Original Article

Molecular characterization and phylogenetic analysis of porcine epidemic diarrhea virus isolates in Eastern China

Chunyan Jiang¹, Xiaoju Zhang¹, Jianfeng Han², Haijian He¹, Chaoying Zhang¹, Hongbing Zhang¹, Jingjing Jin¹, Liang Wang³, Bingqian Ge¹, Yanli Wang¹, Yongjie Liu⁴

¹ Department of Animal Production, Faculty of Agriculture and Bioengineering, Jinhua Polytechnic, Jinhua, Zhejiang, China

² Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

³ Department of Agriculture and Forestry Engineering, Shanxi Yuncheng Agricultural Vocational And Technical College, Yuncheng, Shanxi, China

⁴ College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, Jiangsu, China

Abstract

Introduction: Porcine epidemic diarrhea virus (PEDV) is one of the most common viral pathogens causing swine diarrhea.

Methodology: We performed a genetic evolution analysis of the S1 gene of endemic PEDV strains in Eastern China. The S1 genes of 37 PEDVpositive samples were amplified and sequenced, and compared to the standard CV777 strain, 120 nucleotides were found to have mutations. Results: The nucleotide and deduced amino acid homologies between the sequences and those of the CV777 strain were 90%–91% and 88.2%– 90%, respectively, and their homologies to the vaccine strain were 88.6%–89.7% and 86.2%–87.8%, respectively. Genetic evolution and variation analyses indicated that the 37 PEDV strains belonged to genogroup 2-1, while the CV777 strain, vaccine strain, and earlier Chinese

strains all belonged to genogroup 1-1.

Conclusions: The newly emerged clinical PEDV strains indicate that the PEDV CV777 vaccine currently used in China may not fully protect pigs from infection with recent epidemic strains, and will require the development of new vaccine strains.

Key words: Swine diarrhea; porcine epidemic diarrhea virus; genetic evolution analysis; S1 gene.

J Infect Dev Ctries 2018; 12(12):1124-1133. doi:10.3855/jidc.9898

(Received 25 March 2018 - Accepted 21 November 2018)

Copyright © 2018 Jiang *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Diarrhea is one of the most significant diseases currently threatening the pig industry in China. The causes of diarrhea are complicated, and several pathogens can be involved. The common viral pathogens are porcine epidemic diarrhea virus (PEDV), porcine transmissible gastroenteritis virus (TGEV), and porcine rotavirus serotype A (GARV), all of which have similar clinical manifestations. PEDV has the highest infection and incidence rates among these pathogens, and causes acute enteritis and watery diarrhea in pigs. PEDV was first reported in 1978 in Belgium and the UK [1,2]. It was isolated from infected pigs for the first time in 1980 in China, and has since become quite common. Since October 2010, many provinces in China have witnessed outbreaks of diarrhea due to PEDV among pig herds [3], and PEDV has caused substantial economic losses. The pig industry in Eastern China has been greatly impacted by porcine diarrhea. PEDV is an RNA virus. The spike (S) protein of PEDV is a glycosylated protein that is involved in viral pathogenesis, which contains distinct S1 and S2 domains. The S protein attaches to host cellular receptors, resulting in viral entry by membrane fusion [4-7] and stimulating the production of neutralizing antibodies [4-8]. Mutations in the PEDV S gene are associated with growth adaptation in vitro and the attenuation of virulence in vivo [9,10]. Mutations play an important role in the structure of the S gene, and in determining isolate diversity, the actual epidemiological characteristics of the infection, and the incidence of epidemics causes by mutated strains [11-13]. S gene sequence analysis is critical for assessing the effectiveness of existing vaccines and for developing additional vaccines. Nucleotide sequence comparison has demonstrated that the S1 domain has a higher mutation rate than the S2 domain and is characterized by higher diversity. In addition, the S1 subunit can bind to specific receptors [14]. According to phylogenetic analyses of the S gene, the PEDV

strains were divided into two distinct clusters, genogroup 1 (G1; classical) and genogroup 2 (G2; field epidemic or pandemic) [12,15], and S-INDEL strains [16]. Most epidemic strains are in the G2 group [15]. The present study was performed to analyze S1 gene variations in prevalent PEDV strains and discovered new clinical strains which warrant the development of new vaccine strain.

Methodology

Sources of samples and viruses

A total of 387 samples were used for the diarrhea virus analysis, including feces and small intestine tissue, were collected from 387 individual diarrheic pigs on 38 pig farms in 4 regions of Eastern China (Zhejiang, Jiangsu, Fujian Provinces, as well as Shanghai) from April 2013 to January 2016. PEDVpositive samples were obtained from the Animal Disease Control Center laboratory of Jinhua Polytechnic. All animal work was approved by Jinhua Polytechnic School Animal Care and Use Committee and the methods were carried out in accordance with the approved guidelines.

Reagents, instruments and primers

PrimeScript One-Step reverse transcription polymerase chain reaction (RT-PCR) Kit Ver.2, RNAiso Plus (total RNA extraction reagent), TaKaRa Ex Taq Hot Start, and DL2000 DNA markers were purchased from Takara Bio Biotechnology (Dalian, China). Agarose were purchased from Sangon Biotech (Shanghai, China). Chloroform, isopropanol, and ethanol were purchased from Shanghai Chemical Reagent (Shanghai, China). The S1000TM Thermal Cycler, Bio-Rad Gel DocTM XR+ UV gel imaging analysis system, and the nucleic acid electrophoresis instrument were purchased from Bio-Rad (Hercules, CA, USA).

Nucleotide sequences of the PCR primers of PEDV-S1 were designed by State Key Laboratory of Agricultural Microbiology in China as follows: 5'-

Forward:

CCACCATGAAGTCTTTAACTTACTTCTGGT-3', Reverse: 5'-GAAATTGGCTGTTCATGACTC-3'. Correctly sized PCR products were 1497 bp, and were verified by agarose gel electrophoresis and DNA sequencing. The primers were synthesized by Suzhou Genewiz Bio Technology (Suzhou, China).

Sample pretreatment

The samples were collected from the carcasses of pigs that had died of known or suspected viral diarrhea.

Tissue segments of the small intestine (1-2 cm)were placed in homogenate tubes, treated with 250 µL of phosphate-buffered saline, and subjected to highspeed homogenization. This was followed by freezing and thawing three times and centrifugation at $13,400 \times$ g for 5 minutes. Finally, 200 µL of each supernatant was stored at -20°C until analyzed.

Each fecal sample was diluted 10-fold with 10 mM phosphate-buffered saline, vortexed, and centrifuged for 5 minutes at $13,400 \times g$. The supernatants were stored at -20°C.

RNA extraction

All supplies used for RNA extraction, including deionized water, were treated with 0.1% diethyl pyrocarbonate (DEPC) to avoid RNase contamination. Each liquid sample (200 μ L) was combined with 1 mL RNAiso Plus and RNA was extracted according to the manufacturer's instructions.

One-step RT-PCR

The extraction of PEDV template RNA was performed following the PrimeScript One-Step RT-PCR kit instructions. The reaction included 2 µL of PrimeScript One-Step enzyme mix (including PrimeScript reverse transcriptase, TaKaRa Ex Tag Hot Start, and RNase inhibitor), 25 µL of 2× One-Step buffer (including reaction buffer and dNTP mixture (final concentration, 400 µM)), 1 µL each of the forward and reverse primers (10 µM), 5 µL template RNA, and DEPC water to 50 µL.

The thermal cycling profile was as follows: 50°C for 30 minutes; 94°C for 2 minutes; 35 cycles of 94°C for 30 seconds, 54°C for 30 seconds, and 72°C for 1.5 minutes; and 72°C for 10 minutes. The PCR products were electrophoresed in 1.5% agarose gels.

Sequencing and sequence alignment of PCR products

The selection criteria for the studied samples: First of all, they must be PEDV-positive samples by rapid detection method [17] from 387 samples. Secondly, their PEDV S1 gene can be successfully amplified and sequenced. Thirdly, the regional representation was also considered. The PEDV S1-positive PCR products were sent to Suzhou Genewiz Bio Technology for sequencing, sequence alignment, and confirmation of the sequencing results using NCBI BLAST software. Gene sequences were analyzed with DNAMAN (LynnonBiosoft), MegAlign (DNASTAR, Inc.), Vector NTI 11.5 (Invitrogen Corporation), and MEGA (Molecular Evolutionary Genetics Analysis) 6.0 software.

Results

Amplification and analysis of S1 gene sequences

The PEDV-positive rate in the tested samples was 54.5% (211/387) by rapid detection method (Table 1). In this study, according to the selection criteria, 37 strains were finally established. The PEDV S1 genes from 37 selected PEDV-positive samples were amplified by RT-PCR. The amplified S1 sequences were 1494–1497 nucleotides (nt) long; these sequences encoded amino acids (aa) 1–497 and were positioned between nt 20634 and 22131 of the total PEDV S gene sequence. The S1 sequences were compared on the NBCI website, and the target sequence was confirmed.

The 37 epidemic strains from Eastern China were compared with the CV777 and vaccine strains using BioEdit software, and comparatively, nucleotide mutations, insertions, and deletions were observed in all epidemic strains. A 1-nt gene deletion occurred at 217 (A), and a 6-nt deletion occurred at 475–480 (CGTGAT). One-nucleotide gene insertions occurred at 166 (G) and 205 (G), and an 11-nt insertion occurred at 171–181 (AACCAGGGTGT). With the exception of strain SX-2013, all of the strains had a 3 nt (ATA) gene insertion at 416–418 (Figure 1).

Table 1. The PEDV-	positive/negative san	nple number and 37	epidemic PEDV	strains selected.
	poblici e megani e ban		oprovinno i LLD -	

Farms No.	Farms Name	Samples number	Positive number	Negative number	Selected strain number	Selected virus strain name
1	LS	12	3	9	1	LS-2013
2	JX	12	10	2	1	JX-2013
3	JSA	12	4	8	2	JS-2013-1, JS-2013-2
4	YF	10	8	2	0	
5	HZA	13	5	8	3	HZ-2013-1, HZ-2013-2, HZ-2013-3
6	JK	10	4	6	0	
7	GL	9	2	7	0	
8	QZ	9	4	5	1	QZ-2013
9	XSA	10	8	2	3	XS-2013-1, XS-2013-2, XS-2013-3
10	JC	9	5	4	0	
11	ZM	10	6	4	0	
12	CX	11	9	2	1	CX-2013
13	DQ	9	7	2	1	DQ-2013
14	SX	14	6	8	1	SX-2013
15	SH	7	4	3	1	SH-2013
16	LYA	8	5	3	1	LY-2013
17	NB	8	6	2	1	NB-2014
18	HZB	10	10	0	2	HZ-2014-1, HZ-2014-2
19	QF	10	8	2	0	
20	YY	10	6	4	1	YY-2014
21	ZJ	11	7	4	1	ZJ-2014
22	JSB	11	6	5	1	JS-2014
23	JDA	8	6	2	2	JD-2014-1, JD-2014-2
24	ZQ	10	4	6	0	
25	WC	10	5	5	2	WC-2014-1, WC-2014-2
26	DJ	15	3	12	0	
27	LYB	8	4	4	1	LY-2014
28	DX	9	6	3	2	DX-2015-1, DX-2015-2
29	RM	10	4	6	0	
30	XSB	12	7	5	2	XS-2015-1, XS-2015-2
31	HF	10	7	3	0	
32	LX	10	6	4	1	LX-2015
33	PJ	12	5	7	2	PJ-2015-1, PJ-2015-2
34	LK	12	4	8	0	
35	LYC	11	5	6	2	LY-2015-1, LY-2015-2
36	JZ	8	5	3	0	
37	JDB	12	6	6	1	JD-2015
38	HH	5	1	4	0	
Total		387	211	176	37	

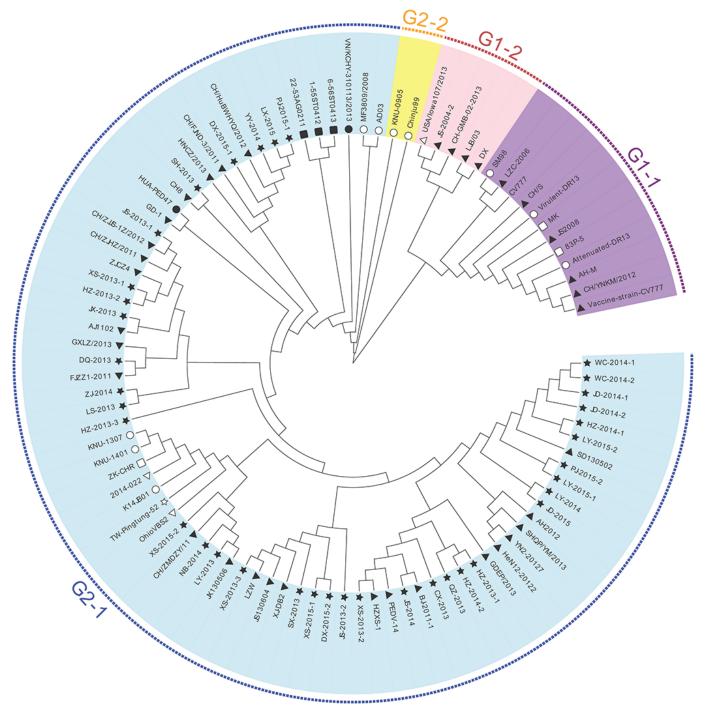
Figure 1. Analysis and	comparison of the	nucleotide sequence	s of the S1	genes of 37 epidemic strains.

