Complete genome sequences of three sub-genotype 2.1b isolates of classical swine fever virus in China

Chunxiao Liu^{1, 2}, Mingliang Li¹, Xingwang Yin¹, Hongliang Zhang², Lirun Xiang², Hongyue Zhai¹, Congcong Wang¹, Yunchao Kan¹, Lunguang Yao¹, Zhijun Tian², Chaoliang Leng¹

¹Henan Key Laboratory of Insect Biology in Funiu Mountain,

Henan Provincial Engineering Laboratory of Insects Bio-reactor,

China-UK-NYNU-RRes Joint Laboratory of Insect Biology, Nanyang Normal University, Nanyang 473061, China

²State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute,

Chinese Academy of Agricultural Sciences, Harbin 150069, China

tzj@hvri.ac.cn; lenghan1223@126.com

Received: October 22, 2017 Accepted: March 15, 2018

Abstract

Introduction: Classical swine fever (CSF) has caused severe economic losses in pig production in many countries. Recent CSF outbreaks in China are mainly associated with sub-genotype 2.1 of CSF virus (CSFV). Although there is abundant information regarding 2.1 isolates, few data are available on whole-genome analysis. **Material and Methods:** The biological and genome characteristics of three recently emerged Chinese CSFV isolates, *i.e.* SD2014-1, SD2014-2, and SD2014-3, were fully analysed. **Results:** Sequence analysis showed that the isolates shared 83.4%–95.0% nucleotide identity with eight other CSFV isolates. In addition, the 5' untranslated region (5'UTR) and the non-structural (NS) proteins NS3, NS4A, and NS4B were more conserved than other regions of the genome. Phylogenetic analysis based on the complete genome sequences or full-length structural protein E2 gene sequences revealed that the three isolates belonged to sub-genotype 2.1b. In addition, several unique molecular characteristics of the 5'UTR, 3'UTR, and E2 were identified. **Conclusion:** The genomic variations of the three isolates will support further analysis of virulence determinants and the evolutionary trend of CSFV.

Keywords: classical swine fever virus, complete genomic analysis, sub-genotype 2.1b, genomic variations.

Introduction

Classical swine fever (CSF) is a highly contagious, lethal, and widespread swine disease caused by CSF virus (CSFV) and is classified by the Office International des Epizooties (OIE) as a notifiable disease (5). CSFV is an enveloped, single-stranded, positivesense RNA virus belonging to the Pestivirus genus within the Flaviviridae family. The family also contains bovine viral diarrhoea virus (BVDV) and border disease virus (BDV) (9, 19). The CSFV genome is approximately 12.3 kb and contains a large open reading frame (ORF) encoding a polyprotein of 3,898 amino acids, with a 5' untranslated region (UTR) and a 3'UTR at either end (18). The polyprotein undergoes viral and cellular proteolysis to produce four structural proteins (C, E0 or E^{ms}, E1, and E2) and eight non-structural proteins (Npro, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (18).

Because of its utility in tracking the virus origin and developing effective control strategies against CSF, many studies focus on sequence diversity analysis of CSFV, particularly the sequence variation associated with virulence changes (8). The sequence comparability of the 5'UTR (150 nt), E2 (190 or 1119 nt), and NS5B (409 nt) have been extensively used for genetic typing and investigating CSFV variation (16, 20, 21). Currently, it has been confirmed that the full-length E2 gene is statistically reliable in phylogenetic analysis (22). At the genome level, CSFV isolates are classified into three genotypes (1, 2, and 3) and 11 sub-genotypes (1.1, 1.2, 1.3, 1.4, 2.1, 2.2, 2.3, 3.1, 3.2, 3.3, and 3.4) (21, 23). Sub-genotype 2.1 isolates have been further divided into 2.1a, 2.1b, and 2.1c (4, 11, 20). In mainland China, CSF outbreaks are largely due to the circulation of several genotypes of CSFV isolates (1.1, 2.1, 2.2, and 2.3) (26), and sub-genotype 2.1b has predominated since the 1990s (7, 22, 26). Recently, a new sub-genotype 2.1d

© 2018 C. Liu et al. This is an open access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivs license (http://creativecommons.org/licenses/by-nc-nd/3.0/) was confirmed by our laboratory (30). Newly emerged CSFV isolates in China in 2014–2015 have been characterised (13). Here, the complete genome sequences of three field CSFV isolates, *i.e.*, SD2014-1, SD2014-2, and SD2014-3, originating from intensive pig farms in Shandong Province, China, were described.

Material and Methods

Clinical samples and virus isolation. A total of eight clinical samples, including the lung, lymphatic nodes, and spleen, were collected from pigs suspected to have CSFV infections during 2014. The animals were from large-scale pig farms in Shandong Province, China. Tissue samples were homogenised using a TissueLyser II (Qiagen, Germany) in Dulbecco's modified Eagle medium (Gibco, USA). The tissue homogenates or sera were centrifuged at $10,000 \times g$ for 15 min. The supernatant was passed through a 0.22 µm filter and transferred to swine testis (ST) cells which were grown for 36-48 h, and the remaining samples were kept at -70°C until the total RNA was extracted. The cells were subsequently incubated at 37°C with 5% CO₂ for 3-5 days. Viral stocks were harvested from infected cells by three cycles of freezing and thawing and stored at -70°C.

Primer design and RT-PCR. Based on the published known sequence of CSFV Zj0801 (GenBank accession no. FJ529205), six pairs of primers were designed (Table 1). Total RNA was extracted from tissue homogenates or serum samples using a viral RNA extraction kit (Qiagen). Reverse transcription (RT)-PCR was performed using the One Step RT-PCR Kit (Qiagen) according to the manufacturer's instructions. A total of $8 \,\mu\text{L}$ of the RNA template was added to $42 \,\mu\text{L}$ of the RT-PCR master mix, including 1 µL each of the primer sets. The reaction conditions were as follows: 95°C for 5 min, 30 cycles of denaturation (95°C for 30 s), annealing at 56°C for 30 s, elongation at 72°C for 2.5 min, and final extension at 72°C for 10 min. Based on the results from the RT-PCR and viral isolation attempts, three samples from three independent pig farms were selected to determine the full-length genomic sequences of CSFV. The three CSFV isolates were designated as SD2014-1, SD2014-2, and SD2014-3.

Genome cloning and sequencing. RT-PCR products were analysed *via* 1% agarose gel electrophoresis. The target fragments were excised from the gels for purification using a Gel Extraction Kit (OMEGA, USA) at a later stage. The purified PCR products were cloned into a pMD18-T vector (TaKaRa, Dalian, China). Recombinant clones were sent to Comate Bioscience (Jinlin, China) for sequencing.

Phylogenetic analysis. The entire genome sequences of SD2014-1, SD2014-2, and SD2014-3 were obtained after sequencing. Phylogenetic trees were constructed with MEGA (version 7) using the neighbour-joining method with 1,000 bootstraps (12). The evolutionary trees of the three CSFV isolates and other known isolates worldwide were determined based on complete genomic sequences or full-length E2 gene sequences (Fig. 1).

Nucleotide and amino acid analysis. The complete genome sequences and deduced amino acid sequences of SD2014-1, SD2014-2, and SD2014-3 were analysed and their homologies with other eight representatives of CSFV strains were compared using DNAstar (DNAstar Inc., USA) (Tables 2 and 3). To explore the genetic variations of SD2014-1, SD2014-2, and SD2014-3, the nucleotide sequences of the 5'UTR and the 3'UTR and the amino acid sequences of E2 were fully analysed, together with 33 other CSFV isolates from China and other countries.

