016); CSFV (Lowings et al., 1996); ASFV (King et al., 2003); PCV2 (Dinh et al., 2021); PCV3 (Chen et al., 2017); PCV4 (Nguyen et al., 2022); PRV, PPV and JEV (Xu et al., 2012); APPV (Postel et al., 2016) and *M. suis* (Hoelzle et al., 2003).

Realtime PCR for PCV3 detection

The Realtime-PCR assays were performed with primer pairs F: 5′-CGCATAAGGGTCGTCTTGGA-3′; R:5′-CMGCTCAGCAAACAAAAACTATGTTC-3′ and probe 5′-FAM-TCCAGGCGCCGTCTAGATCTATGGC-BHQ1–3′. The reaction conditions were set based on the protocols used in a previous study (Yuan et al., 2020).

PCV3 sequencing and analysis

The full genome of this PCV3 strain was then amplified by PCR using two primer pairs, including PCV3-74F (CACCGTGTGAGTGGATATAC), PCV3-1144R (CACCCCAACGCAATAATTGTA) and PCV3-genome-2-F (TTGCACTTGTGT ACAATTATTGCG), and PCV3-genome-2-R (ATCTTC AGGACACTCGTAGCACCAC) (Fu et al., 2017; Ku et al., 2017). The PCR products were then purified using the ExoSAP-IT™ PCR Product Cleanup kit (Thermo Fisher Scientific, USA) following the manufacturer's recommendations. After purification, the products were sequenced by a oversea laboratory (Macrogen, Korea).

To analyze the sequences, the nucleotide BLAST (blastn) and Protein BLAST (blastp) algorithms available at http://blast.ncbi.nlm.nih.gov/Blast.cgi were used to find percentage identities. Sequence alignments were performed using Muscle. For phylogenetic analysis, Neighbor-Joining trees were constructed with the Maximum Composite Likelihood (ML) model using the software tool MEGA (version 10.1). To assess the statistical support for each node in the resulting trees, the data were bootstrapped 1000 times using the bootstrap method.

RESULTS

The detection of PCV3, *M. suis* and other pathogens

PCV3 and *M. suis* were identified in 54.5% (6/11) and 45.4% (5/11) of sows with reproductive disorders, respectively. No co-infection of PCV3 and *M. suis* was observed in the surveyed sows and aborted fetuses, and no other pathogens detected in these sows. Among the 5 sows without reproductive disorders in the same group, none tested positive for pathogens PCV3, PCV2, PCV4, ADV, PPV, PRRS, CSFV, APPV, JEV, ASFV and *M. suis*. The frequency of detecting PCV3 in miscarriage fetal samples (heart, lung) was 83.33%, with a mean Ct qPCR value of 31±3.0, higher than those of sow specimens included colostrum (33.33%, mean Ct 28±5.6), oropharyngeal fluid (33.33%, mean Ct

31±0.0), vulvar fluid (16.67%, mean Ct 34±0.0), and serum (0%) (Table 2). Furthermore, the detection rate of PCV3 in piglets with clinical manifestations from the surveyed sows were 20% at 4 weeks of age and 0% at 1st, 2nd, and 3rd weeks of age.

Table 2 Real-time PCR results of PCV3 detected in sows and fetuses

Type of samples	PCR result	qPCR
	Positive rate (%)	**Mean Ct-value (±*SD*)**
Aborted fetuses (heart, lung)	83.33	31±3
Colostrum	33.33	28±5.6
Serum	0	-
Oropharyngeal fluid	33.33	31±0
Vuval fluid	16.67	34±0

Clinical features and lesions

A total of 16/300 (5.3%) sows in litters 2-4th had clinical reproductive disorders in which 9/300 (3%) sows revealed miscarriage (6/9 sows with stillborn and mummification) and 10/300 (3.33%) sows revealed dermatitis. The most of fetal tissues had marked signs of autolysis; in some fresh stillborn showed mild multifocal interstitial pneumonia and systemic lymphoplasmacytic periarteritis particular endocarditis and myocarditis.

Whole genome analysis of PCV3

The complete genome sequences of DTD-PS5338-2/DongNai_2022 PCV3 strains are available in GenBank under accession numbers OR059205. Sequence identities with other reference PCV3 sequences ranged from 97.85% to 99.35%, and they were 98.85% identical to NNH/DN8/2018 (MT847027) PCV3 strains previously reported in Vietnam (Table 3). The whole genome sequence of the PCV3 DTD-PS5338-2/DongNai_2022 strain had 2000 bases in length. Phylogenetic analyses of the complete genome of PCV3 in this study indicated that the PVC3 strain clusters into the PCV3b subtype.