			170) 180	190	200	210	220	230 240
			1			II]] .	.	
CV777 vaccine CV777	161 161	TAGTA-	IGAA		CTCTTCTAGCTGG				GCGTTCATGGTATT 2:
SX-2013	161	TGG	••••	ACCAGGGTGT	AA A. CT		CAACC.		
XS-2013-3	161	T.GG		ACCAGGGTGT	.AAA. CT	CTGG	.CAACC		C 2
NB-2014	161	T.GG		AAACAGGGTGT	.AAA.CT	стсс	.CAACC		C 2
SH-2013	161	T.GG		AACCAGGGTGT AACCAGGGTGT		стес			C 2
HZ-2014-1 JD-2014-1	161 161	T .GG		ACCAGGGTGT AACCAGGGTGT		CTGG			
JD-2014-1	161	.T.GG		AACCAGGGTGT		СТСС			
WC-2014-1	161	T.GG		AACCAGGGTGT	.AAA.CT				C 2
WC-2014-2	161	T.GG		AACCAGGGTGT		стес			C 2
LY-2014 DX-2015-1	161 161	о.т. С.		AACCAGGGTGT AACCAGGGTGT		CTGG			
DX-2015-1 DX-2015-2	161			ACCAGGGTGT		CTGG			
XS-2015-1	161	.T.GG		AACCAGGGTGT		CTGG			
XS-2015-2	161	T.GG		AACCAGGGTGT		стсс			C 2
LX-2015	161	T.GG		AACCAGGGTGT	TAAA.CT	CTGG	.CAACC		C 2
PJ-2015-1 PJ-2015-2	161 161	T.G G		AACCAGGGTGT AACCAGGGTGT	TAAA.CT	CTGG	.CAACC		
LY-2015-1	161	T.GG		ACCAGGGTGT		CTGG			
LY-2015-2	161	T.GG		AACCAGGGTGT	.AAA. CT	Стес	.CAACC		C 2
JD-2015	161	T.GG		AACCAGGGTGT	.AAA.CT	стес	.CAACC		c 2
CX-2013 JX-2013	161 161	T .GG		AACCAGGGTGT AACCAGGGTGT	AA.A.CT	CTGG			
HZ-2013-2	161	T.GG		ACCAGGGTGT AACCAGGGTGT		CTGG			
HZ-2013-1	161	.T.GG		ACCAGGGTGT	.AAA.CT	Сте	.CAACC		
HZ-2013-3	161	T.GG		AACCAGGGTGT		Стсс			C 2
JS-2013-1	161	T.GG		ACCAGGGTGT		Стес			C 2
JS-2013-2 QZ-2013	161 161	T.GG		AACCAGGGTGT	AAA.CT	Стес			
XS-2013-1	161	T.GG		ACCAGGGIGI		CTCG			
XS-2013-2	161	T.GG		AACCAGGGTGT	.AA A. CT	Стс	.CAACC		C 2
YY-2014	161	T.GG		AACCAGGGTGT	TAAA.CT	Стс	.CAACC		c 2
LY-2013 DQ-2013	161 161	Т.GG		AACCAGGGTGT AACCAGGGTGT		CTGG			
LS-2013	161	T.GG		ACCAGGGTGT		CTGG			
ZJ-2014	161	.T.GG		AACCAGGGTGT	.AAA.CT	Стсс	.CAACC		c 2
HZ-2014-2	161	T.GG		AACCAGGGTGT	.AAA.CT	стсс	.CAACC		C 2
JS-2014	161	T.GG		AACCAGGGTGT	.AAA.CT	omdo	CAACC		C 2
					•				
			410		430	440	450	460	470 480
	200		1		430 • • • • • • • • • • •	440	450	460	470 480
CV777 Vaccine CV777	388 388	TGGGCC	1	IGTTAATG	430 ATGTTACAACAGG	440	450 	460 . GCCATTCCAG	470 480
CV777 vaccine CV777 SX-2013	388 388 400		1	IGTTAATG	430 • • • • • • • • • • •	440 TCGTAACTGCC	450 	460 . SCCATTCCAG	470 480
vaccine CV777 SX-2013 XS-2013-3	388 400 400	TGGGCC	1	GTTA	430 ATGTTACAACAGG	440 TCGTAACTGCC T T	450 II. TATTCAACAAAG C TT.	460 	470 480 CTTATATCCGTGAT 41
vaccine CV777 SX-2013 XS-2013-3 NB-2014	388 400 400 400	TGGGCC	CTAC	ГGTTA АТG 	430 ATGTTACAACAGG	440	450 . TATTCAACAAA(C T T T	460 	470 480
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013	388 400 400 400 400	TGGGCC	CTAC	ГGTTA АТG АТА АТА АТА АТА	430 I.I.I. ATGTTACAACAGG	440 TCGTAACTGCC T. T. T.	450 	460 	470 480 CTTATATGCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014	388 400 400 400	TGGGCC	CTAC	ГGTTA — АТG АТА АТА АТА АТА АТА АТА	430 ATGTTACAACAGG	440 TCGTAACTGCC T 	450 TATTCAACAAA(.CT T T T T T	460 	470 480
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2	388 400 400 400 400 400 400 400	TGGGCC	CTAC	IGTTA	430 . ATGTTACAACAGG	440 TCGTAACTGCC T. T. T. T. T. T.	450 TATTCAACAAA(460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-1	388 400 400 400 400 400 400 400 400	TGGGCC	I CTAC	IGTTA	430 I.I.I. ATGTTACAACAGG	440 TCGTAACTGCC T. T. T. T. T. T. T. T. T.	450 TATTCAACAAAO .C. .T. .T. .T. .T. .T. .T. .T.	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-1 WC-2014-2	388 400 400 400 400 400 400 400 400 400	TGGGCC	I CTAC	IGTTZ ATG .C. ATA	430 II	440 1	450 TATTCAACAAA(C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-1	388 400 400 400 400 400 400 400 400	TGGGCC	I CTAC	IGTTZ ATG .C. ATA	430 I.I.I. ATGTTACAACAGG	440 1	450 TATTCAACAAA(C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-1 WC-2014-2 LY-2014 DX-2015-1 DX-2015-2	388 400 400 400 400 400 400 400 400 400 4	TGGGCC	I CTAC 	IGTTA ATG IGTTA ATG	430 I.I.I. ATGTTACAACAGG	440 1	450 TATTCAACAAAO .C. .T. .T. .T. .T. .T. .T. .T.	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-2 LY-2014 UX-2015-1 DX-2015-2 XS-2015-1	388 400 400 400 400 400 400 400 400 400 4	TGGGCC	I CTAC	IGTTZ ATG IC. ATA .C. ATA	430 I.I.I.ATGTTACAACAGG	440 1	450 TATTCAACAAA C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-2 WC-2014-2 WC-2014-2 LY-2014 DX-2015-1 DX-2015-1 XS-2015-1 XS-2015-2	388 400 400 400 400 400 400 400 400 400 4	TEGECC	I CTAC	IGTTZ ATG IC	430 ATGTTACAACAGG		450 TATTCAACAAA(C	460 	470 480 CTTATATCCGTGAT 44
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-2 LY-2014 UX-2015-1 DX-2015-2 XS-2015-1	388 400 400 400 400 400 400 400 400 400 4	TGGGCC	I CTAC .C .C .C .C .C .C .C .C .C .C .C .C .C .C .C .C .C .C	IGTTZ ATG IC. ATA C. ATA	430 I.I.I.ATGTTACAACAGG	440 1	450 	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-1 WC-2014-2 LY-2014 DX-2015-1 DX-2015-1 ZS-2015-1 PJ-2015-1 PJ-2015-2	388 400 400 400 400 400 400 400 400 400 4	TEGGCC	I CTAC .C	IGTTA	430 I.I.I.ATGTTACAACAGG		450 TATTCAACAAA C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 41
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-2 LY-2014 WC-2014-2 LY-2014 DX-2015-1 DX-2015-2 LXS-2015-2 LX-2015 PJ-2015-2 LY-2015-1	388 400 400 400 400 400 400 400 400 400 4	TEGGCC	I CTAC 	IGTTZ ATG IC	430 ATGTTACAACAGG		450 TATTCAACAAA(C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-2 LY-2014 DX-2015-1 DX-2015-2 LX-2015-1 PJ-2015-2 LY-2015-1 LY-2015-2	388 400 400 400 400 400 400 400 400 400 4	TGGGCC	I CTAC 	IGTTZ	430 I.I.I ATGTTACAACAGG		450 	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-2 WC-2014-2 LY-2015 DX-2015-1 DX-2015-1 PJ-2015-2 LX-2015 PJ-2015-2 LY-2015-2 LY-2015-2 JD-2015	388 400 400 400 400 400 400 400 400 400 4	TEGGCC	I CTAC C C C C C C C	IGTTA	430 ATGTTACAACAGG		450 TATTCAACAAAO .C. .T. .T. .T. .T. .T. .T. .T.	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-2 LY-2014 DX-2015-1 DX-2015-2 LX-2015-1 PJ-2015-2 LY-2015-1 LY-2015-2	388 400 400 400 400 400 400 400 400 400 4	TGGGCC	I CTAC 	IGTTZ	430 ATGTTACAACAGG		450 TATTCAACAAA(C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 LY-2014-1 WC-2014-2 LY-2014-1 WC-2014-2 LY-2015-1 DX-2015-2 LX-2015-1 PJ-2015-2 LY-2015-2 LY-2015-2 JD-2015 CX-2013 HZ-2013-2	388 400 400 400 400 400 400 400 400 400 4	TGGGCC	I CTAC .C	IGTTZ	430 I.I.I ATGTTACAACAGG		450 	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-2 WC-2014-2 LY-2014 DX-2015-1 DX-2015-1 PJ-2015-2 LX-2015 PJ-2015-2 LY-2015 CX-2013 JX-2013 HZ-2013-2 HZ-2013-1	388 4000 4000 4000 400 4000 400 400 400 400	TEGGCC	I CTAC 	IGTTZ	430 I I I ATGTTACAACAGG		450 TATTCAACAAAO .C. .T. .T. .T. .T. .T. .T. .T.	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-2 WC-2014-1 WC-2014-2 LY-2014 DX-2015-1 DX-2015-1 DX-2015-2 LX-2015-1 PJ-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 LY-2015-2 LY-2015-2 LY-2015-2 LY-2015-1 LY-2015-2 LY-2013-2 LY-2	$388 \\ 400 $	TEGGCC	I CTAC 	IGTTZ	430 I.I.I.ATGTTACAACAGG		450 TATTCAACAAAG C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-2 WC-2014-2 LY-2014 DX-2015-1 DX-2015-1 PJ-2015-2 LX-2015 PJ-2015-2 LY-2015 CX-2013 JX-2013 HZ-2013-2 HZ-2013-1	388 4000 4000 4000 400 4000 400 400 400 400	TGGGCC	I CTAC 	IGTTZ	430 I I I ATGTTACAACAGG		450 TATTCAACAAA C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 WC-2014-2 WC-2014-2 WC-2014-2 LY-2014 DX-2015-1 DX-2015-1 PJ-2015-2 LX-2015 PJ-2015-2 LY-2015-1 PJ-2015-2 LY-2015-2 LY-2015-2 LY-2015-2 LY-2015-2 LY-2015-2 LY-2015-2 JD-2015 CX-2013 JX-2013 HZ-2013-2 HZ-2013-2 JS-2013-2 QZ-2013	$388 \\ 400 $	TEGGCC	I CTAC 	IGTTZ	430 I I I ATGTTACAACAGG		450 TATTCAACAAAG .C. .T. .T. .T. .T. .T. .T. .T.	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-2 WC-2014-2 LY-2014 DX-2015-1 DX-2015-1 DX-2015-2 LX-2015-1 PJ-2015-2 LY-2015-1 LY-2015-2 LY-2013-2 LY-2013-3 JS-2013-1 JS-2013-2 LY-2	$388 \\ 400 $	TEGGCC	I CTAC 	IGTTZ	430 I.I.I.ATGTTACAACAGG		450 TATTCAACAAAG C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-2 WC-2014-1 WC-2014-2 LY-2014 WC-2014-2 LY-2015-1 DX-2015-1 PJ-2015-2 LX-2015-1 PJ-2015-2 LY-2015-1 LY-2015-1 LY-2015-2 LY-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 LY-2015-2 LY-2015-1 LY-2015-2 LY-2013-1 LY-2	$388 \\ 400 $		I CTAC 	IGTTZ	430 ATGTTACAACAGG		450 TATTCAACAAA C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-2 WC-2014-2 WC-2014-2 WC-2014-2 LY-2015-1 DX-2015-1 DX-2015-1 PJ-2015-2 LX-2015 PJ-2015-2 LY-2015 CX-2013 JZ-2013-2 HZ-2013-1 HZ-2013-2 HZ-2013-1 JS-2013-2 QZ-2013 XS-2013-1 XS-2013-2 YY-2014	$388 \\ 400 $	TGGGCC	I CTAC .C	IGTTZ	430 .I.I.I ATGTTACAACAGG		450 TATTCAACAAAO .C. .T. .T. .T. .T. .T. .T. .T.	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-2 WC-2014-2 LY-2014-2 WC-2014-2 LY-2014-2 LY-2015-1 DX-2015-2 XS-2015-1 PJ-2015-2 LY-2015-1 PJ-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 JD-2015 CX-2013 JX-2013 JX-2013-1 JS-2013-1 JS-2013-1 XS-2013-1 XS-2013-1	$\begin{array}{c} 388\\ 400\\ 400\\ 400\\ 400\\ 400\\ 400\\ 400\\ 4$		I CTAC 		430 ATGTTACAACAGG		450 TATTCAACAAAG C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-1 WC-2014-2 LY-2014 DX-2015-1 DX-2015-1 DX-2015-2 LX-2015-1 PJ-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 JD-2015 CX-2013 JX-2013 JX-2013-1 JS-2013-1 JS-2013-1 XS-2013-1 XS-2013-1 XS-2013-1 XS-2013-1 XS-2013-1 XS-2013-1 XS-2013-2 YY-2014 LY-2013 DQ-2013 LS-2013	$\begin{array}{c} 388\\ 400\\ 400\\ 400\\ 400\\ 400\\ 400\\ 400\\ 4$			IGTTZ ATG IGTTZ ATG C. ATA C. ATA	430 ATGTTACAACAGG		450 TATTCAACAAA C. T. T. T. T. T. T. T. T. T. T	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-2 WC-2014-2 WC-2014-2 WC-2014-2 LY-2014 DX-2015-1 DX-2015-1 DX-2015-2 LX-2015 PJ-2015-2 LY-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 JD-2015 CX-2013 JX-2013-1 HZ-2013-1 JS-2013-2 QZ-2013 XS-2013-2 QZ-2013 XS-2013-2 YY-2014 LY-2013 LS-2014 LS-2014 LS-2013 LS-2013 LS-2013 LS-2013 LS-2013 LS-2013 LS-2014	$\begin{array}{c} 388\\ 400\\ 400\\ 400\\ 400\\ 400\\ 400\\ 400\\ 4$		I CTAC C C C C C C C	IGTTZ	430 .I.I.I ATGTTACAACAGG		450 TATTCAACAAAO .C. .T. .T. .T. .T. .T. .T. .T.	460 	470 480 CTTATATCCGTGAT 4
vaccine CV777 SX-2013 SX-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 WC-2014-1 WC-2014-2 LY-2014 DX-2015-1 DX-2015-1 DX-2015-2 LX-2015-1 PJ-2015-2 LY-2015-1 LY-2015-2 LY-2015-1 LY-2015-2 JD-2015 CX-2013 JX-2013 JX-2013-1 HZ-2013-3 JS-2013-1 JS-2013-2 QZ-2013 XS-2013-1 XS-2013-2 YY-2014 LY-2013 DQ-2013 DQ-2013 LS-2013	$\begin{array}{c} 388\\ 400\\ 400\\ 400\\ 400\\ 400\\ 400\\ 400\\ 4$		I CTAC C .c. .c. .c. .c. .c. .c. .c. .c.	IGTTZ	430 ATGTTACAACAGG		450 TATTCAACAAAG .C. .T. .T. .T. .T. .T. .T. .T.	460 	470 480 CTTATATCCGTGAT 4

