Transboundary and Emerging Diseases

RAPID COMMUNICATION

Different Lineage of Porcine Deltacoronavirus in Thailand, Vietnam and Lao PDR in 2015

K. Saeng-chuto[1], A. Lorsirigool[1], G. Temeeyasen[1], D. T. Vui[2], C. J. Stott[1], A. Madapong[1], T. Tripipat[1], M. Wegner[3], M. Intrakamhaeng[4], W. Chongcharoen[5], A. Tantituvanont[5], P. Kaewprommal[6], J. Piriyapongsa[6] and D. Nilubol[1]

[1] Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand
[2] Virology Section, Department of Animal Health, National Center for Veterinary Diagnosis, Hanoi, Vietnam
[3] Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand
[4] Department of Veterinary Public Health, Faculty of Veterinary Medicine, Mahasarakham University, Mahasarakham, Thailand
[5] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand
[6] Genome Technology Research Unit, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathumthani, Thailand

Keywords:
porcine deltacoronavirus; Thailand; Vietnam; Lao PDR

Correspondence:
D. Nilubol. Department of Veterinary Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand. Tel.: +662 218 9583;
Fax: +662 251 1656;
E-mail: dachrit@gmail.com

Received for publication April 3, 2016

doi:10.1111/tbed.12585

Summary

Porcine deltacoronavirus (PDCoV) was detected by RT-PCR in 12 of 97 (12.4%) intestinal samples collected during 2015 from piglets with diarrhoea in Thailand, Vietnam and Lao PDR. Spike, membrane and nucleocapsid genes were characterized, and phylogenetic analyses demonstrated that PDCoV isolates from Thai and Lao PDR form a novel cluster, separated from US and China isolates, but relatively were more closely related to China PDCoV than US isolates. Vietnam PDCoVs, however, were grouped together with US PDCoV. The analyses of amino acid changes suggested that they were from different lineage.

Introduction

Porcine deltacoronavirus (PDCoV) is a novel pathogen in the family *Coronaviridae*, genus *Deltacoronavirus*, causing enteric disease characterized by watery diarrhoea similar to porcine epidemic diarrhoea (PED) and transmissible gastroenteritis (TGE) (Jung et al., 2015). PDCoV was first discovered in Hong Kong in 2012, during a study to identify novel coronaviruses (Woo et al., 2012). In February 2014, PDCoV was first detected and reported in Ohio, United States, in association with PED cases. The retrospective investigation demonstrated the presence of PDCoV in the USA as early as 2013 (Sinha et al., 2015). Since then, PDCoV has been detected in most pig-producing states of the USA (Marthaler et al., 2014; Wang et al., 2014; Homwong et al., 2016). Recently, PDCoV was identified for the first time in South Korea and China (Lee and Lee, 2014; Song et al., 2015), and the identification of PDCoV in China was dated back to 2004 (Dong et al., 2015). Increased identification of PDCoV raises concerns regarding the epidemiology and pathogenicity of this virus. We herein report the identification and molecular characterization of PDCoV identified from piglets with clinical diarrhoea in swine farms in South-East Asian countries (SEAC) including Thailand, Vietnam, Lao People's Democratic Republic (Lao PDR) and Philippines.

Materials and Methods

Samples and the detection method

Ninety-seven intestinal samples were collected during 2015 from clinically ill piglets from commercial pig farms with diarrhoea outbreaks in Thailand, Vietnam, Lao PDR and Philippines. Of 97 samples, 68, 10, 6 and 13 were from

Table 1. Results of the detection of porcine deltacoronavirus (PDCoV), porcine epidemic diarrhoea virus (PEDV) and transmissible gastroenteritis virus (TGEV) in intestinal samples by RT-PCR. Samples were collected in 2015 from pig farms in Thailand, Lao PDR, Vietnam and Philippines

