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Research Article

# Nasal Swab– a New Tool for the Detection of Porcine Respiratory Disease Complex in Natural Infected Pigs

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## Abstract

The objectives of the present study were to determine whether nasal swab could be used for the detection of PRDC in naturally infected pigs. Fifteen piglets were used in this study. Serum, oral fluid, nasal swab, lung, tonsil and pulmonary lymph node were collected from each pig. Most common PRDC pathogens are porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae, Pasteurella multocida, Actinobacillus pleuropneumonia* (APP) were examined by nested RT-PCR and multiplex PCR. Among 15 pigs, PRRSV was detected in nasal swabs from 12 pigs, oral fluids from 11 pigs, and serum from only 3 pigs. The ability to detect PRRS virus by nasal swab could be compare with tonsil and lungs which is consider as the first choice of sample. In addition, other pathogens such as PCV2, *Mycoplasma* and APP could also be detected in nasal swab. As many pathogens cause PRDC in pigs could be detected in nasal swabs, therefore nasal swab could be considered as a preferable sample for the detection of PRDC in pigs. PRRS virus was consistently detected in nasal swab it could be explained by the replication of PRRS virus in lymphoid tissue in the nasal cavity then shed into nasal discharge.

**Keywords:** PRDC; Piglets; PRRS; Nasal Swab; Nasal Associated Lymphoid Tissue

## Abbreviations

PRDC: Porcine Respiratory Disease Complex; PRRS: Porcine Reproductive and Respiratory Syndrome; SIV: Swine Influenza Virus; PCV2: Porcine Circovirus Type 2 (PCV2); APP: *Actinobacillus pleuropneumonia*; NALT: Nasal Associated Lymphoid Tissues; TISH: Tyramide-Based *In Situ* Hybridization

## Introduction

Respiratory infection is major concern in piglets and result in substantial economic impact. Porcine respiratory disease complex (PRDC), cause by interaction of viral, bacterial, and adverse management conditions, which is characterized by respiratory symptoms and poor growth in piglets. PRDC has a multifactorial etiology, the most common isolated pathogens are porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae, Pasteurella multocida, Actinobacillus pleuropneumonia* (APP) [1,2]. PRDC can transmission via direct contact (semen, saliva, urine, milk, and faeces) or indirect rout. Factors for indirect transmission include insects, transport vehicles, and aerosols. Aerosol is considering as one of the important way for PRRS transmission. In experimental condition, PRRSV can transmitted for the distance from 0.5 to 180 m [3]. Oral fluids and nasal swabs play an important role for the transmission of diseases.

Since long ago, serum had been considered as optional specimen for detection of PRRSV. PRRSV was first detected in oral fluid in 1997 and in nasal swab in 1998 [4]. Recent studies had documented that oral fluid can replace serum sample for the detection of several disease affecting swine industry such as PRRS, PCV2 [5-7,26]. Moreover, oral fluid can be used for the detection of others pathogenic such as influenza virus, porcine circovirus. PRRSV was detected in nasal swabs from boar which inoculated intranasally with the PRRS virus [8]. In experimental condition, nasal swab had also been used for the detection of Mycoplasma hyopneumoniae [9-12], PCV2 [13] and APP [14]. The use of nasal swabs to detect the etiology of PRDC under field condition has not been study yet. Therefore, the objective of this study was to investigate the suitability of nasal swab for the detection of PRDC in natural infected piglets.

## Materials and Methods

Fifteen piglets from 60 to 90 days with respiratory problem were collected for this study. The farm had a persistent problem with respiratory diseases in weaning piglets. Clinical signs varied from sudden death to chronic pneumonia with weight loss and slow growth. The mortality rate of weaning piglets was about 15-30%. Antemortem sampling consisted of collection of whole blood in serum separation tubes, oral fluids, and nasal swabs. Nasal sam-

