

## Detection of PRRSV circulation using oral fluid samples for nursery management in endemically PRRSV-infected farms

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### *Abstract*

Porcine reproductive and respiratory syndrome (PRRS) has become a major swine disease worldwide. Relevant management strategies and diagnostic assays are of importance for PRRS virus (PRRSV) control. The objective of this study was to determine the use of oral fluids for PRRSV monitoring in endemically PRRSV-infected herds. PRRSV RNA and PRRSV-specific antibodies were monitored using oral fluid samples and serum samples in two conventional swine farms in Thailand ('Farm A', a one-site conventional system farm, and 'Farm B', a one-site P0-P1 segregation system farm) during farrowing to nursery periods. Both PRRSV RNA and PRRSV antibodies were detected from 3 to 9 weeks of age in both sample types. Pen-based oral fluid samples were detected positive over 71% when the prevalence of serum PRRSV-positive pigs in the pens was at least 40%. Mean S/P ratios of the oral fluid samples showed significantly higher levels but had similar pattern to the seroprofile of the blood samples. Increased levels of PRRSV antibodies were detected in all groups at 5 to 9 weeks of age. Overall, the positive correlation of both sample types was 0.65 ( $p < 0.001$ ). It should be noted that Farm B had higher production losses in the farrowing and nursery units, concurrently, with higher levels of PRRSV load in both sample types. Oral fluid testing provides convenient and economical approach, better welfare, and satisfied performance to determine PRRS status, especially during nursery period, when there is moderate to high PRRSV prevalence. These objectives could be better achieved and benefit practitioners by using oral fluid testing together with other measurements.

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**Keywords:** management, monitoring, nursery pigs, oral fluid, PRRSV, swine

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## Introduction

Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV), has become one of the most important swine diseases since the first report in the United States (Christianson, 1994). Economic losses in all production stages from PRRSV circulations within and between farms could be observed worldwide (Baron et al., 1992; Hirose et al., 1995; Holtkamp et al., 2011). According to the disease characteristics involving both reproductive and respiratory problems, outbreaks in swine breeding herds could be extremely severe and are usually followed by porcine respiratory disease complex (PRDC) in growing pigs. Although establishment of different PRRS control protocols has been conducted, ongoing problems still occur. The impact of PRRS monitoring strategies on the success of PRRS controls should not be overlooked. Recognition of the benefits of oral fluid use for PRRS monitoring has recently become apparent due to the advantages over the use of serum samples. Implementation of oral-fluid-based PRRS monitoring during the disease controls in breeding herds and various stages of swine production should be explored in order to obtain valuable information for the development of control strategies.

Improvement of PRRS monitoring strategies could be a crucial factor in effective PRRS control program in breeding herds. Utilization of various strategies in sow herd including parity segregation, gilt acclimation and gilt/sow vaccination have been suggested to be effective. Briefly, parity segregation involves minimizing production losses from PRRSV infection in gilts/primiparous sows by separating their gestation and farrowing units from other multiparous pigs (Moore et al., 2005). Similarly, those gilt management strategies aim to reduce the PRRSV shedding and horizontal transmission before moving those acclimatized gilts to the sow herd and choosing effective monitoring strategies can be vital in PRRS control. The best method used for monitoring must demonstrate precise information on PRRS status. Besides, clinical observation, PRRS monitoring program usually involves the degree and timing of PRRSV spreading in the herd. Although sensitivity and specificity are essential, those values are not the only key determinants in choosing the monitoring method. Simplicity, cost and animal welfare should also be taken into account since these measurements could affect the overall efficacy of the management strategies.

Oral fluid sample is an alternative diagnostic sample for PRRS monitoring and control program in breeding herds (Kittawornrat et al., 2014; Trang et al., 2014). Previously, serum samples have long been used in PRRS monitoring. Several studies suggested that using oral fluids could bring considerable benefits to general PRRS surveillance (Cuong et al., 2014; De Regge and Cay, 2016). Previous data indicated that oral fluids provided a longer detection period and yielded better sensitivity for PRRSV detection than other methods (Kittawornrat et al., 2010; Goodell et al., 2013). Blood collection is invasive and could cause traumatic tissue injury to animals, while oral fluid collection hardly causes tissue injury. However, specific protocol for using oral fluid samples to monitor PRRSV

transmission in farrowing to nursery units is necessary to yield the full use for PRRS control program in nursery pigs.

In this study, comparison of results obtained from oral fluid- and serum-based methods was investigated. Quantitative PRRSV RNA and PRRSV-specific antibody parameters were compared and analyzed. The comparison was done in two selected farms with different management systems with and without parity segregation system.

## Materials and Methods

**Ethics statement:** The study obtained ethical approval from Chulalongkorn University Animal Care and Use Committee, Chulalongkorn University (IACUC number 1531020).

**Trial farms:** The study was conducted at 2 farrowing to finishing swine farms (Farm A and Farm B) using crossbred (Large White x Landrace x Duroc) pigs. The selected farms are located in the central part of Thailand, where intensive farming density is evident, and were diagnosed as PRRSV-positive farms, based on PRRSV-specific antibody responses by ELISA.