				No. of positive samples (%)			
Countries	Provinces	No. of farms	No. of samples	PDCoV	PEDV	TGEV	No. of PDCoV-positive farms[a] (%)
Thailand	Chonburi	3	8	3 (37.5%)	8 (100.0%)	0 (0%)	1 (33.3%)
	Ratchaburi	5	22	1 (4.5%)	20 (90.9%)	0 (0%)	1 (20%)
	Saraburi	3	6	1 (16.7%)	6 (100.0%)	0 (0%)	1 (33.3%)
	Lopburi	1	3	0 (0%)	3 (100.0%)	0 (0%)	0 (0%)
	Buriram	3	5	0 (0%)	10 100.0%)	0 (0%)	0 (0%)
	Chachoengsao	1	2	0 (0%)	2 (100.0%)	0 (0%)	0 (0%)
	Nakhon Ratchasima	3	8	0 (0%)	3 (37.5%)	0 (0%)	0 (0%)
	Nakhon Pathom	5	14	0 (0%)	10 (71.4%)	0 (0%)	0 (0%)
Vietnam	Dong Nai	2	3	1 (33.3%)	3 (100.0%)	0 (0%)	1 (50%)
	Baria	2	2	1 (50.0%)	2 (100.0%)	0 (0%)	1 (50%)
	Long An	2	2	0 (0%)	2 (100.0%)	0 (0%)	0 (0%)
	Binh Duong	1	3	0 (0%)	3 (100.0%)	0 (0%)	0 (0%)
Lao PDR	Khammouane	2	6	5 (83.3%)	5 (83.3%)	0 (0%)	1 (50%)
Philippines	Luzon	2	13	0 (0%)	5 (69.2%)	0 (0%)	0 (0%)
Total		35	97	12 (12.4%)	82 (84.5%)	0 (0%)	6 (17.14%)

[a]All PDCoV-positive farms were PEDV positive.

Thailand, Vietnam, Lao PDR and Philippines, respectively (Table 1). The sampling locations are shown in the Fig. 1.

Intestinal samples from Thailand were collected from 24 farms in the western (Ratchaburi and Nakhon Pathom), eastern (Chonburi and Chachoengsao), middle (Saraburi and Lopburi) and north-eastern (Buriram and Nakhon Ratchasima) regions, representing four major swine-producing areas of Thailand. Samples from Vietnam were from seven pig farms in Dong Nai, Baria, Long An and Binh Duong provinces in the southern region. Samples from Lao PDR were from two pig farms in Khammouane, a province in the northern region sharing a border with Nakhon Phanom, a province in the north-eastern region of Thailand. Samples from Philippines were from two farms on Luzon Island.

Nucleotide sequencing

Total RNA was extracted from intestinal samples using Nucleospin® RNA Virus (Macherey-Nagel Inc., Bethlehem, PA, USA) in accordance with the manufacturer's instructions. cDNA was synthesized from extracted RNA using random hexamers with commercial kit M-MuLV Reverse Transcriptase (New England BioLabs Inc., Ipswich, MA, USA). To screen for the presence of PDCoV, PCR amplification was performed on cDNA using specific primers for membrane (M) and nucleocapsid (N) genes of PDCoV as previously described (Wang et al., 2014). The detection of other porcine coronaviruses, including PEDV and TGEV, was performed following the previously described protocols using specific primers for spike (S) gene of PEDV (Park et al., 2007) and specific primers for N gene of TGEV (Kim et al., 2000).

Positive PDCoV samples were selected and further characterized for complete S, M and N genes using specific primers as described in Table A1. The specific PCR bands were purified by Nucleospin Gel and PCR Clean-up kit (Macherey-Nagel Inc.). The purified PCR products were sequenced. Sequencing was performed by First BASE Laboratories Inc. (Selangor, Malaysia) using an ABI Prism 3730XL DNA sequencer (Applied Biosystems Inc., Carlsbad, CA, USA).

Genetic and phylogenetic analyses

Phylogenetic analyses of the S, M and N genes of the PDCoV isolates were separately constructed together with 23 other PDCoV isolate sequences available in GenBank (Table A2). Bayesian maximum clade credibility trees were analysed using Bayesian Markov Chain Monte Carlo (MCMC) method in BEAST 1.8.3 (Drummond and Rambaut, 2007) with Yang 96 model (Yang, 1996) provided in the BEAST. Tree prior was set as coalescent:constant size (Kingman, 1982). The MCMC chains were run for at least 300 million generations and sampled every 10 000 states. Over 30 000 generated trees were annotated using TreeAnnotator 1.8.3 with 10% burn-in, maximum clade credibility tree and median heights nodes. Tree images were generated using FigTree 1.4.2 (Rambaut, 2014) with decreasing order nodes.

