Original Article

Genetic Characterization and Phylogenetic Analysis of Porcine Circovirus Type 2 in Thai Pigs with Porcine Circovirus Associated Diseases (PCVAD) during 2007-2010

Suphattra Jittimanee Suparlark Nuntawan Na Ayudhya Roongtham Kedkovid Komkrich Teankum Roongroje Thanawongnuwech*

Abstract

Porcine circovirus type 2 (PCV2) was first recognized as a causative agent of post weaning multisystemic syndrome or PMWS in Canada in 1991. Subsequently, it has been reported in almost all intensive pig production countries, and currently all associated diseases linked to PCV2 are included under the term porcine circovirus associated diseases or PCVAD. PCV2 can be divided into 2 major genotypes referred to genotype 1 (PCV2b) and genotype 2 (PCV2a). In Thailand, PMWS was first reported in 1998. However, only one isolate of Thai PCV2 from our group has been submitted to GenBank (AY864814). Therefore, the objective of this study was to determine the genetic characterizations of 12 ORF2 of PCV2 isolates from Thai pigs with PCVAD during 2007-2010. Twelve Thai PCV2 sequences were analyzed together with 19 representative ORF2 sequences. Interestingly, all 12 recent Thai PCV2 sequences belong to genotype 1 consisting of subgroup 1A/B (10/12, 83.33%) and 1C (2/12, 16.67%). In summary, based on genetic characterization and phylogenetic analysis, a few specific substitution patterns were observed in amino acid positions and genotype 1, subgroup 1A/B was predominated in Thai pigs with PCVAD in this study. More samples are needed to investigate the existence of genotype 2.

Keywords: genetic characterization, phylogenetic analysis, pigs, porcine circovirus type 2, Thailand

Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Henri Dunant Rd., Bangkok, 10330, Thailand
Corresponding author E-mail: *Roongroje.T@Chula.ac.th*

บทคัดย่อ

ลักษณะทางพันธุกรรมและการวิเคราะห์วงศ์วานวิวัฒนาการของเชื้อเซอร์โคไวรัสชนิดที่สอง ที่แยกได้จากสุกรของประเทศไทยซึ่งป่วยด้วยกลุ่มอาการพีซีวีเอดีในระหว่างปี ค.ศ. 2007-2010

*สุภัทตรา จิตติมณี ศุภฤกษ์ นันทวัน ณ อยุธยา รุ่งธรรม เกษโกวิท คมกฤช เทียนคำ รุ่งโรจน์ ธนาวงษ์นุเวช**

เชื้อเซอร์โคไวรัสชนิดที่สองในสุกรซึ่งเป็นสาเหตุของโรคพีเอ็มดับเบิลยูเอสนั้น พบการระบาดครั้งแรกในประเทศแคนาดาปี พ.ศ. 2534 จากนั้นมีการแพร่ระบาดไปในหลายประเทศ ซึ่งในปัจจุบันได้มีการเรียกชื่อ กลุ่มอาการที่เกิดจากเชื้อเซอร์โคไวรัสชนิดที่สองนี้ว่า กลุ่มอาการพีซีวีเอดี เชื้อเซอร์โคไวรัสชนิดที่สองจำแนกออกเป็นสองชนิด คือ จีโนไทป์ 1 และ จีโนไทป์ 2 ในประเทศไทยมีรายงานการค้นพบเชื้อเซอร์โคไวรัสชนิดที่สองครั้งแรกเมื่อ พ.ศ. 2541 จนถึงปัจจุบันนี้ประเทศไทยมีข้อมูลรหัสพันธุกรรมของเชื้อเซอร์โคไวรัสชนิดที่สองเพียงหนึ่งสายพันธุ์เท่านั้น ดังนั้นการศึกษาครั้งนี้จึงมีจุดประสงค์เพื่อวิเคราะห์และจำแนกลักษณะทางพันธุกรรมของเชื้อเซอร์โคไวรัสชนิดที่สองในส่วนโออาร์เอฟ 2 ที่ได้จากตัวอย่างสุกรไทยที่ป่วยด้วยกลุ่มอาการพีซีวีเอดี ในช่วงปี พ.ศ. 2550 ถึง พ.ศ. 2553 จำนวนทั้งหมด 12 ตัวอย่างเทียบกับสายพันธุ์อื่นๆที่มีการรายงานจากประเทศต่างๆ พบว่าตัวอย่างทั้งหมดอยู่จัดอยู่ในกลุ่ม จีโนไทป์ 1 ซึ่งจำแนกเป็นกลุ่มย่อย 1A/B จำนวน 10 ตัวอย่าง (ร้อยละ 83.33) และ กลุ่มย่อย 1C จำนวน 2 ตัวอย่าง (ร้อยละ 16.67) จึงสรุปผลการวิเคราะห์และการจำแนกลักษณะทางพันธุกรรมของเชื้อเซอร์โคไวรัสชนิดที่สองทั้ง 12 ตัวอย่างด้วยแผนภูมิต้นไม้ไฟโลเจเนติค พบว่ามีการเปลี่ยนแปลงลำดับของกรดอะมิโนเพียงบางตำแหน่งเท่านั้น และยังพบว่าเชื้อเซอร์โคไวรัสชนิดที่สองสายพันธุ์จีโนไทป์ 1 กลุ่มย่อย 1A/B เป็นสายพันธุ์ที่พบมากที่สุดในตัวอย่างสุกรไทยซึ่งป่วยด้วยกลุ่มอาการพีซีวีเอดีที่ใช้ในการศึกษาในครั้งนี้ ในการศึกษาต่อไปควรมีจำนวนตัวอย่างมากขึ้นเพื่อยืนยันการปรากฏของไวรัสจีโนไทป์ 2 ในประเทศไทย

