MOLECULAR EPIDEMIOLOGY AND CHARACTERIZATION OF ROTAVIRUS GROUP A, GROUP C AND PORCINE EPIDEMIC DIARRHEA VIRUS IN PIGS WITH GASTROENTERITIS AMONG THE COMMERCIAL SWINE FARMS IN THAILAND, 2011-2016

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ระบาดวิทยาและการจำแนกเชิงโมเลกุลของเชื้อไวรัสโรต้าเอ, โรต้าซี และเชื้อไวรัสพีอีดีจากสุกรท้องเสียที่แยกได้จากฟาร์มสุกรในประเทศไทย ระหว่างปี พ.ศ 2554-2559

น.ส.สุพรรษา ต่วนทัพ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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เชื้อไวรัสพีอีดี และเชื้อไวรัสโรต้า ก่อให้เกิดอาการท้องเสียในสุกรทั่วโลก โดยเชื้อไวรัสโรต้า เอ และ ซี นั้นเป็นกลุ่มย่อยที่มีรายงานว่าเป็นสาเหตุของลูกสุกรท้องเสียที่พบได้บ่อยที่สุด สำหรับประเทศไทย เชื้อไวรัสพีอีดี และเชื้อไวรัสโรต้า เอ มีการศึกษามาอย่างต่อเนื่องตั้งแต่อดีต แต่ข้อมูลของเชื้อไวรัสโรต้า ซี นั้นยังมีค่อนข้างจำกัด สำหรับการศึกษาในครั้งนี้ เป็นการศึกษาระบาดวิทยาเชิงโมเลกุลของไวรัสพีอีดี และเชื้อไวรัสโรต้า เอ และ ซี จากตัวอย่างอุจจาระ และ ลำไส้จำนวน 769 ตัวอย่าง จากสุกรในช่วงอายุต่างๆที่แสดงอาการท้องเสีย จากหลายจังหวัด ระหว่างปี พ.ศ 2554 ถึง 2559 ตัวอย่างทั้งหมดถูกนำมาเพิ่มจำนวนสารพันธุกรรมวิธี RT-PCR กับคู่ไพรเมอร์เฉพาะเจาะจง และจำแนกลักษณะทางพันธุกรรมด้วยวิธี nucleotide sequencing สำหรับเชื้อไวรัสพีอีดี ทำการศึกษาในส่วนของยีน S, ORF3 และ N, ไวรัสโรต้า เอ ทำการศึกษายีนทั้ง 11 ท่อน และเชื้อไวรัสโรต้า ซี ได้ทำการศึกษาในส่วนยีนเปลือกหุ้ม VP7, VP4 ผลการศึกษาพบว่า เชื้อไวรัสพีอีดี เป็นสาเหตุที่ก่อให้เกิดอาการท้องเสีย 19.9% (153/769) เชื้อไวรัสโรต้า เอ 9.5% (73/769) และ เชื้อไวรัสโรต้า ซี 6.6% (51/769) การติดเชื้อร่วมกันระหว่างไวรัสโรต้า เอ และโรต้า ซี เท่ากับ 21.6% (11/51), ระหว่างไวรัสพีอีดี กับ ไวรัสโรต้า ซี เท่ากับ 7.8% (4/51) และการติดเชื้อร่วมกันทั้ง 3 ชนิดเท่ากับ 5.8%, ช่วงอายุสุกรตั้งแต่ 1 สัปดาห์ถึง 8 สัปดาห์ ตรวจพบการติดเชื้อไวรัสโรต้ามากที่สุด ในขณะที่ในช่วงอายุน้อยกว่า 4 สัปดาห์ตรวจพบไวรัสพีอีดีมากที่สุด โดยฤดูกาลไม่มีผลต่อความชุกของเชื้อไวรัส ผลการศึกษาแยกจีโนไทป์ของไวรัสโรต้า พบว่า เชื้อไวรัสโรตา เอ พบจีโนไทป์ G9 และ P[13] มากที่สุด สำหรับเชื้อโรต้า ซี พบจีโนไทป์ G1 และ P[5] มากที่สุด นอกจากนี้ การศึกษา genome constellation ของไวรัสโรต้า เอ จำนวน 24 สายพันธุ์ พบว่ามีรูปแบบการเรียงตัวเป็นแบบ Wa-like genotype โดยมียีนบางท่อนแสดงความคล้ายคลึงกับสายพันธุ์ที่แยกได้จากมนุษย์ สำหรับเชื้อไวรัสพีอีดีที่สามารถทำการศึกษาได้ครบทั้ง 3 ยีนนั้น มีจำนวน 95 สายพันธุ์ ผลการศึกษาลักษณะทางพันธุกรรมของเชื้อพบว่ามีการเปลี่ยนแปลงเล็กน้อยจากสายพันธุ์ที่มีการตรวจพบในประเทศไทยมาก่อน ทำให้ลำดับพันธุกรรมยังคงมีความคล้ายคลึงต่อกันสูง นอกจากนี้ ยังแสดงความคล้ายคลึงกับสายพันธุ์ที่แยกได้จากประเทศจีนอีกด้วย และเชื้อไวรัสพีอีดี จำนวน 8 สายพันธุ์ พบการขาดหายไปในบางตำแหน่งของลำดับกรดอะมิโนในส่วนยีน N กล่าวโดยสรุปว่า จากการศึกษาถึงการเปลี่ยนแปลงในระดับพันธุกรรมนี้ ช่วยให้ข้อมูลพื้นฐานเชิงโมเลกุลของสายพันธุ์ไวรัสพีอีดีและไวรัสโรต้าที่พบในประเทศไทย

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Supansa Tuanthap : MOLECULAR EPIDEMIOLOGY AND CHARACTERIZATION OF ROTAVIRUS GROUP A, GROUP C AND PORCINE EPIDEMIC DIARRHEA VIRUS IN PIGS WITH GASTROENTERITIS AMONG THE COMMERCIAL SWINE FARMS IN THAILAND, 2011-2016. Advisor: Prof. Alongkorn Amonsin, D.V.M., Ph.D. Co-advisor: Prof. Yong Poovorawan, M.D.

The most frequent viruses associated with pig gastroenteritis have been previously reported as porcine epidemic diarrhea virus (PEDV) and rotavirus (RV). Rotavirus is an important cause of diarrhea in piglets and pigs worldwide, and group A (RVA) and group C (RVC) are mostly affected. In Thailand, studies on RVA and PEDV have been reported periodically, whereas information on RVC is still limited. In this study, 769 samples (fecal and intestinal content) from pigs with diarrhea were collected from pig herds located in difference provinces throughout Thailand between 2011 and 2016. The specimens were tested using virus-specific RT-PCR to detect the gene encoding capsid protein VP7 and VP4 for RVC, complete 11 gene segments of RVA and S, ORF3, N genes for PEDV. Sequencing analyses showed that 6.6% (51/769) of samples were positive for RVC, 9.5% (73/769) for RVA and 19.9% (153/769) for PEDV. Co-infections of RVA/RVC accounted for 21.6% (11/51) of samples and of PEDV/RVC for 7.8% (4/51) of samples, while only three samples (3/51) or 5.8% tested positive for all three viruses. RV was detected in piglets up to 8 weeks old, while PEDV was often demonstrated from newborn up to 4 weeks of age. Infection severities were not associated with seasonality, since the virus was detected throughout the year. From our study, the G9 and P[13] as the dominant genotypes for RVA, while predominant genotypes of RVC were G1 and P[5]. Furthermore, genome constellation of the Thai RVA strains showed the predominance of Wa-like genotype with significant of reassortment between the porcine and human RVA strains. Comparison of 95 PEDV-positive samples indicated those Thai strains had close genetic relationship and resembled to previously identified PEDV from Thailand and China. Interestingly, eight Thai PEDV strains possessed the amino acid deletions in the N protein. Our findings provide substaintial information in regional rotavirus and PEDV strains in field circulations and may assist inclusions of suitable strains for future vaccine development.

Field of Study:	Biomedical Sciences	Student's Signature .....
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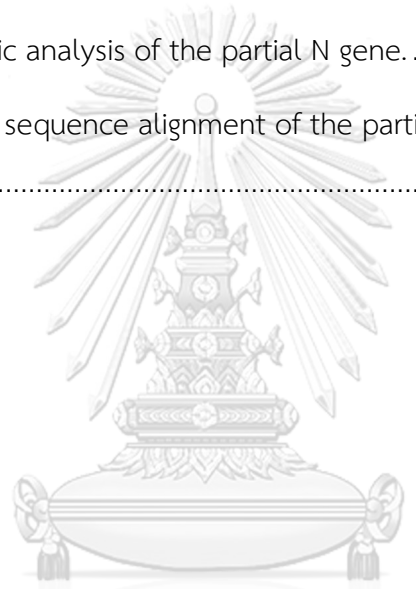


List of Abbreviations

Abbreviation	Full name
ºC	degree Celsius
P-value	probability value
µl	microliter
aa	amino acid
BLAST	basic local alignment search tool
bp	base pair
DLP	double layer particle
ds-RNA	double stranded RNA
g	grams
ex.	samples
HBGAs	histo-blood group antigens
MEGA	molecular evolutionary genetics analysis
min	minute
N	nucleocapsid gene
NCBI	The National Center for Biotechnology Information
ng	nanogram
nm	nanometer
NSP	non-structural protein
nt	nucleotide
ORF3	open reading frame 3 gene
PCR	polymerase chain reaction
PEDV	porcine epidemic diarrhea virus
RCWG	Rotavirus Classification Working Group

RNA	ribosomal nucleic acid
RT	reverse transcription
RV	rotavirus
RVA	rotavirus group A
RVC	rotavirus group C
S	spike gene
sec	seconds
ssRNA	single stranded RNA
TGE	transmissioble gastroenteritis virus
TLP	triple layer particle
VP	viral protein
wks	weeks

CHAPTER I
GENERAL INTRODUCTION

Background and rationale

The viral gastroenteritis associated with high morbidity and mortality rate in suckling and post-weaning piglets are porcine epidemic diarrhea virus (PEDV) and rotavirus. Among these viruses could be a single infection or mixed infections which the naturally infected pigs displayed the similarity of fecal appearances and symptoms. Moreover, the pathogenesis of rotavirus and PEDV infection are similar because the target cells of viral replication are the villous enterocytes in the animal intestine. Blunting of the villi of infected enterocytes and atrophy results in electrolyte imbalance, dehydration due to intestinal malabsorption, osmotic irregularities, watery diarrhea and eventually death.

Rotavirus (RV) contains segmented double-stand RNA genome which genetic reassortments and interspecies transmission naturally occur upon mixed RV strain infection. RV is frequently responsible for diarrhea in humans and animals which porcine RVA strains are one source of new emerging RV genotypes found in human and animal. In general, porcine rotavirus (PRV) infection is associated with gastroenteritis in pigs of all ages but neonatal piglets to 3 weeks and post-weaning periods are frequently infected, resulting in poor growth performance, increased morbidity and mortality rates. Nevertheless, the disease severity depending on the age of infected host.

Nowadays, the Rotavirus Classification Working Group or RCWG classified rotavirus into 8 serogroups based on the basis of major antigenic differences that predominantly reside in the VP6, including A, B, C, D, E, H, I and J. In pig, rotavirus group A (RVA) is most commonly associated with gastroenteritis in piglets while non-

group A RV (group B, E and H) often causes sporadic infection. However, recently rotavirus group C were also frequently reported as a cause of diarrhea in young piglet less than 1 week and preferred in asymptomatic pigs.

Porcine epidemic diarrhea virus (PEDV) is a member of family *Coronaviridae*. PEDV infection causes of acute watery diarrhea, vomiting and severe dehydration in pigs of all age but the infection of younger piglets are usually more severe than the older pigs (especially less than 1 week old). The sudden death is often found from metabolic acidosis symptoms in newborn pigs. PEDV infection significantly contributes to economic loss in pig herds around the world because disease prevention and control programs have not been successful.

The pathogenesis of rotavirus and PEDV infection are more or less similar. The target cells of virus replication are the villous enterocytes, results in cell lysis and led to villi blunting and atrophy. The incubation period of virus infections are slightly short and the onset diarrhea can occur between 18-96 hours post-infection. Infected piglets get severe an electrolyte imbalance, dehydration from intestinal malabsorption, osmotic irregularities, watery diarrhea and eventually death. It causes tremendously losses to the pig industry worldwide up to now including Thailand.

Therefore **the aims of this study** were to:

1. To study the molecular epidemiology of the rotavirus group A, group C and the porcine epidemic diarrhea virus in pigs with clinical diarrhea in Thailand during year 2011-2016.
2. To investigate the co-infection of rotavirus group A, group C and porcine epidemic diarrhea virus and to compare the clinical data of those with and without co-infection.

3. To determine the genotype and the reassortment pattern of rotavirus group A based on a complete genotype constellation and compare to the rotavirus group A identified in human infection.

To achieve all objectives, the study have been performed in pigs with gastroenteritis on Thai swine farms, from year 2011-2016 as follow in 4 parts;

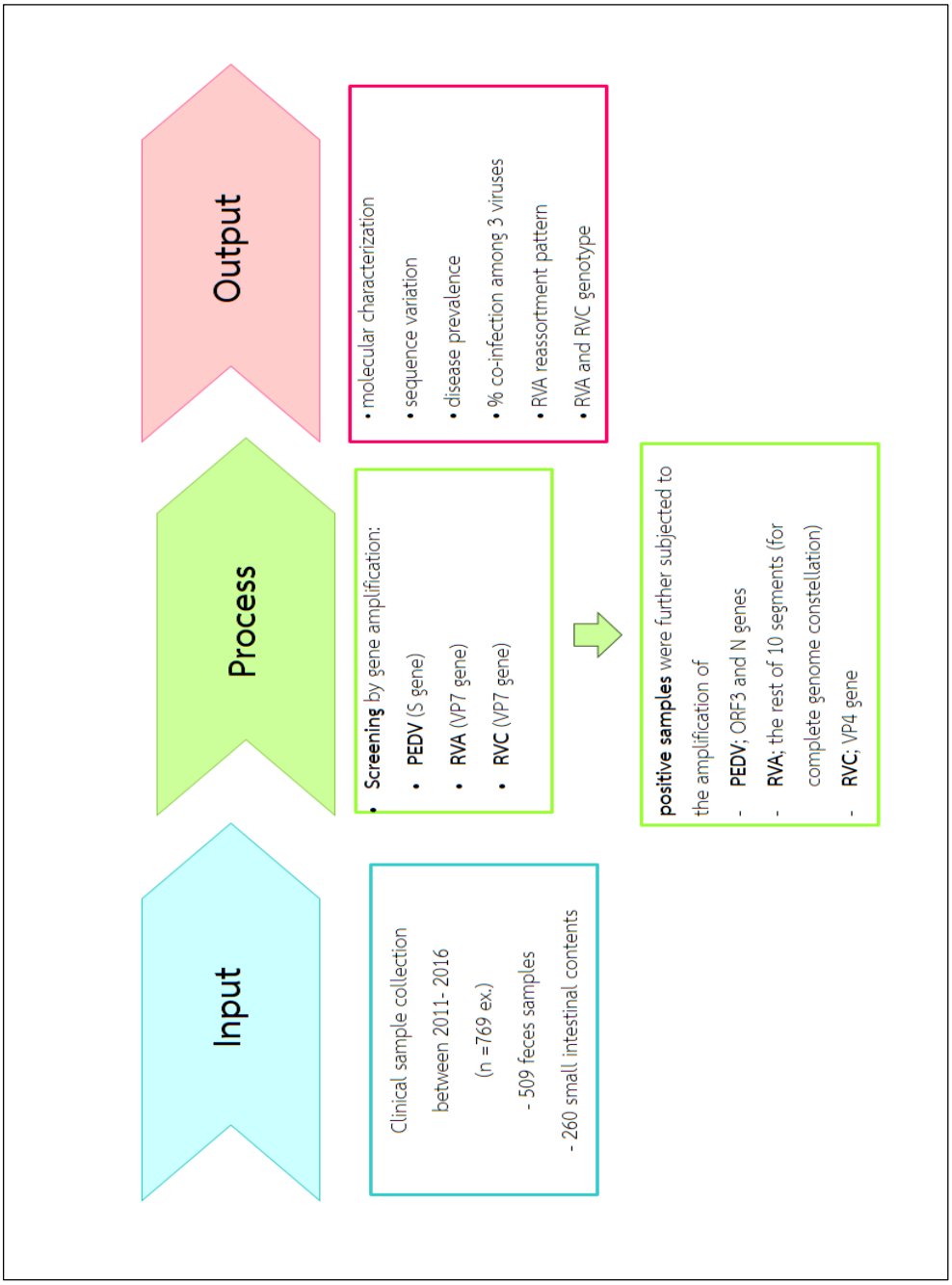
Part 1: The sensitivity and specificity tests of new designed primers for rotavirus group A and group detection.

Part 2: Molecular epidemiology of porcine rotavirus group C and viral co-infection among rotavirus group A, group C and porcine epidemic diarrhea virus.

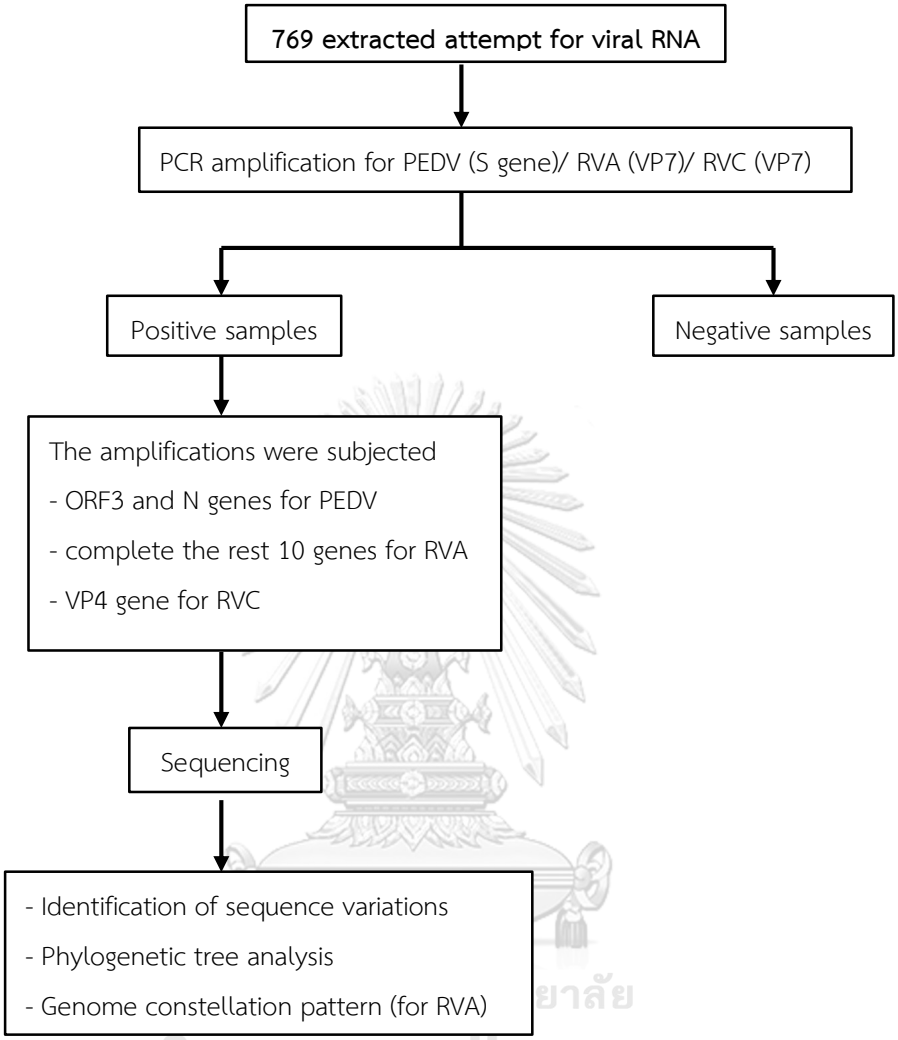
Part 3: Molecular characterization and genome constellation of Thai porcine rotavirus group A.

Part 4: Molecular epidemiology and characterization of spike, ORF3 and nucleocapsid genes of porcine epidemic diarrhea virus.

Conceptual framework



The work diagram



Ethical Consideration

This study have been approved by The Institutional Animal Care and Use Committee (IACUC permission number 1731020) and the Institutional Biosafety Committee (IBC permission number 1731008) of Chulalongkorn University.

CHAPTER II
LITERATURE REVIEW

Characteristic of virus

- **Rotavirus (RV)**

Rotavirus is member of family *Reoviridae*, genus *Rotavirus*. Rotavirus particle size is 75 nm in diameter, icosahedral in shape, non-envelope RNA virus with triple layer capsid structure (Figure 1). A total genome size is approximately 18,522 base pairs with 11 segments of double stranded RNA (ds RNA), each segment encodes only 1 protein except segment 11 which is able to encode 2 non-structural proteins in some species. In porcine rotavirus, the structural proteins comprise with VP1, VP2, VP3, VP4, VP6 and VP7, while non-structural proteins comprise NSP1-NSP5 (1).

The outer layer forms by VP7 and VP4 proteins, while the intermediate layer is formed by VP6 protein and the inner layer (core) is formed by VP1, VP2, and VP3 proteins (1). The complete particle resembles a wheel under electron microscope. Only triple layer virus is infectious (2). Based on the serological differences and diverse virus types, VP6 was used in the classification of rotavirus into 8 serogroups (group A, B, C, D, E, H, I and J) (The cut-off value at 53% amino acid level of VP6 protein) (3-7).

Porcine rotaviruses (PRV) are divided into 5 serogroups (A, B, C, E and H). Group A is the major cause of diarrhea (10-70%) affecting piglets between 1 and 3 weeks of age. Groups B is detected sporadically, while group C commonly causes diarrhea in suckling pigs less than 1 week of age (8-10). Group E has only been detected in pigs in the United Kingdom (11). The new group H has been recently discovered in 2011 (4, 12).

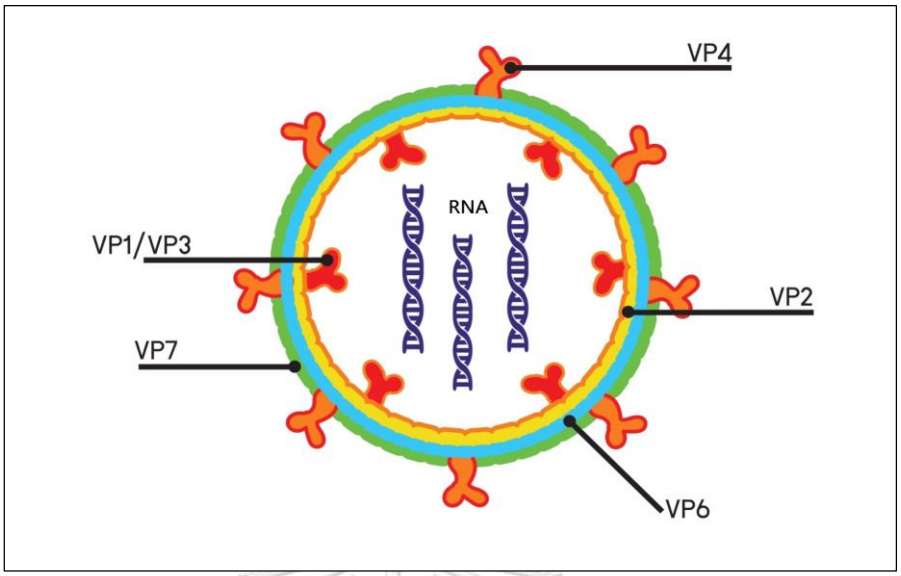


Figure 1. Structure organization of mature RV particle (TLP).

The outer capsid layer represent the VP7 are green and the VP4 spikes are orange. The internal VP6 protein layer is blue and the core VP2 layer in yellow. The flower-shaped VP1–VP3 transcription complex inside of the VP2 layer is colored red (adapt from Pesavento 2006) (13).

Gene function

- **Rotavirus replication**

The function of VP7 and VP4 genes of mature RV particle (triple layer particle, TLP) associated with cell attachment on host cell surface via sialo-glycans or histo-blood group antigens (HBGAs), followed by cellular receptors mediated interactions (such as Integrins and Hsc70) and entranced to host cell by endocytosis. The low calcium of the endosome induced the uncoated of outer layer and release those double-layered particles (DLPs) into the cytoplasm. VP1, VP2 and VP3 genes activate the DLPs rounds for mRNA transcription and protein translation. Cellular protein synthesis is inhibited by NSP3, which NSP3 bind to consensus sequence at 3'end of mRNA (UGUGACC) replace RNA-binding protein (named PABA) and forms dimer for eIF4G cellular protein binding, resulting PABA disable to bind 3'end of mRNA.

NSP2, NSP5, VP1, VP2 and VP3 mediated RNA genome replication within viroplasm structure (double stranded RNA synthesis, RNA packaging and new DLP formation). NSP4 serves as intracellular receptor of endoplasmic reticulum (ER) for those new DLPs, then DLPs go through inside ER. The transient enveloped particles are also found in the ER. Removal of the transient membrane and assembly of the outer capsid proteins VP4 and VP7 and complete virus particle maturation (TLPs; triple layer particle). The progeny virions are released by a non-classical vesicular transport mechanism through cell lysis (Figure 2) (14).

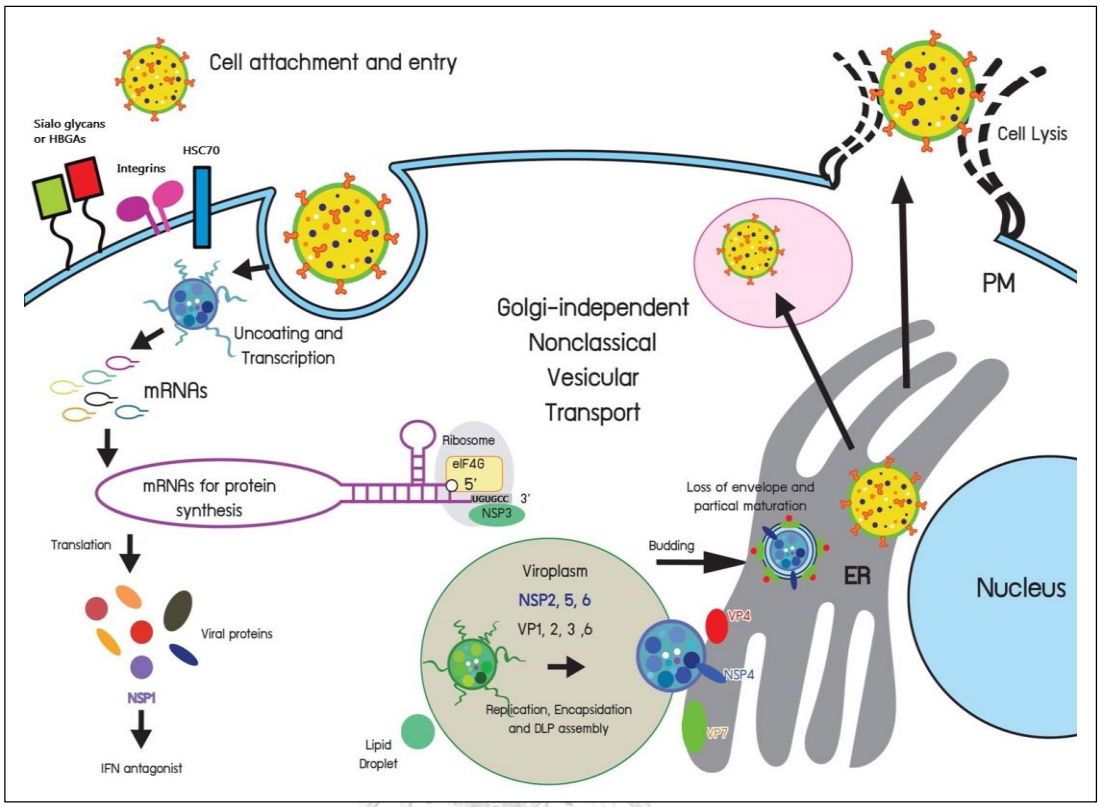


Figure 2. The scheme of rotavirus replication cycle.
(adapt from Hu et al. 2012) (14))

- **Host innate immunity**

VP4 and VP7 genes are main antigenic properties which induced production of host neutralizing antibodies (15, 16). NSP1 acts as IFN antagonist by IRF3/5/7 and β-TrCP degradation, block NF-kB activation by Zinc-binding motif at N-terminal domain. The production of several cytokines, chemokines, intermediated signaling molecules or secondary antiviral proteins are inhibited. Meanwhile, NSP4 acts as enterotoxin activity which its pathway associated to diarrhea and vomiting in infected host (17, 18).

Rotavirus genome segments and their encode proteins are shown in Table 1.

Table 1. Rotavirus genome segments and their encode proteins (3, 13, 14, 17, 19).

Rotavirus segment	segment length (bp)		Encoded proteins	Protein symbol	Location in virus particles	Function
	RVA*	RVC**				
1	3302	3290	VP1	R	Core	RNA-dependent RNA polymerase, ssRNA binding, complex with VP3
2	2690	2736	VP2	C	Core	RNA binding, required for replicase activity of VP1
3	2591	2145	VP3	M	Core	Guanylytransferase, methyltransferase, ssRNA binding, complex with VP1
4	2362	2246	VP4	P	Outer capsid	Neutralizing antigen, viral attachment, homotrimer, protease-enhanced infectivity, virulence, putative fusion region
5	1581	1235	NSP1	A or V	NSP	Interferon antagonist, RNA binding
6	1356	1352	VP6	I	Intermediate	Subgroup antigen, require for transcription, trimer
7	1104	1348	NSP3	T	NSP	Inhibits host translation, acidic dimer, bind 3'end of mRNAs
8	1059	995	NSP2	N	NSP	Forms viroplasms with VP1 and NSP5, octamer, RNA binding, NTPase, NDP kinase, RTPase

9	1062	1063	VP7	G	Outer capsid	G-type neutralization antigen, glycoprotein calcium dependent trimer
10	751	613	NSP4	E	NSP	Enterotoxin secreted from cells, virulence, intracellular receptor for DLPs, involves in morphogenesis of TLPs, viroporin, interact with viroplasms and autophagy pathway
11	667	693	NSP5	H	NSP	Protein kinase, RNA binding, forms viroplasm with NSP2, interacts with VP2 and NSP6, phosphoprotein
			NSP6		NSP	Interacts with NSP5, RNA binding

* Strain Wa-liked represent for RVA

** Strain Cowden represent for RVC

VP (viral protein), NSP (non-structural protein), ssRNA (single strand RNA), DLPs (double layer particles), TLPs (triple layer particles)

Rotavirus nomenclature

The Rotavirus Classification Working Group (RCWG) has assigned rotavirus genotypes based on 11 genome segments, displays as Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, which representing the genotypes of VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5, respectively (20). Rotavirus genotype of individual genes have been defined by the nucleotide identity cut-off percentage value which "x" behind capital letters indicating genotype numbers. The summary of cut-off values for 11 rotavirus segments is shown in Table 2.

Table 2. Nucleotide percentage identity cut-off values defining genotypes for 11 rotavirus gene segments.

Gene	% nucleotide identity cut-off value		Genotypes		Name of genotypes
	RVA	RVC	RVA	RVC	
VP7	80%	84%	35 G	13 G	**G**lycosylated
VP4	80%	77%	50 P	21 P	**P**rotease sensitive
VP6	85%	85%	18 I	13 I	**I**nner capsid
VP1	83%	86%	9 R	4 R	**R**NA-dependent RNA polymerase
VP2	84%	84%	9 C	6 C	**C**ore protein
VP3	81%	86%	8 M	6 M	**M**ethyltransferase
NSP1	79%	74%	18 A	9 A	Interferon **A**ntagonist
NSP2	85%	89%	10 N	8 N	**N**TPase
NSP3	85%	80%	12 T	6 T	**T**ranslation enhancer
NSP4	85%	71%	15 E	5 E	**E**nterotoxin
NSP5	91%	79%	11 H	4 H	p**H**osphoprotein

(applied from RotaC 2.0 automated genotyping tool for group A rotaviruses, http://rotac.regatools.be/classificationinfo.html (19, 21).

Rotavirus genotype

- **G/P genotype**

VP7 and VP4 genes can be used to classify G and P genotypes, which the VP7/VP4 combinations (G/P genotypes) have been suggested as host range restriction patterns.

RVA: from 35G and 50P genotype, genotype G1-G2, G3, G4 (VP7) and P[4], P[8] (VP4) are mainly identified in human RVAs, while genotype G3-G4-G5, G9 and P[6], P[7], P[13], P[19], P[23] are typical genotypes in pig (22-24). Nevertheless, some genotype such as G3, G4, G9 and P[6], P[19] were often identified in both pig and human populations (25-27).

RVC: based on VP7 gene has been established into 18 G genotypes, G1 to G18. Porcine RVCs are shown in G1, G3, G5-G10, G12, G13, bovine RVCs exhibited in G2, dog exhibits in G11, human RVCs exhibited in G4. VP4 gene has been established into 21 P genotypes which the common P genotype in pig are P[1] and P[4], human is P[2], bovine is P[3] and canine is P[8]. Common G/P genotypes in pig have been classified into G1, G3, G5, G6 and P[1], while G4 and P[2] genotype has been classified into a single genotype in human (28-32).

- **Other genotypes**

The rest of genotype classification for rotavirus have followed by VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5 genes. For RVA, those genotypes have been established into 18I, 9R, 9C, 8M, 18A, 10N, 12T, 15E, 11H (RotaC 2.0 automated genotyping tool for group A rotaviruses, http://rotac.regatools.be/classificationinfo.html). Meanwhile RVC revealed genotypes into 13I, 4R, 6C, 6M, 9A, 8N, 6T, 5E and 4H (19, 30). However, the data of RVC complete genome in animals are limited.

Genetic reassortment event among animal and human RV strains

Although most rotavirus infection is host species specific but several studies suggested the interspecies reassortment can occur. According to human and animal rotavirus have close evolutionary relationship, this may provide interspecies transmission events, resulting the emergence of new reassortant strains. The occurrence of reassortment events among RV strains are often represented in the outer capsid genes than other gene segments (VP7 (G genotype) and VP4 genes (P genotype). Nevertheless, the study of whole 11 gene segments of RV is provides better understanding in the origins, genetic relationships, gene reassortment or interspecies transmission events among strains (1, 20)

- **RVA**

The unusual rotavirus strains from animals could introduce across the host species barrier into human populations, leading human-to-human transmissions and probably become pandemic infections. Human rotavirus strains are also introduced across into animals as the same pathways (33-35). The genotype variations are mostly reported on the VP7, VP4, VP6 and NSP1 segments (Table 3).

Table 3. Genetic relationship among RVA strains.

The gene constellations of each prototype strains are given a color, for example, Wa strain is denoted blue, DS-1 strain is denoted in pink, AU-1 strain is denoted in orange, and PO-13 strain is denoted in yellow. Some typical porcine genotypes are colored in blue and purple (modified from Martella et al. 2010) (36).