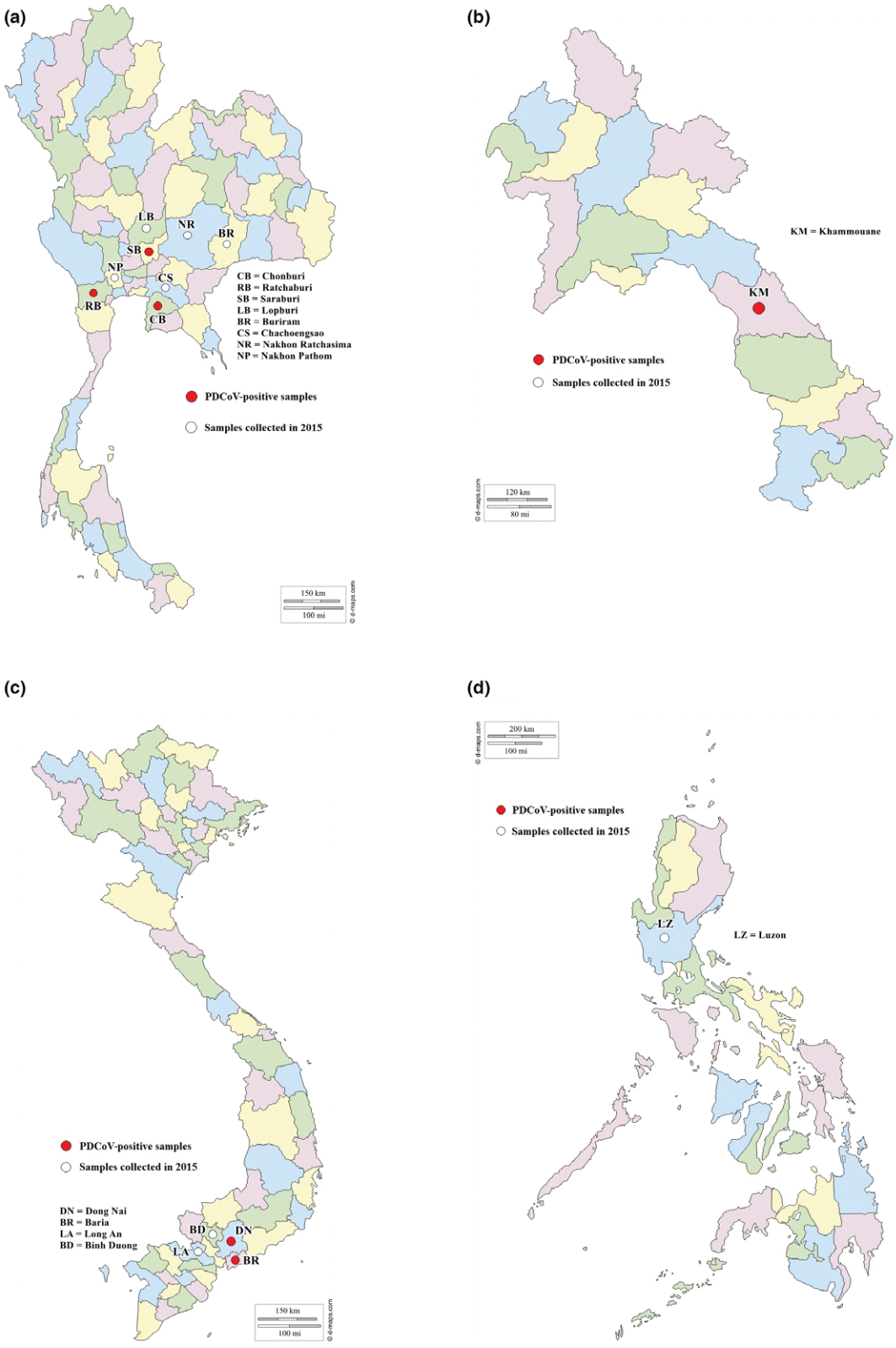

Fig. 1. Geographical distribution of porcine deltacoronavirus (PDCoV) in Thailand (a), Lao PDR (b), Vietnam (c) and Philippines (d). Red dots represent the provinces having PDCoV-positive areas and white dots represent the provinces where samples were collected in 2015.