Farm A is a one-site conventional system farm (without P0-P1 segregation system) having approximately 1,300 sows. Farrowing barn had natural-ventilation facility with 120 farrowing crates. Warming laying area was provided for lactation pigs. Nursery units were designed with evaporative cooling system (EVAP), and stocked 600 pigs (20-25 animals per pen) in each barn. During the farrowing periods, both primiparous (P1) sows and multiparous (P2+) sows were kept in the same barn. After weaning at 24-28 days of age, those sows were mixed in the same nursery barn (Nursery A) until 10 weeks old.

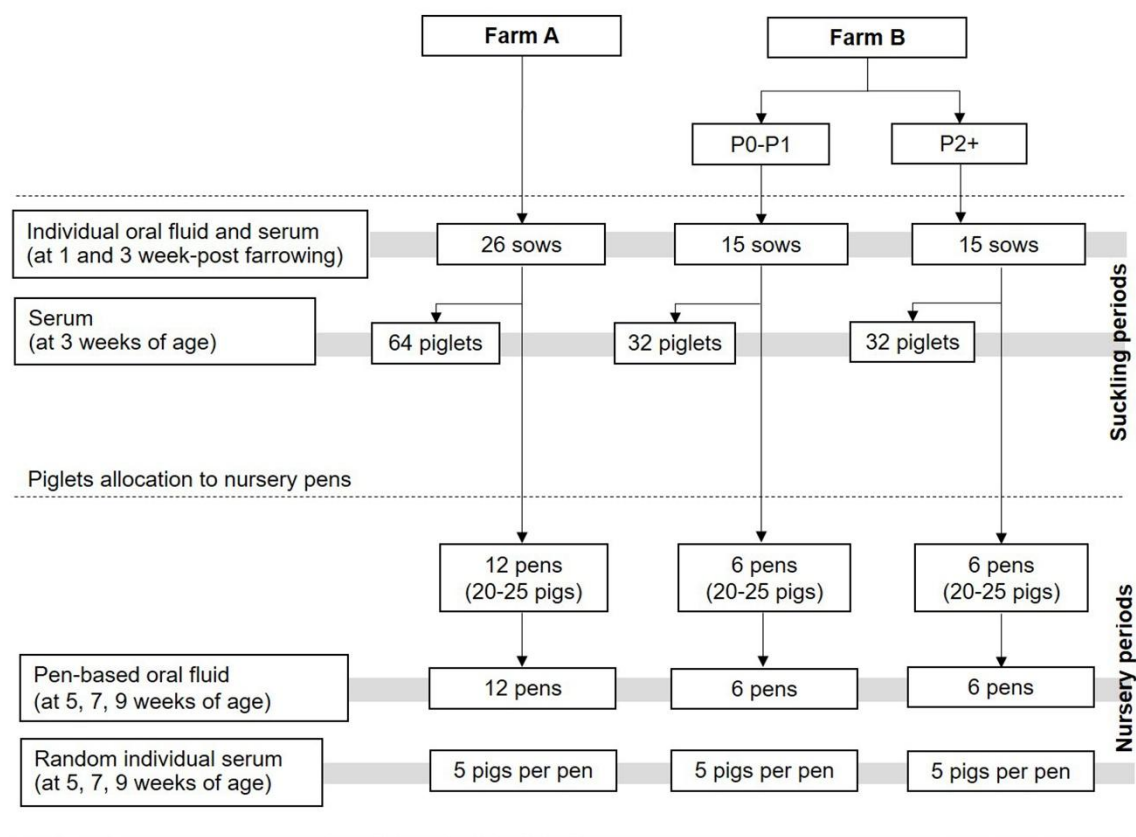
Farm B is a one-site P0-P1 segregation system farm having approximately 5,000 sows. The ventilation of all farrowing and nursery facilities was controlled by the EVAP. Each farrowing barn consisted of 160 farrowing crates with a warming box for piglets on each crate. The nursery barn stocked 700-800 pigs with 20-25 pigs in a pen. P1 and P2+ sows were kept in separate barns during the farrowing periods. After weaning at 24-28 days of age, the weanling piglets of P1 sows were placed in a separate EVAP barn from the piglets of P2+ sows (Nursery B-P1 and Nursery B-P2+, respectively) until 10 weeks old.

P1 sows (n = 11) and P2+ sows (n = 15) from Farm A, and P1 sows (n = 15) and P2+ sows (n = 15) from Farm B that farrowed within the same week were selected and participated in the study. After parturition, all piglets were vaccinated with a modified live virus (MLV) PRRS vaccine (Ingelvac PRRS® MLV, Boehringer Ingelheim) at 2 weeks old in both farms. For further sample collection in the nursery units in the study, 12 nursery pens were randomly selected as monitoring nursery pens for Nursery A. In Farm B, 6 monitoring nursery pens were selected in Nursery B-P1 and Nursery B-P2+ (12 monitoring pens in Farm B).