Prototypes												
Strain name	Host	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
Wa	Human	G1	P[8]	I1	R1	C1	M1	V1	N1	T1	E1	H1
DS-1	Human	G2	P[4]	I2	R2	C2	M2	V2	N2	T2	E2	H2
AU-1	Human	G3	P[9]	I3	R3	C3	M3	V3	N3	T3	E3	H3
PO-13	Avian	G18	P[17]	I4	R4	C4	M4	V4	N4	T4	E4	H4
Reassortants												
Strain name	Host	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
IAL28	Human	G5	P[8]	I1	R1	C1	M1	V1	N1	T1	E1	H1
Se584	Human	G6	P[9]	I2	R2	C2	M2	V3	N2	T1	E2	H3
KTM368	Human	G11	P[25]	I1	R1	C1	M1	V1	N1	T1	E1	H1
L26	Human	G12	P[4]	I2	R2	C2	M2	V2	N1	T2	E2	H1
A131	Porcine	G3	P[7]	I5	R1	C2	M1	V1	N1	T1	E1	H1
Gottfried	Porcine	G4	P[6]	I1	R1	C1	M1	V8	N1	T1	E1	H1
OSU	Porcine	G5	P[7]	I5	R1	C1	M1	V1	N1	T1	E1	H1
A253	Porcine	G11	P[7]	I5	R1	C2	M1	V1	N1	T1	E1	H1
YM	Porcine	G11	P[7]	I5	R1	C1	M1	V8	N1	T1	E1	H1
Cat2	Feline	G3	P[9]	I3	R3	C2	M3	V3	N1	T6	E3	H3
K9	Canine	G3	P[3]	I3	R3	C2	M3	V9	N2	T3	E3	H6

Porcine rotaviruses have been proposed to be origin of several human RV reassortant strains which are mostly detected as cause of human diarrhea (37-39). Porcine RV strains are genetically similar to human Wa-like strains because their

backbone genotype constellation are resembles the human rotavirus strains. Some historical porcine liked human rotavirus strains as shown in Table 4.

Table 4. Some porcine like human RVAs which have been previously published.

Country origin	Porcine-like human reassortant strains	G/P genotype	Reference
Thailand	RVA/Human-wt/THA/MC345/1989/G9P[19]	G9P[19]	(38)
	RVA/Human-wt/THA/MC323/1989/G9P[19]	G9P[19]	(38)
	RVA/Human-wt/THA/CU-B1738-KK/2013/G4P[6]	G4P[6]	(40)
	RVA/Human-wt/THA/KKL-117/2014/G9P[23]	G9P[23]	(41)
	RVA/Human-wt/THA/CMH-S070-13/2013/G9P[19]	G9P[19]	(42)
Japan	RVA/Human-wt/JPN/Ryukyu-1120/2011/G5P[6]	G5P[6]	(43)
Korea	RVA/Human-wt/KOR/CAU12-2/2012/G11P[25]	G11P[25]	(44)
China	RVA/Human-wt/CHN/E931/2008/G4P[6]	G4P[6]	(45)
	RVA/Human-wt/CHN/R1954/2013/G4P[6]	G4P[6]	(45)
India	RVA/Human-wt/IND/RMC321/1990/G9P[19]	G9P[19]	(46)
	RVA/Human-wt/IND/Mani-362/2007/G4P[6]	G4P[6]	(47)
	RVA/Human-wt/IND/Mani-97/2006/G9P[19]	G9P[19]	(47)
Taiwan	RVA/Human-wt/TWN/03-98s140/2009/G4P[6]	G4P[6]	(48)
Belgium	RVA/Human-wt/BEL/BE2001/2009/G9P[6]	G9P[6]	(49)
Argentina	RVA/Human-wt/ARG/Arg4605/2006/G4P[6]	G4P[6]	(50)
	RVA/Human-wt/ARG/Arg4671/2006/G4P[6]	G4P[6]	(50)
Hungary	RVA/Human-wt/HUN/BP271/2000/G4P[6]	G4P[6]	(51)
	RVA/Human-wt/HUN/BP1547/2005/G4P[6]	G4P[6]	(51)
Paraguay	RVA/Human-wt/PRY/1809SR/2009/G4P[6]	G4P[6]	(52)
Ecaudor	RVA/Human-wt/ECU/EC2184/2005/G11P[6]	G11P[6]	(37)

- **RVC**

Unlike RVA, whose genetics are well studied, the complete genetic characterization of RVC are incomplete. Other gene segments except VP7 and VP4 are limited number of sequence databases, while most of full genome sequence data are available for Asian RVC strains. Therefore, the zoonotic potential of RVCs is not completely understood in their evolution to date. Most of reports indicated the reassortant human RVCs bearing the genetic backbone among human strains greater than animal RVC strains (53). However, pig have been suspected to be origin of human RVC reassortant strains and being reservoir for interspecies transmission among animal species involving VP3 gene segment (54-56).

- **Porcine epidemic diarrhea virus (PEDV)**

Porcine epidemic diarrhea virus is the member of family *Coronaviridae*, genus *Alphacoronavirus*. The particle size of the virus is approximately 130 nm in diameter (95-190 nm) and genomic size of 28-32 kb. PEDV morphology under electron microscopy is spherical shape with crown-like spikes (57).

The PEDV genome is a single positive-strand RNA, linear, and non-segmented. The gene arrangement is 5'-Rep-S-ORF3-E-M-N-3' which genome contains 1 non-structural gene, 4 structural genes and 1 accessory gene. The non-structural gene is replicase gene or *Pol* gene encoding polymerase polyprotein with cleavage site. The cleavage site is separated into 15-16 non-structural proteins (NSPs) by specific protease enzymes (58, 59). Some types of non-structural proteins encodes enzyme associated with tRNA processing (tRNA splicing) (60). The 4 structural genes consist of the spike gene (S gene), envelope gene (E gene), membrane gene (M gene) and nucleocapsid gene (N gene) which each gene encoded proteins with similar gene name as S protein, E protein, M protein and N protein, respectively. For necessary gene, the numbers of the accessory gene are different depends on coronavirus

group. For PEDV genome has only an accessory gene which is designated as open reading frame 3 (ORF3 gene).

Gene function

Spike gene encoded S protein which found on the surface of coronavirus particles, making crown-like appearance under electron microscope. S protein contains several domains such as a signal domain (located between aa position 1-18), neutralizing epitopes (located in 4 parts such as position 499-638, 748-755, 764-771 and 1,368-1,374), a transmembrane domain (located between aa position 1,334-1,356 at C-terminal domain) and a short cytoplasmic tail with cysteine-rich residues (60, 61). S protein is the main target of neutralizing antibodies against PEDV (62).

The envelope gene encoded E protein. E protein involves in envelope organization and forms the neck of the viral particle at the final stages of viral budding process. E gene is conserved among members in coronavirus groups but it is not often used for phylogenetic studies because it too short sequence (60, 63).

The membrane gene encoded M protein. M protein is the most common envelope protein that is found on the viral surface. The roles of M protein associate with viral assembly process (S and N protein fusion), induce complement and α-interferon production (63, 64).

The nucleocapsid gene encoded N protein. N protein plays an important role in biological process of virus such as RNA-binding protein, virus RNA synthesis, and cell-mediated immunity production (such as up-regulation of IL-8 expression, NF-kB activation or inhibit interferon production) (65-67).

The open reading frame 3 is accessory gene located between S and E genes which encoded ORF3 protein. ORF3 gene is one of pathogenicity factors and could be a marker for monitoring of nucleotide sequence variation during higher passage adaptation through cell culture (68).

Clinical symptoms and pathogenesis in pigs

- **Rotavirus**

Rotavirus causes acute watery diarrhea in weaning and post-weaning piglets which the most susceptible age is between 1-5 weeks. It is transmitted via fecal-oral route. The target site of rotavirus replication is the villous enterocytes in the small intestine, especially jejunum and ileum, although rotavirus also replicates in the duodenum, cecum and colon. Infection leads to cell lysis, villi blunting and atrophy. The degree of villous atrophy associated with rotavirus strain, serogroup and pig age, in which RVA and RVC showed more severe villous atrophy and pig diarrhea than others (69). There are many stages of villous destruction from rotavirus infection, such as no visible, slight and large lesions. Slight lesions included enterocyte vacuolization or loss, while large lesions are resulting in villous blunting and crypt hyperplasia. The general symptom of rotavirus infection is osmotic diarrhea, enterocyte destruction reduces sodium and water reabsorption includes decrease mucosal disaccharidase enzyme activity, impaired glucose-coupled sodium transport and increase thymidine kinase activity. The undigested carbohydrate, fat, protein are deposit within the intestine, resulting an undigested bolus causing osmotic diarrhea. Moreover, the reduction of $Na^{+}K^{+}$- ATPase activity and glucose-coupled sodium absorption are caused of malabsorption at villi tips (3, 70).

The incubation period is approximately 18–96 hours. At 16-18 hour post-infection, infected villous epithelial cells become degeneration, the most severe of villous atrophy are seen between 24-72 hours. At 48 hour of infection, crypt epithelial cells begin hyperplasia. Lateral villi fusion occurs by 24-168 hour post-infection. However, the age of pig associates with the time of villi regeneration (71). The disease is more severe in newborn and young pigs. However, colostrum from

lactating sows with levels of immunoglobulin could reduce disease severity in neonatal piglets by inducing piglet's gut protective immunity (72).

- **PEDV**

Porcine epidemic diarrhea virus is a cause of per-acute to acute watery diarrhea with high mortality rate in neonatal and sucking piglets. The major route of transmission is fecal-oral. The onset of diarrhea is often found between 12-36 hours after ingestion of PEDV particle. Virus goes directly to the enterocytes of the small intestine and replicates inside within 18 hours post-infection. At 24 hours post-infection, villi shortening and atrophy is found, while crypts of epithelial cells are not affected. The villous height is decreased from 700-900 nm to 200-300 nm, resulting in the reduced ratio of villous height and crypt depth from 6.5-8: 1 to 1.5-2.3: 1. The PEDV particles are found along the length of small intestine and produce the narrow membranous bridge from infected epithelial cell to the membrane of a normal epithelial cell at 120 hours post infection (73).

In field cases, infected pigs either RV or PEDV showed the similarity of disease appearance in clinical sign of diarrhea and degree of the pathogenicity on the small intestine. Congested intestines, thin-walled, dilated and reduce peristalsis were found. The pale gray to yellow watery fluid or white flecks of milk curd deposited within intestinal lumen were often seen. The co-infection among these viruses are possible.

Disease distribution in pig populations

- **Rotavirus**

Rotavirus was first discovered in a bovine calf in 1969, human in 1973, and pigs in 1975 (71).

RVA is the most common causative agents associated with diarrhea in both young human and animals (74, 75). In the pig industry, rotavirus infections have continuously been reported in other countries since 1985 such as England, Brazil, Slovakia, Canada, USA, Japan, Korea, and Thailand with prevalence rate in diarrhea pig is varies between 0.9-89% (Table 5).

RVC was the first identified in 1980s in pig and considered as an enteric pathogen in several countries with moderate prevalence rate between 4.4-46% (such as Japan, South Korea, USA, Canada, Italy, Brazil, Czech and Ireland). RVC have been distributed to human and many animal species. Particularly in pig, RVC infections are often reported in piglets with coinfection with the other viruses rather than a single infection. The infection could be found in asymptomatic pigs (10, 28, 29, 31, 55, 76-81).

Table 5. The prevalence studies of porcine RVA.

Country	Report	Pig age	% RVA prevalence	Method detection
Brazil	(82)	post-weaning	46%	RT-PCR
	(83)	34 days	27%	RT-PCR
	(84)	< 7 to >21 days	53%	PAGE
Canada	(85)	late feeder pigs	6.8%	RT-PCR
EU country	(81)	0-18 wks	0.9%	Real time PCR
Belgium	(76)	<2wks	61%	RT-qPCR
England	(86)	post weaning	80-89%	RT-PCR
USA	(87)	pre-post weaning (>21 d)	62%	RT-qPCR
	(88)	diarrhea pigs	67%	PAGE
Vietnam	(89)	<9 to >52 wks	32.7%	RT-PCR
Thailand	(90)	0-4 wks	23%	RT-PCR
	(91)	0-4 wks	19%	Multiplex RT-PCR
	(92)	piglets	10.7%	RT-PCR
	(93)	7-49 days	22.3%	RT-PCR
	(94)	piglets	89%	PAGE
India	(95)	<3 month	10.8%	PAGE/ RT-PCR
Slovenia	(34)	3 to >10 wks	18%	RT-PCR
Italy	(31)	nursery pigs	16.1%	EM/ immunoenzyme assay
Ireland	(28)	4-5 wks (asymptomatic pig)	6.5%	RT-PCR
Korea	(96)	1-14 days	13.2%	Multiplex RT-PCR
Japan	(75)	suckling pig	67.3%	RT-PCR
		post weaning pig	65.5%	
Germany	(97)	pre-post weaning	20.8%	EM
Hungary	(98)	post weaning	18.6%	ELISA

- **PEDV**

PEDV infection is a major etiologic agent in gastroenteritis pig herds worldwide. The first detection was reported in 1971 in England. In 1978, a prototype strain designated CV777 was experimentally isolated (99). In the past, PEDV outbreak in Europe, North and South American and Australia is rare. In Asia, PEDV outbreaks have been reported in several countries since the 1980s including Japan, Korea, China, Vietnam, Philippines and Thailand (the example of first PEDV reports in each countries were summarized in Table 6). Nevertheless, in 2013 there was a widespread PEDV outbreak in the USA resulting in >95% mortality rate among infected suckling piglets (100).

Table 6. The first reports of PEDV detection in each countries of the regions.

Continent	Country	Year of publication	Reference
America	Canada	1980 , 2016	(101, 102)
	USA and Mexico	2013	(100)
Europe	UK	1971	(103)
	Belgium	1978	(99)
	Spain	1985	(104)
	The Netherlands	1989	(105)
	Czech Republic	1993	(68)
	Hungary	1995	(98)
	Italy	2005	(106)
	Germany	2014	(107)
	France	2014	(108)
	Slovenia	2014	(109)
	Portugal	2014	(110)
	Austria	2014	(111)
Asia	China	1973	(112)
	Japan	1982	(113)
	South Korea	1993	(114)
	Philippines	2007	(115)
	Thailand	1995	(116)
	Vietnam	2013	(117)
	Taiwan	2014	(118)

The pandemic PEDV strain terms

Nowadays, PEDV strains are termed into 2 major groups called genogroup 1 (G1) and 2 (G2). Each genogroups are sub-divided into subgroup 1a and 1b, 2a and 2b which are termed in the classical strain (G1a), the new variant strains or S INDEL strains (G1b), Asian highly virulent strains (G2a) and North American virulent strains (G2b) (119). Original PEDV (represented by the prototypic CV777 strain) was first

reported in the 1970's in Europe and was associated with high morbidity and mortality among infected pigs which was widespread and became endemic disease in several areas out of European countries (99).

The new variant strains or S INDEL strains were firstly denoted in the US in 2014 and subsequently introduced to Asia and Europe. In the N-terminal region of the S protein (S1 region) present d insertions and deletions (S-INDEL) compared to the prototype CV777. One-third of S gene among the new variants G1b shared genetic identity greater than 95% to classical strain (G1a), while the rest of two-third had genetic similarity greater than 99% to highly virulent strains (G2). Therefore, S-INDEL variants appear to be recombination strains among G1a and G2 (120, 121). For example, US-liked strain OH851 (First U.S S-INDEL strain), Indiana1283/2013 and Minisota52 have amino acid insertion at residue 161-162 and 2 deletions at residue 59-62 and residue 140 (121, 122). The deletion within S1 region (at residue 23-230) might responsible for less pathogenic (123, 124). Infection by some S-INDEL variants was reported to produce decreased symptom severity including moderate diarrhea, lower titers of viral shedding, and reduced mortality (124, 125).

The high virulent strains (G2) initially reported as epidemic disease in Asia and have continuously emerged in North America. Nowadays, highly virulent strains become the pandemic strains which have resulted globally severe outbreaks (126). However, S-INDEL and high virulent strains have been suspected as cause of classical PEDV vaccine failure in Asia (127, 128).

Disease epidemiology in Thailand

- **Rotavirus**

- **RVA**

Disease surveillances have been limited within breeding herds of Northern region and were not carried out simultaneously. The previous reports suggested G

genotype as G3, G4, G5, G9, G10 are often combined with P genotypes as P[6], P[7], P[13], P[19], P[23]. Meanwhile, P[27] was proposed as a new novel P genotype in 2001. Due to G/P genotype reports, G4P[6] were commonly detected between 2000-2001 and 2009-2010, G3P[6] were detected between 2002-2005, G3P[23] and G9P[23] were detected between 2006- 2007 (91-94, 129, 130). Otherwise, the porcine RVA genome constellation study in Thailand was rare documented elsewhere (24, 42, 129).

- **RVC**

Report of infection and molecular characterization study of Thai porcine RVC are rarely documented.

- **PEDV**

PEDV is most commonly distributed in Thai pig herds since 1990's. The first PEDV identification was published in 1995 by IFA technique and PEDV was successful expressed the viral cell culture adaptability in Vero cell line in 1997 (116, 131). The outbreak was more deleterious in breeding farms of central Thailand during late year of 2007 to 2008 and then became endemic pathogen in Thai breeding herds. The first molecular characteristics of Thai PEDV (based on M and partial S genes) was published in 2009 and the molecular characterization study of Thai PEDV have periodically monitored (132). The complete S and ORF3 gene characterizations were reported in 2014 and 2 genetic distinct variants of PEDV were observed in 2015 (133, 134). Most of PEDV studies suggested Thai PEDV strains have close genetic relatedness to the Chinese-liked strains. Up to date, several studies during 2017-2018 have been changed from molecular epidemiology and genetic characterization of PEDV into host immune response to against PEDV infection, the monoclonal antibody production, the viral replication in cell line and the viral pseudotyped characterization model for PEDV spike gene (135-139).

Vaccine

- **Rotavirus**

Rotavirus vaccine has been introduced into human populations as prophylaxis to against rotavirus diarrhea since early 1980s (140). Nowadays, human rotavirus vaccines are available as RotaRix (single strain human rotavirus, G1P[8]) and RotaTeq (5 bovine-human reassortant rotaviruses, G1, G2, G3, G4, G6, P[8], P[7]) (141). Both vaccines have been implemented into the immunization program for infant worldwide which vaccine efficacy to prevent severe rotavirus gastroenteritis are shown in the individual (142, 143).

For pig populations, farm managements based on the overall biosecurity are still the main strategy to prevent rotavirus infection within herds. Vaccine usage aims to reduce disease severity and prevent piglet death from rotavirus infection. Only RVA vaccination has been conducted in some countries, while RVC vaccine is not yet available (144). RVA vaccine for pregnant sow could induce high level of rotavirus antibody in passive milk to prevent diarrhea in piglet and reduce significantly viral shedding from their feces. Presently, the licensed porcine RVA vaccines are available as ProSystems series (Merck, Whitehouse Station, NJ), which consist of ProSystems RCE for pregnant sows-gilts, ProSystems ROTA for young piglet and ProSystems TGE/ROTA for nursing piglets (combination attenuated live TGE virus and two major rotavirus) (Table 7). There were limited information on the vaccine efficacy in field study and those vaccines are not available in Thailand.

Table 7. The licensed rotavirus vaccines available in pig.

Product	G type	Others	Vaccine type	Route	Recommended
- ProSystems RCE	G4, G5	*E.coli* pilus antigen (K88, K99, F41, 987P) *Clostridium perfringens* Type C toxoid	Modified lived	IM x 2	Gilt and late term pregnant sow (5 wk before farrowing)
- ProSystems TGE/Rota	G4, G5	TGEV	Modified lived	IM x 2	Late term pregnant sow previously exposed to TGE virus (5 wk before farrowing)
				PO x 2 (2 wk interval) and IM x 1	Gilt and late term pregnant sow not previously exposed to TGE virus
- ProSystems Rota	G4, G5	-	Modified lived	PO x 1 and IM x 1	Young pig (7-10 days)

*PO (oral route), IM (intramuscular injection route)

TGEV (transmissible gastroenteritis virus), PEDV (porcine epidemic diarrhea virus)

- **PEDV**

During 1999 to 2015, most of commercial vaccines have been produced from China, South Korea and Japan. Classical PEDV strains such as CV777, Japan 83P-5, SM98 and Korean DR13 have been used as traditional attenuated vaccines in Asia (145) (Table 8). The immunized sow with attenuated vaccine could successfully

induce lactogenic immunity in their piglets to against classical PEDV strain infections. Afterwards, the new variants have emerged in China in the end of 2010 and introduced into USA in 2013 have the mutations within neutralizing epitopes of S gene which make those strains differed from classical derived vaccine strains. Therefore, US commercial vaccine have been launch in 2014 (100, 120, 146, 147).

Nevertheless, the licensed PEDV vaccine in Thailand has been used in some breeding herds, although PEDV remains circulating in commercial swine herds nationwide.

Table 8. The licensed PEDV vaccines available worldwide.

License country	Company	Product	Virus	Strain	Vaccine type	Adjuvant
South Korea	- CAVAC (ChoongAng Vaccine Laboratories)	SuiShot PT-100	PEDV, TGEV	N/A	killed	aluminium hydroxide
		SuiShot PED	PEDV	N/A	live	none
		SuiShot PED-SM	PEDV	61P	live	none
	- Green Cross Veterinary Products	PT-Vac	PEDV, TGEV	N/A	killed	IMS-1313
		PED oral vaccine	PEDV	DR13	live	none
		PED Guard	PEDV	QIAP1401	killed	montanide gel
		PED Virus Live vaccine	PEDV	KPEDV-9	live	none
		PTR Combined Live vaccine	PEDV, TGEV, RVA	SM98P, 175L, A1	live	none
	- Komipharm International	PRO-VAC PED-Fc	PEDV	SM98P	live	IMS-1313
		PRO-VAC PED	PEDV	SM98P	live	none
		PRO-VAC TP	PEDV, TGEV	SM98P, 175L	killed	Montanide IMS-1313 NPR
		PRO-VAC TRP	PEDV, TGEV, RVA	SM98P 175L, A1	live	none
Japan	- KAKETSUKEN (Chemo-Sero-Therapeutic Research Institute)	SUIMMUGEN TGE/PED	PEDV, TGEV	96P4C6 Vesicle strain	live	N/A
	Nisseiken	NIKEN PED	PEDV	P-5V	live	none
		NIKEN TGE-PED	PEDV, TGEV	P-5V, H-5	live	none
		Mixed Live vaccine				

China	**- CAHIC (China Animal Husbandry)**	PEDV and TGEV vaccine	PEDV, TGEV	ZJ08, HB08	live	N/A
	- Chengdu TECBOND Biological Products	PEDV and TGEV vaccine	PEDV, TGEV	CV777, Chinese strain	killed	aluminium hydroxide
	- Guangdong Wens Dahuanong Biotechnology	PEDV and TGEV vaccine	PEDV, TGEV	N/A	killed	N/A
	- HVRI (Harbin Veterinary Research Institute)	Porcine viral diarrhea triple live vaccine	PEDV, TGEV, RVA	CV777, Huada, G5	live	N/A
	- Pulike Biological Engineering	PEDV and TGEV vaccine	PEDV, TGEV	CV777, Huada	killed	N/A
	- Shanghai Kile Bio-Pharmaceutical	PEDV, TGEV and RVA vaccine	PEDV, TGEV, RVA	CV777, Huada , NX	live	none
		PEDV and TGEV vaccine	PEDV, TGEV	CV777, Huada	live	none
	- Zhejiang Ceva Ebvac Biotech	PEDV and TGEV vaccine	PEDV, TGEV	N/A	killed	aluminium hydroxide
USA	**- Zoetis**	PEDV vaccine	PEDV	N/A	killed	N/A
	- Merck	PEDV vaccine	PEDV	N/A	RNA	N/A

*TGE (transmissible gastroenteritis virus), PEDV (porcine epidemic diarrhea virus), RVA (rotavirus group A), N/A (data not available) (Source; The Center for Food Security and Public Health, Iowa State University)

CHAPTER III

METHODS AND RESULTS

Sensitivity and specificity tests of new designed primers for rotavirus group A and group C detection

Materials and methods

The 2 primer sets of RVA VP7 and RVC VP7 were newly designed for RVA/ RVC detection. The primer sequences of **RVA VP7** was VP7-CU-RVAF: CGGTTAGCTCCTTTTAATGT for forward primer sequence, VP7-CU-RVAR: CATTTCTTCCAATTTACTCGC for reverse primer sequence and the primer sequences of **RVC VP7** was VP7-CU-RVCF: GAAGCTGTCTGACAAACTGG for forward primer sequence, VP7-CU-RVCR: GCCACATGATCTTGTTTACGC for reverse primer sequence. The expected PCR product size of RVA VP7 was 891 bp and was 1046 bp for RVC VP7. The detection primers were determined the impact of a variable under the specificity and sensitivity analysis.

Initially, RVA and RVC positive control from plasmid cloning were quantified for the purified nucleic acid concentrations by NanoDrop spectrophotometer and subsequently converted the nucleic acid concentrations into copy number by formula;

1. **Number of copies** (molecules) = $\frac{\mathbf{x}\ \text{ng} \times 6.0221 \times 10^{23}\ \text{molecule/mole}}{(\mathbf{N} \times 660\ \text{g/ mole}) \times 1 \times 10^{9}\ \text{ng/g}}$

   where

   x = amount of nucleic acid concentration

   N = expected PCR product size

2. **Dilution calculator** = $C_1V_1 = C_2V_2$

   where

   C1 = initial concentration of solution

   V1 = initial volume of solution

   C2 = final concentration of solution

   V2 = final volume of solution

To determine the primer sensitivity, a serial of the 10 fold dilutions (start from 10^9 to 10^0) of known RVA and RVC plasmid concentrations were performed. Each dilutions were served as DNA template for PCR amplification step, following conditions: 35 cycles of amplification steps including denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute for RVA VP7 and 52°C for 1 minute for RVC VP7, extension at 72°C for 90 seconds, and final extension at 72°C for 5 minutes. The amplicons were purified using agarose gel electrophoresis.

For the specificity test, cross-amplification among the primer sets and other reference strains were also verified by PCR amplification method. The reference strains were the common enteric viral pathogens which consist of porcine epidemic diarrhea virus (PEDV), hepatitis E virus (HEV), porcine circovirus type 2 (PCV2) and norovirus genogroup I and II (NOV GI and GII). The PCR conditions followed by sensitivity test.

Results

- **Sensitivity test**

The lower detection limit of the positive plasmids ranging from 10^2 copies/ul for RVA, and 10^3 copies/ul for RVC, respectively (Figure 3a, 3b).

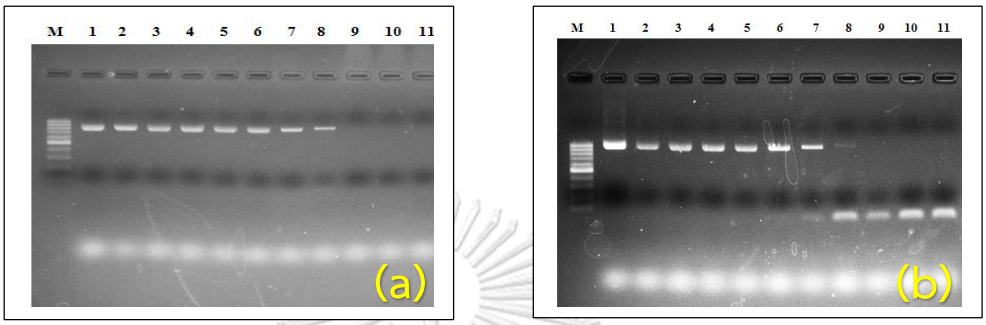


Figure 3. Sensitivity limits of serial dilution of RVA/RVC plasmids.

Lane M, 100-bp DNA ladder; lane 1-10, 10^9 to 10^0; lane 11 negative control. PCR method was able to detect RVA plasmid concentrations at 10^2 copies/ul (a) and RVC plasmid concentration was able to detect at 10^3 copies/ul (b).

- **Specificity test**

The primer sets were able to produce the expected sizes of each proper rotavirus strains. There were no cross-amplification among primer sets to references (Figure 4a, 4b).

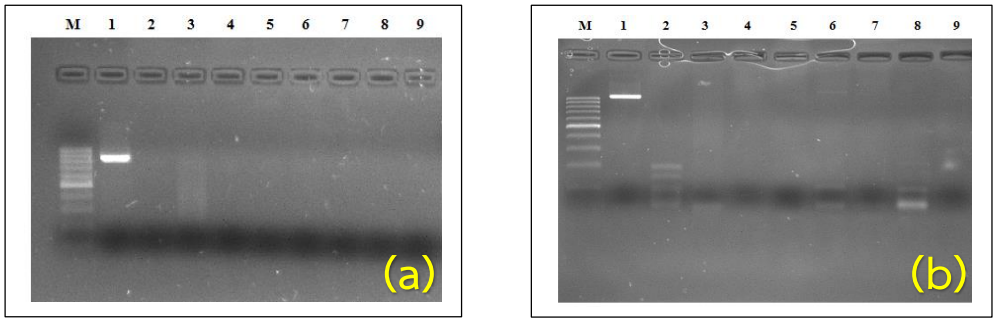


Figure 4. The primers produced the expected sizes of RVA (a) and RVC (b) strains.

Lane M, 100-bp DNA ladder; lane 1 = RVA (a) or RVC (b), lane 2 = RVC (a) or RVA (b), lane 3 = PEDV, lane 4 = HEV, lane 5 = PCV2, lane 6 = NOV GI, lane 7 = NOV GII, lane 8 = negative sample, lane 9 = negative control.

Part 2: Molecular epidemiology of porcine rotavirus group C and viral co-infection among rotavirus group A, group C and porcine epidemic diarrhea virus

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Molecular epidemiology of porcine rotavirus C and viral co-infection among rotavirus group A, group C and porcine epidemic diarrhea virus

Diarrhea is associated with high morbidity and mortality rates in suckling and post-weaning piglets. The frequent viral etiologies are identified as RV and porcine PEDV. Enteric virus replication blunts the villous enterocytes in the small intestine, resulting in electrolyte imbalance, intestinal malabsorption, watery diarrhea, dehydration, and often death (54, 148). Infections generally occur via fecal-oral route and result in significant economic impact due to animal loss, sanitation efforts, and reduced pork production. Without molecular diagnostics, PEDV and rotavirus infections are difficult to ascertain and differentiate as they cause similar clinical symptoms and frequently co-infect pigs. Pigs of all ages are susceptible to these viral infections, which can manifest in different disease severity depending on the age of the animal (149-152). Among the most common to infect swine, RVA affects piglets between one and three weeks of age, while RVC frequently causes diarrhea in pre- and post-weaning piglets (8, 10, 31). However, neonatal and post-weaned piglets are most vulnerable due to the lack of protective immunity (153). Asymptomatic infection in adult pigs further complicate efforts to identify and quarantine sick animals, which are crucial in preventing the spread of infection (10, 28, 76, 80, 81).

Based on the binary classification of Rotavirus Classification Working group (RCWG) that assigned using VP7 (G) and VP4 (P) proteins, genotype G3-G4-G5, G9 and P[6], P[7], P[13], P[19], P[23] are typical genotypes associated with infection in pigs (154). For porcine RVC, the common G genotypes are shown in G1, G3, G5-G10, G12, G13 (28, 29, 155). Due to the common P genotype in pig are P[1] and P[4] (10, 156).

This genetic diversity renders most pig herds susceptible to repeated rotavirus infection. Thus, awareness of the circulating porcine RVC on pig farms is critical in

evaluating the disease burden and the potential impact of widespread infection. RVC infection is currently not well-studied in Thailand due to the lack of disease awareness, vaccine availability, and access to molecular diagnostics in current routine practices.

Materials and methods

Sample collection and preparation

A total of 769 samples (509 feces and 260 small intestine contents) from various ages of pigs with watery diarrhea were submitted to the Livestock Animal Hospital of the Faculty of Veterinary Science, Chulalongkorn University in Nakhon Pathom province between May 2011 and August 2016 for viral testing. Samples were from 2011 (n = 40), 2012 (n = 95), 2013 (n = 87), 2014 (n = 158), 2015 (n = 164), and 2016 (n = 225). These represent archived and convenient samples from 123 commercial pig farms located throughout Thailand (Table 9). Samples were categorized following age groups: 0–6 days, 1–4 weeks (pre-weaning), 4–8 weeks (early nursery), 8–12 weeks (late nursery), >12 weeks (starter–finisher), and sow (both pregnant and lactating).

The small intestine contents (the duodenum and upper part of the jejunum collected from dead piglets, particularly the thin walled area where gas accumulated inside the lumen) and fecal samples were prepared to be 10% (v/v) suspensions in sterile phosphate-buffered saline (0.1 M, pH 7.2), centrifuged at 3,000 *xg* for 20 minutes. The supernatants were collected and kept in -20°C until used.

Table 9. Details of the farm location and pig age for which samples were derived between 2011 and 2016.

Region/ province	Total	0-6 d	1-4 wk	>4-8 wk	>8-12 wk	>12 wk	Sow	N/A
Central	**(173)**							
Lop Buri	2		1	1				
Samut Songkhram	3		3					
Suphan Buri	32	12	18				1	1
Saraburi	16	3	4	8			1	
Phranakhon Si Ayutthaya	6	6						
Nakhon Pathom	114	36	31	16	2	12	5	12
Western	**(316)**							
Kanchanaburi	16	0	8	7				1
Prachuap Khiri Khan	4		4					
Phetchaburi	2		2					
Ratchaburi	294	36	131	89	12	5	11	10
Eastern	**(109)**							
Chon Buri	92	27	21	10	0	34		
Chachoengsao	17	10	1			6		
Northeastern	**(80)**							
Ubon Ratchathani	4	4						
Udon Thani	4		4					
Nakhon Ratchasima	72	10	2	9	15	14	22	
Southern	**(26)**							
Trang	23		9	6				8
Nakhon Si Thammarat	3		3					
Unspecified location	**(65)**	3	2	3		1	1	55

N/A, data not available.

Viral nucleic acid detection and sequencing

A total of 769 viral RNAs were extracted using Ribospin vRD II viral RNA extraction kit (GeneAll, Seoul, Korea) according to the manufacturer's instructions. The partial S gene of PEDV and VP7 gene of RVA/RVC were initially amplified using SuperScript III One- Step RT-PCR System with Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA). RNAs were reverse-transcribed at 48°C for 45 minutes. Cycling parameters were initial denaturation at 95°C for 2 minutes, followed by 35 cycles at 94°C for 30 seconds, 52°C or 55°C for 1 minute, 72°C for 90 seconds, and final extension at 72°C for 5 minutes. Moreover, RVC VP7- positive samples were further subjected to the amplification of VP4 gene. The primer sequences are shown in Table 10. Amplicons were purified using agarose gel electrophoresis and sequenced.

Table 10. Nucleotide primers used in the amplification of PEDV, RVA and RVC in this study.