Based on the complete genome sequences, all PCV3 isolates clustered together and were separated from other members of the genus Circovirus. The new PCV3 strain grouped closely with NNH/DN8/2018, 309/Thailand/2016, KU/1606/2016, CHN/Shanghai/0708/2016, and other related strains (Figure 2).

Table 3 Genome nucleotide identity estimated by blastn analysis considering full-genome sequences available in GenBank.

DTD-PS5338-2/DongNai_2022				DTD-PS5338-2/DongNai_2022			
GenBank seq	%Identity	nt #	E value	GenBank seq	%Identity	nt #	E value
MF162298	99.35	13	0.0	KY354038	98.85	23	0.0
MF589106	99.25	15	0.0	KX458235	98.75	25	0.0
MF589652	99.25	15	0.0	MH367847	98.75	25	0.0
KY421348	99.2	16	0.0	NC031753	98.75	25	0.0
KY996342	99.15	17	0.0	KY996345	98.7	26	0.0
KX966193	99.1	18	0.0	KY778776	98.7	26	0.0
KY418606	99.05	19	0.0	MF079253	98.7	26	0.0
KY865243	98.95	21	0.0	KX778720	98.65	27	0.0
MH603562	98.95	21	0.0	KY865242	98.65	27	0.0
MF589107	98.95	21	0.0	KY421347	98.6	28	0.0
KY996337	98.95	21	0.0	KY924474	98.1	38	0.0
MT847027	98.85	23	0.0	KY924473	97.85	43	0.0
KY075986	98.85	23	0.0				

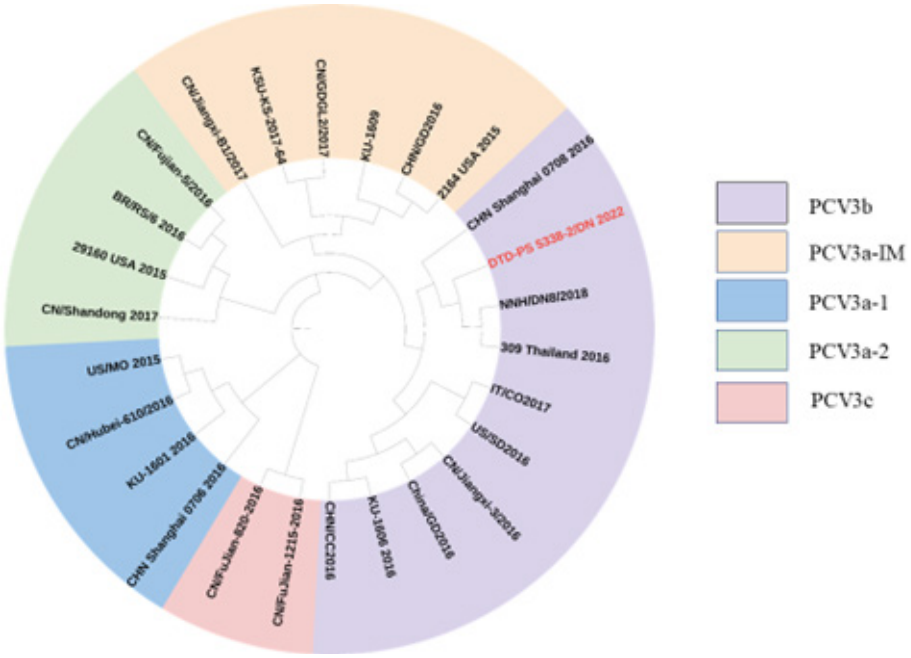

Figure 2 Phylogenetic tree based on full-genome sequences constructed using the Maximum-Likelihood method using the software tool MEGA (version 10.1). To assess the statistical support for each node in the resulting trees, the data were bootstrapped 1000 times using the bootstrap method.