Results

Virus isolation. The tissue homogenates or sera of suspected CSFV-infected pigs were transferred to ST cells for virus isolation. After being identified, the three strains, *i.e.*, SD2014-1, SD2014-2, and SD2014-3, were successfully isolated.

Analysis of full-length genomic sequences. The genomic sequences of SD2014-1 (GenBank accession no. MF149061), SD2014-2 (GenBank accession no. MF149062), and SD2014-3 (GenBank accession no. MF149063) were 12,296, 12,333, and 12,296 nucleotides in length, respectively. The three strains shared 97.7%–98.6% homology between each other (Table 2).

Table 1. Primers used for amplification of gene fragments of CSFV SD2014-1, SD2014-2, and SD2014-3

Fragment	Primer sequence (5'-3') ^a	Position in genome ^a	Product size (bp) ^a
CSFV-A	GTATACGAGGTTAGTTCATTCTCGT	1-2.047	2.047
CSFV-A	GATTACCAGAGAAAGCAACAAGAAT	1-2,047	2,047
CSFV-B	GATAATAGGCCCCGGTAAATTTGAC	2,023-3,313	1.291
CSFV-D	TTTCCTTACAGGTCCCTCGCTAGAG	2,025-5,515	1,291
CSFV-C	AAATGAGACGGGTTACAGGGTA	3,124-4,771	1,648
CSFV-C	CATCCCGTAGATCTCTTCACCTCCA	5,124-4,771	1,048
CSFV-D	CATAGATGAAATAGCTGGCGGGACC	4,564-7,171	2,608
CSI V-D	TAGTGCTCTGCCAGCCTCCACAGTG	4,304-7,171	2,008
CSFV-E	TCTGCTGATATCAGAGGAGCTG	()(()(0)	2 902
CSFV-E	GCTTACCCAGACTTAATGTTTCTAG	6,866–668	2,803
CSFV-F	GCCCTATGTAAGGTCGACACCGCTC	0 572 12 206	2 725
CSFV-F	GGGCCGTTAGGAAATTACCTTAGTC	9,572–12,296	2,725

^a The primer sequence, position in genome and product size with respect to the CSFV Zj0801 (accession no. FJ529205) genome

Table 2. The genome homology of SD2014-1, SD2014-2, and SD2014-3 (%)

Strains	SD2014-1	SD2014-2	SD2014-3
SD2014-1	_	97.7	98.6
SD2014-2	97.7	_	97.9
SD2014-3	98.6	97.9	_

Complete nucleotide sequences of the three isolates were further compared with eight other representative CSFV isolates, including Shimen (AF092448), Paderborn (AY072924), HEBZ (GU592790), HNSD-2012 (JX218094), JSZL (KT119352), CSFV39 (AF407339), Alfort/Tuebingen (J04358), and 94.4/IL/94/TWN (AY646427) (Table 3). The three isolates shared 94.9%–95.0% and 94.7%–94.8% homology with subgenotype 2.1b isolate HEBZ and 2.1d isolate JSZL, respectively; these were the most closely related isolates. In addition, they shared 88.2%–94.2% homology with other genotype 2 isolates, including 2.1a Paderborn, 2.1c HNSD-2012, 2.2 CSFV39, and 2.3 Alfort/Tuebingen. They also shared only 85.5% homology with 1.1 isolate

Shimen and 83.4%–83.5% homology with 3.4 isolate 94.4/IL/94/TWN. In sum, these results indicate that all three isolates belong to sub-genotype 2.1b or 2.1d.

To further examine the genomic variation in the two isolates, their genomic characteristics were analysed in detail. Compared with the eight representative isolates, the 5'UTRs of the three isolates were the most conserved regions in the genomes, which exhibited 96.0%-98.1% homology with 2.1 isolates Paderborn, HEBZ, HNSD-2012 or JSZL, 93.0%-95.4% homology with 2.2 isolate CSFV39 or 2.3 isolate Alfort/Tuebingen, and 91.4%-94.1% homology with 1.1 isolate Shimen or 3.4 isolate 94.4/IL/94/TWN. In addition, the amino acid identities of NS3, NS4A, and NS4B among the three isolates and eight other representative isolates were higher than other regions of the genome, and the homologies were 98.1%-99.4%, 95.2%-100%, and 93.7%-100%, respectively. The detailed identities of the three isolates and eight other representative strain isolates are summarised in Table 3.

Nucleotides	Shimen (1.1) ^a	Paderborn (2.1a) ^a	HEBZ (2.1b) ^a	HNSD-2012 (2.1c) ^a	JSZL (2.1d) ^a	$(2.2)^{a}$ $(2.3)^{a}$		94.4/IL/94/T WN (3.4) ^a
5'UTR	94.1*	97.1*	98.1*	96.2*	96.8*	94.4*	94.6*	91.4*
	92.8**	98.1**	96.8**	96.5**	97.1**	93.0**	95.4**	92.8**
	93.3***	97.6***	97.3***	96.0***	97.6***	93.8***	94.1***	92.2***
N^{pro}	87.1	94.2	95.6	92.9	95.6	87.5	87.9	85.3
	86.9	93.3	94.6	92.1	94.6	87.3	87.5	84.3
	86.9	94.0	95.4	93.1	95.8	88.1	88.5	85.1
С	84.2	94.6	93.6	90.6	95.6	89.2	90.9	84.2
	83.8	94.9	93.3	90.9	95.3	89.6	91.2	83.8
	84.2	94.3	92.6	90.2	94.6	88.9	90.6	84.5
E ^{rns}	84.1	93.0	94.3	92.7	95.0	89.3	90.3	83.1
	84.7	92.5	94.0	92.5	94.9	89.0	90.0	83.4
	84.1	92.5	93.8	92.8	94.6	88.8	90.2	83.7
E1	85.3	93.5	94.5	91.1	94.7	89.1	88.9	81.0
	85.3	93.8	95.2	91.5	95.4	90.8	88.9	81.9
	85.1	94.0	94.7	91.6	95.2	89.9	89.4	81.7
E2	83.3	93.7	93.9	90.2	93.7	87.7	87.8	81.5
	83.6	93.7	94.3	90.2	93.7	87.9	87.9	81.9
	83.6	93.8	94.1	90.3	93.7	87.7	88.0	81.4
P7	79.2	95.2	96.6	94.2	93.7	89.9	89.9	86.0
	81.2	95.2	96.1	94.2	94.2	89.9	89.4	87.0
	80.2	95.2	96.1	94.2	93.2	89.9	89.4	87.0
NS2	82.8	92.7	94.5	91.1	93.8	88.3	88.0	80.4
	82.5	93.1	94.7	91.2	93.4	88.0	88.4	80.1
	82.9	93.0	94.7	91.5	93.8	88.4	88.3	80.2
NS3	86.9	94.9	95.2	93.4	95.5	90.7	90.9	85.8
	86.5	94.7	94.9	93.2	95.3	90.6	90.6	85.2
	97.0	95.1	95.2	93.3	95.7	90.8	91.2	85.4
NS4A	87.8	96.3	95.2	91.0	95.8	90.5	91.5	83.1
	87.3	95.2	94.2	92.1	94.7	88.4	88.4	85.2
	89.4	95.8	94.7	91.5	95.2	89.9	91.0	83.6
NS4B	87.8	93.1	94.6	91.0	94.1	90.0	88.8	84.2
	88.0	93.7	95.0	91.9	95.1	90.6	89.2	85.0
	88.1	93.8	95.3	91.6	94.6	89.9	89.3	84.6
NS5A	85.2	93.2	94.4	91.0	93.6	85.2	90.1	82.3
	85.5	93.4	94.7	90.9	93.8	85.4	90.1	82.4
	85.5	93.4	94.7	90.8	94.0	85.3	90.0	82.5
NS5B	85.0	94.2	95.0	92.5	94.9	85.4	90.1	83.2
	85.1	94.7	95.6	92.7	95.2	85.6	90.4	83.6
	85.0	94.8	95.6	93.2	95.3	85.4	90.4	83.4
3'UTR	85.9	95.7	95.3	93.4	96.1	85.5	95.3	81.9
	85.0	94.8	94.4	93.4	96.1	84.6	94.4	81.9
	84.1	93.5	93.1	93.4	94.8	83.7	93.1	81.1
Complete	85.5	94.0	94.9	92.0	94.7	88.2	89.8	83.4
Complete	85.5	94.1	94.9	92.1	94.7	88.3	89.8	83.6
	85.5	94.2	95.0	92.3	94.8	88.2	90.0	83.5