Primers	Sequence (5' to 3')	Location	Annealing	Amplicon
PEDV S (157)	TTCTGAGTCACGAACAGCCA	1466-1485	55°C	651 bp
	CATATGCAGCCTGCTCTGAA	2097-2116		
RVA VP7 (AB176677.1)	CGGTTAGCTCCTTTTAATGT	33-52	55°C	891 bp
	CATTTCTTCCAATTTACTCGC	903-924		
RVC VP7 (M61101.1)	GAAGCTGTCTGACAAACTGG	17-36	52°C	1046 bp
	GCCACATGATCTTGTTTACGC	1042-1061		
RVC VP4 (158)	GATCRATGGCGTCYTCAC	17-34	55°C	1222 bp
	CCTGATGAATGTAATCCWGGAT	1216-1238		

Analysis of the RVC VP7 and VP4 genes

Nucleotide sequences were assembled and edited using SeqMan II and aligned using BioEdit and ClustralX v.2.0.11 (159). Phylogenetic trees were constructed using MEGA6 software with the maximum likelihood method and 1,000 replicates (160). Reference sequences from the GenBank database were available for inclusion in all phylogenetic trees. Prototypic RVC strain Cowden (G1P[1]) (accession no. M61101.1 for VP7, M74218.1 for VP4), Shintoku (G2P[3]) (accession no. U31750.1 for VP7, U26551.1 for VP4), HF (G3, undetermined P) (accession no. U31748.1) and Bristol (G4P[2]) (accession no. X77257.1 for VP7, X79442.1 for VP4) served as reference strains. Best model fitting was automatically calculated for genetic distances. Bootstrap values >85% were considered significant for the VP7 gene and >80% for the VP4 gene. Nucleotide (nt) sequences of RVC VP7 and VP4 genes were deposited in the GenBank database (Appendix, Table S1).

Results

Viral detection

Between 2011 and 2016, 19.9% (153/769) of the samples tested positive for PEDV. The overwhelming majority of the samples were from 0-6 day-old piglets (Figure 5). RVA was found in 9.5% (73/769) of the samples, while RVC was identified in 6.6% (51/769) of the samples. One-fifth of the samples (21.6%, 11/51) were co-infected with RVA/RVC, most of which were from piglets ≥4-8 weeks of age. Fewer PEDV-positive samples were co-infected with RVC (4/51) (7.8%). Only 3 samples tested positive for all 3 viruses (5.8%).

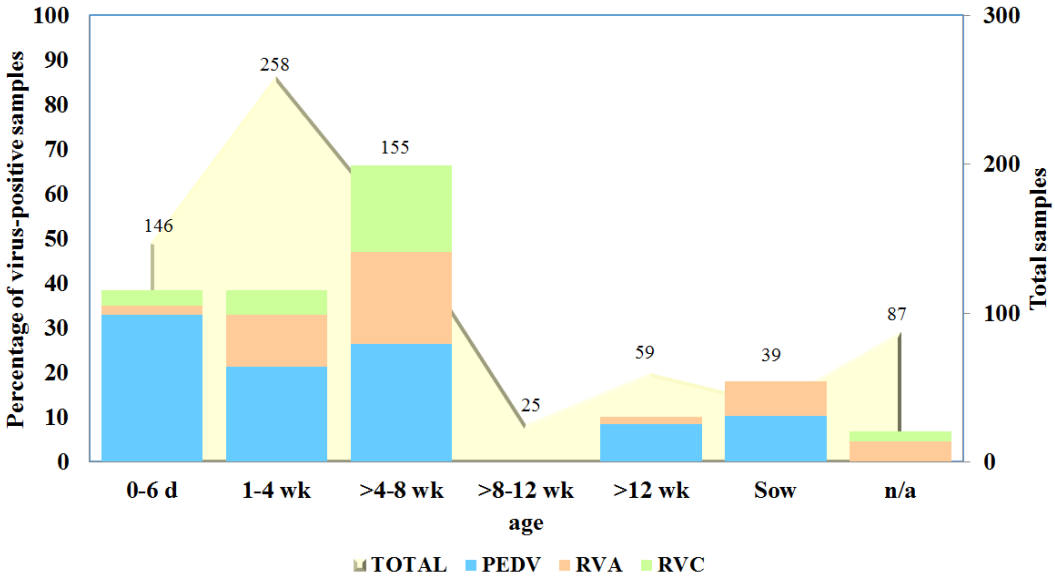

Figure 5. Age distribution of pig samples tested positive for PEDV, RVA and RVC.

Sequence and phylogenetic analysis of the RVC VP7 gene

We sought to focus our study on RVC and therefore examined the G and P genotypes. Sufficient sequences of VP7 were successfully obtained from 47 samples (47/51), most of which were derived from feces (Table 11). The near full-length VP7 sequences were compared to the RVC references available in the GenBank database. Phylogenetic analysis showed that the RVC in this study belonged to genotype G1 (59.6%, 28/47), G3 (2.1%, 1/47), G6 (21.3%, 10/47), and G9 (17%, 8/47) (Figure 6).

The G1 strains were closely related to the prototypic Cowden (86.1-91.7% nt identity). The lone G3 strain RVC/Pig-wt/THA/CU-PY/12/G3 was distantly related to the prototypic HF (78%). The G6 strains shared high identity to a porcine rotavirus strain RVC/Pig-wt/ITA/43/06-16/2005/G6P[x] isolated in Italy in 2005 (88.6-90.9% nt identity) and the G9 strains were closely related to a Vietnamese porcine rotavirus strain (strain RVC/Pig-wt/VNM/14175-22/2016/G9P[7]) (86.3-89.5% nt identity).

Table 11. Porcine RVC strain information.

Year	Strain name	Age of pig (wk)	Sample	Genotype		Co-infect	
				VP7	VP4	RVA	PEDV
2012	RVC/Pig-wt/THA/CU-PY/2012/G3P[x]	1-4	s.i	G3			
2013	RVC/Pig-wt/THA/CU571/2013/G6P[x]	n/a	feces	G6			
	RVC/Pig-wt/THA/CU264-U12/2013/G9P[7]	n/a	feces	G9	P[7]		
2014	RVC/Pig-wt/THA/CU875-1C/2014/G1P[x]	5-8	s.i	G1		+	
	RVC/Pig-wt/THA/CU1035/2014/G1P[x]	1-4	deces	G1			+
	RVC/Pig-wt/THA/CU781-2/2014/G1 P[x]	1-4	s.i	G1			
2015	RVC/Pig-wt/THA/CU-SUN/2015/G9P[x]	5-8	feces	G9		+	
	RVC/Pig-wt/THA/CU-BDN-C/2015/G1P[x]	5-8	feces	G1		+	
	RVC/Pig-wt/THA/CUSB-N/2015/G1P[x]	5-8	feces	G1		+	
	RVC/Pig-wt/THA/CU-CHN/2015/G1P[x]	5-8	feces	G1			
	RVC/Pig-wt/THA/CU4-6C/2015/G1P[x]	5-8	s.i	G1			
	RVC/Pig-wt/THA/CU5-1C/2015/G1P[x]	5-8	s.i	G1			
	RVC/Pig-wt/THA/CU5-3/2015/G1P[x]	5-8	s.i	G1			
	RVC/Pig-wt/THA/CU12/2015/G6P[x]	5-8	feces	G6			
	RVC/Pig-wt/THA/CU13/2015/G9P[x]	1-4	feces	G9			
	RVC/Pig-wt/THA/CU14/2015/G1P[x]	5-8	feces	G1			
	RVC/Pig-wt/THA/CU40/2015/G9P[4]	5-8	feces	G9	P[4]	+	
	RVC/Pig-wt/THA/CU48/2015/G1P[4]	5-8	feces	G1	P[4]		
	RVC/Pig-wt/THA/CU49/2015/G9P[x]	1-4	feces	G9			
	RVC/Pig-wt/THA/CU54/2015/G6P[x]	5-8	s.i	G6			
	RVC/Pig-wt/THA/CU60/2015/G1P[5]	5-8	s.i	G1	P[5]		+
	RVC/Pig-wt/THA/CU62C/2015/G1P[x]	5-8	s.i	G1			
	RVC/Pig-wt/THA/CU68C/2015/G1P[x]	5-8	s.i	G1			
	RVC/Pig-wt/THA/CU69C/2015/G1P[x]	5-8	s.i	G1			
	RVC/Pig-wt/THA/CU74C/2015/G1P[x]	1-4	s.i	G1			+
	RVC/Pig-wt/THA/CU79C/2015/G1P[x]	0-6 d	s.i	G1			+
	RVC/Pig-wt/THA/CU84/2015/G9P[7]	5-8	feces	G9	P[7]	+	+

**continued*

Year	Strain name	Age of pig (wk)	Sample	RVC genotype		Co-infect	
				VP7	VP4	RVA	PEDV
2016	RVC/Pig-wt/THA/CU108C/2016/G1P[x]	5-8	feces	G1			
	RVC/Pig-wt/THA/CU109C/2016/G1P[x]	1-4	feces	G1			
	RVC/Pig-wt/THA/CU111C/2016/G1P[x]	1-4	feces	G1			
	RVC/Pig-wt/THA/CU150C/2016/G1P[x]	5-8	s.i	G1			
	RVC/Pig-wt/THA/CU115C/2015/G1P[x]	5-8	feces	G1			
	RVC/Pig-wt/THA/CU99C/2016/G1P[x]	5-8	feces	G1		**+**	+
	RVC/Pig-wt/THA/CU100C/2016/G1P[x]	5-8	feces	G1		+	+
	RVC/Pig-wt/THA/CU122/2016/G6P[5]	0-6 d	feces	G6	P[5]		
	RVC/Pig-wt/THA/CU123/2016/G6P[5]	0-6 d	feces	G6	P[5]		
	RVC/Pig-wt/THA/CU124/2016/G6P[5]	0-6 d	feces	G6	P[5]		
	RVC/Pig-wt/THA/CU125/2016/G6P[5]	0-6 d	feces	G6	P[5]		
	RVC/Pig-wt/THA/CU135/2016/G6P[5]	1-4	feces	G6	P[5]		
	RVC/Pig-wt/THA/CU136/2016/G6P[x]	1-4	feces	G6		**+**	
	RVC/Pig-wt/THA/CU146C/2016/G6P[x]	5-8	feces	G6			
	RVC/Pig-wt/THA/CU200/2016/G1P[1]	5-8	feces	G1	P[1]	**+**	
	RVC/Pig-wt/THA/CU201C/2016/G1P[x]	1-4	feces	G1			
	RVC/Pig-wt/THA/CU202/2016/G1P[x]	5-8	feces	G1			
	RVC/Pig-wt/THA/CU275C/2016/G9P[x]	1-4	feces	G9			
	RVC/Pig-wt/THA/CU276C/2016/G9P[x]	1-4	feces	G9			
	RVC/Pig-wt/THA/CU330C/2016/G1P[x]	5-8	Feces	G1			

wk (week), d (day), s.i (small intestine)

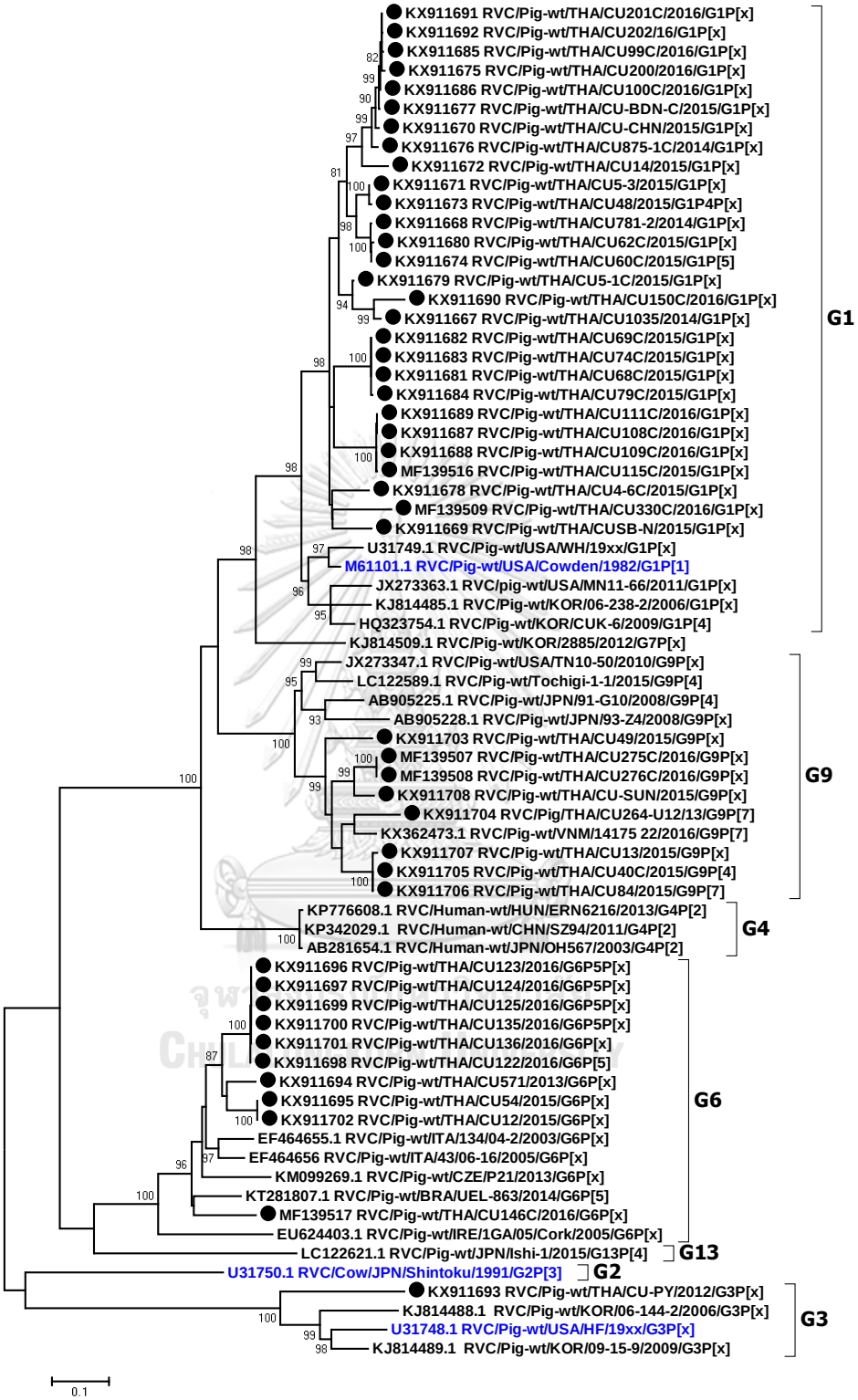

Figure 6. Phylogenetic analysis of the RVC VP7 gene.

Strains identified in this study are shown as dotted. RVC reference strains are blue.

The nearly full-length VP7 sequence encompassing nt 112-952 from the Thai RVC strains encoded amino acid residues 38 to 316. This region spans the variable region 2 (VR2) to variable region 8 (VR8). Genotype G1 and G9 represented three variable sites at residues 39, 53 and 57. Most G6 strains (9/10 strains) had four residue insertion between amino acid positions 245 and 248 (SSSV/SSTL/SSTM/SSSM) towards the carboxyl terminus of VR8 except strain RVC/Pig-wt/THA/CU46C/2016/G6P[x] (Figure 7). Potential N-linked glycosylation sites at residues 67-69 and 225-227 and the putative signal cleavage site at residues 49-50 (A/G-Q) were conserved in all the Thai strains in this study.

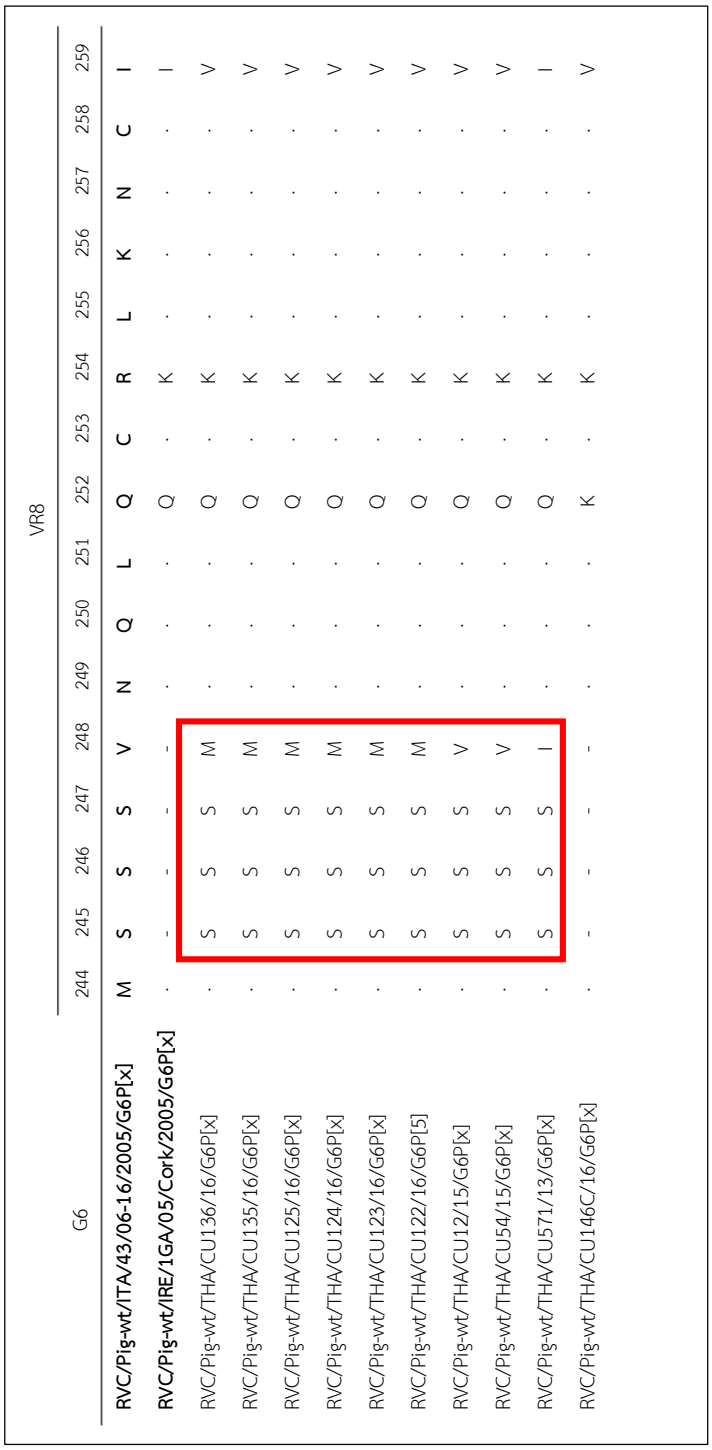

Figure 7. The selected amino acid position of VR8 region for sequence comparison and analysis.

Most Thai G6 strains had four residue insertion between amino acid positions 245 and 248 (covered by the box). RVC G6 reference strains are bold.

Sequence and phylogenetic analysis of the RVC VP4 gene

Partial VP4 gene amplification was subsequently performed for all VP7-positive samples. Phylogenetic analysis of the 11 available sequences of VP4 showed that the majority clustered with the genotype P[5] prototype (Figure 8). Other P genotypes identified were P[1], P[4], and P[7]. In all, there were six G/P combinations (G6P[5], G1P[1], G1P[4], G1P[5], G9P[4] and G9P[7]). The combination G6P[5] predominated in this study (45.5%, 5/11).

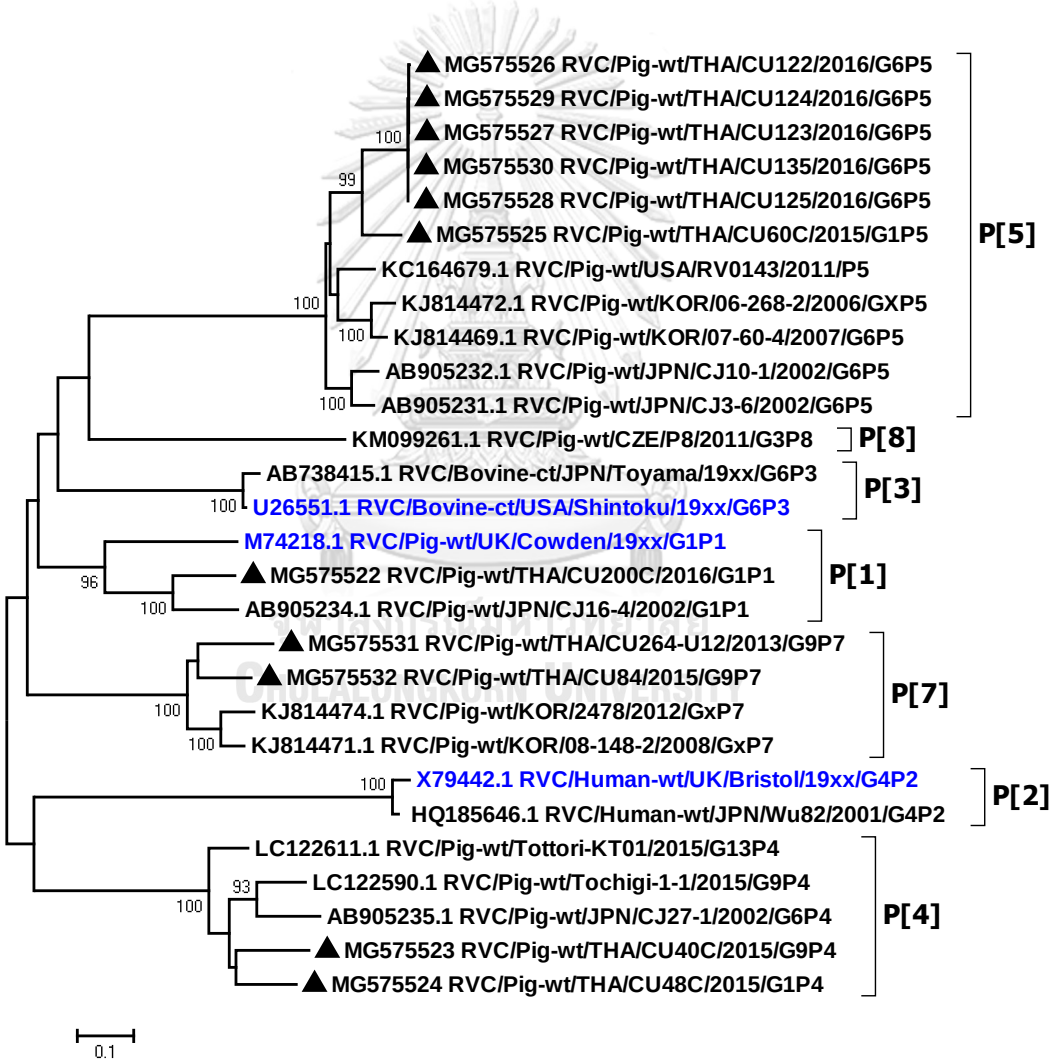

Figure 8. Phylogenetic analysis of the RVC VP4 gene.

Strains identified in this study are indicated with triangles. RVC reference strains are blue.

Analysis of the Thai RVC intra-genotype nt sequences showed between 79.5% (for P[4] strains) and 80.7% (for P[7] strains). Sequence identity for P[5] strains was >99.8%. The deduced amino acid sequences of several representative RVC strains from this study were compared with the amino acid sequences of the prototype strains (Figure 9). The alignment region spanned residues 15 to 385 (based on Cowden numbering). Regions of exceptionally high conservation were more frequent towards the carboxyl than the amino terminus, especially in the last one-third of the sequence. Of interest is the two residues deletion at position 111-112 of strain RVC/Pig-wt/THA/CU200C/2016/G1P[1] compared to the Cowden strain. Other deletions found appeared to be genotype-specific, such as at positions 109-110 for P[4] strains, position 257 for P[4], positions 72, 213, and 214 for P[5], and positions 138-140 for P[7]. Hypervariation such as at positions 228, 236 and 241 were located throughout the sequence.

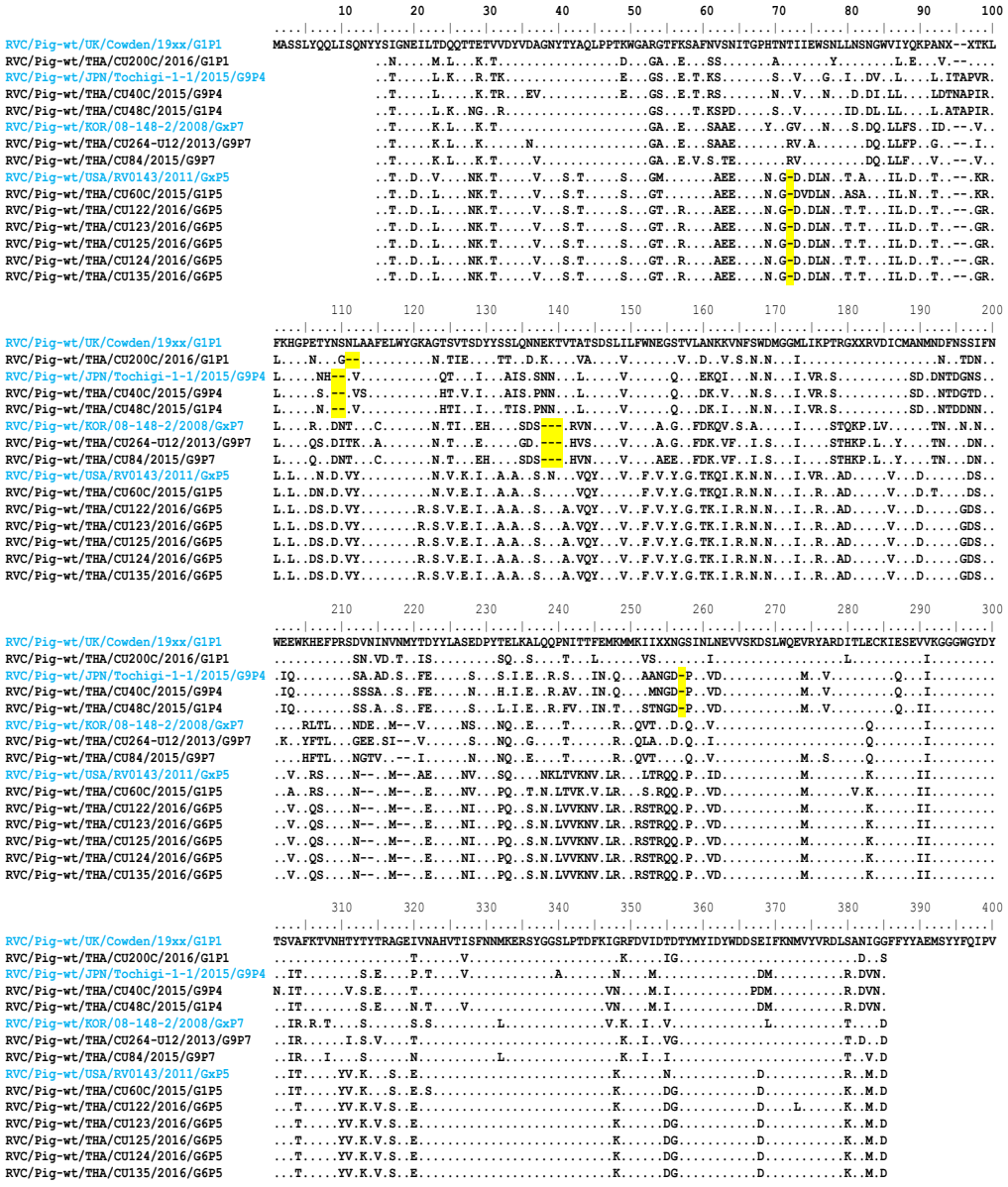

Figure 9. Amino acid alignment of the deduced amino acid residues encoded by the RVC VP4 gene.

Residue positions 15 to 385 were numbered based on the prototype strain Cowden (genotype P[1]). Other reference strains belonging to P[4], P[7] and P[5]. Reference strains are in blue, while strains from this study are black. Dots represent identical residues to the prototypic Cowden. Dash represents unknown amino acids due to missing nucleotides in the alignment.

Part 3: Molecular characterization and genome constellation of Thai porcine rotavirus group A

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Molecular characterization and genome constellation of Thai porcine rotavirus group A

Rotavirus is highly contagious and is frequently responsible for acute gastroenteritis in humans and animals. It is a major cause of diarrhea-associated childhood hospitalization for children younger than 5 years of age (161). On swine farms, RVA infection contributes to a substantial economic loss (154). Complicating management and control of RVA infection are an unpredictable pattern of outbreaks, unknown passive immunity within the nursing herds, feasibility of mass vaccination, and co-infection with other enteric viral pathogens. Therefore, RVA infection remains an important threat to the pig industry.

The Rotavirus Classification Working Group (RCWG) has assigned rotavirus genotypes of the 11 gene segments encoding VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5, which corresponds to genotype designation Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, respectively (20, 162). Genotype G1-G2, G3, G4 (VP7) and P[4], P[8] (VP4) are mainly identified in human RVA, while genotype G3-G4-G5, G9 and P[6], P[7], P[13], P[19], P[23] are typical in infected pigs (22-24). Some genotypes such as G3, G4, G9 and P[6], P[19] frequently infect both pig and human (27).

RVA surveillance in Thailand is performed regionally and previously shows frequent combinations of G3, G4, G5, G9, G10 and P[6], P[7], P[13], P[19], P[23] (24, 90, 92, 163). We previously examined the prevalence of RVA on Thai swine farms and found that a significant number of RVA infection occurs in piglets (164). As detailed knowledge of RVA genome constellation can better facilitate the understanding of rotavirus reassortment patterns and evolution, thus the genetic diversity of 24 Thai porcine RVA strains previously identified between 2011 and 2016 were characterized for genotypic distribution, gene patterns, and the phylogenetic relationship among these strains compared to RVA strains in the vaccine.

Materials and Methods

Samples

A total of 769 samples from diarrheic pigs were submitted to the Livestock Animal Hospital of Veterinary Science, Chulalongkorn University Faculty of Veterinary Science in Nakhon Pathom province between May 2011 and August 2016. Farm locations and provincial origins were previously described in part 2.

Feces (n=509) and small intestinal content (intestinal mucosa of the duodenum and upper part of jejunum from tissues scraping, n=260) were prepared as 10% (w/v) suspension in sterile phosphate-buffered saline (0.1 M, pH 7.2), centrifuged at 3,000 *xg* for 20 minutes. The supernatants were collected and kept in -20°C until used.

Reverse-transcription polymerase chain reaction (RT-PCR), viral nucleic acid detection and sequencing

A total of 769 viral RNAs were extracted using Ribospin vRD II viral RNA purification Kit (GeneAll, Seoul, Korea) according to manufacturer's instructions. Partial VP7 gene was amplified by RT-PCR using SuperScript III One-Step RT-PCR with Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA). Reverse transcription was performed at 48°C for 45 minutes. PCR cycling parameters were initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 30 second, annealing at 55°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

The VP7- positive RNAs (n= 73) were subsequently subjected to partial amplification of gene segments encoding VP1, VP2, VP3, VP4, VP6, NSP1, NSP2, NSP3, NSP4, and NSP5 by RT-PCR using SuperScript III One-Step RT-PCR with Platinum Taq

DNA polymerase (Invitrogen, Carlsbad, CA) (Table 12.). PCR amplicons were resolved and purified using agarose gel electrophoresis. Amplicons were purified using agarose gel electrophoresis and subjected to Sanger sequencing. 24 RVA strains which genome sequencing were successful were included in this study. Nucleotide sequences obtained from this study were deposited in the GenBank database (Appendix, Table S2).

Table 12. Nucleotide primers used in the amplification of RVA genes.

Primers	Sequences (5' to 3')	Location	Annealing	Amplicon
VP7 gene	CGGTTAGCTCCTTTTAATGT	33-52	55°C	891 bp
(AB176677.1)	CATTTCTTCCAATTTACTCGC	903-924		
VP4 gene	GGCTATAAATGGCTTCGCTC	1-21	52 °C	1074 bp
(165)	AATGCTTGTGAATCATCCCAG	1074-1094		
VP6 gene	GTCTTCGACATGGAAGTTC	15-33	46°C	633 bp
(AB779621)	AAACTGYTGAATRTTTGCTGG	627-649		
VP1 gene	GGAAGTAYAATCTAATCTTG	23-42	42°C	1075 bp
(DQ490539.1)	TACATYTCRYYATCHACATC	1078-1098		
VP2 gene	TTTCCDACHATGCCDGTTGA	1598-1617	44°C	1097 bp
(JX406748.1)	GGCGTYTACARYTCRTTCAT	2675-2694		
VP3 gene	GGCTWTTAAAGCARAYTAGTAG	1-23	50°C	630 bp
(166)	TTTRTCTCTRAAYACACGATTTGA	608-631		
NSP1 gene	TTATGAAAAGTCTTGTGGAA	9-28	46°C	545 bp
(JX406751.1)	AAATGARAATGGDGTYTGATT	533-553		
NSP2 gene	TTTTAAAGCGTCTCAGTCG	4-22	48°C	727 bp
(JX406754.1)	AACTCTRTAGTGACCTTTACC	710-730		
NSP3 gene	TTTCAGTGGTTGTTGCTCA	14-32	52°C	995 bp
(JX406753.1)	AGAGGGTYAYGTGWAGATGG	989-1008		
NSP4 gene	AAAGTTCTGTTCCGAGAGA	19-27	52°C	732 bp
(JX406756.1)	AGACCRTTCCTTCCATTAACG	720-740		
NSP5 gene	GCTACAGTGATGTCTCTCAGC	13-33	52°C	580 bp
(GU199491.1)	TTGCGACTTGCTTCATCCTC	579-592		

D=A/G/T; H=A/C/T; R=A/G; Y=C/T

Sequence and phylogenetic analyses

Strain genotypes were determined using the RotaC 2.0 automated genotyping tool (167). Nucleotide sequences were assembled and edited using SeqMan II and aligned using BioEdit and ClustralX v.2.0.11 (159). Reference sequences from the GenBank database were available for inclusion in all phylogenetic trees. Phylogenetic trees were constructed using MEGA6 software with the maximum likelihood method and 1,000 replicates (160). Best model fitting was automatically calculated for genetic distances. Bootstrap values >80% were considered significant.

The deduced amino acid sequences from the Thai RVA strains were compared to those of the RVA strains in the porcine RVA vaccine and reference strains. Sequence identity in percentage was from amino acid comparison unless noted otherwise. For genotype G3, G4, G5, and G9, strain A131 (accession no. L35055), Gottfried (X06759), OSU (X04613), and A2 (AB180971) were used, respectively. The amino acid residue at position 291 was not analyzed because the sequence of the VP7 PCR product did not include this residue. For genotype P[6], P[13], P[19], and P[23], reference strains were Gottfried (M33516), HP140 (DQ003291), 4F (L10359), CMP48/08 (HQ268847), respectively. For genotype I1 and I5, Wa (K02086) and YM (X69487) served as reference strains. Specific reference strains were chosen based on recommendations by the Rotavirus Classification Working Group (RCWG) and when their nucleotide sequences encompassed the same region as the Thai strains in this study (20, 162).

