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Coexistent infection event of porcine reproductive and respiratory syndrome virus, porcine circovirus type 2 and *Pasteurella multocida* in swine

Thammakarn, C.[1*], Pham, H. S. H.[2], Kaewhom, P.[3], Klompanya, A.[4] and Srikijkasemwat, K.[1]

[1]Department of Animal Production Technology and Fisheries, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, 1 Chalongkrung Rd., Ladkrabang, Bangkok 10520, Thailand; [2]Faculty of Animal Science and Veterinary medicine, Hue University of Agriculture and Forestry-Hue University, Thua Thien Hue 0234, Vietnam; [3]Faculty of Agricultural Technology, Burapha University, Sakaeo Campus, Sa Kaeo 27160, Thailand; [4]Public-Private Collaborative Research Center, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, 1 Chalongkrung Rd., Ladkrabang, Bangkok 10520, Thailand.

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Abstract Result elucidated the instances of concurrent infections by crucial pathogens in pigs, which is adversely impacted the herd's health status on a farm located in the Rachaburi province of Thailand. The farm which accommodated a population of 700 sows had found its fattening pig units exhibited unsatisfactory growth performance and an inconsistent size appearance. The serum samples underwent the testing via enzyme linked immunosorbent assay, unveiled a substantial antibody response to the porcine reproductive and respiratory syndrome virus (PRRSV). The finding strongly indicated the prevalence of PRRSV infection within the farm. Furthermore, analysis of lymph node samples confirmed the presence of porcine circovirus type 2 (PCV2) through conventional polymerase chain reaction. The bacterial culture carried out on lung samples which detected an infection caused by *Pasteurella multocida*. In summary, the investigation is conclusively established the occurrence of a mixed infection involving both viruses and bacteria on this farm, specifically PRRSV, PCV2, and *P. multocida*. The report is illustrated the possibility of multiple infections by viruses and bacteria, which can potentially undermine the efficacy of vaccinations and cause severe clinical signs on the farm.

Keywords: *Pasteurella multocida*, PCV2, Pig, PRRSV

Introduction

Thailand's pig production industry has shifted its emphasis towards enhancing production yields. This transformation involves a shift from small

[*]**Corresponding Author**: Thammakan, C.; **Email**: chanathip.th@kmitl.ac.th

scale and extensive operations to large scale and intensive farming, accompanied by the adoption of new business concepts. (Thanapongtharm *et al*., 2016). In the current farming system, there is an increasing concern regarding the stress experienced by animals. This concern arises from the adverse effects of stress on their well-being and overall productivity. Various types of stress are significant, including social stress, environmental stress, metabolic stress, immunological stress, and stress induced by human handling (Martínez-Miró *et al*., 2016).

The intensive farming system often leads to the emergence of diseases, which can become endemic on farms. The primary causative agents of pig diseases continue to be predominantly viruses and bacteria. Crucial viral diseases such as porcine reproductive and respiratory syndrome (PRRS), porcine circovirus type 2 (PCV2), classical swine fever (CSF), foot and mouth disease (FMD), and african swine fever (ASF) remain major concerns and may lead to secondary infections with certain bacteria, exacerbating their severity.These diseases typically circulate within the farm, resulting in increased costs associated with implementing biosecurity measures and the intensive use of drugs and vaccines. The objective was to illustrate the incidence of co-infection by insidious pathogens that can occur in swine farms.

Materials and methods

History taking and clinical findings

A pig farm situated in Rachaburi province, Thailand, had a capacity of approximately 700 sows, yielding approximately 8,000 fattening pigs. The pigs are raised in open-air stalls, with *ad libitum* access to feed and water. In terms of biosecurity, there appears to be no specific disease prevention system in place. Furthermore, no stringent biosecurity and disinfection procedures were observed before entering the production unit. In the finishing unit, pigs exhibited clinical signs of emaciation and ruffled hair. Additionally, coughing was observed in some of the pigs. These animals displayed unsatisfactory growth performance, accompanied by irregular size variations. The pig vaccination program adopted a comprehensive approach, incorporating both mass vaccinations and age-specific protocols. This program included vaccines against PRRS, CSF, PCV2, Pseudorabies, FMD and Porcine epidemic diarrhea (PED) and *Mycoplasma hyopneumoniae* (MH).

Table 1. Vaccine and vaccination schedule utilized on the farm

Pig types	Vaccines	Age at vaccination
Boar	Foot and mouth disease	Every 6 months
Sow	Foot and mouth disease	Every 6 months
	Porcine epidemic diarrhea	Every 4 months
Suckling-Finishing	Porcine reproductive and respiratory Syndrome	2 weeks
	Classical swine fever	3 weeks
	Porcine circovirus type 2	4 weeks
	Mycoplasma hyopneumoniae	4 weeks
	Pseudorabies	5 weeks
	Foot and mouth disease	8 weeks

Blood and tissue sampling

In the finishing unit, the non-uniform pigs on were gathered and housed together in a same pen. Blood sampling was performed on four non-uniform pigs, labeled as samples P1, P2, P3, and P4. Additionally, blood collection was also carried out in another housing unit. A total of six weaned piglets were sampled, with three piglets from each 4- and 7-week-old group. A necropsy was performed on a deceased pig. Tissue samples were collected from lung, mesenteric and mediastinum lymph node. All samples were individually stored in kept in icebox for subsequent diagnosis.