Table 3. Detailed comparison of the full-length genomes of SD2014-1, SD2014-2, and SD2014-3 to other representative CSFV isolates (%)

Table 1 (co	ontinued)							
Amino acid								
N ^{pro}	92.9	97.0	95.2	95.8	96.4	93.5	90.5	91.7
	93.5	96.4	94.6	95.2	95.8	92.9	91.1	91.1
	92.9	97.0	95.2	95.8	96.4	93.5	91.7	91.7
С	91.9	94.9	97.0	92.9	97.0	93.9	92.9	88.9
	90.9	94.9	94.9	92.9	94.9	93.9	92.9	88.9
	90.9	94.9	94.9	92.9	94.9	93.9	92.9	88.9
E ^{rns}	89.9	97.4	97.8	98.2	98.2	95.2	96.0	91.2
	89.9	97.4	97.8	98.2	98.2	95.2	96.0	91.2
	89.9	96.5	96.9	97.4	97.4	94.3	95.2	91.2
E1	92.3	96.4	95.9	94.9	96.4	96.4	96.4	89.2
	93.8	97.4	96.9	95.9	97.4	97.4	97.4	90.8
	93.8	97.4	96.9	95.9	97.4	97.4	97.4	90.8
E2	88.5	95.2	95.7	94.6	95.7	90.1	92.2	88.2
	89.8	96.0	95.7	95.4	95.7	91.4	93.2	89.0
	89.5	96.0	96.5	95.7	96.5	90.9	93.0	89.0
P7	91.3	98.6	95.7	97.1	94.2	95.7	95.7	95.7
	92.8	98.6	95.7	97.1	94.2	95.7	95.7	94.2
	91.3	98.6	95.7	97.1	94.2	95.7	95.7	95.7
NS2	90.6	97.6	97.2	96.9	96.1	95.4	94.7	88.0
	90.8	97.8	97.4	96.7	95.8	95.4	95.0	88.2
	90.6	97.4	97.4	96.7	95.8	95.4	95.0	88.2
NS3	98.4	99.4	98.8	98.8	99.4	98.7	99.1	98.5
	98.1	99.1	98.5	98.5	99.1	98.4	98.8	98.2
	98.4	99.4	98.8	98.8	99.4	98.7	99.1	98.5
NS4A	98.4	100	98.4	95.2	100	96.8	100	95.2
	98.4	100	98.4	95.2	100	96.8	100	95.2
	98.4	100	98.4	95.2	100	96.8	100	95.2
NS4B	96.0	98.3	98.8	99.1	98.6	96.8	98.3	93.9
	96.0	98.3	98.8	99.1	98.6	96.8	98.3	93.9
	95.7	98.0	98.6	98.8	98.3	96.5	98.0	93.7
NS5A	88.6	95.0	95.4	93.2	95.2	88.2	93.4	86.9
	89.2	95.2	95.6	93.4	95.2	88.8	93.2	87.3
	88.8	95.0	95.4	93.2	95.6	88.4	93.0	86.7
NS5B	91.8	96.5	96.6	96.4	97.5	92.5	95.8	89.4
	91.9	97.1	97.6	97.2	98.5	92.6	95.9	89.4
	92.6	97.8	97.9	97.6	98.7	93.3	96.6	90.1

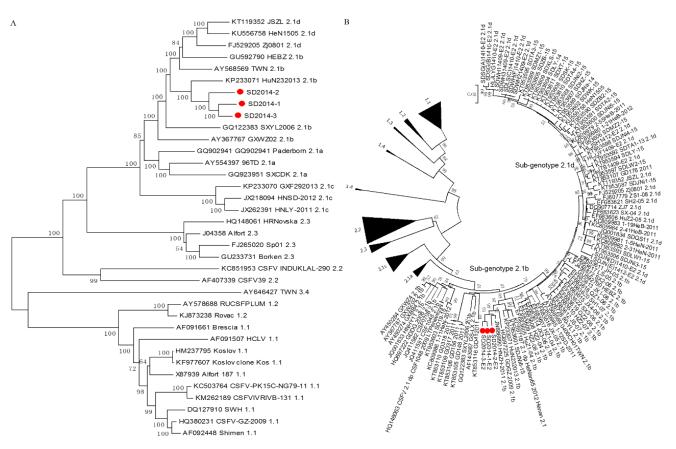


Fig. 1. Phylogenetic analysis of the 3 new isolates (\bullet) and other reference CSFV isolates based on the complete genomic sequences (A) and full-length E2 gene sequences (B)