Results

G, P, and I genotypes

The characterization of 24 RVA-positive samples from Thai swine obtained between 2011 and 2016 showed a predominance of G9 (62.5%, 15/24), followed by G3 (20.8%, 5/24), G4 (12.5%, 3/24), and G5 (4.2%, 1/24) (Figure 10). These Thai strains shared 82.5-100% amino acid identity to one another and 82.4-93.5% amino acid identity to the A2 reference strain. Several strains showed identical nucleotide sequences (RVA/Pig-wt/THA/CU101/2016/G9P[23] and RVA/Pig-wt/THA/CU280-2/2016/G9P[19], and RVA/Pig-wt/THA/CU176/2016/G9P[13] and RVA/Pig-wt/THA/CU232/2016/G9P[13]), all of which were identified in 2016 and were from two adjacent provinces (strain RVA/Pig-wt/THA/CU101/2016/G9P[23] and RVA/Pig-wt/THA/CU280-2/2016/G9P[19], RVA/Pig-wt/THA/CU232/2016/G9P[13] were collected from pig herds located in Ratchaburi province, while RVA/Pig-wt/THA/CU176/2016/G9P[13] was collected from Kanchanaburi province). Of the Thai G4 strains (3/24), they were more closely related to RVA strains of recent years (92.2- 93.7% amino acid identity) than to the prototypic porcine RVA strain Gottfried (80.8-82.8% identity). The 5 Thai G3 strains shared 88.7-99.6% identity and were similar to a Thai porcine strain (RVA/Pig-wt/THA/CMP39/2000/G3P[19]) previously identified as far back as 2000 (91.4-93.3% identity). The only one Thai G5 strain in this study (RVA/Pig-wt/THA/CU181/2016/G5P[13]) was also similar to a previously described Thai porcine strain (RVA/Pig-wt/THA/CMP178/2006/G5P[13] and RVA/Pig-wt/THA/CMP-001-12/2012/G5P[13] greater than 90%) compared to the prototypic porcine RVA strain OSU (81.8% identity).

Four P genotypes were identified in our study, of which P[13] was predominant (50%, 12/24), followed by P[23] (25%, 6/24), P[19] (16.6%, 4/24) and P[6] (8.4%, 2/24). All Thai P[13] strains except RVA/Pig-wt/THA/CU192/2016/G9P[13]

clustered in the same genetic group (Figure 11). Two Thai P[13] strains, RVA/Pig-wt/THA/CU140/2016/G9P[13] and RVA/Pig-wt/THA/CU181/2016/G5P[13] were identified in different provinces but showed identical nucleotide sequences. Interestingly, all Thai P[23] strains in this study shared 93-95.6% amino acid identity to RVA/Human-wt/THA/KKL-117/2014/G9P[23] previously identified in an infant with diarrhea in Thailand. The Thai P[19] strains shared 84.4-86.7% identity among each other and 87.5-96.4% identity with the reference strain RMC321. The Thai P[6] strains (RVA/Pig-wt/THA/CU-L141/2012/G4P[6] and RVA/Pig-wt/THA/CULC-1/2013/G4P[6]) showed closer genetic relatedness to an RVA strain identified from a patient (E931, 94-96.2% identity) than to Gottfried (75.8-76.4%). Overall, the dominant G and P combinations were G9P[13] and G9P[23] (n=6 each), followed by G3P[13] (n=5), G9P[19] (n=3), G4P[6] (n=2), G4P[19] and G5P[13] (n=1 each).

There were 23 strains of I5 genotype (88-95.3% amino acid identity), which clustered with the YM porcine reference strain (Figure 12). Interestingly, one Thai RVA strain (RVA/Pig-wt/THA/CU-L141/2012/G4P6) belonged to I1 genotype along with Gottfried and Wa strains.

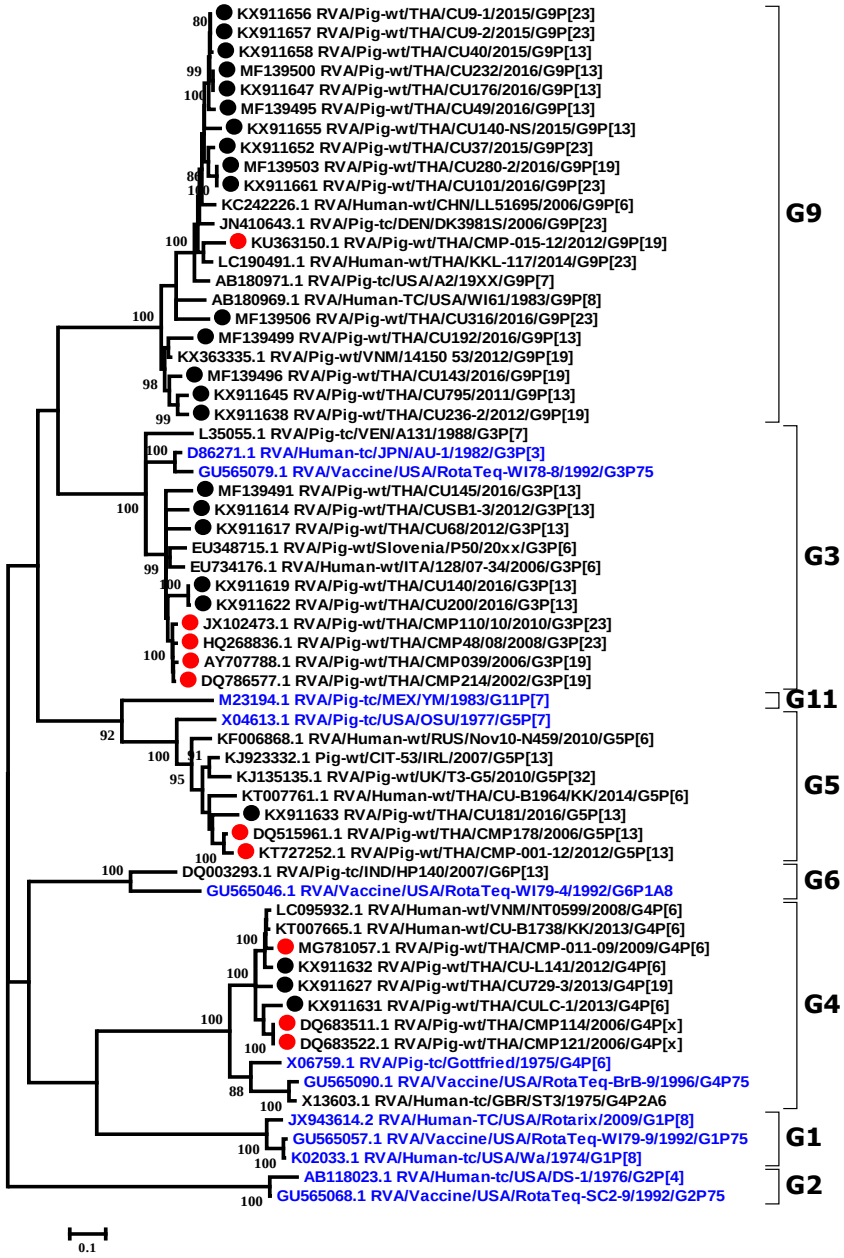

Figure 10. Phylogenetic analysis of the RVA VP7 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

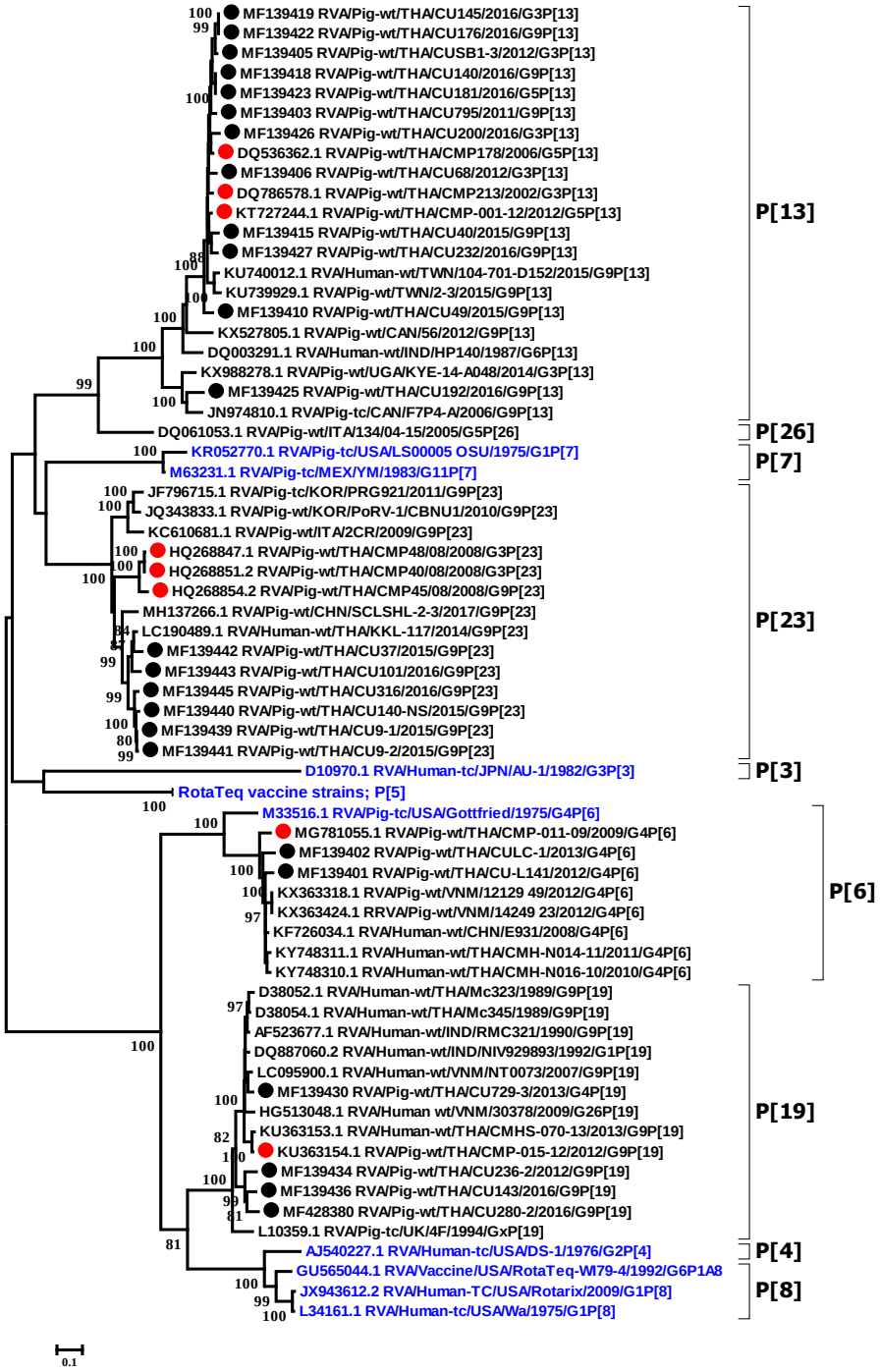

Figure 11. Phylogenetic analysis of the RVA VP4 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

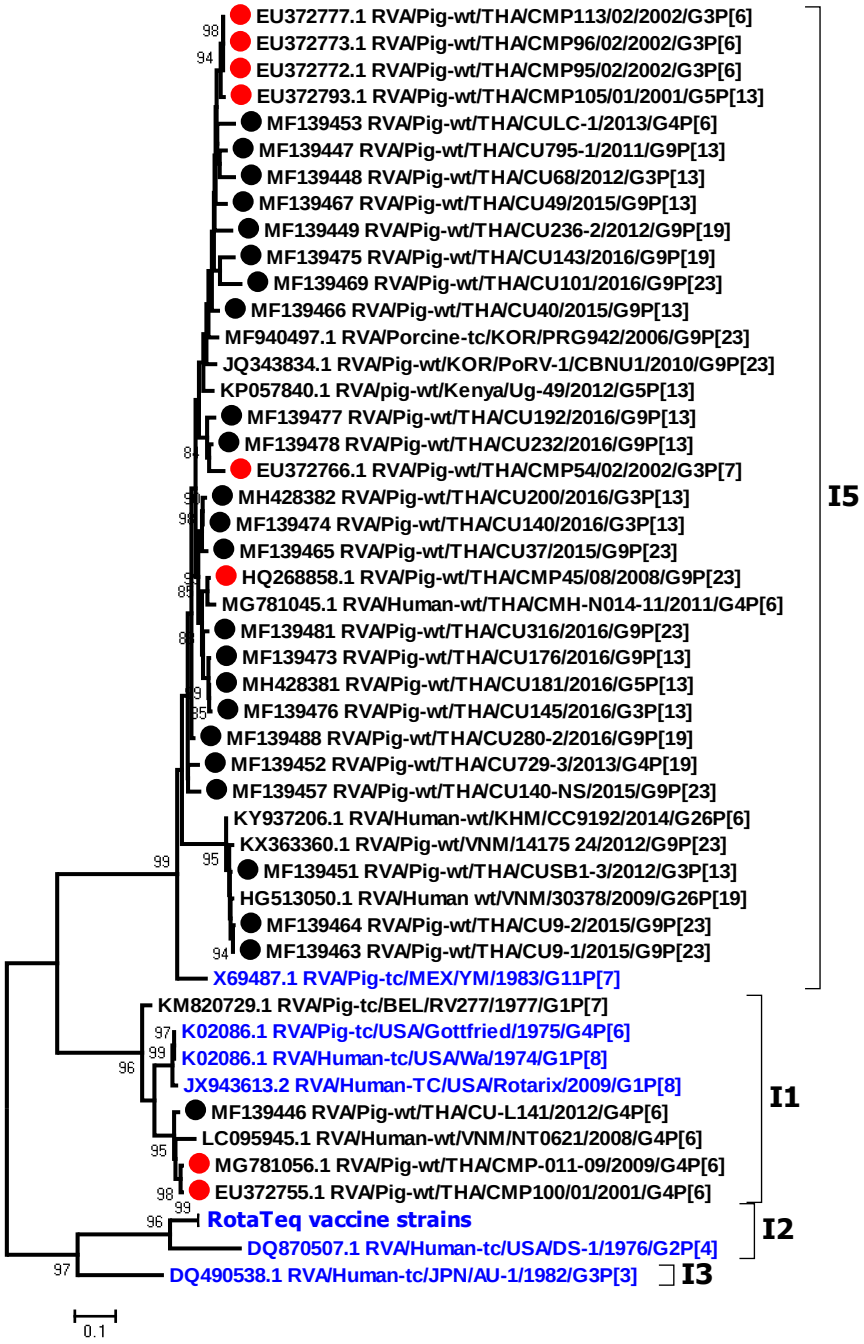

Figure 12. Phylogenetic analysis of the RVA VP6 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

Analysis of other genotypes

The majority of the 24 Thai porcine RVA strains demonstrated a conserved internal genotype constellation of R1-C1-M1-A8-N1-T1-E1-H1. All Thai strains in our study belonged to the R1 genotype. The C genotypes were not generated from 3 strains due to insufficient PCR amplicon, but the remaining 21 strains were C1 genotype and shared 82-86% amino acid identity (86.2-92.8% identity with Gottfried, OSU, and YM strains). Among the Thai M1 genotype, only 3 strains clustered closely with Gottfried (81.6-84.9% identity), OSU (81.8-87.7% identity), and Wa (82.1-85% identity), while the rest clustered in a separate subgroup (Figure 13, 14, 15).

Analysis of the non-structural protein genes showed all strains were A8 genotype (Figure 16, 17, 18, 19, and 20). Although seven of the Thai strains clustered with Gottfried, the majority comprised a separate branch containing relatively more recent RVA strains. Collectively, the Thai strains in these two lineages shared 78.4-85.8% amino acid identity. Most Thai N1 strains (n=23) clustered with Gottfried, but one strain (RVA/Pig-wt/THA/CU192/2016/G9P13) clustered with Wa and OSU. For T genotype, most Thai strains (n=21) grouped with Gottfried, OSU, and Wa in the T1 cluster. The majority of the E genotype (n=23) strains was E1 for which both the human and porcine RVA vaccine strains belonged. One E9 genotype (RVA/Pig-wt/THA/CU143/2016/G9P19) was the only outlier strain and clustered with the reference strain CMP034 previously identified in piglet on a Thai farm. Three Thai H1 genotype strains were identical in nucleotide sequences (RVA/Pig-wt/THA/CU140/2016/G9P13, RVA/Pig-wt/THA/CU200/2016/G3P13, and RVA/Pig-wt/THA/CU280-2/2016/G9P19), which was not surprising since these were farm samples from the same province.

Taken together, the characterization of all 11 segments of RVA from 24 strains identified in Thailand over a 5-year period revealed a dominance of the constellation

Gx-P[x]-I5-R1-C1-M1-A8-N1-T1-E1-H1. With the exception of I and A genotypes, this pattern resembles the prototypic RVA strain of Wa lineage (which possesses I1 and A1).

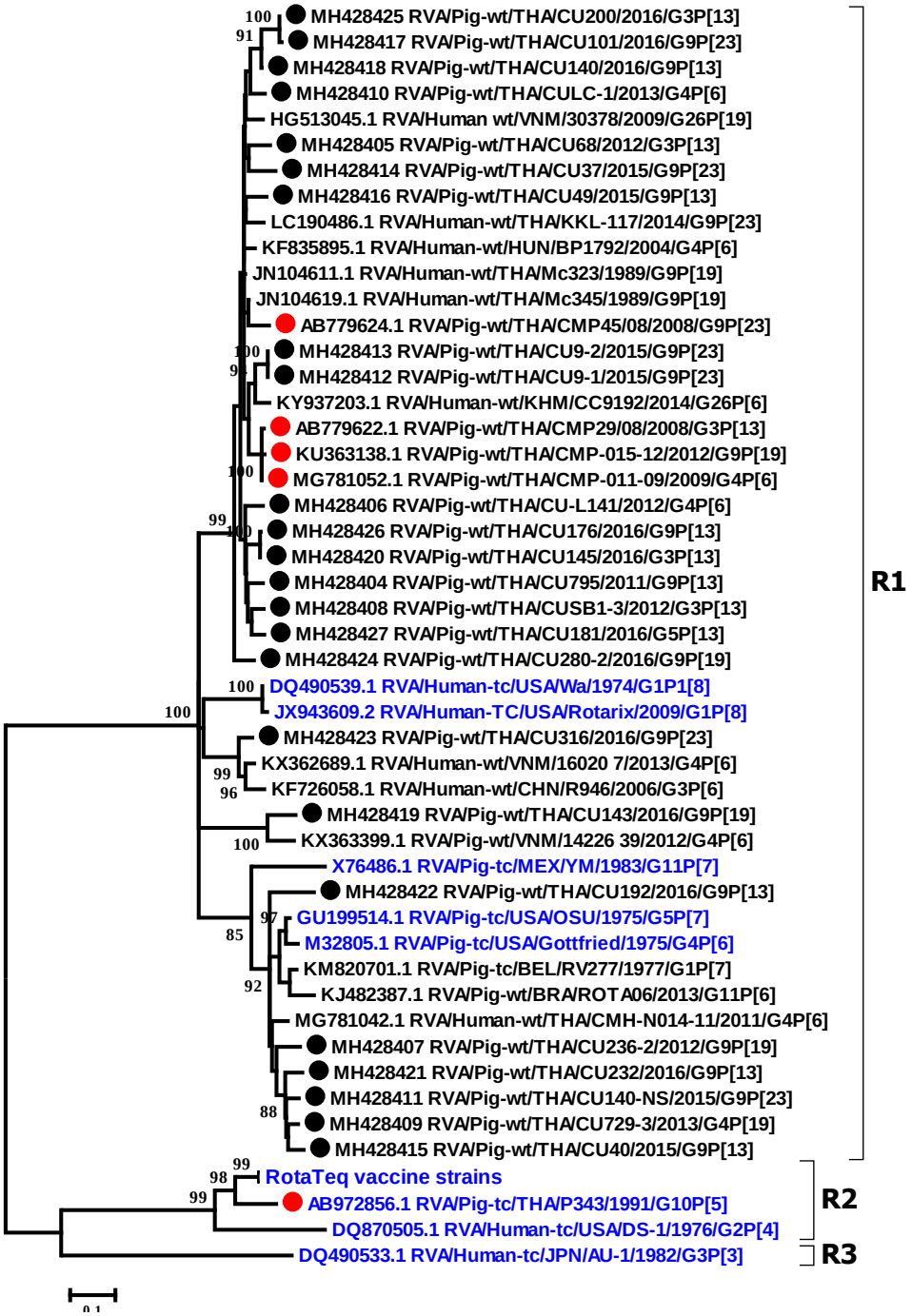

Figure 13. Phylogenetic analysis of the RVA VP1 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

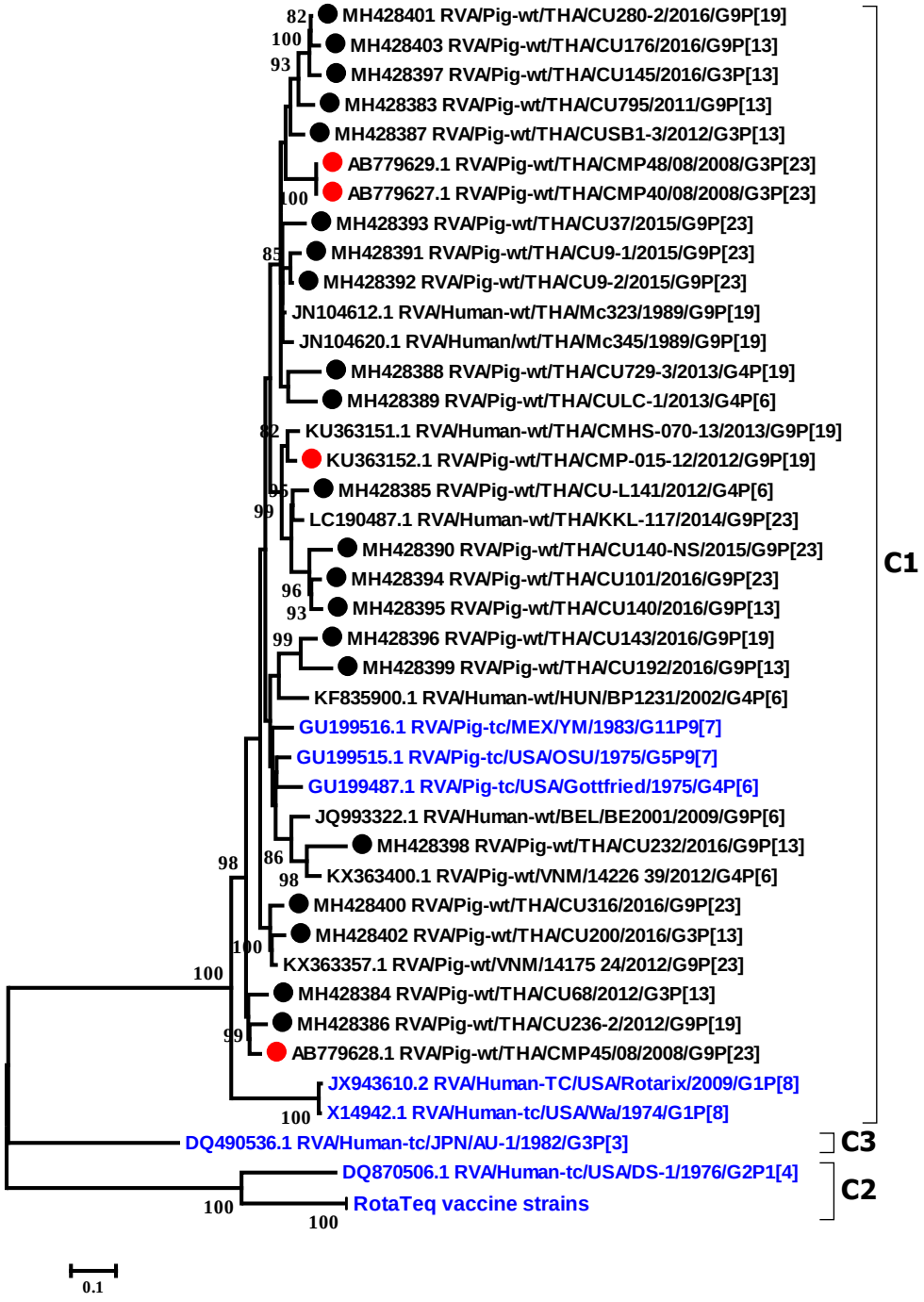

Figure 14. Phylogenetic analysis of the RVA VP2 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

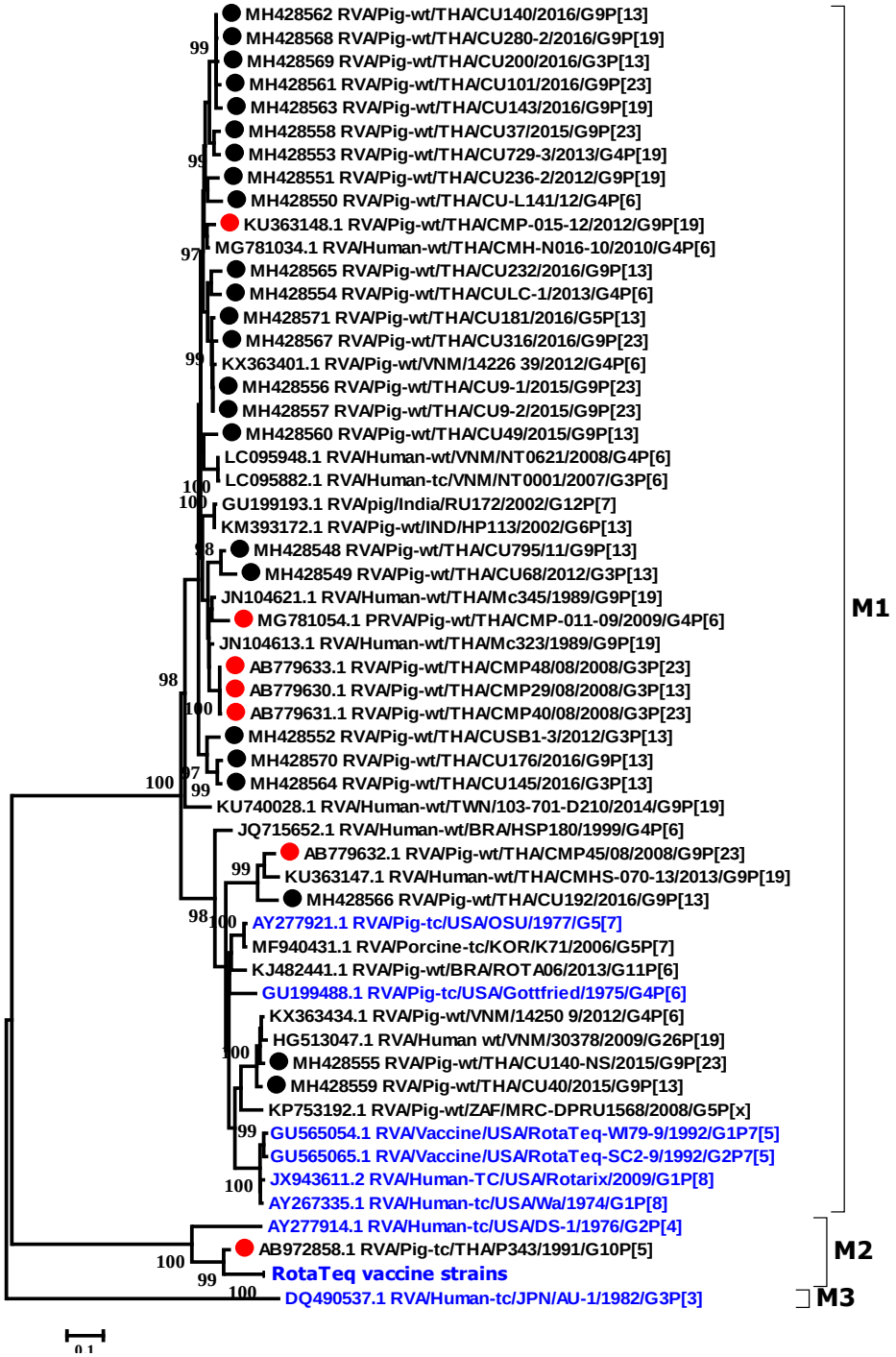

Figure 15. Phylogenetic analysis of the RVA VP3 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

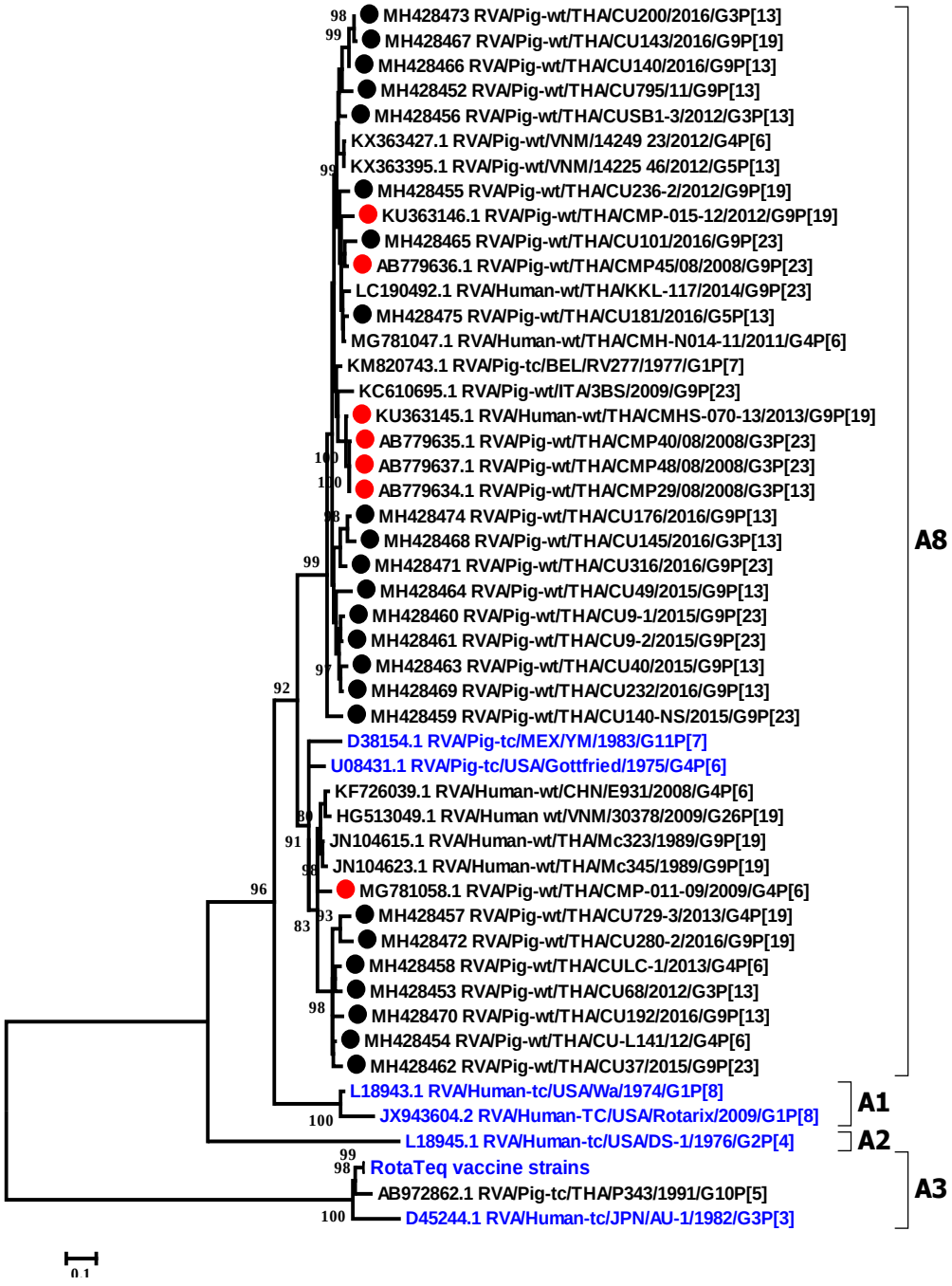

Figure 16. Phylogenetic analysis of the RVA NSP1 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

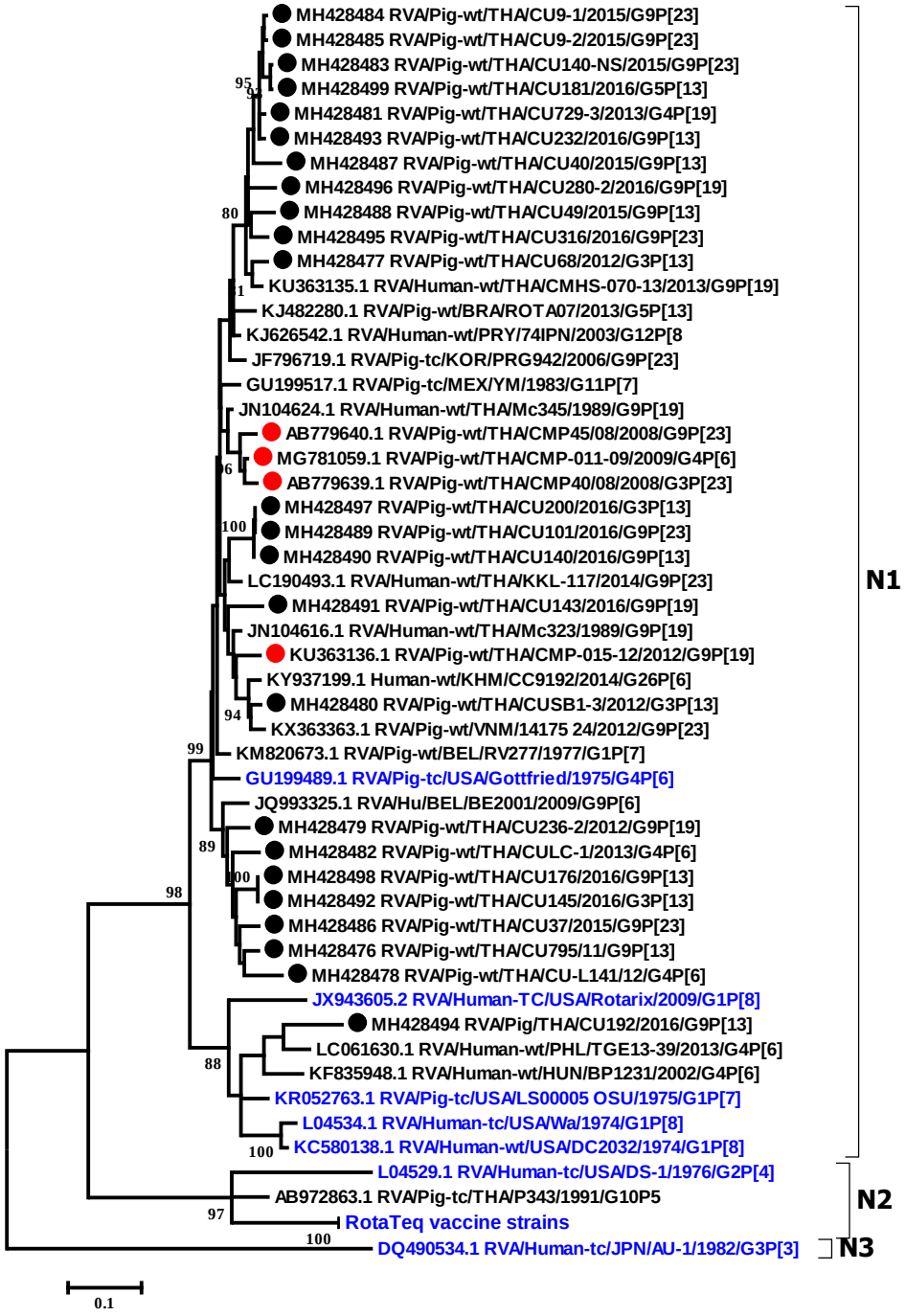

Figure 17. Phylogenetic analysis of the RVA NSP2 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