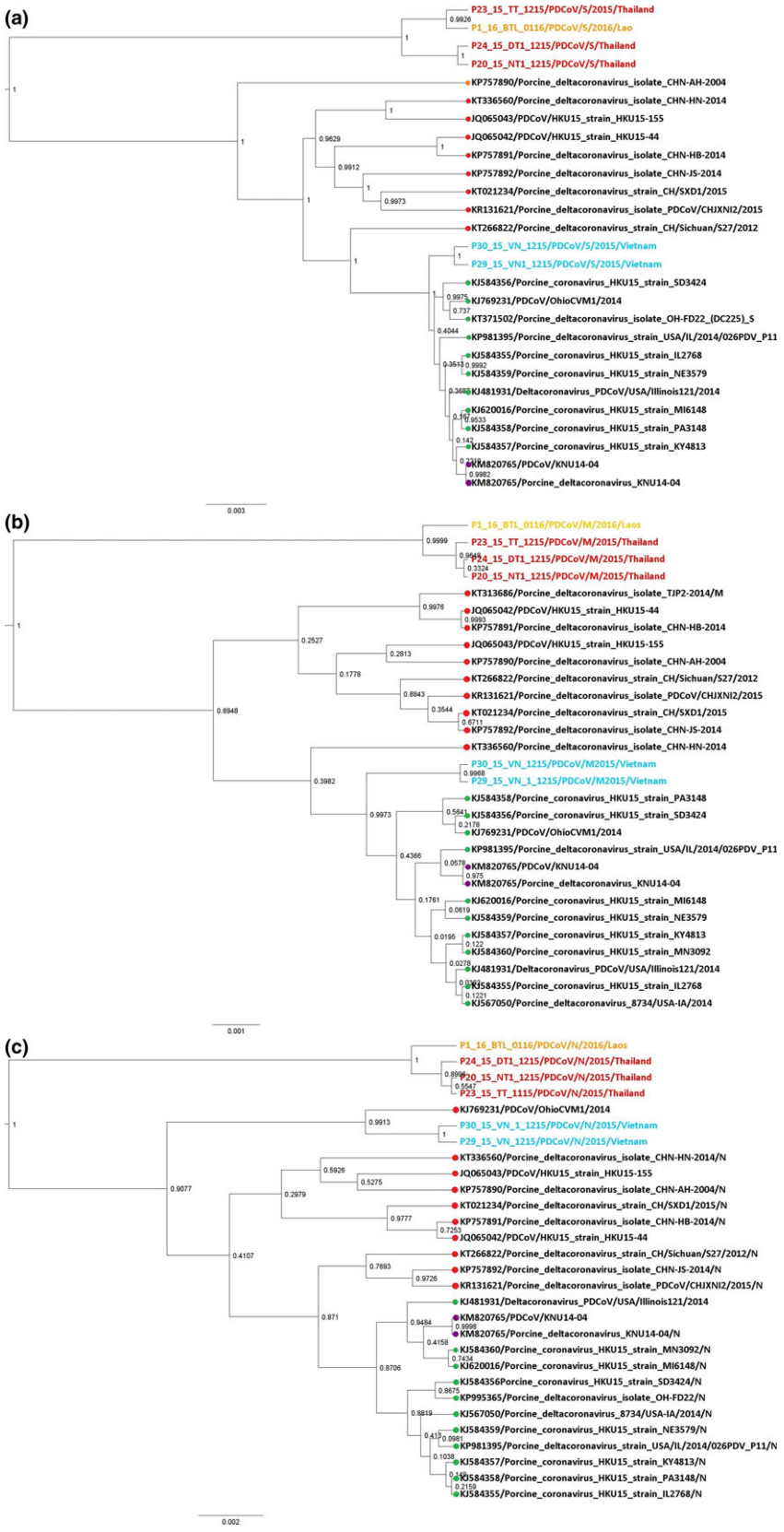

Fig. 2. Bayesian phylogenetic analysis of spike (a), membrane (b) and nucleocapsid (c) gene. Red, yellow and blue represent PDCoV isolates from Thailand, Lao PDR and Vietnam, respectively. Red, green and purple dots represent PDCoV isolated from China, USA and Korea, respectively. The reference sequences obtained from GenBank are indicated by strain name and accession number.

Table 2. Comparison of the nucleotide and amino acid sequence identities (%) based on S, M and N genes from Thai, Laos and Vietnam isolates with USA and China PDCoV groups

		US PDCoV isolates		China PDCoV isolates	
PDCoV isolates	Genes	Nucleotide (%)	Amino acid (%)	Nucleotide (%)	Amino acid (%)
Thai	S	96.0–96.4	98.2–99.1	95.5–96.8	98.5–99.1
	M	97.8–98.3	99.5	98.0–98.7	99.5
	N	97.6–98.1	98.2–99.1	97.8–98.7	98.5–99.1
Laos	S	96.0–96.4	98.2–99.1	95.5–96.8	98.5–99.1
	M	97.8–98.3	99.5	98.0–98.7	99.5
	N	97.6–98.1	98.2–99.1	97.8–98.7	98.5–99.1
Vietnam	S	99.3–99.7	98.8–99.4	98.2–99.5	98.5–99.7
	M	99.3–99.6	100	98.9–99.5	99.5–100
	N	98.4–99.2	98.8–99.4	98.2–98.9	98.5–99.7

Results and Discussions

Porcine epidemic diarrhoea (PED) has been endemic in SEAC since 2007 with continued sporadic outbreaks with lower severity of clinical disease compared to the pandemic outbreak in 2007–2009 (Temeeyasen et al., 2014; Vui et al., 2014). Since the emergence of PED, several pig farms in SEAC have experienced sporadic outbreaks of diarrhoea in piglets more than once a year. The causative agent was considered to be a variant of PEDV. The role of PDCoV in the outbreak, although suspected, was not investigated at that time. PDCoV was suspected when rebreaks of clinical enteric disease similar to PED occurred every two months in some herds, which is too frequent compared to the period of six-month protection reported earlier (Goede et al., 2015). PDCoV has since been investigated in addition to the detection of PEDV.

