Original Article

Efficacy of a Chimeric PCV2 Vaccine: a Field Trial

Termsitthi Paphavasit[1] Philip Lehrbach[2] Wichian Navasakuljinda[2]
Roongtham Kedkovid[1] Sitthichok Lacharoje[1] Roongroje Thanawongnuwech[1]
Komkrich Teankum[1*]

Abstract

Field trial of a Chimeric porcine circovirus type 2 (PCV2) (Suvaxyn PCV2®, Fort Dodge Animal Health, USA) vaccine was performed in order to investigate the efficacy on decreasing pathological lesions and PCV2 viremia in a PCV2-affected swine herd in Thailand. A herd was selected based on history and serological data. Three-week-old pigs (n = 200) were equally divided into two groups. At 4 weeks old, the pigs (n = 100) were injected intramuscularly with 2 ml of Suvaxyn PCV2® vaccine and the non-vaccinated pigs were injected with 2 ml of normal saline. Sera were collected from 20 pigs per group at the age of 4, 5, 7, 9, 12 and 15 weeks old for assessing PCV2-specific antibodies and PCV2 genetic material by serology and polymerase chain reaction (PCR) tests. Results of PCV2 specific antibodies revealed high titers at 4 weeks of age and declined at 5-7 weeks old in both groups indicating the maternal derived antibodies. Seroconversion was observed in the vaccinated pigs at 9 weeks of age suggesting antibody response to vaccination. In the non-vaccinated pigs, PCV2 seroconversion was detected at 12 weeks, indicating antibody response to natural infection. The PCV2 genetic material was not detected in the vaccinated pigs before 15 weeks of age, while PCV2 DNA was present in the sera of the non-vaccinated pig at all time points. Postmortem examination (n = 20) at 16 weeks of age revealed that the histopathological lesions of the lymph nodes of the vaccinated pigs were less severe than those of the non-vaccinated pigs. The average of lymph node/body weight ratio in the vaccinated pigs was lower than those in the non-vaccinated pigs, but was not statistically significant. The results suggest that Suvaxyn PCV2® was able to induce PCV2-specific antibodies and subsequently reduced PCV2 viremia and PCV2-associated pathological lesions.

Keywords : chimeric vaccine, efficacy, lesion, porcine circovirus type 2, viremia

[1]Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330 Thailand
[2]Fort Dodge Animal Health, Thailand
[*]Corresponding author: E-mail : komkrich.t@chula.ac.th

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บทคัดย่อ

ประสิทธิภาพของไคเมอริควัคซีนป้องกันโรคเซอร์โคไวรัสในสุกรในภาคสนาม

เติมสิทธิ ปภาวสิทธิ์[1] ฟิลลิป แลร์บาค[2] วิเชียร นวสกุลจินดา[2] รุ่งธรรม เกษโกวิท[1] สิทธิโชค ลาชโรจน์[1]
รุ่งโรจน์ ธนาวงษ์นุเวช[1] คมกฤช เทียนคำ[1*]

การทดลองใช้ไคเมอริควัคซีน (Suvaxyn PCV2® บริษัท ฟอร์ท ดอดจ์ แอนิมัล เฮ็ลธ์ อเมริกา)ป้องกันโรคเซอร์โคไวรัสในสุกรในภาคสนามได้จัดทำขึ้นเพื่อทดสอบประสิทธิภาพของวัคซีน ในการลดรอยโรคทางพยาธิวิทยาและการติดเชื้อในกระแสเลือดในฟาร์มสุกรที่พบปัญหาการติดเชื้อเซอร์โคไวรัสในประเทศไทย โดยทำการคัดเลือกฟาร์มสุกรโดยอาศัยประวัติของฟาร์มและข้อมูลทางซีรัมวิทยา นำลูกสุกรหย่านมอายุ 3 สัปดาห์ จำนวน 200 ตัวเข้าสู่การทดลอง โดยแบ่งออกเป็น 2 กลุ่มเท่าๆ กัน เมื่ออายุ 4 สัปดาห์ ทำการฉีดวัคซีน Suvaxyn PCV2® ปริมาณ 2 มล. เข้ากล้ามเนื้อในขณะที่สุกรกลุ่มควบคุมได้รับการฉีดน้ำเกลือในปริมาณเท่ากัน จากนั้นเก็บตัวอย่างซีรัมจากสุกร กลุ่มละ 20 ตัว ในช่วงอายุ 4 5 7 9 12 และ 15 สัปดาห์ เพื่อศึกษาทางซีรัมวิทยาและตรวจหาเชื้อเซอร์โคไวรัสชนิดที่ 2 โดยวิธีปฏิกิริยาลูกโซ่โพลิเมอร์เรส จากผลการทดลองพบระดับการตอบสนองทางภูมิคุ้มกันต่อเชื้อเซอร์โคไวรัส ชนิดที่ 2 ในสุกรทั้งสองกลุ่มสูงขึ้นในช่วงอายุ 4 สัปดาห์และลดลงในช่วง 5 ถึง 7 สัปดาห์ ซึ่งอาจเป็นผลจากการลดลงของภูมิคุ้มกันที่ถ่ายทอดจากแม่สุกร แต่เมื่อสุกรมีอายุ 9 สัปดาห์สุกรกลุ่มที่ได้รับวัคซีนมีการตอบสนองของภูมิคุ้มกันต่อเชื้อเซอร์โคไวรัสชนิดที่ 2 ในระดับที่สูงขึ้นซึ่งน่าจะเป็นผลมาจากการได้รับวัคซีน ในสุกรที่ไม่ได้รับวัคซีนจะมีการตอบสนองของระดับภูมิคุ้มกันที่ 12 สัปดาห์ซึ่งบ่งบอกถึงการติดเชื้อโดยธรรมชาติ ผลการตรวจหาเชื้อเซอร์โคไวรัสชนิดที่ 2 ในซีรัมพบว่าสุกรกลุ่มที่ได้รับวัคซีนตรวจไม่พบเชื้อเซอร์โคไวรัสชนิดที่ 2 จนกระทั่งอายุ 15 สัปดาห์ แต่ในสุกรที่ไม่ได้รับวัคซีนกลับตรวจพบเชื้อเซอร์โคไวรัสชนิดที่ 2 ได้ในทุกช่วงอายุ จากการชันสูตรสุกรกลุ่มละ 20 ตัว เมื่ออายุ 16 สัปดาห์ พบว่ารอยโรคทางจุลพยาธิวิทยาของต่อมน้ำเหลืองในสุกรที่ได้รับวัคซีนมีความรุนแรงน้อยกว่าสุกรที่ไม่ได้รับวัคซีน การศึกษาน้ำหนักสัมพัทธ์ของต่อมน้ำเหลืองพบว่าในสุกรกลุ่มที่รับวัคซีนมีแนวโน้มที่ค่าน้ำหนักสัมพัทธ์ของต่อมน้ำเหลืองจะน้อยกว่าสุกรที่ไม่ได้รับวัคซีน ผลการศึกษาครั้งนี้พบว่าวัคซีนดังกล่าว มีประสิทธิภาพในการลดการติดเชื้อในเลือดของสุกรและลดความรุนแรงของรอยโรคทางพยาธิวิทยาได้