Most of the strains contained the following mutations: ten bases from G to A (G4A, G80A, G208A, G209A, G256A, G388A, G481A, G590A, G1066A, and G1362A), 29 bases from T to C (T13C, T14C, T71C, T86C, T144C, T149C, T213C, T240C, T250C, T303C, T342C, T390C, T408C, T413C, T462C, T469C, T531C, T600C, T630C, T636C, T742C,

T753C, T1074C, T1082C, T1086C, T1107C, T1188C, T1245C, and T1458C), 30 bases from C to T (C42T, C50T, C78T, C92T, C137T, C140T, C191T, C203T, C246T, C252T, C255T, C336T, C386T, C450T, C550T, C559T, C603T, C642T, C678T, C710T, C801T, C903T, C921T, C987T, C1125T, C1320T, C1356T, C1464T, C1467T, and C1491T), four bases

Figure 2. Phylogenetic tree of the S1 genes of 37 epidemic and reference PEDV strains. The 37 endemic PEDV strains are marked by black stars, Vietnam strains by black circles, Thailand strains by black squares, Chinese local strains isolated in recent years by black triangles, Japanese strains by white squares, South Korea strains by white circles, Taiwan strains by white star, and American and Canadian strains by white triangles. Virus CV777 is the Chinese strain JN599150, attenuated by cell passage.



from A to C (A79C, A207C, A683C, and A900C), seven bases from T to G (T81G, T259G, T491G, T741G, T811G, T945G, and T1375G), eight bases from C to A (C85A, C134A, C184A, C601A, C632A, C840A, C909A, and C951A), 12 bases from A to G (A128G, A165G, A204G, A257G, A389G, A484G, A591G, A604G, A607G, A631G, A792G, and A822G), four bases from T to A (T143A, T183A, T188A, and T258A), four bases from G to T (G163T, G267T, G297T, and G535T), seven bases from G to C (G190C, G202C, G214C, G487C, G688C, G915C, and G943C), two bases from C to G (C244G and C260G), and three bases from A to T (A392T, A483T, and A666T).

Genetic evolution analysis of PEDV epidemic strains

Fifty-eight S1 genes were selected from the GenBank database as reference sequences for genetic evolution analysis. Mega6.0 software was used to construct a neighbor joining phylogenetic tree. Genogroup 2 (G2; field epidemic or pandemic) consisted of 77 strains. G2-1 included the 37 endemic strains, 24 strains isolated from China in the last five years, a strain from the USA (OhioVBS2), a Canadian strain (2014-022), a Japanese strain (ZK-CHR), five Korean strains (K14JB01, KNU-1401, KNU-1307, MF3809/2008, and AD03), two Vietnamese strains (VN-KCHY-310113-2013 and HUA-PED47), three Thai strains (22-53AG0211, 1-55ST0412, and 6-56ST0413), and one Taiwanese strain (TW-Pingtung-52). G2-2 consisted of two Korean strains, Chinju99 and KNU-0905.

Genogroup 1 (G1; classical) consisted of 17 reference strains. G1-1 was composed of two Japanese strains (MK, 83P-5), three Korean strains (SM98, virulent Korea DR13, and attenuated DR13), the standard PEDV strain (CV777), the vaccine strain CV777, and five strains isolated in China (LZC-2006, CH/S, JS2008, AH-M, and CH/YNKM/2012). G1-2 contained one American strain (Iowa107) and four Chinese strains (JS-2004-2, CH-GMB-02-2013, LJB/03 and DX; Figure 2).

Nucleotide and amino acid homology analyses of the PEDV partial S1 gene

Among the endemic and reference strains, the nucleotide sequence homology was 96.12% integrally. The homologies between the 37 endemic strains and the CV777 reference and vaccine strains were 90%–91% and 88.6%–89.7%, respectively. The 37 endemic strains had the highest homologies to the reference strains K14JB01 (Korea), TW-Pingtung-52 (Taiwan), 2014-022 (Canada), and OhioVBS2 (USA; all 99.3%).

However, homology was lowest with the vaccine CV777 strain, at only 88.6%–89.7%. The homology to the reference strain JS2008, which was isolated in China in 2008, was 88.9%–90.1%, while the homology between the three strains isolated from China more recently, AH2012, BJ-2011-1, and PEDV-14, was 97.1%–100%.

A similar phenomenon was observed at the amino acid level. The homology of the amino acid sequences was 95.71% as a whole. The 37 endemic strains had highest homologies to the reference strains K14JB01 (Korea) and OhioVBS2 (USA; both 99.8%), whereas homology to the vaccine CV777 strain was lower, at 86.2%–87.8%.

Analysis of the deduced amino acid sequences of the partial SI gene

In this study, the amplified partial S1 gene was 1497 nt, encoding 495-497 aa. The PJ-2015-1, PJ-2015-2, and LY-2015-1 genes encoded 495 aa proteins, the SX-2013, XS-2015-2, LX-2015, LY-2015-2, JD-2015, and DX-2015-1 genes encoded 496 aa proteins, and all other strains encoded 497 aa proteins. DNAMAN software was used to analyze the aa results, and as shown in Figure 3, compared with the CV777 reference strain, the epidemic strains had a total of 44 mutation sites, including five aa insertions (NQGV inserted at 57-60 and N inserted at 143), and two aa deletions (at 166–167). The S1 gene had five hypervariable regions: at 26-28 (from QST to SAN), at 54-56 (from SMN to IGE), at 67-71 (from GTGIE to AGQHP), at 160-167 (from YMRDGKDI to HMSEHS--), and at 204-206 (from RRS to SGG); two two-aa mutations (at 133-134 from DN to SI, at 250-251 from DS to EP); and a number of single aa mutations (I4T, I123T, P14S, S61N, S63T, L81V, Y83H, D85R, V141A, A182S, H187Y, L190F, L350F, R200K, T214E, Y231S, T240I, D318Q, E373Q, and S462A). In addition, the 37 epidemic strains had different aa mutation sites and deletions. The deletion and insertion sites were similar to those of K14JB01 (the Korean strain) and OhioVBS2 (the American strain).

Prediction of S1 protein glycosylation sites and epitopes

The N-glycosylation sites of the S1 protein from the 37 strains were predicted using NetNGlyc 1.0(CBS Prediction Servers) [18]. S1 protein of CV777 vaccine strain has 11 N-glycosylation sites (56NSSS, 126NKTL, 228NCSG, 111NTSA, 211NVTS, 319NDTF, 259NDST, 295NQTM, 339NLSF, 346NSSD, 420NFTG). Compared with the CV777

vaccine strain, in SX-2013 S1 protein, six aa mutations (N56E, S113N, N126I, S230I, M298I, and F322S) and one insertion (NQGV at 57–60) resulted in the destruction of the N-glycosylation site. And the mutation of six other aa (S61N, S63T, S117N, I119T, F326S, and K380N) increased the number of N-glycosylation sites. Figure 4 shows 10 predicted N-glycosylation sites (61NSTW, 117NATA, 214NVTS, 262NDST, 298NQTI, 322NDTS, 342NFSF, 349NSSN, 379NSTV, 423NFTG) of the SX-2013 S protein.