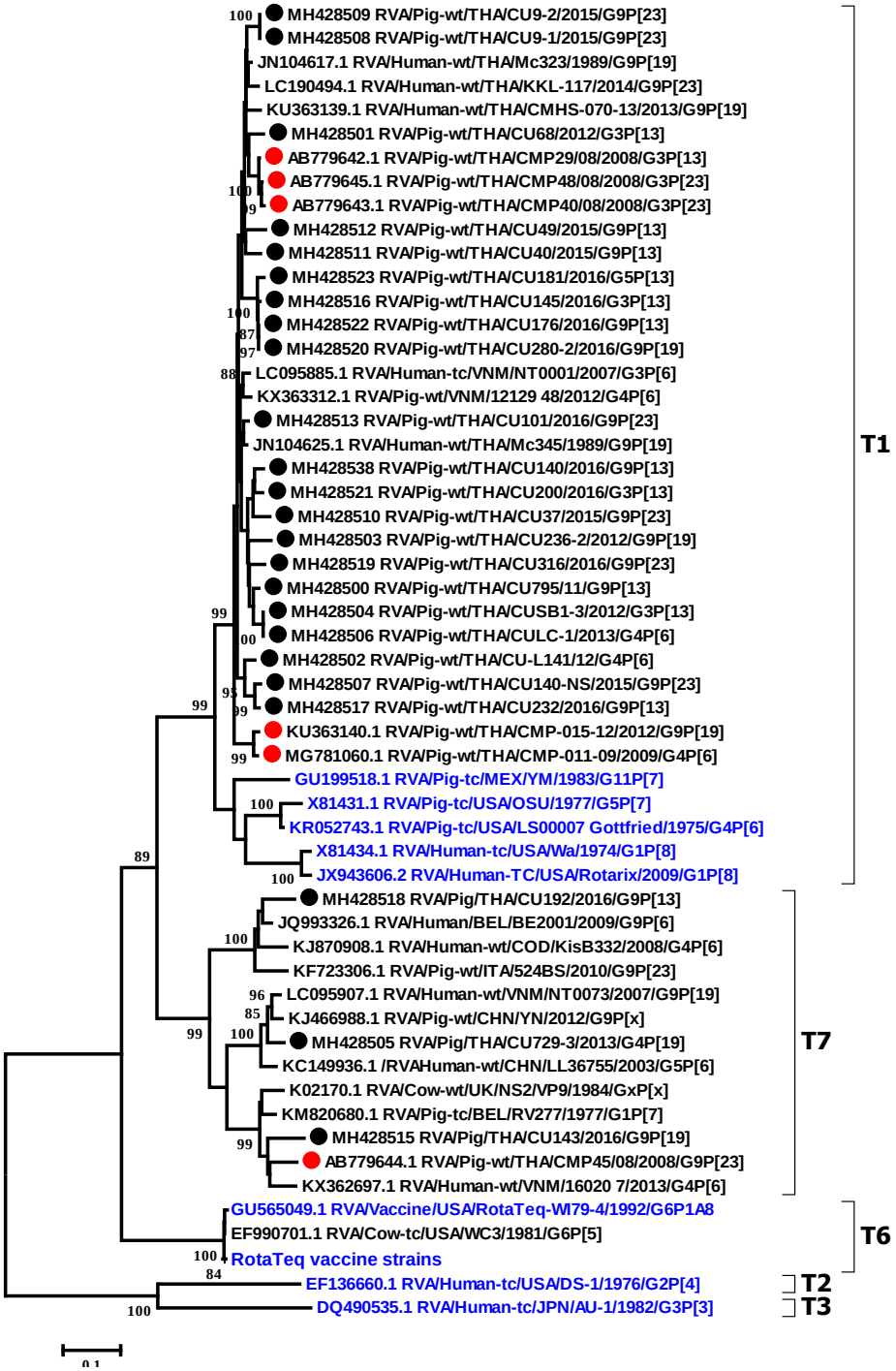

Figure 18. Phylogenetic analysis of the RVA NSP3 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

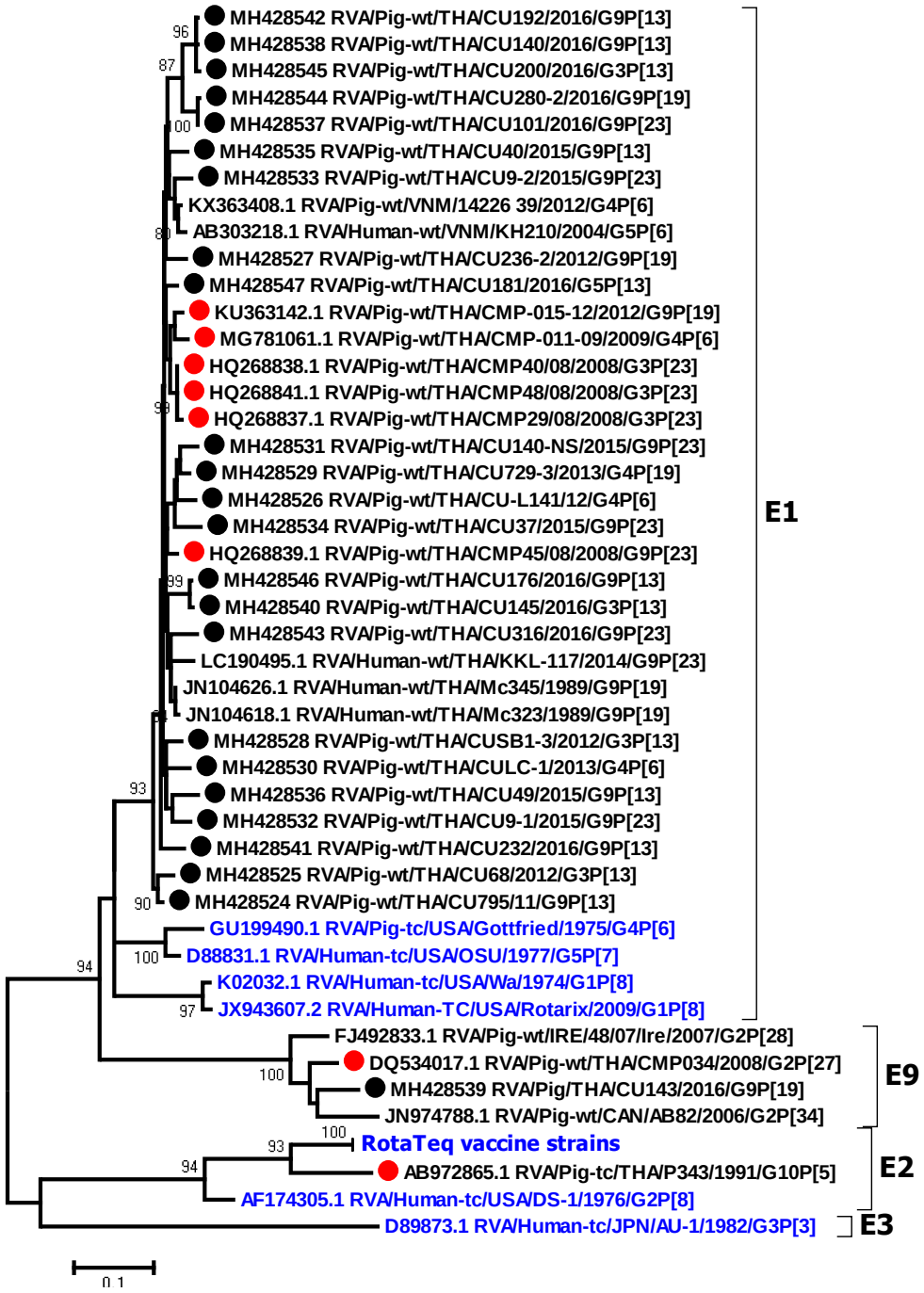

Figure 19. Phylogenetic analysis of the RVA NSP4 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

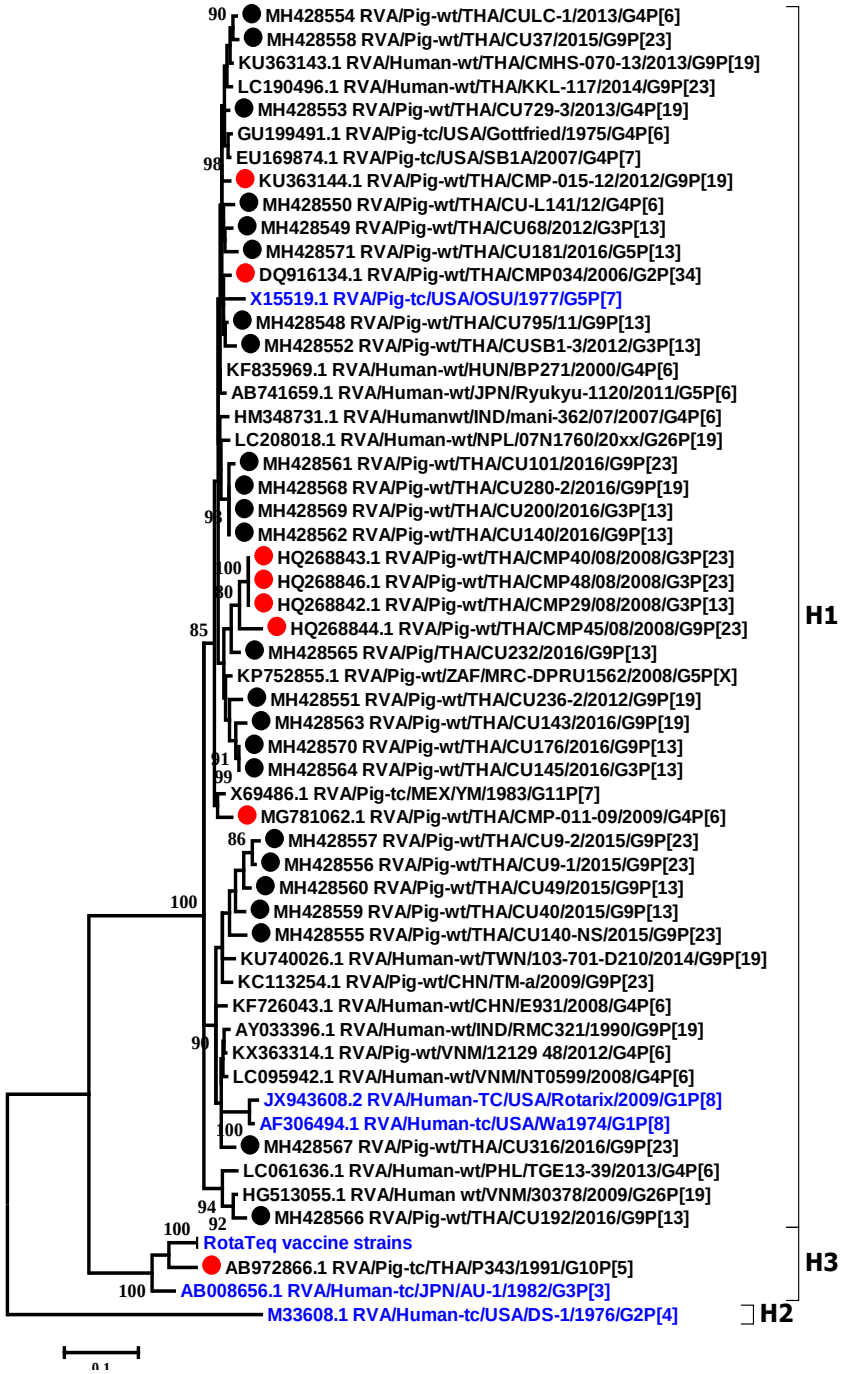

Figure 20. Phylogenetic analysis of the RVA NSP5 gene.

The nucleotide sequences of the Thai strains (dotted) were compared to those of the RVA reference and vaccine strains (blue).

In summary, most of 24 strain genotype constellations for VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 were dominantly displayed as the Wa-like backbone of Gx-P[x]-I1/I5-R1-C1-M1-A8-N1-T1/T7-E1/E9-H1. The general information (age of infected pigs, sample origin and provinces) and genotype constellations of 24 RVA porcine strains were summarized in Table 13.

Table 13. The information of 24 Thai RVA porcine strains.

Strain	Age	Sample	Prov	VP7	VP4	VP6
RVA/Pig-wt/THA/CU68/2012/G3P[13]	3 d	s.i.	NP	G3	P[13]	I5
RVA/Pig-wt/THA/CUSB1-3/2012/G3P[13]	6 wk	feces	SB	G3	P[13]	I5
RVA/Pig-wt/THA/CU145/2016/G3P[13]	5 wk	feces	KB	G3	P[13]	I5
RVA/Pig-wt/THA/CU140/2016/G3P[13]	6 wk	feces	RB	G3	P[13]	I5
RVA/Pig-wt/THA/CU200/2016/G3P[13]	6 wk	feces	RB	G3	P[13]	I5
RVA/Pig-wt/THA/CU-L141/2012/G4P[6]	2 wk	s.i.	RB	G4	P[6]	I1
RVA/Pig-wt/THA/CULC-1/2013/G4P[6]	1 wk	feces	NP	G4	P[6]	I5
RVA/Pig-wt/THA/CU729-3/2013/G4P[19]	2 wk	feces	RB	G4	P[19]	I5
RVA/Pig-wt/THA/CU181/2016/G5P[13]	2 wk	feces	SP	G5	P[13]	I5
RVA/Pig-wt/THA/CU795/2011/G9P[13]	5 d	s.i.	NP	G9	P[13]	I5
RVA/Pig-wt/THA/CU40/2015/G9P[13]	6 wk	feces	CB	G9	P[13]	I5
RVA/Pig-wt/THA/CU49/2015/G9P[13]	3 wk	feces	CB	G9	P[13]	I5
RVA/Pig-wt/THA/CU176/2016/G9P[13]	4 wk	feces	KB	G9	P[13]	I5
RVA/Pig-wt/THA/CU232/2016/G9P[13]	6 wk	feces	RB	G9	P[13]	I5
RVA/Pig-wt/THA/CU192/2016/G9P[13]	6 wk	feces	RB	G9	P[13]	I5
RVA/Pig-wt/THA/CU236-2/2012/G9P[19]	2 wk	feces	RB	G9	P[19]	I5
RVA/Pig-wt/THA/CU280-2/2016/G9P[19]	4 wk	feces	RB	G9	P[19]	I5
RVA/Pig-wt/THA/CU143/2016/G9P[19]	6 wk	feces	KB	G9	P[19]	I5
RVA/Pig-wt/THA/CU9-1/2015/G9P[23]	4 wk	s.i.	CB	G9	P[23]	I5
RVA/Pig-wt/THA/CU9-2/2015/G9P[23]	4 wk	s.i.	CB	G9	P[23]	I5
RVA/Pig-wt/THA/CU140-NS/2015/G9P[23]	6 wk	feces	CB	G9	P[23]	I5
RVA/Pig-wt/THA/CU37/2015/G9P[23]	10 d	feces	RB	G9	P[23]	I5
RVA/Pig-wt/THA/CU101/2016/G9P[23]	5 wk	feces	RB	G9	P[23]	I5
RVA/Pig-wt/THA/CU316/2016/G9P[23]	24 d	feces	NR	G9	P[23]	I5

** Continued*

Strain	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Pig-wt/THA/CU68/2012/G3P[13]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CUSB1-3/2012/G3P[13]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU145/2016/G3P[13]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU140/2016/G3P[13]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU200/2016/G3P[13]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU-L141/2012/G4P[6]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CULC-1/2013/G4P[6]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU729-3/2013/G4P[19]	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Pig-wt/THA/CU181/2016/G5P[13]	R1	-	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU795/2011/G9P[13]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU40/2015/G9P[13]	R1	-	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU49/2015/G9P[13]	R1	-	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU176/2016/G9P[13]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU232/2016/G9P[13]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU192/2016/G9P[13]	R1	C1	M1	A8	N1	T7	E1	H1
RVA/Pig-wt/THA/CU236-2/2012/G9P[19]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU280-2/2016/G9P[19]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU143/2016/G9P[19]	R1	C1	M1	A8	N1	T7	E9	H1
RVA/Pig-wt/THA/CU9-1/2015/G9P[23]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU9-2/2015/G9P[23]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU140-NS/2015/G9P[23]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU37/2015/G9P[23]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU101/2016/G9P[23]	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Pig-wt/THA/CU316/2016/G9P[23]	R1	C1	M1	A8	N1	T1	E1	H1

d, day; wk, week; s.i., small intestine; Prov, provincial origin of Thai RVA strains, CB, Chon Buri; KB, Kanchanaburi; NP, Nakhon Pathom; NR, Nakhon Ratchasima; RB, Ratchaburi; SB, Saraburi; SP, Suphan Buri.

Comparison of the antigenic epitopes on VP7 between the Thai porcine and the vaccine strains

Amino acid variance on the antigenic epitopes of VP7 (namely region 7-1a, 7-1b and 7-2) among circulating RVA strains can influence the effectiveness of the vaccine used in the field. To determine residue differences on these important epitopes seen on the 24 Thai RVA strains, we compared them to Gottfried, OSU, and the other reference RVA strains (Figure 21). The Thai G3 strains demonstrated conservative amino acid change at N123D, and less so at T147A and N221A, compared to prototypic A131 strain. Comparison of the three Thai G4 strains to Gottfried showed common changes at S87T, I129V and A213N. Strain RVA/Pig-wt/THA/CU729-3/2013/G4P[19] possessed six residue changes, the most of any G4 strains. Comparison with OSU showed that the only Thai G5 strain displayed changes at T96N, V129I, E130D, I212V, S242N, G146A, and A221T. For most Thai G9 strains, they differed from the reference strain at N100D and K212T, with RVA/Pig-wt/THA/CU316/2016/G9P23 possessing the maximum of six residue variance.

Strain	7-1a														7-1b								7-2									
	87	91	94	96	97	98	99	100	104	123	125	129	130	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
A131-G3	T	T	N	N	S	W	K	D	Q	N	A	V	D	Q	D	T	N	S	N	K	D	A	T	L	S	E	N	G
RVA/Pig/THA/CU68/2012/G3P[13]	.	.	.	.	.	.	.	.	.	D	.	.	.	.	.	A	S	.	.	.	.	.	.	.	.	.	A	.
RVA/Pig/THA/CUSB1-3/2012/G3P[13]	.	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.
RVA/Pig/THA/CU140/2016/G3P[13]	.	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.
RVA/Pig/THA/CU145/2016/G3P[13]	.	.	.	.	.	.	.	.	.	D	.	I	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.
RVA/Pig/THA/CU200/2016/G3P[13]	.	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.
Gottfried-G4	S	T	N	N	E	W	K	D	Q	N	L	I	D	Q	N	T	A	D	T	R	A	S	G	E	S	T	S	G
RVA/Pig/THA/CU-L141/2012/G4P[6]	T	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	N	N	.	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CULC-1/2013/G4P[6]	T	.	.	.	.	.	.	.	.	.	.	V	.	.	.	.	N	.	.	.	.	.	.	Q	.	.	G	.
RVA/Pig/THA/CU729-3/2013/G4P[19]	T	.	.	.	.	.	.	.	.	.	.	V	.	.	.	V	N	.	.	.	T	.	.	.	.	.	N	.
OSU-G5	N	T	A	T	K	W	T	E	Q	D	A	V	E	Q	D	I	N	D	S	K	D	G	N	L	S	T	A	G
RVA/Pig/THA/CU181/2016/G5P[13]	.	.	.	N	.	.	.	.	.	.	.	I	D	.	.	V	.	.	N	.	.	A	.	.	.	.	T	.
A2-G9	T	T	G	T	E	W	K	N	Q	D	A	I	D	Q	N	K	A	D	N	K	D	S	T	L	S	E	N	G
RVA/Pig/THA/CU795/2011/G9P[13]	.	.	.	.	.	.	.	D	.	.	.	.	N	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CU236-2/2012/G9P[19]	.	N	.	.	.	.	.	D	.	.	.	V	.	.	.	T	.	.	.	.	.	.	.	.	.	.	S	.
RVA/Pig/THA/CU9-1/2015/G9P[23]	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CU9-2/2015/G9P[23]	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CU37/2015/G9P[23]	.	.	.	A	.	.	.	D	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CU40/2015/G9P[13]	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.
RVA/Pig/THA/CU49/2015/G9P[13]	.	.	.	A	.	.	.	D	.	.	.	.	.	.	.	P	.	.	S	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CU101/2016/G9P[23]	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CU140-NS/2015/G9P[23]	.	.	.	.	.	.	.	D	.	.	.	.	N	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CU143/2016/G9P[19]	.	.	.	A	.	.	.	D	.	.	.	.	.	.	.	T	.	.	.	.	T	.	.	.	.	.	S	.
RVA/Pig/THA/CU176/2016/G9P[13]	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CU192/2016/G9P[13]	.	.	.	.	S	.	.	D	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	S	.
RVA/Pig/THA/CU232/2016/G9P[13]	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CU280-2/2016/G9P[19]	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
RVA/Pig/THA/CU316/2016/G9P[23]	.	.	R	A	.	.	.	D	.	N	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	S	.

Figure 21. Residue differences in the VP7 antigenic regions between the Thai strains and the RVA reference/vaccine strains.

Residue positions for each region are numbered. Identical residues are indicated by dots.

Part 4. Molecular epidemiology and characterization of spike, ORF3 and nucleocapsid genes of porcine epidemic diarrhea virus

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Molecular epidemiology and characterization of spike, ORF3 and nucleocapsid genes of porcine epidemic diarrhea virus

PEDV contributes to enteropathogenic diarrhea especially among suckling piglets and significant economic impact on the swine industry worldwide (148, 168). PEDV-infected pigs experience watery diarrhea, vomiting and severe dehydration, which leads to 50%-90% mortality among susceptible piglets (169, 170). Transmission is frequently fecal-oral, but infections via airborne and fomites have been documented (171-173).

PEDV is a single-stranded positive-sense RNA virus belonging to the family *Coronaviridae*, genus *Alphacoronavirus* (119). PEDV genome comprises 6 genes as *Pol*, spike, open reading frame 3, envelope, membrane and nucleocapsid genes (68).

The widely-accepted classification of PEDV is based on the S gene sequence, which categorizes PEDV genotypes into two genogroups (G1 and G2). Each genogroup is further sub-divided into 2 subgroups (G1a, G1b, G2a, and G2b). Classical strains are designated G1a, while the new variant strains (S-INDEL) strains belong to G1b. The highly virulent Asian and North American strains are designated G2a and G2b, respectively (119). The original PEDV (represented by the prototypic CV777 strain) was first reported in the 1970's in Europe and was associated with high morbidity and mortality among infected pigs (99). The S-INDEL strains were first reported in the U.S. in 2014 and were subsequently introduced to Asia and Europe. These strains contain insertions and deletions in the N-terminal region of the S protein (S1 region) compared to the prototype CV777. One-third of the S gene among the new variants G1b shared greater than 95% identity to the classical G1a strain, while the remaining two-thirds possessed greater than 99% similarity to highly virulent G2 strains. Therefore, S-INDEL variants resemble recombination strains between G1a and G2 (120, 121). Infection by some S-INDEL variants was reported to

produce decreased symptom severity including moderate diarrhea, lower titers of viral shedding, and reduced mortality (124, 125). The highly virulent G2 strains caused an epidemic in Asia and have been identified in North America and elsewhere around the world (126).

Thailand has a major pork production industry in Southeast Asia. Despite improved animal husbandry, farm management, vaccination, and boosting of lactogenic immunity, PEDV outbreaks continue to occur on swine farms. PEDV situations in Thailand have continuously emerged since 1990s, in which the first report based on molecular characterization of PEDV has been published in the NCBI database in 2009. The previously identified Thai PEDV strains had close genetically related to Chinese field strains (JS-2004-2), so Chinese strain was hypothesized to be the ancestor of Thai PEDV strains (132). Loss of piglets due to PEDV infection necessitates constant epidemiological surveillance to monitor transmission effectively. The molecular epidemiology of Thai PEDV was periodically reported which most of the studies involved with S, M, ORF3 gene sequence characterizations and full-length genome analysis (132-134, 174).

S gene sequence had high variations and it encompassed several neutralizing epitopes (located in 4 parts of amino acid position including position 499-638, 748-755, 764-771 and 1,368-1,374). The amplification of large size of the whole S gene was complicated, while neutralizing epitopes had high variations. As this region is subjected to the immune pressure from the host and sequence variations, then are frequently used for PEDV diagnosis and genetic diversity investigation (126, 133, 175-177). However, the CO-26K equivalent (COE) domain (aa position 504-643) were observed throughout this most distal region of the S protein (123, 178).

ORF3 is accessory and a transmembrane protein, which possesses a potassium ion channel function (179). ORF3 gene is one of pathogenicity factors

which ORF3 gene variation have been used in molecular epidemiological studies of PEDV (68, 120, 180).

Because of N protein is highly conserved among PEDV strains and is abundant in virus infected cell at the early stage of infection. N gene is able to use for accuracy diagnosis at early PEDV infection and differentiating close genetic relatedness strains (128, 181, 182).

Therefore, to determine the genetic relationship among the current and past PEDV strains in Thailand compared to the global strains, we characterized the S, ORF3, and N genes and evaluated the deduced amino acid sequence variations in 95 PEDV strains from commercial swine farms throughout the country.

Materials and Methods

Samples

A total of 769 samples from diarrheic pigs were submitted to the Livestock Animal Hospital of Veterinary Science, Chulalongkorn University Faculty of Veterinary Science in Nakhon Pathom province between May 2011 and August 2016. Farm locations and provincial origins were previously described in part 2.

Feces (n=509) and small intestinal content (intestinal mucosa of the duodenum and upper part of jejunum from tissues scraping, n=260) were prepared as 10% (w/v) suspension in sterile phosphate buffered saline (0.1 M, pH 7.2), centrifuged at 3,000 *xg* for 20 minutes, and clarified filtrates collected.

Reverse-transcription polymerase chain reaction (RT-PCR), viral nucleic acid detection and sequencing

A total of 769 viral RNAs were extracted using Ribospin vRD II viral RNA purification Kit (GeneAll, Seoul, Korea) according to manufacturer's instructions. Partial S gene was amplified by RT-PCR using SuperScript III One-Step RT-PCR with Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA). Reverse transcription was performed at 48°C for 45 minutes. PCR cycling parameters were initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 30 second, annealing at 55°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

The PEDV S- positive RNAs (n=153) were subsequently subjected to amplify of ORF3 and N genes by RT-PCR using SuperScript III One-Step RT-PCR with Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA) with annealing temperatures of 51°C and 55°C, respectively (Table 14). Amplicons were purified using agarose gel electrophoresis and subjected to Sanger sequencing. 95 PEDV strains which genome

sequencing were successful were included in this study. Nucleotide sequences obtained from this study were deposited in the GenBank database (Appendix, Table S3).

Table 14. Nucleotide primers used to amplify the PEDV genes.

Primers	Sequences (5' to 3')	Location	Annealing	Amplicon
S gene	TTCTGAGTC ACGAACAGCCA	1466-1485	55°C	651 bp
(157)	CATATGCAGCCTGCTCTGAA	2097-2116		
	CTAAACAGAAACTTTATGGCTT	79-100	55°C	760 bp
N gene	ATGTCTTTGAGGTCACGTTC	907-926		
(DQ355223.1)	CTTCTCAGAACAGAGGAGG	650-668	55°C	848 bp
	GTGTCACCACCATCAACAG	1358-1376		
ORF3 gene	CGAAGCTTTTGAAAAGGTCC	18-37	51°C	755 bp
(GU372734.1)	GGAAAAAGAGTACGAAAAGCC	752-772		

Nucleotide and amino acid sequence analyses

Nucleotide sequences were assembled and edited using SeqMan II and aligned using BioEdit and ClustralX v.2.0.11 (159). Genetic relatedness among the Thai PEDV strains from this study (n=95) was compared to previously identified strains in Thailand, global strains, and PEDV vaccine strains. Phylogenetic trees were constructed using the maximum likelihood method and 1,000 bootstrap replicates implemented in MEGA6 (160). Best model fitting was automatically calculated for genetic distances. Bootstrap values >80% were considered significant. Vaccine strains in which sequences were available for inclusion in all three phylogenetic trees were CV777 (Belgian strain), attenuated DR13 (Korean strain), and 96P4-C6 (Japanese strain). Deduced amino acid sequences of the Thai strains were also compared to those of the vaccine strains and reported as amino acid identity unless otherwise stated. Residue position numbering was based on CV777.

Results

Because of flanking the CO-26K equivalent (COE) domain is one of several epitope regions which had high variation, so their variations may effect on viral neutralization that could be a good referral for PEDV genetic diversity, then the partial S gene primer flanking the CO-26K equivalent (COE) domain was used to screen all those samples for PEDV.

The result present 153 out of 769 samples tested positive for the PEDV S gene and 95 samples yielded sufficient amplification products for further sequence analysis of the partial S, ORF3, and N genes. Mostly PEDV-positive samples were derived from animals aged between 3 days to 8 weeks. A 15-week old pig and two lactating sows were the only three exceptions. PEDV distributions were mostly detected in the central part of Thailand (The province origins of 95 PEDV strains were summarized in Appendix, Table S4).

Analysis of the S gene

Based on genetic analysis, the S sequences clustered into two major groups (G1 and G2) (Figure 22). While the historical CV777 strain was assigned in the G1-1 group, the majority of the Thai PEDV strains in this study (92/95) either clustered with previously identified Thai strains or with some of the common vaccine strains such as KPEDV-9 and attenuated DR13 were assigned in the G1-3 group. Three Thai PEDV strains (NP-68/12, NP-65/14, and RB38/15) were genetically distinct from others and formed a G1-2 cluster with Thai PEDV strains AGPED0609.1, AGPED0609.2 previously identified in 2011.

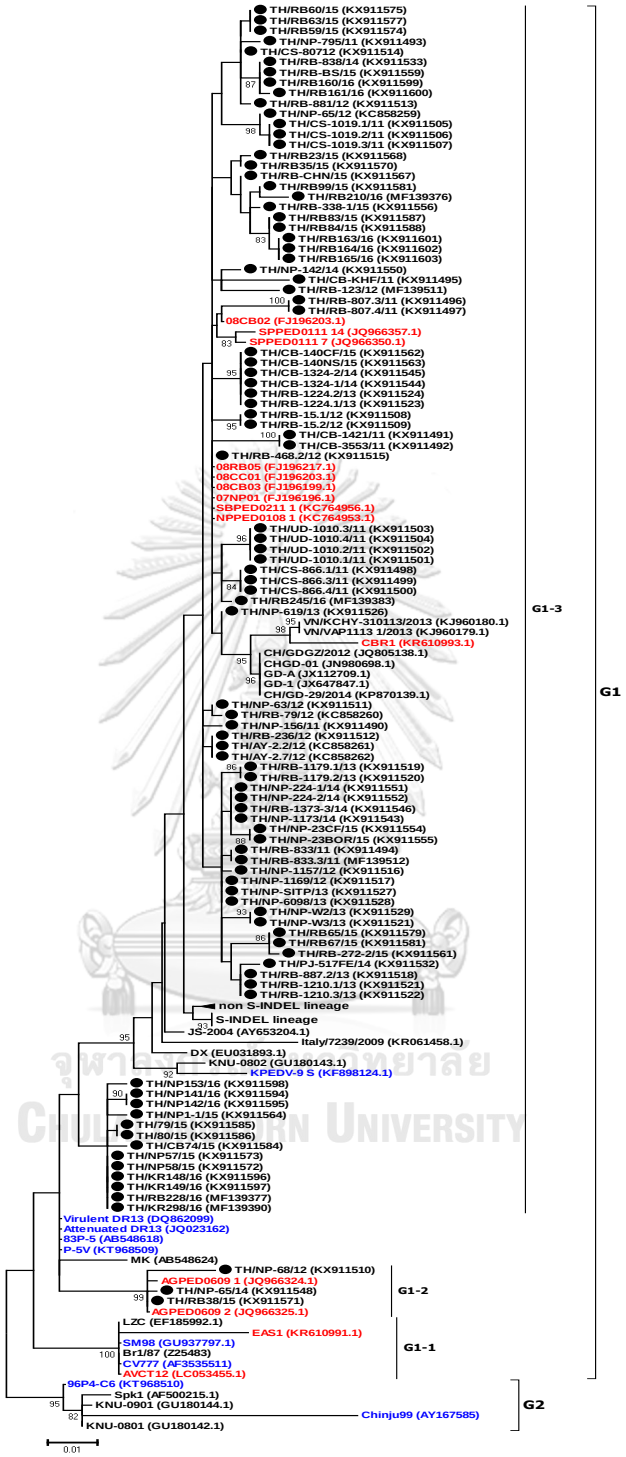


Figure 22. Phylogenetic analysis of the partial S gene.

Previously identified PEDV in Thailand from past years (red) and 95 strains identified in this study (dotted) were compared to the vaccine strains (blue).

The deduced amino acid residues of the S protein showed that Thai PEDV strains consistently shared amino acid sequence identity to the PEDV strains reported on swine farms in Vietnam (92.3-99.4% identity), Japan (93.8-98.7% identity), China (92.8-99.2% identity), Korea (91.9-99.4% identity) and the U.S. (93.6-98.7% identity). Moreover, the Thai strains shared 92.8-96.6% identity to the prototypic strain CV777 and 93.7-98.5% identity to other vaccine strains (Table 15). A Thai PEDV strain designated RB-468.2/12 shared 100% identity to Thai strains from other studies (08RB05, 08CC01, 08CB03, 07NP01, SBPED0211.1 and NPPED0108.1), all of which were previously identified in or before 2011.