คำสำคัญ : ไคเมอริควัคซีน ประสิทธิภาพ รอยโรค เซอโคไวรัสชนิดที่ 2 สุกร การติดเชื้อในกระแสเลือด

[1]ภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพ 10330
[2]บริษัท ฟอร์ท ดอดจ์ แอนิมัล เฮ็ลธ์ (ไทยแลนด์) จำกัด
[*]ผู้รับผิดชอบบทความ E-mail : komkrich.t@chula.ac.th

Introduction

Porcine circovirus (PCV) belonging to the family *Circoviridae* is a small non-enveloped virus with a capsid size of 17 nm. Porcine circovirus type 1 (PCV1) and type 2 (PCV2) are widespread in the swine populations. PCV1 does not cause clinical disease and is generally considered as a non-pathogenic virus. In contrast, PCV2 is a causative agent of postweaning multisystemic wasting syndrome (PMWS), a multi-factorial new emerging disease in swine (Mankertz et al., 2004). PCV2 has also been associated with other pathological conditions in pigs including porcine dermatitis and nephropathy syndrome (PDNS), reproductive failures, porcine respiratory disease complex (PRDC), proliferative and necrotizing pneumonia and congenital tremor (Segales et al., 2004). Those pathological conditions currently known as porcine circovirus associated diseases (PCVAD) were first recognized in North America in 1991. Later, PCVAD has been reported worldwide in Europe and Asia in most swine raising countries (Allan and Ellis, 2000; Fenaux et al., 2004; Chae, 2005). Clinical signs of PCVAD include progressive weight loss, dyspnea, enlargement of superficial inguinal lymph nodes, pallor, jaundice and diarrhea (Darwich et al., 2004; Segales et al., 2004).

In Thailand, a PCV2 retrospective study detected using immunohistochemistry identified that the first PCV-infected case was as early as in 1993. Later using formalin-fixed, paraffin-embedded (FFPE) tissues from suspected cases occurring during 2000 - 2002 demonstrated approximately 38.76% of PCV2 infection (50/129). The presence of PCV2 antigen was seen primarily in the lymph nodes 40.70% (Banlunara et al., 2002). Recent data based on swine diagnosis annual report in 2006-2008 from the Livestock Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University (unpublished data) found increasing incidence of PMWS and PDNS in the cases of swine systemic infection (58.23 %, n = 79). This indicates that PCV2 is one of the major causative agents of swine disease in the Thai swine industry.

The Suvaxyn PCV2® (Fort Dodge Animal health, USA) is a Chimeric PCV1-2 vaccine containing immunogenic capsid gene cloned of PCV2 into the backbone of the non-pathogenic PCV1 (Fenaux et al., 2004). Vaccination with the Chimeric PCV1-2 significantly reduced viremia and decreasing risk of clinical diseases experimentally (Fenaux et al., 2004). Suvaxyn PCV2® when administered 1-shot to 3 week-old pigs was able to prevent PCV2 viremia and was able to prevent the development of microscopic lesions in the lymphoid tissues when pigs were challenged 4 months after vaccination (Urniza et al., 2006). However, the swine farms in Thailand have different conditions and farm managements from other countries. The field trial in Thailand would give useful information to the Thai farmers before the implementation of PCV2 vaccine in the farm management.

The objective of this study was to investigate the efficacy of Suvaxyn PCV2® vaccine on pathological lesions and viremic condition in a PCV2 affected farm in Thailand.