คำสำคัญ: ลักษณะทางพันธุกรรม การวิเคราะห์วงศ์วานวิวัฒนาการ สุกร เซอร์โคไวรัสชนิดที่สอง ประเทศไทย

ภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ถนนอังรีดูนังต์ ปทุมวัน กรุงเทพฯ 10330

***ผู้รับผิดชอบบทความ E-mail:** Roongroje.T@Chula.ac.th*

Introduction

Porcine circovirus type 2 (PCV2) was first recognized as a causative agent of postweaning multisystemic wasting syndrome (PMWS), a multi-factorial disease in swine in Canada in 1991 (Harding and Clark, 1997). Subsequently, it has been reported in almost all intensive pig production countries worldwide (Allan and Ellis, 2000; Chae, 2005). PCV2 causes several clinical and pathological conditions in pigs including porcine respiratory disease complex (PRDC), reproductive failures, porcine dermatitis and nephropathy syndrome (PDNS), proliferative and necrotizing pneumonia and congenital tremor (Darwich et al., 2004; Chae, 2005). Currently, these associated diseases and conditions linked to PCV2 are called porcine circovirus associated diseases (PCVAD).

PCV2 belonging to the family *Circoviridae*, is a smallest mammalian, non-enveloped, single-stranded DNA virus enclosing a circular genome about 1.76 kb (Mankertz et al., 1997). The genome of PCV2 contains 3 major open reading frames (ORFs): ORF1, ORF2 and ORF3. The Cap protein is the main structural and major immunogenic of PCV2, which is encoded by ORF2. As a result, ORF2 is commonly used for reconstruction of the phylogenetic tree similar to the whole PCV2 genome study (Olvera et al., 2007)

Several studies suggested that PCV2 could be divided into 2 major genotypes (Carman et al., 2006; Cheung et al., 2007; Ma et al., 2007; Takahagi et al., 2008; Kim et al., 2009). Recently, both genotypes were proposed and referred to PCV2a (PCV2-genotype 2) and PCV2b (PCV2-genotype 1). However, PCV2c genotype has been described, but only found in Denmark (Segales et al., 2008). Interestingly, the virulence of PCV2a and PCV2b isolates was similar in the conventional SPF pig model, but the virulence of the isolates within the same cluster differed (Opriessnig et al., 2008). Alternatively, PCV2 can be classified into 8 subgroups 1A to 1C and 2A to 2E (Olvera et al., 2007), but those were not associated with the disease conditions or geographic areas. Recently, a new type of PCV referred to PCV1/2a was reported and later found to be a chimeric virus containing ORF1 of PCV1 and ORF2 of PCV2a in Canada in 2009 (Gagnon et al., 2010).

In Thailand, PMWS caused by PCV2 was firstly reported in 1998 (Tantilertcharoen et al., 1999)

and a PCV2 retrospective study in Thailand identified PCV-infected case occurring as early as in 1993 (Kiatipattanasakul-Banlunara et al., 2002). It should be noted that only one Thai PCV2 isolate from our group has been submitted to GenBank (AY864814) in 2004 and it was classified into subgroup 1C (Manokaran et al., 2008; Wiederkehr et al., 2009). However, genetic information about PCV2 in Thai swine herds has still been unavailable. Therefore, the objective of this study was to determine the genetic characterizations of ORF2 genome of current 12 PCV2 isolates from Thai pigs with PCVAD during 2007-2010.