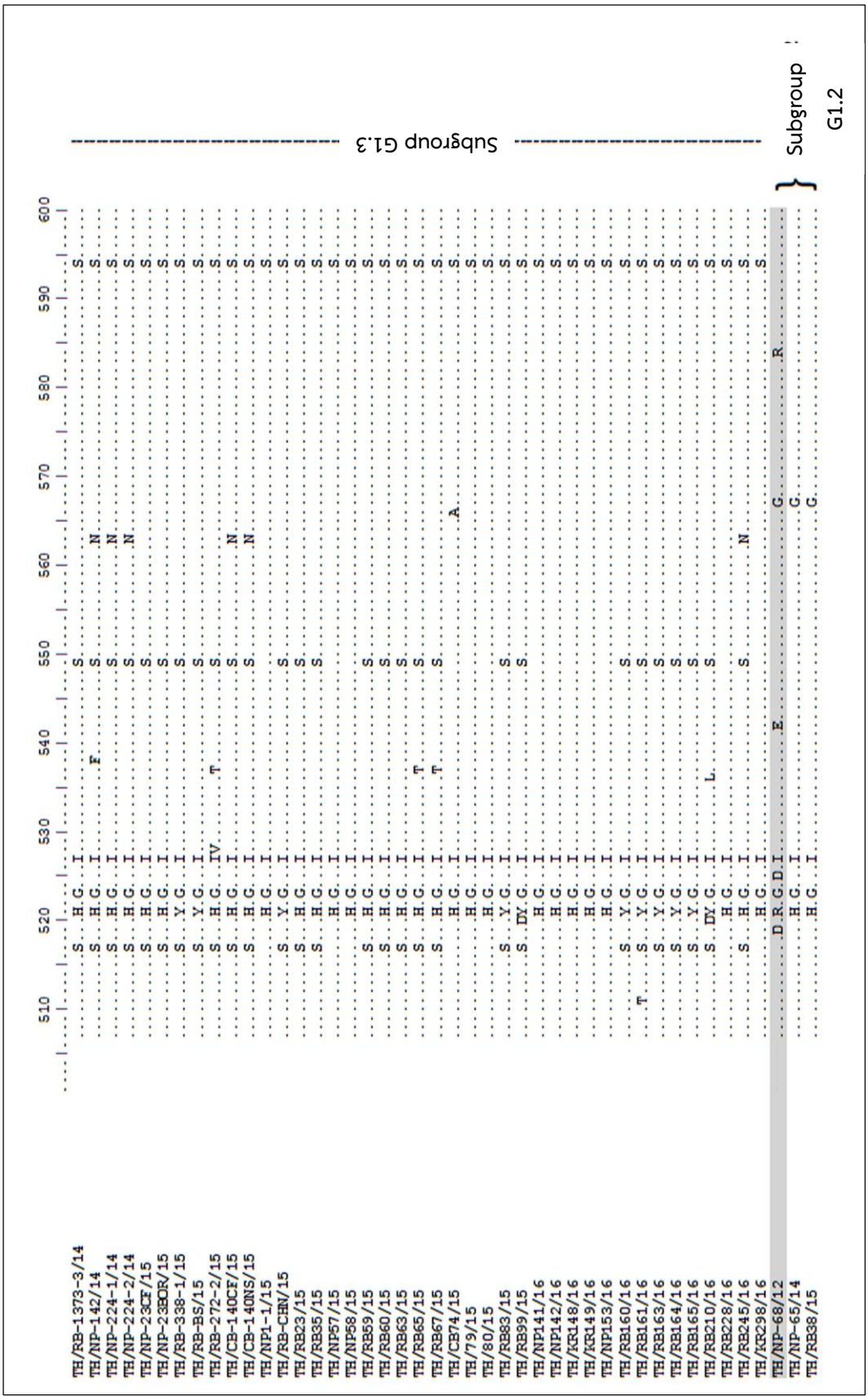
Table 15. Comparison of amino acid identities between the Thai PEDV and the six vaccine strains.

	Amino acid identity of Thai PEDV strains		
Vaccine strains	**S gene (COE domain)**	**ORF3 gene**	**N gene**
CV777	92.8-96.6%	95.8-97%	94.3-95.7%
Attenuated DR13	94.8-98.5%	96.7-98.1%	95.1-97%
83P-5	94.8-98.5%	n/a	95.2-97%
P-5V	94.8-98.5%	96.7-98.1%	n/a
KPEDV-9	93.7-97.2%	n/a	n/a
94P4C6	95.8-97%	95-96.5%	94.5-96.3%

n/a = sequence not available

Alignment of amino acid sequences encompassing positions 507 to 689 revealed that all 95 Thai strains differed from CV777 at residues S523G, V527I, and I635V. Additionally, all 92 Thai strains from the G1-3 group also possessed A517S, L521H, T549S, L612F, and I667F. The three G1-2 Thai strains were primarily characterized by S567G, G594S and M641I, of which NP-68/12 differed most from CV777 at 14 residues over this region (Figure 23).

Subgroup G1.3


Subgroup G1.3
Subgroup G1.2

Subgroup G1.3

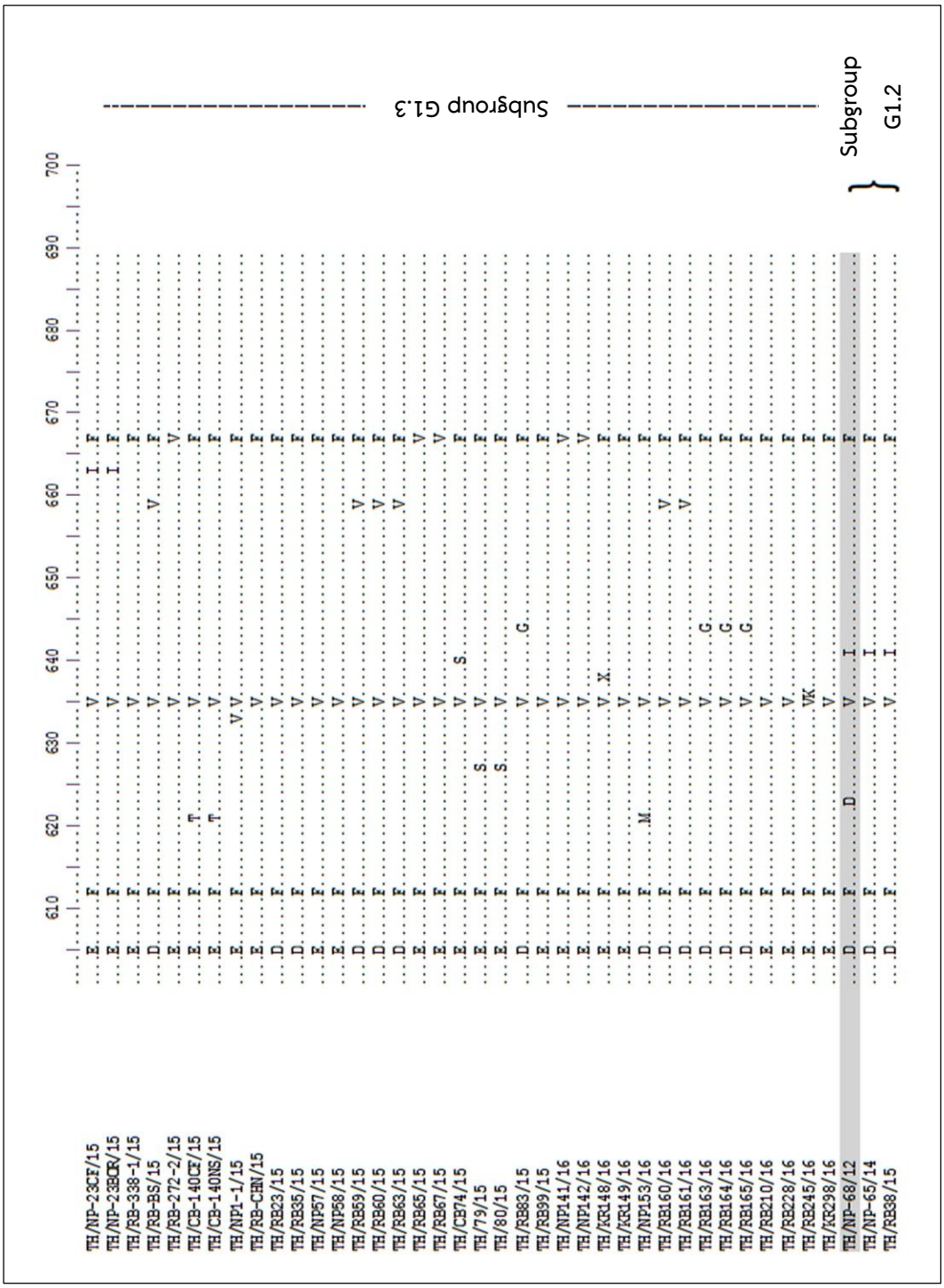


Figure 23. Amino acid sequence alignment of the partial S gene encompassing the COE domain of the Thai PEDV strains and the prototypic CV777.

Numbers indicate residue position. Identical residues are dotted. Strain NP-68/12 differed most from CV777 which showed in highlight.

Analysis of the ORF3 gene

Analysis of the entire ORF3 gene showed that it was phylogenetically divided into two groups. A number of the Thai PEDV strains (12/95) belonged to the G1 group with CV777 and the attenuated DR13 strain (Figure 24). The majority of the Thai strains (83/95) were more closely related to the previous Thai strains compared to the vaccine strains and were grouped in G2. This group also contained strains with S-INDEL. Deduced amino acid sequences showed that the Thai PEDV strains shared 94.4%-100% amino acid identity with each other, 95.8%-97% identity to CV777, and 95%-98.1% identity to the other vaccine strains. In particular, Thai G1 strains in this study shared 99.7%-99.8% identity to a Chinese YC/2013 strain from 2013, and 98.8-99.1% identity to a previous Thai strain CBR1 identified in 2014. Meanwhile, the Thai G2 strains in this study shared 95.8%-100% identity to previous Thai strains (identified between 2008- 2012). All Thai strains were similar to CV777 in their absence of deletions of residues 82-98 and 138-139 in the ORF3 protein compared to other vaccine strains (Table 16).

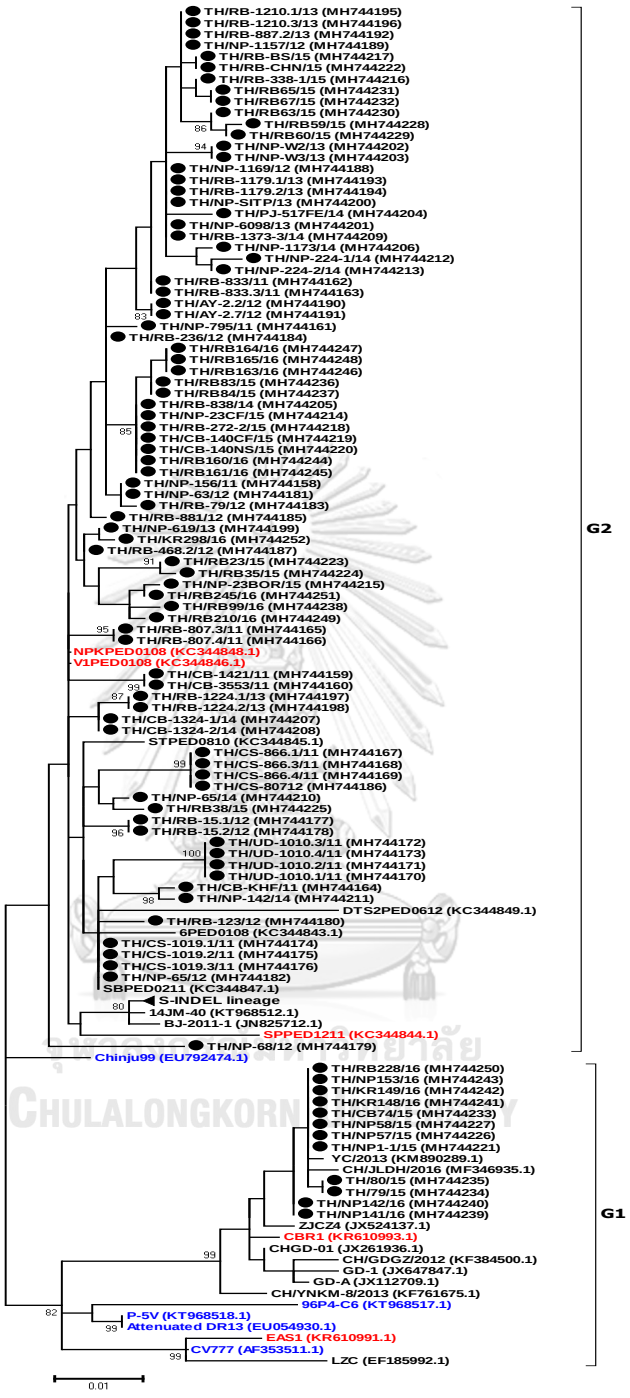


Figure 24. Phylogenetic analysis of the complete ORF3 gene.

Previously identified PEDV in Thailand from past years (red) and 95 strains identified in this study (dotted) were compared to the vaccine strains (blue).

Table 16. Residue differences within the ORF3 of the Thai PEDV, CV777, and other vaccine strains.

	Position of amino acid point mutations			
Strain	**82-98**	**138-139**	**165**	**182**
Thai PEDV strains	YCPLLYYCGA**F**LDATIIC	YY	V	Q/H
CV777	YCPLLYYCGALLDATIIC	YY	S	H
Vaccine strains*	deletion	deletion	F	H

*vaccine strains = attenuated DR13, 96P4-C6, P-5V

Analysis of the N gene

While CV777 and other vaccine strains clustered within the G1 group, all Thai PEDV strains in this study appeared to have diverged and branched off as a separate group (Figure 25). Although all Thai strains in this study shared 96-100% amino acid identity, the Thai strains belonging to G3-1 (83/95) are genetically close to the reference strains previously identified in the U.S. (OH851) and China (CH/ZMDZY/11) (97.1%-99.7% amino acid identity). In the G3-2 group, the Thai strains TH/NP1-1/15 and TH/CB74/15 showed identical nucleotide sequences to the Vietnamese strain CT3. Next, we analyzed the deduced amino acid sequences of the Thai strains, which spanned residues 13 to 406 (out of 441 residues) of the N protein. Sequence alignment with CV777 showed that eight Thai strains possessed several residue deletions in the middle of the N protein encompassing positions 241 to 251 in addition to several residue differences (Figure 26). Four Thai strains (TH/UD-1010.1/11, TH/UD-1010.2/11, TH/UD-1010.3/11, and TH/UD-1010.4/11) were missing residues R241 and H242. Three Thai strains (TH/RB160/16, TH/RB161/16, and TH/RB-838/14) were missing residues K243 and Q244. Finally, residue E251 was absent in TH/NP-68/12.

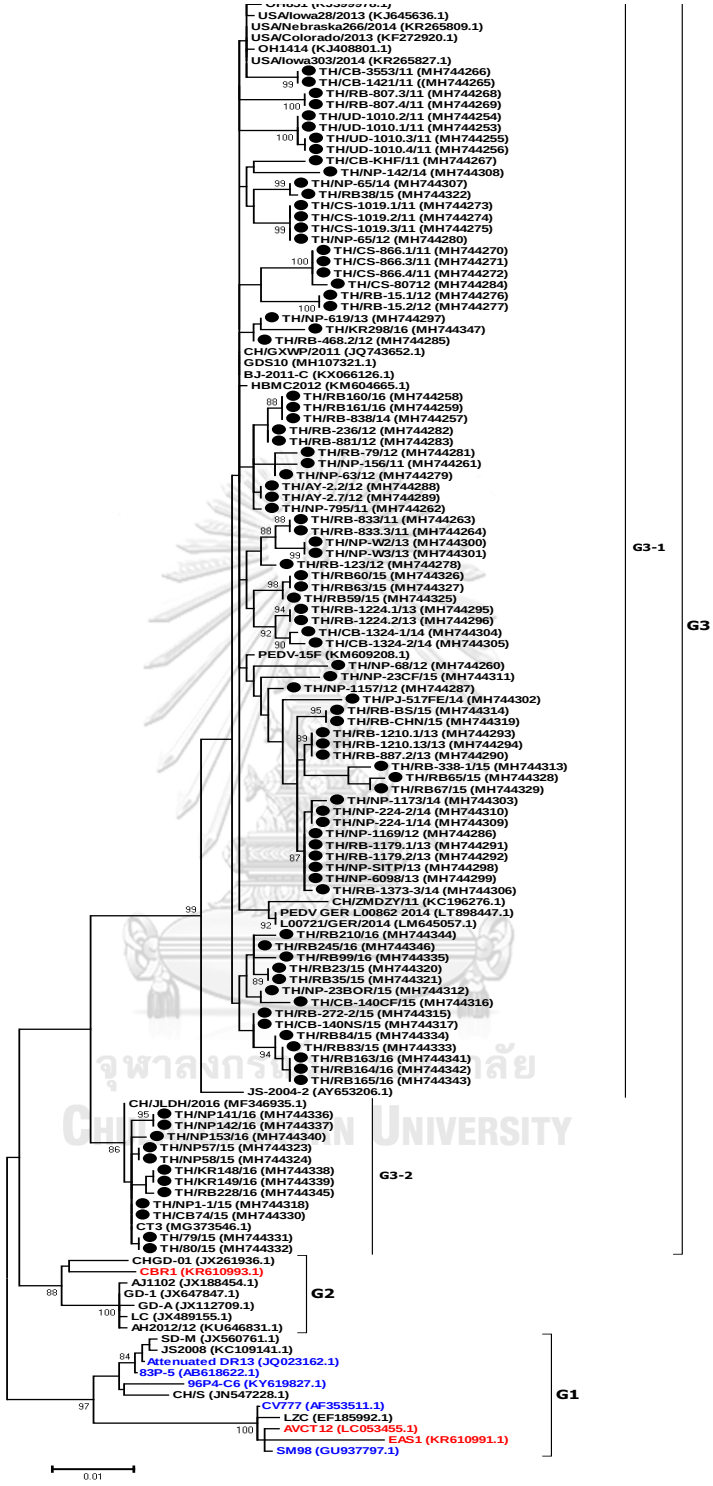


Figure 25. Phylogenetic analysis of the partial N gene.

Previously identified PEDV in Thailand from past years (red) and 95 strains identified in this study (dotted) were compared to the vaccine strains (blue).

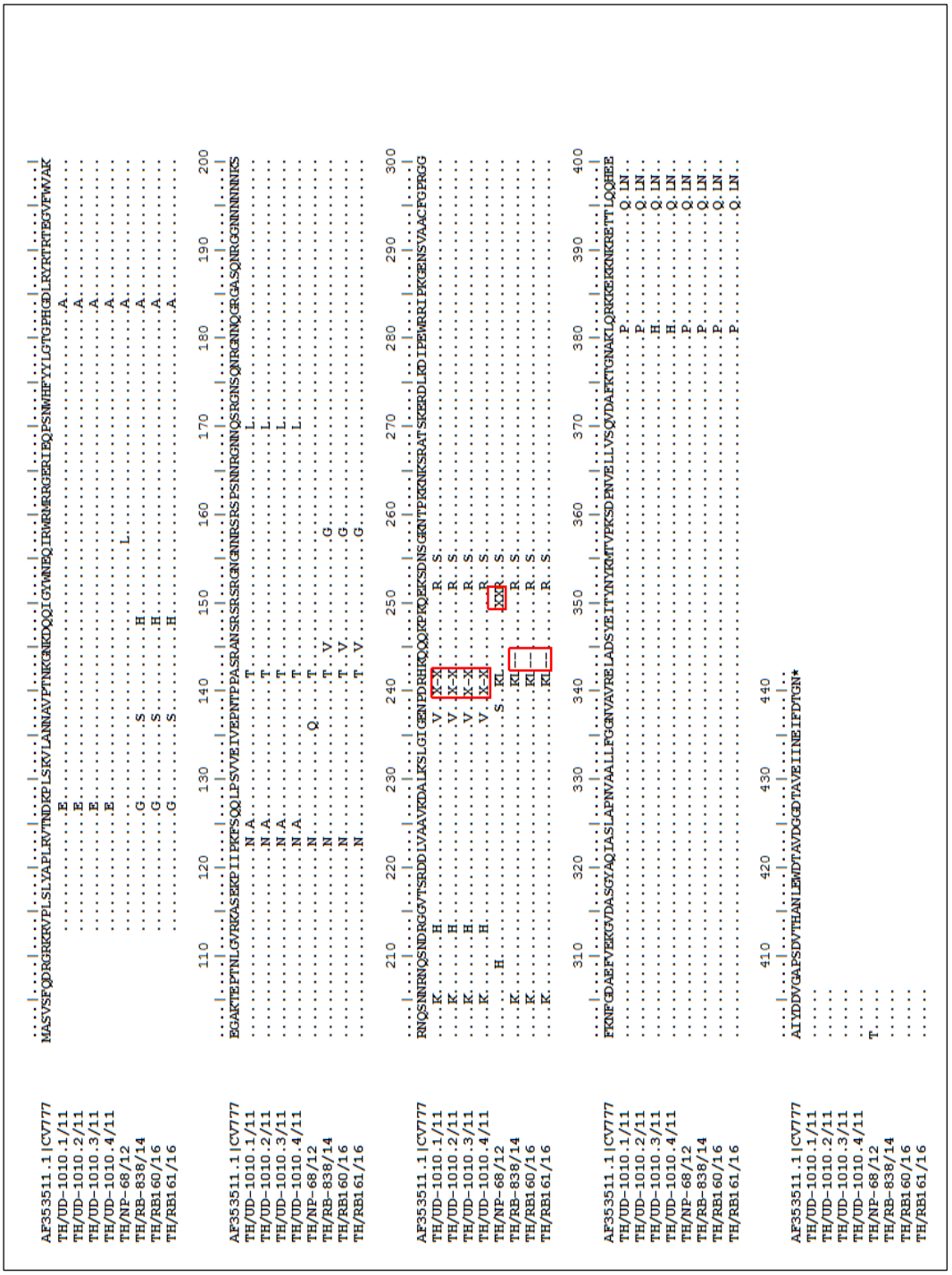


Figure 26. Amino acid sequence alignment of the partial N gene from Thai PEDV strains with deletions.

Numbers indicate residue position. Identical residues are dotted. Deletions are noted with dashed lines covered by the boxes.

CHAPTER IV
DISCUSSION AND CONCLUSSION

The viral etiology of diarrhea in pigs is not practically investigated on Thai pig farms, which often contributes to the failure to prevent and contain disease transmission among herds. It is believed that animals co-infected with more than one enteric viruses experienced increased intestinal epithelium damage and/or viral replication, which result in more severe diarrhea. For over five consecutive years from 2011 to 2016, this study demonstrated rotavirus from symptomatic piglets and pigs in low occurrence rate around 6.6% for RVC and 9.5% for RVA, while 19.9% of the samples were positive for PEDV.

RVC infections were previously based on detection with a lower prevalence rate than RVA in symptomatic piglets with diarrhea (28, 81, 183, 184). Meanwhile, most epidemiological study of porcine rotavirus prevalence in Thailand involved only RVA (approximately 10-23%), whereas porcine RVC was limited (90, 93, 129, 130). Otherwise, previous studies of PEDV often reported on genetic characterization of emerging strains than providing the occurrence rate within Thai swine herds (133, 134, 174).

In the case of mixed infection among rotavirus and PEDV have been found often, the intestinal epithelium damage and/or viral replication were increased, resulting in more severe diarrhea (31, 185, 186). In this study, we found cases of co-infections between PEDV and rotavirus in younger piglets (<4 weeks old), which often showed a higher morbidity rate. Likewise, several previous studies reported that younger piglets showed higher morbidity and mortality than older pigs (34, 170, 187). It was possibly that PEDV infection could contribute to the rapid recovery of enterocytes and have an influence in rapid disease recovery (188).

Rotavirus infections appeared most frequently in pigs older than 4-8 weeks old, while samples from pigs younger than 4 weeks were increased for PEDV positivity. Samples from older pigs (> 12 weeks) and sows were susceptibility to PEDV infections but rotavirus positivity was declined. Nevertheless, there were instances of co-infection with PEDV and RVA in sows even though they are usually asymptomatic in either diseases. The possibility of multiple enteric viruses circulating and persisting within swine herds might be increased from vertical transmission.

For seasonal factor, most of the previous studies indicated the rotavirus infection was frequently found in the winter season. Thailand is located in the tropics, with warm weather all year round enhancing rotavirus could be detected throughout the year in this study. Nevertheless, there are several studies suggested rotavirus infection is less seasonally influenced in the tropical zone, because the relatively high humidity may facilitate increased rotavirus infection (189-191).

The genetic diversity among Thai RV and PEDV strains were also determined. For RVC, strain RVC/Pig/THA/CU146C/16/G6P[x] separated out of the G6 genotype clusters and also shared lower nucleotide identity among Thai strains (82.2-84.4%). The amino acid sequence of RVC/THA/CU146C/16/G6P[x] lacked 4 amino acid residues between positions 245 and 246, this finding is in concordance with an Irish RVC strain (strain 1GA/05/Cork) in a 2008 study (28). Apart from strain RVC/Pig/THA/CU146C/16/G6P[x], we found the G6 genotype has four amino acid additions at the carboxy-terminus of the variable region VR8 (between residues 245 and 248), these findings were related to several strains from Italy (strain 344/04-7, 43/06-16, 43/06-22 and 134/04-2), the Czech Republic (strain CZE/P8/2011) and Japan (strain CJ3-6) (29, 31, 78). However, there are no reports linking this substitution with any disease severity.

The N protein is abundantly expressed during the early stages of PEDV infection and is therefore a preferred target for PEDV detection. Sequence variations

on the N gene could also be used to evaluate PEDV genetic diversity and relationship among circulating PEDV strains. Our phylogenetic analysis of the near-complete N gene showed that the Thai strains genetically grouped with the recent PEDV strains from the U.S. and China.

S protein encompassed the CO-26K equivalent (COE) domain (amino acid residue 507 to 689) of Thai PEDVs had the most sequence variation. As this region is subjected to the immune pressure from the host, sequence variations were observed throughout this most distal region of the S protein in PEDV (123, 178). The analysis of the S sequence from strains in the U.S. between 2016 and 2017 has identified 3 new emergent strains (termed S1 NTD-del PEDV variants). These variants possessed several large deletions within S1 N-terminal domain and differed from the original U.S. S INDEL (176). Nevertheless, high variation region of the antigenic epitope (COE domain) of Thai strains could not present deleted region but the different period of sampling (2011-2016) may present the trend of PEDV genotype diversities. Most of the Thai strains in this study belonged to the high virulent G2a lineage. Several Thai strains (23/95), especially those from 2013 to 2015, were genetically related to the new variant G2b strains (Chinese-like strains and U.S.-like strains). Overall, Asian high virulent strains seem to be more dominant genotype in this study.

Furthermore, 8 Thai PEDVs possess amino acid deletions within N gene sequences which to our knowledge has not been previously reported. These deletions involved residues located within the immunodominant region of the N protein between residues 136 and 289 previously shown to be associated with NF-kB activation pathway (65). These missing residues encompassing position 241 to 251 have not been previously characterized in other PEDV or alphacoronaviruses even though the N gene has the greatest sequence similarities to TGEV and human coronaviruses (HCV 229E and HCV NL63) (192, 193). Therefore, residue deletions in

this region may indicate an evolving flexible domain with no major functional significance.

For RVA, we identified the genetic diversity of circulating RVA and atypical genotypes in some segments which might refer to inter-species reassortants. Although several Thai strains in this study resembled the genome constellation of the prototypic Gottfried (Gx-P[x]-I1-R1-C1-M1-A8-N1-T1-E1-H1), the phylogenetic analysis of the nucleotide sequences showed notable strain differences. For example, a Thai RVA strain (CU-L141/2012/G4P[6]) with human-like G genotype of constellation **G4-P[6]-I1**-R1-C1-M1-A8-N1-T1-E1-H1 was identified from a two-week-old piglet. The variants of constellation **G4-P[6]**-I5-R1-C1-M1-A8-N1-T1-E1-H1 (strain CULC-1/2013/G4P[6]), **G4-P[19]**-I5-R1-C1-M1-A8-N1-**T7**-E1-H1 (strain CU236-2/2012/G9P[19]), G9-P[13]-I5-R1-C1-M1-A8-N1-**T7**-E1-H1 (strain CU192/2016/G9P[13]), and G9-P[19]-I5-R1-C1-M1-A8-N1-**T7-E9**-H1 (strain CU143/2016/G9P[19]) strongly suggest RVA genome reassortment during RVA co-infection and inter-species transmission. Typically, genotypes G5, I5, A1/A8, T1, and E1 are identified with porcine RVA, while G4, I1, T7 and E9 genotypes are more rare (1, 51). Interestingly, only one Thai strain in this study was G5, and genotype A1 was absent among our strains. Meanwhile, the G9P[19] and G9[P23] combination identified in this study were previously described in Thai children with diarrhea and on swine farms in northern Thailand (2009) and reportedly possessed identical genome constellations consistent with possible zoonotic transmission (41, 42). However, previously reported presented genotype P[19] in combination with G5, and G9 were associated with porcine infection, while in combination with G1 and G3 is typically associated with human (26, 38, 42, 93, 144).

According to pigs are suspected reservoir for RVA transmission to human, as close contact between farm animals and pig handlers enable atypical RVA reassortants to emerge (35, 36, 194). The surveillance of porcine RVA has important

implications for human health. Previous studies have concluded that some human RVA strains were likely of porcine origin, for example strains Mc323/1989/G9P[19] and Mc345/1989/G9P[19] isolated in 1989, CMH-S070-13/2013/G9P[19] or KKL117/2014/G9P[23] (26, 41, 42). All strains have historically derived from Thai patients and also had genetic relatedness in the majority of the non-G/P genotypes to the Thai porcine RVA strains in this study (>90% amino acid identity).

Furthermore, the antigenic epitope regions provided important clues to the potential vaccine effectiveness, in which the variations on these region may affect viral neutralization and vaccine escapes. Therefore, the characterizing amino acid differences in the antigenic epitope regions were also observed. For RVA vaccine, the licensed porcine RVA vaccine such as the ProSystems ROTA for young piglet is not widely applicable on Thai swine farms (Merck, Whitehouse Station, NJ). Our study found that G3, G4, G5 and G9 genotypes possessed limited amino acid differences among the Thai and the vaccine strains. The amino acid identity of the Thai G4 strains was 80.8-82.8% compared to Gottfried, which was less than between the Thai G9 strains and A2 (82.4-93.5%). Although VP7 antigenic epitopes are critical to induce neutralizing antibody against rotavirus infection, antigenic epitopes on VP4 protein is also involved (195). Characterization of the latter among the porcine Thai and the vaccine strains were not included because there were no P[7] in this study and the two P[6] strains were too few to make any comparison meaningful.

PEDV vaccine, we analyzed only S protein residue 507 to 689, which this region referred to as the CO-26K equivalent (COE) domain is one of several PEDV neutralizing epitopes (123, 178). Thus, variations on the epitope region seen in circulating PEDV strains may affect viral neutralization and vaccine escapes. Our observation that the vaccine strains are phylogenetically distant from the majority of the circulating PEDV strains might reflects the relatively low vaccine efficacy afforded

by some of the current vaccine strains, of which CV777, 83P-5, SM98 and DR13 have been used in attenuated vaccines in Asia (128, 145).

There is no available RVC vaccine in this present, thus the sequence identity among field strains and prototype strains were determined instead. The genetic relationship among those strains were quite low, such as the Cowden strain was between 59.6 and 66%, for the human strain Bristol was 52.2-62.7% and for bovine strain Shintoku was 59.5-66.1%.

After all, none of the pigs in which our samples were derived were prior vaccinated for RVA or PEDV, thus the genotypes identified in this study were naturally circulating and did not result from vaccine-escaped variants. Nevertheless, the analysis of the residue changes within antigenic epitopes of Thai circulating strains alone may not accurately predict potential vaccine escapes but the other epitope region variations, serological assays and/or animal experiment study should be further conducted.

In fact, pig farmers often feed pregnant sows with either minced intestinal content from infected piglets or diluted sow feces as a low-cost way of eliciting immunity against porcine enteric disease virus infections within the Thai pig herds. This practice may inadvertently foster periodic outbreaks of both rotavirus and PEDV, which causes recurrent infection within the same herd and perpetuates its circulation on pig farms. Taken together, the common practice of restocking new susceptible pigs with improper gilt acclimatization, insufficient colostrum intake in newborn piglets, insufficient immunization of pregnant sow or keeping of older pigs with asymptomatic infection may serve as a reservoir and spread the infection subclinically. Vertical transmission is also possible as PEDV RNA has been detected in the milk of infected lactating sows (181, 196). Moreover, this observation is consistent with the potential transmission of field strains across farms due to animal transportation and trade throughout the region of commercial pig farms. There were

several Thai PEDV and rotavirus strains had identical amino acid sequences or belonging to the same subtypes, although those strains derived from pigs of different herds, regions, ages, and year of the collection.

This study has some limitations. Samples in our study were primarily submitted from pigs in central Thailand where the majority of pig farms are located. Therefore surveillance of farms in other parts of the country was incomplete. Moreover, the analysis could have revealed additional diversity of strains circulating in Thailand because PEDV strains with missing S, ORF3 or N gene sequences did not include in this analysis. Likewise, the identification of additional genome constellations of RVA may have been missed as only 24 strains were analyzed in this study.

Due to the fact that we were not able to sequence all the VP4 gene of RVC (from a total of 47 RVC strains), there were probably issues such as high variability in the region that we used for amplification (VP8 segment, aa positions 1-231) or RNA degradation from long-term sample storage (158). P[5] genotype was the predominant genotype because the P[5] strains were collected from the same herd and the same period, it was probably those strains that had high genetic similarity rather than other genotypes such as P[4] or P[7]. Nevertheless, our multi-year study provides additional knowledge regarding the diversity of PEDV and rotavirus in this region, which is essential for further vaccine development from suitable field strains.

Conclusion

The finding provide information about rotavirus and PEDV surveillance and molecular characteristics based on several gene segments that might be useful for a better understanding of the re-occurring, genetic variation among strains, or of the possibility of interspecies transmission for RVA. As a results, most of field Thai RVA, RVC and PEDV strains had close genetic relationship and resembled previous reports

from Thailand and Asian countries. This finding was suggestive that those Thai variants have evolved from the same ancestors and remain circulating within Asian countries. The vaccination prone to be effective tool for disease prevention and control in commercial pig herd, even though the circulating strains differed significantly from the classical vaccine strains in both RVA and PEDV. Therefore, the continuous investigations of this study provide important insight into regional rotavirus and PEDV strains in circulation, which may assist inclusions of suitable strains for future vaccine development to reduce the severity of the economic loss in the swine industry.

APPENDIX

Table S1. Accession number of 47 RVC strains.