ples were collected by inserting a sterile swab 2 cm into each of the nostril as previously described [15]. The swab was place in a sterile plastic tube containing 1 mL phosphate buffered saline (PBS). Oral fluid was taken by absorbing in the cotton rope as described in [16,17]. PRRS RNA in sera, nasal swabs, oral fluids were extracted by QIAamp Viral RNA Mini (QIAGEN, MD, USA) in accordance with the manufacture's instruction. PRRSV was detected by nested RT-PCR. The PCR primer pairs used for nested RT-PCR were 13586F (5'-GTGGTATTTGGCAATGTGTC-3')/14652R (5'-CTCCAGGTTTC-TATGGCTGA-3') amplifying 1067bp of ORF4-6 region of PRRSV as the first RT-PCR and P420F (5'-CCATTCTGTTGGCAATTTGA-3')/ P620R (5'-GGCATATATCATCACTGGCG-3') amplify 713bp or 716bp as the second PCR [18,19]. The first RT-PCR was performed with AccessQuick™ RT-PCR System (Promega, WI, USA) and the 2nd PCR was performed with GoTaq Green Master Mix (Promega, WI, USA). DNA extraction and PCR process for PCV2, *Mycoplasma hyopneumoniae*, and *Actinobacillus pleuropneumoniae* were performed as described in previous study [20]. RNA extraction and multiplex RT-PCR to detect SIV were performed as described elsewhere [21].

To examine the pathogenesis of nasal rout infection for PRRSV, the pigs were euthanized and necropsy were performed. Nasal associated lymphoid tissues (NALT) was collected as described by Kuper [22]. The area of NALT tissues (Figure 1) were carefully separated from the bone and fixed in 4% paraformaldehyde. Lung and tonsil were also collected. After fixation tissues were processed, and embedded in paraffin by standard histologic procedures. Histopathological changes were examined by hematoxylin eosin (HE) stain. PRRSV was performed with tyramide-based in situ hybridization (TISH) using antisense cRNA probes specific for PRRSV RNA were synthesized from the ORF-7 gene sequence, as described previously [23]. Digoxigenin-labeled cRNA probes were prepared using a DIG RNA labeling Kit (Roche Diagnostics GmbH, Mannheim, Germany). ISH for PRRSV was performed according to Tanizaki [24].

## Results and Discussion

### Gross and microscopic findings

At necropsy, multifocal areas of consolidation were disseminated through the pulmonary lobes and were accompanied by edema of the interlobular connective tissue in the cranial lobes with many fibrinous adhesions to the thoracic wall. There were enlargements of the pulmonary lymph nodes. Microscopic examination of lungs reveals that most of the pigs have interstitial pneumonia and/or fibrinosuppurative bronchopneumonia. Interstitial pneumonia characterized by alveolar septal infiltration with mononuclear cells, type II pneumocyte hypertrophy and hyperplasia, and alveolar exudate consisting of mixed mononuclear cells and necrotic debris.

The nasal cavity was cut horizontally, make block and do HE staining. The NALT area were found in the circle as shown in figure 1.

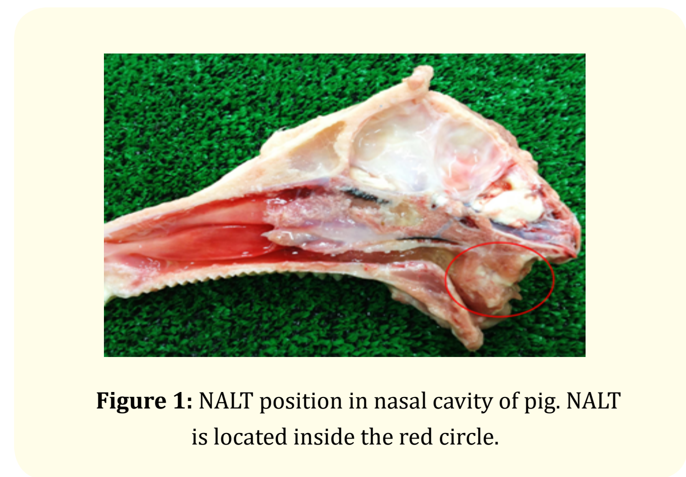

**Figure 1:** NALT position in nasal cavity of pig. NALT is located inside the red circle.

To evaluate the suitability of nasal swabs as a potential sample for diagnostic PRDC infection in pigs, sample of serum, oral fluid, nasal swabs were collected from 15 pigs before necropsy and examined for PRRSV by nRT-PCR; PCV2, APP and *Mycoplasma hyopneumoniae* by PCR. The results were described in table 1.