Materials and Methods

Herd status: Based on history and necropsy reports, a PCV2-affected herd containing 3,200 sows in Prachinburi province, Thailand was selected for this experiment. Three pigs (9-week-old, n = 1 and 16-week-old pigs, n = 2) submitted to the Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University were diagnosed as PCVAD on October 6th, 2006. To examine the serological status of this herd, preliminary survey was performed in January 2007. Serum samples (sows and gilts, n = 20; nursery and finisher pigs at 3, 5, 7, 9, 12, 16, 20, 24 weeks of age, n = 5 per group) were tested by a modified indirect ELISA based on the recombinant ORF2 capsid protein of PCV2 (Fort Dodge Animal Health, Biological Research & Development and Regulatory Affairs). The presence of PCV2 genetic materials in serum samples was examined by polymerase chain reaction (PCR).

Animal experiment: The protocol used in this study was approved by the committee on laboratory animal care, Faculty of Veterinary Science, Chulalongkorn University, No. 0731038. Two hundred, weaned, 3-week-old pigs were randomly divided into two groups, (Group A and Group B; 100 pigs per group). The pigs from each group were equally divided into ten subgroups (A1 - A10 and B1- B10) and were housed in small pens in the same building. At 4 weeks of age, group-A pigs were vaccinated intramusculary with 2 ml of Suvaxyn PCV2® vaccine and group-B pigs were injected intramusculary with 2 ml of normal saline.

Clinical parameters: All pigs were clinically examined by an animal caretaker on the farm once weekly without knowing of group affiliation. For the production parameter, individual live body weight of the studied pigs was measured at 4 and 16 weeks of age. Calculation of average daily weight gain (ADWG) was performed as the difference of the body weights between the two weighting timepoints divided by the number of days between that two timepoints. All animals were monitored weekly for clinical signs of porcine respiratory disease complex (PRDC), as described previously (Halbur et al., 1995). The body condition scores were also recorded (Straw et al., 1999).

Hematological study: EDTA-stabilized blood samples were obtained (1 pig/pen, n = 10) at 4, 5, 7, 9, 12 and 15 weeks of age for complete blood count (CBC) and differential cell count.

Serological examination: Serum samples were collected (2 pigs/pen, n =20) at 4, 5, 7, 9, 12 and 15 weeks of age for PCV2 antibody detection using a modified indirect ELISA based on the recombinant ORF2 capsid protein of PCV2 (Fort Dodge Animal health, Biological Research & Development and Regulatory Affairs). Bound antibodies

were detected with TMB peroxidase substrate (KPL®, MD, USA). The absorbance of each well was read by the spectrophotometer at 650 nm. The (S/P) ratios over 0.070 are considered positive. Antibodies against PRRSV in the same sera were also detected using a commercial ELISA test kit (IDEXX Labs, Inc., USA). PRRSV positive sample is considered when the S/P ratios are over 0.4.

Postmortem examination

Gross pathology: During the study, the animals showing clinical signs or died during the experiment were necropsied and the organs were collected for histopathology, immunohistochemistry, bacteriology and PCR detection for PCV2 involvement.

At 16 weeks of age (84 days post vaccination), the trial was terminated and 20 pigs per group were randomly selected and euthanized using pentobarbital sodium (CEVA Sante Animale, France). Complete necropsy was performed and significant gross lesions were recorded. Pulmonary lesions were observed and scored (Thanawongnuwech et al., 2004). The degree of lymph nodes enlargement (superficial inguinal lymph nodes, tracheobronchial lymph nodes and mesenteric lymph nodes) ranged from 0-3 (0: normal size, 1: one time larger than normal, 2: two times larger than normal, 3: three times larger than normal) was estimated (Fenaux et al., 2004). Weight of the superficial inguinal lymph nodes was measured and lymph node/body weight ratio was calculated individually in each necropsied pig. Organ samples including lymphoid tissues (tracheobronchial lymph nodes, mesenteric lymph nodes, superficial inguinal lymph nodes, spleen and ileum), lung, liver and kidney were collected for histopathology, immunohistochemistry, bacteriology and PCR for PCV2 detection.

Histopathology: The collected tissues were routinely processed for histopathology. Sections from lung, lymph nodes, and ileum were scored for the severity of lesions according to Halbur et al.(1995) and Fenaux et al. (2004). Lung scores were ranged from 0-4 (0: normal, 1: mild interstitial pneumonia, 2: moderate multifocal interstitial pneumonia, 3: moderate diffuse interstitial pneumonia and 4: severe diffuse interstitial pneumonia). Depletion of lymphoid tissue was observed and scored ranging from 0-3 (0: no lymphoid depletion, 1: mild lymphoid depletion, 2: moderate multifocal lymphoid depletion and 3: severe lymphoid depletion). The degree of histiocytic replacement (HR) in the follicles was scored ranging from 0-3 (0: no replacement to 3: severe replacement) (Fenaux et al., 2004).

