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AN IN VITRO VIRUCIDAL EFFICACY OF DISINFECTANTS AGAINST PORCINE CIRCOVIRUS TYPE 2

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เหวียน ทิ มินห์ ตรัง : ประสิทธิภาพในการฆ่าไวรัสของยาฆ่าเชื้อในหลอดทดลองต่อเชื้อเซอร์โคไวรัสไทป์สองในสุกร (AN IN VITRO VIRUCIDAL EFFICACY OF DISINFECTANTS AGAINST PORCINE CIRCOVIRUS TYPE 2) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: สุพจน์ วัฒนะพันธ์ศักดิ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: พรชลิต อัศวชีพ, 64 หน้า.

การศึกษาครั้งนี้มีจุดประสงค์เพื่อประเมินประสิทธิภาพของยาฆ่าเชื้อที่มีขายอยู่ในท้องตลาด เพื่อใช้ในการฆ่าเชื้อ เซอร์โคไวรัสชนิดที่สอง ชนิดย่อยเชื้อ ซัปไทป์บี (พีซีวีทูบี) ในหลอดทดลอง โดยกลุ่มของยาฆ่าเชื้อที่ทดสอบมี 3 กลุ่มใหญ่ คือ ยาฆ่าเชื้อกลุ่มอ๊อกซิไดส์ซิ่งเอเจนส์ กลุ่มควอเตอร์นารี่แอมโมเนี่ยมคอมพาวส์ กลุ่มไอโอดีน และสารเคมีอีก 2 ชนิด คือ ด่าง และกลุ่มอัลดีไฮด์ โดยยาฆ่าเชื้อทุกชนิดเจือจางด้วยน้ำกลั่น และน้ำกระด้าง ที่เตรียมขึ้นใหม่ทุกครั้ง และมีการเติม 5% ซีรั่มตัวอ่อนลูกวัว เพื่อเปรียบเสมือนมีการปนเปื้อนของสารอินทรีย์ในยาฆ่าเชื่อ ความเข้มข้นสุดท้ายของยาฆ่าเชื้อและความเข้มข้นของไวรัสถูกปรับให้มีสัดส่วนเป็น 0.5, 1, และ 2 เท่าของความเข้มข้นที่แนะนำตามแต่ละบริษัท หลังจากไวรัสและยาฆ่าเชื้อแต่ละชนิดสัมผัสกันเป็นเวลา 1, 10, 30 นาที และ 12 ชั่วโมง ความเป็นพิษของยาฆ่าเชื้อจะถูกทำให้หมดไปโดยผ่าน ดีท็อกซิไฟล์ คลอรั่ม ก่อนที่จะนำสารละลายไวรัสนั้น ไปตรวจดูความมีชีวิตของไวรัสด้วยวิธี ไอพีเอ็มเอ และ ไอเอฟเอ ผลพบว่า ที่ 12 ชั่วโมง ไวรัสยังสามารถมีชีวิตอยู่ได้ ในกลุ่มของยาฆ่าเชื้อ กลุ่มควอเตอร์นารี่แอมโมเนี่ยมคอมพาวส์ หมายเลข 6 และ 7 กลุ่มไอโอดีน หมายเลข 8 และ 9 แต่ถ้าเป็นกลุ่มของยาฆ่าเชื้อที่มีการรวมกันของกลูตารอลดีไฮด์และกลุ่มควอเตอร์นารี่แอมโมเนี่ยมคอมพาวส์ จะสามารถฆ่าเชื้อพีซีวีทูบีสอง ได้ภายใน 30 นาที แต่จากผลการทดลองครั้งนี้พบว่า ยาฆ่าเชื้อกลุ่มอ๊อกซิไดส์ซิ่งเอเจนส์ หมายเลข 1,2, และ3 ให้ผลดีที่สุดในการฆ่าเชื้อ พีซีวีทูบีได้หมดภายในระยะเวลา 10 นาที ซึ่งภายในกลุ่มอ๊อกซิไดส์ซิ่งเอเจนส์ พบว่า ยาที่มีส่วนผสมของ โพแทสเซี่ยม เปอร์ออกซีโมโนซัลเฟต และโซเดี่ยม ไดคลอโรไอโซไซยานูเลต ที่ความเข้มข้น 1:200 ให้ผลดีที่สุดในการฆ่าเชื้อ พีซีวีทูบี ลงมาถึง 5 ล๊อก 10 ทีซีไอดี50 ในเวลาเพียง 1 นาที

ภาควิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....

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NGUYEN THI MINH TRANG: AN IN VITRO VIRUCIDAL EFFICACY OF DISINFECTANTS AGAINST PORCINE CIRCOVIRUS TYPE 2. ADVISOR: SUPHOT WATTANAPHANSAK, D.V.M., M.Sc., Ph.D., CO-ADVISOR: PORNCHALIT ASSAVACHEEP, D.V.M., M.Sc., Ph.D, 64 pp.

The objective of this study was to evaluate in vitro the virucidal activity against PCV2b of nine commercial disinfectants which were divided into three groups consisting of oxidizing products, QAC and iodine products, and two chemical substances including an alkali agent and aldehyde agent. Each disinfectant was freshly diluted in distill water and hardness of water as conducting the disinfectant test procedure. Five percent of fetal bovine serum were added as organic matter. After mixing the disinfectant with the stock of PCV2b, the final concentrations were 0.5x, 1x, 2x of the products' labelled dilutions. After 1, 10, 30-minute and 12-hour contact time between the virus and the disinfectant, the mixture was detoxified by flowing through a detoxification column. The infectivity of the collected virus was determined by indirect immunoperoxidase monolayer assay and indirect immunofluorescence assay. Viability of PCV2b was illustrated after 12-hour exposure to QAC products (disinfectants 6 and 7) and iodine products (disinfectants 8 and 9). PCV2b inactivation was found after 30-minute contacting with a combination of glutaraldehyde and QAC (disinfectants 4 and 5). Oxidizing products were the most effective disinfectants (disinfectants 1, 2 and 3) against PCV2b after 10-minute contact time. Of oxidizing products, a combination of potassium peroxomonosulfate and sodium dichloro isocyanurate (1:200), which significantly reduced PCV2b titer (5 $\log_{10}TCID_{50}ml^{-1}$) after 1-minute exposure, indicated the best virucidal product against PCV2b.

Department:	Veterinary Medicine	Student's Signature	.....
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CONTENTS

Page

THAI ABSTRACT iv

ENGLISH ABSTRACT v

ACKNOWLEDGEMENTS vi

CONTENTS vii

LIST OF TABLES ix

LIST OF FIGURES ix

ABBREVIATION x

CHAPTER 1 INTRODUCTION 1

Background and significance of the problem 1

CHAPTER 2 LITERATURE REVIEW 3

2.1 Porcine Circovirus 3

2.1.1 Discovery of PCV viruses 3

2.1.2 Genomic characteristics 4

2.1.3 Genomic analysis 5

2.1.4 Porcine Circovirus Associated Diseases (PCVAD) 6

2.1.5 Distribution of porcine circovirus associated diseases (PCVAD) in Thailand 8

2.1.6 Shedding and transmission of PCV2 9

2.1.7 To control and prevent the spread of PCVAD 10

2.1.8 Biological and physiochemical properties of PCV2 12

2.2 Methods of testing disinfectant 15

2.2.1 Viral suspension tests 16

2.2.2 Development of diagnose techniques to determine viral survival 17

Page

2.2.3 Immunofluorescence assay (IFA) and Immunoperoxidase assay (IPA) 19

2.3 Activity of disinfectant against viruses 20

2.3.1 Group of disinfectants and their mechanism of action 21

CHAPTER 3 MATERIALS AND METHODS.....25

3.1 Virus preparation 25

3.1.1 Indirect immune-peroxidase monolayer assay (IPMA) 25

3.1.2 Indirect immunofluorescence assay (IFA) 26

3.2 Disinfectants 26

3.3 Evaluating efficacy of disinfectants.....27

3.4 Viral titration.....29

CHAPTER 4 RESULTS.....30

4.1 Reduction in PCV2b titer after exposure to 11 disinfectants.....30

4.2 Comparison of IPMA and IFA to positive sample (undetectable PCV2b) after exposure to five commercial disinfectants 36

CHAPTER 5 DISCUSSION.....39

REFERENCES 44

APPENDIX A 58

VITA.....64

LIST OF TABLES

Table 3. 1 Active ingredients and recommended dilution of disinfectants 28

Table 4. 1 Reduction in PCV2b titer after exposure to nine commercial disinfectants 32

Table 4. 2 Agreement on positive results (PCV2b inactivation) of PMA and IFA.....36

LIST OF FIGURES

Figure 2. 1 Distribution of porcine circovirus type 2 in Thailand 9

Figure 2.2 The gray and red area representing the capsid and the nucleic acid of a typical PCV2 virus [86].....13

Figure 2. 3 In comparison the resistance of PCV2, CAV and the other non-enveloped virus to pasteurization. PCV2 (■, graph series 1), and CAV (▲, series 2), other viruses (○): parvovirus B19 (graph series 7) and canine parvovirus (CPV; series 5), CPV (series 3) and B19 (graph series 8), human hepatitis A virus (HAV; series 4) and human poliovirus 1 (PV; series 6) [12].....14

Figure 4. 1: PCV2b retention after 1-minute exposure to disinfectants 1, 2 and 3 31

Figure 4. 2: PCV2b retention after 1-, 10- and 30-minute exposure to disinfectants 4, 5, 6 and 7.....33

Figure 4. 3: PCV2b retention after 1-, 10-, 30-minute and 12-hour exposure to disinfectant 8 and 9 34

Figure 4. 4: PCV2b retention after 1-, 10-, 30-minute and 12-hour exposure to 9 disinfectants.....35

Figure 4. 5 The infectivity of PCV2b examined by IPMA and IFA.....38

ABBREVIATION

A.O.A.C.	Association of Official Analytical Chemists
AFNOR	Association French Normalization Organization Regulation
$CaCO_3$	Calcium carbonate
CAM	Chorioallantoic membrane
CEN	European Committee for Standardization
CO_2	Carbon dioxide
^0C	Degree celsius
DGHM	German Society for Hygiene and Microbiology
DISF	Disinfectant protocols
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EST	European suspension test
H_2O_2	Hydrogen peroxide
HRP	Horseradish peroxidase-labelled antibodies
IFA	Immunofluorescence assay
Ig	Immunoglobulin
IIFA	Indirect immunofluorescence assay
IIPA	Indirect immune-peroxidase assay
IPA	Immune-peroxidase assay
OECD	Organization for Economic Co-operation and Development
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PCV	Porcine circoviruses
PCV1	Porcine circovirus type 1
PCV2	Porcine circovirus type 2
PCVAD	Porcine circovirus associated disease
PDNS	Porcine dermatitis and nephropathy syndrome
pH	Logarithmic measure of hydrogen ion concentration

PMWS	Postweaning multisystemic wasting syndrome
PPV	Porcine parvovirus
PRDC	Porcine respiratory disease complex
PRRS	Porcine reproductive & respiratory syndrome
PRV	Pseudorabies virus
QAC	Quaternary ammonium compound
QCT	Quantitative carrier test
QSDT	Quantitative surface disinfection test
RT-PCR	Reverse transcriptase polymerase chain reaction
SDIC	Sodium dichloro isocyanurate
SPF	Specific-pathogen free
SFV	Classical swine fever virus
SST	Standardization suspension test
$TCID_{50}$	Tissue culture infectious dose 50
USA	The United States of America
UV	Ultra violet
µl	microliter
Rep	replication
Cap	capsid
DW	Distil water
HW	Hardness of water
1/2x	½ recommended dilution
1x	recommended dilutions

CHAPTER 1

INTRODUCTION

Background and significance of the problem

Porcine circovirus type 2 (PCV2) is an important pathogenic agent that could impair the immune system. PCV2 viruses are of the postweaning multisystemic wasting syndrome (PMWS) and play a crucial role of coinfection with various pathogens leading to the outbreak of disease complexes in swine [1]. Those diseases include PMWS, porcine respiratory disease complex (PRDC), porcine dermatitis and nephropathy syndrome (PDNS), enteritis and reproductive failure. Pig farms affected by the concurrence of PCV2 and other pathogens, such as PRRS, PPV, etc., could suffer economic loss and take a long time to eradicate [2, 3]. Pigs naturally infected with PCV2 commonly excrete the virus through nasal, oral and fecal secretions [4]. Experimentally, PCV2 concurrent with PRRSV or PPV led to more severe conditions in pigs and more PCV2 was shed in the environment [5, 6]. Although direct and indirect transmission of PCV2 plays different levels of risk factors, the horizontal transmission has been recognized as a prominent route related to PCV2 spread. Particularly, PMWS might occur in healthy pigs direct contacting with PMWS infected pigs or secretions from infected pigs [7-9]. Currently, PCV2 commercial vaccines widely used in pig farms could reduce mortality, viral load and increase growth performances [10]. However, PCV2 viruses are still ubiquitous in the environment because pigs continuously shed viruses after immunization [9]. Therefore, a combination of vaccination and strictly biosecurity could be more effective to control and prevent the spread of several diseases and syndromes associated with PCV2 in pig farms [2].