In the study, 97 intestinal samples were submitted to the laboratory in 2015 for PEDV diagnosis and therefore were tested for three viral pathogens including PEDV, TGEV and PDCoV. Of 97 intestinal samples tested, 12 samples (12.4%) were positive for PDCoV, 82 samples (84.5%) were positive for PEDV, and none were positive for TGEV (Table 1). Samples positive for PDCoV were also positive for PEDV. Three, two and one farms in Thailand, Vietnam and Lao PDR, respectively, were positive with both PED and PDCoV. Of 12 PDCoV-positive samples, five, two and five samples were from farms in Thailand, Vietnam and Lao PDR, respectively. Only PEDVs were present in samples from Philippines. Interestingly, PDCoV was detected in all four swine-producing areas in Thailand. The locations and numbers of farms in each country were presented in Table 1.

Six samples (three from Thailand, two from Vietnam and one from Lao PDR) were selected for further complete sequencing of S, M and N genes. Sequences have been deposited in GenBank under accession nos. KU87047 9–KU870484. The genetic analyses demonstrated that S, M and N genes of three Thai PDCoV (P20_15_NT1_1215, P23_15_TT_1215 and P24_15_DT1_1215), one Lao PDCoV (P1_16_BTL_0116) and two Vietnam PDCoV (P29_15_VN_1_1215 and P30_15_VN_1215) isolates are 3477-3480, 651 and 1026 nucleotide (nt) in length, encoding for 1159-1160, 127 and 342 amino acids, respectively.

To demonstrate the genetic relationship between Thai, Lao and Vietnam, and the previously reported China and US PDCoV isolates, phylogenetic analyses of S, M and N genes were separately constructed and the results of all three genes demonstrated that PDCoVs from Thailand and Lao PDR form their own cluster, separated from China and US PDCoV (Fig. 2). Based on S and M genes, Vietnam PDCoV isolates are grouped together with US PDCoV, separated from Thai and Lao PDCoV. Vietnam PDCoV isolates are closely related to the US isolates than China PDCoV. The results based on the phylogenetic analyses of S, M and N genes suggested that PDCoVs from Thailand and Lao PDR are from different lineage compared to Vietnam PDCoV (Fig. 2).

The percentage of nucleotide and amino acid similarities between Thai, Lao and Vietnam, and the previously reported China and US PDCoV isolates, are displayed in Table 2. Based on S, M and N genes, the three Thai PDCoV isolates were more highly homologous to Lao PDCoV than Vietnam PDCoV with nucleotide and amino acid similarities at 99.8% and 100%, respectively. Thai and Lao PDCoV isolates relatively were more closely related to China PDCoV than US isolates. Based on S gene, Thai and Lao isolates shared nucleotide and amino acid similarities at 95.5–96.8% and 98.5–99.1%, respectively, with China PDCoV, as well as sharing nucleotide and amino acid similarities at 96.0–96.4% and 98.2–99.1%, respectively, to US PDCoV. Similar to S gene results, the M and N genes of Thai and Lao PDCoV shared nucleotide and amino acid similarities at 98.0-98.7% and 99.5%, and 97.8–98.7% and 98.5–99.1%, respectively, with China PDCoV, and shared nucleotide and amino acid similarities at 97.8–98.3% and 99.5%, and 97.6–98.1% and 98.2–99.1%, respectively, to US PDCoV isolates. In contrast, Vietnam PDCoV isolates were more homologous to US PDCoV isolates than China isolates. Based on S gene, Vietnam PDCoV isolates shared nucleotide

and amino acid similarities at 99.3–99.7% and 98.8–99.4%, respectively, to US isolates, while nucleotide and amino acid similarities with China PDCoV at 98.2–99.5% and 98.5–99.7%, respectively. Based on M and N genes, Vietnam PDCoV isolates shared nucleotide and amino acid similarities at 99.3–99.6% and 100%, and 98.4–99.2% and 98.8–99.4%, respectively, to US PDCoV, while nucleotide and amino acid similarities with China PDCoV at 98.9–99.5% and 99.5–100%, and 98.2–98.9% and 98.5–99.7%, respectively.