Materials and Methods

Field samples: Clinical samples (serum, tonsil or lymph nodes) from different farms in high pig density provinces of Thailand submitted to Chulalongkorn University-Veterinary Diagnostic Laboratory (CU-VDL) and Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University during 2007-2010 were included in this study. These samples were kept in -80°C until performing DNA extraction and PCR. Viral DNA was extracted from lymphoid tissue homogenates and serum samples using NucleoSpin Extract Viral DNA Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions.

PCR amplification of ORF 2 gene: A full-length ORF2 gene of PCV2 was amplified by PCR with forward primer, PCV2-f1 (5'-CCA TGC CCT GAA TTT CCA TA-3') and reverse primer PCV2-r1 (5'-ACA GCG CAC TTC TTT CGT TT-3') published by Takahagi et al. (2008), in a 50 µl reaction mixture. The amplification reaction was performed with an initial step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 1 min and a final extension step at 72°C for 7 min. The PCV2 positive samples of 702 nt were used for DNA sequencing.

Viral sequences and phylogenetic analysis: The PCR products were separated by 1.5% agarose gel electrophoresis and purified with NucleoSpin Extract II (Macherey-Nagel, Düren, Germany) for viral sequences. DNA sequencing was carried out by 1st BASE Company (Singapore) with primers used in the previous PCR reaction. A total of 12 ORF2 sequences from Thai pigs with PCVAD during 2007-2010 were obtained and translated into amino acid sequences for analysis. The 12 Thai PCV2 sequences were analyzed together with 19 representative ORF2 sequences reported in GenBank including 16 reference strains of PCV2a and PCV2b, the former Thai isolate in 2004 (THA_01NP1, AY864814), PCV2c ORF2 sequence (EU148503) and PCV1 (AY193712) shown in table 1. A phylogenetic trees was constructed by MEGA 4 software (Tamura et al., 2007) based on the sequence of ORF2 to determine the distribution and evolutionary trend of PCV2 in Thailand using the neighbor-joining (NJ) method with 1000 bootstrapping replicates (Saitou and Nei, 1987).

Table 1 Classification of 12 Thai PCV2 isolates compared with 8 PCV2 reference strains in the same group by nucleotide and deduced amino-acid sequences of ORF2

Accession number	Nucleotide sequence (262-267)	Amino acid sequence (86-87)	Genotype	subgroup	Country/area	% NT identity[2]
FJ905468	**CCCCGC**	**PR**	**PCV2b**	**1A/B**	**Korea**	**100**
AY484409	**CCCCGC**	**PR**	**PCV2b**	**1A/B**	**Netherland**	**100**
THA_07NP88	CCCCGC	PR	PCV2b	1A/B	Nakhon Pathom	98.9/98.6
THA_07NP144	CCCCGC	PR	PCV2b	1A/B	Nakhon Pathom	99.6/99.6
THA_08NP113	CCCCGC	PR	PCV2b	1A/B	Nakhon Pathom	99.8/99.8
THA_08NP266	CCCCGC	PR	PCV2b	1A/B	Nakhon Pathom	99.8/99.8
THA_10SB01	CCCCGC	PR	PCV2b	1A/B	Saraburi	99.6/99.6
THA_10SB02	CCCCGC	PR	PCV2b	1A/B	Saraburi	99.8/99.4
THA_10SB03	CCCCGC	PR	PCV2b	1A/B	Saraburi	99.4/99.4
THA_10SB04	CCCCGC	PR	PCV2b	1A/B	Saraburi	99.1/99.1
THA_10NP01	CCCCGC	PR	PCV2b	1A/B	Nakhon Pathom	99.5/99.5
THA_10AY01	CCCCGC	PR	PCV2b	1A/B	Ayudhya	99.3/99.3
AY864814 (01NP1)	**CCCCTC**	**PL**	**PCV2b**	**1C**	**Thailand**	**100**
EU302140	**CCCCTC**	**PL**	**PCV2b**	**1C**	**Indonesia**	**100**
THA_09NP165	CCCCTC	PL	PCV2b	1C	Nakhon Pathom	98.4/98.6
THA_09NP290	CCCCTC	PL	PCV2b	1C	Nakhon Pathom	97.7/98.1
AY256455	**AAAATC**	**KI**	**PCV2a**	**2C**	**Hungary**	**100**
AY322004	**AAAATC**	**KI**	**PCV2a**	**2D**	**France**	**100**
EU148503	**CCCCTC**	**PL**	**PCV2c**	**-**	**Denmark**	**100**
AY193712	**ATCTTC**	**IF**	**PCV1**	**-**	**China**	**100**

[1]Boldface letters are reference strains

[2]Compared with the reference nucleotide sequences in the same group

Results

Genetic characterization: All 12 Thai ORF2 of PCV2 sequences in this study had a genome length of 702 nt and revealed nucleotide identities ranged between 97.7-99.8% (Table 1), indicating no significant differences between PCV2 genotype. However, the nucleotide substitutions in the ORF2 gene among the 12 Thai PCV2 sequences compare to the former Thai

PCV2 sequences in 2004 (THA_01NP1, AY864814) were observed. These substitutions led to amino acid changes located between amino acid positions 46-90, 121-131 and 190-191 (Fig. 1).