Moreover, PCV2 viruses are small non-enveloped viruses, and classified as highly resistant viruses. Pasteurization and dry-heat treatment could not be effective methods to inactivate PCV2. According to O' Dea et al., 2007 and Welch et al., 2006, PCV2 was only destroyed by wet-heat treatment for 15 minutes at 80^0C [11, 12]. In addition, PCV2 is only killed by oxidizing agents and sodium hydroxide compound after incubated for 10 minutes [13]. Some *in vitro* studies showed that oxidizing agents and

combinations of aldehyde quaternary ammonium compound only reduced PCV2 titers but the infectivity of the PCV2 virus still remains [14]. In Thailand, there was only a study showing a significant effectiveness of a substance, namely, potassium peroxomonosulfate against PCV2 in field condition [15]. The result showed that the compound could significantly reduce the amount of PCV2 in the environment. For those reasons, selecting a proper disinfectant to apply in biosecurity strategies is very crucial to minimize the transmission of PCV2 in the farming. However, little information is available on the efficacy of disinfectants against the PCV2 Thai isolate *in vitro*. Therefore, we would like to exam different disinfectants against PCV2 Thai isolates in Thailand.

Objectives

To evaluate the *in vitro* virucidal efficacy of commercial disinfectants against PCV2b virus.

Hypothesis

Some commercial disinfectants can inactivate PCV2b Thai isolate under control condition in the laboratory expected outcomes.

CHAPTER 2

LITERATURE REVIEW

2.1 Porcine Circovirus

Porcine circoviruses (PCV) included porcine circovirus type 1 (PCV1) and porcine circovirus type 2 (PCV2) are small non-enveloped, single-stranded and circular DNA genome, belonging to the genus *circovirus* of the family *circoviridae* [1]. PCV1 is apathogenic virus in pigs. PCV2 is a causative agent of several diseases and syndromes referred to as porcine circovirus associated disease (PCVAD). PCVAD include post-weaning multisystemic wasting syndrome (PMWS) [16, 17], porcine respiratory disease complex (PRDC) [16, 18], dermatitis and nephropathy syndrome (PDNS), PCV2-enteritis and PCV2-reproductive failure [19].

2.1.1 Discovery of PCV viruses

Porcine circovirus (PCV) was initially described as a contaminant in the continuous pig kidney cell culture PK/15 (ATCC-CCL 33) [20]. According to analyzing sedimentation, nuclease sensitivity, and seroprevalence, PCV were discovered as a new vertebrate small virus, which is characterized as a non-envelop and covalently closed, circular, single-stranded DNA virus, measuring 1.76 kb in size [21]. In comparison to organization and morphology, porcine circovirus together with other three novel circoviruses, which include psittacine beak and feather disease virus (BFDV) [22], chicken anemia virus [23] and pigeon circovirus [24]were classified in a newly recognized virus family, *circoviridae.* Although the prevalence of PCV was detected in swine population of many countries via several serological surveys [25] experimental infection of PCV on pigs did not develop clinical signs [26]. The fact that circovirus DNAs extracted from tissue samples of North American and European pigs, having clinical signs of wasting, shared 76% homology comparing to previously identified PK-15- associated PCV. This demonstrated the appearance of a new type of pathogenic PCV designated as PCV2, while the original PCV was referred to as PCV1 [27].

2.1.2 Genomic characteristics

PCV1 consist of 1759 nucleotides [21] less than 9 nucleotides comparing with PCV2 (1768 nt) [28]. Both PCV1 and PCV2 possess three open reading frame (ORF) which include ORF1 (942bp), ORF2 (699bp) [28] and ORF3 [29-31]. ORF1 encodes rep and rep' proteins responsible for viral replication function [31, 32]. ORF2 encodes a structural protein, a capsid protein, which plays an immunogenic function [33], while ORF3 encodes a nonstructural protein causing apoptosis and having pathological function [29].

The largest ORF1, which encodes a major structural protein (36 kD), contains an origin of viral replication and viral replication initiator rep protein. A sequence CGGCAGCGG/TCAG detected twice reveals a characteristic of rolling replication of PCVs [30] [32]. Moreover, total nucleotide sequence of PCV ORF1 revealed that ORF1 is a conserved sequence with 83% nucleotide homology, and 86% amino acid homology [28]. According to mapping of genome organization of PCVs, two noncoding regions consisting of 83 and 44 bp divided two major ORFs (ORF1 and ORF2) into an opposite orientation. Although ORF2 of PCV1 and PCV2, with 699 bp long encoding a 233 amino acid sequence (28kDa), was identified being similar [28], ORF2, which is more variable than ORF1 [34], shares 65% DNA homology and 67% amino acid homology [28, 34]. Furthermore, ORF2 contains a conserved basic amino acid sequence at N-terminus being similar with a major structural protein of chicken anemia [33]. This demonstrated the origin of PCV2 as well [28, 33].

Mapping the location of ORFs revealed that ORF3 belongs to ORF1, but opposite orientation [32]. Analyzing transcription and transfection of ORF3 demonstrated that ORF3 is not essential for PCVs replication but apoptosis [29]. ORF3 is responsible for PCV2-induced apoptosis by activating the initiator caspase 8 leading to activate the effector caspase 3, which is regulated by apoptosis signal-regulating kinase 1 (ASK1) [35]. In additionally, the finding of a novel PCV2 gene, ORF4 (180bp) located within ORF1, coincided with orientation of ORF3 is also nonessential to PCV2 replication but suppresses caspase activity and regulates $CD4^+$ and $CD8^+$ lymphocytes [36].

2.1.3 Genomic analysis

According to analyzing the whole genome of PCV1 and PCV2, the PCV1 and PCV2 reveal around 80% genomic homology [27, 37], while genomic homology within PCV2 strains shares 94% to 100% [38-40]. Moreover, NJ method was used to analyze 148 PCV2 sequences. The result indicated that PCV2 could be divided into two groups (group 1 and group 2) and eight clusters (1A to 1C and 2A to 2E). Group 1 (1767 nucleotides) and group 2 (1768 nucleotides) are differentiated by a single indel, just after a stop codon (genome position 1033) of the cap gene. For eight clusters, cluster 1B has a possible recombinant origin. The cluster 1C possesses a capsid gene, in which one amino acid (lysine) is encoded by a codon to be inserted prior to a stop codon (genome position 1036). Analyzing and comparing selective pressure of all parts of PCV2 genome, cap and rep genes reveal that cap gene is less pressure than rep gene. Therefore, cap gene has a high level of variation and being a suitable phylogenetic and epidemiological marker [41].

In addition, analyzing 45 PCV2 genomes were isolated from both PMWS–affected herds and non-affected herds in the year 2003 and 2004 in Denmark. The result showed that all isolates was belonged to group 1 and having highly homology (99.4% to 100%). However, a retrospectively phylogenetic analysis of PCV2 genomes isolated from the year 1980 (GenBank accession nr. EU148503), 1987 (EU148504), 1990 (EU148505) was identified being a new group (group 3). Some PCV2 isolates from the year 1993 (EU148506) and 1996 (EU148507) were ordered in group 2. Nucleotide sequence analysis of PCV2 isolates in Denmark indicated that group 1 showed higher genomic homology with group 3 (nearly 95%) than group 2 (91 to 93.6%). The predominant differences between these groups were detected in capsid protein, leading to low cross protection in pigs between group 1 and 2. These findings demonstrated that PCV2 genomes changed over time and globally shifted from group 2 to group 1 (from 2003), which is evaluated being a potentially pathogenic group in many countries [38, 40, 42, 43].

The genotype of PCV2s continuously substitute within PCV2 group 1. Namely PCV2a was commonly found from 2007 to 2009 in Portugal, prior to the detection of PCV2b

[39]. Moreover, subtyping PCV2 DNA derived from colostrum and serum of both sows and pre-suckling piglets indicated that PCV2a&b have been presented. Yet PCV2b is predominant and more virulent comparing to PCV2a [44], even among PCV2b subtypes having different virulence [45]. The concurrence of both PCV2a&b were frequently detected in both healthy herds and affected herds [46, 47]. The prevalence of PCV2a&b could lift up PCV2 replication and is a cause of vaccine failure [47]. Sequencing the whole genome of PCV2a&b isolates revealed 95.7% resemblance. While analyzing DNA sequences of two PCV2b isolates showed that there are only two base pairs different, resulting in 99.9% nucleotide sequence identify. Sequencing capsid genes of PCV2a&b revealed 92.2% similar that encode 93% of amino acid resemblance, while replication genes were less variable (98.5%) [45].

Although PCV2b have been predominant worldwide, PCV2c was detected in Danish and Indonesia [38, 40]. PCV2d with 1766 bp was shifted from PCV2a&b and become a predominant PCV2 strain in China [48]. Recently PCV2b/1C has been prevalent in pig population in Shangdong province, China [49]. PCV2e has been prevalent parallel to a prime PCV2b in Thailand as well [50]. In combination of these findings and according to analyzing nucleotide substitution of PCV2 genomes in many countries, nucleotide substitution rate of PCV2s was placed on the order of 1.2 to $3.12x10^{-3}$ and $6.57x\ 10^{-3}$ substitutions/site/year [43, 51, 52]. PCV2 viruses were recognized being single stranded DNA viruses having highest rate of mutation. Furthermore, it is likely that PCV2 have been spread via asymptomatic animals and meat traded between countries over the world [40, 51, 53].

2.1.4 Porcine Circovirus Associated Diseases (PCVAD)

The term post-weaning multisystemic wasting syndrome (PMWS) was designated being a new disease in swine herds in many countries in the 1990s. Results of several retrospective surveys indicated that PMWS has been found as early as years of 1986 in Spain and Switzerland, 1989 in Japan, 1993 in Thailand, 1969 in Belgium, 1973 in Northern Ireland and 1985 in Canada [54, 55]. The disease was commonly found in pigs with 5 to 12 week of age. The infected pigs developed several forms of clinical signs including progressive weight loss, tachypnea, dyspnea, jaundice, diarrhea, and

pallor. Moreover, the microscopic lesions were commonly found in interstitial pneumonia, lymphadenopathy, hepatitis, and nephritis, myocarditis, enteritis, and pancreatitis. To determine the presence of PCV2 in tissue samples, immunohistochemistry and in situ hybridization assays were used. The results showed that the virus can be detected in several viscera, including lung, liver, kidney, lymph nodes, spleen, tonsils and ileal-Peyer's patches [16, 56, 57]. However, the coinfection of other pathogenic agents consisting of parvovirus (PPV), porcine reproductive and respiratory syndrome (PRRS) virus could trigger PCV2 replication, leading to the development of PCVAD [16].

According to an experimental study, pig inoculated with both PCV2 and PPV developed more severe conditions comparing to only PCV2 or PPV infection. These clinical signs consist of hepatomegaly and enlarged kidneys, severe macrophage infiltration, syncytia formation and numerous cytoplasmic and nuclear amphophilic inclusion bodies in lymphoid tissues. Granulomatous lesions are obvious in liver, lung, kidney, pancreas, myocardium, intestines, testis, brain and salivary, thyroid and adrenal glands [58]. Moreover, in one case-control study found that the mortality rate in nursery pigs was dramatically increased when PCV2 concurrent infection with at least one of other pathogens, such as *Mycoplasma hyopneumoniae*, PRRS, and *Escherichia coli* K88 [59]. These indicated that co-factors play an important role to develop severe PMWS clinical signs.

Porcine dermatitis and nephropathy syndrome (PDNS) is a skin syndrome that associated with PCV2 infection. The acute skin lesions were started with a round to irregular, red to purple macules and papules. The distribution of lesions expanded to the perineal area of the hindquarters, limbs, dependent parts of the abdomen and thorax, and margins of the ears. Porcine reproductive and respiratory syndrome virus (PRRSV) were often detected in macrophages sited around blood vessels of kidneys and skin tissues by immunohistochemistry. That evidence demonstrated that the concurrence of PCV2 virus and PRRSV in pig caused PDNS [60, 61].

In respective aspects, several cases of abortion, PCV2 was isolated from the fetus of late term abortion sows, especially from litters showing mummified, macerated, autolyzed, and fresh stillborn piglets. Moreover, PCV2 can replicate in embryos before

21 days, leading to embryonic death ([26, 62]. These indicated that PCV2 could be a cause of reproductive failure with the vertical transmission [19].

2.1.5 Distribution of porcine circovirus associated diseases (PCVAD) in Thailand

According to a study on detection of PCV2 from submitted pig carcass and sick pigs to Chulalongkorn University diagnostic laboratory Nakhonpathom, from 2006 - 2010, there was approximately 34-65 percent of necropsied pigs infected with PCV2 throughout the year. The percentage of PCV2 infected pigs reached a peak in October [63]. Nursery pigs of the extensive farming system in central Thailand were more frequently infected with PCV2 or co-infected with other viruses as PRRSV and CSFV than the suckling pigs. Moreover, in a recent study, fecal swab samples and whole blood samples were collected from 5-10 week old piglets from negative-PMWS and PMWS-affected farms located in eastern, north-eastern, western and central parts of Thailand (figure 2.1). Of 37.14% fecal swab samples, 7.14% positive with PCV2 viruses were determined as negative-PMWS farms and 50% positive with PCV2 viruses as PMWS-affected farms. There was 57.14% of PCV2 viremia found through examining whole blood samples derived from PMWS-affected farms as well. Viremia was not found in negative-PMWS farms. This results in PCV2 viruses found in PMWS-affected farms (67.14%) were by far, higher than negative-PMWS farms (7.14%). According to analyzing ORF2 sequences of Thai PCV2 isolates, phylogenetic trees revealed that predominant genotypes of PCV2 viruses were PCV2b (group 1) with clusters 1A/B and 1C (80%). A few PCV2 isolates found were PCV2a and PCV2e [50, 64, 65].