The signal peptide cleavage site and number were predicted to check whether signal peptide has been affected which is relevant to the protein's targeting at virus surface using SignalP 4.1 (CBS Prediction Servers) [19] online signal peptide prediction software. The results showed that the 37 epidemic strains and the CV777 standard strain all contained only one signal peptide. The signal peptide of the PJ-2015-1 strain spanned aa l–26, with the cleavage site at aa 26; the rest of the strains contained signal peptides from aa l–19, with cleavage at aa 19.

Discussion

The major symptom of diarrheic piglets caused by PEDV is watery, gray-yellow, or gray diarrhea, which is sometimes preceded by vomiting. Dehydration in piglets is serious, with death occurring within 2–4 d, and a nearly 100% mortality rate. In a laboratory epidemiological survey, porcine epidemic diarrhea (PED) was much more prevalent from November to January in 2010–2012 [20]. The incidence of PEDV in piglets was 10%–100%, and the mortality rate reached 40%–92.58% on some PEDV-exposed pig farms. In the present study, the prevalence of PEDV was as high as 54.5%, and was the main viral pathogen for diarrhea in Eastern China, consistent with reports on PEDV in other provinces in the past five years [21-23].

In this study, G2 was divided into two subgroups including G2-1 and G2-2 by evolutionary tree software based on certain algorithms, reflecting the genetic distance and the branch of evolution. G2-2 consisted of two Korean strains, Chinju99 and KNU-0905.The 37 strains were concentrated in the G2-1 subgroup, and all contained mutations, deletions, and insertions (mainly

Figure 3. Analysis and comparison of the amino acid sequences of the S1 genes of 37 epidemic strains.