The amino acid substitutions of each gene between PDCoV isolates from each country are showed in Technical Fig. A1a–c. Based on S, M and N genes, Thai and Lao PDCoV isolates had 23–26, 1 and 4–5 amino acid substitutions, respectively, compared to China PDCoV. Moreover, Thai and Lao PDCoV isolates had 25–28, 1 and 4–5 amino acid substitutions at S, M and N genes, respectively, compared to US PDCoV. In contrast, Vietnam PDCoV had only 2–4 and 1–2 amino acid substitutions compared to both China and US PDCoV isolates based on S and N genes, respectively, but no amino acid substitution was observed in M gene.

In conclusion, the study reported the identification of PDCoV in SEAC including Thailand, Lao PDR, Vietnam and Philippines. The PDCoVs isolated from Thailand and Lao PDR form their own cluster, separated from China and US PDCoV, but relatively were more closely related to the isolates from China than to US PDCoV. In contrast, the PDCoVs isolated from Vietnam were more closely related to the isolates from the USA. The results of the study suggested that the viruses from these three SEAC might originate from different ancestors. The identification of PDCoV in SEAC suggests that the virus may have been in this region for some time, but has not been detected due to greater focus on PEDV variants. Although PDCoV was not detected in Philippines, it does not mean the virus was not there due to limited sample numbers from this area. The origin and source of introduction into Thailand, Lao PDR and Vietnam are still questionable. The viruses could have been in this region for some time, and continuously evolved until separated into different lineage, or the viruses were introduced from different ancestors or sources. Further retrospective investigations are urgently needed to elucidate source and evolution. In addition, further analysis and molecular epidemiology based on the complete genome sequence, and pathogenicity studies of PEDV and PDCoV co-infection are urgently needed.

Acknowledgements

This work was supported by the National Research Council of Thailand and Agricultural research development agency (Public organization), and partial funding was provided by Special Task Force for Activating Research (STAR), Swine Viral Evolution and Vaccine Research (SVEVR), Chulalongkorn University.

Conflict of Interest

The authors declare that there are no conflict of interests.

References

Dong, N., L. Fang, S. Zeng, Q. Sun, H. Chen, and S. Xiao, 2015: Porcine Deltacoronavirus in Mainland China. *Emerg. Infect. Dis.* 21, 2254–2255.

Drummond, A. J., and A. Rambaut, 2007: BEAST: bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214.

Goede, D., M. P. Murtaugh, J. Nerem, P. Yeske, K. Rossow, and R. Morrison, 2015: Previous infection of sows with a "mild" strain of porcine epidemic diarrhea virus confers protection against infection with a "severe" strain. *Vet. Microbiol.* 176, 161–164.

Homwong, N., M. C. Jarvis, H. C. Lam, A. Diaz, A. Rovira, M. Nelson, and D. Marthaler, 2016: Characterization and evolution of porcine deltacoronavirus in the United States. *Prev. Vet. Med.* 123, 168–174.

Jung, K., H. Hu, B. Eyerly, Z. Lu, J. Chepngeno, and L. J. Saif, 2015: Pathogenicity of 2 porcine deltacoronavirus strains in gnotobiotic pigs. *Emerg. Infect. Dis.* 21, 650–654.

Kim, O., C. Choi, B. Kim, and C. Chae, 2000: Detection and differentiation of porcine epidemic diarrhoea virus and transmissible gastroenteritis virus in clinical samples by multiplex RT-PCR. *Vet. Rec.* 146, 637–640.

Kingman, J. F. C., 1982: The coalescent. *Stoch. Proc. Appl.* 13, 235–248.

Lee, S., and C. Lee, 2014: Complete genome characterization of Korean porcine deltacoronavirus strain KOR/KNU14-04/2014. *Genome Announc.* 2, e01191–14.

Marthaler, D., Y. Jiang, J. Collins, and K. Rossow, 2014: Complete genome sequence of strain SDCV/USA/Illinois121/2014, a porcine deltacoronavirus from the United States. *Genome Announc.* 2, e00218–14.

Park, S. J., H. J. Moon, J. S. Yang, C. S. Lee, D. S. Song, B. K. Kang, and B. K. Park, 2007: Sequence analysis of the partial spike glycoprotein gene of porcine epidemic diarrhea viruses isolated in Korea. *Virus Genes* 35, 321–332.

Rambaut, A., 2014: *FigTree*. University of Edinburgh, Edinburgh. Available at http://tree.bio.ed.ac.uk/software/figtree/ (accessed September 19, 2016).