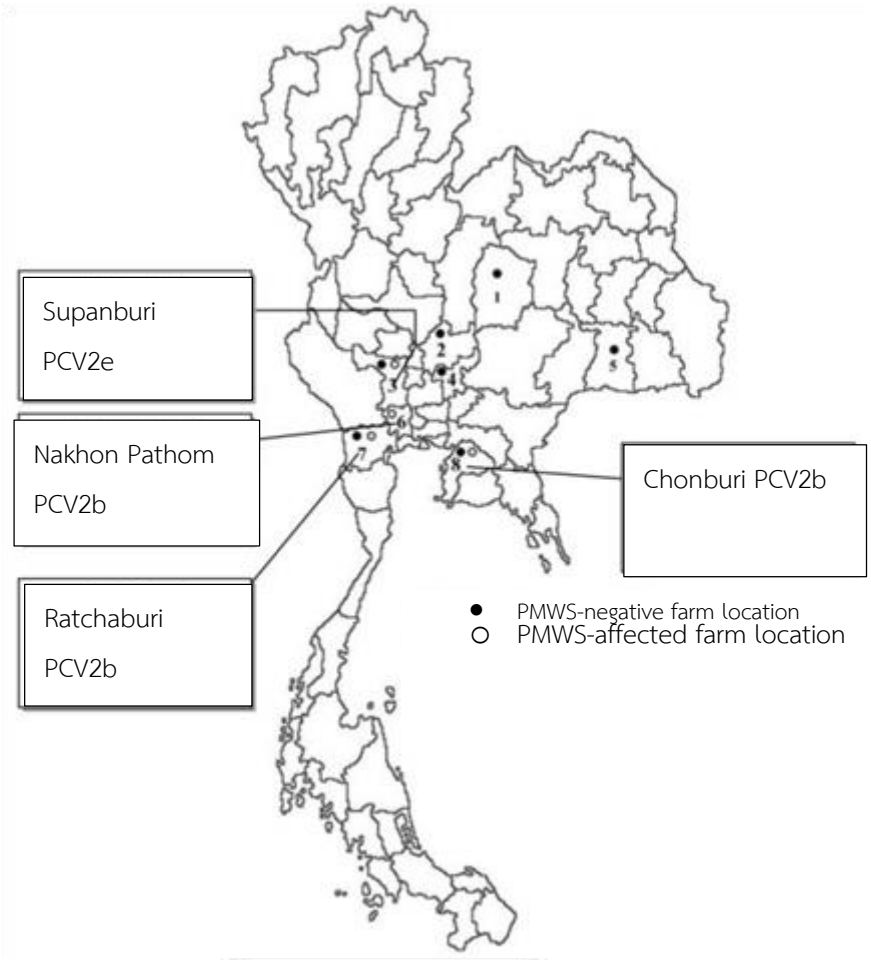


Figure 2. 1 Distribution of porcine circovirus type 2 in Thailand

2.1.6 Shedding and transmission of PCV2

According to a case–control study, the seroprevalence of other pathogens, including Aujeszky's disease virus (ADV), porcine parvovirus (PPV), porcine reproductive and respiratory virus (PRRSV), swine influenza virus (SIV), *Actinobacillus pleuropneumoniae* (Ap), *Lawsonia intracellularis* (law), *Mycoplasma hyopneumoniae* (Myc), *Salmonella spp*. (salm), *Pasteurella multocida* (PMT), was observed parallel to monitoring PMWS occurrence in PMWS affected farms in Spain and Denmark. The PMWS might be mild or severe or asymptomatic, depending on the herd immunity of those farms. PMWS cases occurred in a wide range of age, from nursery to fattening stage that correlated with PCV2 load increased and maternal antibodies minimized. Additionally, the more severe disease express, the more PCV2 is shed in pig environment [8]. Infected pigs continuously shed PCV2 viruses via oral, nasal secretions, urine and feces [4, 66]. PCV2

shedding through colostrum was suggested as well [67]. Subsequently, PCV2 has frequently detected in air samples and waste water collected from pig farms [68, 69]. Although direct and indirect transmission of PCV2 plays different levels of risk factors, the horizontal transmission has been recognized as a prominent route related to PCV2 spread. The most potential factor associated with PMWS spread is nose to nose contact between healthy pigs and PMWS infected pigs or close exposure to secretions of infected pigs [7-9, 70]. The movement of live pigs from affected to healthy farms is considered to be a way in which PCV2 spread from farm to farm. An experiment with newborn cross-fostered piglets also showed the speedy spread of PCV2 from infected to naïve pigs [71]. Feeding uncooked positive PCV2 tissues consisting of lung, lymph node, bone marrow, is another important route of PCV2 spread that pigs become viremic after 7 days of ingestion [72]. PCV2 shedding in semen was demonstrated in several studies in which boars experimentally inoculated or naturally infected with PCV2. The results showed that PCV2 DNA in semen was initially found after 5 days of inoculation. PCV2 shedding prolonged until 47 days after inoculation but 27.3 weeks in cases of boars naturally infected with PCV2. This evidence indicated a potential risk of vertical transmission through artificial insemination [73, 74].

2.1.7 To control and prevent the spread of PCVAD

Since inactivated PCV2 vaccine (Circo vac, Merial) was initially introduced to the market, three other subunit vaccines (Circoflex, Boehringer Ingelheim; Circumvent, Intervet/Merck; Porcillis PCV, Schering-Plough/Merck) and another inactivated chimaeric PCV1/2 vaccine (Fostera PCV; Pfizer Animal Health) were subsequently developed to protect pig herds against PCVAD [75-78]. Due to the fact that maternal PCV2 serological profiles might be various between pig farms, almost commercial vaccines focus on protecting piglets against early infection of PCV2, with the exception to one vaccine (Circo vac), which is used to stimulate acquired immunity in sows. Acquired immunity could retain at least 13 weeks after immunization [79] demonstrated the efficacy of vaccination against PCV2 infection in growing and fattening stages as well. According to several studies investigated on the effectiveness of PCV2 commercial vaccines against PCVAD. The results showed that all PCV2

commercial vaccines could reduce mortality rate in growing pigs, viral load, as well as PCV2 shedding in PMWS affected farms. Furthermore, growth performance and antibody titers specific to PCV2 are significantly increased [80-82]. Although the more innovative vaccines were built up, the more efficacy of vaccines against PCVAD achieved, retaining PCV2 viremia among immunized pigs indicated a drawback of PCV2 vaccination [79, 83].

Alarcon et al (2011) and (2013) conducted several studies on 147 English farms in which risk factors and levels of PMWS occurrences were considered for 5 years. Total 5 strategies consisting of PCV2 vaccination, improving of biosecurity measures, adjusting diet for the grower, reducing of stocking density, total depopulation and repopulation were carried out to control the spread of PMWS or PCV2 subclinical infection. Basing on a good or a poor biosecurity application, one or a combination of those strategies was selected to be monitored. Results of the study indicated that for a good biosecurity condition, PCV2 vaccination was a relatively possible option making the most economic benefit in both severe PMWS affected farms and moderately PMWS affected farms. For affected farms with poor biosecurity conditions, PCV2 vaccination combining with improvement of biosecurity measures showed the best option that brought the most profitable, particularly, in severe PMWS affected farms. Other strategies, such as a combination of vaccination and balance diet, biosecurity measures and diets, which were ranked in the second or the third options due to less economic efficiency, recommended as complemented strategies basing on circumstances of particular affected farms [2, 3].

Patterson et al (2011a) evaluated the effectiveness of four disinfectant protocols in reducing the amount of PCV2 contaminated in 1:61 scale model livestock trailer, followed by a determination whether PCV2 persisted in the model possible infect to SPF pigs. Four disinfectant compounds represented to four disinfectant protocols (DISF), consisting of quaternary ammonium compound (DISF 1), oxidizing agent containing potassium peroxomonosulfate (DSF2), glutaraldehyde and quaternary ammonium compound (DSF3), DISF4 is a combination of DISF2 followed by adding sodium hypochlorite compound. Sampling on the surface of trailers was carried out before washing, after washing, after applying disinfectant protocol and after exposure

to animals. After exposure to the disinfected trailers, serum samples were also collected weekly for 7 weeks. The results showed that washing significantly reduced the amount of PCV2. After disinfection, PCV2 DNA was detected in trailers, ranging from 1.5 $\log_{10}$ to 5.3 $\log_{10}$. However, both PCV2 viremia and seroconversion were not found in pigs after exposure to disinfected trailers. This result led to a conclusion that selecting a proper disinfectant applying to transport vehicles might reduce PCV2 transmission [84].

2.1.8 Biological and physiochemical properties of PCV2

Porcine circoviruses are small, non-enveloped, icosahedral viruses. The viruses possess a single capsid formed from 60 subunits in which each unit is a flat pentameric morphological unit [85]. According to analyzing crystal structure of the capsid, PCV2 subunits fold is characterized as a canonical viral jelly roll, which is composed of two sheets containing four antiparallel β strands. The loop, which is formed by the interaction between residues of subunits, alternate the length of β strands to form residues of the loop. Long loop are crucial to characterize the feature of the capsid and interact with neighboring loops to stabilize the capsid. The loops on the surface of the capsid contribute to form the icosahedral 3-fold axes and two protrusion, icosahedral 5- and 2- fold axes, in which several residues, located on the loops or on the capsid shell, play as epitopes. The epitopes are identified by monoclonal antibodies and used to differentiate 8 genetic clusters (PCV2b-1A to PCV2b-1C and PCV2a-2A to PCV2a-2E) and 2 genotypes (PCV2a and PCV2b). The common epitopes between PCV2a and PCV2b locating in between the amino acids 48 and 233/234 interact with antibodies [86, 87].

According to analyzing the capsid of $PCV2^{N12}$ VLPs via cryo-EM image, the strongest density is near the 3-fold axes, while the weakest is near the 2-fold axes, with the presence of the sulfates. It is predicted that a heparin sulfate binds to the highly conservation PCV2 sequence forming the hydrophilic pocket, near the 2-fold axes [86, 88]. In addition, PCV2 used heparin sulfate and chondroitin sulfate B as receptors to entry into the host cells [89]. Due to the heat stable property of heparin sulfate binding sites, porcine circoviruses are recognized as heat resistant viruses. Hence, PCV1 remains

stable at pH 3 and 70^0C for 15 minutes as well as treated by chloroform [90].

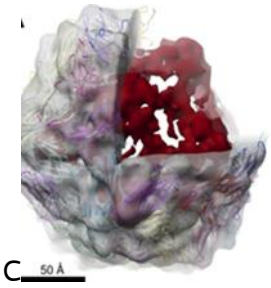


Figure 2.2 The gray and red area representing the capsid and the nucleic acid of a typical PCV2 virus [86]

A study applied such standards inactivating viruses, such as pasteurization (10hr at 60^0C) and dry heat treatment (80^0C for 72hr) to treat PCV2. The results showed that PCV2 viral titer was reduced 1.33 logs by pasteurization and 0.75 logs by dry heat treatment. PCV2 relatively resist to extreme dry heat treatment (at 120^0C for 30 minutes) but remarkably reduced (3.5logs) by wet heat treatment, as the temperature increased up to 80^0C [12]. PCV2 in suspended cell cultures could be inactivated at 80^0C for 15 minutes as well. This evidence showed that the temperature used to inactivate PRRSV or envelope RNA virus could fail in killing PCV2 [11]. In comparison to other non-enveloped viruses, the resistance pattern of CAV showed similar to PCV2 [12]. Hepatitis A virus and canine parvovirus, with the similar pattern of resistance to dry heating, could be inactivated at 80^0C after 24h or 90^0C before 6h. Canine parvovirus was completely killed at 80^0C after 48h or 10h at 90^0C [91]. The effectiveness of dry heat treatment was also found to inactivate bovine parvovirus [92]. Parvovirus B19 was rapidly inactivated by wet heating as well [93, 94]. Those evidence showed that circoviruses are the most resistant to heat temperature comparing to other non-enveloped viruses (Figure 2). Kim et al., (2009) also illustrated that PCV2 could survive in a wide range of pH and only inactivated at pH12.

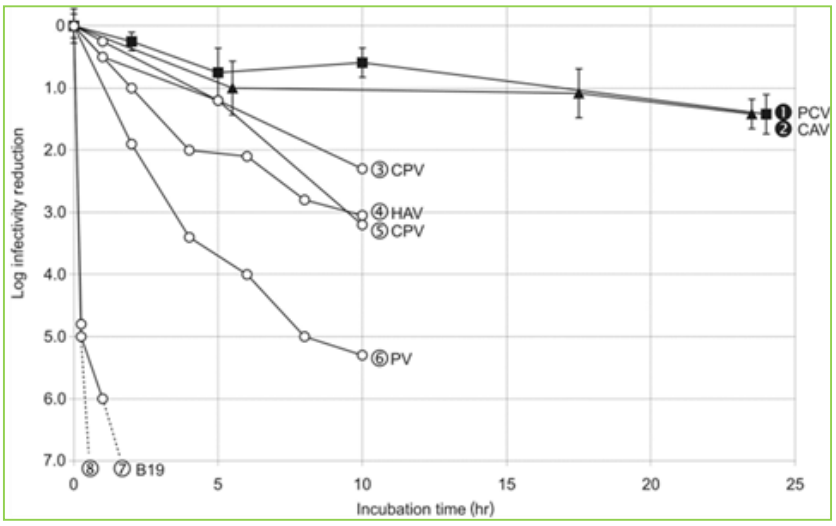


Figure 2. 3 In comparison the resistance of PCV2, CAV and the other non-enveloped virus to pasteurization. PCV2 (■, graph series 1), and CAV (▲, series 2), other viruses (○): parvovirus B19 (graph series 7) and canine parvovirus (CPV; series 5), CPV (series 3) and B19 (graph series 8), human hepatitis A virus (HAV; series 4) and human poliovirus 1 (PV; series 6) [12].

