CO-INFECTION OF PRRSV, PCV2 AND PASTEURELLA MULTOCIDA IN A PIG FARM AT RACHABURI PROVINCE OF THAILAND

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ABSTRACT

This report aims to illustrate co-infection of important pathogens in pigs and affected to herd health status of a farm located at Rachaburi province of Thailand. The farm has capacity about 700 sows with 8,000 fattening pigs. Fattening pigs in the farm showed unsatisfied growth performance as well as ununiform size appearance. To investigate the health problem occurred with the farm, blood sampling was carried out from 4 finishers in an accumulated ununiform pen. Necropsy was conducted from a dead pig of the same pen for further diagnosis by Real-Time polymerase chain reaction (Real-Time PCR) and bacterial culture. Serum was tested by Enzyme Linked Immunosorbent Assay (ELISA). The obtained results from ELISA diagnosis revealed that high antibody response to Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) found in all serum samples (S/P ratio ≥ 0.4) which can be interpreted that PRRSV infection raised in this farm. The result from lymph node samples showed positive Porcine Circovirus type 2 (PCV2) detection by Real-Time PCR. Bacterial culture from lung sample found infected by Pasteurella multocida. Overall, it was determined that mix of infection between viruses and bacteria occurred in this farm, PRRSV, PCV2 and Pasteurella multocida. This report is illustrated that the mutuality of infection could breakthrough acquire immunity from vaccination, affected to health status and production of the farm.

Keywords: Co-infection, Pasteurella multocida, PCV2, PRRSV

INTRODUCTION

In Thailand, the commercial pig production system started since 1960s when the first commercial pig breeds were imported from the United Kingdom by the Department of Livestock Development (DLD) and then from the United States by Kasetsart University. In the present, pig farming has changed aims for increasing in production yield. The production systems transform from extensive, small-scale systems towards intensive and integrative large-scale as well as introduction of modern technologies and farm management (Thanapongtharm et al., 2016). Under intensive system, there has been concerned about animal stress on due to the undesirable consequences that stress produces in the normal physiology of pigs and its effects on their welfare and general productive performance. The most important types of stress including social, environmental, metabolic, immunological and due to human handling
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(Martínez-Miró et al., 2016), lead to diseases emerge and cause endemic in farms. The diseases usually circulate in farm and cause of increasing of cost for biosecurity system as well as drug and vaccines application intensively.

This report aims to illustrated that the mutuality of infection with insidious pathogens, viruses and bacteria which can be possible occurred in the farm that has unsatisfied biosecurity system. The conspire of infection can harmful to herd health status and affected to production efficiency.

MATERIALS AND METHODS

Clinical History

A commercial pig farm located at Rachaburi province of Thailand, has production capacity about 700 sows which can be produce around 8,000 fattening pigs. Pigs raise in the stall with opened air system. Feed and water were provided ad libitum. In the aspect of biosecurity, no specific disease preventing system was observed. There is no any disinfection procedure prior access to the production area of the farm.

The pigs in finishing unit showed the signs of emaciation, ruffle hair with skinny appearance as well as coughing found in some pigs. The pigs have unsatisfied growth performance as well as ununiform size occurred.

Figure 1. Pig in the finishing unit showed ununiform size (A), piglet had ataxia and incoordination signs (B).

Vaccination program for sow units was performed by mass vaccination method, including vaccines against Porcine Epidemic Diarrhea (PED) and Foot and Mouth Disease (FMD). While vaccines against Porcine Reproductive and Respiratory Syndrome (PRRS), Classical Swine Fever (CSF), Porcine Circovirus type 2 (PCV2), Mycoplasma hyopneumoniae (MH), Aujeszky ’s Disease (AD) and FMD were applied for fattening pigs (Table 1).
Table 1. The vaccination program of sow and fattening pigs of the farm.