Strain name	Accession number	
	VP7	VP4
RVC/Pig-wt/THA/CU-PY/12/G3P[x]	KX911693	-
RVC/Pig-wt/THA/CU571/13/G6[x]	KX911694	-
RVC/Pig-wt/THA/CU264-U12/13/G9P[7]	KX911704	MG575531
RVC/Pig-wt/THA/CU875-1C/14/G1[x]	KX911676	-
RVC/Pig-wt/THA/CU1035/14/G1[x]	KX911667	-
RVC/Pig-wt/THA/CU781-2/14/G1[x]	KX911668	-
RVC/Pig-wt/THA/CUSB-N/15/G1[x]	KX911669	-
RVC/Pig-wt/THA/CU-SUN/15/G9[x]	KX911708	-
RVC/Pig-wt/THA/CU-CHN/15/G1[x]	KX911670	-
RVC/Pig-wt/THA/CU5-3/15/G1[x]	KX911671	-
RVC/Pig-wt/THA/CU14/15/G1[x]	KX911672	-
RVC/Pig-wt/THA/CU48/15/G1P[4]	KX911673	MG575524
RVC/Pig-wt/THA/CU60/15/G1P[5]	KX911674	MG575525
RVC/Pig-wt/THA/CU200/16/G1P[1]	KX911675	MG575522
RVC/Pig-wt/THA/CU875-1C/14/G1[x]	KX911676	-
RVC/Pig-wt/THA/CU-BDN-C/15/G1[x]	KX911677	-
RVC/Pig-wt/THA/CU4-6C/15/G1[x]	KX911678	-
RVC/Pig-wt/THA/CU5-1C/15/G1[x]	KX911679	-
RVC/Pig-wt/THA/CU62C/15/G1[x]	KX911680	-
RVC/Pig-wt/THA/CU68C/15/G1[x]	KX911681	-
RVC/Pig-wt/THA/CU69C/15/G1[x]	KX911682	-
RVC/Pig-wt/THA/CU74C/15/G1[x]	KX911683	-
RVC/Pig-wt/THA/CU79C/15/G1[x]	KX911684	-
RVC/Pig-wt/THA/CU99C/16/G1[x]	KX911685	-
RVC/Pig-wt/THA/CU100C/16/G1[x]	KX911686	-
RVC/Pig-wt/THA/CU108C/16/G1[x]	KX911687	-
RVC/Pig-wt/THA/CU109C/16/G1[x]	KX911688	-
RVC/Pig-wt/THA/CU111C/16/G1[x]	KX911689	-

**continued*

Strain name	Accession number	
	VP7	VP4
RVC/Pig-wt/THA/CU150C/16/G1[x]	KX911690	-
RVC/Pig-wt/THA/CU201C/16/G1[x]	KX911691	-
RVC/Pig-wt/THA/CU202/16/G1[x]	KX911692	-
RVC/Pig-wt/THA/CU-PY/12/G3[x]	KX911693	-
RVC/Pig-wt/THA/CU571/13/G6[x]	KX911694	-
RVC/Pig/THA/CU115C/15/G1P[x]	MF139516	-
RVC/Pig/THA/CU330C/16/G1P[x]	MF139509	-
RVC/Pig-wt/THA/CU54/15/G6[x]	KX911695	-
RVC/Pig-wt/THA/CU123/16/G6P[5]	KX911696	MG575527
RVC/Pig-wt/THA/CU124/16/G6P[5]	KX911697	MG575528
RVC/Pig-wt/THA/CU122/16/G6P[5]	KX911698	MG575526
RVC/Pig-wt/THA/CU125/16/G6P[5]	KX911699	MG575529
RVC/Pig-wt/THA/CU135/16/G6P[5]	KX911700	MG575530
RVC/Pig-wt/THA/CU136/16/G6[x]	KX911701	-
RVC/Pig-wt/THA/CU12/15/G6[x]	KX911702	-
RVC/Pig-wt/THA/CU49/15/G9[x]	KX911703	-
RVC/Pig-wt/THA/CU40/15/G9P[4]	KX911705	MG575523
RVC/Pig-wt/THA/CU84/15/G9P[7]	KX911706	MG575532
RVC/Pig-wt/THA/CU13/15/G9[x]	KX911707	-
RVC/Pig-wt/THA/CU-SUN/15/G9[x]	KX911708	-

Table S2. Accession number of 24 RVA strains.

Strain name	Accession number		
	VP7	VP4	VP6
RVA/Pig-wt/THA/CU795/2011/G9P[13]	KX911645	MF139403	MF139447
RVA/Pig-wt/THA/CU68/2012/G3P[13]	KX911617	MF139406	MF139448
RVA/Pig-wt/THA/CU-L141/2012/G4P[6]	KX911632	MF139401	MF139446
RVA/Pig-wt/THA/CU236-2/2012/G9P[19]	KX911638	MF139434	MF139449
RVA/Pig-wt/THA/CUSB1-3/2012/G3P[13]	KX911614	MF139405	MF139451
RVA/Pig-wt/THA/CU729-3/2013/G4P[19]	KX911627	MF139430	MF139452
RVA/Pig-wt/THA/CULC-1/2013/G4P[6]	KX911631	MF139402	MF139453
RVA/Pig-wt/THA/CU140-NS/2015/G9P[23]	KX911655	MF139440	MF139457
RVA/Pig-wt/THA/CU9-1/2015/G9P[23]	KX911656	MF139439	MF139463
RVA/Pig-wt/THA/CU9-2/2015/G9P[23]	KX911657	MF139441	MF139464
RVA/Pig-wt/THA/CU37/2015/G9P[23]	KX911652	MF139442	MF139465
RVA/Pig-wt/THA/CU40/2015/G9P[13]	KX911658	MF139415	MF139466
RVA/Pig-wt/THA/CU49/2015/G9P[13]	MF139495	MF139410	MF139467
RVA/Pig-wt/THA/CU101/2016/G9P[23]	KX911661	MF139443	MF139469
RVA/Pig-wt/THA/CU140/2016/G9P[13]	KX911619	MF139418	MF139474
RVA/Pig-wt/THA/CU143/2016/G9P[19]	MF139496	MF139436	MF139475
RVA/Pig-wt/THA/CU145/2016/G3P[13]	MF139491	MF139419	MF139476
RVA/Pig-wt/THA/CU232/2016/G9P[13]	MF139500	MF139427	MF139478
RVA/Pig-wt/THA/CU192/2016/G9P[13]	MF139499	MF139425	MF139477
RVA/Pig-wt/THA/CU316/2016/G9P[23]	MF139506	MF139445	MF139481
RVA/Pig-wt/THA/CU280-2/2016/G9P[19]	MF139503	MH428380	MF139487
RVA/Pig-wt/THA/CU200/2016/G3P[13]	KX911622	MF139426	MH428382
RVA/Pig-wt/THA/CU176/2016/G9P[13]	KX911647	MF139422	MF139473
RVA/Pig-wt/THA/CU181/2016/G5P[13]	KX911633	MF139423	MH428381

**continued*

Strain name	Accession number			
	VP1	VP2	VP3	NSP1
RVA/Pig-wt/THA/CU795/2011/G9P[13]	MH428404	MH428383	MH428428	MH428452
RVA/Pig-wt/THA/CU68/2012/G3P[13]	MH428405	MH428384	MH428429	MH428453
RVA/Pig-wt/THA/CU-L141/2012/G4P[6]	MH428406	MH428385	MH428430	MH428454
RVA/Pig-wt/THA/CU236-2/2012/G9P[19]	MH428407	MH428386	MH428431	MH428455
RVA/Pig-wt/THA/CUSB1-3/2012/G3P[13]	MH428408	MH428387	MH428432	MH428456
RVA/Pig-wt/THA/CU729-3/2013/G4P[19]	MH428409	MH428388	MH428433	MH428457
RVA/Pig-wt/THA/CULC-1/2013/G4P[6]	MH428410	MH428389	MH428434	MH428458
RVA/Pig-wt/THA/CU140-NS/2015/G9P[23]	MH428411	MH428390	MH428435	MH428459
RVA/Pig-wt/THA/CU9-1/2015/G9P[23]	MH428412	MH428391	MH428436	MH428460
RVA/Pig-wt/THA/CU9-2/2015/G9P[23]	MH428413	MH428392	MH428437	MH428461
RVA/Pig-wt/THA/CU37/2015/G9P[23]	MH428414	MH428393	MH428438	MH428462
RVA/Pig-wt/THA/CU40/2015/G9P[13]	MH428415	-	MH428439	MH428463
RVA/Pig-wt/THA/CU49/2015/G9P[13]	MH428416	-	MH428440	MH428464
RVA/Pig-wt/THA/CU101/2016/G9P[23]	MH428417	MH428394	MH428441	MH428465
RVA/Pig-wt/THA/CU140/2016/G9P[13]	MH428418	MH428395	MH428442	MH428466
RVA/Pig-wt/THA/CU143/2016/G9P[19]	MH428419	MH428396	MH428443	MH428467
RVA/Pig-wt/THA/CU145/2016/G3P[13]	MH428420	MH428397	MH428444	MH428468
RVA/Pig-wt/THA/CU232/2016/G9P[13]	MH428421	MH428398	MH428445	MH428469
RVA/Pig-wt/THA/CU192/2016/G9P[13]	MH428422	MH428399	MH428446	MH428470
RVA/Pig-wt/THA/CU316/2016/G9P[23]	MH428423	MH428400	MH428447	MH428471
RVA/Pig-wt/THA/CU280-2/2016/G9P[19]	MH428424	MH428401	MH428448	MH428472
RVA/Pig-wt/THA/CU200/2016/G3P[13]	MH428425	MH428402	MH428449	MH428473
RVA/Pig-wt/THA/CU176/2016/G9P[13]	MH428426	MH428403	MH428450	MH428474
RVA/Pig-wt/THA/CU181/2016/G5P[13]	MH428427	-	MH428451	MH428475

**continued*

Strain name	Accession number			
	NSP2	NSP3	NSP4	NSP5
RVA/Pig-wt/THA/CU795/2011/G9P[13]	MH428476	MH428500	MH428524	MH428548
RVA/Pig-wt/THA/CU68/2012/G3P[13]	MH428477	MH428501	MH428525	MH428549
RVA/Pig-wt/THA/CU-L141/2012/G4P[6]	MH428478	MH428502	MH428526	MH428550
RVA/Pig-wt/THA/CU236-2/2012/G9P[19]	MH428479	MH428503	MH428527	MH428551
RVA/Pig-wt/THA/CUSB1-3/2012/G3P[13]	MH428480	MH428504	MH428528	MH428552
RVA/Pig-wt/THA/CU729-3/2013/G4P[19]	MH428481	MH428505	MH428529	MH428553
RVA/Pig-wt/THA/CULC-1/2013/G4P[6]	MH428482	MH428506	MH428530	MH428554
RVA/Pig-wt/THA/CU140-NS/2015/G9P[23]	MH428483	MH428507	MH428531	MH428555
RVA/Pig-wt/THA/CU9-1/2015/G9P[23]	MH428484	MH428508	MH428532	MH428556
RVA/Pig-wt/THA/CU9-2/2015/G9P[23]	MH428485	MH428509	MH428533	MH428557
RVA/Pig-wt/THA/CU37/2015/G9P[23]	MH428486	MH428510	MH428534	MH428558
RVA/Pig-wt/THA/CU40/2015/G9P[13]	MH428487	MH428511	MH428535	MH428559
RVA/Pig-wt/THA/CU49/2015/G9P[13]	MH428488	MH428512	MH428536	MH428560
RVA/Pig-wt/THA/CU101/2016/G9P[23]	MH428489	MH428513	MH428537	MH428561
RVA/Pig-wt/THA/CU140/2016/G9P[13]	MH428490	MH428514	MH428538	MH428562
RVA/Pig-wt/THA/CU143/2016/G9P[19]	MH428491	MH428515	MH428539	MH428563
RVA/Pig-wt/THA/CU145/2016/G3P[13]	MH428492	MH428516	MH428540	MH428564
RVA/Pig-wt/THA/CU232/2016/G9P[13]	MH428493	MH428517	MH428541	MH428565
RVA/Pig-wt/THA/CU192/2016/G9P[13]	MH428494	MH428518	MH428542	MH428566
RVA/Pig-wt/THA/CU316/2016/G9P[23]	MH428495	MH428519	MH428543	MH428567
RVA/Pig-wt/THA/CU280-2/2016/G9P[19]	MH428496	MH428520	MH428544	MH428568
RVA/Pig-wt/THA/CU200/2016/G3P[13]	MH428497	MH428521	MH428545	MH428569
RVA/Pig-wt/THA/CU176/2016/G9P[13]	MH428498	MH428522	MH428546	MH428570
RVA/Pig-wt/THA/CU181/2016/G5P[13]	MH428499	MH428523	MH428547	MH428571

Table S3. Accession number of 95 PEDV strains.

Strain name	Accession number		
	S	ORF3	N
TH/NP-156/11	KX911490	MH744158	MH744261
TH/CB-1421/11	KX911491	MH744159	MH744262
TH/CB-3553/11	KX911492	MH744160	MH744263
TH/NP-795/11	KX911493	MH744161	MH744264
TH/RB-833/11	KX911494	MH744162	MH744265
TH/RB-833.3/11	MF139512	MH744163	MH744266
TH/CB-KHF/11	KX911495	MH744164	MH744267
TH/RB-807.3/11	KX911496	MH744165	MH744268
TH/RB-807.4/11	KX911497	MH744166	MH744269
TH/CS-866.1/11	KX911498	MH744167	MH744270
TH/CS-866.3/11	KX911499	MH744168	MH744271
TH/CS-866.4/11	KX911500	MH744169	MH744272
TH/UD-1010.1/11	KX911501	MH744170	MH744253
TH/UD-1010.2/11	KX911502	MH744171	MH744254
TH/UD-1010.3/11	KX911503	MH744172	MH744255
TH/UD-1010.4/11	KX911504	MH744173	MH744256
TH/CS-1019.1/11	KX911505	MH744174	MH744273
TH/CS-1019.2/11	KX911506	MH744175	MH744274
TH/CS-1019.3/11	KX911507	MH744176	MH744275
TH/RB-15.1/12	KX911508	MH744177	MH744276
TH/RB-15.2/12	KX911509	MH744178	MH744277
TH/NP-68/12	KX911510	MH744179	MH744260
TH/RB-123/12	MF139511	MH744180	MH744278
TH/NP-63/12	KX911511	MH744181	MH744279

*continued

Strain name	Accession number		
	S	ORF3	N
TH/NP-65/12	KC858259	MH744182	MH744280
TH/RB-79/12	KC858260	MH744183	MH744281
TH/RB-236/12	KX911512	MH744184	MH744282
TH/RB-881/12	KX911513	MH744185	MH744283
TH/CS-80712	KX911514	MH744186	MH744284
TH/RB-468.2/12	KX911515	MH744187	MH744285
TH/NP-1169/12	KX911517	MH744188	MH744286
TH/NP-1157/12	KX911516	MH744189	MH744287
TH/AY-2.2/12	KC858261	MH744190	MH744288
TH/AY-2.7/12	KC858262	MH744191	MH744289
TH/RB-887.2/13	KX911518	MH744192	MH744290
TH/RB-1179.1/13	KX911519	MH744193	MH744291
TH/RB-1179.2/13	KX911520	MH744194	MH744292
TH/RB-1210.1/13	KX911521	MH744195	MH744293
TH/RB-1210.3/13	KX911522	MH744196	MH744294
TH/RB-1224.1/13	KX911523	MH744197	MH744295
TH/RB-1224.2/13	KX911524	MH744198	MH744296
TH/NP-619/13	KX911526	MH744199	MH744297
TH/NP-SITP/13	KX911527	MH744200	MH744298
TH/NP-6098/13	KX911528	MH744201	MH744299
TH/NP-W2/13	KX911529	MH744202	MH744300
TH/NP-W3/13	KX911530	MH744203	MH744301
TH/PJ-517FE/14	KX911532	MH744204	MH744302
TH/RB-838/14	KX911533	MH744205	MH744257
TH/NP-1173/14	KX911543	MH744206	MH744303
TH/CB-1324-1/14	KX911544	MH744207	MH744304
TH/CB-1324-2/14	KX911545	MH744208	MH744305

**continued*

Strain name	Accession number		
	S	ORF3	N
TH/RB-1373-3/14	KX911546	MH744209	MH744306
TH/NP-65/14	KX911548	MH744210	MH744307
TH/NP-142/14	KX911550	MH744211	MH744308
TH/NP-224-1/14	KX911551	MH744212	MH744309
TH/NP-224-2/14	KX911552	MH744213	MH744310
TH/NP-23CF/15	KX911554	MH744214	MH744311
TH/NP-23BOR/15	KX911555	MH744215	MH744312
TH/RB-338-1/15	KX911556	MH744216	MH744313
TH/RB-BS/15	KX911559	MH744217	MH744314
TH/RB-272-2/15	KX911561	MH744218	MH744315
TH/CB-140CF/15	KX911562	MH744219	MH744316
TH/CB-140NS/15	KX911563	MH744220	MH744317
TH/NP1-1/15	KX911564	MH744221	MH744318
TH/RB-CHN/15	KX911567	MH744222	MH744319
TH/RB23/15	KX911568	MH744223	MH744320
TH/RB35/15	KX911570	MH744224	MH744321
TH/RB38/15	KX911571	MH744225	MH744322
TH/NP57/15	KX911573	MH744226	MH744323
TH/NP58/15	KX911572	MH744227	MH744324
TH/RB59/15	KX911574	MH744228	MH744325
TH/RB60/15	KX911575	MH744229	MH744326
TH/RB63/15	KX911577	MH744230	MH744327
TH/RB65/15	KX911579	MH744231	MH744328
TH/RB67/15	KX911581	MH744232	MH744329
TH/CB74/15	KX911584	MH744233	MH744330
TH/79/15	KX911585	MH744234	MH744331
TH/80/15	KX911586	MH744235	MH744332

**continued*

Strain name	Accession number		
	S	ORF3	N
TH/RB83/15	KX911587	MH744236	MH744333
TH/RB84/15	KX911588	MH744237	MH744334
TH/RB99/16	KX911591	MH744238	MH744335
TH/NP141/16	KX911594	MH744239	MH744336
TH/NP142/16	KX911595	MH744240	MH744337
TH/KR148/16	KX911596	MH744241	MH744338
TH/KR149/16	KX911597	MH744242	MH744339
TH/NP153/16	KX911598	MH744243	MH744340
TH/RB160/16	KX911599	MH744244	MH744258
TH/RB161/16	KX911600	MH744245	MH744259
TH/RB163/16	KX911601	MH744246	MH744341
TH/RB164/16	KX911602	MH744247	MH744342
TH/RB165/16	KX911603	MH744248	MH744343
TH/RB210/16	MF139376	MH744249	MH744344
TH/RB228/16	MF139377	MH744250	MH744345
TH/RB245/16	MF139383	MH744251	MH744346
TH/KR298/16	MF139390	MH744252	MH744347

Table S4. The details of provincial origin and year collection of 95 PEDV strains.

	2011 (n=19)	2012 (n=15)	2013 (n=12)	2014 (n=12)	2015 (n=22)	2016 (n=15)
NP (25)	TH/NP-156/11 TH/NP-795/11	TH/NP-68/12 TH/NP-63/12 TH/NP-1169/12 TH/NP-1157/12 TH/NP-65/12	TH/NP-SITP/13 TH/NP-6098/13 TH/NP-W2/13 TH/NP-W3/13 TH/NP-619/13	TH/NP-65/14 TH/NP-142/14 TH/NP-224-1/14 TH/NP-224-2/14 TH/NP-1173/14	TH/NP-23CF/15 TH/NP-23BOR/15 TH/NP1-1/15 TH/NP57/15 TH/NP58/15	TH/NP141/16 TH/NP142/16 TH/NP153/16
RB (43)	TH/RB-833/11 TH/RB-833.3/11 TH/RB-807.3/11 TH/RB-807.4/11	TH/RB-15.1/12 TH/RB-15.2/12 TH/RB-123/12 TH/RB-79/12 TH/RB-236/12 TH/RB-468.2/12 TH/RB-881/12	TH/RB-887.2/13 TH/RB-1179.1/13 TH/RB-1179.2/13 TH/RB-1210.1/13 TH/RB-1210.3/13 TH/RB-1224.1/13 TH/RB-1224.2/13	TH/RB-838/14 TH/RB-1373-3/14 TH/RB-338-1/15 TH/RB-272-2/15	TH/RB-BS/15 TH/RB-CHN/15 TH/RB23/15 TH/RB35/15 TH/RB38/15 TH/RB59/15 TH/RB60/15	TH/RB99/16 TH/RB160/16 TH/RB161/16 TH/RB163/16 TH/RB164/16 TH/RB165/16 TH/RB210/16 TH/RB228/16 TH/RB245/16
CS (7)	TH/CS-1019.1/11 TH/CS-1019.2/11 TH/CS-1019.3/11 TH/CS-866.1/11 TH/CS-866.3/11 TH/CS-866.4/11	TH/CS-80712/12				
CB (8)	TH/CB-1421/11 TH/CB-3553/11 TH/CB-KHF/11			TH/CB-1324-1/14 TH/CB-1324-2/14	TH/CB-140CF/15 TH/CB-140NS/15 TH/CB74/15	

**continued*

	2011 (n=19)	2012 (n=15)	2013 (n=12)	2014 (n=12)	2015 (n=22)	2016 (n=15)
UD (4)	TH/UD-1010.1/11 TH/UD-1010.2/11 TH/UD-1010.3/11 TH/UD-1010.4/11					
AY (2)		TH/AY-2.2/12 TH/AY-2.7/12				
PJ (1)				TH/PJ-517FE/14		
NR (3)						TH/KR148/16 TH/KR149/16 TH/KR298/16
n/a (2)					TH/79/15 TH/80/15	

NP (Nakorn Pathom), RB (Ratchaburi), CS (Chachoengsao), CB (Chonburi), UD (Udon Thani), AY (Phra Nakhon Si Ayutthaya), PJ (Prachuap Khiri Khan), NR (Nakhon Ratchasima), n/a (unknown farm location)

REFERENCES

1. Matthijnssens J, Ciarlet M, Heiman E, Arijs I, Delbeke T, McDonald SM, et al. Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. J Virol. 2008;82(7):3204-19.

2. Estes MK KA. Rotaviruses. In: Knipe DM HP, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, editor. Fields Virology 5th ed. Philadelphia: Kluwer/Lippincott; Williams and Wilkins; 2007. p. 1917–74.

3. Estes M.K., and Greenberg H.B. Rotaviruses. In: David M. Knipe, and Peter M. Howley, editor. Fields Virology. 6th Edition: Lippincott Williams & Wilkins; 2013. p. 1347-401.

4. Molinari BL, Lorenzetti E, Otonel RA, Alfieri AF, Alfieri AA. Species H rotavirus detected in piglets with diarrhea, Brazil, 2012. Emerging infectious diseases. 2014;20(6):1019-22.

5. Matthijnssens J, Otto PH, Ciarlet M, Desselberger U, Van Ranst M, Johne R. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. Arch Virol. 2012;157(6):1177-82.

6. Banyai K, Kemenesi G, Budinski I, Foldes F, Zana B, Marton S, et al. Candidate new rotavirus species in Schreiber's bats, Serbia. Infect Genet Evol. 2017;48:19-26.

7. Mihalov-Kovacs E, Gellert A, Marton S, Farkas SL, Feher E, Oldal M, et al. Candidate new rotavirus species in sheltered dogs, Hungary. Emerg Infect Dis. 2015;21(4):660-3.

8. Gouvea V, Allen JR, Glass RI, Fang ZY, Bremont M, Cohen J, et al. Detection of group B and C rotaviruses by polymerase chain reaction. J Clin Microbiol. 1991;29(3):519-23.

9. Médici K.C. , Barry A.F. . Porcine rotavirus groups A, B, and C identified by polymerase chain reaction in a fecal sample collection with inconclusive results by polyacrylamide gel electrophoresis. J Swine Health Prod. 2011;19(3):146–50.

10. Marthaler D, Rossow K, Culhane M, Collins J, Goyal S, Ciarlet M, et al.

Identification, phylogenetic analysis and classification of porcine group C rotavirus VP7 sequences from the United States and Canada. Virology. 2013;446(1-2):189-98.

11. Chasey D, Bridger JC, McCrae MA. A new type of atypical rotavirus in pigs. Archives of virology. 1986;89(1-4):235-43.

12. Wakuda M, Ide T, Sasaki J, Komoto S, Ishii J, Sanekata T, et al. Porcine rotavirus closely related to novel group of human rotaviruses. Emerging infectious diseases. 2011;17(8):1491-3.

13. Pesavento JB, Crawford SE, Estes MK, Prasad BV. Rotavirus proteins: structure and assembly. Curr Top Microbiol Immunol. 2006;309:189-219.

14. Hu L, Crawford SE, Hyser JM, Estes MK, Prasad BV. Rotavirus non-structural proteins: structure and function. Curr Opin Virol. 2012;2(4):380-8.

15. Offit PA, Blavat G. Identification of the two rotavirus genes determining neutralization specificities. J Virol. 1986;57(1):376-8.

16. Clarke E, Desselberger U. Correlates of protection against human rotavirus disease and the factors influencing protection in low-income settings. Mucosal Immunol. 2015;8(1):1-17.

17. Estes MK, and Greenberg HB. Rotaviruses. In: David M. Knipe, and Peter M. Howley, editor. Fields virology. sixth ed. Philadelphia: Wolters Kluwer - Lippincott Williams & Wilkins; 2013. p. 1347–401.

18. Graff JW, Mitzel DN, Weisend CM, Flenniken ML, Hardy ME. Interferon regulatory factor 3 is a cellular partner of rotavirus NSP1. J Virol. 2002;76(18):9545-50.

19. Soma J, Tsunemitsu H, Miyamoto T, Suzuki G, Sasaki T, Suzuki T. Whole-genome analysis of two bovine rotavirus C strains: Shintoku and Toyama. J Gen Virol. 2013;94(Pt 1):128-35.

20. Matthijnssens J, Ciarlet M, Rahman M, Attoui H, Banyai K, Estes MK, et al. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch Virol. 2008;153(8):1621-9.

21. Rojas MA, Goncalves JLS, Dias HG, Manchego A, Santos N. Identification of two novel Rotavirus A genotypes, G35 and P[50], from Peruvian alpaca faeces. Infect Genet Evol. 2017;55:71-4.

22. Lorenzetti E, da Silva Medeiros TN, Alfieri AF, Alfieri AA. Genetic heterogeneity of

wild-type G4P[6] porcine rotavirus strains detected in a diarrhea outbreak in a regularly vaccinated pig herd. Vet Microbiol. 2011;154(1-2):191-6.

23. Miyazaki A, Kuga K, Suzuki T, Kohmoto M, Katsuda K, Tsunemitsu H. Genetic diversity of group A rotaviruses associated with repeated outbreaks of diarrhea in a farrow-to-finish farm: identification of a porcine rotavirus strain bearing a novel VP7 genotype, G26. Vet Res. 2011;42:112.

24. Okitsu S, Khamrin P, Thongprachum A, Kongkaew A, Maneekarn N, Mizuguchi M, et al. Whole-genomic analysis of G3P[23], G9P[23] and G3P[13] rotavirus strains isolated from piglets with diarrhea in Thailand, 2006-2008. Infect Genet Evol. 2013;18:74-86.

25. Chitambar SD, Arora R, Chhabra P. Molecular characterization of a rare G1P[19] rotavirus strain from India: evidence of reassortment between human and porcine rotavirus strains. J Med Microbiol. 2009;58(Pt 12):1611-5.

26. Ghosh S, Urushibara N, Taniguchi K, Kobayashi N. Whole genomic analysis reveals the porcine origin of human G9P[19] rotavirus strains Mc323 and Mc345. Infect Genet Evol. 2012;12(2):471-7.

27. Theuns S, Heylen E, Zeller M, Roukaerts ID, Desmarets LM, Van Ranst M, et al. Complete genome characterization of recent and ancient Belgian pig group A rotaviruses and assessment of their evolutionary relationship with human rotaviruses. J Virol. 2015;89(2):1043-57.

28. Collins PJ, Martella V, O'Shea H. Detection and characterization of group C rotaviruses in asymptomatic piglets in Ireland. J Clin Microbiol. 2008;46(9):2973-9.

29. Moutelikova R, Prodelalova J, Dufkova L. Diversity of VP7, VP4, VP6, NSP2, NSP4, and NSP5 genes of porcine rotavirus C: phylogenetic analysis and description of potential new VP7, VP4, VP6, and NSP4 genotypes. Arch Virol. 2015;160(7):1715-27.

30. Niira K, Ito M, Masuda T, Saitou T, Abe T, Komoto S, et al. Whole genome sequences of Japanese porcine species C rotaviruses reveal a high diversity of genotypes of individual genes and will contribute to a comprehensive, generally accepted classification system. Infect Genet Evol. 2016;44:106-13.

31. Martella V, Banyai K, Lorusso E, Bellacicco AL, Decaro N, Camero M, et al. Prevalence of group C rotaviruses in weaning and post-weaning pigs with enteritis. Vet Microbiol. 2007;123(1-3):26-33.

32. Medici MC, Abelli LA, Martinelli M, Martella V, Dettori G, Chezzi C. Molecular characterization of group C rotaviruses detected in children in Italy. J Clin Virol. 2009;44(1):62-5.

33. Matthijnssens J, De Grazia S, Piessens J, Heylen E, Zeller M, Giammanco GM, et al. Multiple reassortment and interspecies transmission events contribute to the diversity of feline, canine and feline/canine-like human group A rotavirus strains. Infect Genet Evol. 2011;11(6):1396-406.

34. Steyer A, Poljsak-Prijatelj M, Barlic-Maganja D, Marin J. Human, porcine and bovine rotaviruses in Slovenia: evidence of interspecies transmission and genome reassortment. The Journal of general virology. 2008;89(Pt 7):1690-8.

35. Midgley SE, Banyai K, Buesa J, Halaihel N, Hjulsager CK, Jakab F, et al. Diversity and zoonotic potential of rotaviruses in swine and cattle across Europe. Vet Microbiol. 2012;156(3-4):238-45.

36. Martella V, Banyai K, Matthijnssens J, Buonavoglia C, Ciarlet M. Zoonotic aspects of rotaviruses. Vet Microbiol. 2010;140(3-4):246-55.

37. Banyai K, Esona MD, Kerin TK, Hull JJ, Mijatovic S, Vasconez N, et al. Molecular characterization of a rare, human-porcine reassortant rotavirus strain, G11P[6], from Ecuador. Arch Virol. 2009;154(11):1823-9.

38. Urasawa S, Hasegawa A, Urasawa T, Taniguchi K, Wakasugi F, Suzuki H, et al. Antigenic and genetic analyses of human rotaviruses in Chiang Mai, Thailand: evidence for a close relationship between human and animal rotaviruses. J Infect Dis. 1992;166(2):227-34.

39. Do LP, Nakagomi T, Otaki H, Agbemabiese CA, Nakagomi O, Tsunemitsu H. Phylogenetic inference of the porcine Rotavirus A origin of the human G1 VP7 gene. Infect Genet Evol. 2016;40:205-13.

40. Chieochansin T, Vutithanachot V, Phumpholsup T, Posuwan N, Theamboonlers A, Poovorawan Y. The prevalence and genotype diversity of Human Rotavirus A circulating in Thailand, 2011-2014. Infect Genet Evol. 2016;37:129-36.

41. Komoto S, Tacharoenmuang R, Guntapong R, Ide T, Sinchai P, Upachai S, et al. Identification and characterization of a human G9P[23] rotavirus strain from a child with diarrhoea in Thailand: evidence for porcine-to-human interspecies transmission. J Gen

Virol. 2017;98(4):532-8.

42. Yodmeeklin A, Khamrin P, Chuchaona W, Kumthip K, Kongkaew A, Vachirachewin R, et al. Analysis of complete genome sequences of G9P[19] rotavirus strains from human and piglet with diarrhea provides evidence for whole-genome interspecies transmission of nonreassorted porcine rotavirus. Infect Genet Evol. 2017;47:99-108.

43. Komoto S, Maeno Y, Tomita M, Matsuoka T, Ohfu M, Yodoshi T, et al. Whole genomic analysis of a porcine-like human G5P[6] rotavirus strain isolated from a child with diarrhoea and encephalopathy in Japan. J Gen Virol. 2013;94(Pt 7):1568-75.

44. Than VT, Park JH, Chung IS, Kim JB, Kim W. Whole-genome sequence analysis of a Korean G11P[25] rotavirus strain identifies several porcine-human reassortant events. Arch Virol. 2013;158(11):2385-93.

45. Zhou X, Wang YH, Ghosh S, Tang WF, Pang BB, Liu MQ, et al. Genomic characterization of G3P[6], G4P[6] and G4P[8] human rotaviruses from Wuhan, China: Evidence for interspecies transmission and reassortment events. Infect Genet Evol. 2015;33:55-71.

46. Das S, Varghese V, Chaudhuri S, Barman P, Kojima K, Dutta P, et al. Genetic variability of human rotavirus strains isolated from Eastern and Northern India. J Med Virol. 2004;72(1):156-61.

47. Mukherjee A, Ghosh S, Bagchi P, Dutta D, Chattopadhyay S, Kobayashi N, et al. Full genomic analyses of human rotavirus G4P[4], G4P[6], G9P[19] and G10P[6] strains from North-eastern India: evidence for interspecies transmission and complex reassortment events. Clin Microbiol Infect. 2011;17(9):1343-6.

48. Hwang KP, Wu FT, Banyai K, Wu HS, Yang DC, Huang YC, et al. Identification of porcine rotavirus-like genotype P[6] strains in Taiwanese children. J Med Microbiol. 2012;61(Pt 7):990-7.

49. Zeller M, Heylen E, De Coster S, Van Ranst M, Matthijnssens J. Full genome characterization of a porcine-like human G9P[6] rotavirus strain isolated from an infant in Belgium. Infect Genet Evol. 2012;12(7):1492-500.

50. Degiuseppe JI, Beltramino JC, Millan A, Stupka JA, Parra GI. Complete genome analyses of G4P[6] rotavirus detected in Argentinean children with diarrhoea provides evidence of interspecies transmission from swine. Clin Microbiol Infect. 2013;19(8):E367-

71.

51. Papp H, Laszlo B, Jakab F, Ganesh B, De Grazia S, Matthijnssens J, et al. Review of group A rotavirus strains reported in swine and cattle. Vet Microbiol. 2013;165(3-4):190-9.

52. Martinez M, Galeano ME, Akopov A, Palacios R, Russomando G, Kirkness EF, et al. Whole-genome analyses reveals the animal origin of a rotavirus G4P[6] detected in a child with severe diarrhea. Infect Genet Evol. 2014;27:156-62.

53. Marton S, Deak J, Doro R, Csata T, Farkas SL, Martella V, et al. Reassortant human group C rotaviruses in Hungary. Infect Genet Evol. 2015;34:410-4.

54. Chang KO, Nielsen PR, Ward LA, Saif LJ. Dual infection of gnotobiotic calves with bovine strains of group A and porcine-like group C rotaviruses influences pathogenesis of the group C rotavirus. J Virol. 1999;73(11):9284-93.

55. Jeong YJ, Park SI, Hosmillo M, Shin DJ, Chun YH, Kim HJ, et al. Detection and molecular characterization of porcine group C rotaviruses in South Korea. Vet Microbiol. 2009;138(3-4):217-24.

56. Yamamoto D, Ghosh S, Kuzuya M, Wang YH, Zhou X, Chawla-Sarkar M, et al. Whole-genome characterization of human group C rotaviruses: identification of two lineages in the VP3 gene. J Gen Virol. 2011;92(Pt 2):361-9.

57. Ducatelle R, Coussement W, Pensaert MB, Debouck P, Hoorens J. In vivo morphogenesis of a new porcine enteric coronavirus, CV 777. Archives of virology. 1981;68(1):35-44.