Laboratory diagnosis

The blood samples were processed to separate the serum and then subjected to antibody detection against the PRRS virus (PRRSV) using enzyme-linked immunosorbent assay (ELISA), specifically the PRRS X3 Ab kit (IDEXX, USA), following the kit's instruction. Furthermore, the serum collected from the weaned group underwent additional testing through the virus neutralization process, employing the neutralizing peroxidase-linked antibody assay (NPLA), as described by Terpstra *et al*. (1984), to detect antibodiy to classical swine fever virus (CSFV).

The pooled sample of mesenteric and mediastinum lymph nodes was utilized for PCV2 detection. DNA extraction was carried out using the Isolate II Genomic DNA kit (BIOLINE, UK), followed by subsequent processing through conventional polymerase chain reaction (PCR). The primers p1 (5'-CAC GGA TAT TGT AGT CCT GGT-3') and p2 (5'-CGC ACC TTC GGA TAT ACT GTC-3'), as detailed by Cao *et al*. (2005), were employed for this purpose.

The lung tissue was dispatched to a private sector diagnostic center (Betagro Science Center Co., Ltd.), situated in Patumthani province, Thailand, Subsequently, they underwent bacterial culture following the laboratory's protocol.

Results

Serological diagnosis

The results obtained from the ELISA were presented in terms of sample-to-positive (S/P) ratio, which ranged from 0.443 to 2.629. These values indicate a seroconversion of antibodies against PRRSV. This finding suggests the presence of a PRRSV challenge within this group, as illustrated in Figure 1.

The results obtained from the NPLA assay of the weaned groups indicated that the geometric mean titer (GMT) was within the expected range of antibody responses typically observed in vaccinated pigs, consistent with the findings of Direksin *et al*. (2016). Remarkably, the GMT was higher in the 4-week-old group (25.39) when compared to the 7-week-old group (16.00), as detailed in Table 2.

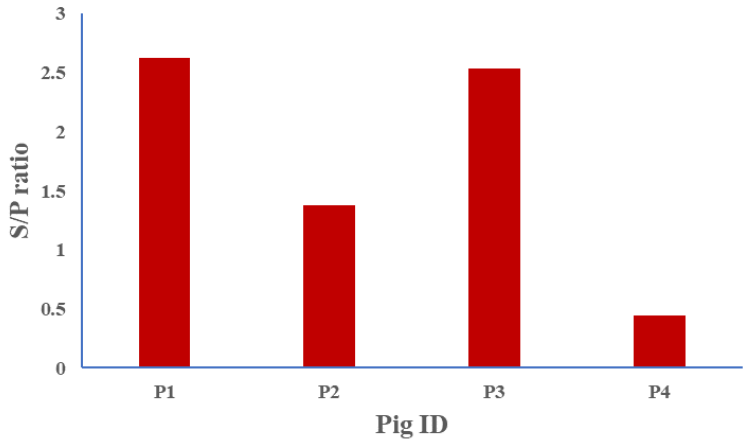

Figure 1. The individual antibodies against PRRSV in four non-uniformly sized pigs were evaluated using ELISA and are presented in terms of the S/P ratio

The pigs assigned the numbers P1, P2, P3, and P4, exhibited S/P ratios of 2.629, 1.381, 2.54, and 0.443, respectively. The interpretation criteria are in line with the manufacturer's reference, where an S/P ratio ≥ 0.4 indicates a positive antibody response, while an S/P ratio of < 0.4 signifies sero-negativity.

Table 2. Virus neutralization titers against CSFV in weaned piglets

Age of piglets	Number of pigs counted in each virus neutralization titer									
	2	4	8	16	32	64	128	256	Mean	GMT
4 weeks	-	-	1	-	1	1	-	-	4.67	25.39
7 weeks	1	-	1	-	-	-	-	1	4.00	16.00

GMT = Geometric mean titer

Molecular diagnosis of PCV2 detection

The results obtained from the pooled lymph node samples revealed a detected band at 494 base pairs (bp), which corresponds to the ORF2 gene of PCV2. These results confirm the presence of PCV2 in the lymph nodes.