8									
CV777	PST. IV PWLLIPVIL	TISLPODUTRCOST	FRRFFSKFNVQAPAVVVLGGYLPS	MN SSSWYCCTC	TERASGUNGTELSYT	DSGOGFETG	SCYOLVLHKATN	ATARLETCO	122
vaccine CV777			FRRFFSKFNVQAPAVVVLGGYLPS						122
SX-2013			FRRFFSKFNVQAPAVVVLGGYLPI						126
XS-2013-3	KGI WYFWI FI DVI	TISLPODUTROSANTN	FRRFFSKENVQAPAVVVLGGYLPI	GENOGUNSTWYCAGO	HPTASCVHGIEVSHI	RCHGFEIGISOEPEDP	SCYOLVI-HKATN CNTN	ATARLETCO	126
NB-2014			FRRFFSKENVQAPAVVVLGGYLPI						126
SH-2013	CINVENT DIDUI	TICI PODUTRCEANTN	FREFESKENVQAPAVVVLGGYLPI	CENCCUNSTWYC ACC	UP DACCUUCTEI CHT	RECHEFFICIE OF PERE	SCYOLVI UKATN DNIM	ARADIPICO	126
HZ-2014-1			FRQFFSKFNVQAPAVVVLGGYLP						126
JD-2014-1	CINVENT PIDUI	TICLEODUTRCEANTN	FREFSKENVQAPAVVVLGGYLPI	CENCCUNCTWYCho	HETASGVHGIEVSHI	CHCEFTCTCOFPEDD	SCYOLVI UKATN CNTH	ADDIDICO	126
WC-2014-2	CITY FUI DI DUI	TICIDODUTRCEANTN	FRRFFSKENVOAPAVVVLGGYLPI	CENCOUNS TWICKOU	HPTACCUUCTEVCHT	CHCEFTCTCOFPEDD	SCYOLYL HYDEN	ADDIDICO	126
LY-2014-2			FREFESKENVQAPAVVVLGGYLPI						126
DX-2015-1			FRRFFSKENVQAPAVVVLGGYLPI						126
XS-2015-2			FRRFFSKFNVQAPAVVVLGGYLPI						126
LX-2015			FRRFFSKENVQAPAVVVLGGYLPI						126
PJ-2015-1			FRRFFSKENVQAPAVVVLGGYLPI						125
LY-2015-2			FROFFSKENVOAPAVVVLGGYLPI						126
JD-2015			FRRFFSKENVQAPAVVVLGGYLPI						126
CX-2013	KOL WY FWI FI PUL	TISL PODUTROSANTN	FRRFFSKENVQAPAVVVLGGYLPI	GENOGUNSTWYCAGO	HPTASCVHCIEVSHI	RCHCFEIGISOEPEDP	SCYOLVLHKATN CNTN	ATARLETCO	126
JX-2013			FRRFFSKENVQAPAVVVLGGYLPI						126
HZ-2013-2	KGT TV FWI FI DVI	TISL PODUTROSANTN	FREFESKENVQAPAVVVLGGYLPI	GENOGUNSTWYCAGO	HPTASCVHCIEVSHI	RCHGFFIGTSOFPEDP	SCYOLVLHKATN CNTN	ATARLETCO	126
HZ-2013-1			FRRFFSKFNVQAPAVVVLGGYLPI						126
JS-2013-1			FRRFFSKENVQAPAVVVLGGYLPI						126
0Z-2013	KST. TYFWI. FLPVI	TISLPODUTRCSANTN	FRRFFSKFNVQAPAVVVLGGYLPI	GENOGUNSTWYCAGO	HPTASGVHGTEVSHT	REGREETETS	SCYOLYLHKATN CNTN	ATARLETCO	126
YY-2014	KSLTYFWLFLPVL	TLSLPODVTRCSANTN	FRRFFSKFNVQAPAVVVLGGYLPI	GENOGVNSTWYCAGO	HPTASGVHGIFLSHI	RGGHGFEIGISOEPEDP	SGYOLYLHKATN GNTN	ATARLETCO	126
LY-2013	KSLTYFWLFLPVL	TLSLPODUTRCSANTN	FRRFFSKFNVQAPAVVVLGGYLPI	GENOGVNSTWYCAGO	HPTASGVHGIEVSHI	RGGHGFEIGISOEPEDP	SGYOLYLHKATN GNAN	ATARLETCO	126
DO-2013			FRRFFSKFNVQAPAVVVLGGYLPI						126
LS-2013			FRRFFSKFNVQAPAVVVLGGYLPI						126
ZJ-2014			FRRFFSKFNVQAPAVVVLGGYLPI						126
JS-2014	KSLTYFWLFLPVL	TLSLPODVTRCSANTN	FRRFFSKFNVQAPAVVVLGGYLPI	GENOGVNSTWYCAGO	HPTASGVHGIFVSHI	RGGHGFEIGISOEPFDP	SGYOLYLHKATN GNTN	ATARLEICO	126
Consensus	l vfwl lpvl	tllq trc n	fr ffskfnvgapavvvlggylp	s wyc	sgvhgif s i		sqyqlylhkatn n	a arlricq	
Consensus						g gfeig qepfdp		a arlricq	
CV777	FPDNKTLGPTVN.I	DVTTGRNCL <mark>F</mark> NKAIPAY	RDGKDIVVGITWDNDRVTVFADK	IYHFYLKNDWSRVA	RCYNRRSCAMO	g gfeig qepfdp PTYY <mark>M</mark> LNVTSAGEDGIY	YEPCTANCTGYAANVFATDS	a arlricq NGHIPEGFS	251
CV777 vaccine_CV777	FPDNKTLGPTVN.I FPDNKTLGPTVN.I	DVTTGRNCL <mark>F</mark> NKAIPAY DVTTGRNCLSNKAIPA.	RDGKDIVVGITWDNDRVTVFADK LQDGKNIVVGITWDNDRVTVFADK	IYHFYLKNDWSRVA1 IYHFYIKNDWSRVA1	TRCYNRRSCAMO <mark>YV</mark> YT TRCYNKRSCAMO <mark>YV</mark> YT	g gfeig qepfdp PTYYMLNVTSAGEDGIY PTYYMLNVTSAGEDGIY	YEPCTANCTGY <mark>A</mark> ANVFATDS YEPCTANCSGY <mark>A</mark> ANVFATDS	a arlricq NGHIPEGFS NGHIPEGFS	250
CV777 vaccine_CV777 SX-2013	FPDNKTLGPTVN.I FPDNKTLGPTVN.I FP <mark>SI</mark> KTLGPTAN.I	OVTTGRNCL <mark>E</mark> NKA I PAY OVTTGRNCLSNKA I PA OVTTGRNCL <mark>E</mark> NKA I PA	RDGKDIVVGITWDNDRVTVFADK LODGKNIVVGITWDNDRVTVFADK MSEHS.VVGITWDNDRVTVF5DK	IYHFYLKNDWSRVA IYHFYIKNDWSRVA IYYFY KNDWSRVV	RCYNRRSCAMQ <mark>YV</mark> YT RCYNKRSCAMQYVYT KCYN <mark>SGG</mark> CAMQYVYE	g gfeig qepfdp PTYYMLNVTSAGEDGIY PTYYMLNVTSAGEDGIY PTYYMLNVTSAGEDGIS	YEPCTANCTGYAANVFATDS YEPCTANCSGYAANVFATDS YOPCTANCTGYSANVFATER	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253
CV777 vaccine_CV777 SX-2013 XS-2013-3	FPDNKT LGPTVN.I FPDNKT LGPTVN.I FPSIKT LGPTANI FPSIKT LGPTANI	OVTTGRNCLENKAIPAY OVTTGRNCLSNKAIPA OVTTGRNCLENKAIPA OVTTGRNCLENKAIPA	MRDGKDIVVGITWDNDRVTVFADK LQDGKNIVVGITWDNDRVTVFADK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK	IYH FYLKNDWSRVA IYH FYIKNDWSRVA IYH FY KNDWSRVV IYY FY KNDWSRVV IYY FY KNDWSRVA	RCYNRRSCAMQYYYT RCYNKRSCAMQYYYT KCYN <mark>SGGCAMQYYY</mark> E KCYN <mark>SGGCAMQYYY</mark> E	g gfeig gepfdp PTYYMLNVTSAGEDGIY PTYYMLNVTSAGEDGIY PTYYMLNVTSAGEDGIS PTYYMLNVTSAGEDGIS	YEPCTANCT GYAANV FAT DS YEPCTANCS GYAANV FAT DS Y OPCTANCT GYSANV FAT E Y OPCTANCT GYSANV FAT E P	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254
CV777 vaccine_CV777 SX-2013 XS-2013-3 NB-2014	FPDNKT LG PTVN . I FPDNKT LG PTVN . I FPSIKT LG PTAN . I FPSIKT LG PTANNI FPSIKT LG PTANNI FPSIKALG PTANNI	DVTTGRNCLENKAIPAY DVTTGRNCLSNKAIPA DVTTGRNCLENKAIPA DVTTGRNCLENKAIPA DVTTGRNCLENKAIPA	MRDGKDIVVGITWDNDRVTVFADK LQDGKNIVVGITWDNDRVTVFADK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK	IYH FYL KN DWS R VA IYH FYI KN DWS R VA IYY FY KN DWS R VV IYY FY KN DWS R VA IYY FY F KN DWS R VA	RCYNRRSCAMQYVYT RCYNKRSCAMQYVYT KCYN <mark>SGC</mark> CAMQYVYE KCYNSGCAMQYVYE KCYNSGCAMQYVYE	g gfeig qepfdp PTYYMLNVTSAGEDGIY PTYYMLNVTSAGEDGIY PTYYMLNVTSAGEDGIS PTYYMLNVTSAGEDGIS PTYYMLNVTSAGEDGIS	YEPCTANCT GYAANV FATDS YEPCTANCS GYAANV FATDS YOPCTANCT GYSANV FATE P YOPCTANCT GYSANV FATE P YOPCTANCT GYAANV FATE P	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254
CV777 vaccine_CV777 SX-2013 XS-2013-3 NB-2014 SH-2013	FPDNKT LGPTVN.F FPDNKT LGPTVN.F FPSIKT LGPTANF FPSIKT LGPTANN FPSIKALGPTANNI FPSIKT LGPTANNI	DVTTGRNCLFNKA I PAY DVTTGRNCLSNKA I PA DVTTGRNCLFNKA I PA DVTTGRNCLFNKA I PA DVTTGRNCLFNKA I PA DVTTGRNCLFNKA I PA	RDGKDIVVGITWDNDRVTVFADK LODGKNIVVGITWDNDRVTVFADK MSEHSVVGITWDNDRVTVFDK MSEHSVVGITWDNDRVTVFDK MSEHSVVGITWDNDRVTVFDK	IYHFYLKNDWSRVA IYHFYIKNDWSRVA IYFYFKNDWSRVA IYYFYFKNDWSRVA IYYFYFKNDWSRVA IYYFYFKNDWSRVA	IRCYNRRSCAMQYVYT IRCYNKRSCAMQYVYT KCYNSGCAMQYVYE KCYNSGCAMQYVE KCYNSGCAMQYVE KCYNSGCAMQYVE	g gfeig qepfdp PTYYLLNVTSAGEDGIY PTYYLLNVTSAGEDGIS PTYYLLNVTSAGEDGIS PTYYLLNVTSAGEDGIS PTYYLLNVTSAGEDGIS PTYYLLNVTSAGEDGIS	YEPCTANCT GYAANV FAT DS YEPCTANCS GYAANV FAT DS YOPCTANCT GYSANV FAT EP YOPCTANCT GYSANV FAT EP YOPCTANCT GYAANV FAT EP YOPCTANCT GYAANV FAT EP	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254
CV777 vaccine_CV777 SX-2013 XS-2013-3 NB-2014 SH-2013 HZ-2014-1	FPDNKT LG PTVN. FPDNKT LG PTVN. FPSIKT LG PTAN. FPSIKT LG PTANN FPSIKT LG PTANN FPSIKT LG PTANN FPSIKT LG PTANN	DVTTGRNCLENKAIPA DVTTGRNCLENKAIPA DVTTGRNCLENKAIPA DVTTGRNCLENKAIPA DVTTGRNCLENKAIPA DVTTGRNCLENKAIPA	R DGKDIVVGITWDNDRVTVFADK LQDGKNIVVGITWDNDRVTVFADK MSEHSVVGITWDNDRVTVFDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK	IYH FYL KNDWSR VA IYH FYI KNDWSR VA IYY FY FKNDWSR VA IYY FY FKNDWSR VA IYY FY FKNDWSR VA IYY FYF KNDWSR VA	RCYNRRSCAMQYYYT RCYNRRSCAMQYYYT CYNSGCAMQYYYE CCYNSGCAMQYYE CCYNSGCAMQYYE CCYNSGCAMQYYE CCYNSGCAMQYYE	g gfeig qepfdp PTYYN LNVTSAGEDGIY PTYYN LNVTSAGEDGIY PTYYN LNVTSAGEDGIS PTYYN LNVTSAGEDGIS PTYYN LNVTSAGEDGIS PTYYT LNVTSAGEDGIS PTYYN LNVTSAGEDGIS	YEPCTANCT GYAANV FAT DS YEPCTANCS GYAANV FAT DS YOCCTANCT GYSANV FAT E P YOPCTANCT GYSANV FAT E P YOPCTANCT GYAANV FAT E P YOPCTANCT GYAANV FAT E P YOPCTANCT GYAANV FAT E P	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254
CV777 vaccine_CV777 sx-2013 xS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1	FPDNKT LG PTVN.F FPDNKT LG PTVN.F FPSIKT LG PTANU FPSIKT LG PTANNT FPSIKT LG PTANNT FPSIKT LG PTANNT FPSIKT LG PTANNT	OVTTGRNCLENKA I PAY OVTTGRNCLSNKA I PA OVTTGRNCLENKA I PA OVTTGRNCLENKA I PA OVTTGRNCLENKA I PA OVTTGRNCLENKA I PA OVTTGRNCLENKA I PA OVTTGRNCLENKA I PA	RDGKDIVVGITWDNDRVTVFADK LDDGKNIVVGITWDNDRVTVFADK MSEHS.VVGITWDNDRVTVFDK MSEHS.VVGITWDNVTVFSDK MSEHS.VVGITWDNVTVFSDK MSEHS.VVGITWDNVTVFSDK MSEHS.VVGITWDNVTVFSDK	IYH FYL KNDWSRVA IYH FYL KNDWSRVA IYH FYL KNDWSRVA IYY FY FKNDWSRVA IYY FY FKNDWSRVA IYY FY FKNDWSRVA IYY FY FKNDWSRVA IYY FY FKNDWSRVA	RCYNRRSCAMOYVYT RCYNKRSCAMOYVYT RCYNSGGCAMOYVY RCYNSGGCAMOYVY RCYNSGGCAMOYVY RCYNSGGCAMOYVY RCYNSGGCAMOYYY RCYNSGGCAMOYYY	g gfeig qepfdp PTYYMLNVTSAGEDGIY PTYYMLNVTSAGEDGIY PTYYMLNVTSAGEDGIS PTYYMLNVTSAGEDGIS PTYYMLNVTSAGEDGIS PTYYMLNVTSAGEDGIS PTYYMLNVTSAGEDGIS PTYYMLNVTSAGEDGIS	Y E P CTANCT G YMANV FAT DS Y E P CTANC G YMANV FAT DS Y E P CTANC G YMANV FAT F Y D P CTANC G YMANV FAT F	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254
CV777 vaccine_CV777 SX-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 WC-2014-2	FPDNKT LGPTVN.F FPDNKT LGPTVN.F FPS IKT LGPTANNT FPS IKT LGPTANNT FPS IKT LGPTANNT FPS IKT LGPTANNT FPS IKT LGPTANNT FPS IKT LGPTANNT	DVTTGRNCL NKAI PAY DVTTGRNCL NKAI PA DVTTGRNCL NKAI PA DVTTGRNCL NKAI PA DVTTGRNCL NKAI PA DVTTGRNCL NKAI PA DVTTGRNCL NKAI PA DVTTGRNCL NKAI PA	R DGK DI VYGI TWDN DR VTV FADK LD DGKNIVVGI TWDN DR VTV FADK MSE HSVVGI TWDN DR VTV FDK SE HSVVGI TWDN DR VTV FDK MSE HSVVGI TWDN DR VTV FDK	IYH FYLKNDWSRVA IYH FYLKNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA	RCYNRRSCAMO Y VYT RCYNSGGCAMO Y VYT KCYNSGGCAMO Y VY KCYNSGGCAMO Y VY KCYNSGGCAMO Y VY KCYNSGGCAMO Y VY KCYNSGGCAMO Y VY KCYNSGGCAMO Y VY KCYNSGGCAMO Y VY	g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGI PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS	Y EPCTANCT GY ANV FAT DS Y EPCTANCS GY ANV FAT DS Y EPCTANCIG YS ANV FAT EP Y EPCTANCIG YS ANV FAT EP Y EPCTANCIG YANV FAT EP Y EPCTANCIG YA ANV FAT EP Y EPCTANCIG YA ANV FAT EP Y EPCTANCIG YA ANV FAT EP	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254
CV777 vaccine_CV777 SX-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 WC-2014-2 LY-2014	FPDNKILGFTVN.F FPDNKILGFTVN.F FPSIKILGFTANI FPSIKILGFTANN FPSIKILGFTANN FPSIKILGFTANN FPSIKILGFTANN FPSIKILGFTANN FPSIKILGFTANN FPSIKILGFTANN	DVTTGRNCL NKAIPA VTTGRNCL NKAIPA VTTGRNCL NKAIPA VTTGRNCL NKAIPA VTTGRNCL NKAIPA VTTGRNCL NKAIPA VTTGRNCL NKAIPA VTTGRNCL NKAIPA VVTTGRNCL NKAIPA	R DGKDIVVGITWDNDRVTVFADK LDDGKNIVVGITWDNDRVTVFADK MSEHSVVGITWDNDRVTVFDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK	IYH FYL KNDWSRVA IYH FYL KNDWSRVA IYH FY FKNDWSRVA IYH FY FKNDWSRVA IYY FY FKNDWSRVA IYY FY FKNDWSRVA IYY FY FKNDWSRVA IYY FY FKNDWSRVA IYY FY FKNDWSRVA IYY FY FKNDWSRVA	RCYNRRSCAMO Y YY RCYNRRSCAMO Y YY RCYNSGCAMO Y YY	g gfeig qepfdp PTYYN LNVTSAGBOGIY PTYYN LNVTSAGBOGIY PTYYN LNVTSAGBOGI PTYYN LNVTSAGBOGI PTYYN LNVTSAGBOGI PTYYN LNVTSAGBOGI PTYYN LNVTSAGBOGI PTYYN LNVTSAGBOGI PTYYN LNVTSAGBOGI	YEPCTANCTGY ANV FATDS YEPCTANCSGY ANV FATDS YEPCTANC GYSANV FAT P YEPCTANC GYSANV FAT P YEPCTANC GYANV FATE P YEPCTANC GYANV FAT P YEPCTANC GYANV FAT P YEPCTANC GYANV FAT P	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 SX-2013-3 NB-2014 SH-2014 HZ-2014-1 JD-2014-1 WC-2014-2 LY-2014 DX-2015-1	FPDNKILGPTVN.F FPDNKILGPTVN.F FPSIKILGPTNN FPSIKILGPTNNI FPSIKILGPTNNI FPSIKILGPTNNI FPSIKILGPTNNI FPSIKILGPTNNI FPSIKILGPTNNI FPSIKILGPTANNI	DVTTGRNCLSNKAIPA DVTTGRNCLSNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA	R OGK DIVYGITWDNDRVTVFADK LODGKNIVVGITWDNDRVTVFADK NSEHVVGITWDNORVTVFSDK NSEHVVGITWDNCVTVFSDK NSEHVVGITWDNCVTVFSDK NSEHVVGITWDNCVTVFSDK NSEHVVGITWDNDRVTVFSDK NSEHVVGITWDNDRVTVFSDK NSEHVVGITWDNDRVTVFSDK	IYH FY L KNDWS RVA IYH FY I KNDWS RVA IYY FY KNDWS RVA	RCYNRRSCAMO Y WY RCYNKRSCAMO Y WY RCYNSGCAMO Y WY KCYNSGCAMO Y WY RCYNSGCAMO Y WY	g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS	Y EPCTANCT GY ANN FAT DS Y EPCTANC GY ANN FAT DS Y EPCTANC GY ANN FAT EP Y EPCTANC GY ANN FAT EP	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-1 UC-2014-2 LY-2014 LY-2014 XS-2015-2	FPDNK LGTVN. FPDNK LGTVN. FPDNK LGTVN. FPDNK LGTNN FPSIK LGTNN FPSIK LGTNN FPSIK LGTNN FPSIK LGTNN FPSIK LGTNN FPSIK LGTNN FPSIK LGTNN FPSIK LGTNN FPSIK LGTNN	DVTTGRNCLSNKAIPA VVTTGRNCLSNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA	R DGKDIVVGITWDNDRVTVFADK LDDGKNIVVGITWDNDRVTVFADK MSEHS.VVGITWDNDRVTVFDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK	IYH FY L KNDWSR VA IYH FY I KNDWSR VA IYH FY RNDWSR VA	RCYNRRSCAMO Y YY RCYNRRSCAMO Y YY KCYNG GCAMO Y YY KCYNG GCAMO YY KCYNG GCAMO YY KCYNG GCAMO Y YY KCYNG GCAMO Y YY KCYNG GCAMO YY KCYNG GCAMO YY KCYNG GCAMO YY	g gfeig qepfdp PTYYN LNVTSAGEDGIY PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGIS PTYYN LNVTSAGEDGIS PTYYN LNVTSAGEDGIS PTYYN LNVTSAGEDGIS PTYYN LNVTSAGEDGIS PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGIS PTYYN LNVTSAGEDGIS PTYYN LNVTSAGEDGIS	Y EPCTANCT GY ANV FAT DS Y EPCTANCS GY ANV FAT DS Y DPCTANC GYSANV FAT E Y DPCTANC GYSANV FAT E Y DPCTANC GYANV FAT E	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 SX-2013- XS-2014-3 NB-2014 SH-2014 HZ-2014-1 JD-2014-1 JD-2014-1 UY-2014-2 LY-2014 DX-2015-1 XS-2015-2 LX-2015	FPDNKILGFTVN.F FPDNKILGFTVN.F FPDNKILGFTVN.F FPSIKILGFTNNN FPSIKILGFTNNN FPSIKILGFTNNN FPSIKILGFTNNN FPSIKILGFTNNN FPSIKILGFTNNN FPSIKILGFTNNN FPSIKILGFTNNN FPSIKILGFTNNN