SD2014-1 seq TAALAC GAAAAT TATAAAA TCT SD2014-2 seq C - T SD2014-3 seq C - T SD2014-3 seq C - T
SD2014-2.seq C T SD2014-3.seq C T A SD2014-3.seq C T A SD2014-3.seq C T KF977607 Kosbv cbne Kos 11 seq C T X87939 Albrt 187 1 1.seq C T AF091507 HCIV 1.1.seq C T AF091661 Breach 1.1 seq C T
SD2014-2.seq C T SD2014-3.seq C T A- SD2014-3.seq C. T A- KF977607 Kosbv chne Kos 11.seq C. T X87939 Alfort 187 1 1.seq C. T AF091507 HCIV 1.1.seq C. T AF091601 Breech 11.seq C. T AF092448 Shinen 11.seq C. T D2127910 SWH 1.1.seq C. T HQ380231 CSFV-6Z-2009 11.seq C. T HQ280231 CSFV-PK15C-NG79-11 11.seq C. T KC503764 CSFV-PK15C-NG79-11 11.seq C. T HM237795 Kosbv 1.1.seq C. T KW262189 CSFVIRIMB-131 11.seq C. T HM237795 Kosbv 1.1.seq C. T KW373238 Rovac 12.seq C. G. T AY578688 RUCSFPIIM 12.seq C. G. T AY578688 RUCSFPIIM 21.seq C. G. C Q902941 Pachebon 2.1a.seq C. G. C Q902941 Pachebon 2.1a.seq C. G. C
SD2014-3 seq C
KF977607 Kosbv cbne Kos 11 seq C T
AF091507 HCIV 1.1.seq CT
AF091661 Bresch 11.seq C. T
AF092448 Shinen 11.seq C. D2127910 SWH 11.seq C. HQ380231 CSFV-GZ-2009 11.seq C. HQ380231 CSFV-GZ-2009 11.seq C. T KC503764 CSFV-PK15C-NG79-11 11.seq C. T KM262189 CSFVJRJB-131 11.seq C. T HM237795 Koshv 1.1.seq C. T KW37238 Rovac 12.seq C. T AY576688 RUCSFPIJM 12.seq C. T AY576688 RUCSFPIJM 12.seq C. T AY576689 79 GTD 2.la.seq C. C. T GQ902941 Padeiborn 2.la.seq C. C. C. GQ922951 SXCDK 2.la seq C. C. AY568569 0406CH01TWN 2.lb.seq C. T AY568569 0406CH01TWN 2.lb.seq C. T Q122383 SXYI2006 2.lb.seq C. C. C
1.1-1.2 isolates DQ127910 SWH 11.æq C.
HQ 380231 CSFV-GZ-2009 11.seq C. 1 1 1 1 1 1 1 1 1 1 1 1 1 1<
HQ 380231 CSFV-GZ-2009 11.seq C. 1 1 1 1 1 1 1 1 1 1 1 1 1 1<
KM262189 CSFVIRI/B-131 11 seq C. T HM237795 Kosbv 11 seq C. T KW873238 Rovac 12 seq C. T AV578688 ROCSFPIIM 12 seq C. T AV554397 96ID 2.1a seq C. C. T Q2902941 Pacheborn 2.1a seq C. CT GQ9293951 SXCDK 2.1a seq C. CT KP233071 HuN232013 2.1b seq C. AV568569 0406CH01TWN 2.1b seq C. T Q122383 SXY12006 2.1b seq C. C
HM237795 Kosbv 1 1.seq C. T
K1873238 Rovac 1 2.seq C T
AY578688 RUCSEPILM 12 seq C T
AX554397 96ID 2.1a.seq CGCT T. GQ902941 Padeborn 2.1a.seq CCT GQ923951 SXCDK 2.1a.seq CCA KP233071 HuN232013 2.1b.seq CCA AY568569 0406CH01TWN 2.1b.seq CT- GU592790 HEBZ 2.1b.seq CT- GU592790 HEBZ 2.1b.seq CC- Q122383 SXYI2006 2.1b.seq CC-
GQ902941 Padeborn 2.1a.æq C CT
G2923951 SXCDK 2.la seq C. CA KP233071 HuN232013 2.lb.seq C. - AY568569 0406CH01TWN 2.lb.seq C. T GU592790 HEBZ 2.lb.seq C. C. T GU122383 SXYI2006 2.lb.seq C. C. C.
KP233071 HuN232013 21bæq C. -
AY568569 0406CH01TWN 2.lb.æq C. T- GU592790 HEBZ 2.lb.æq - 2 1a-2 1d isolates G2122383 SXYI2006 2.lb.æq C. C-
GU592790 HEBZ 2.1b.æq 2 1a-2 1d isolates Gu122383 SXYI2006 2.1b.æq C C
2 1a-2 1d isolates @2122383 SXYI2006 21b.seq CC
Z 1a-Z 10 ISOIATES
AY367767 GXWZ02 2 lb seq CC
KP233070 GXF292013 2.1c.seq CT
JX218094 HNSD-2012 2 1 c.æq C TT
JX262391 HNIX-2011 21c.æq CTC
FU529205 Zj0801 2.1d.æq C
KTI 19352 JSZL 2 ld seq C – G A
KU556758 HeW1505 2.1d.æq CAA
AF407339 CSFV39 22 seq C
KC851953 CSFV_NDUKLAL-290 22 seq CC.T
FJ265020 Sp01 2.3 seq
2.2-2.3, 3.4 isolates J04358 Affort 2.3 seq
GU233731 Bonken 2.3.seqCT
HQ148061 HRNovska 2.3 seq C - T A
$LAY646427 \text{ TWN } 34 \text{ seq} \qquad \qquad C C C A$

Fig. 2. Sequence alignments of 5'UTR of the 3 new CSFV isolates and 32 reference isolates. Some mutation or deletion regions of these isolates are indicated by red boxes (\square) and described in detail in the text

Majoniy	TTTT-TTATT'	TTTTTA	ACAGCACTTT	AGCTGGAAGGAA	AAATTC
	60 70 80	170	180	190	200
SD2014–1 <i>.</i> seq				G	
SD2014-2.seq	······································				
SD2014-3.seq					
AF092448 Shinen 1.1 seq	^T T	т.		A	т
AF091507 HCLV 1.1.seq					
AF091661 Brescia 1.1 seq	·				
KC503764 CSEV-PK15C-NG79-11 11.seq	T.T-TTT				
KM262189 CSFVIJRIJB-131 11 seq	C.T-TT				
KJ873238 Rovac 1.2.seq	C.T.TATTTATTTAGA				
AY578688 RUCSFPLUM 1.2 seq	T.TATTTATTTAGA				
AY367767 GXWZ02 2 1b.seq					
KP233070 GXF292013 2.1c.seq	······································				
KT119352 JSZL 2.1d.seq					A
AF407339 CSFV39 2.2.seq	······································				

Fig. 3. Sequence alignments of 3'UTR of the 3 new CSFV isolates and 11 reference isolates. Some mutation or insertion regions of these isolates are indicated by red boxes (□) and described in detail in the text

Phylogenetic analysis. To understand the genetic relationships among SD2014-1, SD2014-2, SD2014-3, and other CSFV isolates, phylogenetic trees were produced using 36 CSFV entire genome sequences or 205 full-length E2 gene sequences (Fig. 1). CSFV isolates were divided into genotypes 1, 2, and 3. Genotype 2 isolates were further divided into subgenotypes 2.1, 2.2, and 2.3, and sub-genotype 2.1 isolates could be further subdivided into 2.1a, 2.1b, 2.1c, and 2.1d. The three new isolates, *i.e.*, SD2014-1, SD2014-2, and SD2014-3, belonged to 2.1b.

Sequence analysis of UTRs. The 5'UTR and 3'UTR have been documented to be crucial regulatory

elements in the CSFV genome (10, 28). Within the CSFV genome, the 5'UTR of CSFV is the most conserved region. Compared with those of other subgenotype isolates, the 5'UTRs of SD2014-2 and SD2014-3 had a nucleotide T deletion at position 44 (T⁴⁴), while the 5'UTR of SD2014-1 had a nucleotide substitution at the very same position (T/C⁴⁴A) (Fig. 2). In addition, different CSFV isolates showed nucleotide A, T, C, or deletions at this position, while some isolates in the same sub-genotype showed different nucleotides at position 44 (Fig. 2). Similarly to most CSFV isolates, the 5'UTRs of SD2014-1 and SD2014-2 had two continuous nucleotide A deletions at positions 357–358, while the 5'UTR of SD2014-3 had only one nucleotide A deletion at these positions (Fig. 2).

The 3'UTRs of all three new isolates were similar to most of the CSFV isolates. However, the HCLV vaccine strain had 12 continuous nucleotide insertions (TTTTTTTTTTT) at positions 66-77 compared with the reference Shimen strain (Fig. 3). Similarly, the other two strains, RUCSFPLUM (AY578688) and Rovac (KJ873238), had 13 nucleotide insertions (TATTTATTTAGAT) at these positions (Fig. 3). Among the 14 analysed CSFV isolates, the CSFV-PK15C-NG79-11 (KC503764) and CSFV/IVRI/VB-131 (KM262189) strains had five and three poly T-nucleotide insertions, respectively (Fig. 3). In addition, compared with those of sub-genotype 1.1 isolates, the 3'UTRs of most sub-genotype 2.1 isolates, including the three new isolates, had two discontinuous nucleotide T deletions at positions 173 and 199, respectively (Fig. 3).