Immunohistochemistry (IHC): Paraffin sections from lymphoid tissues (superficial inguinal lymph nodes, tracheobronchial lymph nodes, mesenteric lymph nodes and ileum) of the studied pigs were screened for the presence of PCV2 antigen. Briefly, sections were cut (4 μm) and placed on 3-aminopropyltriethoxysilan-treated slides and were then incubated at 60°C for 10 min. After deparaffinization, the sections were treated with 0.1% trypsin at 37°C for 30 min and washed in phosphate-buffered saline (PBS). Endogenous peroxidase activity was eliminated by using 0.3% H_2O_2 in methanol (2 ml: 200 ml) for 30 min at room temperature. After washing, the slides were incubated with 0.01% bovine serum albumin (BSA) at 37°C for 30 min. The sections were then incubated with primary antibody, 1:500 dilution of polyclonal rabbit anti-PCV2 antibody (Fort Dodge Animal health, Biological Research & Development and Regulatory Affairs) then incubated at 4°C overnight. After washing, the slides were incubated with 1:400 biotinylated goat anti-rabbit IgG antibody (KPL, MD,USA) at 37°C for 35 min, followed by incubation with avidin-biotin complex peroxidase solution (ABC, Dako, Denmark) at 37°C for 35 min. The immunoreactivity was detected in 3, 3'-diaminobenzidine-4HCl (DAB) substrate (Sigma, USA). Sections were then counterstained with hematoxylin (Banlunara et al., 2002).

Polymerase chain reaction (PCR)

DNA extraction: Superficial inguinal lymph node, mesenteric lymph node and tracheobronchial lymph node from each pig were pooled as one sample in each necropsied pig (n = 20). Organ samples including lung, spleen, liver and ileum from each pig were also pooled as one sample. Extraction of DNA from pooled organs and pooled lymph nodes was performed using a commercial DNA extraction kit (ChargeSwitch® gDNA Tissue Kits, Invitrogen®, California, USA). Serum samples from the same pen (n = 2), collected as mentioned above, were pooled for DNA extraction using a commercial DNA extraction kit (Viral Nucleic Acid Extraction Kit, RBC

Bioscience®).

PCR detection of PCV2: To detect PCV2 DNA in the pooled organs and sera, forward and reverse primers were designed to amplify product of the ORF gene specific for the Open reading frame 1 (ORF1) encoding for the replication protein. The amplification was performed in 20-µl reaction mixture containing 10 µl of a commercial master mix (Go taq® Green Master Mix, Promega®, Madison, USA), 0.5 µl of forward primers (ATG CCC AGA AAG AAT GGA AGA AG) and reverse primers (AGG TCA CTC CGT TGT CCT TGA GAT C), 3 µl of DNA template and distilled water 6 µl to yield a final volume of 20 µl. Amplification conditions contained 1 cycle with initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 20 sec. annealing at 56°C for 20 sec. and extension at 72°C for 20 sec. with a final extension at 72°C for 2 min. PCR products were separated by electrophoresis using 1.5% agarose gel. The gels were stained with 10% ethidium bromide and visualized under UV transilluminator.

Statistical analysis: Statistical analysis for the histopathology and immunohistochemistry was performed using Mann-Whitney rank sum test. Averages daily weight gain and means S/P ratio from ELISA were analyzed by Student's t-test.

Results

Herd status before an experiment: Necropsy of three submitted pigs (9-week-old, n = 1 and 16-week-old pigs, n = 2) revealed generalized lymphadenopathy and icteric mucous membrane in all pigs. Histopahologically, severe lymphoid depletion with the presence of intracytoplasmic inclusion bodies in macrophages and giant cells in the lymphoid tissues were seen in all animals examined. PMWS associated with PCV2 infection was suggested. In addition, the serological profile from this farm revealed high prevalence of PCV2 infection both in the sows (90%, 18/20) and in the growing pigs (40/40, 100%). In piglets, the average PCV ELISA titers were high possibly due to maternal-derived antibody at 3 weeks of age and then declined at about 5 weeks. The titers were again gradually increased from 9 weeks to 24 weeks of age (Figure 1) probably due to natural PCV2 infection. PCR detection of PCV2 from pooled sera in January 2007 showed positive results at 9, 12, 16 and 24 but not at 3 or 5 weeks of age.

Clinical score and production parameters: In both groups, respiratory signs (coughing and dyspnea) were frequently observed during 5 to 9 weeks of age, and were subsided later until the end of the experiment. The body condition scores (BCS) were similar as well as average daily weight gain (ADWG) (380 g/d) (Table 1). In this experiment, total mortality rate for the vaccinated pigs was 23% and 22% for the non-vaccinated pigs. The mortality rates for both groups of pigs were high during 5 to 9 weeks of age due to secondary bacterial infection and later were subsided within a month. At 14 weeks of age, some animals in both groups were died from salmonellosis and hemolytic *E. coli* infection (Figure 2).

Hematological and serological results: Anemic condition was not observed in both groups of pigs and the total white blood cell count (WBC) was similar. However, mild leukopenia (WBC < 9,000 cells/µl) was detected in vaccinated pigs at 4 weeks (n = 1) and 9 weeks of age (n = 1), and in non-vaccinated pigs at 4 weeks (n = 2) and 9 weeks of age (n = 1).

PCV2 antibodies: The average S/P ratio of PCV2 antibodies were high at 4 weeks of age and then declined at about 5 weeks in both groups indicating the declining of the maternal derived antibodies between 4 and 7 weeks of age. The seroconversion was observed in the vaccinated group at 9 weeks of age (Figure 3). In the non-vaccinated group, PCV2 seroconversion was detected at 12 weeks of age.

PRRSV antibodies: Seroconversion of the PRRSV antibodies was observed at 5 weeks of age in both groups and remained seropositive through the end of the experiment (Figure 4).