Furthermore, the infectivity of PCV2 after exposure to various chemical compounds in suspension test was determined by few previous studies. Results of Royer et al (2001) showed that after 10-minute exposure, the oxidizing compound is the most effective to inactivate PCV2, with approximately 4log reduction. The infectivity of PCV2 could be reduced by 3 logs by aldehyde quaternary ammonium and quaternary ammonium compounds, followed by 2 log PCV2 reduction by phenol compound. No significantly PCV2 reduction (less than 2 logs) was found in the contact between PCV2 and Iodine, formaldehyde and chlorhexidine [95]. According to increasing the exposure time up to 30 minutes, the survival pattern of PCV2 was relatively similar to the previous study [14]. However, a recent study showed that some oxidizing compounds comprising a mixture of potassium peroxomonosulfate and sodium chloride, and sodium hypochlorite could completely inactivate PCV2 within 10 minutes. PCV2 was undetectable after 30 minutes exposure to a formaldehyde and quaternary ammonium compound, and a glutaraldehyde and quaternary ammonium compound [13]. In Thailand, there was one field study using potassium peroxomonosulfate and sodium dichloro isocyanurate for controlling PCV2 under field condition. The result

showed that the compounds significantly reduced the amount of PCV2 load in pig environment [15].

2.2 Methods of testing disinfectant

Nowadays, a variety of methodologies, which have been progressed for 150 years, were carried out to evaluate the efficacy of disinfectants over the world. Retrospectively, a preparation of infected carrier and suspension test were initially built up by Robert Koch and Rideal –Walker respectively [96, 97]. Subsequently, a variety of national suspension standards including the qualitative suspension test of the German Society for Hygiene and Microbiology DGHM, the quantitative suspension test of the Dutch Committee on Phytopharmacy, the use-dilution method of the A.O.A.C. and the Kelsey and Sykes' test were evolved in several laboratories around the world. In addition, bactericidal activity of those national standards was compared by Reybrouck et al (1975). There was a conclusion that the technique of each disinfectant efficacy test has its own characteristic. It was impossible to establish a correlation between the efficacy results of those standards [98]. The conflict of those methods was continuously tracked by European Committee for Standardization (CEN), with the objective to design a reference method acceptable for all member countries. After reevaluated by 10 laboratories and source of variation figured out, a more evolved standardization suspension test (SST) was consulted and published as European suspension test (EST), which is widely used up to now .

The limitation of suspension test is initially recognized as a general test in which determined effectiveness of disinfectants against microorganisms is by far from the practical condition and provides poorly predicting the efficacy of disinfectants in field condition. Carrier and surface tests are in the line with further methods to evaluate more reliable the efficacy of disinfectants. Carrier and surface tests were recognized as practical tests divided into two sub-methods in which microorganisms are dried on carriers or carriers are rinsed in a microorganism suspension, followed by exposure to disinfectants [99]. However, several technical factors pertaining to various surface tests leading to divergent results were mentioned by Reybrock et al [100]. An extensive comparison of these tests consisting of the DGHM test of the German Society for

Hygiene and Microbiology, the AFNOR test NF-T 72-190 of the French Association for Standardization, the quantitative carrier test (QCT) of Van Klingeren, the Leuven test and the quantitative surface disinfection test (QSDT) was carried out. These factors including inoculum preparation, organic load, exposure time, recovery procedure, and interpretation were consulted to figure out the diverse results [100, 101].

Furthermore, a requirement of disinfectants in the use - condition urged to figure out test methods in which efficacy of disinfectant is more appropriate to field conditions. Manufacturers also need to demonstrate the efficacy of their disinfectant products at certain concentrations. Subsequently, recommended dilutions are labeled for in-use conditions. Therefore, a guideline of test methods was built up by the European Committee for Standardization (CEN) to satisfy those demand. According to the guideline, a set of test methods consists of 3 phases in which phase 1 is a suspension test evaluating the general efficacy of disinfectant products. Phase 2 is subdivided into 2 steps in which the suspension test is used in step 1. Phase 2 step 2 with a surface test is applied as a practical test. Both phase 1 and phase 2 are performed in the laboratory. Phase 3 is on the progress serving for a field test [102].

Nowadays, globally distribution of disinfectant products has been mainly under the regulation of the European Committee for standardization (CEN) in Europe, Environmental Protection Agency (EPA) in the USA and globally the Organization for Economic Co-operation and Development (OECD).

2.2.1 Viral suspension tests

It is noted that the act of viruses to chemical disinfectants is by far from the bacterial act. Normally, viruses have a broader spectrum of stability, leading to high resistance against disinfectants. It is due to the properties of viruses, enveloped or non-enveloped viruses and classified lipophilic or hydrophilic viruses [103]. It is likely that evaluating the efficacy of disinfectants against viruses is based on both selecting test methods and representative viruses. Standard test methods, specific to virucidal tests, issued by international legal associations such as CEN, EPA and OECD are effectiveness. Moreover, reference viruses commonly selected to simulate standard tests represented groups of viruses such as poliovirus, parvovirus SV 40, adenovirus and vaccinia virus [102, 104].

According to the legal guidelines for suspension tests in which test parameters such as temperature, organic load, and volume ratios are kept unchanged. There is at least 4 log_{10} viral reduction, representing approximately 99.99% of virus inactivation; then disinfectant is accepted as a virucidal.

Although the suspension test belonging to phase 2 step 1 is a basic test having some limitations, the efficacy of disinfectants against viruses from suspension tests could not represent to the efficacy of disinfectants applying to field conditions but comparative to recommended concentrations that are labeled in disinfectant products [101]. To demonstrate the efficacy of disinfectants in practical conditions, suspension test must be the former test followed by a surface or carrier test. Moreover, suspension tests are recognized as an initiate test taken part in the procedure of evaluating the efficacy of disinfectant products. Although a number of log_{10} virus reduction ($3log_{10}$) in the surface test is commonly smaller than in suspension test, the result of virus reduction in suspension test is utilized as a positive control in surface test [99, 101, 102, 105].

2.2.2 Development of diagnose techniques to determine viral survival

Since disinfectant test methods had been introduced to prevent the infection and transmission of microorganism by Robert Koch [96]. At that time, the major problem of the test methods was hard to determine exactly the time bacteria exposure to disinfectants. Mimicking primary test methods targeting on bacteria and fungi, virucidal test were modified to deal with previous problems found in bactericidal tests. The first alternative virucidal test in which virucidal activity of 17 virucidal agents against two representative viruses, such as influenza A virus and vaccinia virus, was selected to investigate by Groupe'. Serial 10-fold dilution method was applied to immediately terminate the act of disinfectant on those viruses after 10 minutes of exposure at room temperature or 37^0C in a water bath. The infectivity of viruses after exposure to disinfectant agents was determined by infecting the allantoic cavity of egg embryos [106-108]. Subsequently, a comparison between suspension test and carrier test, with mimicking Groupe's study, was carried out by Wright. Wright performed a study on 20 disinfectants against vesicular stomatitis virus. Some new points in which suspension virus was placed on the surface of the chorioallantoic membrane (CAM) comparing to

injecting into the allantoic cavity were figured out in the study, leading to different results. The former, the CAM infection ($10^{7.5}$) was more effective than the latter, the allantoic infection ($10^{5.4}$). Suspension test was also more effective than carrier test. Particularly, porcelain cylinders soaked in virus suspension then dried without disinfection comparing to porcelain cylinders soaked in virus suspension without dried. The former, the virus titer was by far lower (approximately $10^{1.7}$) than the later (greater than $10^{6.5)}$ [107]. Determining the infectivity of the virus after exposure to disinfectants was also conducted in host animal consisting of mice, guinea pig [109].

The evolution of the cell line and other technologies brought a remarkable change in virucidal test methods in which virus after exposure to disinfectant was infected into cell culture. Subsequently, the very low titer of virus infectivity was detected by laboratory assays. However, cytotoxicity, when cell culture is applied to the disinfectant test, is critical. Therefore, several detoxified methods were carried out. Of that letheen broth and skim milk were used as neutralizers. Gaustad conducted a study using quaternary ammonium compound, phenolic, iodophor against Herpes simplex virus, MP strain, poliovirus, type 1 Brunhidle strain, vaccinia virus and WR strain. Letheen broth was used to detoxify cytotoxicity to HEp-2. The results showed that Letheen broth was significantly effective to inactivate toxicity from those disinfectant compounds [110] but less efficient to eliminate aldehyde compounds. In another study, an effective neutralizer as skim milk was simulated to neutralize the toxicity of various disinfectant compounds [111]. High-speed centrifugation (150,000xg for 3h at 4^0C) was also found to eliminate the virus from hand solution samples [112]. Eventually, the majority of virucidal tests utilized gel filtration and Sephadex LH20 as the optimal detoxification methods to any disinfectant compound [13, 113, 114].

When cell culture was initially introduced in virucidal tests, a plaque assay was used to determine viral titer [111, 115]. PCR and RT-PCR were also used to prove the presence of virus after detoxification [15, 84]. However, more reliable and most commonly used assays to determine the infectivity of virus in disinfectant tests are indirect immunofluorescent assay and indirect immune-peroxidase monolayer assay [13, 14, 114].

2.2.3 Immunofluorescence assay (IFA) and Immunoperoxidase assay (IPA)

Immunofluorescence assay (IFA) and immune-peroxidase assay (IPA) are of the longitudinal standard techniques applied in both clinical laboratories and scientific research worldwide. For IFA, fluorescent-labeled antibodies are used to detect specific target antigens directly. Indirect immunofluorescence assay (IIFA) are more complicated than IFA due to a two-step technique, in which a primary antibody (unlabeled antibody) binds to the target antigen. Of the following steps, fluorescent-labeled secondary antibodies is used to detect the first antibody [116]. However, to avoid nonspecific antibody binding, retain the location and antigenicity of the immune complexes, several techniques which include preparing proper quality and concentration of the labelled antibody, separating tissue samples and handling are considered. Moreover, UV light and a suitable plain light avoiding the fluorescence image to be faded are required [116]. To cope with several limitations of IF methods, IP methods are built up. The principle of the Immuno-peroxidase assay (IPA), indirect immune-peroxidase assay (IIPA) and immune-peroxidase monolayer assay (IPMA) is similar to IF methods but enzyme-labeled antibodies are altered, rather than fluorescent-labeled antibodies. Due to the alterative of enzymes conjugating to antibodies, particularly, horseradish peroxidase-labelled antibodies (HRP), the image outcome of the IP method is visible under the electronic microscopy, coping with the need of UV microscopy for fluorescence detection [117]. Moreover, IIPA and IPMA methods have more advantages than IP method due to increased sensitivity and the small amount of peroxidase conjugate required [118]. .

Basing on detecting target antigens placed in tissues or antibodies generated by the immune system, IF and IP methods are adjustable methods which are used to stain not only frozen tissues (IF), paraffin wax tissues (IP), biopsies and serum of various species but also tissue culture (IFA and IPMA) [119-121]. Therefore, IF and IP methods are widely utilized in immune-histopathologic evaluation to prove the presence of various potential pathogens in various infected tissues [122-125]. According to several studies using IF and IP staining for immunoglobulins (IgG, IgA, IgM) in human renal biopsy specimens, the results demonstrated a high agreement between IF and IP methods in

detecting immunoglobulin prevalence [119, 120]. In routine diagnosis, IIF, IIP and IPMA are utilized to screen sero-prevalence, neutralizing antibodies of various diseases, clearance of the infection etc. [82, 126-129].

For measuring antibody titer of PCV2, IPMA was proven as a standard technique and widely used to determine antibody titers in several studies [75, 128, 129]. In comparison of IPMA and neutralizing antibody (NA) assay to quantity PCV2 antibodies, there is a positive correlation between two methods [129]. Finding the correlation between IPMA and three commercial ELISAs for detection of PCV2 antibodies was carried out by Pileri et al., (2014). The results indicated that there were high sensitivity (90%) and highest specificity (100%) between IPMA and ELISAs [130].

2.3 Activity of disinfectant against viruses

Disinfectants are chemical compounds classified as antimicrobials due to the antimicrobial activity of disinfectants. However, disinfectants have a broad spectrum activity that focuses on multiple targets on the surface of microorganisms, comparing to antibiotics mainly affecting to intracellular targets. In practice hygiene, disinfectants have been widely used to reduce or inactivate pathogens existing on the surface of facilities. A successful disinfectant protocol could be effective to control and prevent the spread of contagious diseases, particularly, in cases of the outbreaks of diseases. However, the effectiveness of disinfectants against viruses could vary due to the interaction between chemical properties of disinfectants and bio-physiochemical properties of viruses [131]. Enveloped viruses which have a membrane containing lipid bilayer are classified as lipophilic viruses. Therefore, there is an affinity between those viruses and disinfectants having lipophilic properties, such as phenols, quaternary ammonium compounds, biguanides (chlorhexidine). This results in enveloped viruses susceptible to those disinfectants. While some virucidal agents such as glutaraldehyde, hypochlorite, hydrogen peroxide, iodine, chlorine which strongly interact with amino and sulfhydryl groups are effective against non-enveloped viruses [131-133]. In addition, some disinfectants, such as iodine and chlorine, could inactivate almost enveloped viruses and non-enveloped viruses. However, in general, various

modifications of concentrations of disinfectants could change the efficacy of disinfectants against those viruses [134].