Amino acid analysis of E2. The E2 protein is essential for virus attachment and entry into the target cell and is also an important virulence determinant of CSFV (24). Consequently, the E2 amino acid sequences of the three new isolates and 33 reference isolates were compared and analysed (Fig. 4). There were some unique molecular characteristics for the new isolates, including the amino acid W at position 132 (W¹³²), E²²⁸, I²⁸³, and L³⁴³ of SD2014-1 and L¹² of SD2014-2. In addition, the three new isolates also had some consistent molecular characteristics, including E³¹, Y²¹⁰ and K^{277} . Consistently with most 2.1b and 2.1d isolates, the new isolates had an amino acid substitution at position 182 (L¹⁸² W). In contrast, at position 195, the new isolates were amino acid V, consistent with 1.1 isolates, and all other sub-genotype isolates were amino acid T. Additionally, at position 364, SD2014-1 was V, SD2014-2 was L, SD2014-3 was A, and other sub-genotype isolates were I.

Discussion

CSF is one of the most important viral diseases of pigs in a number of countries, including China (17, 30). In recent decades, the C-strain vaccine has played a vital role in the control or elimination of CSF, and large CSF outbreaks have been rare (17, 30). However, sporadic outbreaks occur continuously in many regions of the world (30). As an RNA virus, CSFV has high error rates during replication, and genetic variability can allow the virus to easily evade the host immune response; therefore, it is important to surveil the genetic diversity of CSFV, track the virus origin and undertake effective control strategies. In the present study, the complete genome sequences of three CSFV strains isolated in 2014 were sequenced and analysed. The results will help to better understand the molecular diversity of CSFV isolates circulating in China.

	Majoniy	RLACKEDYRYAISSTNEIGPLGAEGLTTT	WKEYNH	PIGW	IGVIECTA
		10 20 3	0 1	30	140
	SD2014-1 pm	RLSCKEDYRYAISSTNEIGPLGAEGLTTT	WEBYNH	PIWW	IGVIECTA
	SD2014-2 pm	L.		G.	
	SD2014-3 pm			G.	
	AF092448 Shinen 1.1 pro	A	.KS.	G.	
	AF091507 HCLV 1.1 pro		.KSÇ	G.	
	AF091661 Brescia 1.1 pro	ANLL	.QÇ	G.	
	X87939 Alfort 187 1 1 pro	Q.ALG			
	DQ127910 SWH 1.1.pm		.KS.	G.	
1.1-1.2 isolates	HM237795 Kosbv1.1.pm	A			
1.1-1.2 ISUIdles	HQ380231 CSEV-GZ-2009 1 1 pro	A	.KS.	G.	
	KC503764 CSEV-PK15C-NG79-11 1.1.pm		.KSY	G.	
	KF977607 Kosbv clane Kos 11 pro	AL	.к	G.	
	KM262189 CSEV JARI VB-131 1 1 pro		.KSY	G.	
	KJ873238 Rovac 1.2 pro	AHKFS	.к	G.	v
	AY578688 RUCSEPLUM 12 pro	AHKL	.к	G.	v
	GQ902941 Paderborn 2.1.a.pro		.к	G.I	мн
	GQ923951 SXCDK 2.1apmo		.к	G.	
	AY554397 96ID 2.1a.pro	н	.к	G.	
	GQ122383 SXY12006 2.1bpm		.к	G.	
	GU592790 HEBZ 2.1bpm		.RS.	G.	
	KP233071 HuN232013 2.1bpm		.к		
2.1a-2.1d isolates	AY367767 GXWZ02 2 1b pro		.к	G.	
2.10 2.10 1500005	JX218094 HNSD-2012 2 Jcpm				
	JX262391 HNLY-2011 2.1 cpm				
	KP233070 GXE292013 2.1cpm	R			
	FJ529205 Zj0801 2.1dpm	КК			
	KTI 19352 JSZL 2.1d pro	· · · · · · · · · · · · · · · · · · ·			
	KU556758 HeN1505 pm				
	KC851953 CSFV_NDUKIAL-290 22 pro				
	AF407339 CSFV39 2.2 E2 pro	ADL			
	FI265020 Sp01 2.3 pm	ALL			
2.2-2.3, 3.4 isolates	GU233731 Borken 2.3 pro	ALL			
,	HQ148061 HRNovska 2.3 pro	AL			
	J04358 Alfort 2.3 pro	ALL			
	AY646427 TWN 3.4 pro	AGL	KD.S.	G.	