Phylogenetic analyses: The phylogenetic analysis in this study reconstructed from the 12 Thai ORF2 sequences during 2007-2010 along with 19 ORF2 sequences published in GenBank database representing all PCV2 genotypes shown in Fig. 2. All 12 Thai PCV2 sequence belonged only to genotype 1 (PCV2b) according to the classification proposed by Grau-Roma et al. (2008). Based on the subgroup terminology described previously (Olvera et al., 2007), nucleotides 262-267 and amino acids 88-89 of ORF2 were compared and classified. The nucleotide sequences "CCCCGC", "CCCCTC" and "AAAATC" are the signatures motif for PCV2b subgroup 1A/B, 1C and PCV2a, respectively. The amino acid "PR" was enclosed with subgroup 1A/B, while the PL and KI were related with subgroup 1C and PCV2a (Cheung et al., 2007). All 12 Thai PCV2 genotype 1 were divided into 2 subgroups consisting of 1A/ B (10/12, 83.33%) and 1C (2/12, 16.67%). This results indicates that the predominant Thai PCV2 in this study is genotype 1 (100%) particularly subgroup 1A/B (83.33%). It should be noted that previously found subgroup 1C (01NP1) in 2004 was still observed (09NP165 and 09NP290) in 2009 in the same province.

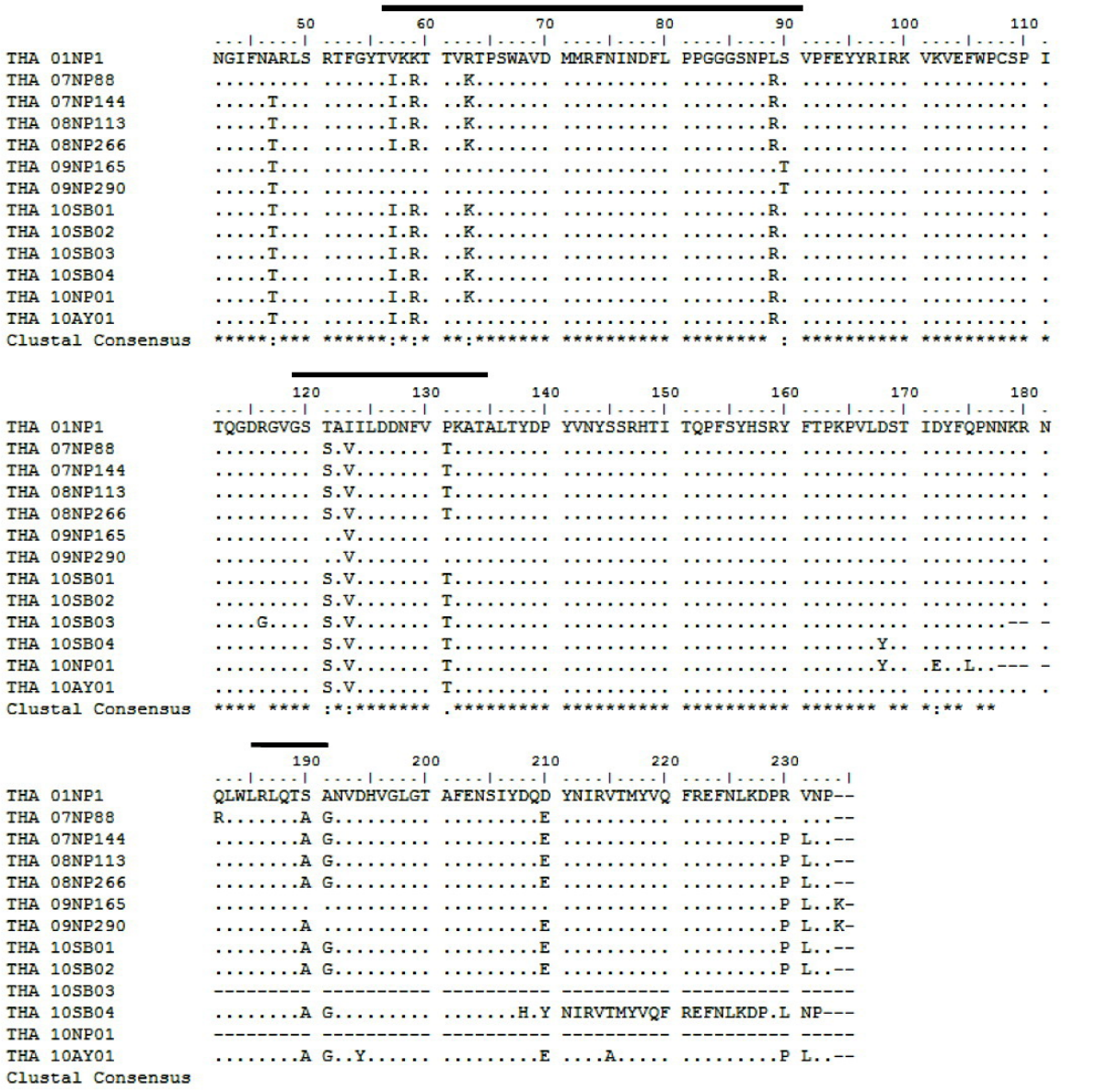

Figure 1 Alignment of amino acids predicted from the ORF2 nucleotide sequences of the 12 Thai PCV2 sequences during 2007-2010 compared with the former Thai sequences in 2004 (01NP1) revealing amino acid changes located between amino acid positions 46-90, 121-131 and 190-191. Regions reported by Grau-Roma et al. (2008) as more heterogenic are highlighted in black lines.