**Oral fluid and serum collections:** Oral fluid and serum collections were done in the same manner for both farms (Figure 1). In the farrowing units, oral fluid and

serum samples were individually collected from sows at one and 3 weeks post farrowing (WPF). Only serum samples were collected from the piglets (32 pigs on each sow group) at 3 weeks of age. For individual oral fluid sampling, 100% cotton ropes, 2.0 cm in diameter, were hung in each stall at shoulder height of the sows for 20-30 minutes. The ropes were carefully collected to

prevent cross-contamination from the other pigs. Oral fluids were extracted from the ropes by mechanical compression and then transferred into 50-ml tubes. Blood samples were collected by single-use blood collection systems (Monovette® 9ml Z, Sarstedt AG & Co, Germany) from the jugular vein. All samples were stored at -80°C until assayed.



**Figure 1** Flow diagram summarizing sample collection in suckling and nursery periods

In the nursery units, pen-based oral fluid samples and individual serum samples were collected at 5, 7, 9 weeks of age from pigs in the assigned monitoring nursery pens. Pen-based oral fluid collection was done by hanging 2 cotton ropes (100%) of 1.0-cm diameter in the monitoring pens. Oral fluids were then extracted from the ropes and stored as previously described. Blood samples were collected from 5 pigs in each monitoring pen.

**Quantification of PRRSV RNA:** PRRSV RNA was extracted from the serum and oral fluid samples using NucleoSpin® RNA virus kit (MACHEREY-NAGEL GmbH & Co. KG, Germany). Viral RNA extraction from the serum samples were done as described in the manufacturer's instructions, whereas the extraction from the oral fluid samples was performed with a modified method by using larger volumes of 200 µl, instead of 150 µl. Finally, copy number of viral RNA was examined using previously described TaqMan® probe-based real-time RT-PCR, with primers and probes specific for PRRSV nucleoprotein gene (ORF7) (Egli et al., 2001). RT-PCR mixture (25 µl) was based on SuperScript™ III Platinum® One-step Quantitative RT-PCR system (Invitrogen, Carlsbad, California, USA); RT-PCR were performed on Corbett Rotor-Gene™

6000 (Qiagen) real-time PCR machine. Copy number of the viral RNA was calculated using standard curve method. For each test, samples with a Ct < 33 were considered positive (Sirisereewan et al., 2017).

**Detection of PRRSV-specific antibody:** Anti-PRRSV antibody detection was performed on the serum samples using IDEXX PRRS X3 ELISA test kit (IDEXX laboratories, Inc., Westbrook, ME, USA). Oral fluid samples were tested by a commercial PRRSV Antibody Test Kit for Oral Fluids (IDEXX PRRS OF, IDEXX laboratories, Inc., The Netherlands). ELISA was performed as described in the manufacturer's instructions. S/P ratio value of greater than 0.4 was considered positive.

**Performance monitoring of sows and nursery pigs:** The reproductive performance parameters of the sows and performance index of nursery pigs were recorded on a computerized recording system for swine herds (PigLive® software, Kasetsart University) (Udomprasert et al., 1993). Production parameters related to PRRSV infection were monitored from farrowing until weaning.

**Data analysis:** Statistical analyses were done using GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA, USA). Statistical analysis of variance (ANOVA) followed by Tukey's multiple comparison tests was done to compare each variable of interest between monitoring periods and sample types. Viral titers and S/P ratios of oral fluid and serum samples of sows were presented as copy number means from individual sows. In the nursery unit, the oral fluid results were calculated from pen-based oral fluid samples of the monitoring pens, and the serum analysis was calculated in all monitoring pens of the same nursery unit. Association between PRRSV RNA detection rate from pen-based oral fluid samples and positive percentages based on five serum samples in the monitoring pen was done using data from all 3 nursery units. The Pearson's correlation coefficient was used to determine the relationship of virus concentration and average S/P ratios yielded from the serum and oral fluid samples. Statistical significance was set at  $p < 0.05$ .

## Results

**Detection of PRRSV RNA in oral fluid and serum samples:** An overview of PRRSV concentration and the proportion of PRRSV-positive in serum and oral fluid specimens on each farm are displayed in Figure 2. PRRSV RNA quantification from the oral fluid and serum samples of lactating sows were done twice, at 1 and 3 weeks after parturition. In both farms, viral RNA was not detected in all tested samples from the sows. PRRSV was first detected in the serum samples at 3 weeks of age (pre-weaning period). For PRRSV monitoring in nursery pigs, direct comparison between the oral fluid and serum samples was done in three nursery units: Nursery A, Nursery B-P1, and Nursery B-P2+ (Table 1). The pen-based oral fluid samples showed significantly higher viral titers than the serum samples in Nursery B-P1 at 5 weeks old and Nursery B-P2+ at 5 and 9 weeks old. However, the serum samples showed significantly higher viral titers in Nursery A at 7 weeks old, and Nursery B-P1 at 7 and 9 weeks old. In addition, positive correlations were estimated between both sample types in Nursery-A ( $r = 0.57$ ;  $p < 0.001$ ), Nursery-B-P1 ( $r = 0.80$ ;  $p < 0.001$ ) and Nursery-B-P2+ ( $r = 0.75$ ;  $p < 0.001$ ). Moreover, no differences in the proportion of PRRSV positive were found between the pen-based oral fluid and serum samples over the monitoring periods. The association between PRRSV detection rate of pen-based oral fluid sample and percentages of serum-positive pigs within a pen is shown in Table 2. The detection rate of the pen-based oral fluids increased when the serum prevalence increased. Over 71% of the PRRSV-positive pens were identified by pen-based oral fluids when the serum percentages of PRRSV-positive pigs in the pens had at least 40%.