	Majority	ACKT	GGNW:	ICAK(æΡV	TTTGGQVI	KQCRW	CGPDI	KEPDGI	PHYPIC	KCII	JAN	ET
		180		190		200		210	ı	220		z	30
	SD2014-1pm	YCKW	GGNW'	ICVK0	DPV	VYMGGQVI	KOCRWO	CGITYI	KEPDGI	PHYPIC	KCII	EN	ET
	SD2014-2pm					. R						А.	
	SD2014-3pm											А.	
	AF092448 Shinen 11 pro	••••L										Α.	
	AF091507 HCLV 1.1.pro AF091661 Bresch 1.1.pro	••• L										.А. Л	••
	X87939 Alfort 187 1 1 pro												
	DQ127910 SWH 1.1.pro	L										A.	
1.1-1.2 isolates	HM237795 Kosby 1.1.pro	L			Е.	. T. L.		D.	N			v.	
1.1-1.2 ISUIdles-	HQ380231 CSFV-GZ-2009 1.1 pro	L										А.	
	KC503764 CSFV-PK15C-NG79-11 1.1 pro	••••L											
	KF977607 Koosby clane Kos 1.1 pro KM262189 CSFV JARIVB-131 1.1 pro	••••				. T. L.						. V . 7	
	KU873238 Rovac 1 2 pro	L				ть.						.А. А.	
	AY578688 RUCSEPIUM 12 pro	L				ть.						Α.	
	GQ902941 Padenbonn 2.1.a.puo	L				т.т						А.	
	GQ923951 SXCDK 2 lapmo	L				т.т							
	AY554397 96ID 2.1a.pro	L				т.ті						т.	
	GQ122383 SXY12006 2.1bpm	н г				Т.К					· V	.A.	
	GU592790 HEBZ 2.1bpm KP233071 HuN232013 2.1bpm					Т						А.	
	AY367767 GXWZ02 2 1bpro					т						A.	
2.1a-2.1d isolates	JK218094 HNSD-2012 2 1cpm	L				т.т						А.	
	JK262391 HNLY-2011 21cpro	L				А.Т						.А.	
	КР233070 GXF292013 2.1срю	L				т.т						А.	
	FJ529205 Zj0801 2.1dpm					Т						А.	
	KT119352 JSZL 2.1d.pro KU556758 HeN1505 pro		1			т						А.	
	KC851953 CSFV NDUKIAL-290 22 pro	H.L	1			TIL						A	
	AF407339 CSFV39 22 E2 pto	н. г			А.	т. т		D.				А.	.1
	FJ265020 Sp01 2.3 pm					T.R						т.	
2.2-2.3, 3.4 isolates	GU233731 Borken 2 3 pro	н г										.т.	
	HQ148061 HRNovska 2.3 pro	H.L.				т.к т.к						.т.	
	J04358 Alfort 2.3 pro AY646427 TWN 3.4 pro	N.RL				T.T						A.	
	III010127 INIT SHEED	H.KD											
			-								L		
	Mariprity	LDGRI	LGPMP	_	EIVS	SAGPVRK		VLVV	VALLGG	RYVLWL	IVIY	IVI	TEQLA
	Majaday		LGPMP	CRPK		SAGPVRK	AEF\	VLVV				IVI	
		270		CRPK 280)	SAGPVRK 290	AEF 340		350	3	60		370
	SI2014-1pm	270	lgpmp	CRPK 280 CKPK) E119	SAGPVRK 290 SAGPVRK	AEF 340 AEL	(ATAA	350 VALLGG	3 RYVLWL	60 IVIY		370
	ST2014-1pm ST2014-2pm	270		CRPK 280 CKPK) E119	SAGPVRK 290	AEF 340 AEL	VLVV	350 VALLGG	3 RYVLWL	60 IVIY		370
1	SI2014-1pm	270	LGPMP	CRPK 280 CKPK) EIIS	SAGPVRK 290 SAGPVRK	AEF 340 AEL F.		350 VALLGG	3 RYVLWL	60 IVTY		370
	SI2014-1pmo SI2014-2pmo SI2014-3pmo AF092448 Shinen 1.1pmo AF091507 HCIV 1.1pmo	270 LDGRI S.E.	LGPMP	CRPK 280 CKPK) EIIS V. V. V.	SAGPVRK 290 SSAGPVRK	AEF 340 AEL .F. .F.	VLVV	350 VALLGG	3 RYVLWL	60 IVTY	VII L A IV.	370
	S12014-1pro S12014-2pro S12014-3pro Ar092448 Shinen 1.1pro Ar091507 HCIV 1.1pro Ar091661 Brescia 1.1pro	270 LDGRI S.E. S.E.	LGPMP	CRPK 280 CKPK .R .R) EIIS - V - V - V	SAGPVRK 290 SAGPVRK	AEFV 340 AELV . F. . F. . F.		350 VALLGG	3 RYVLWL	60 IVTY	VII L A IV.	370
	SI2014-1 pro SI2014-2 pro SI2014-3 pro AF092448 Shin en 1.1 pro AF091507 HCIV 1.1 pro AF091661 Brescin 1.1 pro X87939 Alfort 187 1.1 pro	270 LDGRI S.E. S.E. S.E.	LGPMP	CRPK 280 CKPK .R .R .R) EIIS - V - V - V - V - V	SAGPVRK 290 SAGPVRK	AEF 340 AEL . F. . F. . F. . F.		350 VALLGG	3 RYVLWL	60 IVTY	VII L A IV.	370
	SD2014-1pm SD2014-2pm SD2014-3pm AF092448 Shin en 1.1pm AF091661 Brescin 1.1pm AF091661 Brescin 1.1pm X87939 ABort 187 1.1pm DQ127910 SWH 1.1pm	270 LDGRJ S.E. S.E. S.E. S.E.	LGPMP	CRPK 280 CKPK .R .R .R .R) EIIS - V - V - V - V - V - V	SAGPVRK 290 SAGPVRK	AEF 340 AEL F. F. F. F.		350 VALLGG	3 RYVLWL	60 IVTY	VII L A IV.	370
1.1-1.2 isolates	SI2014-1 pro SI2014-2 pro SI2014-3 pro AF092448 Shin en 1.1 pro AF091507 HCIV 1.1 pro AF091661 Brescin 1.1 pro X87939 Alfort 187 1.1 pro	270 LDGRJ S.E. S.E. S.E. S.E.	LGPMP	CRPK 280 CKPK .R .R .R .R .R) EIIS V V V V V V	SAGPVRK 290 SSAGPVRK	AEF 340 AEL F. F. F. F. F.	VVLVV	350 VALLGG	3 RYVLWL	60 IVTY	VII L A IV.	370
1.1-1.2 isolates-	SD2014-1pm SD2014-2pm SD2014-3pm AF092448 Shinen 1.1pm AF091507 HCIV 1.1pm AF091661 Brescin 1.1pm X87939 Alfort 187 1.1pm Dj227910 SWH 1.1pm HM237795 Koshv 1.1pm	270 LDGRI S.E. S.E. S.E. S.E. S.E.	LGPMP	CRPK 280 CKPK .R .R .R .R .R .R .R) EIIS - V - V - V - V - V - V - V - V - V - V	SAGPVRK	AEF 340 AELV .F. .F. .F. .F. .F. .F.		350 VALLGG	3 RYVLWL		VII L IV. IV. IV. IV. IV. IV.	370 .TEQLA
1.1-1.2 isolates-	SU2014-1pio SU2014-2pio SU2014-3pio Ar092448 Shinen 11pio Ar091661 Brescin 11pio Ar091661 Brescin 11pio X87939 Alfort 187 11pio DQ127910 SWH 11pio HM237795 Kosbv 11pio HQ380231 CSFV-GZ-2009 11pio KC503764 CSFV-PK15C-NG79-11 11pio KF977607 Kosbv chine Kos 11pio	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRIPK 280 CKIPK .R .R .R .R .R .R .R .R .R) EII: V V V V V V V V V V V V V V V	SAGPVRK 290 SAGPVRK	AEF 340 AEL F. F. F. F. F. F. F. F. F. F.	VLVV	350 VALLGG	3 RYVLWL	60 IVTY	VII L A IV. IV. IV. IV. IV. IV. IV.	370 .TEQLA
1.1-1.2 isolates-	SD2014-1pm SD2014-2pm SD2014-3pm AF092448 Shin en 11pm AF091661 Bresch 11pm AF091661 Bresch 11pm X87939 Abott 187 11pm DQ127910 SWH 11pm HM237795 Koshv 11pm HM237795 Koshv 11pm HM237795 Koshv 11pm KC503764 CSFV-FK15C-NG79-11 11pm KK577707 Koshv chne Kos 11pm KM262189 CSFV JARIVB-131 11pm	270 LDGRU S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRPK 280 CKPK .R .R .R .R .R .R .R .R .R .R) EIIS . V . V . V . V . V . V . V . V . V . V	SAGPVRK 290 SSAGPVRK	AEF 340 AEL F. F. F. F. F. F. F. F. F. F.	VEVV	350 VALLGG	3 RYVLWL	60 IVTY	VII L A IV. IV. IV. IV. IV. IV. IV. IV.	370 JTEQLA