Sinha, A., P. Gauger, J. Zhang, K. J. Yoon, and K. Harmon, 2015: PCR-based retrospective evaluation of diagnostic samples for emergence of porcine deltacoronavirus in US swine. *Vet. Microbiol.* 179, 296–298.

Song, D., X. Zhou, Q. Peng, Y. Chen, F. Zhang, T. Huang, T. Zhang, A. Li, D. Huang, Q. Wu, H. He, and Y. Tang, 2015: Newly emerged porcine deltacoronavirus associated With diarrhoea in swine in China: identification, prevalence and

full-length genome sequence analysis. *Transbound. Emerg. Dis.* 62, 575–580.

Temeeyasen, G., A. Srijangwad, T. Tripipat, P. Tipsombatboon, J. Piriyapongsa, W. Phoolcharoen, T. Chuanasa, A. Tantituvanont, and D. Nilubol, 2014: Genetic diversity of ORF3 and spike genes of porcine epidemic diarrhea virus in Thailand. *Infect. Genet. Evol.* 21, 205–213.

Vui, D. T., N. Tung, K. Inui, S. Slater, and D. Nilubol, 2014: Complete genome sequence of porcine epidemic diarrhea virus in Vietnam. *Genome Announc.* 2, e00753–14.

Wang, L., B. Byrum, and Y. Zhang, 2014: Porcine coronavirus HKU15 detected in 9 US states, 2014. *Emerg. Infect. Dis.* 20, 1594–1595.

Woo, P. C., S. K. Lau, C. S. Lam, C. C. Lau, A. K. Tsang, J. H. Lau, R. Bai, J. L. Teng, C. C. Tsang, M. Wang, B. J. Zheng, K. H. Chan, and K. Y. Yuen, 2012: Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. *J. Virol.* 86, 3995–4008.

Yang, Z., 1996: Maximum-Likelihood Models for Combined Analyses of Multiple Sequence Data. *J. Mol. Evol.* 42, 587–596.

Appendix

Table A1. Primers used for the complete spike, membrane and nucleocapsid gene sequences of porcine deltacoronavirus and specific primer of PEDV, TGEV

Primers	Primer sequence (5′-3′)	Sizes (bp)
PDCoV_S1_F	ATGCAGAGAGCTCTATTGATTATGACC	961
PDCoV_S1_R	CTTCGCCAAAATCCATGTGTGCAG	
PDCoV_S2_F	CAATAGCATGCCAGCGCTCTTCTCA	923
PDCoV_S2_R	TGGTATTTCAACTTCGCCATCGTATAG	
PDCoV_S3_F	CATCCACATTACAGAATACTCGAC CA	979
PDCoV_S3_R	TGAGTAACATATGCATTAAGTGCAGC	
PDCoV_S4_F	CATTATCACACCTGACTGCACAGCT	1005
PDCoV_S4_R	CTAC CATTC CTTAAACTTAAAGGAC G	
PDCoV_M-F	ATCCTC CAAGGAGGCTATGC	494
PDCoV_M-R	GC GAATTCTGGATC GTTGTT	
PDCoV_N-F	TTTCAGGTGCTCAAAGCTCA	695
PDCoV_N-R	GC GAAAAGCATTTC C TGAAC	
PEDV-F	TTCTGAGTCACGAACAGCCA	651
PEDV-R	CATATGCAGCCTGCTCTGAA	
TGEV-F	GATGCCGAC CAGATAGAAGT	612
TGEV-R	GCAATAGGGTTGCTTGTACC	

Table A2. Twenty-three isolates of porcine deltacoronavirus (PDCoV) were used to be templates of genetic analyses in the study

No.	Isolates	Year	Place of isolation	Accession #
1	HKU15-155	2012	Hong Kong	JQ065043
2	8734/USA-IA	2014	Iowa, USA	KJ567050
3	IL2768	2014	Ohio, USA	KJ584355
4	NE3579	2014	Nebraska, USA	KJ584359
5	SD3424	2014	South Dakota, USA	KJ584356
6	KY4813	2014	Kentucky, USA	KJ584357
7	PA3148	2014	Pennsylvania, USA	KJ584358
8	MN3092	2014	Minnesota, USA	KJ584360
9	Illinois121/2014	2014	Illinois, USA	KJ481931
10	MI6148	2014	Michigan, USA	KJ620016
11	026PDV	2015	Illinois, USA	KP981395
12	OhioCVM1	2015	Ohio, USA	KJ769231
13	OH-FD22N	2015	Ohio, USA	KP995365
14	CHN-HN-2014	2015	China	KT336560
15	KNU14-04/2014	2014	South Korea	KM820765
16	CHN-AH-2004	2004	China	KP757890
17	CHN-JS-2014	2014	China	KP757892
18	CH/SXD1/2015	2015	China	KT021234
19	CHJXNI2/2015	2015	China	KR131621
20	CH/Sichuan/S27/2012	2012	China: Sichuan	KT266822
21	TJP2-2014/M	2014	China	KT313686
22	HKU15-44	2012	China: Hong Kong	JQ065042
23	CHN-HB-2014	2014	China	KP757891