FPSIKILGFTNNN	DVTTGRNCL NKAIPA VVTTGRNCL NKAIPA VVTTGRNCL NKAIPA VVTTGRNCL NKAIPA VVTTGRNCL NKAIPA VVTTGRNCL NKAIPA VVTTGRNCL NKAIPA VVTTGRNCL NKAIPA VVTTGRNCL NKAIPA VVTTGRNCL NKAIPA	R DGKDIVVGITWDNDRVTVFADK LDDGKNIVVGITWDNDRVTVFADK MSEHSVVGITWDNDRVTVFDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK MSEHSVVGITWDNDRVTVFSDK	IYH FY L KNDWS RVA IYH FY L KNDWS RVA IYY FY KNDWS RVA IYH FY KNDWS RVA IYH FY KNDWS RVA IYH FY KNDWS RVA	RCYNRRSCAMO YYT RCYNRRSCAMO YYT KCYNBGGCAMO YYE KCYNBGGCAMO YYE KCYNBGGCAMO YYE KCYNGGCAMO YYE KCYNGGCAMO YYE KCYNGGCAMO YYE KCYNGGCAMO YYE KCYNGGCAMO YYE KCYNGGCAMO YYE	g gfeig qepfdp PTYYN LNVTSAGEDGIY PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYL LNVTSAGEDGI	YEPCTANCTGY ANV FATDS YEPCTANCSGYANV FATDS YEPCTANC GYANV FATD YEPCTANC GYANV FATE YEPCTANC GYANV FATE	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 WC-2014-2 LY-2014 DX-2015-1 XS-2015-2 LX-2015 PJ-2015-1	PP DNK LG TYN I FP DNK LG TYN I FP DNK LG PTNN I FP DNK LG PTNN I FP DNK LG PTNNI FP DNK LG PTNNI	DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA	R DGK DI VYGI TWDN DR VTV FADK LD DGKNIVVGI TWDN DR VTV FADK MSE HE. VYGI TWDN DR VTV FDK SE HE. VYGI TWDN DR VTV FDK MSE HE. VYGI TWDN DR VTV FDK	IYH FY L KNDWS VM IYH FY L KNDWS VW IYH FY L KNDWS RVW IYH FY KNDWS RVM IYH FY KNDWS RVM	RCYNRRSCAMO Y YT RCYNRRSCAMO YYT KCYNBGGAMO YY KCYNBGGAMO YY KCYNBGGAMO YY KCYNBGGAMO YY KCYNBGGAMO YY KCYNBGGAMO YY KCYNBGGAMO YY KCYNBGGAMO YY KCYNBGGAMO YY KCYNBGGAMO YY	g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGI PTYYM LNVTSAGEDGI	Y E PCTANCT GY ANN FAT DS Y E PCTANC GY ANN FAT DS Y D PCTANC GY SANN FAT D Y D PCTANC GY SANN FAT E Y D PCTANC GY SANN FAT E Y D PCTANC GY ANN FAT E	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 SX=2013-3 NB=2014 SH=2013 HZ=2014=1 JD=2014=1 JD=2014=1 UY=2014=1 UY=2014=2 LY=2014 LY=2015=1 LY=2015=2	FPDNKILGTVN. FPDNKILGTVN. FPDNKILGTNN. FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN	DVTTGRNCL NKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA	R DGKDIVVGITWDNDRVTVFADK LDDGKNIVVGITWDNDRVTVFADK MSEHS.VVGITWDNDRVTVFDK SEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK MSEHS.VVGITWDNDRVTVFSDK	IYH FY L KNOWSR VA IYH FY I KNOWSR VA IYH FY KNOWSR VA	RCYNRRSCAMO Y YT RCYNRGCAMO Y YT KCYNGGCAMO Y YY KCYNGGCAMO YY	g gfeig qepfdp PTYYN LNVTSAGEDGIY PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYL LNVTSAGEDGI	Y E P CTANCT G Y ANV FAT D S Y E P CTANCS G Y ANV FAT D S Y D CTANC G YS ANV FAT E P Y D CTANC G YS ANV FAT E P Y D CTANC G YANV FAT E P Y D CTANC G YANN FAT E P	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 XS-2013-3 NB-2014 SH-2014-1 JD-2014-1 WC-2014-2 LY-2014 DX-2015-1 XS-2015-1 LX-2015 LX-2015-1 LY-2015-2 JD-2015	PP DNK LG TYN . FP DNK LG TYN . FP DNK LG TYN . FP SIX LG TYN .	DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA	R DGK DI VYGITWDN DRYTY FADK LD DGKNIVYGITWDN DRYTY FADK NSE HE., VYGITWDN CYTY FS NSE HE., VYGITWD CYTY FS NSE HE., VY	IY HEY L KNOWSK VA IY HEY I KNOWSK VA IY HEY I KNOWSK VA IY HEY KNOWSK VA	RCYNRRSCAMO YYT RCYNRGCAMO YYT KCYNGGCAMO YY KCYNGGCAMO YY	g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS	Y E PCTANCT GY ANN FAT DS Y E PCTANCT GY ANN FAT DS Y D PCTANCT GYSANN FAT E Y D PCTANCT GYSANN FAT E Y D PCTANCT GYSANN FAT E Y D PCTANCT GYANN FAT E	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 XS-2013-3 NB-2014 HZ-2014-1 JD-2014-1 JD-2014-1 WC-2014-2 LY-2014 LY-2014 XS-2015-2 LX-2015-2 JD-2015-2 JD-2015 CX-2013	PPDNK LGTVN.I FPDNK LGTVN. FPDNK LGTNN. FPBIK LGTNN FPBIK LGTNN	DVTTGRNCL NKAIPA VTTGRNCL NKAIPA	R DGKDIVVGITWDNDRVTVFADK LDDGKNIVVGITWDNDRVTVFADK MSEHEVVGITWDNDRVTVFDK SEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK	IYH FY L KNDWSRVA IYH FY I KNDWSRVA IYH FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA IYH FY KNDWSRVA IYY FY KNDWSRVA IYY FY KNDWSRVA	RCYNRRSCAMO Y YY RCYNRRSCAMO Y YY KCYNGGCAMO YY	g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS	Y E PCTANCT GY ANV FAT DS Y E PCTANCS GY ANV FAT DS Y D PCTANC GYSANV FAT P Y D PCTANC GYSANV FAT P Y D PCTANC GYANV FAT P Y D PCTANC GYANV FAT P Y D PCTANC GYANN FAT P Y D PCTANC GYANV FAT P	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 SX-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 WC-2014-2 LY-2014 DX-2015-2 LX-2015 PJ-2015-2 LX-2015 JD-2015 CX-2013 JX-2013	PP DNK LG TYN . FP DNK LG TYN . FP DNK LG TYN . FP SIX LG TYN . FP SIX LG TNN FP SIX LG TNN	DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA	B OGK DI VYGITWDN DR VTV FADK LODGKNIVVGITWDN DR VTV FADK NSEHVYGITWDN VTV FSDK MSEHVYGITWDN CVTV FSDK	IY HEY LENDWSRVM IY HEY I KNDWSRVM IY HEY I KNDWSRVM IY HEY KNDWSRVM	RCYNRRSCAMO Y YT RCYNRGCAMO Y YT RCYNGGCAMO Y YT RCYNGGCAMO Y Y RCYNGGCAMO Y Y RCYNGGCAMO YY RCYNGGCAMO YY RCYNGGCAMO Y Y RCYNGGCAMO Y Y	g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS	Y D PCTANCT GY ANN FAT DS Y D PCTANCT GY ANN FAT DS Y D PCTANCT GY ANN FAT D Y D PCTANCT GY ANN FAT D	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 XS-2013-3 NB-2014 HZ-2014-1 JD-2014-1 JD-2014-1 WC-2014-2 LY-2014 XS-2015-2 LX-2015-2 JJC-2015-2 JD-2015 CX-2013	PP DNK LG TVN . FP DNK LG TVN . FP DNK LG TVN . FP DNK LG TNN . FP SIK LG TNN .	DVTTGRNCLSNKAIPA VVTTGRNCLSNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA VVTTGRNCLNKAIPA	R DGK DI VVGITWDN DR VTV FADK LD DGKNIV GITWDN DR VTV FADK NGE HE. VVGITWDN DR VTV FDK SEHE. VVGITWDN DR VTV FDK NGE HE. VVGITWDN DR VTV FDK	IYH FY L KNDWS VM IYH FY L KNDWS VW IYH FY L KNDWS RVW IYH FY KNDWS RVM IYH FY KNDWS RVM	RCYNRRSCAMO YYT RCYNRRSCAMO YYT KCYNBGCAMO YY KCYNBGCAMO YY	g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS	Y E PCTANCT GY ANV FAT DS Y E PCTANC GY ANV FAT DS Y PCTANC GYSANV FAT D Y PCTANC GYSANV FAT P Y PCTANC GYANV FAT P	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 UC-2014-1 UC-2014-2 LY-2014 DX-2015-1 XS-2015-2 LX-2015 FJJ-2015-1 LY-2015-2 JD-2015 CX-2013 JX-2013-2	FPDNKILGTVN. FPDNKILGTVN. FPDNKILGTNN. FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN FPDIKILGTNN	DVTTGRNCL NKAIPA VTTGRNCL NKAIPA	R DGKDIVVGITWDNDRVTVFADK LDDGKNIVVGITWDNDRVTVFADK MSEHS.VVGITWDNDRVTVFDK MSEHS.VVGITWDNCVTVFSDK MS	IYH FY L KNOWSR VA IYH FY I KNOWSR VA IYH FY KNOWSR VA	RCYNRRSCAMOLYYT RCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY KCYNBGCAMOLYY	g gfeig qepfdp PTYYN LNVTSAGEDGIY PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYL LNVTSAGEDGI	Y E PCTANCI GY ANN FAT DS Y E PCTANCI GY ANN FAT DS Y PCTANCI GY ANN FAT D Y PCTANCI GY ANN FAT P Y PCTANCI GY ANN FAT P	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 SX-2013-3 NB-2014 HZ-2013-1 JD-2014-1 JD-2014-1 UC-2014-1 UY-2014-2 LY-2014 LY-2015-1 LY-2015-2 JD-2015-2 JD-2015 CX-2013 JX-2013 HZ-2013-2 HZ-2013-1	PP DNK LG TVN . FP DNK LG TVN . FP DNK LG TVN . FP DNK LG TNN . FP SIK .	DVTTGRNCLSNKAIPA DVTTGRNCLSNKAIPA DVTTGRNCLSNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA DVTTGRNCLNKAIPA	R DGK DI VVGITWDN DR VTV FADK LD DGKNIV GITWDN DR VTV FADK NGE HE. VVGITWDN DR VTV FDK SEHE. VVGITWDN DR VTV FDK NGE HE. VVGITWDN DR VTV FDK	IY HEY L KNOWSK VA IY HEY I KNOWSK VA IY HEY I KNOWSK VA IY HEY KNOWSK VA	RCYNRRSCAMO Y YT RCYNRGGAMO YY KCYNGGCAMO YY	g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS	Y E PCTANCT GY ANN FAT DS Y E PCTANCT GY ANN FAT DS Y PCTANCT GYSANN FAT E Y PCTANCT GYSANN FAT E	a arlricq NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 XS-2013-3 NB-2014 SH-2014-1 JD-2014-1 UC-2014-2 LY-2014 DX-2015-1 XS-2015-1 LY-2015-1 LY-2015-1 LY-2015-2 JD-2015 CX-2013 HZ-2013-1 JS-2013-1	PPDNK LGTVN. FFDNK LGTVN. FFDNK LGTNN. FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN FFBIKLGTNN	DVTTGRNCL NKAIPA VTTGRNCL NKAIPA	R DGKDIVVGITWDNDRVTVFADK LDDGKNIVVGITWDNDRVTVFADK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNRVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK MSEHEVVGITWDNTVTVFDK	I'H FY I KNDWSRVA I'H FY I KNDWSRVA I'Y FY KNDWSRVA	RCYNRRSCAMO Y YY RCYNRRSCAMO Y YY RCYNRGGAMO YY RCYNBGGAMO YY	g gfeig qepfdp PTYYN LNVTSAGDGIY PTYYN LNVTSAGDGIY PTYYN LNVTSAGDGI PTYYN LNVTSAGDGI	Y E PCTANCT GY ANV FAT DS Y E PCTANCS GY ANV FAT DS Y PCTANC GYSANV FAT E Y PCTANC GYSANV FAT E Y PCTANC GYANV FAT E	a arlricq NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 SX-2013-3 NB-2014 HZ-2014-1 JD-2014-1 JD-2014-1 WC-2014-2 LY-2014 LY-2014 SX-2015-2 LX-2015-1 LY-2015-1 LY-2015-2 JD-2015 CX-2013 JX-2013-2 HZ-2013-1 JS-2013-1 JS-2013-1	PP DNK LG TYN . FP DNK LG TYN . FP DNK LG TYN . FP DNK LG TYN . FP SIKLE FTNN FP SIKLE FTNN FP SIK LG TNN FP SIK LG TNN	DUTTGRNCLSNKAIPA DUTTGRNCLSNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA DUTTGRNCLNKAIPA	R DGK DI VYGITWDN DRYTY FADK LD DGKNIVYGITWDN DRYTY FADK NGE HE., VYGITWDN CYTY FS SE HE., VYGITWDN CYTY FS NGE HE., VYGITWDN	IY HEY LEND WSR VM IY HEY I KNDWSR VM IY HEY KNDWSR VM	RCYNRRSCAMO Y YT RCYNRRSCAMO Y YT RCYNRGCAMO Y YT RCYNRGCAMO Y Y RCYNRGCAMO Y Y RCYNRGCAMO Y Y RCYNRGCAMO Y Y RCYNRGCAMO Y Y RCYNRGCAMO Y Y RCYNRGCAMO Y Y RCYNGGCAMO Y Y	g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS	Y D PCTANC GY ANN FAT DS Y D PCTANC GY ANN FAT DS Y D PCTANC GY ANN FAT D Y D PCTANC GY ANN FAT D	a arlricq NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 xS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 WC-2014-2 LY-2014 DX-2015-1 XS-2015-2 LX-2015 FJ-2015-1 LY-2015-2 JD-2015 CX-2013 JZ-2013 HZ-2013-1 JS-2013-1 QZ-2013 YY-2014	PP DNK LG TVI . I FP DNK LG TVI . I FP DNK LG TVI . I FP DNK LG TVN . I FP DNK LG TNN FP DNK LG TNN	DVTTGRNCL NKAIPA VTTGRNCLNKAIPA	B OGK DI VYGITWDN DRYTY FADK LODGKNIVYGITWDN DRYTY FADK NSEH VYGITWDN CYTY FSOK MSEH VYGITWDN CYTY FSOK	IYH FY L KNDWSR VA IYH FY L KNDWSR VA IYH FY L KNDWSR VA IYH FY KNDWSR VA IYY FY KNDWSR VA	RCYNRRSCAMO Y YT RCYNRRSCAMO YYT KCYNBGCAMO YY KCYNBGCAMO YY	g gfeig qepfdp Pry Yi LNVTSAGEDGIY Pry Yi LNVTSAGEDGIS Pry YI LNVTSAGEDGIS	Y E PCTANCI GY ANV FAT DS Y E PCTANCIGYANV FAT DS Y PCTANCI GYANV FAT DS Y PCTANCI GYANV FAT E Y PCTANCI GYANV FAT E	a arlricq NGHIPEGFS	250 253 254 254 254 254 254 254 254 254 254 254
CV777 vaccine_CV777 SX-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 JD-2014-2 LY-2014 DX-2015-1 LY-2015-2 LX-2015-2 LX-2015-1 LY-2015-2 JD-2015 CX-2013 JX-2013 HZ-2013-1 QZ-2013-1 QZ-2013 YY-2014 LY-2013	PP DNK LG TVN . FP DNK LG TVN . FP DNK LG TVN . FP DNK LG TVN . FP SIK LG TNN FP SIK LG TNN FF SIK FF SIK LG TNN FF SIK FF FF SIK FF SIK FF	DUTTGRNCL NKAIPA DUTTGRNCL NKAIPA	R DGK DI VYGITWDN DR YTY FADK LD DGKNIVYGITWDN DR YTY FADK MSE HE., VYGITWDN DR YTY FDK MSE HE., VYGITWDN TYTY FDK MSE HE., VYGITWDN TYTY FDK MSE HE.,	IY H FY I KNDWS VN IY H FY I KNDWS VN IY H FY I KNDWS RVN IY H FY KNDWS RVN IY FY KNDWS RVN		g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS	Y E PCTANCT GY ANN FAT DS Y E PCTANC GY ANN FAT DS Y E PCTANC GYSANN FAT E Y D PCTANC GYSANN FAT E Y D PCTANC GYANN FAT E	a arlricq NGHIPEGFS	250 253 254
CV777 vaccine_CV777 SX-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 UY-2014-1 UY-2014-1 UY-2014-2 LY-2014-2 LY-2015-1 LY-2015-2 JD-2015-1 LY-2015-2 JD-2015 CX-2013 JX-2013 HZ-2013-1 JS-2013-1 JS-2013-1 QZ-2013 YY-2014 LY-2013 DQ-2013	PPDNK LG TVN. I FPDNK LG TVN. I FPDNK LG TVN. I FPDNK LG TNN FPDIK LG TNN	DVTTGRNCL NKAIPA VTTGRNCL NKAIPA	R DGKDIVVGITWDNDRVTVFADK LDDGKNIVVGITWDNDRVTVFADK MSEHEVVGITWDNDRVTVFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFDK MSEHEVVGITWDNTFTFTFDK MSEHEVVGITWDNTFTFTFDK MSEHEVVGITWDNTFTFTFNK MSEHEVVGITWDNTFTFTFNK MS	I'H FY I KNDWSRVA I'H FY I KNDWSRVA I'Y FY KNDWSRVA	RCYNRRSCAMO Y YY RCYNRRSCAMO Y YY RCYNRGGCAMO Y YY KCYNGGCAMO YY	g gfeig qepfdp PTYYN LNVTSAGEDGIY PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYN LNVTSAGEDGI PTYYL LNVTSAGEDGI	Y E PCTANCT GY ANV FAT DS Y E PCTANCS GY ANV FAT DS Y PCTANCS GY ANV FAT DS Y PCTANC GYSANV FAT P Y PCTANC GYANV FAT P	a arlricq NGHIPEGFS	250 253 254 256
CV777 vaccine_CV777 XS-2013-3 NB-2014 SH-2013 HZ-2014-1 JD-2014-1 WC-2014-2 LY-2014 DX-2015-1 XS-2015-2 LX-2015-1 LY-2015-2 JD-2015 CX-2013 JX-2013-1 JX-2013-1 QZ-2013 YY-2014 LY-2013 DQ-2013 DQ-2013 LS-2013	PPDNK LG TVN. I FPDNK LG TVN. I FPDNK LG TVN. I FPDNK LG TNN FPDIK LG TNN	DVTTGRNCL NKAIPA VTTGRNCL NKAIPA	R DGK DI VYGITWDN DRYTY FADK LD DGKNIVYGITWDN DRYTY FADK NGE HE VYGITWDN CYTY FS KG DGK DI VYGITWDN CYTY FS	IY HEY LENDWSRVE IY HEY I KNDWSRVE IY HEY I KNDWSRVE IY HEY KNDWSRVE	RCYNRRSCAMO Y YY RCYNRGCAMO YY RCYNGGCAMO YY	g gfeig qepfdp PTYYM LNVTSAGEDGIY PTYYM LNVTSAGEDGIS PTYYM LNVTSAGEDGIS	Y E PCTANCT GY ANV FAT DS Y E PCTANCS GY ANV FAT DS Y PCTANCS GY ANV FAT DS Y PCTANC GYSANV FAT P Y PCTANC GYANV FAT P	a arlricq NGHIPEGFS	250 253 254 256