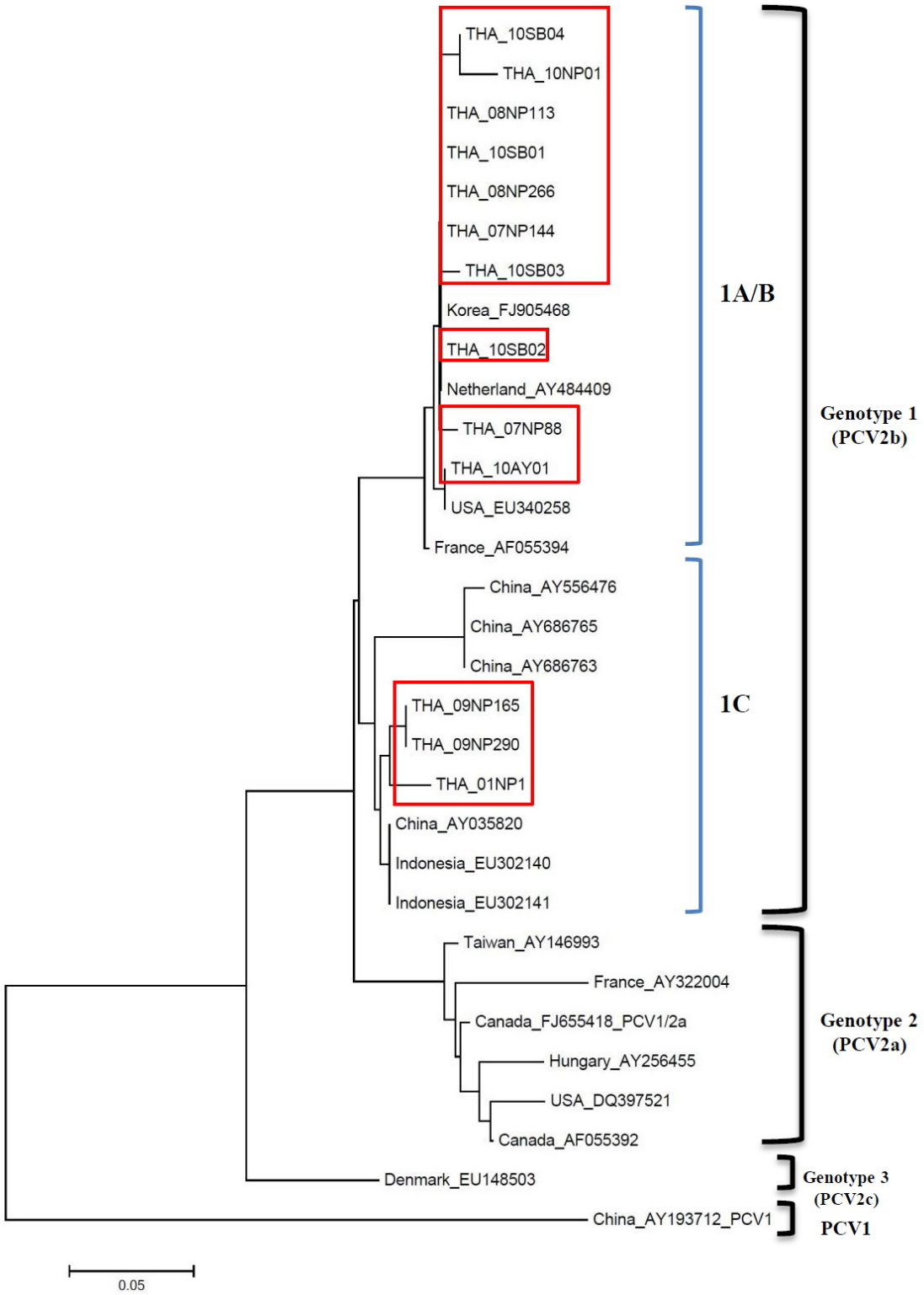

Figure 2 Phylogenetic tree based on neighbor-joining (NJ) method is constructed from the 12 Thai PCV2 sequences during 2007-2010 in this study with 19 ORF2 of PCV2 sequences reported in GenBank including 16 reference strains of PCV2a and PCV2b, the former Thai isolate (THA_01NP1), PCV2 group 3 (PCV2c) sequence (EU148503) and PCV1 (AY193712) strain.

Discussion

Porcine circovirus type 2 (PCV2) is divided into 2 major genotypes based on sequencing analysis. However, several nomenclature have been used such as group I and II, pattern 321 and 422, SG3 and SG1/SG2, group 1 and 2, and Group A and B (Olvera et al., 2007; Segales et al., 2008; Timmusk et al., 2008). Recently, both genotypes were proposed and referred to PCV2a and PCV2b (Segales et al., 2008). In Thailand, PMWS was firstly reported in 1998 (Tantilertcharoen et al., 1999). It should be noted that only one Thai PCV2 isolate belonging to genotype 1 was submitted to GenBank (AY864814) in 2004. In this study, we characterized and reconstructed phylogenetic analysis of 12 Thai ORF2 of PCV2

sequences collected from pigs with PCVAD during 2007-2010 to accurately determine the cluster relationships. All 12 Thai PCV2 isolates in this study were closely related (The nucleotide identities ranged between 97.7-99.8%) based on ORF 2 sequence. The main position of amino acid replacements among Thai ORF2 sequences in this study were located at amino acid positions 46-90, 121-131 and 190-191 within heterogenic regions (Fig. 1) similar to previous reports (Larochelle et al., 2002; Grau-Roma et al., 2008). The phylogenetic analysis of the 12 Thai PCV2 isolates revealed that all studied Thai PCV2 sequences belonged to genotype 1 (Fig. 2), according to previous classification (Grau-Roma et al., 2008).