2.3.1 Group of disinfectants and their mechanism of action

Aldehyde (R-CHO)

The nature structure of aldehyde contains an alkyl group (R-) and an aldehyde group (-CHO). The modification of alkyl group correspond to the substitution of the chemical properties of aldehydes. Mono-aldehydes (R-CHO), with the simplest R- group, except to formaldehyde, might not have antimicrobial activity due to the fact that they are easily metabolized to acids and lose the function to adhere to the cell. Di-aldehydes (CHO-CHO) have more stable antimicrobial activities than mono-aldehydes. Due to the substantially antimicrobial properties, glutaraldehyde and formaldehyde are commonly used for disinfection purpose [131, 135].

In molecular aspect, aldehydes tend to react with amines and sulfhydral groups of proteins and nucleic acids. According these chemical mechanisms, aldehydes could interfere the enzymatic activity, the synthesis and the function of the nucleic acids, which subsequently restrict other biochemical and physiological events of the cell. For formaldehyde, particularly, at a high concentration, it not only interacts with amino group, forming inter- and intramolecular crosslinking with phenolic and indole residues of protein amino group but also react with DNA and RNA. However, RNA-formaldehyde interaction is stronger. Glutaraldehyde has similar antimicrobial activity to formaldehyde but more effective due to the fact that glutaraldehyde possesses two aldehyde groups on the same molecule. The hydrophobicity on the hydrophilicity plays an important role in the penetration of aldehydes into the cell. The reaction of glutaraldehyde with protein significantly increases not only due to alkaline pH but also the presence of cations as Ca and Mg, and organic matter. However, the requirement of the concentration of glutaraldehyde depends on enveloped (lipophilic) or non-enveloped (hydrophilic) viruses. More than 1% of glutaraldehyde is effective against non-enveloped viruses but approximately 0.2% of glutaraldehyde could inactivate enveloped viruses [131, 135].

Halogen- releasing agent

Chlorine releasing agents (CRAs)

The most important types of chlorine releasing agents are sodium hypochlorite, chlorine dioxide and sodium dichloroisocyanurate (NaDCC). Both sodium hypochlorite and sodium dichloroisocyanurate are widely used for hard surface disinfection. The advantage of NaDCC is that it releases higher concentration of available chlorine and being less susceptible to inactivation by organic matter. In water, the equilibrium of hypochlorite ion, OCl^-, Na^+ and hypochlorous acid base on the modification of the pH. Between pH 4 and 7, HOCl is predominant, whereas above pH 9, ClO^- predominant. It is considered that CRAs are highly active oxidizing agents destroying the cellular activity of protein, particularly, at low pH, in which the activity of CRAs is maximal. This corresponds with the highest percentage of undissolved HOCl, which could disrupt the oxidative phosphorylation. Furthermore, CRAs could inactivate viruses by disrupting the nucleic acids and modifying the morphology of the capsid proteins [131, 135].

Iodine and Iodine releasing agents

Povidone-iodine and poloxamer-iodine are widely used for disinfection purposes. The antimicrobial activity of iodine and iodine releasing agents are rapid and similar to chlorine. Rapid penetrating and attacking key groups of proteins, nucleotides and fatty acids of the microorganisms, leading to the cell death, are antimicrobial activity of iodine group. The reaction of iodine with bacteria and enveloped viruses is similar, whereas, non-enveloped viruses are less sensitive, particularly, parvovirus [131, 135].

Quaternary Ammonium compounds (QACs)

Quaternary ammonium compounds are cationic agents (surfactants, surface-active agents), which reduce the surface tension and form micelles, leading to dispersing in the liquid. According to the low toxicity and being able to be formulated for specific application and target organisms, QACs are widespread used for cleaning, sanitizing and disinfecting surface. QACs comprise a central nitrogen and four branches of R groups, forming the cation portion. The common negatively charged anion portion (-X) is chlorine or bromine, which links to the nitrogen forming the QACs salts. The R group can be a branch of carbon chain, an aromatic ring or including the number of nitrogen

atoms. The nature of the R groups, which help to classify QACs, modify the antimicrobial activity of QACs.

The action of QACs mainly consist of penetrating the cell wall of microorganisms, reacting with cytoplasmic membrane (lipid or protein) leading to membrane disorganization. Furthermore, QACs can degrade proteins and nucleic acids and autolyze enzymes. The antimicrobial activity of QACs eventually results in the cell death [136]. For enveloped viruses, QACs effect on lipid but not for non-enveloped viruses. Morphological change inactivating viruses is a further antimicrobial activity of products basing on QACs [131].

Peroxygens

Hydrogen (H_2O_2) is a clear and colorless liquid that is considered as environmentally friendly product due to that fact that it can rapidly degrade into the innocuous products as water or oxygen. Commercial concentrations of H_2O_2 range from 3 – 90% that are widely used for disinfection, sterilization and antisepsis purposes. H_2O_2 have a broad spectrum efficacy against bacteria, viruses, yeasts and bacterial spores. Higher concentration of H_2O_2 (10% - 30%) and longer contact time are required for sporicidal activity. H_2O_2 produces hydroxyl group which targets sulfhydryl groups and double bonds of the essential cell components like lipid, protein and DNA [131].

Peracetic acid (PAA) (CH_3COOOH) is also environmentally friendly products that not only is able to degrade into acetic acid and oxygen but also being decomposed by peroxidase. Similarity to H_2O_2 targeting sulfhydryl groups and sulfur bonds to denature protein, enzymes and increase permeability but PAA has more potential efficacy against bacteria, fungi, bacteria spores and viruses at low concentrations (<0.3%). Particularly, PAA remains the antimicrobial efficacy in the presence of organic load [131, 135].

Phenols, bis-phenols and halophenols

In generally, depending on the compound, phenols and other groups of phenols are widely used for antiseptic, disinfection and preservation purposes for many years. Due to the membrane-active properties, phenols are considered as protoplasmic poisons. Phenols cause leakage to intracellular constituents, including the release of K^+ leading to damaging the cell membrane. Furthermore, phenols act only at the point when

pairs of daughter cell separating. The young bacterial cells are more sensitive than the older cells to phenols.

Triclosan and hexachlorophene (Bis-phenols) are widely used for disinfection purpose as well. Triclosan primarily effects on the cytoplasmic membrane while hexachlorophene inhibits the membrane bound part of the electron transport chain. Chloroxylenol (Halophenols) effects on microbial membrane being considered as bactericidal, exception to *pseudomonas aeruginosa* and many molds are resistant.

CHAPTER 3

MATERIALS AND METHODS

3.1 Virus preparation

The PCV2b virus used in this study was isolated from a pig farm having clinical signs of PMWS and identified by the previous study [65]. The virus was propagated by infecting one-day old SW cells. The infected cell monolayer, maintained in minimal essential medium (MEM), supplemented with 7% fetal bovine serum, was incubated for 5 days at 37^0 C in 5% CO_2 incubator. The infectivity of PCV2 was confirmed by indirect immunofluorescent antibody assay (IFA) at the 6^{th} day of the inoculation. Subsequently, the virus was harvested by three times frozen and thawed, and centrifuged at 8,000xg for 20 minutes. The supernatant was collected and stocked in 50ml tubes in -80^0C until used. The concentration of PCV2b virus titer was quantified by using a titration assay, indirect immune-peroxidase monolayer assay (IPMA) as described by Labarque et al (2000).

3.1.1 Indirect immune-peroxidase monolayer assay (IPMA)

The infectivity and quantification of PCV2b virus was determined by an indirect immune-peroxidase monolayer assay (IPMA) as described by Labarque et al (2000). Briefly, twenty percent of SW cells were cultured in 96-well plates for two days. After washed 3 times with sterilized PBS, 100µl of serial 10 fold dilutions of the PCV2b virus was added into each well. After incubated for 1 hour, the infected cell was maintained with MEM supplemented with 2% fetal bovine serum for 60 hours at 37^0C in 5% CO_2 incubator. The medium was discarded; and fifty percent of acetone in methanol was subsequently fixed in the cell monolayer. The infected plates were kept in - 20 until used.

The IPMA is a standard assay to determine the viral titers. Mouse monoclonal antibody and sheep-anti-mouse IgG peroxidase conjugate were specifically used to detect PCV2b virus. Briefly, the cell monolayer plates were rehydrated for 40 minutes. Then 100µl of mouse monoclonal antibody (1:1000) in PBS with 5% skim milk was added. The infected plate was put in a moisture chamber for one hour, following to wash with

phosphate buffered saline (PBS) (pH:7.4) for 3 times. Subsequently, 100µl of sheep-anti-mouse IgG peroxidase conjugate (1:500 diluted in PBS with 5% skim milk) was added and incubated for one hour. After three times washing, a substrate solution of 3-amino-9-ethylcarbazole in 0.05 M acetate buffer with 0.05% H_2O_2 was added to each well. After 20 minutes at room temperature incubation, the plate was then washed three times with tap water. The IPMA plates were read under inverted-microscope.

3.1.2 Indirect immunofluorescence assay (IFA)

The infectivity of PCV2b was confirmed and titrated as described by Horlen et al (2008a), with slightly modification [137]. To determine the infectivity and viability of PCV2b, 500µl of MEM containing SW-infected cells were collected, after infected with PCV2b for 6 days. The viral suspension was centrifuged at 11,000xg for 5 minutes. Then, the pellet was smeared on a slide and dry at room temperature. The slide was fixed with acetone. The fixed slide was rehydrated with distill water for 5 minutes, then 1-milliliter of monoclonal antibody (1:1000) diluted in phosphate buffered saline (PBS) was added on the surface. Subsequently, the slide was incubated in moisture chamber at 37^0C for 1 hour. After 3-time washing with PBS, 500µl (1:200) fluorescein-labeled anti-mouse IgG diluted in PBS was added. Further steps, the slide was incubated in a moisture chamber for 1 hour, following to washing with tap water for three times. Then, the stained slide was screened under the fluorescent scope.

3.2 Disinfectants

Nine commercial disinfectants (disinfectants 1-9) and two chemical substances against PCV2 were tested in *vitro* condition. For each disinfectant, the product composition and authorized concentration are expressed as the ratio of active ingredients (%) and the portion of disinfectant diluted in distill water and hardness of water. Disinfectants were classified into three groups including oxidizing products (disinfectants 1-3), QAC products (disinfectant 4-7) and iodine products (disinfectants 8-9), alkali (chemical substance 10) and aldehyde (chemical substance 11). The information of disinfectants was listed in table 3.1.

3.3 Evaluating efficacy of disinfectants

Each disinfectant was freshly diluted with distill-water and hardness of water at a concentration of 400 ppm as described by Wattanaphansak et al (2010) [138]. The final concentration of each disinfectant was adjusted to obtain 0.5x, 1x, and 2x of the disinfectant's recommended dilutions. A mixture of disinfectant/virus was incubated at room temperature for four-time points including 1 minute, 10 minutes, 30 minutes, and 12 hours. Five percent of fetal bovine serum supplemented as organic load were immediately added to the mixture. The mixture was detoxified by using Sephadex LH-20 bead (GE Healthcare Bio-Sciences) detoxification column, as described by Royer et al (2001), with some modification. Briefly, two milliliters of a slurry containing 25 percent of Sephadex LH-20 beads were filled in each 3 ml empty syringe with its bottom covered by a cotton bud. The syringe was placed into a 15 ml tube. Five hundred microliters of the mixture were added to the top of the syringe. The mixture passed through the gel and the cotton into the 15 ml tube. The detoxified virus collected had been 10-fold diluted in MEM prior to the viral infectivity was determined. The titers of collected virus were screened by IPMA as described previously.

Table 3. 1 Active ingredients and recommended dilution of disinfectants

Dis.	Tradename	Class of disinfectant	Active ingredients	Labelled dilution
1	Virusnip™ Novartis Animal Health	Oxidizing agent	potassium peroxymonosulfate 50%; and sodium Dichloroisocyanurate 5%	1:100
2	Virkon®S Antec international	Oxidizing agent	Sodium dodecyl benzene sulphonate 13.17%; Potassium monopersulfate 49.4% Available chlorine 10%	1:100
3	Clorox Bleach Clorox	Oxidizing agent	Sodium hypochlorite 8.25% Other ingredients 91.75% Available chlorine 7.85%	1:21.5
4	Ultraxide® Thailand	Glutaraldehyde and QAC	Glutaraldehyde 15%; Alkyl benzyl dimethyl ammonium chloride (QAC) 10%	1:70
5	Omnicide® MSD animal health	Glutaraldehyde and QAC	Glutaraldehyde 15%; Alkyl dimethyl benzyl ammonium chlorides 10%	1:60
6	Omniclean® MSD animal health	QAC and alcohol	Alkyl benzyl dimethyl ammonium chloride 5% and Polyethoxylated alkyl alcohol 6%	1:200
7	Astop®200 Thailand	QAC	Dodecyl dimethyl ammonium chloride 20%	1:2000
8	Biocide®30 Imported product from England	Iodine complex and acids	Ethylated alcohol-iodine complex 2.840%; Sulfuric acid 9.423%; Phosphoric acid 9.530%	1:125
9	Iophor® Thailand	Povidone-iodine	Povidone-iodine 1.5%	1:95
10	Sodium hydroxide	Alkali	Sodium hydroxide	2%
11	Formalin	Aldehyde	Formaldehyde	1:100

Dis.: Disinfectant

3.4 Viral titration

A total number of detoxified PCV2 samples were 264 samples. The infectivity of PCV2b in each sample was examined by using IPMA with 4 replications. The tissue culture infective dose ($TCID_{50}$/ml) described by Reed and Muench (1938) [139] was used to determine the virus titers. Excel software was utilized to calculate $TCID_{50}$/ml; the data was then transformed to $\log_{10}$ $TCID_{50}ml^{-1}$. Four $\log_{10}$ $TCID_{50}ml^{-1}$ reduction was considered as 99.99% of inactivated virus [113]. The disinfectant test procedure was repeated twice in cases of detoxified samples having the results with undetectable PCV2b in the first test. Subsequently, all volume of the collected samples was used to infect SW cells as described above. The infectivity of PCV2b virus was confirmed by using IFA as described previously.