DISCUSSION

In this study, PCV3 and *M. suis* were found in sows with reproductive disorders, suggesting their potential involvement in causing such conditions. Interestingly, no tested pathogens were found in the sows without reproductive disorders. PCV3 was detected at a higher frequency in miscarriage samples compared to other sample types, indicating its potential association with fetal tissues. Although PCV3 was highly detected in damaged fetal tissues, piglets from the same group of sow were only found to be PCV3 positive at week 4. Phylogenetic analysis revealed that the PCV3 strains in this study were closely related to previously reported strains in Vietnam.

PCV3 has been associated with various health issues in pigs, including reproductive disorders, porcine dermatitis and nephropathy syndrome (PDNS), myocarditis, multisystem inflammation, and pneumonia (Phan et al., 2016; Ku et al., 2017; Palinski et al., 2017; Shen et al., 2018). In this study focusing on reproductive failure cases, PCV3 was the most frequently detected while other pathogens such as PPRS, CSFV, PCV2, PCV4, PPV, APPV, ASFV and JEV were not found in the current case. Co-infection of PCV3 and PRV was observed, and a significant number of viruses, aside from PCV-3, have been recognized as potential causative agents of reproductive disease in previous studies (Hermann et al., 2005; Brunborg et al., 2007; Madson et al., 2009; Truyen, 2019).

These findings underscore the potential of PCV3 as a pathogen capable of causing reproductive problems, as suggested by other researchers (Arruda et al., 2019; Mora-Díaz et al., 2020; Temeeyasen et al., 2021). PCV3 has been previously reported in pigs with reproductive failures, including cases of abortions and stillborn piglets (Arruda et al., 2019; Mora-Díaz et al., 2020;

Temeeyasen et al., 2021). Notably, mummified fetuses showed a high rate of PCV3 infection, higher than that observed in other sample types such as colostrum, oropharyngeal fluid, vulvar fluids, and serum.

Approximately 33% of the tested sows shed PCV3, indicating its presence in these animals. Early PCV3 infection, possibly transmitted through colostrum, may have implications for pigs in later stages of production. Recent studies have also suggested a potential involvement of PCV3 in porcine respiratory disease complex (PRDC) in grower pigs (Kedkovid et al., 2018). In this study, PCV3 infection was observed at 4 weeks of age, suggesting that PCV3 infection during the suckling period might lead to more severe outcomes during the nursery and grower periods.

Phylogenetic analysis, a widely used tool for characterizing PCV3 sequences, has shown that PCV3 can be divided into different subtypes based on the analysis of various genetic regions (Franzo et al., 2018; Fu et al., 2018; Fux et al., 2018; Li et al., 2018; Franzo et al., 2019). In this study, the isolate strains were found to have high nucleotide identity when compared to previously reported PCV3 sequencing. The PCV3 strains identified in this study belong to the PCV3b subtype, consistent with findings from previous studies in Vietnam (Nguyen et al., 2021).

Furthermore, *M. suis* was detected in sows with clinical reproductive disorders, and there was no evidence of co-infection between it and PCV3. The impact of *M. suis* infection can be very significant, in particular for the breeding herd in the period around farrowing (Biondo et al., 2009; Strait et al., 2012; Hoelzle et al., 2014; Stadler et al., 2014). In sows, acute *M. suis* infection can result in sudden death due to coma and hypoglycemia, or present as a subacute illness with reduced fertility, increased estrus, and lactation disturbances (Brownback, 1981; Henderson et al., 1997; Messick, 2004; Strait et al., 2012). Studies have also observed an increase in stillbirth rates among *M. suis*-positive gilts (Brissonnier et al., 2020). To date, there have been no reports of the simultaneous detection of *M. suis* and PCV3 in reproductive disorders on farms. Therefore, the study of the interaction mechanism and the role between PCV3 and *M. suis* has important implications for elucidation in the etiology of reproductive disorders.

Overall, this study provides valuable insights into the presence and genetic characteristics of PCV3 and *M. suis* in cases of reproductive disorders in sows. The findings support the potential role of PCV3 in causing reproductive issues and highlight the need for further research and monitoring to better understand the complication among pathogens with reproductive health in swine. Such knowledge will contribute to the development of effective control and management strategies in pig production systems.

CONCLUSIONS

This study provides valuable insights into the incidence and characteristics of PCV3 and *M. suis* infections in cases of reproductive disorders in sows at a breeding farm in Vietnam. The findings contribute to our understanding of among pathogens potential causes for reproductive failure in pigs.

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CONFLICT OF INTEREST

We have no conflict of interest.

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