mutations and insertions) compared with the CV777 vaccine strain and older Chinese isolates. PEDV evolutionary strains were prevalent in China, as with the exception of earlier isolated strains such as the CH/S, LJB/03, DX, and JS-2004-2, the 37 strains obtained in this study were, in terms of homology and evolutionary relationships, more similar to strains isolated in recent years in South Korea (K14JB01, KNU-1401, and KNU-1307), Vietnam (VN-KCHY-310113-2013 and HUA-PED47), Thailand (22-53AG0211, 1-55ST0412, and 6-56ST0413), the USA (OhioVBS2), and Canada (2014-022). Thus, PEDV undergoes continuous variation, and the strains isolated after 2013 are different from earlier strains (such as JS-2004-2), consistent with similar reports [24,25]. Moreover, strains recently isolated in Asia, the USA [26-31], Canada [32], and Europe [16] display high homology. Although the route of transmission is not clear, it has been theorized that contamination of feed or feed ingredients [32,33] or equipment and vehicles used to transport pigs [27] are the main routes of transmission, although further investigation and surveillance are required. However, the homology between the recent endemic strains and the PEDV vaccine strain currently in widespread use is not high, which may impact the protective effect of the vaccine.

The prediction of the N-glycosylation sites of the PEDV S protein has important biological significance [32]. A large number of aa mutations have been reported in the S1 region [28]. In this study, the S1 proteins of the 37 strains were all predicted to have ten glycosylation sites and 41 amino acid mutations compared with the CV777 vaccine strain which has eleven glycosylation sites. In contrast, the two strains from Vietnam had seven glycosylation sites, six strains (CV777, USA-Iowa107-2013, virulent DR13. attenuated DR13, vaccine strain CV777, and JS2008) had 11, and two strains from South Korea (SM98, 83P-5) had 12. Some amino acid mutations and insertions changed the N-glycosylation sites. Because the S1 domain is the major epitope region, these sites may alter the antigenicity and pathogenicity of the strains, and

Figure 4. Predicted glycosylation sites of the (SX-2013 and CV777 vaccine stains) S1 protein.

Name: SX-2013 Length: 497	
KSLTYFWLFLPVLSTLSLPQDVTRCSANTNFRRFFSKFNVQAPAVVVLGGYLPIGENQGVNSTWYCAGQHPTASGVHGIF	80
VSHIRGGHGFEIGISQEPFDPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANDVTTGRNCLFNKAIPAHMSEHS	160
VVGITWDNDRVTVFSDKIYYFYFKNDWSRVVTKCYNSGGCAMQYVYEPTYYMLNVTSAGEDGISYQPCTANCIGYSANVF	240
ATEPNGHIPEGFSFNNWFLLSNDSTLVHGKVVSNQPLLVNCLLAIPKIYGLGQFFSFNQTIDGVCNGAAVQRAPEALRFN	320
INDTSVILAEGSIVLHTALGTNFSFVCSNSSNPHLATFAIPLGATQVPYYCFLKVDTYNSTVYKFLAVLPPTVREIVITK	400
YGDVYVNGFGYLHLGLLDAVTINFTGHGTDDDVSGFWTIASTNFVDALIEVQGTAIQRILYCDDPVSQLKCSQVAFDLDD GFYPISSRNLLSHEQPI	480
NN.	80
N.	160
N.	240
N	320
.NN	400
	480
	560
Name: vaccine-CV777 Length: 494	
TPLIYFWLFLPVLLTLSLPODVTRCOSTINFRFFSKFNVOAPAVVVLGGYLPSMNSSSWYCGTGIETDSGVHGIFLSYI	80
DSGOGFEIGISOEPFDPSGYOLYLHKATNGNTSAIARLRICOFPDNKTLGPTVNDVTTGRNCLSNKAIPALODGKNIVVG	160
ITWDNDRVTVFADKIYHFYIKNDWSRVATRCYNKRSCAMOYVYTPTYYMLNVTSAGEDGIYYEPCTANCSGYAANVFATD	240
SNGHIPEGFSFNNWFLLSNDSTLLHGKVVSNOPLLVNCLWAIPKIYGLGOFFSFNOTMDGVCNGAAAORAPEALRFNIND	320
TFVILAEGSIVLHTALGTNLSFVCSNSSDPHKAIFTIPLGVTEVPYYCFLKVDTYKSTVYKFLAVLPPTVKEIVITKYGD	400
	400
VYVNGFGYLHLGLLDAVTINFTGHGTDDDVSGFWTVASTNFVDALIEVQGTAIQRILYCDDPVSQLKCSQVSFDLDDGFY PISSRNLLSHEOPI	480
N	80
	160
	240
	320
	400
	400
	480 560
	500

Asn-Xaa-Ser/Thr sequences in the sequence output below are highlighted in blue; Asparagines predicted to be N-glycosylated are highlighted in red.

further studies addressing this are required. However, signal peptide prediction results for the 37 strains were similar to the CV777 strain, indicating that the signal peptide and cleavage site were stable.