The stock PCV2b virus was diluted in a series of 10-fold dilutions for eight concentrations. Each level of viral dilutions was transferred to infect SW cells. The $TCID_{50}$ results transformed to $\log_{10}$ $TCID_{50}ml^{-1}$ were recorded as the viral control.

According to Reed-Muench method, the dilution of the virus would infect 50% of the test animals. The virus titer of the stock virus is recorded as an infectious dose 50 (ID_{50}). For cell-culture, tissue culture infectious dose 50 ($TCID_{50}$) was used to express 50% of the cell culture infected by the stock virus.

Formula to calculate tissues culture infectious dose 50 ($TCID_{50}$) [139]

$$\mathrm{TCID50} = \frac{(\%\ \text{mortality at dilution next above } 50\%) - 50\%}{(\%\ \text{mortality at dilution next above } 50\%) - (\text{mortality at dilution next below})}$$

CHAPTER 4

RESULTS

4.1 Reduction in PCV2b titer after exposure to 11 disinfectants

According to the three levels of disinfectant concentrations and the length of contact time between the virus and disinfectants, the effectiveness of nine commercial disinfectants against PCV2b Thai isolate measured by the reduction of PCV2b titer is summarized in table 4.1. Viral titers were reported as $\log_{10}TCID_{50}\ ml^{-1}$. The efficacy of disinfectants evaluated according to tested concentrations including 1/2x, 1x, and 2x disinfectant's recommended concentrations. For each concentration of disinfectants, the results of PCV2 titer reduction after exposure to disinfectants were recorded; PCV2b titer without contacting with those disinfectants treated was used as viral controls. The viral control titer was relatively high and remained stable during the test procedure, approximately 7.67 $\log_{10}$ $TCID_{50}$ ml^{-1}. At 1/2x of recommended concentrations, all disinfectant suspension was clear color. Only a mixture of glutaraldehyde and QAC (disinfectant 5) at 1x and 2x recommended concentrations have the opalescence, precipitation. This disinfectant contains a combination of $2.14gL^{-1}$ of glutaraldehyde and $1.43gL^{-1}$ of alkyl benzyl dimethyl ammonium chloride. The cytotoxicity was found in 2x tested concentrations of the oxidizing compound (disinfectant 2).

In general, PCV2b titer reduction was not significantly different between two experiments in which all tested disinfectants were diluted in distill water and hardness of water. This is due to the active ingredients of commercial disinfectants are cared for coping with the impact of environment factors as organic matter and hardness of water to the efficacy of the products.

For oxidizing product, (disinfectants 1, 2, and 3), showed a strong reduction of PCV2b titer even though tested at the lowest concentration (1/2x) (Table 4.1 & Figure 4.1). At 1-minute contacting to PCV2b, the titer was reduced ranging from approximately 3 $\log_{10}\ TCID_{50}ml^{-1}$ to 5 $\log_{10}$ $TCID_{50}$ ml^{-1}. For disinfectant 1, it is a combination of $2.5gL^{-1}$ of potassium peroxomonosulfate and 0.25g L^{-1} of sodium dichloro isocyanurate. At

1/2x recommended dilution, it could reduce approximately 5$log_{10}TCID_{50}ml^{-1}$ PCV2b titers after 1-minute exposure. On the other hand, disinfectants 2 and 3, containing a combination of 0.66gL^{-1} sodium dodecyl benzene sulfonate and 2.47gL^{-1} potassium mono persulfate (disinfectant 2); 1.92gL^{-1} sodium hypochlorite (disinfectant 3), could reduce approximately 3$log_{10}TCID_{50}ml^{-1}$ to 4$log_{10}TCID_{50}ml^{-1}$ PCV2b titers. When the concentration of tested disinfectant increased up to 1x and 2x, the oxidizing products (disinfectants 1, 2, 3) completely inactivated PCV2b. Moreover, when the contact time prolonged up to 10- and 30 minutes, PCV2b inactivation was found at all tested concentrations. The results illustrated disinfectant 1 is the most effective oxidizing product against PCV2b, comparing to other oxidizing products

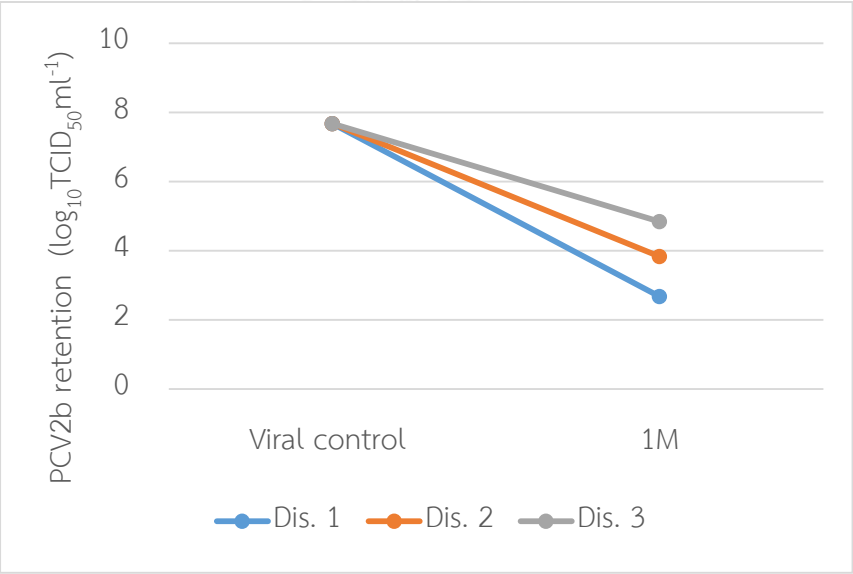


Figure 4. 1: PCV2b retention after 1-minute exposure to disinfectants 1, 2 and 3

Table 4. 1 Reduction in PCV2b titer after exposure to nine commercial disinfectants

		1M		10M		30M		12H	
Disinfectant	Con.	DW	HW	DW	HW	DW	HW	DW	HW
1	1:200	5.00	5.00	7.67	7.67	7.67	7.67	NA	NA
	1:100	7.67	7.67	7.67	7.67	7.67	7.67	NA	NA
	1:50	7.67	7.67	7.67	7.67	7.67	7.67	NA	NA
2	1:200	4.34	3.34	7.67	7.67	7.67	7.67	NA	NA
	1:100	7.67	7.67	7.67	7.67	7.67	7.67	NA	NA
	1:50[a]	7.67	7.67	7.67	7.67	7.67	7.67	NA	NA
3	1:43	2.67	3.00	7.67	7.67	7.67	7.67	NA	NA
	1:21.5	3.07	3.91	7.67	7.67	7.67	7.67	NA	NA
	1:10.75	7.67	7.67	7.67	7.67	7.67	7.67	NA	NA
4	1:140	2.67	2.67	4.67	4.00	5.00	5.00	7.67	7.67
	1:70	3.07	3.57	5.00	5.00	7.67	7.67	NA	NA
	1:35	7.67	7.67	7.67	7.67	7.67	7.67	NA	NA
5	1:120[b]	1.67	2.67	4.67	4.00	7.67	7.67	7.67	7.67
	1:60[bc]	1.67	2.67	5.00	5.00	7.67	7.67	7.67	7.67
	1:30[bc]	2.67	3.34	7.67	7.67	7.67	7.67	7.67	7.67
6	1:400	1.67	2.67	2.67	3.17	3.67	4.00	5.67	5.67
	1:200	2.67	2.67	4.34	3.67	4.00	4.00	5.67	6.17
	1:100	2.34	2.67	3.67	3.17	4.34	4.34	6.17	6.17
7	1:4000	1.34	1.34	2.67	2.67	3.17	2.17	2.67	2.67
	1:2000	2.34	2.17	2.17	2.67	3.17	3.17	6.17	6.17
	1:1000	2.17	2.67	3.00	3.00	3.00	2.67	6.17	6.17
8	1:250	1.67	1.67	2.34	1.34	3.67	3.00	3.34	3.17
	1:125	3.17	3.17	2.67	2.67	2.67	2.67	4.17	4.17
	1:62.5	3.17	3.17	2.67	3.67	4.67	3.67	5.34	5.34
9	1:190	1.67	1.67	2.67	1.34	1.67	2.67	4.34	4.67
	1:95	3.67	3.00	4.00	3.34	2.67	2.34	4.17	4.17
	1:47.5	3.67	4.00	4.00	3.00	2.67	3.67	6.17	6.17
Viral Control		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[a] **:** Cytotoxicity

[b]**:** Opalescence of the mixture: disinfectants diluted with virus suspension

[c]**:** Precipitation of the mixture: disinfectants diluted with virus suspension

1M: 1 minute; 10M: 10 minutes; 30M: 30 minutes; 12H: 12 hours

DW: distill water; HW: hardness of water

NA: did not perform IPMA; Con.: tested concentrations

For QAC products (disinfectants 4, 5, 6, 7), at the lowest concentrations and 1- minute exposure, QACs could reduce from 1.34 to 2.67 $log_{10}TCID_{50}ml^{-1}$PCV2b titers (Table 4.1 & Figure 4.2). Regarding to disinfectants 4 and 5, they contain similar active ingredients which is 2.14gL^{-1} of glutaraldehyde and 1.43gL^{-1} of alkyl benzyl dimethyl ammonium chloride. The results for a combination of glutaraldehyde and QAC showed that the PCV2b viral reduction was found in similar pattern. PCV2b titer reduced approximately from 1.67 to 5.00 $log_{10}TCID_{50}$ ml^{-1} and then completely inactivated after 30-minute exposure at all concentrations. For disinfectant 6, it contains 250mgL^{-1} Alkyl benzyl dimethyl ammonium chloride and 300mgL^{-1}polyethoxylated alkyl alcohol. It could reduce approximately 4$log_{10}TCID_{50}ml^{-1}$ PCV2b titer after a 30-minute exposure. However, 1.5 - 2 $log_{10}TCID_{50}ml^{-1}$ PCV2b retention after 12-hour exposure indicated that remaining disinfectant 6 did not completely inactivate PCV2b. For disinfectant 7, it contains 1 gL^{-1} dodecyl dimethyl ammonium chloride, reduced from 2.67 to 6.17 $log_{10}TCID_{50}ml^{-1}$ PCV2 titer after 12-hour exposure. In the other aspect, approximately 5.00 $log_{10}TCID_{50}ml^{-1}$ PCV2b retention at 1/2x labelled dilution indicated that disinfectant 7 diluted at the lowest tested concentration was not virucidal against PCV2b, considered as a negative control, equivalent to viral control.

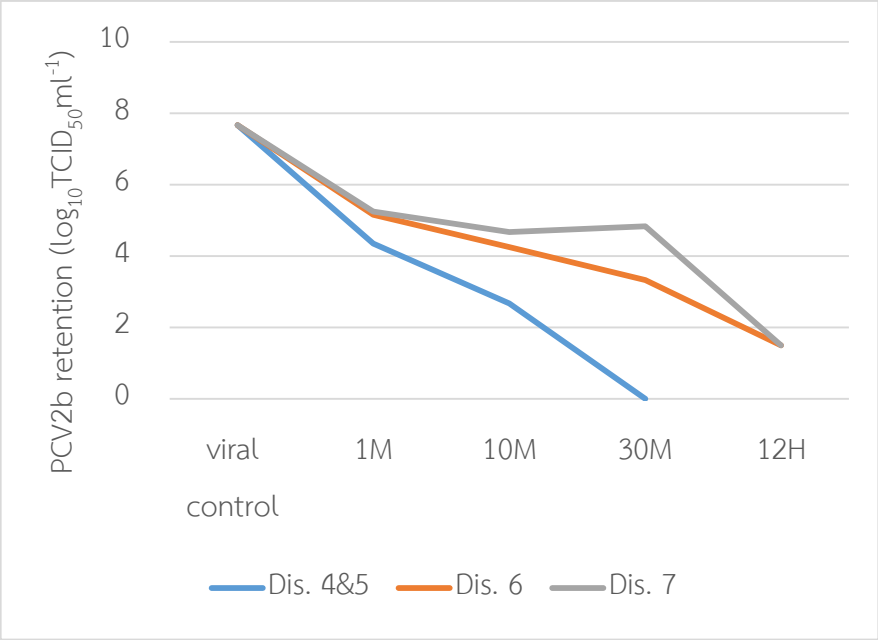


Figure 4. 2: PCV2b retention after 1-, 10- and 30-minute exposure to disinfectants 4, 5, 6 and 7

For iodine products (disinfectants 8 and 9), it contains 0.11gL^{-1} ethylated alcohol-iodine complex, 0.38gL^{-1} sulfuric acid, and 0.38gL^{-1} phosphoric acid (disinfectant 8); and 0.1gL^{-1} povidone-iodine (disinfectant 9), reduced approximately 3.00 to 3.67 $log_{10}TCID_{50}ml^{-1}$PCV2b titer after 30-minute exposure. Moreover, disinfectants 8 and 9 did not kill PCV2b after 12-hour exposure (Figure 4.4). In addition, approximately 3 $log_{10}TCID_{50}ml^{-1}$PCV2b PCV2b retention illustrated that PCV2b are not susceptible to iodine products.