**Serology test:** An overview of the antibody titers and the proportion of positive results in serum and oral fluid on each farm is displayed in Figure 3. Mean S/P ratios of the oral fluid samples were significantly higher, but in similar pattern, than those of the serum samples. Similarly, the average S/P ratio of both sample

types declined significantly from 1 to 3 weeks post farrowing. In the nursery period, a total of 360 pigs from 72 pens were tested by ELISA and the results indicated that PRRSV antibodies in the pigs increased at 5 to 9 weeks of age. All pen-based oral fluid samples were found positive to PRRSV, corresponding well with the serum results depending on the percentage of serum positive-pigs in each pen. Correlations between the average S/P ratios in the serum and oral fluid samples using Pearson's correlation coefficient were 0.85, 0.87, and 0.72 ( $p < 0.001$ ) in Farm A, Farm B-P1, and Farm B-P2+, respectively.

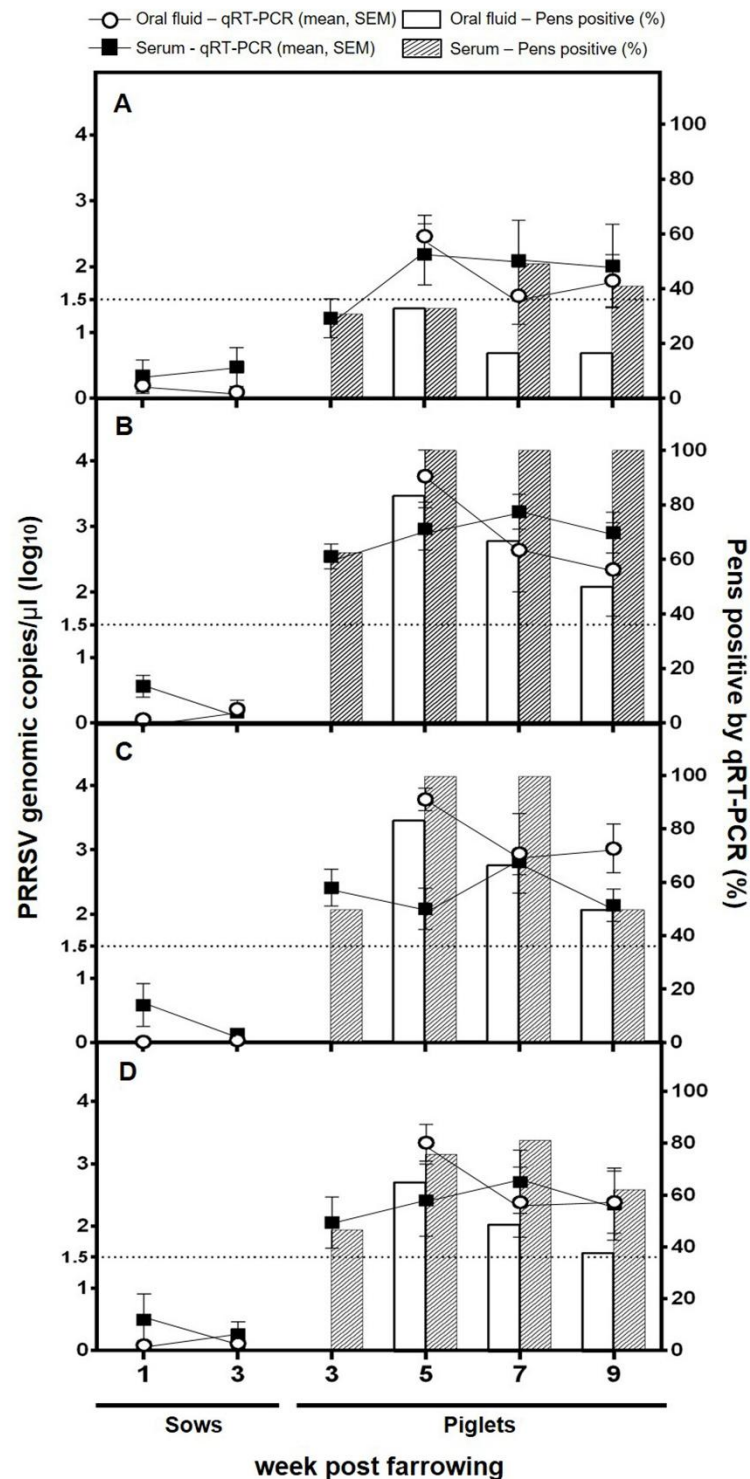
**Production parameters:** Computerized performance parameters of the sows, pre-weaning piglets, and nursery pigs are summarized in Table 3. In Farm A, following the farrowing period, two enrolled P1 sows became sick by post-partum dysgalactia syndrome and were culled before weaning. In Farm B, one enrolled P1 sow was also culled before weaning. Percentages of stillbirth increased significantly in Farm B-P1 sows compared to the data of Farm A sows. Pre-weaning mortality was high in all sow groups of both farms. Lactation length and wean-to-first-service interval were longer in Farm B due to the clinical condition of the animals. Data yielded from Nursery A were in acceptable ranges. In contrast to Farm A, clinical signs were observed in the nursery pigs from both P1 and P2+ sows of Farm B, including depression, respiratory distress and emaciation, correlating well with the higher viral loads shown in both oral fluid and serum samples (Figure 2). ADG, FCR, FCG and mortality rates of Farm B nursery pigs were also below the baseline data.