Pathology of pigs died during experiment

Necropsy of pigs from both groups died during 5 and 9 weeks of age showed generalized lymphadenopathy and severe fibrinopurulent polyserositis. *Pasteurella multocida* and *Streptococcus suis* were frequently isolated from those cases. At 14 weeks of age, *Salmonella* spp. and hemolytic *E. coli* were isolated from the three vaccinated pigs died in this period. Histopathologically, severe fibrinopurulent pneumonia concurrent with lymphoid depletion and necrosis of lymphoid tissues was observed from both groups.

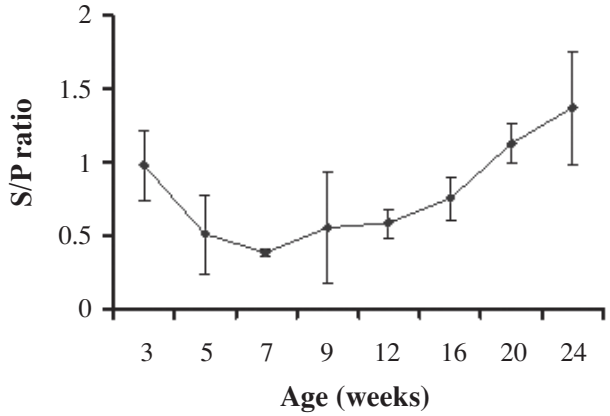

Figure 1 Mean S/P ratio of PCV2 ELISA with standard deviation in the nursery and growers as monitored in January 2007.

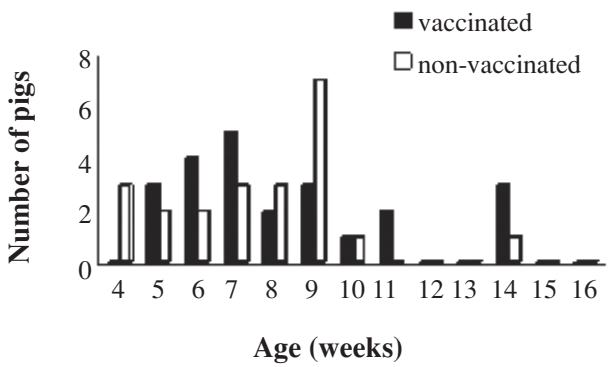

Figure 2 Number of pigs died during the vaccination experiment.

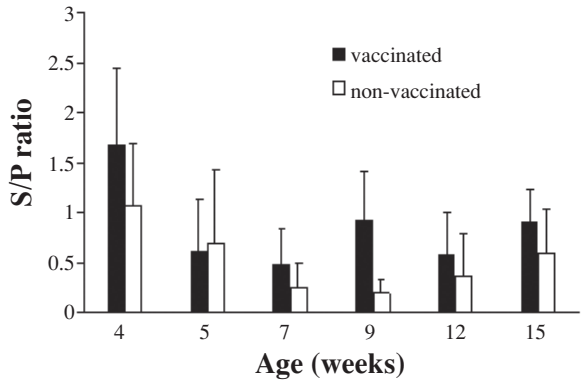

Figure 3 Mean S/P ratios of PCV2 ELISA with standard deviation in the vaccinated and non-vaccinated pigs.

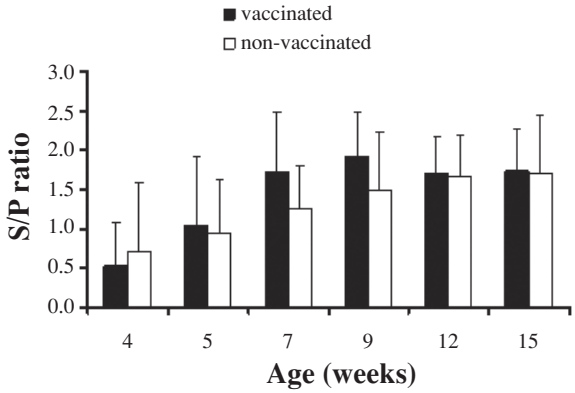

Figure 4 Mean S/P ratio of PRRSV ELISA with standard deviation in the vaccinated and non-vaccinated pigs.

Table 1 Mean score of respiratory signs and body condition in the experimental pigs

Age	Vaccinated group		Non-vaccinated group	
(weeks)	Respiratory signs	BCS	Respiratory signs	BCS
5	2.45 ± 1.932	2.45 ± 0.826	2.58 ± 1.645	2.74 ± 1.098
6	2.40 ± 2.037	2.30 ± 0.923	1.74 ± 1.548	3.05 ± 0.848
7	2.11 ± 2.378	2.26 ± 0.933	1.72 ± 1.809	2.83 ± 0.786
8	2.94 ± 2.235	2.33 ± 0.907	1.38 ± 0.719	2.69 ± 0.479
10	1.41 ± 1.460	2.88 ± 0.781	0.88 ± 1.544	3.38 ± 0.719
11	0.77 ± 0.903	3.47 ± 1.125	0.73 ± 1.580	4.06 ± 0.961
12	1.00 ± 1.323	3.53 ± 0.874	0.47 ± 0.640	3.40 ± 0.828
13	0.65 ± 1.222	3.17 ± 0.883	0.33 ± 0.488	4.06 ± 1.223
14	0.33 ± 0.488	4.07 ± 1.223	0.20 ± 0.561	4.20 ± 0.676
15	0.33 ± 0.816	3.67 ± 1.047	0.13 ± 0.352	3.53 ± 0.640

Values are expressed as a mean score ± S.E. (standard error) BCS: body condition score, n = 20

Immunohistochemistry for PCV2 was positively confirmed in two cases from non-vaccinated pigs. Detection of PCV2 DNA by PCR revealed positive results in both vaccinated pigs (3 of 5 cases) and non-vaccinated pigs (6 of 7 cases).