| Pig | Nasal swab | | | | Serum | | | | Oral fluid | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PRRS | PCV2 | Myco | APP | PRRS | PCV2 | Myco | App | PRRS | PCV2 | Myco | APP |
| 1 | + | - | + | - | - | - | - | - | + | - | - | - |
| 2 | + | - | - | + | - | - | - | - | + | - | - | |
| 3 | + | + | + | - | - | - | - | - | + | - | - | - |
| 4 | + | + | - | - | + | - | - | - | + | - | - | - |
| 5 | + | - | - | - | + | - | - | - | + | - | - | - |
| 6 | + | - | - | - | - | - | - | - | + | - | - | - |
| 7 | - | - | - | - | + | - | - | - | + | - | - | - |
| 8 | + | - | + | + | - | - | - | - | + | - | - | - |
| 9 | + | + | - | - | - | - | - | - | - | - | - | - |
| 10 | + | - | - | - | - | - | - | - | + | - | - | - |
| 11 | + | - | - | - | - | - | - | - | + | - | - | - |
| 12 | + | + | + | - | - | - | - | - | - | + | - | - |
| 13 | - | + | - | - | - | - | - | - | - | + | - | - |
| 14 | - | + | + | - | - | - | - | - | - | - | - | - |
| 15 | + | - | - | - | - | - | - | - | + | - | - | - |
| | 12 | 6 | 5 | 2 | 3 | 0 | 0 | 0 | 11 | 2 | 0 | 0 |

**Table 1:** Detection of PRDC etiology in nasal swab, serum and oral fluid samples by PCR.

Among 15 pigs, PRRSV was detected in nasal swabs from 12 pigs, oral fluids from 11 pigs, and serum from only 3 pigs. The virus was detected in 2 of 15 serum samples compare to 12 of 15 nasal swab samples. Mycoplasma and PCV2 can be detected in 5 and 6 of 15 nasal swab samples, respectively. Mycoplasma was not detected in serum and oral fluid. The PRRS virus RNA consistently detected in oral fluid, tonsil and nasal swab. Nasal swab was the most consistently detected PRRS virus then follow by oral fluid. Interestingly, serum was considered as traditional sample for PRRS but in this study only 3 of 12 PRRS positive pigs were detected in serum. It may be explained that these pigs are not at early stage of infection.

Traditionally, tonsil, lung and pulmonary lymph node were considered as perfect sample for detection of the PRDC pathogen [3,6,11,16]. Therefore, in this study tonsil, lung and pulmonary lymph node also collected and examined for the PRDC etiology to compare with the antemortem sample such as nasal swab, serum, oral fluid. The results were described in table 2.

| Pig | Tonsil | | | | Lung | | | | Pulmonary lymph node | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PRRS | PCV2 | Myco | App | PRRS | PCV2 | Myco | App | PRRS | PCV2 | Myco | App |
| 1 | + | - | - | - | + | - | + | - | + | - | - | - |
| 2 | - | - | - | - | - | - | - | + | - | - | - | - |
| 3 | + | + | - | - | + | + | - | - | + | + | - | - |
| 4 | + | + | - | - | + | + | - | - | + | + | - | - |
| 5 | + | + | - | - | + | + | - | - | + | + | - | - |
| 6 | + | - | - | - | + | - | - | - | + | - | - | - |
| 7 | + | + | - | - | + | - | - | - | + | - | - | - |
| 8 | + | - | - | - | + | - | - | - | + | - | - | - |
| 9 | + | + | - | - | - | + | - | - | - | + | - | - |
| 10 | + | + | - | - | + | + | - | - | + | + | - | - |
| 11 | + | + | - | - | + | - | - | - | + | + | + | - |
| 12 | - | + | - | - | + | + | - | - | - | + | + | - |
| 13 | - | + | - | - | - | + | - | - | + | + | + | - |
| 14 | + | - | - | - | + | - | + | - | - | - | - | - |
| 15 | + | - | - | - | + | - | - | - | + | - | - | - |
| | 12 | 9 | 0 | 0 | 12 | 7 | 2 | 1 | 11 | 8 | 3 | 0 |

**Table 2:** Detection of PRDC etiology in tonsil, lung and pulmonary lymph node.

PRRS virus was detected in tonsils and lungs of 12 among 15 necropsy piglets. PCV2 and mycoplasma were able to detect in tonsil, lung and pulmonary lymph node. The result shown that both viruses and bacterial can be detected in nasal swab samples therefore, nasal swab could be alternative to serum, oral fluid as well as the necropsy tissue such as lung, tonsil, lymph node for the detection of PRDC. Therefore, nasal swab is potential sample for the detection of both bacterial and virus causing PRDC in pigs.