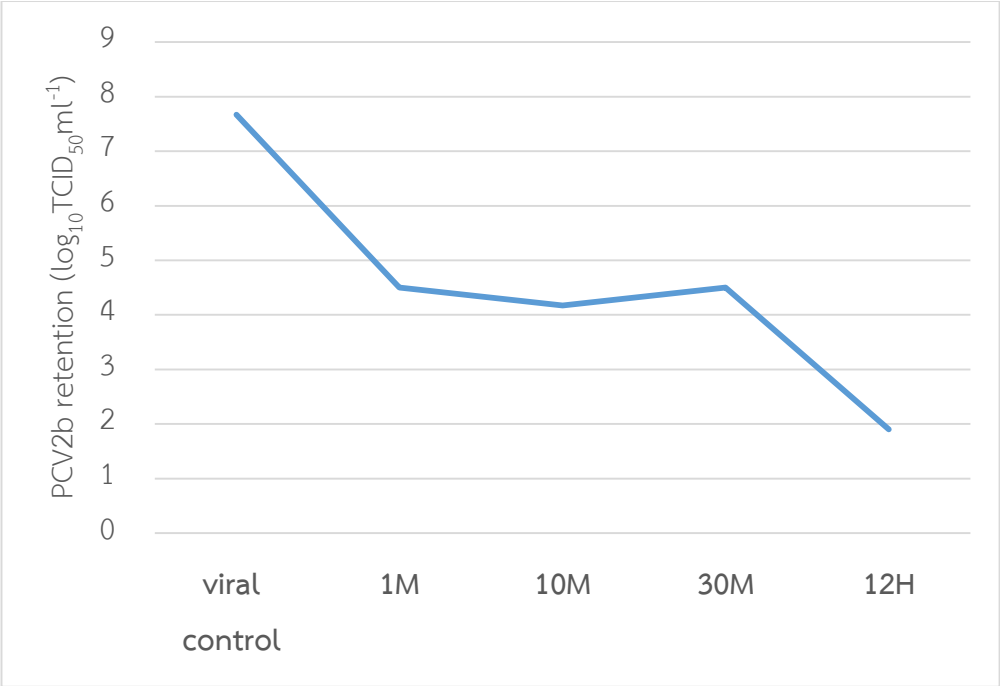


Figure 4. 3: PCV2b retention after 1-, 10-, 30-minute and 12-hour exposure to disinfectant 8 and 9

Regarding to three groups of disinfectants being tested, after 10-minute contact time, although a combination of glutaraldehyde and QAC (disinfectant 4 and 5) reduced approximately 5$log_{10}TCID_{50}ml^{-1}$ PCV2b titer, oxidizing products (disinfectant 1, 2, 3), which completely inactivated PCV2b, demonstrated the most virucidal products against PCV2.

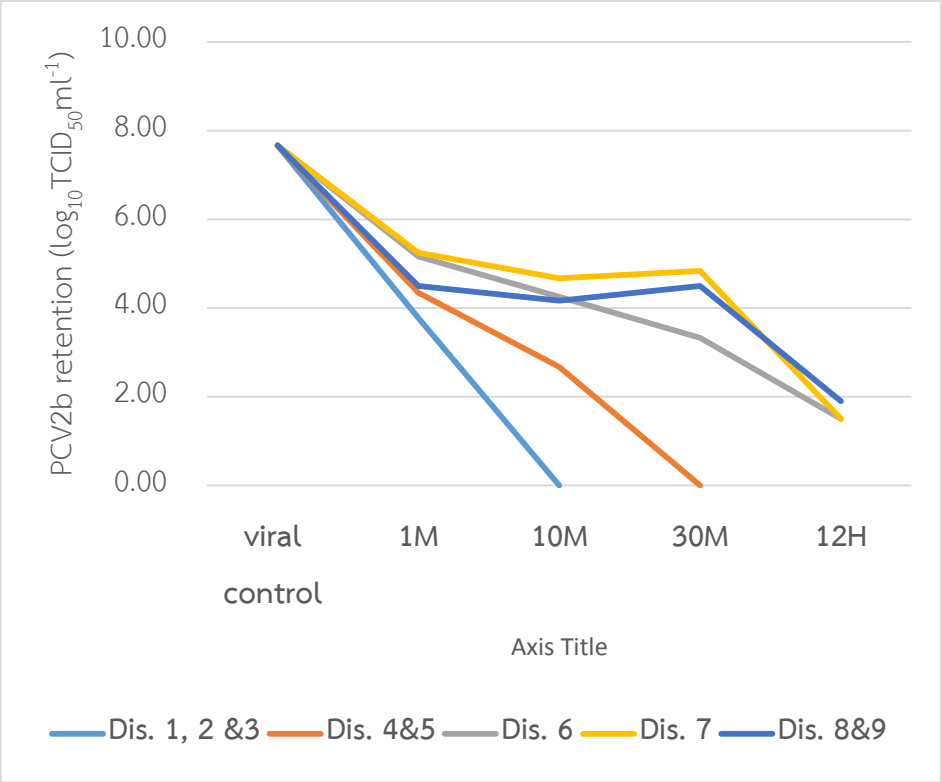


Figure 4. 4: PCV2b retention after 1-, 10-, 30-minute and 12-hour exposure to 9 disinfectants

For alkali and aldehyde agents (disinfectant 10 and 11), there were 1%, 2% and 4% of alkali agent (disinfectant 10) and 0.5%, 1% and 2% of aldehyde (disinfectant 11) being tested. PCV2 was inactivated after 1-minute exposure to all concentrations of the agents.

4.2 Comparison of IPMA and IFA to positive sample (undetectable PCV2b) after exposure to five commercial disinfectants

IPMA and IFA were used to confirm the viability of the PCV2b in the prepared samples after detoxification (PCV2b inactivation). The results were showed in table 4.2. There was 100% agreement between both assays in examining PCV2b infection.

Table 4. 2 Agreement on positive results (PCV2b inactivation) of PMA and IFA

Dis.	Time point	IPMA			IFA		
		1/2x	1x	2x	1/2x	1x	2x
1	1’	NA	-	-	-	-	-
	10’	-	-	-	-	-	-
	30’	-	-	-	-	-	-
2	1’	NA	-	-	-	-	-
	10’	-	-	-	-	-	-
	30’	-	-	-	-	-	-
3	1’	NA	NA	-	NA	NA	-
	10’	-	-	-	-	-	-
	30’	-	-	-	-	-	-
4	1’	NA	NA	-	NA	NA	-
	10’	NA	NA	-	NA	NA	-
	30’	NA	-	-	NA	-	-
5	1’	NA	NA	NA	NA	NA	NA
	10’	NA	NA	NA	NA	NA	NA
	30’	-	-	-	-	-	-

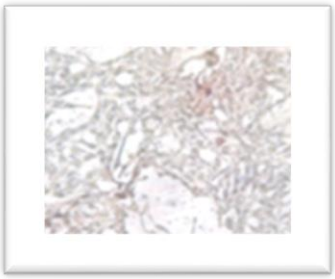
IPMA: indirect immune-peroxidase monolayer assay
IFA: Indirect immunofluorescence assay
Dis.: Disinfectant
1/2x: half recommended dilution

NA: did not perform IPMA and IFA

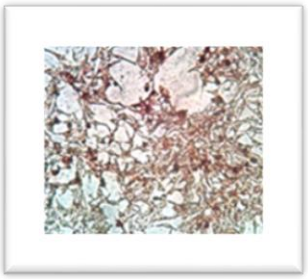
(-): PCV2b inactivation

The infectivity of PCV2b after exposure to disinfectants

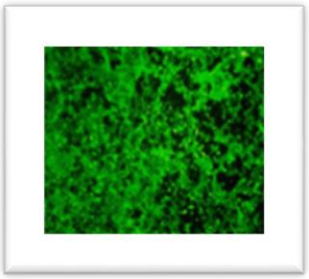
The infectivity of PCV2b without exposure to disinfectants (viral control)



PCV2b positive (IPMA)

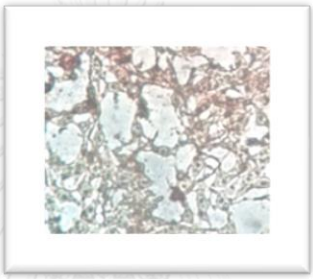


PCV2 positive (IPMA)

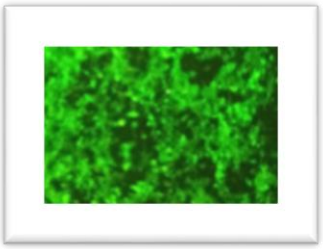


PCV2b positive (IFA)

PCV2b positive (IPMA)

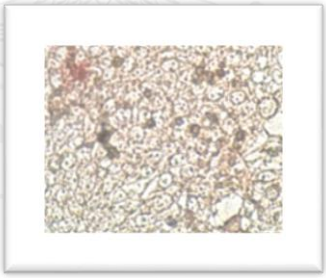


PCV2 positive (IPMA)

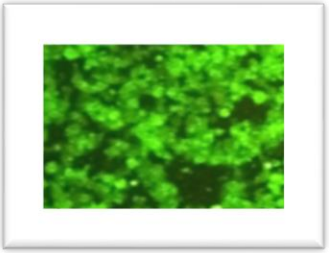


PCV2b positive (IFA)

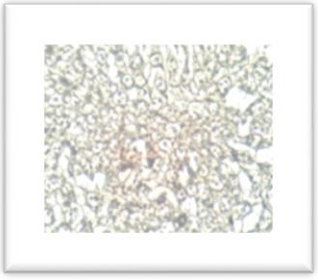
PCV2b positive (IPMA)



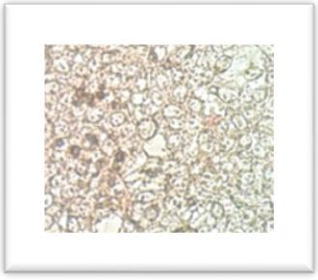
PCV2b positive (IPMA)



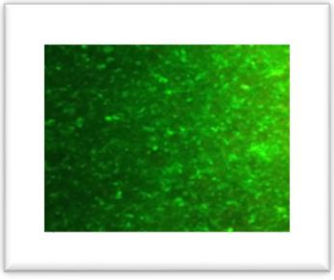
PCV2b positive (IFA)



PCV2b positive (IPMA)



PCV2b positive (IPMA)



PCV2b positive (IFA)

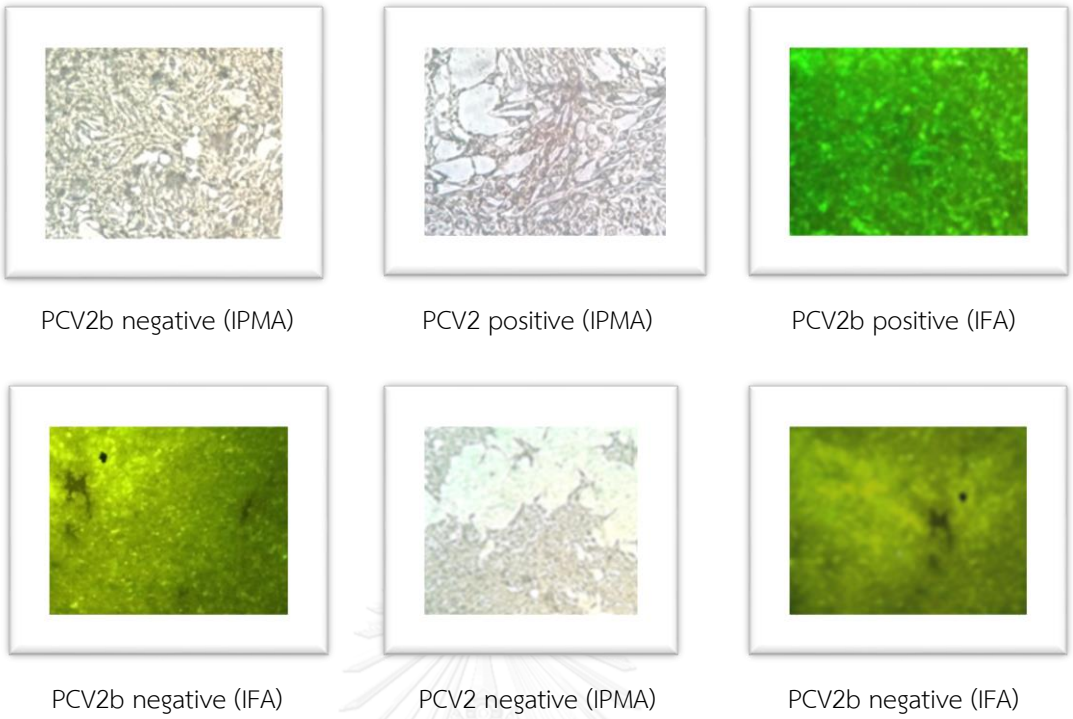


Figure 4. 5 The infectivity of PCV2b examined by IPMA and IFA

The pictures of SW cells stained by IPMA or IFA were shown in the Figure 4.6. The red color or fluorescence color indicated the viability of PCV2b infecting in SW cells, while the pictures without signatures of the colors, representing the collected samples which PCV2b was completely inactivated, or the SW cells were not infected by PCV2b, treating as cell control.