Previous studies revealed that both genotypes were associated with PCVAD-affected and non-affected herds (Cheung et al., 2007; Carman et al., 2008; Grau-Roma et al., 2008; Horlen et al., 2008; Wiederkehr et al., 2009). However PCV2b is currently prevailing in naturally occurring infections (Takahagi et al., 2008; Timmusk et al., 2008; Cortey et al., 2010). Although the virulence of PCV2a and PCV2b isolates was similar in the SPF pig model, the virulence of isolates within the same cluster was different (Opriessnig et al., 2008). On the other hand, many recent publications have reported a shift from PCV2a to PCV2b genotype that might be related to the occurrence of PMWS outbreaks in Canada (Gagnon et al., 2007), Sweden (Timmusk et al., 2008), Switzerland (Wiederkehr et al., 2009) and Spain (Cortey et al., 2010), indicating that PCV2b may be more virulent than PCV2a.

All 12 Thai PCV2 isolates in this study were classified within only one PCV2 genotype (genotype 1: 1A/B and 1C) similar to several countries such as Cuba (genotype 1: 1A) and Indonesia (genotype 1: 1C). Interestingly 10 Thai PCV2 sequences were located within subgroup A/B (10/12, 83.33%) together with the other PCV2 sequences from Korea (FJ905468), the Netherlands (AY484409) and USA (EU340258). These PCV2 isolates subgroup A/B were found in PCVAD pigs from Nakhon Pathom (2007, 2008 and 2010), Saraburi (2010) and Ayudhya province (2010). The other 2 Thai PCV2 sequences (THA_09NP165 and THA_09NP290) from Nakhon Pathom province were classified within subgroup 1C (2/12, 16.67%) similar to the previous Thai PCV2 isolate (THA_01NP1, AY864814), a Chinese isolate (AY035820) and 2 Indonesian isolates (EU302140 and EU302141) which were found in Indonesian pigs imported to Singapore (Manokaran et al., 2008).