Postmortem examination: At the end of the experiment (16 weeks of age), necropsies were performed on 20 pigs in each group. Grossly, mild cranioventral pneumonia (vaccinated group, 5/20; non-vaccinated group, 4/20) and mild to moderate chronic pleuritis in both groups of pigs (vaccinated group, 4/20; non-vaccinated group, 2/20) were recorded. The average pneumonic lung scores did not differ between groups (vaccinated group = 1.05±2.942 and non-vaccinated group = 1.15±2.907). The enlargement of the superficial inguinal lymph nodes of vaccinated pigs was ranged from 1 to 2 times. In contrast to the vaccinated pigs, enlargement of superficial inguinal lymph nodes of non-vaccinated pigs were ranged from 1 to 3 times (Table 2). Additionally, the average of lymph node/body weight ratio in vaccinated pigs ($38.5x10^{-5}$) was lower than those in the non-vaccinated pigs ($45.4x10^{-5}$), but it was not statistically significant. One pig from the vaccinated group (No. 7A1) had severe edema of perirenal and periureteral areas with moderate diffuse petechial hemorrhagic nephritis (Figure 5A). One animal from the non-vaccinated group had mild enlarged kidney with multifocal white foci (Figure 5B).

Histopathology: Microscopic examination of lymphoid tissue revealed variable degrees of lymphoid depletion and histiocytic and granulomatous inflammation (Figure 6) in both groups. Intracytoplasmic inclusion bodies could not be observed in any sample tested. In the vaccinated pigs, mean scores of lymphoid depletion and histiocytic replacement in the lymphoid organs were lower than those in the non-vaccinated pigs, especially in the mesenteric lymph nodes and Peyer's patches (Table 3). In lungs, interstitial pneumonia with granulomatous inflammation was observed in 3 vaccinated pigs and 7 non-vaccinated pigs. In kidneys, severe diffuse lymphocytic interstitial nephritis was sometimes observed in both groups (pig No. 7A1 and 5B1). Mild to moderate degree of lymphocytic infiltration in renal pelvis was often seen in both groups.

Detection of PCV2 antigen in lymphoid tissues by IHC: PCV2 antigen was detected in the lymphoid tissues in both groups using IHC. In general, positivity of PCV2 antigen was observed in macrophages, lymphocytes and occasionally in multinucleated giant cells. In ileum, PCV2 antigen was detected not only in Peyer's patches but also in lymphocytes in lamina propia and sometimes in epithelial cells. In vaccinated pigs, mild degree (+1) of positive labeling was observed in the superficial inguinal lymph node, tracheobronchial lymph nodes, mesenteric lymph nodes (7 of 20 pigs; 35%), and in the Payer's patches (3 of 20 pigs; 15%). In the non-vaccinated pigs, mild to moderate degree of PCV2-positive cells (+1 and +2) was seen in the superficial inguinal lymph nodes (12 of 20 pigs; 60%). Low amount of PCV2 positive cells (+1) was detected in the tracheobronchial lymph nodes (7/20, 35%), mesenteric lymph nodes (8/20, 40%) and Peyer's patches (5/20, 25%). Averaged scores of PCV2 antigen detection in lymphoid organs are shown in Table 4.

Polymerase chain reaction (PCR)

PCR detection in pooled sera: Interestingly, none of PCV2 genetic material was detected in the vaccinated pigs before 15 weeks of age (Figure 7). In contrast to the vaccinated pigs, PCV2 DNA was detected in non-vaccinated pigs at 4 weeks (20%, 2/10), 5 weeks (70%, 7/10), 7 weeks (50%, 5/10), 9 weeks (100%, 10/10), 12 weeks (30%, 3/10) and 15 weeks of age (30%, 3/10).

PCR detection in the pooled lymph nodes and pooled organs: PCR detection of PCV2 in the pooled lymph nodes revealed 75% (15/20) positive results in the vaccinated pigs, whereas 100% (20/20) of the samples from the non-vaccinated pigs were positive. In addition, PCR detection of PCV2 in the pooled organs revealed 70% (14/20) positive results in the vaccinated pigs and 90% (18/20) positive in the non-vaccinated pigs.

Discussion

PCVAD is now considered as one of the most important disease complexes in pigs worldwide. The prevalence of PCV2 infection in Thailand is increasing (Banlunara et al., 2002). Although strict biosecurity measures to control the disease have been implemented in the field, but the disease situation did not improve as expected. Therefore, several PCV 2 vaccines have been

introduced to overcome the disease.