## Discussion

As expected, oral fluid collection was easy to perform in the farrowing sows and the nursery pigs. The workers could handle by themselves without causing stress or injury to the animals. It should be noted that the failure to collect oral fluid samples from the suckling pigs was probably related to the untrained younger age piglets. Previous studies described 97% success rate on oral fluid collection from 3-week-old, trained pigs (Kittawornrat et al., 2014; White et al., 2014). In nursery period, pen-based oral fluid collection could be collected as frequently as needed after being trained. In addition, pen-based collection costs less because of the use of less sample number and also increases the sensitivity of the samples.

After farrowing, no evidence of PRRSV viremia or shedding was found in all sows tested. However, viremia was found in 3-week-old piglets in all groups. It should be speculated that sample collection in the absence of clinical signs might have low sensitivity compared to the collection from sick sows. In addition, PRRSV viremia in sows is difficult to detect because PRRS viremic duration is rather short in immunized animals, particularly in repeatedly immunized sows. The low proportion of PRRSV positive piglets in Farm A might be related to the MLV vaccination at 2 weeks of ages or possibly the presence of low level of vertical transmission (Balka et al., 2016). However, Farm B pigs had higher viral loads possibly

due to having higher levels of both vertical and horizontal transmission together with MLV vaccination with the presence of clinical diseases



**Figure 2** PRRSV quantitative RT-PCR ( $\log_{10}$  genomic copies per  $\mu\text{l}$ ) of Farm A (a), Farm B-P1 (b), Farm B-P2+ (c) and the overall samples (d) in sows and their piglets from serum and oral fluid samples. All results are expressed as pen-based results for the real-time RT-PCR test. The horizontal line at 1.5  $\log_{10}$  genomic copies per  $\mu\text{l}$  represents the cut-off for positive samples (Ct of < 33).

In the nursery period, oral fluid samples could be very promising for PRRSV monitoring, especially when the prevalence of PRRSV infection is moderate to high. It has been suggested previously that the prevalence of PRRSV infection is a crucial factor for

a successful use of oral fluids in PRRSV monitoring (Olsen et al., 2013; De Regge and Cay, 2016; Strugnelli, 2010). This is also true in our situations. In the present study, it was found that over 70% of the PRRSV-infected pens could be detected by the pen-based oral

fluid samples when at least 40% of the pigs in the pen showed viremia. On the other hand, when the prevalence of PRRSV is low, such as in Farm A, pen-based oral fluid might be insufficient. In our study, approximately 42% of the PRRSV-infected pens (mostly from farm A) showed negative results using the pen-based oral fluid samples. However, most of these pens (approximately 80%) had low prevalence of viremic pigs (20%, 1 in 5). The detection rate of oral fluid samples in our study seems to be lower compared with other studies. De Regge and Cay (2016) demonstrated that when the serum prevalence within pens exceeded 30%, detection rate in oral fluids could reach 100%. The major reason explaining the difference

in the detection rates could be the difference of the phase of infection. The present study focused on 3- to 7-week-old piglets, representing an early phase of infection, while the other study included approximately 8-28 weeks of age and a late phase of infection. It has been shown previously that during the later phase of PRRSV infection, the virus could be found frequently more in the oral fluids (Decorte et al., 2015). Therefore, when viremia declines together with high virus shedding in oral fluids, detection rate of the oral fluid in infected pen could be higher. Our study indicated that in the early phase of infection, oral fluids could still be useful with maximum benefit when the prevalence of viremic pigs is moderate to high.