<table>
<thead>
<tr>
<th>Vaccines</th>
<th>Sow Age at vaccination time</th>
<th>Fattening Age at vaccination time</th>
</tr>
</thead>
<tbody>
<tr>
<td>PED</td>
<td>Every 4 months</td>
<td>-</td>
</tr>
<tr>
<td>FMD</td>
<td>Every 6 months</td>
<td>8 weeks</td>
</tr>
<tr>
<td>PRRS</td>
<td>-</td>
<td>2 weeks</td>
</tr>
<tr>
<td>CSF</td>
<td>-</td>
<td>3 weeks</td>
</tr>
<tr>
<td>PCV2</td>
<td>-</td>
<td>4 weeks</td>
</tr>
<tr>
<td>MH</td>
<td>-</td>
<td>4 weeks</td>
</tr>
<tr>
<td>AD</td>
<td>-</td>
<td>5 weeks</td>
</tr>
</tbody>
</table>

PED = Porcine Epidemic Diarrhea, FMD = Foot and Mouth Disease, PRRS = Porcine Reproductive and Respiratory Syndrome, CSF = Classical Swine Fever, PCV2 = Porcine Circovirus type 2, MH = Mycoplasma hyopneumoniae, AD = Aujeszky’s Disease

Sampling and Tissue Collection

The uniform pigs in this farm were collected and kept into a same house, which are divided into several pens. To investigate the health problem occurred with the farm, one among these pens was selected for diseases investigation, blood sampling was carried out from 4 pigs in the same pen (assigned as sample number 001, 002, 003, 004). On the other hand, blood collecting was also performed at another house, from totally 6 weaned piglets, three piglets from 4-week-old group and three piglets from 7-week-old group. The collected blood was stored in ice box then delivered to laboratory.

Necropsy was conducted from a dead pig found in the same group of blood collection. The tissue collection was taken from several organs including lung, spleen, kidney, mesenteric lymph node and mediastinum lymph node. Each sample was stored separately in plastic bags then kept in ice box for further diagnosis.

Laboratory Diagnosis

Blood and tissue samples were sent to Betagro Science Center Co., Ltd., a private sector which served for laboratory diagnosis, located at Patumthani province, Thailand. Serum was separated from blood and subjected for antibody against PRRS virus (PRRSV) detection by Enzyme Linked Immunosorbent Assay (ELISA) using IDEXX PRRS X3 Ab Test (IDEXX®, USA) regarding to manufacturer’s protocol. The mesenteric and mediastinum lymph node were pooled and subjected for tested by Real-Time polymerase chain reaction (Real-Time PCR) method regarding to laboratory protocol (undisclosed). The protocol for PRRSV testing by ELISA and Real-Time PCR for PCV2 detection were accredited by National Bureau of
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Agricultural Commodity and Food Standards (ACFS) and Department of Medical Sciences (DMSc) of Thailand, accreditation number are AC 07-29-9999-0045-000 and 1092/49 respectively. The lung tissue was conducted for bacterial culture regarding to laboratory protocol (undisclosed). The serum incurred from weaned group was further tested by serum neutralization using neutralizing peroxidase-linked antibody (NPLA) assay (Terpstra et al. 1984) against swine fever virus ALD strain.

RESULTS AND DISCUSSION

Serological Test

The obtained result from ELISA diagnosis showed in term of sample to positive ratio (S/P ratio) which high value of antibody against PRRSV was illustrated, could be interpreted that PRRSV infection raised in this group (Table 2).

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>S/P ratio</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>2.629</td>
<td>Positive</td>
</tr>
<tr>
<td>002</td>
<td>1.381</td>
<td>Positive</td>
</tr>
<tr>
<td>003</td>
<td>2.54</td>
<td>Positive</td>
</tr>
<tr>
<td>004</td>
<td>0.443</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*Reference report number 180000254285

S/P ratio ≥ 0.4 = positive, < 0.4 = negative

The result obtained from NPLA assay evinced that the geometric mean titer (GMT) of the weaned group fall in the normal range of antibody response of vaccinated pig (Direksin et al. 2016) higher in 4-week-old than 7-week-old (Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>SN-Titerb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>4 weeks</td>
<td></td>
</tr>
<tr>
<td>7 weeks</td>
<td>1</td>
</tr>
</tbody>
</table>

*Reference report number 180000257800

bSN-Titer = Serum Neutralization Titer using neutralizing peroxidase-linked antibody (NPLA) assay

cGMT = Geometric Mean Titer
Molecular and Bacterial Diagnosis

The results from pooled lymph node showed positive PCV2 detection by Real-Time PCR. While bacterial culture from lung sample found infected by *Pasteurella multocida* (Table 4).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Methods of diagnosis</th>
<th>Result</th>
<th>Reference report No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph nodes</td>
<td>Real-Time PCR</td>
<td>PCV2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180000254287</td>
</tr>
<tr>
<td>Lung</td>
<td>Bacterial culture</td>
<td><em>Pasteurella multocida</em></td>
<td>180000255236</td>
</tr>
</tbody>
</table>

<sup>a</sup>PCV2 = Porcine Circovirus type 2

Respiratory disease in pigs is common in modern pig farming and is often referred to as porcine respiratory disease complex (PRDC). PRDC results from infection with various combinations of primary and secondary respiratory pathogens. There is multifactorial disease caused by environmental conditions, population size, management system, genetics etc. also play critical roles in the outcome of PRDC. There are several viral and bacterial pathogens commonly associated with PRDC including PRRSV, swine influenza virus (SIV), PCV2, *Mycoplasma hyopneumoniae* and *Pasteurella multocida* (Opriessnig *et al.*, 2011).