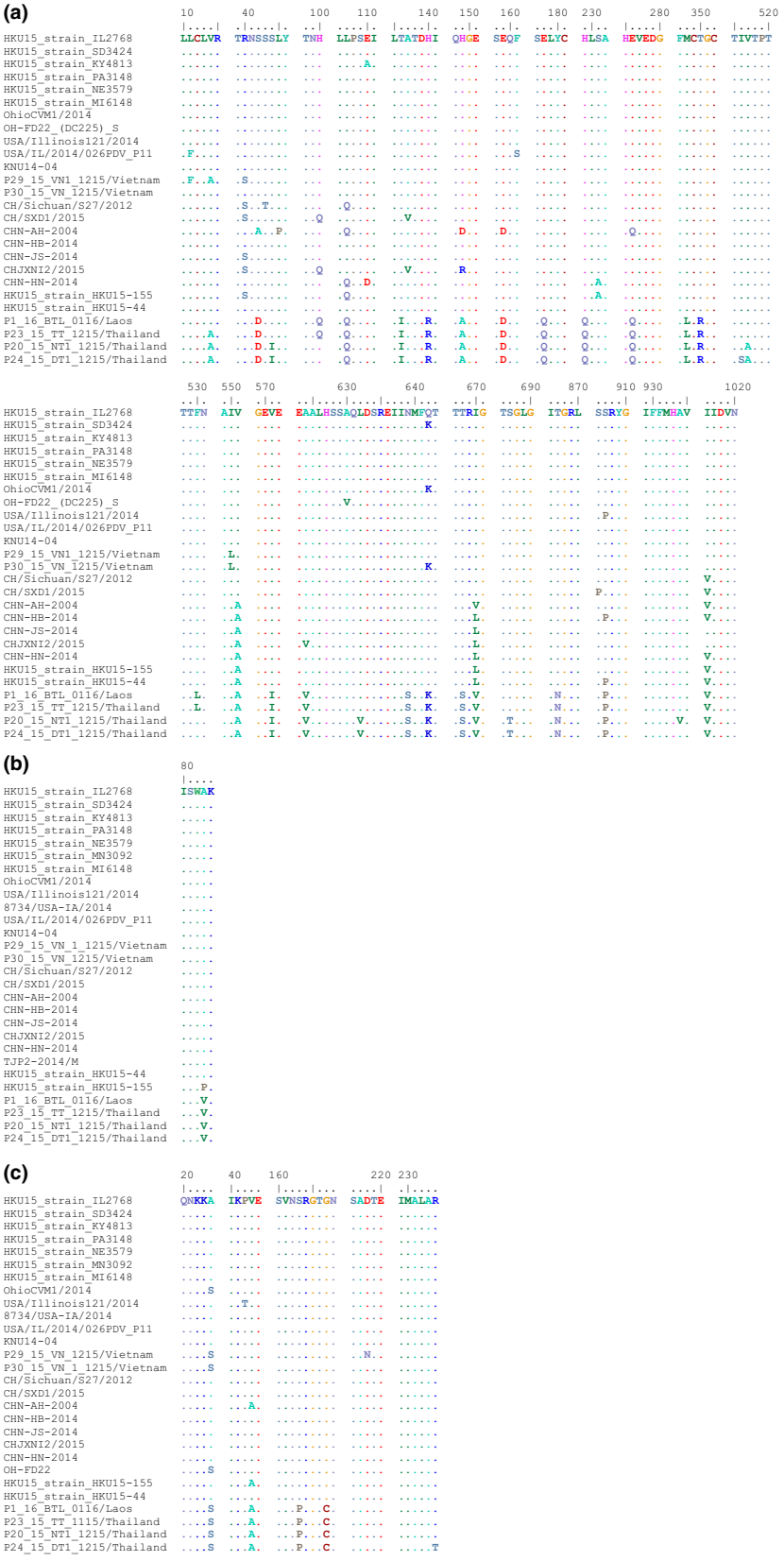

Fig. A1. The substitutions of PDCoV isolates from Thailand, Laos PDR, and Vietnam, base on spike (a), membrane (b) and nucleocapsid (c) gene.