1.1-1.2 isolates-	SD2014-1 pao SD2014-2 pao SD2014-3 pao AF092448 Shin en 1.1 pao AF091507 HCIV 1.1 pao AF091507 HCIV 1.1 pao AF091661 Bresch 1.1 pao DJ27910 SWH 1.1 pao HM237795 Kosbv 1.1 pao HM237795 Kosbv 1.1 pao HM237795 Kosbv 1.1 pao KC503764 CSFV-FK15C-NG79-11 1.1 pao KF977607 Kosbv chne Kos 1.1 pao KM262189 CSFV JRR VB-131 1.1 pao KG873238 Rovac 1.2 pao	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRPK 286 CKPK 	EIIS V V V V V V V V V V V V V	SAGPVRK 290 SSAGPVRK	AEF 340 AEL F. F. F. F. F. F. F. F. F. F.		350 VALLGG	3 RYVLWL		VII L A IV. IV. IV. IV. IV. IV. IV. IV. IV.	370 TEQLA
1.1-1.2 isolates	SD2014-1pm SD2014-2pm SD2014-3pm AF092448 Shinen 11pm AF091661 Bresch 11pm AF091661 Bresch 11pm X87939 Abott 187 11pm DQ127910 SWH 11pm HM237795 Koshv 11pm HM237795 Koshv 11pm HM237795 Koshv 11pm KC503764 CSFV-FK15C-NG79-11 11pm KK577707 Koshv chne Kos 11pm KM262189 CSFV JARIVB-131 11pm	270 LDGRU S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRPK 286 CKPK 	EIIS V V V V V V V V V V V V V	SAGPVRK 290 SAGPVRK M.	AEF 340 AEL F. F. F. F. F. F. F. F. F. F.		350 VALLGG	3 RYVLWL		VII L A IV. IV. IV. IV. IV. IV. IV. IV. IV. I	370 TEQLA
1.1-1.2 isolates	SD2014-1 pio SD2014-2 pio SD2014-2 pio SD2014-3 pio AF092448 Shinen 1.1 pio AF091507 HCDV 1.1 pio AF091507 HCDV 1.1 pio DQ127910 SWH 1.1 pio DQ127910 SWH 1.1 pio HM237795 Kosbv 1.1 pio HM237795 Kosbv 1.1 pio HM237795 Kosbv 1.1 pio KC503764 CSFV-FK15C-NG79-11 1.1 pio KC9777607 Kosbv chine Kos 1.1 pio KM262189 CSFV JKTVB-131 1.1 pio KM262189 Roxec 1.2 pio AY578688 RUCSFPIJM 1.2 pio	270 LDGRJ S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRPK 286 CKPK 	E III: V.	SAGPVRK 290 SAGPVRK M.	AEF 340 AEL .F. .F. .F. .F. .F. .F. .F. .F		350 VALLGG	3 RYVLWL	60 IVIY	VII L A IV. IV. IV. IV. IV. IV. IV. IV. IV. I	370 .TEQLA
1.1-1.2 isolates	SD2014-1 pao SD2014-2 pao SD2014-3 pao AF092448 Shin en 1.1 pro AF091661 Brescin 1.1 pro AF091661 Brescin 1.1 pro X87939 ABort 187 1.1 pro DQ127910 SWH 1.1 pro HM237795 Kosbov 1.1 pro HM237795 Kosbov 1.1 pro HM237795 Kosbov 1.1 pro KC503764 CSFV-FK15C-NG79-11 1.1 pro KC503764 CSFV-FK15C-NG79-11 1.1 pro KM262189 CSFV JMR1VB-131 1.1 pro KM262189 CSFV JMR1VB-131 1.1 pro KG873238 Rovac 1.2 pro GQ902941 Padaibon 2.1 a pro GQ902351 SXCDK 2.1 a pro AX578688 JUCSFPLIM 1.2 pro GQ902351 SXCDK 2.1 a pro AX554397 960D 2.1 a pro	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRPK 286 CKPK 	E III V V V V V V V V V V V V V	SAGPVRK 290 SSAGPVRK	AEF - F - F - F - F - F - F - F -		350 VALLGG	3 RYVIWI	60 IVTY	VII L A IV. IV. IV. IV. IV. IV. IV. IV. IV. I	370 TEQLA P. P.
1.1-1.2 isolates	SD2014-1 pao SD2014-2 pao SD2014-2 pao SD2014-3 pao AF092448 Shin en 1.1 pao AF091507 HCIV 1.1 pao AF091661 Brescin 1.1 pao DJ27910 SWH 1.1 pao HM237795 Kosbv 1.1 pao HM237795 Kosbv 1.1 pao HM237795 Kosbv 1.1 pao HM237795 Kosbv 1.1 pao KC503764 CSFV-FK15C-NG79-11 1.1 pao KF977607 Kosbv chne Kos 1.1 pao KM252189 CSFV JMR1V8-131 1.1 pao KB73238 Rovac 1.2 pao AV578688 RUCSFPILM 1.2 pao GQ902941 Rachabom 2.1 a pao GQ902951 SXDK 2.1 a pao AV554397 961D 2.1 a pao GQ122383 SX12006 2.1 b pao	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRPK 286 CKPK .R	E III: - V - V - V - V - V - V - V - V	SAGPVRK 290 SSAGPVRK	AEF F.		350 VALLGG	3 RYVIWI		V11 L IV. IV. IV. IV. IV. IV. IV. IV. IV. I	370 JTEQLA
1.1-1.2 isolates	SD2014-1 pao SD2014-2 pao SD2014-2 pao SD2014-3 pao AF092448 Shinen 1.1 pao AF091507 HCDV 1.1 pao AF091507 HCDV 1.1 pao AF091507 HCDV 1.1 pao AF091507 HCDV 1.1 pao BD227910 SWH 1.1 pao HD237795 Kosbv 1.1 pao HD237795 Kosbv 1.1 pao HD237795 Kosbv 1.1 pao HD237795 Kosbv 1.1 pao KCS03764 CSFV-FK15C-NG79-11 1.1 pao KCS03764 CSFV-FK15C-NG79-11 1.1 pao KE9777607 Kosbv char Kos 1.1 pao KM252189 CSFV JKRU8-131 1.1 pao KM252189 CSFV JKRU8-131 1.1 pao KM2578688 HUCSFPHIM 1.2 pao GQ902941 Padabom 2.1 a pao GQ902941 Padabom 2.1 a pao GQ902951 SXCDK 2.1 a pao GQ122383 SXM2006 2.1 b pao GU122383 SXM2006 2.1 b pao	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP 	CRPK 280 CKPK R R R R R R	E III V V V V V V V V V V V V V	SAGPVRK 290 SAGPVRK M.	AEF(- F) - F, - F,		350 VALLGG	3 RYVIWI	60 IVTY	VII L IV. IV. IV. IV. IV. IV. IV.	370 JTEQLA
	SI2014-1 pio SI2014-2 pio SI2014-3 pio AF092448 Shinen 11 pio AF091507 HCIV 1.1 pio AF091507 HCIV 1.1 pio AF091507 HCIV 1.1 pio DQ127910 SWH 1.1 pio HQ23793 Alfort 187 11 pio HQ23795 Koshv 1.1 pio HQ2360231 CSFV-GZ-2009 11 pio KC503764 CSFV-FK15C-NG79-11 1.1 pio KC503764 CSFV-FK15C-NG79-11 1.1 pio KC970238 Roxe: 1.2 pio AV578688 RUCSFPILM 12 pio GQ902941 Padabom 2.1a pio GQ902941 Padabom 2.1a pio GQ902941 Padabom 2.1a pio GQ122383 SW12006 2.1b pio GQ592790 HBIZ 2.1b pio KP233071 HuN232013 2.1b pio	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRPK 280 CKPK R R R R R R	E I I S - V - V - V - V - V - V - V - V	SAGPVRK 290 SAGPVRK	AEFV 340 . F. . F.		350 VALLGG	3 RYVLWL	60 IVTYY	VII L A IV. IV. IV. IV. IV. IV. IV.	370 TEQLA P.P.P.