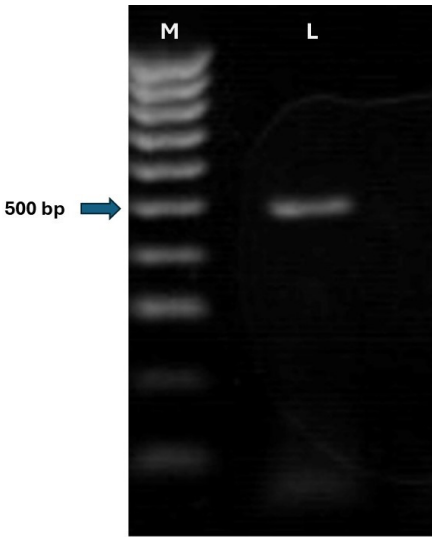

Figure 2. The PCR detection results unveiled a 494 bp PCR product on the agarose gel. In the gel image, "M" represents the 100 bp DNA ladder, while "L" denotes the pooled lymph node samples

Bacterial culture from tissue samples

Bacterial culture from the lung sample revealed an infection with *Pasteurella multocida* (Reference report number: 180000255236).

Discussion

Respiratory diseases (commonly known as porcine respiratory disease complex; PRDC) are frequently encountered in commercial pig farms. This disease results from concurrent infections involving primary and secondary

respiratory pathogens, including swine influenza virus (SIV), PRRSV, PCV2, MH, and *Pasteurella multocida*, which can be influenced by various factors such as environmental conditions, population size, management systems, genetics, and other contributing factors. Several pathogens including virus and bacteria are associated with PRDC. (Opriessnig *et al*., 2011).

The schedule for vaccination typically varies among farms, especially in Thailand, depending on factors such as the farm's health status, disease conditions, and location. In this specific case, the sow vaccination program included a limited range of vaccines, while a more comprehensive vaccination was observed for piglets. However, the results obtained indicated the presence of three pathogens in the investigated pigs. Among these, PRRSV holds significant importance in the pig production industry. Many areas in Thailand have reported the circulation of PRRSV in farms (Thanapongtharm *et al*., 2014; Olanratmanee *et al*., 2015; Poonsuk *et al*., 2016). Consequently, a PRRS vaccine is incorporated into the vaccination program, even though PRRSV still posed a challenge on the farm, as evidenced by a positive result in the ELISA test.

Similar to PRRSV, PCV2 has emerged as a significant pathogen globally, including in Thailand (Jantafong *et al*., 2011; Jittimanee *et al*., 2011; Thangthamniyom *et al*., 2017; Thammakarn *et al*., 2022). It boasts one of the highest evolutionary rates among DNA viruses (Karuppannan and Opriessnig, 2017). Consequently, vaccines play a pivotal role in controlling this disease. As mentioned earlier, PCV2 is one of the pathogens associated with PRDC (Ellis *et al*., 2000). Therefore, the lymph nodes were subjected to PCV2 identification through PCR, while the lung tissue underwent bacterial culture. A positive result was obtained from PCV2 identification, while *Pasteurella multocida* was isolated from lung tissue. In this instance, PRRSV was identified as coexisting with PCV2, leading to increased severity. This observation underscores the enhanced synergistic effects during infections (Fan *et al*., 2013).

In addition, serum samples were randomly collected from the weaned pigs to investigate whether CSF might have been a factor in the observed health issues. The results indicated no discernible interference with the immune response to CSF. This suggests that CSFV was not challenged on the farm, at least within the weaned population. While the results from the non-uniform size group indicated the presence of PRRSV and PCV2 challenges on the farm, these infections did not appear to affect the antibody response to CSFV following vaccination. This corresponds with Lim *et al*. (2016), which suggested that both PRRSV and PCV2 might influence the replication of the virus contained in CSF vaccine, but CSF antibody was not adversely affected. Nevertheless, it is worth noting that Huang *et al*. (2011) reported that the infection of PCV2 could reduce the efficacy of the CSF vaccine, although no interference was observed in the

weaned pigs. This variation could be attributed to the timing of vaccination, which likely occurred before the infection with PRRSV and PCV2. (Oliver-Ferrando *et al*., 2016). The presence of *Pasteurella multocida* infection often observed in the case of pneumonia, also strongly associated with respiratory problems (Choi *et al*., 2003). While the PRRSV can potentially facilitate secondary infections. However, this role remains uncleared (Carvalho *et al*., 1997).

In terms of biosecurity, maintaining robust measures is important for disease prevention. However, this particular farm appears to lack these fundamental principles. Notably, there is no disinfection procedure in place before entering the farm, which facilitates the easy penetration of pathogens. Despite the use of numerous vaccines, the combination of infections with a high pathogen load has affected the overall health status of the farm.

In conclusion, the results from serological examination, molecular diagnosis, and bacterial culture have conclusively demonstrated that the farm was infected with a minimum of three pathogens. It has been established that a combination of infections involving both viruses and bacteria has presented, specifically PRRS, PCV2, and *Pasteurella multocida*. The results underscore the significance of understanding how co-infections can potentially compromise the effectiveness of vaccination efforts, consequently impacting the health status and productivity of the farm.

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