In this study, the efficacy of Suvaxyn PCV2® in a PCV2-affected herd in Thailand was evaluated and the results demonstrated that the vaccine could successfully reduce PCV2 viremia. Similar to the previous study of the Suvaxyn PCV2® efficacy in the specific pathogen free pigs, the significant reduction of viremia and the risk of clinical diseases were also observed (Fenaux et al., 2004; Urniza

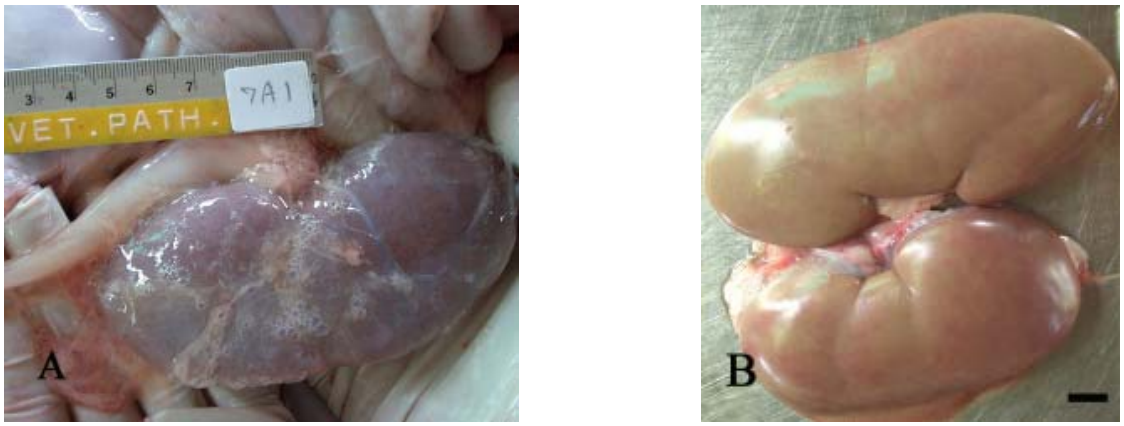

Figure 5 Gross lesions of the experimental pigs. A: Perirenal and periureteral edema with hemorrhagic nephritis (vaccinated pig, No. 7A1), B: Mild enlarged kidney with multifocal to diffuse white foci (non-vaccinated pig, No.5B1) Bar = 1 cm.

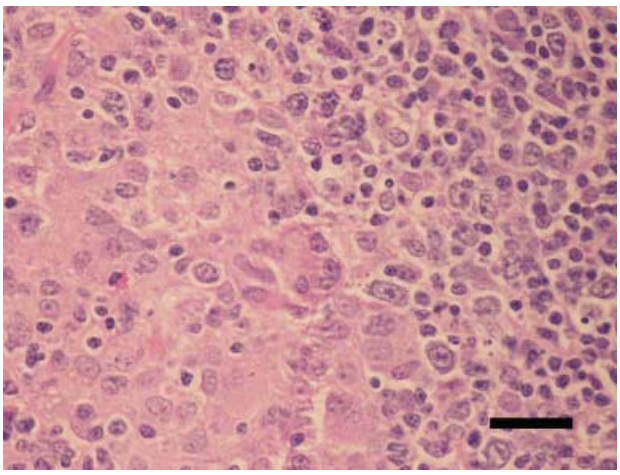

Figure 6 Mesenteric lymph node (non-vaccinated pig; No. 2b2). Severe histiocytic replacement in lymphoid follicle. Hematoxilin & Eosin staining, Bar = 25 μm.

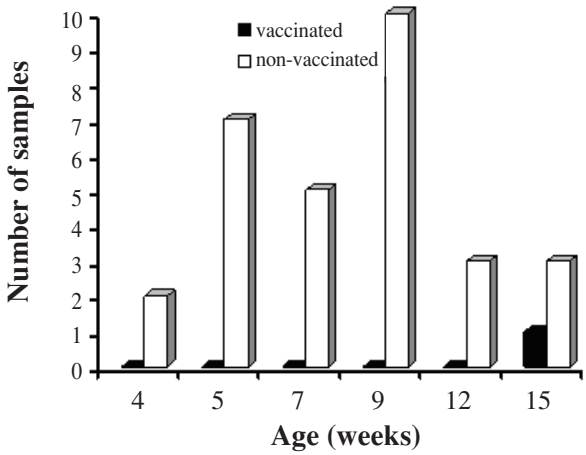

Figure 7 Detection of PCV2 DNA in pooled sera.

Table 2 Number of pigs with enlargement of superficial inguinal lymph nodes

	Superficial inguinal lymph node enlargement (times)			
	1	**1.5**	**2**	**3**
Vaccinated group	9	7	4	0
Non-vaccinated group	4	7	8	1

n = 20

Table 3 Mean scores of lymphoid depletion and histiocytosis

Mean scores of lymphoid depletion in the lymphoid organs				
Group	**Superficial inguinal**	**Tracheobronchial**	**Mesenteric**	**Payer's patches**
Vac	2.75 ± 0.099*	1.70 ± 0.164	1.10 ± 0.143[a]	0.65 ± 0.167[a]
Non-vac	2.70 ± 0.105	2.00 ± 0.162	1.90 ± 0.191[b]	1.40 ± 0.152[b]
Mean scores of histiocytosis in lymphoid organs				
Group	**Superficial inguinal**	**Tracheobronchial**	**Mesenteric**	**Payer's patches**
Vac	2.45 ± 0.135	1.80 ± 0.156	1.20 ± 0.172[a]	0.75 ± 0.204[a]
Non-vac	2.75 ± 0.099	2.15 ± 0.167	1.85 ± 0.167[b]	1.65 ± 0.167[b]

*standard error, [a,b]different superscripts in the same column means statistically different (p<0.05), Vac: vaccinated group, Non-vac: non-vaccinated group, n = 20