Nevertheless, the sequence and phylogenetic analyses performing in this study did not show any evidence of recombination as reported in PCV type 2 isolated in Hong Kong, Korea and USA (Ma et al., 2007; Choi and Chae, 2008; Hesse et al., 2008). However, a few amino acid replacements among Thai ORF2 sequences in this study were observed. Due to the high nucleotide substitution rate of PCV2 compared to other single-stranded DNA viruses, it was estimated approximately $1.2x10^3$ substitutions/site/year (Firth et al., 2009). Therefore, the emerging of any new PCV2 genotype is possible in the future. Since the samples in this study were collected from the highest pig density provinces located in central Thailand, the results yielded in this study can demonstrate at least 2 introductions of PCV2 genotype 1 into Thailand. Imported swine breeders and semen appear to be the major route or transmission. Another evidence of introducing new virus strain into the swine herds is using improper killed chimeric vaccine in Canada (Gagnon et al., 2010). It should be noted that no chimeric virus or no PCV2a was found in this study even though that killed chimeric vaccine has been commercially available in Thailand a few years ago (Paphavasit et al., 2009). In order to yield the accurate data, more PCV2 strains from different parts of the country should be examined for more epidemiological information of PCV2 in the Thai swine herds.

In summary, based on the sequences and phylogenetic analysis of all current 12 Thai PCV2 isolates, two subgroups: 1A/B and 1C of genotype 1 were found in Thailand. A few specific substitution patterns in each subgroup were observed in amino acid positions. This report revealed that PCV2 genotype 1 was predominated in Thai pigs with PCVAD. This finding provides some useful information about PCV2molecular epidemiology in Thailand with at least 2 introductions into the country.

Acknowledgements

We would like to thank the Royal Golden Jubilee Ph.D. Program from the Thailand Research Fund and Chulalongkorn University for S. Jittimanee (PHD/0252/2550) Ph.D. Program in Veterinary Pathobiology financial support. This study was supported by grants from National Research University from Chulalongkorn University (Health Cluster-HR1164A7) and Rachadapiseksompoch Endowment Fund from Chulalongkorn University (CU cluster Emerging H-15-75-53).

References

Allan, G.M. and Ellis, J.A. 2000. Porcine circoviruses: A review. J Vet Diagn Invest. 12(1): 3-14.

Carman, S., McEwen, B., DeLay, J., van Dreumel, T., Lusis, P., Cai, H. and Fairles, J. 2006. Porcine circovirus-2 associated disease in swine in Ontario (2004 to 2005). Can Vet J. 47(8): 761-762.

Chae, C. 2005. A review of porcine circovirus 2-associated syndromes and diseases. Vet J. 169(3): 326-336.

Cheung, A.K., Lager, K.M., Kohutyuk, O.I., Vincent, A.L., Henry, S.C., Baker, R.B., Rowland, R.R. and Dunham, A.G. 2007. Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. Arch Virol. 152(5): 1035-1044.

Choi, K.S., Chae, J.S. 2008. Genetic characterisation of porcine circovirus type 2 in Republic of Korea. Res Vet Sci. 84: 497-501.

Cortey, M., Pileri, E., Sibila, M., Pujols, J., Balasch, M., Plana, J. and Segalés, J. 2011. Genotypic shift of porcine circovirus type 2 from PCV-2a to PCV-2b in Spain from 1985 to 2008. Vet J. 187(3): 363-368.

Darwich, L., Segales, J. and Mateu, E. 2004. Pathogenesis of postweaning multisystemic wasting syndrome caused by porcine circovirus 2: An immune riddle. Arch Virol. 149(5): 857-874.

Firth, C., Charleston, M.A., Duffy, S., Shapiro, B. and Holmes, E.C. 2009. Insights into the evolutionary history of an emerging livestock pathogen: porcine circovirus 2. J Virol 83(24): 12813-12821.

Gagnon, C.A., Tremblay, D., Tijssen, P., Venne, M.H., Houde, A. and Elahi, S.M. 2007. The emergence of porcine circovirus 2b genotype (PCV-2b) in swine in Canada. Can Vet J. 48: 811-819.

Gagnon, C.A., Music, N., Fontaine, G., Tremblay, D. and Harel, J. 2010. Emergence of a new type of porcine circovirus in swine (PCV): A type 1 and type 2 PCV recombinant. Vet Microbiol. 144(1-2): 18-23.

Grau-Roma, L., Crisci, E., Sibila, M., Lopez-Soria, S., Nofrarias, M., Cortey, M., Fraile, L., Olvera, A. and Segalés, J. 2008. A proposal on porcine circovirus type 2 (PCV2) genotype definition and their relation with postweaning multisystemic wasting syndrome (PMWS) occurrence. Vet Microbiol. 128(1-2): 23-35.