**Table 1** Number of serum and oral fluid samples positive in real-time RT-PCR for PRRSV within each sampling pen by age of pigs (weeks)

Farm	Pen	Detection of PRRSV using real-time RT-PCR by age of pigs (weeks)					
		5		7		9	
		Oral fluid	Serum	Oral fluid	Serum	Oral fluid	Serum
A	1	+	0/5	+	2/5	-	1/5
	2	+	2/5	-	1/5	-	0/5
	3	-	0/5	-	0/5	-	1/5
	4	-	0/5	-	1/5	+	0/5
	5	-	0/5	-	0/5	-	0/5
	6	-	1/5	-	0/5	-	0/5
	7	-	0/5	-	1/5	-	1/5
	8	-	0/5	-	0/5	+	2/5
	9	-	1/5	-	0/5	-	0/5
	10	+	0/5	-	0/5	-	1/5
	11	-	0/5	-	1/5	-	0/5
	12	+	1/5	+	1/5	-	0/5
B-P1	1	+	2/5	-	1/5	-	1/5
	2	+	2/5	+	2/5	-	1/5
	3	+	3/5	-	2/5	+	2/5
	4	-	3/5	+	3/5	+	2/5
	5	+	1/5	+	3/5	+	3/5
	6	+	4/5	+	4/5	-	2/5
B-P2+	1	+	1/5	+	1/5	-	1/5
	2	+	1/5	+	2/5	-	0/5
	3	+	2/5	+	2/5	+	1/5
	4	+	1/5	-	1/5	-	0/5
	5	+	1/5	-	1/5	+	2/5
	6	-	2/5	+	3/5	+	0/5

**Table 2** Percentages of PRRSV detection by modified real-time RT-PCR using pen-based oral fluid samples based on prevalence of serum PRRSV-positive pigs within a pen\*

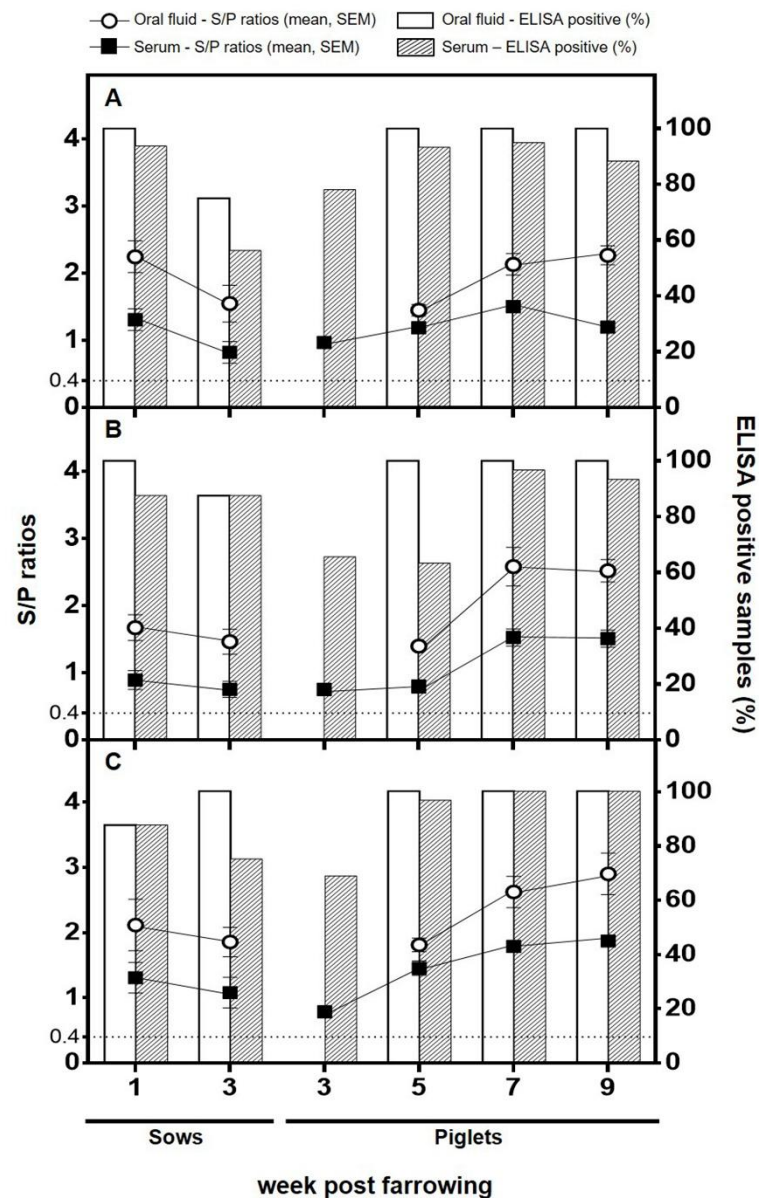
Prevalence of serum PRRSV-positive pigs within a pen (%)	PRRSV positive pen-based oral fluid samples <sup>†</sup>			
	Farm A (n = 36)	Farm B-P1 (n = 18)	Farm B-P2+ (n = 18)	Total
0	14.29% (3/21)	0	33% (1/3)	16.67% (4/24)
20 (1+)	16.67% (2/12)	25.00% (1/4)	67% (6/9)	36.00% (9/25)
40 (2+)	100.00% (3/3)	71.43% (5/7)	80% (4/5)	80.00% (12/15)
60 (3+)	NA	80.00% (4/5)	100% (1/1)	83.33% (5/6)
80 (4+)	NA	100.00% (2/2)	NA	100.00% (2/2)
100 (5+)	NA	NA	NA	NA

\* Data were evaluated based on samples from nursery periods at 5, 7, 9 weeks of age.