Table 4 Immunohistochemical detection (%) of PCV2 antigen in lymphoid organs

Mean scores of PCV2 detection in lymphoid organs by IHC				
Group	**Superficial inguinal**	**Tracheobronchial**	**Mesenteric**	**Payer's patches**
Vac	0.35 ± 0.109*	0.35 ± 0.109	0.35 ± 0.109	0.15 ± 0.082
Non-vac	0.65 ± 0.131	0.35 ± 0.109	0.40 ± 0.112	0.25 ± 0.099
Number of pigs with positive IHC				
Vac	7/20 (35%)	7/20 (35%)	7/20 (35%)	3/20 (15%)
Non-vac	12/20[a] (60%)	7/20 (35%)	8/20 (40%)	5/20 (25%)

*standard error; No statistically significant difference between groups, [a]one pig in group B revealed moderate degree of PCV2 antigen detection (score = 2), Vac: vaccinated group, Non-vac: non-vaccinated group, n = 20

et al., 2006; Opriessnig et al., 2008). The field trial performed in the US also showed promising results (Connor and Elsener, 2007).

The study of disease status before the experiment had shown that the selected pig herd was affected with PCVAD. The high prevalence of PCV2 infection of this herd was also confirmed by serological profile in most of the pigs including the sows, nursery and growing pigs. The averaged S/P ratio and seroprevalence were high in the gilts, which probably due to the gilt acclimatization process. In the piglets, the declining of the maternal derived antibodies was seen between 3 to 7 weeks of age. The PCV2 seroconversion was later observed from 9 to 24 weeks of age indicating that the piglets were naturally infected with PCV2 between 5-9 weeks of age when their maternal immunity was declined. The same pattern of maternal immunity decay was also observed previously (McKeown et al., 2005). Similar to the study in a large Canadian farrow to finishing barn, seroconversion of the piglets to PCV2 was observed during 10 and 15 weeks of age indicating the transmission during the nursery period (MacIntosh et al., 2006). High seroprevalence of PCV2 in the grower and finisher pigs and the presence of PCV2 in the sera at 9, 12, 16 and 24 weeks of age were highly suggested of active circulation of PCV2 in this farm.

During the experiment, coughing and dyspnea were more frequently observed in pigs from both groups between 5 to 8 weeks of ages, when the animals were susceptible for PCV2 and PRRSV infection resulting in increased secondary bacterial infection and high mortality. The evidence of PCV2 infection in these pigs was confirmed by IHC and PCR. Additionally,

co-infection with PRRSV as observed by serology could also contribute to the onset and exacerbation of the clinical diseases in those pigs. These complex diseases in the nursery resulted in poor body condition scores and high mortality rate in both groups. These results were differed from the previous study of Suvaxyn PCV2® in which PCV2 vaccination can significantly reduce the mortality rate (Connor and Elsener, 2007).

Similar to the preliminary data from this farm, the waning pattern of the maternal derived PCV2 antibodies was similarly seen in both experimental groups. After vaccination, seroconversion was observed at 9 weeks of age suggesting the induction of PCV2 antibodies within 4-5 weeks post vaccination. The same pattern was also seen in the previous study when the vaccinated pigs showed seroconversion to PCV2 within 4 to 6 weeks post vaccination (Fenaux et al., 2004). In the non-vaccinated pigs, PCV2 seroconversion was detected later at about 12 weeks of age. This might be due to natural PCV2 exposure after weaning.

Macroscopically, enlargement of the superficial inguinal lymph nodes of the non-vaccinated pigs was greater than those in the vaccinated pigs. Moreover, the average of lymph node/body weight ratio in the vaccinated pigs was tended to be lower than those in the non-vaccinated pigs but not statistically different, suggesting that the vaccinated pigs might have mild lesions of the lymph nodes as similar to the previous report (Fenaux et al., 2004). Histopathologically, lymphoid depletion and histiocytosis were observed in both groups. However, mean scores of lymphoid depletion and histiocytosis in those lymphoid organs of the vaccinated group were lower than those in the non-vaccinated group, especially in the mesenteric lymph nodes and Peyer's patches. Interestingly, granulomatous pneumonia was frequently observed in the non-vaccinated pigs than that in the vaccinated pigs. These findings indicated that histological lesions of the vaccinated pigs were less severe than those in the non-vaccinated pigs (Fenaux et al., 2004).

Numbers of pigs with positive immunohistochemical detection in the superficial inguinal lymph node in the vaccinated pigs (7/20) were lower than those in the non-vaccinated pigs (12/20). Although it was not statistically significant, the mean IHC scores of PCV2 detection in the vaccinated pigs were lower than those in the non-vaccinated pigs. In addition, PCR detection of PCV2 in the pooled lymph nodes in the vaccinated pigs also revealed the lower percentages of positive results. These results suggested that the vaccinated pigs may be able to clear the viral burden in their lymphoid tissues leading to the reduction of the lesions in the lymph nodes. However, the IHC detection of PCV2 may not be a good tool for evaluating the severity of the PCVAD since low amount of PCV2 antigen could not be detected in the chronic stage of infection represented by the granulomatous inflammation in the lymphoid tissue (Opriessnig et al., 2007).

In conclusion, this is the first trial of PCV2 vaccine in the field condition in Thailand, which demonstrated that Suvaxyn PCV2® was able to induce PCV2 antibody and subsequently reduce PCV2 viremia and to a lesser extend of the pathological lesions. However, effective managements and strict biosecurity should also be implemented to control other complicated factors. Co-infection with PRRSV and subsequently complicated with secondary bacterial infection may have some impacts of this study.

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