### Pathogenesis of nasal infection

In order to examine the pathogenesis of PRRS by nasal infection rout the NALT were collected and make the serial section, one for HE staining and one for TISH. Picture of NALT ISH showed positive at the area of the lymphoid tissue (Figure 2 and Figure 3).

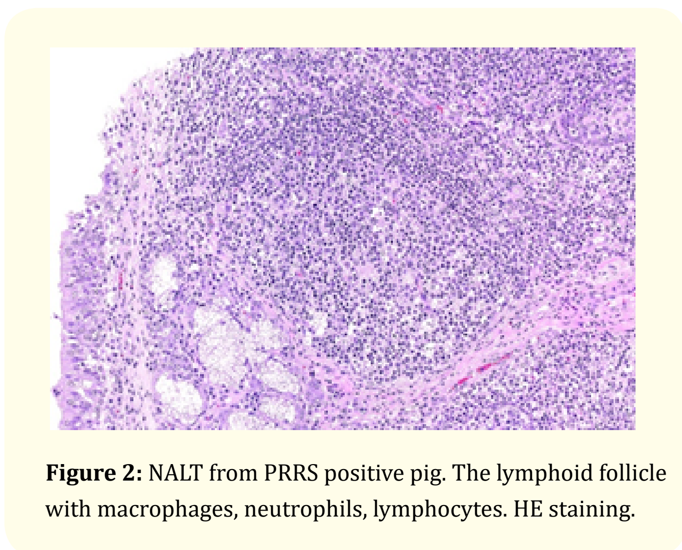

**Figure 2:** NALT from PRRS positive pig. The lymphoid follicle with macrophages, neutrophils, lymphocytes. HE staining.

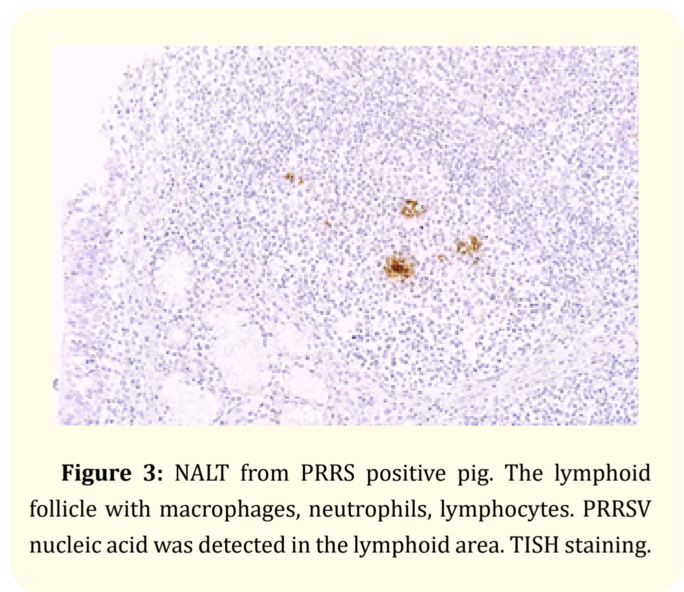

**Figure 3:** NALT from PRRS positive pig. The lymphoid follicle with macrophages, neutrophils, lymphocytes. PRRSV nucleic acid was detected in the lymphoid area. TISH staining.

Respiratory tract is a common replication site of many viruses and bacterial [25,26]. The results suggested that PRRS virus was replicated in the lymphoid tissue in the nasal cavity and the virus was spread to the nasal discharge.

## Conclusions

As many pathogens cause PRDC in pigs could be detected in nasal swabs, therefore nasal swab could be an alternative choice of sample for the detection of PRDC in pigs. PRRS virus detected in nasal swab could be explain by the virus first replicated in the lymphoid tissue in the nasal cavity then shed virus to the nasal discharge.

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## Conflict of Interest

The authors declared that there is no conflict of interest.

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