<sup>†</sup> NA = not available

Previous studies have already demonstrated the positive correlation of ELISA results between individual serum and pen-based oral fluid samples (Decorte et al., 2015; Kuiek et al., 2015; De Regge and Cay, 2016). Similar to those previous studies, the mean

S/P ratios in the oral fluid samples showed significantly higher levels than those in the serum samples. However, both sample types had similar pattern. The higher levels of S/P ratios in oral fluid samples were reported in association with the IDEXX PRRS Oral Fluids Ab Test (IDEXX, 2013).



**Figure 3** Mean S/P ratios of Farm A (a), Farm B-P1 (b) and Farm B-P2+ (c) in sows and their piglets from serum and oral fluid samples. Sow oral fluid and serum ELISA results are presented as the mean of individual samples. In nursery units, mean S/P ratios of oral fluid samples were expressed as pen-based results. S/P ratios of > 0.4 are considered positive.

**Table 3** Performance indices of pigs in farrowing and nursery units (mean  $\pm$  SEM)\*

Farm units	Parameters	Farm A <sup>†</sup>	Farm B	
		Mixed parity	P1	P2+
Farrowing	No. of total born/litter	11.19 $\pm$ 0.90	11.75 $\pm$ 1.15	11.75 $\pm$ 1.10
	No. of born alive litter size	10.55 $\pm$ 0.93	10.45 $\pm$ 1.03	10.80 $\pm$ 0.98
	Stillborn (%)	4.77 $\pm$ 1.83 <sup>a</sup>	10.46 $\pm$ 3.28 <sup>b</sup>	5.91 $\pm$ 1.93 <sup>a,b</sup>
	Mummies (%)	0.91 $\pm$ 0.50	0.57 $\pm$ 0.57	2.21 $\pm$ 1.28
	No. of pigs weaned/litter	9.53 $\pm$ 0.53	9.30 $\pm$ 0.50	9.54 $\pm$ 0.54
	Weaning weight (kg)	6.93 $\pm$ 0.19	7.43 $\pm$ 0.31	8.77 $\pm$ 0.18
	Pre-weaning mortality (%)	9.70 $\pm$ 2.47	11.02 $\pm$ 3.72	11.59 $\pm$ 4.04
	Lactation length (days)	25.80 $\pm$ 0.23	29.27 $\pm$ 0.22	28.13 $\pm$ 0.39
	Wean-to-first-service interval (days)	4.84 $\pm$ 0.41	5.67 $\pm$ 0.72	7.88 $\pm$ 1.87
Nursery	ADG (g)	428.00	180.00	194.00
	FCR	1.26	1.97	1.82
	FCG (THB)	29.00	43.64	40.80
	Culled and Mortality (%)	1.90	14.70	11.10

\* Statistical analyses were performed among Farm A, Farm B-P1 and Farm B-P2+ using the same parameter (within a row) in the farrowing unit with different superscript letters (a and b) ( $p < 0.05$ ).

<sup>†</sup> Nursery pigs in Farm A were from sows of mixed parity.

PRRSV antibodies in the studied sows showed a decline in S/P ratios from 1 to 3 WPF possibly due to the recovery from stress after farrowing. Generally, vertical transmission and evidence of positive weaning pigs should not be seen in the absence of new or re-infections in sows (Cano et al., 2008). However, some sows in Farm B-P1 and P2+ had S/P ratios increased at 3 WPF (25% and 37.5% in oral fluid samples; 12.5% in serum samples of both groups). Those sows might have concurrent infections causing PRRSV circulation in the farrowing unit, especially in Farm B situation. Additionally, their litters had PRRSV RT-PCR-positive (50-63%) associated with the production losses in the farrowing units compared to Farm A data. However, litters should not be used as the only sample size because the prevalence of infection within litter varies (Graham et al., 2013). The production parameters showed higher pre-weaning mortality and had higher numbers of culled sows after weaning associated with the increased levels of average wean-to-first-service interval and sows in heat by 7 days after weaning. Concurrently, high detection levels of PRRSV RNA and PRRS antibodies were found both in serum and oral fluid samples at 5 to 9 weeks of age. It could be speculated that post-weaning infection occurred concurrently with production loss and increased feed costs in nursery periods. Albina et al. (1994) has shown that maternal antibodies persisted until 4-8 weeks. Therefore, it should be speculated that confounding factors from concurrent infection with PRRSV and other diseases might affect the production performances in both Farm B-P1 and P2+ groups.

Based on the objective of this study, P0-P1 segregation and conventional management farms were chosen to determine the use of oral fluid testing for PRRSV monitoring before and after weaning. This study demonstrated that PRRSV status could be monitored using oral fluid samples for both ELISA and RT-PCR tests in case of persistent infection when having moderate to high prevalence. Unexpectedly, the detection of virus circulation and herd immunity were higher in the P0-P1 segregation than in the mixed parity management farm, corresponding with the poor production parameters. This is possible due to poor biosecurity management, lack of workers and short distance between each group of Farm B (Madec et al., 2001; Papatsiros, 2012). In addition, P0-P1 segregation needs high layer, costs and biosecurity program (Dee, 1997). These data suggested that disease prevalence surveys for monitoring disease problem is necessary in the field situation together with other parameters.