58. Masters PS, Kuo L, Ye R, Hurst KR, Koetzner CA, Hsue B. Genetic and molecular biological analysis of protein-protein interactions in coronavirus assembly. Adv Exp Med Biol. 2006;581:163-73.

59. Putics A, Gorbalenya AE, Ziebuhr J. Identification of protease and ADP-ribose 1"-monophosphatase activities associated with transmissible gastroenteritis virus non-structural protein 3. The Journal of general virology. 2006;87(Pt 3):651-6.

60. Woo PC, Huang Y, Lau SK, Yuen KY. Coronavirus genomics and bioinformatics analysis. Viruses. 2010;2(8):1804-20.

61. Kang H, Feng M, Schroeder ME, Giedroc DP, Leibowitz JL. Putative cis-acting stem-loops in the 5' untranslated region of the severe acute respiratory syndrome

coronavirus can substitute for their mouse hepatitis virus counterparts. J Virol. 2006;80(21):10600-14.

62. Sun D, Feng L, Shi H, Chen J, Cui X, Chen H, et al. Identification of two novel B cell epitopes on porcine epidemic diarrhea virus spike protein. Vet Microbiol. 2008;131(1-2):73-81.

63. Masters PS. The molecular biology of coronaviruses. Adv Virus Res. 2006;66:193-292.

64. Zhang Z, Chen J, Shi H, Chen X, Shi D, Feng L, et al. Identification of a conserved linear B-cell epitope in the M protein of porcine epidemic diarrhea virus. Virol J. 2012;9:225.

65. Cao L, Ge X, Gao Y, Ren Y, Ren X, Li G. Porcine epidemic diarrhea virus infection induces NF-kappaB activation through the TLR2, TLR3 and TLR9 pathways in porcine intestinal epithelial cells. J Gen Virol. 2015;96(Pt 7):1757-67.

66. Shi D, Lv M, Chen J, Shi H, Zhang S, Zhang X, et al. Molecular characterizations of subcellular localization signals in the nucleocapsid protein of porcine epidemic diarrhea virus. Viruses. 2014;6(3):1253-73.

67. Shan Y, Liu ZQ, Li GW, Chen C, Luo H, Liu YJ, et al. Nucleocapsid protein from porcine epidemic diarrhea virus isolates can antagonize interferon-lambda production by blocking the nuclear factor-kappaB nuclear translocation. J Zhejiang Univ Sci B. 2018;19(7):570-80.

68. Song D, Park B. Porcine epidemic diarrhoea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. Virus Genes. 2012;44(2):167-75.

69. Shaw DP, Morehouse LG, Solorzano RF. Experimental rotavirus infection in three-week-old pigs. Am J Vet Res. 1989;50(11):1961-5.

70. Ramig RF. Pathogenesis of intestinal and systemic rotavirus infection. J Virol. 2004;78(19):10213-20.

71. Yuan L, Stevenson GW, Saif LJ. Rotavirus and Reovirus. In: Barbara E. Straw JJZ, Sylvie D' Allaire and David J. Taylor (Ed.). , editor. Disease of swine. 9th ed. Iowa: Blackwell Publishing; 2006. p. 435-54.

72. Janke BH, Morehouse LG, Solorzano RF. Single and mixed infections of neonatal pigs with rotaviruses and enteroviruses: clinical signs and microscopic lesions. Can J Vet

Res. 1988;52(3):364-9.

73. Ducatelle R, Coussement W, Charlier G, Debouck P, Hoorens J. Three-dimensional sequential study of the intestinal surface in experimental porcine CV 777 coronavirus enteritis. Zentralbl Veterinarmed B. 1981;28(6):483-93.

74. Morilla A, Arriaga C, Ruiz A, Martinez AG, Cigarroa R, Valazquez A. Association between diarrhoea and shedding of group A and atypical groups B to E rotaviruses in suckling pigs. Ann Rech Vet. 1991;22(2):193-200.

75. Katsuda K, Kohmoto M, Kawashima K, Tsunemitsu H. Frequency of enteropathogen detection in suckling and weaned pigs with diarrhea in Japan. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 2006;18(4):350-4.

76. Theuns S, Conceicao-Neto N, Zeller M, Heylen E, Roukaerts ID, Desmarets LM, et al. Characterization of a genetically heterogeneous porcine rotavirus C, and other viruses present in the fecal virome of a non-diarrheic Belgian piglet. Infect Genet Evol. 2016;43:135-45.

77. Will LA, Paul PS, Proescholdt TA, Aktar SN, Flaming KP, Janke BH, et al. Evaluation of rotavirus infection and diarrhea in Iowa commercial pigs based on an epidemiologic study of a population represented by diagnostic laboratory cases. J Vet Diagn Invest. 1994;6(4):416-22.

78. Suzuki T, Hasebe A, Miyazaki A, Tsunemitsu H. Analysis of genetic divergence among strains of porcine rotavirus C, with focus on VP4 and VP7 genotypes in Japan. Virus Res. 2015;197:26-34.

79. Stipp DT, Alfieri AF, Lorenzetti E, da Silva Medeiros TN, Possatti F, Alfieri AA. VP6 gene diversity in 11 Brazilian strains of porcine group C rotavirus. Virus Genes. 2015;50(1):142-6.

80. Saif LJ, Bohl EH, Theil KW, Cross RF, House JA. Rotavirus-like, calicivirus-like, and 23-nm virus-like particles associated with diarrhea in young pigs. J Clin Microbiol. 1980;12(1):105-11.

81. Zhou W, Ullman K, Chowdry V, Reining M, Benyeda Z, Baule C, et al. Molecular investigations on the prevalence and viral load of enteric viruses in pigs from five European countries. Vet Microbiol. 2016;182:75-81.

82. Molinari BL, Possatti F, Lorenzetti E, Alfieri AF, Alfieri AA. Unusual outbreak of post-weaning porcine diarrhea caused by single and mixed infections of rotavirus groups A, B, C, and H. Veterinary microbiology. 2016;193:125-32.

83. Barbosa BR, Bernardes NT, Beserra LA, Gregori F. Molecular characterization of the porcine group A rotavirus NSP2 and NSP5/6 genes from Sao Paulo State, Brazil, in 2011/12. TheScientificWorldJournal. 2013;2013:241686.

84. Barreiros MA, Alfieri AA, Alfieri AF, Medici KC, Leite JP. An outbreak of diarrhoea in one-week-old piglets caused by group A rotavirus genotypes P[7],G3 and P[7],G5. Veterinary research communications. 2003;27(6):505-12.

85. Wilhelm B, Leblanc D, Leger D, Gow S, Deckert A, Pearl DL, et al. Farm-level prevalence and risk factors for detection of hepatitis E virus, porcine enteric calicivirus, and rotavirus in Canadian finisher pigs. Can J Vet Res. 2016;80(2):95-105.

86. Chandler-Bostock R, Hancox LR, Payne H, Iturriza-Gomara M, Daly JM, Mellits KH. Diversity of group A rotavirus on a UK pig farm. Veterinary microbiology. 2015;180(3-4):205-11.

87. Marthaler D, Homwong N, Rossow K, Culhane M, Goyal S, Collins J, et al. Rapid detection and high occurrence of porcine rotavirus A, B, and C by RT-qPCR in diagnostic samples. Journal of virological methods. 2014;209:30-4.

88. Janke BH, Nelson JK, Benfield DA, Nelson EA. Relative prevalence of typical and atypical strains among rotaviruses from diarrheic pigs in conventional swine herds. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 1990;2(4):308-11.

89. Pham HA, Carrique-Mas JJ, Nguyen VC, Ngo TH, Nguyet LA, Do TD, et al. The prevalence and genetic diversity of group A rotaviruses on pig farms in the Mekong Delta region of Vietnam. Veterinary microbiology. 2014;170(3-4):258-65.

90. Yodmeeklin A, Khamrin P, Chuchaona W, Saikruang W, Kongkaew A, Vachirachewin R, et al. Great genetic diversity of rotaviruses detected in piglets with diarrhea in Thailand. Arch Virol. 2016;161(10):2843-9.

91. Saikruang W, Khamrin P, Chaimongkol N, Suantai B, Kongkaew A, Kongkaew S, et al. Genetic diversity and novel combinations of G4P[19] and G9P[19] porcine rotavirus strains in Thailand. Vet Microbiol. 2013;161(3-4):255-62.

92. Okitsu S, Khamrin P, Thongprachum A, Maneekarn N, Mizuguchi M, Ushijima H. Predominance of porcine P[23] genotype rotaviruses in piglets with diarrhea in northern Thailand. J Clin Microbiol. 2011;49(1):442-5.

93. Maneekarn N, Khamrin P, Chan-it W, Peerakome S, Sukchai S, Pringprao K, et al. Detection of rare G3P[19] porcine rotavirus strains in Chiang Mai, Thailand, provides evidence for origin of the VP4 genes of Mc323 and Mc345 human rotaviruses. J Clin Microbiol. 2006;44(11):4113-9.

94. Pongsuwanna Y, Taniguchi K, Chiwakul M, Urasawa T, Wakasugi F, Jayavasu C, et al. Serological and genomic characterization of porcine rotaviruses in Thailand: detection of a G10 porcine rotavirus. J Clin Microbiol. 1996;34(5):1050-7.

95. Dubal ZB, Bhilegaonkar KN, Barbuddhe SB, Kolhe RP, Kaur S, Rawat S, et al. Prevalence and genotypic (G and P) determination of porcine group A rotaviruses from different regions of India. Tropical animal health and production. 2013;45(2):609-15.

96. Song DS, Kang BK, Oh JS, Ha GW, Yang JS, Moon HJ, et al. Multiplex reverse transcription-PCR for rapid differential detection of porcine epidemic diarrhea virus, transmissible gastroenteritis virus, and porcine group A rotavirus. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc. 2006;18(3):278-81.

97. Wieler LH, Ilieff A, Herbst W, Bauer C, Vieler E, Bauerfeind R, et al. Prevalence of enteropathogens in suckling and weaned piglets with diarrhoea in southern Germany. J Vet Med B Infect Dis Vet Public Health. 2001;48(2):151-9.

98. Nagy B, Nagy G, Meder M, Mocsari E. Enterotoxigenic Escherichia coli, rotavirus, porcine epidemic diarrhoea virus, adenovirus and calici-like virus in porcine postweaning diarrhoea in Hungary. Acta Vet Hung. 1996;44(1):9-19.

99. Pensaert MB, de Bouck P. A new coronavirus-like particle associated with diarrhea in swine. Arch Virol. 1978;58(3):243-7.

100. Stevenson GW, Hoang H, Schwartz KJ, Burrough ER, Sun D, Madson D, et al. Emergence of Porcine epidemic diarrhea virus in the United States: clinical signs, lesions, and viral genomic sequences. J Vet Diagn Invest. 2013;25(5):649-54.

101. Ojkic D, Hazlett M, Fairles J, Marom A, Slavic D, Maxie G, et al. The first case of porcine epidemic diarrhea in Canada. Can Vet J. 2015;56(2):149-52.

102. Turgeon DC, Morin M, Jolette J, Higgins R, Marsolais G, DiFranco E. Coronavirus-like particles associated with diarrhea in baby pigs in Quebec. Can Vet J. 1980;21(3):100-xxiii.

103. Wood EN. An apparently new syndrome of porcine epidemic diarrhoea. Vet Rec. 1977;100(12):243-4.

104. Carvajal A, Lanza I, Diego R, Rubio P, Carmenes P. Evaluation of a blocking ELISA using monoclonal antibodies for the detection of porcine epidemic diarrhea virus and its antibodies. J Vet Diagn Invest. 1995;7(1):60-4.

105. Pijpers A, van Nieuwstadt AP, Terpstra C, Verheijden JH. Porcine epidemic diarrhoea virus as a cause of persistent diarrhoea in a herd of breeding and finishing pigs. Vet Rec. 1993;132(6):129-31.

106. Martelli P, Lavazza A, Nigrelli AD, Merialdi G, Alborali LG, Pensaert MB. Epidemic of diarrhoea caused by porcine epidemic diarrhoea virus in Italy. Vet Rec. 2008;162(10):307-10.

107. Hanke D, Jenckel M, Petrov A, Ritzmann M, Stadler J, Akimkin V, et al. Comparison of porcine epidemic diarrhea viruses from Germany and the United States, 2014. Emerg Infect Dis. 2015;21(3):493-6.

108. Grasland B, Bigault L, Bernard C, Quenault H, Toulouse O, Fablet C, et al. Complete genome sequence of a porcine epidemic diarrhea s gene indel strain isolated in france in december 2014. Genome Announc. 2015;3(3).

109. Toplak I, Ipavec M, Kuhar U, Kusar D, Papic B, Koren S, et al. Complete Genome Sequence of the Porcine Epidemic Diarrhea Virus Strain SLO/JH-11/2015. Genome Announc. 2016;4(2).

110. Mesquita JR, Hakze-van der Honing R, Almeida A, Lourenco M, van der Poel WH, Nascimento MS. Outbreak of Porcine Epidemic Diarrhea Virus in Portugal, 2015. Transbound Emerg Dis. 2015;62(6):586-8.

111. Steinrigl A, Fernandez SR, Stoiber F, Pikalo J, Sattler T, Schmoll F. First detection, clinical presentation and phylogenetic characterization of Porcine epidemic diarrhea virus in Austria. BMC Vet Res. 2015;11:310.

112. Jinghui F, Yijing L. Cloning and sequence analysis of the M gene of porcine epidemic diarrhea virus LJB/03. Virus Genes. 2005;30(1):69-73.

113. Takahashi K, Okada K, Ohshima K. An outbreak of swine diarrhea of a new-type associated with coronavirus-like particles in Japan. Nihon Juigaku Zasshi. 1983;45(6):829-32.

114. Kweon C, Kwon B, Jung T, Kee Y, Hur D, Hwang E. Isolation of porcine epidemic diarrhea virus (PEDV) in Korea. Korean J Vet Res. 1993;33:249–54.

115. Paraguison-Alili R, Domingo CY. Phylogenetic tracking of current porcine epidemic diarrhea virus (PEDV) strains in the Philippines. Arch Virol. 2016;161(9):2601-4.

116. Srinuntapunt S, Trongwongsa L, Antarasena C, Sangsuwan W, Prommuang P. Porcine epidemic diarrhea in Trang Province. J Thai Vet Med Assoc. 1995;46:11-9.

117. Duy T, Toan T, Puranaveja S, Thanawongnuwech R. Genetic characterization of porcine epidemic diarrhea virus (PEDV) isolates from southern Vietnam during 2009-2010 outbreaks. Thai J Vet Med. 2013;41:55-64.

118. Lin CN, Chung WB, Chang SW, Wen CC, Liu H, Chien CH, et al. US-like strain of porcine epidemic diarrhea virus outbreaks in Taiwan, 2013-2014. J Vet Med Sci. 2014;76(9):1297-9.

119. Lin CM, Saif LJ, Marthaler D, Wang Q. Evolution, antigenicity and pathogenicity of global porcine epidemic diarrhea virus strains. Virus Res. 2016;226:20-39.

120. Wang S, Cheng X, Chen S, Lin F, Jiang B, Zhu X, et al. Classification of emergent U.S. strains of porcine epidemic diarrhea virus by phylogenetic analysis of nucleocapsid and ORF3 genes. J Clin Microbiol. 2014;52(9):3509-10.

121. Huang YW, Dickerman AW, Pineyro P, Li L, Fang L, Kiehne R, et al. Origin, evolution, and genotyping of emergent porcine epidemic diarrhea virus strains in the United States. MBio. 2013;4(5):e00737-13.

122. Oka T, Saif LJ, Marthaler D, Esseili MA, Meulia T, Lin CM, et al. Cell culture isolation and sequence analysis of genetically diverse US porcine epidemic diarrhea virus strains including a novel strain with a large deletion in the spike gene. Vet Microbiol. 2014;173(3-4):258-69.

123. Hou Y, Lin CM, Yokoyama M, Yount BL, Marthaler D, Douglas AL, et al. Deletion of a 197-Amino-Acid Region in the N-Terminal Domain of Spike Protein Attenuates Porcine Epidemic Diarrhea Virus in Piglets. J Virol. 2017;91(14).

124. Murakami S, Miyazaki A, Takahashi O, Hashizume W, Hase Y, Ohashi S, et al.

Complete Genome Sequence of the Porcine Epidemic Diarrhea Virus Variant Tottori2/JPN/2014. Genome Announc. 2015;3(4).

125. Vlasova AN, Marthaler D, Wang Q, Culhane MR, Rossow KD, Rovira A, et al. Distinct characteristics and complex evolution of PEDV strains, North America, May 2013-February 2014. Emerg Infect Dis. 2014;20(10):1620-8.

126. Lee C. Porcine epidemic diarrhea virus: An emerging and re-emerging epizootic swine virus. Virol J. 2015;12:193.

127. Sun D, Wang X, Wei S, Chen J, Feng L. Epidemiology and vaccine of porcine epidemic diarrhea virus in China: a mini-review. J Vet Med Sci. 2016;78(3):355-63.

128. Song D, Moon H, Kang B. Porcine epidemic diarrhea: a review of current epidemiology and available vaccines. Clin Exp Vaccine Res. 2015;4(2):166-76.

129. Khamrin P, Maneekarn N, Peerakome S, Chan-it W, Yagyu F, Okitsu S, et al. Novel porcine rotavirus of genotype P[27] shares new phylogenetic lineage with G2 porcine rotavirus strain. Virology. 2007;361(2):243-52.

130. Chan-It W, Khamrin P, Saekhow P, Pantip C, Thongprachum A, Peerakome S, et al. Multiple combinations of P[13]-like genotype with G3, G4, and G5 in porcine rotaviruses. J Clin Microbiol. 2008;46(4):1169-73.

131. Antarasena C, Trongwongsa L, Jearasuk P, Prommuang P, Rujkwan S, editors. Isolation of porcine epidemic diarrhea virus in vero cell cultures. 35th Kasetsart University annual conference; 1997 3-5 Febuary 1997; Bangkok, Thailand.

132. Puranaveja S, Poolperm P, Lertwatcharasarakul P, Kesdaengsakonwut S, Boonsoongnern A, Urairong K, et al. Chinese-like strain of porcine epidemic diarrhea virus, Thailand. Emerg Infect Dis. 2009;15(7):1112-5.

133. Temeeyasen G, Srijangwad A, Tripipat T, Tipsombatboon P, Piriyapongsa J, Phoolcharoen W, et al. Genetic diversity of ORF3 and spike genes of porcine epidemic diarrhea virus in Thailand. Infect Genet Evol. 2014;21:205-13.

134. Cheun-Arom T, Temeeyasen G, Tripipat T, Kaewprommal P, Piriyapongsa J, Sukrong S, et al. Full-length genome analysis of two genetically distinct variants of porcine epidemic diarrhea virus in Thailand. Infect Genet Evol. 2016;44:114-21.

135. Jaru-Ampornpan P, Jengarn J, Wanitchang A, Jongkaewwattana A. Porcine Epidemic Diarrhea Virus 3C-Like Protease-Mediated Nucleocapsid Processing: Possible

Link to Viral Cell Culture Adaptability. J Virol. 2017;91(2).

136. Rattanapisit K, Srijangwad A, Chuanasa T, Sukrong S, Tantituvanont A, Mason HS, et al. Rapid Transient Production of a Monoclonal Antibody Neutralizing the Porcine Epidemic Diarrhea Virus (PEDV) in Nicotiana benthamiana and Lactuca sativa. Planta Med. 2017;83(18):1412-9.

137. Srijangwad A, Stott CJ, Temeeyasen G, Senasuthum R, Chongcharoen W, Tantituvanont A, et al. Immune response of gilts to single and double infection with porcine epidemic diarrhea virus. Arch Virol. 2017;162(7):2029-34.

138. Temeeyasen G, Sinha A, Gimenez-Lirola LG, Zhang JQ, Pineyro PE. Differential gene modulation of pattern-recognition receptor TLR and RIG-I-like and downstream mediators on intestinal mucosa of pigs infected with PEDV non S-INDEL and PEDV S-INDEL strains. Virology. 2018;517:188-98.

139. Wanitchang A, Saenboonrueng J, Srisutthisamphan K, Jongkaewwattana A. Characterization of influenza A virus pseudotyped with the spike protein of porcine epidemic diarrhea virus. Arch Virol. 2018;163(12):3255-64.

140. Zissis G, Lambert JP, Marbehant P, Marissens D, Lobmann M, Charlier P, et al. Protection studies in colostrum-deprived piglets of a bovine rotavirus vaccine candidate using human rotavirus strains for challenge. J Infect Dis. 1983;148(6):1061-8.

141. Vesikari T. Rotavirus vaccination: a concise review. Clin Microbiol Infect. 2012;18 Suppl 5:57-63.

142. Santos VS, Marques DP, Martins-Filho PR, Cuevas LE, Gurgel RQ. Effectiveness of rotavirus vaccines against rotavirus infection and hospitalization in Latin America: systematic review and meta-analysis. Infect Dis Poverty. 2016;5(1):83.

143. Wu JY, Zhou Y, Zhang GM, Mu GF, Yi S, Yin N, et al. Isolation and characterization of a new candidate human inactivated rotavirus vaccine strain from hospitalized children in Yunnan, China: 2010-2013. World J Clin Cases. 2018;6(11):426-40.

144. Naseer O, Jarvis MC, Ciarlet M, Marthaler DG. Genotypic and epitope characteristics of group A porcine rotavirus strains circulating in Canada. Virology. 2017;507:53-63.

145. Gerdts V, Zakhartchouk A. Vaccines for porcine epidemic diarrhea virus and

other swine coronaviruses. Vet Microbiol. 2017;206:45-51.

146. Van Diep N, Sueyoshi M, Norimine J, Hirai T, Myint O, Teh APP, et al. Molecular characterization of US-like and Asian non-S INDEL strains of porcine epidemic diarrhea virus (PEDV) that circulated in Japan during 2013-2016 and PEDVs collected from recurrent outbreaks. BMC Vet Res. 2018;14(1):96.

147. Li R, Qiao S, Yang Y, Su Y, Zhao P, Zhou E, et al. Phylogenetic analysis of porcine epidemic diarrhea virus (PEDV) field strains in central China based on the ORF3 gene and the main neutralization epitopes. Arch Virol. 2014;159(5):1057-65.

148. Jung K, Saif LJ. Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis. Vet J. 2015;204(2):134-43.

149. Ciarlet M, Conner ME, Finegold MJ, Estes MK. Group A rotavirus infection and age-dependent diarrheal disease in rats: a new animal model to study the pathophysiology of rotavirus infection. J Virol. 2002;76(1):41-57.

150. Neog BK, Barman NN, Bora DP, Dey SC, Chakraborty A. Experimental infection of pigs with group A rotavirus and enterotoxigenic Escherichia coli in India: gross, histopathological and immunopathological study. Vet Ital. 2011;47(2):117-28.

151. Pott J, Stockinger S, Torow N, Smoczek A, Lindner C, McInerney G, et al. Age-dependent TLR3 expression of the intestinal epithelium contributes to rotavirus susceptibility. PLoS Pathog. 2012;8(5):e1002670.

152. Riepenhoff-Talty M, Lee PC, Carmody PJ, Barrett HJ, Ogra PL. Age-dependent rotavirus-enterocyte interactions. Proc Soc Exp Biol Med. 1982;170(2):146-54.

153. Bohl EH, Saif LJ, Theil KW, Agnes AG, Cross RF. Porcine pararotavirus: detection, differentiation from rotavirus, and pathogenesis in gnotobiotic pigs. J Clin Microbiol. 1982;15(2):312-9.

154. Vlasova AN, Amimo JO, Saif LJ. Porcine Rotaviruses: Epidemiology, Immune Responses and Control Strategies. Viruses. 2017;9(3).

155. Rahman M, Banik S, Faruque AS, Taniguchi K, Sack DA, Van Ranst M, et al. Detection and characterization of human group C rotaviruses in Bangladesh. J Clin Microbiol. 2005;43(9):4460-5.

156. Jiang B, Gentsch JR, Tsunemitsu H, Saif LJ, Glass RI. Sequence analysis of the gene encoding VP4 of a bovine group C rotavirus: molecular evidence for a new P

genotype. Virus Genes. 1999;19(1):85-8.

157. Kim SY, Song DS, Park BK. Differential detection of transmissible gastroenteritis virus and porcine epidemic diarrhea virus by duplex RT-PCR. J Vet Diagn Invest. 2001;13(6):516-20.

158. Diaz-Salinas MA, Romero P, Espinosa R, Hoshino Y, Lopez S, Arias CF. The spike protein VP4 defines the endocytic pathway used by rotavirus to enter MA104 cells. J Virol. 2013;87(3):1658-63.

159. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23(21):2947-8.

160. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30(12):2725-9.

161. Collaborators GBDDD. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect Dis. 2017;17(9):909-48.

162. Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Banyai K, Brister JR, et al. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Arch Virol. 2011;156(8):1397-413.

163. Khamrin P, Okitsu S, Ushijima H, Maneekarn N. Novel nonstructural protein 4 genetic group in rotavirus of porcine origin. Emerg Infect Dis. 2008;14(4):686-8.

164. Tuanthap S, Phupolphan C, Luengyosluechakul S, Duang-In A, Theamboonlers A, Wattanaphansak S, et al. Porcine rotavirus C in pigs with gastroenteritis on Thai swine farms, 2011-2016. PeerJ. 2018;6:e4724.

165. Winiarczyk S, Paul PS, Mummidi S, Panek R, Gradzki Z. Survey of porcine rotavirus G and P genotype in Poland and the United States using RT-PCR. J Vet Med B Infect Dis Vet Public Health. 2002;49(8):373-8.

166. Silva FD, Espinoza LR, Tonietti PO, Barbosa BR, Gregori F. Whole-genomic analysis of 12 porcine group A rotaviruses isolated from symptomatic piglets in Brazil during the years of 2012-2013. Infect Genet Evol. 2015;32:239-54.

167. Maes P, Matthijnssens J, Rahman M, Van Ranst M. RotaC: a web-based tool for the complete genome classification of group A rotaviruses. BMC Microbiol. 2009;9:238.

168. Choudhury B, Dastjerdi A, Doyle N, Frossard JP, Steinbach F. From the field to

the lab - An European view on the global spread of PEDV. Virus Res. 2016;226:40-9.

169. Madson DM, Magstadt DR, Arruda PH, Hoang H, Sun D, Bower LP, et al. Pathogenesis of porcine epidemic diarrhea virus isolate (US/Iowa/18984/2013) in 3-week-old weaned pigs. Vet Microbiol. 2014;174(1-2):60-8.

170. Shibata I, Tsuda T, Mori M, Ono M, Sueyoshi M, Uruno K. Isolation of porcine epidemic diarrhea virus in porcine cell cultures and experimental infection of pigs of different ages. Vet Microbiol. 2000;72(3-4):173-82.

171. Lowe J, Gauger P, Harmon K, Zhang J, Connor J, Yeske P, et al. Role of transportation in spread of porcine epidemic diarrhea virus infection, United States. Emerg Infect Dis. 2014;20(5):872-4.

172. Sasaki Y, Alvarez J, Sekiguchi S, Sueyoshi M, Otake S, Perez A. Epidemiological factors associated to spread of porcine epidemic diarrhea in Japan. Prev Vet Med. 2016;123:161-7.

173. Alonso C, Goede DP, Morrison RB, Davies PR, Rovira A, Marthaler DG, et al. Evidence of infectivity of airborne porcine epidemic diarrhea virus and detection of airborne viral RNA at long distances from infected herds. Vet Res. 2014;45:73.

174. Stott CJ, Temeeyasen G, Tripipat T, Kaewprommal P, Tantituvanont A, Piriyapongsa J, et al. Evolutionary and epidemiological analyses based on spike genes of porcine epidemic diarrhea virus circulating in Thailand in 2008-2015. Infect Genet Evol. 2017;50:70-6.

175. Zhang J, Chen Q, Gauger PC, Harmon KM, Yoon KJ. Reply to "classification of emergent U.S. strains of porcine epidemic diarrhea virus by phylogenetic analysis of nucleocapsid and ORF3 genes". J Clin Microbiol. 2014;52(9):3511-4.

176. Su Y, Hou Y, Prarat M, Zhang Y, Wang Q. New variants of porcine epidemic diarrhea virus with large deletions in the spike protein, identified in the United States, 2016-2017. Arch Virol. 2018;163(9):2485-9.

177. Van Diep N, Norimine J, Sueyoshi M, Lan NT, Hirai T, Yamaguchi R. US-like isolates of porcine epidemic diarrhea virus from Japanese outbreaks between 2013 and 2014. Springerplus. 2015;4:756.

178. Chang SH, Bae JL, Kang TJ, Kim J, Chung GH, Lim CW, et al. Identification of the epitope region capable of inducing neutralizing antibodies against the porcine epidemic

diarrhea virus. Mol Cells. 2002;14(2):295-9.

179. Teeravechyan S, Frantz PN, Wongthida P, Chailangkarn T, Jaru-Ampornpan P, Koonpaew S, et al. Deciphering the biology of porcine epidemic diarrhea virus in the era of reverse genetics. Virus Res. 2016;226:152-71.

180. Kaewborisuth C, He Q, Jongkaewwattana A. The Accessory Protein ORF3 Contributes to Porcine Epidemic Diarrhea Virus Replication by Direct Binding to the Spike Protein. Viruses. 2018;10(8).

181. Li W, Li H, Liu Y, Pan Y, Deng F, Song Y, et al. New variants of porcine epidemic diarrhea virus, China, 2011. Emerg Infect Dis. 2012;18(8):1350-3.

182. Kubota S, Sasaki O, Amimoto K, Okada N, Kitazima T, Yasuhara H. Detection of porcine epidemic diarrhea virus using polymerase chain reaction and comparison of the nucleocapsid protein genes among strains of the virus. J Vet Med Sci. 1999;61(7):827-30.

183. Marthaler D, Jiang Y, Collins J, Rossow K. Complete Genome Sequence of Strain SDCV/USA/Illinois121/2014, a Porcine Deltacoronavirus from the United States. Genome Announc. 2014;2(2).

184. Theuns S, Vyt P, Desmarets LMB, Roukaerts IDM, Heylen E, Zeller M, et al. Presence and characterization of pig group A and C rotaviruses in feces of Belgian diarrheic suckling piglets. Virus Res. 2016;213:172-83.

185. Amimo JO, Vlasova AN, Saif LJ. Detection and genetic diversity of porcine group A rotaviruses in historic (2004) and recent (2011 and 2012) swine fecal samples in Ohio: predominance of the G9P[13] genotype in nursing piglets. J Clin Microbiol. 2013;51(4):1142-51.

186. Ishimaru Y, Nakano S, Nakano H, Oseto M, Yamashita Y. Epidemiology of group C rotavirus gastroenteritis in Matsuyama, Japan. Acta Paediatr Jpn. 1991;33(1):50-6.

187. Annamalai T, Saif LJ, Lu Z, Jung K. Age-dependent variation in innate immune responses to porcine epidemic diarrhea virus infection in suckling versus weaned pigs. Vet Immunol Immunopathol. 2015;168(3-4):193-202.

188. Jeong YJ, Matthijnssens J, Kim DS, Kim JY, Alfajaro MM, Park JG, et al. Genetic diversity of the VP7, VP4 and VP6 genes of Korean porcine group C rotaviruses. Vet Microbiol. 2015;176(1-2):61-9.

189. Cook SM, Glass RI, LeBaron CW, Ho MS. Global seasonality of rotavirus

infections. Bull World Health Organ. 1990;68(2):171-7.

190. Levy K, Hubbard AE, Eisenberg JN. Seasonality of rotavirus disease in the tropics: a systematic review and meta-analysis. Int J Epidemiol. 2009;38(6):1487-96.

191. Sumi A, Rajendran K, Ramamurthy T, Krishnan T, Nair GB, Harigane K, et al. Effect of temperature, relative humidity and rainfall on rotavirus infections in Kolkata, India. Epidemiol Infect. 2013;141(8):1652-61.

192. Lin CM, Gao X, Oka T, Vlasova AN, Esseili MA, Wang Q, et al. Antigenic relationships among porcine epidemic diarrhea virus and transmissible gastroenteritis virus strains. J Virol. 2015;89(6):3332-42.

193. Bridgen A, Duarte M, Tobler K, Laude H, Ackermann M. Sequence determination of the nucleocapsid protein gene of the porcine epidemic diarrhoea virus confirms that this virus is a coronavirus related to human coronavirus 229E and porcine transmissible gastroenteritis virus. J Gen Virol. 1993;74 (Pt 9):1795-804.

194. Martel-Paradis O, Laurin MA, Martella V, Sohal JS, L'Homme Y. Full-length genome analysis of G2, G9 and G11 porcine group A rotaviruses. Vet Microbiol. 2013;162(1):94-102.

195. Greenberg HB, Estes MK. Rotaviruses: from pathogenesis to vaccination. Gastroenterology. 2009;136(6):1939-51.

196. Sun RQ, Cai RJ, Chen YQ, Liang PS, Chen DK, Song CX. Outbreak of porcine epidemic diarrhea in suckling piglets, China. Emerg Infect Dis. 2012;18(1):161-3.

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2. Intharasongkroh D, Sa-Nguanmoo P, Tuanthap S, Thongmee T, Duang-In A, Klinfueng S, Chansaenroj J, Vongpunsawad S, Theamboonlers A, Payungporn S, Chirathaworn C and Poovorawan Y. 2017. Hepatitis E Virus in Pork and Variety Meats Sold in Fresh Markets. Food Environ Virol 9(1):45-53. Epub 2016/09/02. doi:

10.1007/s12560-016-9258-0. PubMed PMID: 27580911.

3. Chansaenroj J, Tuanthap S, Thanusuwannasak T, Duang-In A, Klinfueng S, Thaneskongtong N, Vutithanachot V, Vongpunsawad S, and Poovorawan Y. 2017. Human enteroviruses associated with and without diarrhea in Thailand between 2010 and 2016. PloS one 12 (7):e0182078.

4. Tuanthap S, Phupolphan C, Luengyosluechakul S, Duang-In A, Theamboonlers A, Wattanaphansak S, Vongpunsawad S, Amonsin A and Poovorawan Y. 2018. Porcine rotavirus C in pigs with gastroenteritis on Thai swine farms, 2011-2016. PeerJ 6: e4724. 10.7717/peerj.4724

5. Tuanthap S, Vongpunsawad S, Luengyosluechakul S, Sakkaew P, Theamboonlers A, Amonsin A, and Poovorawan Y. 2019. Genome constellations of 24 porcine rotavirus group A strains circulating on commercial Thai swine farms between 2011 and 2016. PLoS One 14:e0211002. 10.1371/journal.pone.0211002.

6. Tuanthap S, Vongpunsawad S, Phupolphan C, Duang-in A, Wattanaphansak S, Assavacheep P, Theamboonlers A, Luengyosluechakul S, Amonsin A, Poovorawan Y. 2019. Analysis of the spike, ORF3, and nucleocapsid genes of porcine epidemic diarrhea virus circulating on Thai swine farms, 2011–2016. PeerJ 7:e6843. 10.7717/peerj.6843