Conclusion

Given the increasing incidence of swine PEDV in China, PEDV coinfection with other viral pathogens will further increase the incidence of PED, which occurs widely in China. A diarrhea epidemic occurred despite immunization with attenuated or inactivated diarrhea triple vaccine (PEDV+TGEV+GARV) or bivalent vaccine (PEDV+TGEV) at 43.5% of pig sites [34], indicating that the protective effect of the current PEDV vaccine needs further improvement [35]. But the CV777 vaccine is G1 genogroup and samples are G2 genogroup, like Sato et al. [36] show partial protection against G2 genogroup with G1 genogroup vaccine. Because the vaccine protection can depend on vaccine strain and other factors such as coinfection with other virus, inactivation method of virus or vaccine administration way etc. There are large variations in the S1 genes of Eastern China PEDV strains compared with the Chinese vaccine strain CV777, indicating rapid gene mutation in epidemic PEDV strains. More effective surveillance and management strategies need to be developed for porcine diarrhea. Our study also highlights the need to improve current vaccines and simultaneously take steps to enhance the immunity of sows to control the spread of pathogens to piglets. Future work is needed to develop a new vaccine strain different from CV777 based on our present study such as mix of inactivated virus G1+G2 genogroup to enhance the vaccine efficacy against newly emerged PEDV.

Acknowledgements

We thank the local veterinarians and farmers for their technical help during the survey and their assistance in sample collection and information support. This work was supported by the Science and Technology Research Project grants "Study on the relationship between piglet diarrhea and the health index of sows" and "Epidemiological investigation and comprehensive prevention and control strategy of viral diarrhea in piglets" (2014-2-001 and 2014-2-003) from Jinhua City, Zhejiang Province.

Authors' contributions

JCY, ZXJ and HJF carried out the studies, participated in collecting data, and drafted the manuscript. HHJ, ZCY, ZHB and JJJ performed the statistical analysis and participated in its design. WL, GBQ, WYL and LYJ helped to draft the manuscript. All authors read and approved the final manuscript.

References

- 1. Chasey D, Cartwright SF (1978) Virus-like particles associated with porcine epidemic diarrhoea. Res Vet Sci 25: 255-256.
- Pensaert MB, de Bouck P (1978) A new coronavirus-like particle associated with diarrhea in swine. Arch Virol 58: 243-247.
- Sun RQ, Cai RJ, Chen YQ, Liang PS, Chen DK, Song CX (2012) Outbreak of porcine epidemic diarrhea in suckling piglets, China. Emerg Infect Dis 18: 161-163.
- 4. Duarte M, Laude H (1994) Sequence of the spike protein of the porcine epidemic diarrhea virus. Gen Viro 175: 1195-1200.
- Li BX, Ge JW, Li YJ (2007) Porcine aminopeptidase N is a functional receptor for the PEDV coronavirus. Virology 365: 166-172.
- 6. Ma G, Feng Y, Gao F, Wang J, Liu C, Li Y (2005) Biochemical and biophysical characterization of the transmissible gastroenteritis coronavirus fusion core. Biochem Biophys Res Commun 337: 1301-1307.
- Yeo SG, Hernandez M, Krell PJ, Nagy EE (2003) Cloning and sequence analysis of the spike gene of porcine epidemic diarrhea virus Chinju99. Virus Genes 26: 239-246.
- Chang SH, Bae JL, Kang TJ, Kim J, Chung GH, Lim CW, Laude H, Yang MS, Jang YS (2002) Identification of the epitope region capable of inducing neutralizing antibodies against the porcine epidemic diarrhea virus. Mol Cells 14: 295-299.
- Park SJ, Song DS, Ha GW, Park BK (2007) Cloning and further sequence analysis of the spike gene of attenuated porcine epidemic diarrhea virus DR13. Virus Genes 35: 55-64.
- Sato T, Takeyama N, Katsumata A, Tuchiya K, Kodama T, Kusanagi K (2011) Mutations in the spike gene of porcine epidemic diarrhea virus associated with growth adaptation *in vitro* and attenuation of virulence *in vivo*. Virus Genes 43: 72-78.
- Puranaveja S, Poolperm P, Lertwatcharasarakul P, Kesdaengsakonwut S, Boonsoongnern A, Urairong K, Kitikoon P, Choojai P, Kedkovid R, Teankum K, Thanawongnuwech R (2009) Chinese-like strain of porcine epidemic diarrhea virus, Thailand. Emerg Infect Dis 15: 1112-1115.
- 12. Lee DK, Park CK, Kim SH, Lee C (2010) Heterogeneity in spike protein genes of porcine epidemic diarrhea viruses isolated in Korea. Virus Res 149: 175-182.
- Park SJ, Moon HJ, Yang JS, Lee CS, Song DS, Kang BK, Park BK (2007) Sequence analysis of the partial spike glycoprotein gene of porcine epidemic diarrhea viruses isolated in Korea. Virus Genes 35: 321-332.
- 14. Spaan W, Cavanagh D, Horzinek MC (1988) Coronaviruses: structure and genome expression. J Gen Virol 69: 2952.
- Chen J, Liu X, Shi D, Shi H, Zhang X, Li C, Chi Y, Feng L (2013) Detection and molecular diversity of spike gene of porcine epidemic diarrhea virus in China. Viruses 5: 2601-2613.
- Pensaert MB, Martelli P (2016) Porcine epidemic diarrhea: A retrospect from Europe and matters of debate. Virus Res 226: 1-6.
- 17. Zhang K, Liu X, Ku X, Cheng S, He Q (2011) Establishment and clinical application of a multiplex reverse transcription PCR for porcine epidemic diarrhea virus, porcine transmissible gastroenteritis virus and porcine group A rotavirus. Chin J Ani Vet Sci 42 Suppl 1: 47-50.

- Gupta R, Brunak S (2002) Prediction of glycosylation across the human proteome and the correlation to protein function. Pac Symp Biocomput 7: 310-322.
- Petersen TN, Brunak S, von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 8: 785-786.
- Zhang C, Chen X, Zhao L, Ni B, Wu Y, Chen L, Zhou L, Xu H (2013) Epidemiologicalinvestigation of and control strategies for swine viral diarrhea. China Ani Health Insp 30: 47-48. [Article in Chinese]
- Liu Y, Zhao H, Wang Z, Wu G, He Q (2013) Molecular epidemiological investigation of swine viral diarrhea. China Ani Husb Vet Med 40: 204-207. [Article in Chinese]
- 22. Yang X, Huo JY, Chen L, Zheng FM, Chang HT, Zhao J, Wang XW, Wang CQ (2013) Genetic variation analysis of reemerging porcine epidemic diarrhea virus prevailing in central China from 2010 to 2011. Virus Genes 46: 337-344.
- Zhang Z, Li L, Wang S, Liu S, Wu F, Zheng H, Li L, Zhang Y, Li X (2012) The identification and pathogenicity of porcine epidemic disease virus variant. Chin J Vet Sci 32: 1423-1428. [Article in Chinese]
- Choi JC, Lee KK, Pi JH, Park SY, Song CS, Choi IS, Lee JB, Lee DH, Lee SW (2014) Comparative genome analysis and molecular epidemiology of the reemerging porcine epidemic diarrhea virus strains isolated in Korea. Infect Genet Evol 26: 348-351.
- 25. Fan B, Yu Z, Pang F, Xu X, Zhang B, Guo R, He K, Li B (2017) Characterization of a pathogenic full-length cDNA clone of a virulent porcine epidemic diarrhea virus strain AH2012/12 in China. Virology 500: 50-61.
- Marthaler D, Jiang Y, Otterson T, Goyal S, Rossow K, Collins J (2013) Complete genome sequence of porcine epidemic diarrhea virus strain USA/Colorado/2013 from the United States. Genome Announc 1: e00555-13.
- 27. Lowe J, Gauger P, Harmon K, Zhang J, Connor J, Yeske P, Loula T, Levis I, Dufresne L, Main R (2014) Role of transportation in spread of porcine epidemic diarrhea virus infection, United States. Emerg Infect Dis 20: 872-874.
- Wang L, Byrum B, Zhang Y (2014) New variant of porcine epidemic diarrhea virus, United States, 2014. Emerg Infect Dis 20: 917-919.
- 29. Cima G (2013) Fighting a deadly pig disease. Industry, veterinarians trying to contain PED virus, new to the US. J Am Vet Med Assoc 243: 469-470.

- Stevenson GW, Hoang H, Schwartz KJ, Burrough ER, Sun D, Madson D, Cooper VL, Pillatzki A, Gauger P, Schmitt BJ, Koster LG, Killian ML, Yoon KJ (2013) Emergence of porcine epidemic diarrhea virus in the United States: clinical signs, lesions, and viral genomic sequences. J Vet Diagn Invest 25: 649-654.
- Jung K, Wang Q, Scheuer KA, Lu Z, Zhang Y, Saif LJ (2014) Pathology of US porcine epidemic diarrhea virus strain PC21A in gnotobiotic pigs. Emerg Infect Dis 20: 662-665.
- 32. Pasick J, Berhane Y, Ojkic D, Maxie G, Embury-Hyatt C, Swekla K, Handel K, Fairles J, Alexandersen S (2014) Investigation into the role of potentially contaminated feed as a source of the first-detected outbreaks of porcine epidemic diarrhea in Canada. Transbound Emerg Dis 61: 397-410.
- 33. Dee S, Clement T, Schelkopf A, Nerem J, Knudsen D, Christopher-Hennings J, Nelson E (2014) An evaluation of contaminated complete feed as a vehicle for porcine epidemic diarrhea virus infection of naive pigs following consumption via natural feeding behavior: proof of concept. BMC Vet Res 10: 176.
- Li W, Li H, Liu Y, Pan Y, Deng F, Song Y, Tang X, He Q (2012) New variants of porcine epidemic diarrhea virus, China, 2011. Emerg Infect Dis 18: 1350-1353.
- 35. Meng F, Ren Y, Suo S, Sun X, Li X, Li P, Yang W, Li G, Li L, Schwegmann-Wessels C, Herrler G, Ren X (2013) Evaluation on the efficacy and immunogenicity of recombinant DNA plasmids expressing spike genes from porcine transmissible gastroenteritis virus and porcine epidemic diarrhea virus. PLoS One 8: e57468.
- Sato T, Oroku K, Ohshima Y, Furuya Y, Sasakawa C (2018) Efficacy of genogroup 1 based porcine epidemic diarrhea live vaccine against genogroup 2 field strain in Japan. Virol J 15: 28.

Corresponding author

Chunyan Jiang, PhD Wuzhou street No. 1188, Wucheng district, Jinhua Zhejiang Province, P.R. China 321007 Tel: 86-579-82230787 Fax: 86-579-82230787 Email: chyjiang@hotmail.com

Conflict of interests: No conflict of interests is declared.