Generally, the vaccination program in pigs may difference in each farm especially in Thailand, depending to the health status and diseases condition and location of each farm. In the present case, vaccination program of sow containing not much kinds of vaccines, while tight vaccination program was observed in piglet especially before weaning. However, the obtained results showed that 3 pathogens found together in investigated pigs. The PRRSV is crucial for pig’s production industry. Many areas in Thailand have this virus circulates in the farms (Nilubol *et al.*, 2012; Thanapongtharm *et al.*, 2014; Olanratmanee *et al.*, 2015; Poonsuk *et al.*, 2016). Hence the vaccine against this virus was determined for using including the present farm. However, even the vaccine was included in the program, PRRSV was still challenged in the farm, as investigated by ELISA test with positive result.

Same as PRRSV, PCV2 has recently become an important pathogen across the world including in Thailand. It has the highest evolution rate among DNA viruses (Karuppannan & Opriessnig, 2017) Thus vaccine plays important role for this disease controlling. As mention above, PCV2 is one of PRDC pathogens possible causes respiratory disease. Thus, the lymph nodes therefore asked for PCV2 identification by Real-Time PCR. While the lung
tissue was further worked by bacterial cultured. The PCV2 identification was reported as positive and *Pasteurella multocida* was found by bacterial culture.

In this farm, the PRRSV found as co-infection with PCV2. The PRRSV infection followed by PCV2 infection enhanced the replication of both viruses, led to more severe clinical signs and lesions, indicating greater synergistic effects during the sequential infection (Fan *et al.*, 2013).

On the other hand, the serum was also random collected from weaned population. It was subjected for ruled out the causative of this health status, whether classical swine fever was involved in this problem or not. The result indicated that no interference evidence with immunity response to classical swine fever which can be interpreted that classical swine fever virus (CSFV) may not challenge in the farm, at least in the weaned group. Although the result of ununiform size group illustrated that PRRSV and PCV2 challenged in this farm, but it was no effected with antibody response to swine fever after vaccination, this is in accordance with the report of Lim *et al.* (2016) which illustrated that co-infection with PRRSV/PCV2 may affect the replication or activity of the CSF vaccine virus in pigs vaccinated with the LOM strain (as the strain used in this farm), but CSFV antibody levels were not negatively affected. However, according to Huang *et al.* (2011) which reported that PCV2 infection can decrease the efficacy of classical swine fever vaccine, but no interference effect found in weaned group of this farm. It is possible due to the vaccination program which applied when the percentage of viremic pigs was minimal, triggering an effective humoral immune response before the peak of infection (Oliver-Ferrando *et al.*, 2016).

The infection of *Pasteurella multocida* can be found in case of PRDC as mentioned. This bacterial frequently isolated from the lungs of pneumonic pigs and is thought to play a central role in porcine pneumonic pasteurellosis. This bacterium is one of most frequently identified specie, also high association with respiratory diseases in pigs (Choi *et al.*, 2003).

Although the challenging of PRRSV in farm can possible facilitate secondary infection. But the role of PRRSV as initiator of secondary diseases still undefined (Carvalho *et al.*, 1997) as the result in this report could not indicate that the co-infection was caused by PRRSV.

In the aspect of biosecurity, it is crucial for diseases prevention in all livestock farms. This farm lack for this basic theory. There is no any disinfection procedure prior to enter to the farm. This condition facilitates pathogens penetrate to this farm easily. Although there are many
vaccines used in this farm, but the mix of infections and high pathogens load can affect to health status of the farm.

CONCLUSIONS

Overall, the result from laboratory diagnosis revealed that the farm was infected with at least 3 pathogens. It was determined that there are mix of infection between viruses and bacteria occurred, PRRS, PCV2 and Pasteurella multocida. This report is illustrated that the mutuality of infection could breakthrough acquire immunity from vaccination, affected to health status and production of the farm.

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