### Conclusion

The present study demonstrated that oral fluid samples could be used for monitoring PRRSV infection status for planning management strategies in both P0-P1 segregation and conventional management farms. Oral fluid testing provides better economical approach and animal welfare while being cost-effective. Oral fluid samples could be used to determine the timing of infection in sow herd and in nursery period based on results from ELISA and RT-PCR tests. However, the sensitivity of PRRSV detection is acceptable with some limitations compared to the

use of serum samples. These objectives could be better achieved and benefit practitioners by using oral fluid testing together with other measurements.

### Acknowledgements

The authors are grateful to the staff of Veterinary Diagnostic Laboratory and the graduate students in the Veterinary Pathobiology program, Faculty of Veterinary Science, Chulalongkorn University assisting in this study. This research is financially supported by the 100<sup>th</sup> Anniversary Chulalongkorn University Fund for Doctoral Scholarship and the National Research University Project, Office of Higher Education Commission (NRU-59-PPS023-HR).

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## บทคัดย่อ

### การตรวจหาสถานะของโรคพรีอาร์อาร์เอสจากตัวอย่างน้ำลายเพื่อใช้ในการวางแผนการจัดการสุกร อนุบาลในฟาร์มที่มีโรคพรีอาร์อาร์เอสเป็นโรคประจำถิ่น

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โรคพรีอาร์อาร์เอส (PRRS) เป็นโรคที่มีความสำคัญต่อระบบการเลี้ยงสุกรทั่วโลก การจัดการและการวินิจฉัยโรคที่จำเพาะจึงมีความสำคัญในการควบคุมเชื้อไวรัสพรีอาร์อาร์เอส (PRRSV) วัตถุประสงค์ของการศึกษานี้เพื่อประเมินประสิทธิภาพการใช้ตัวอย่างน้ำลายสุกรในการตรวจหาสถานะโรคพรีอาร์อาร์เอสในฟาร์มที่เป็นโรคประจำถิ่น โดยตรวจหาสารพันธุกรรมของเชื้อไวรัสพรีอาร์อาร์เอสและแอนติบอดีที่เกิดจากการติดเชื้อไวรัสพรีอาร์อาร์เอสจากตัวอย่างน้ำลายและซีรัมในช่วงคลอดจนถึงอนุบาล จากฟาร์มสุกรแบบครบวงจรในประเทศไทย จำนวน 2 ฟาร์ม (ฟาร์มเอ, conventional system และฟาร์มบี, P0-P1 segregation system) พบว่าสามารถตรวจพบสารพันธุกรรมและแอนติบอดีต่อเชื้อไวรัสพรีอาร์อาร์เอสในตัวอย่างทั้งสองชนิดที่อายุ 3 ถึง 9 สัปดาห์ และสามารถตรวจพบผลบวกมากกว่า 71% ของตัวอย่างน้ำลายรายคอก แม้ว่าสุกรในคอกให้ผลบวกจากตัวอย่างซีรัมเพียง 40% ทั้งนี้ พบว่าค่าเฉลี่ย S/P ratios จากตัวอย่างน้ำลายมีระดับสูงกว่าตัวอย่างซีรัมอย่างมีนัยสำคัญ แต่มีรูปแบบการตอบสนองที่เหมือนกัน พบการเพิ่มขึ้นของระดับแอนติบอดีต่อเชื้อไวรัสพรีอาร์อาร์เอสที่อายุ 5 จนถึง 9 สัปดาห์ในสุกรทุกกลุ่ม ในการประเมินยังพบว่าผลจากตัวอย่างน้ำลายมีความสัมพันธ์ในเชิงบวกกับตัวอย่างซีรัมอย่างมีนัยสำคัญทางสถิติที่  $r = 0.65$  ( $p < 0.001$ ) นอกจากนี้ ในฟาร์มบีพบความเสียหายในเล้าคลอดและเล้าอนุบาล ซึ่งสอดคล้องกับการพบเชื้อไวรัสพรีอาร์อาร์เอสปริมาณสูงในตัวอย่างทั้งสองชนิด วิธีการตรวจวินิจฉัยจากตัวอย่างน้ำลายเป็นวิธีที่คำนึงถึงสวัสดิภาพสัตว์ สะดวก ประหยัด และให้ผลการตรวจเป็นที่น่าเชื่อถือ สำหรับใช้สำรวจสถานะโรคพรีอาร์อาร์เอส โดยเฉพาะอย่างยิ่งในช่วงอนุบาลที่มีความชุกของโรคพรีอาร์อาร์เอสในระดับปานกลางถึงมาก สรุปได้ว่าการใช้ตัวอย่างน้ำลายสามารถให้ผลการตรวจที่ดีและเป็นประโยชน์สำหรับเกษตรกร โดยเฉพาะเมื่อใช้วินิจฉัยร่วมกับวิธีการทดสอบอื่น ๆ

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