Harding, J.C.S. and Clark, E.G. 1997. Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). Swine Health Prod. 5: 201-203.

Hesse, R., Kerrigan, M. and Rowland, R.R.R. 2008. Evidence for recombination between PCV2a and PCV2b in the field. Virus. Res. 132: 201-207.

Kim, H.H., Park, S.I., Hyun, B.H., Park, S.J., Jeong, Y.J., Shin, D.J., Chun, Y.H., Hosmillo, M., Lee, B.J., Kang, M.I. and Cho, K.O. 2009. Genetic diversity of porcine circovirus type 2 in Korean pigs with postweaning multisystemic wasting syndrome during 2005-2007. J Vet Med Sci. 71(3): 349-353.

Larochelle, R., Magar, R. and D'Allaire, S. 2002. Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) strains from cases presenting various clinical conditions. Virus Res. 90(1-2): 101-112.

Ma, C.M., Hon, C.C., Lam, T.Y., Li, V.Y., Wong, C.K., de Oliveira, T. and Leung, F.C. 2007. Evidence for recombination in natural populations of porcine circovirus type 2 in Hong Kong and mainland China. J Gen Virol. 88(6): 1733-1737.

Mankertz, A., Persson, F., Mankertz, J., Blaess, G. and Buhk, H.J. 1997. Mapping and characterization of the origin of DNA replication of porcine circovirus. J Virol. 71(3): 2562-2566.

Manokaran, G., Lin, Y.-N., Soh, M.-L., Lim, E.A.-S., Lim, C.-W. and Tan, B.-H. 2008. Detection of porcine circovirus type 2 in pigs imported from Indonesia. Vet Microbiol. 132(1-2): 165-170.

Olvera, A., Cortey, M. and Segalés, J. 2007. Molecular evolution of porcine circovirus type 2 genomes: phylogeny and clonality. Virology. 357(2): 175-185.

Opriessnig, T., Ramamoorthy, S., Madson, D.M., Patterson, A.R., Pal, N., Carman, S., Meng, X.J. and Halbur, P.G. 2008. Differences in virulence among porcine circovirus type 2 isolates are unrelated to cluster type 2a or 2b and prior infection provides heterologous protection. J Gen Virol. 89(Pt 10): 2482-2491.

Paphavasit, T., Lehrbach, P., Navasakuljinda, W., Kedkovid, R., Lacharoje, S., Thanawongnuwech, R. and Teankum, K. 2009. Efficacy of a chimeric PCV2 vaccine: A field trial. Thai J Vet Med. 39(2): 145-155.

Pérez, L.J., de Arce, H.D. and Frías, M.T. 2010. Genetic characterization and phylogenetic analysis of porcine circovirus type 2 strains present in Cuban swine herds. Res Vet Sci. 89(2): 301-305.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 4(4): 406-425.

Segalés, J., Olvera, A., Grau-Roma, L., Charreyre, C., Nauwynck, H., Larsen, L., Dupont, K., McCullough, K., Ellis, J., Krakowka, S., Mankertz, A., Fredholm, M., Fossum, C., Timmusk, S., Stockhofe-Zurwieden, N., Beattie, V., Armstrong, D., Grassland, B., Baekbo, P. and Allan, G. 2008. PCV-2 genotype definition and nomenclature. Vet Rec. 162(26): 867-868.

Takahagi, Y., Nishiyama, Y., Toki, S., Yonekita, T., Morimatsu, F. and Murakami, H. 2008. Genotypic change of porcine circovirus type 2 on Japanese pig farms as revealed by restriction fragment length polymorphism analysis. J Vet Med Sci. 70(6): 603-606.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 24(8): 1596-1599.

Tantilertcharoen, R., Kiatipattanasakul, W. and Thanawongnuwech, R. 1999. Report of circovirus infection in pig in Thailand. Thai J Vet Med. 29: 73-83 (in Thai).

Timmusk, S., Wallgren, P., Brunborg, I., Wikström, F., Allan, G., Meehan, B., McMenamy, M., McNeilly, F., Fuxler, L., Belák, K., Põdersoo, D., Saar, T., Berg, M. and Fossum, C. 2008. Phylogenetic analysis of porcine circovirus type 2 (PCV2) pre- and post-epizootic postweaning multisystemic wasting syndrome (PMWS). Virus Genes. 36(3): 509-520.

Wiederkehr, D.D., Sydler, T., Buergi, E., Haessig, M., Zimmermann, D., Pospischil, A., Brugnera, E. and Sidler, X. 2009. A new emerging genotype subgroup within PCV -2b dominates the PMWS epizooty in Switzerland. Vet Microbiol. 136(1-2): 27-35.