CHAPTER 5

DISCUSSION

Porcine circoviruses type 2 have been recognized as a highly resistant virus and being one of the most important viral pathogens that could cause a significantly economic loss in pig farms [140, 141]. In Thailand, according to a previous study carried out from 2006 to 2010, PCV2 prevalence was various from 34% to 65% [63]. Comparing to other routes of PCV2 transmission, an immense amount of PCV2 viruses predominantly spread between pigs through close contact or nose to nose contact [7, 9]. This results in potential PCVAD spread within a susceptible herd. Furthermore, PCV2 viruses also found ubiquitous in pig environment due to PCV2 vaccination or disinfection that failed in eliminating PCV2 viruses [71, 75, 84][71, 75, 84][71, 75, 84][71, 75, 84][71, 75, 84][71, 75, 84][71, 75, 84][71, 75, 84][71, 75, 84]. However, the spread of PCVAD might not occur due to a remarkable PCV2 reduction after vaccination or disinfection [15].

Failure in reducing PCV2 viruses of disinfection might be due to several causes such as physical cleaning before disinfection [15], environmental conditions and selecting a proper disinfectant. Of those, using a good disinfectant is prominent. Particularly, PCV2 virus is one of the hardest viruses to heat treatment and pasteurization [12] and extreme tolerent in acid environment (pH2). According to previous studies, the resistance of PCV2 viruses to a variety of commercial disinfectants has been an enormous concern due to several evidences of disinfectant test results, in which only some disinfectant compounds could completely inactivate PCV2 viruses [13, 84, 114]. In addition, an optimal disinfectant concentration and contact time must be considered as well.

In the present study, nine commercial disinfectants divided into three groups, which include oxidizing, povidone-iodine, and QAC products, are effective against various viruses and commonly used in Thai Market being examined. Basing on the guideline of European suspension test (EST), the results of the present study revealed a relative agreement on the efficacy of nine commercial disinfectants and two chemical substances (disinfectants 10 and 11) against PCV2b virus as comparing to previous studies [13, 14, 114]. Apart from the present study, 1-minute contact time was not

examined in previous studies. A short contact time examined in this study revealed valuble results in the efficacy of disinfectants against the PCV2. Although the control PCV2b titer was relatively high (7.67 $\log_{10}$ $TCID_{50}ml^{-1}$), oxidizing products at 1x and 2x labelled-recommended dilutions and double labeled concentrations were able to eliminate PCV2b virus after 1-minute exposure.

Retaining PCV2b virus after 1-minute exposure to oxidizing products was found at ½x labelled-recommended dilutions. These results provided the evidence to compare the virucidal activity of the individual oxidizing compound. Oxidizing agents as potassium mono persulphate, potassium peroxomonosulfate (PMP) and sodium hypochlorite not only interact with sulfhydryl (-SH) and sulfur (S-S) bonds leading to denature proteins, enzymes but also damage viral genome. Oxidizing agents are also volatile agents that probably inactivate target microorganisms in the short contact time [131]. Sodium dodecyl benzene sulfonate is a surfactant agent, which probably increases the hydrophilicity of the solution [142]. Sodium dichloro isocyanurate (SDIC) is a chlorine-releasing agent, which is able to increase the concentration of available chlorine and being less susceptible to inactivation of organic loads. In water, SDIC is able to generate hypochlorite, which is the most effective form of chlorine, damaging cellular activity of proteins and inhibiting DNA synthesis [131]. Hypochlorite is also found in linearity of the regeneration by PMP [143]. Therefore, a combination of potassium peroxomonosulfate 50% and sodium dichloro isocyanurate 5% (disinfectant 1) showed higher performance in reducing PCV2b virus titer (5$\log_{10}$ $TCID_{50}ml^{-1}$), comparing to hypochlorite agent alone (disinfectant 3) (3$\log_{10}$ $TCID_{50}ml^{-1}$) or a compound of potassium mono persulphate 49.4% and sodium dodecyl benzene sulfonate 13.17% (disinfectant 2) (4$\log_{10}$ $TCID_{50}ml^{-1}$). Subsequently, all tested concentrations of oxidizing products (disinfectants 1, 2, 3) completely eliminated PCV2b after 10-minute exposure. These results are consistent with previous studies that oxidizing products are the most effective chemical compounds against PCV2 viruses [13-15, 84, 114].

The second effective disinfectants against PCV2b virus after 1-minute exposure are disinfectants 4 and 5 containing the similar combination of QAC and glutaraldehyde, glutaraldehyde 15% and alkyl benzyl dimethyl ammonium chloride (QAC) 10%. This combination was able to reduce approximately 2$\log_{10}TCID_{50}$ ml^{-1} to 3.5 $\log_{10}TCID_{50}$ ml^{-}

[1] PCV2b viral titers. In comparison with QAC (disinfectant 7) alone or a formula with a combination of QAC and alcohol (disinfectant 6), both were able to reduce approximately 1.5$\log_{10}TCID_{50}$ ml^{-1} to 2.5 $\log_{10}TCID_{50}$ ml^{-1} PCV2b viral titers. To elucidate the results, chemical activity of individual agents of QAC products was regarded.

Aldehydes as glutaraldehyde and formaldehyde are surface-active agents, which probably disrupt nucleic acids and denature proteins, namely residual lysine on capsid surface of viruses [131, 144]. Moreover, glutaraldehyde considered is more efficacious in the present of organic loads and hard water than formaldehyde. The optimal disinfection of glutaraldehyde depends on optimal pH, higher than 7.00, and temperature increased [145].

QACs as cationic membrane-active agents, with hydrophobic activity, tend to interact with phospholipids and proteins in the cytoplasmic membrane of bacteria and plasma membrane of yeast impairing permeability. For envelope virus, QACs predominantly remove the envelope and limitedly destroy virus particles [146]. Considering to non-envelop viruses, QACs are able to form micelle [131, 136, 146]. The disinfectant efficacy of QACs also depends on their generations. Later generations are more germicidal due to more tolerent to organic loads [136, 145]. Both glutaraldehyde and QACs have a higher performance as the contact time prolonged and concentration increased [136, 147].

Therefore, a combination of glutaraldehyde and QAC (disinfectant 4 and 5) revealed more effective in reducing the infectivity of the PCV2b virus, comparing to QAC alone (disinfectant 7) or QAC combining with alcohol (disinfectant 6). For disinfectant 4 and 5 the former, showed higher performance than the later. This is due to the fact that the former containing a QAC with the 4th generation, comparing to the QAC with 3th generation of the later [136]. For disinfectant 7 and disinfectant 6, the former, with the 4th generation of QAC, the later containing a compound of the 3rd generation of QAC and Polyethoxylated alkyl alcohol had relatively similar performance in reducing PCV2b titer. This is because the combination of QAC and another organic solvent probably increase the effectiveness of QAC against PCV2b virus.

Subsequently, strong proofs of QACs (disinfectant 4 and 5) in eliminating PCV2 was found after 10 and 30-minute contacting with the virus. A remarkable PCV2b titer

reduction (approximately $6\log_{10}TCID_{50}ml^{-1}$) was also found when disinfectant 6 and disinfectant 7 contacting with the PCV2 virus after 12 hours. The efficacy results of QAC compounds against the PCV2b virus in the present study agreed with previous studies. In previous studies, mean titer of PCV2 virus reduced approximately 3log after a 10-minute exposure to QACs [114]. The pcv2 virus, subsequently, was eliminated or reduced when the contact times prolonged up to 30-minute or 24 hours respectively [13].

Two least effective disinfectants against the PCV2 virus in the present study are disinfectant 8 and 9, containing compounds of povidone-iodine 1.5% (disinfectant 9) and Ethylated alcohol-iodine complex 2.840%; Sulphuric acid 9.423%; Phosphoric acid 9.530% (disinfectant 8). These products probably reduced from approximately $2\log_{10}$ $TCID_{50}ml^{-1}$ to maximum $4\log_{10}$ $TCID_{50}ml^{-1}$ PCV2b titer after a 30-minute exposure. Approximately $3\log_{10}TCID_{50}ml^{-1}$ PCV2b retained after 12-hour exposure. This result is relatively consistent with a previous study. The mean titer of PCV2 virus reduced approximately 2log after a 10-minute exposure to this type of disinfectants [114]. In previous study, the results showed that povidone-iodine compound was the most effective against non-enveloped viruses (swine vesicular disease virus and African horse sickness virus), envelop viruses like vesicular stomatitis virus, African swine fever virus, equine viral arteritis virus, and porcine reproductive and respiratory syndrome virus [146]. However, the result showed iodine compound was not effective against the PCV2 virus, comparing with another previous study [14]. The conflict results in the efficacy of iodine compounds against PCV2 viruses and other viruses may be due to different generations of iodine products, the types, and quality of stock viruses in each study and other test conditions [14, 114]. In addition, iodine-iodophor compounds considered are favored to attack to key groups of proteins possessing free sulfur acids cysteine and methionine and degrade nucleoprotein of viruses [131, 146].

Of nine commercial disinfectants to be tested, oxidizing products were the most effective disinfectant against the PCV2b. In recent field study, although the PCV2 virus was not eliminated, significantly reduction in the number of PCV2 DNA copies in swab samples of oral fluid and surrounding environment was found after treated with disinfectant 1 (1:200) [15]. Comparing to disinfectant 2, all concentrations of

disinfectant 1 (1:100, 1:200, and 1:400), were by far more effective against classical swine fever virus (SFV) and pseudorabies virus (PRV) [148].

In comparison to other noneveloped viruses, at 1% of hypochloride, iodine product and glutaraldehyde agent could completely inactivated CAV in tissue culture, after 10-minute exposure [149]. While various disinfectants could completely inactivate pseudorabies and transmissible gastroenteritis viruses, PPV was only inactivated after 5-minute incubating with hypochloride [150]. Those results coincided with the result of the present study, leading to making a conclusion that disinfectant 1 is the best disinfectant against a variety of contagious viral pathogens in animals. This evidence indicated that PCV2 virus is a highly resistant virus as well.

In conclusion, of three groups of disinfectants being tested in the present study, oxidizing products were the most effective against the PCV2b virus at all tested concentrations. A combination of glutaraldehyde and QAC placed on the second disinfectants against PCV2b after 10 and 30-minute exposure. Retaining PCV2b virus after 12-hour challenging with QAC alone, a QAC combining with alcohol and iodine products demonstrated the least effective groups of disinfectants against the PCV2b virus.

It seems that several variances, consisting of test conditions, quality of stock virus and the experience in testing procedure, might affect the results of tested disinfectant efficacy. The results relatively agreed with previous studies and provided a valuable information about the efficacy of some known disinfectants and new disinfectants against the PCV2b virus. Although the results might not reflect exactly a real efficacy of disinfectants, in particular, in various hygiene targets in the field condition, they complemented the specific information about the levels of virucidal activities of those disinfectants against a highly resistant virus as PCV2 virus. Disinfectants having a strong evidence of virucidal might help to select the most effective disinfectant appling in hygiene strategies, in which variety of crucial pathogens are significantly reduced or eliminated. Therefore, it is suggested that PCV2 virus could be used as a surrogate virus to test virucidal activity of new commercial disinfectants. Moreover, we suggest that an investigation of the efficacy of the effective disinfectants should be carried out in the field conditions, particularly, in combination strategies between vaccination and hygiene application.

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APPENDIX A

The infectivity of PCV2b examined by IPMA after 1-minute exposure to nine commercial disinfectants

		1/2D				1/2H				1D				1H				2D				2H			
3		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
	10^{-3}	+	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
4		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+		-	-	+	-	-	-
	10^{-3}	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
1		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
2		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-2}	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-3}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
7		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-3}	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-4}	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
8		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

	10^{-3}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-4}	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-2}	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	-	-	+	-	-	-
	10^{-3}	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-4}	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+
	10^{-3}	+	+	+	-	+	+	-	-	+	+	-	-	-	-	-	-	+	+	+	-	+	+	-	-
	10^{-4}	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-
	10^{-3}	+	+	+	-	+	+	+	-	+	+	+	-	-	+	+	-	+	+	-	-	-	-	-	-
	10^{-4}	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The infectivity of PCV2b examined by IPMA after 10-minute exposure to nine commercial disinfectants

3		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	-	-	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	10^{-2}	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-3}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1		1/2D				1/2H				1D				1H				2D				2H			

		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
2		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
7		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-3}	+	-	-	-	+	-	-	-	+	+	+	+	+	-	-	-	+	-	-	-	+	+	-	-
	10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
8		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
	10^{-3}	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-	+	+	-	-	+	-	-	-
	10^{-4}	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
9		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-2}	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	-	+	-	-	-	+	+	+	-
	10^{-3}	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-4}	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
6		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	-	+	+	+	+
	10^{-3}	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		1/2D				1/2H				1D				1H				2D				2H			
5		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The infectivity of PCV2b examined by IPMA after 30-minute exposure to five commercial disinfectants

7		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10^{-2}	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
	10^{-3}	+	+	-	-	+	+	+	-	-	-	-	+	+	-	-	-	+	-	-	-	+	+	-	-
	10^{-4}	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
	10^{-2}	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-
	10^{-3}	+	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-
	10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	10^{-2}	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-
	10^{-3}	+	+	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-	-
	10^{-4}	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-5}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	+	+	+	-
	10^{-2}	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	10^{-3}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The infectivity of PCV2b examined by IPMA after 12-hour exposure to four commercial disinfectants

7		1/2D	1/2H	1D	1H	2D	2H

		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-
	10^{-2}	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-3}	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	-	+	+	+	-
	10^{-2}	+	+	-	-	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	10^{-3}	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	+	-	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
	10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6		1/2D				1/2H				1D				1H				2D				2H			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
	10^{-1}	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	-	+	-	-	-	+	-	-	-
	10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Publication

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