1.1-1.2 isolates	SD2014-1 pao SD2014-2 pao SD2014-2 pao SD2014-3 pao AF092448 Shinen 1.1 pao AF091507 HCDV 1.1 pao AF091507 HCDV 1.1 pao AF091507 HCDV 1.1 pao AF091507 HCDV 1.1 pao BD227910 SWH 1.1 pao HD237795 Kosbv 1.1 pao HD237795 Kosbv 1.1 pao HD237795 Kosbv 1.1 pao HD237795 Kosbv 1.1 pao KCS03764 CSFV-FK15C-NG79-11 1.1 pao KCS03764 CSFV-FK15C-NG79-11 1.1 pao KE9777607 Kosbv char Kos 1.1 pao KM252189 CSFV JKRU8-131 1.1 pao KM252189 CSFV JKRU8-131 1.1 pao KM2578688 HUCSFPHIM 1.2 pao GQ902941 Padabom 2.1 a pao GQ902941 Padabom 2.1 a pao GQ902951 SXCDK 2.1 a pao GQ122383 SXM2006 2.1 b pao GU122383 SXM2006 2.1 b pao	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRPK CKPK CKPK R	E I I S E I I S - V - V - V - V - V - V - V - V	SAGPVRK 290 SAGPVRK	AEF - F. -		350 VALLGG	3 RYVLWL		VII L A IV. IV. IV. IV. IV. IV. IV.	370 TEQLA
	S12014-1 pio S12014-2 pio S12014-3 pio AF092448 Shin en 1.1 pio AF091507 HCIV 1.1 pio AF091507 HCIV 1.1 pio Dj27910 SWH 1.1 pio H237795 Kosbv 1.1 pio H237795 Kosbv 1.1 pio H237795 Kosbv 1.1 pio H2380231 CSFV-GZ-2009 1.1 pio KC503764 CSFV-FK15C-NG79-11 1.1 pio KF977607 Kosbv chine Kos 1.1 pio KM262189 CSFV JMR V8-131 1.1 pio KB73238 Rovac 1.2 pio AK578688 RUCSFPIIM 1.2 pio G2902941 Rachebom 2.1 a pio G2923951 SXDK 2.1 a pio AK554397 961D 2.1 a pio G2122383 SX12006 2.1 b pio G2122383 SX12006 2.1 b pio G223071 HuX232013 2.1 b pio AX367767 GW202 2.1 b pio JX218094 HNSD-2012 2.1 c pio JX262391 HNLY-2011 2.1 c pio	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRP K 286 286 4 7 8 8 8 8 7 8 7 8 7 8 7 8 7 8 7 8 7 8	E II :	SAGPVRK 290 SAGPVRK	A EFV 340 A EIV - F. - F. - F. - F. - F. - F. - F. - F.	VLVV	350 VALLGG	3 RYVIWI		VII I IV. IV. IV. IV. IV. IV.	370 JTEQLA
	S12014-1 pio S12014-2 pio S12014-3 pio AF092448 Shinen 1.1 pio AF091507 HCIV 1.1 pio AF091507 HCIV 1.1 pio D127910 SWH 1.1 pio D127910 SWH 1.1 pio H233795 Kosbv 1.1 pio H2380231 CSFV-GZ-2009 1.1 pio KC503764 CSFV-FK15C-NG79-11 1.1 pio KF977607 Kosbv chine Kos 1.1 pio KM252189 CSFV JKRUB-131 1.1 pio KM252189 CSFV JKRUB-131 1.1 pio KM252189 CSFV JKRUB-131 1.1 pio KM2578688 HUCSFPHIM 1.2 pio G2022911 Padabom 2.1 a pio G2022911 Padabom 2.1 a pio G2022911 Padabom 2.1 a pio G2122383 SX12006 2.1 b pio G1592790 HEBZ 2.1 b pio KP23071 HuN22013 2.1 b pio J218094 HNSD-2012 2.1 c pio J221803 HNIX-2011 2.1 c pio KP233070 GXF292013 2.1 c pio	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP 	CRPK 286 CKPK 	E II : . V . V . V . V . V . V . V	SAGPVRK 290 SAGPVRK	A EFV 340 4 EIV F.	VI.VV	350 VALLGG	3 RYVIWI	60 IVIY	VII L A IV. IV. IV. IV. IV. IV. IV.	370 JTEQLA P.P.P.F.
	S12014-1 pio S12014-2 pio S12014-3 pio AF092448 Shin en 1.1 pio AF091661 Bresch 1.1 pio AF091661 Bresch 1.1 pio M237795 Koshv 1.1 pio PM237795 Koshv 1.1 pio PM237795 Koshv 1.1 pio PM237795 Koshv 1.1 pio PM237795 Koshv 1.1 pio KC503764 CSFV-GZ-2009 1.1 pio KC503764 CSFV-FRISC-NG79-11 1.1 pio KF977607 Koshv chne Kos 1.1 pio KM262189 CSFV JRTVB-131 1.1 pio KM262189 CSFV JRTVB-131 1.1 pio KM262189 CSFV JRTVB-131 1.1 pio KM262189 CSFV JRTVB-131 1.1 pio G202241 Padahom 2.1 a pio G202241 Padahom 2.1 a pio G202241 Padahom 2.1 a pio G202241 Padahom 2.1 a pio G2022351 SXCDK 2.1 a pio G202241 Padahom 2.1 a pio G20223351 SXCDK 2.1 a pio G202241 Padahom 2.1 a pio G20241 Padahom 2.1 a pio G20241 Padahom 2.1 a pio G2024241 Pada	270 LDGRJ S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRPK 280 	E III: - V - V - V - V - V - V - V - V	SAGPVRK 290 SAGPVRK M.	AEFV AEFV . F. . F.	VI.VV	350 VALLGG	3 RYVLWL		VII L A IV. IV. IV. IV. IV. IV. IV.	370 TEQLA P.P.P. F.
	S12014-1 pio S12014-2 pio S12014-3 pio AF092448 Shin en 11 pio AF091661 Bresch 11 pio AF091661 Bresch 11 pio Q127910 SWH 11 pio H237795 Koshv 11 pio H237795 Koshv 11 pio H237075 Koshv 11 pio H2380231 CSFV-6Z-2009 11 pio KC503764 CSFV-FK15C-NG79-11 11 pio KC977607 Koshv chne Kos 11 pio KM262189 CSFV JRTVB-131 11 pio KC97238 Roxec 12 pio AV578688 RUCSFPLIM 12 pio G2902941 Padabon 21 a pio G2902941 Padabon 21 a pio G292351 SCDK 21 a pio AV554397 961D 2.1 a pio G222383 SX12006 21 b pio G1592790 HEEZ 21 b pio G226391 FNLV2 21 b pio AV367767 G3W202 2 1 b pio AV367767 CSW202 2 1 b pio AV367767 CSW202 2 1 b pio KP233071 HNLV22013 2 1 c pio KP233071 GXF292013 2 1 c pio KP233070 GXF292013 2 1 c pio KP234070 GXF292013 2 1 c pio	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E		CRPK 280 R. R. R. R. R. R. R. R. R. R. R. R. R.	E III: V.	SAGPVRK 290 SAGPVRK	AEFV F.	·····	350 VALLGG	3 RYVIWI		VII L A IV. IV. IV. IV. IV. IV. IV. IV	370 TEQLA P. P. F.
	S12014-1 pio S12014-2 pio S12014-3 pio AF092448 Shin en 1.1 pio AF091661 Bresch 1.1 pio AF091661 Bresch 1.1 pio M237795 Koshv 1.1 pio PM237795 Koshv 1.1 pio PM237795 Koshv 1.1 pio PM237795 Koshv 1.1 pio PM237795 Koshv 1.1 pio KC503764 CSFV-GZ-2009 1.1 pio KC503764 CSFV-FRISC-NG79-11 1.1 pio KF977607 Koshv chne Kos 1.1 pio KM262189 CSFV JRTVB-131 1.1 pio KM262189 CSFV JRTVB-131 1.1 pio KM262189 CSFV JRTVB-131 1.1 pio KM262189 CSFV JRTVB-131 1.1 pio G202241 Padahom 2.1 a pio G202241 Padahom 2.1 a pio G202241 Padahom 2.1 a pio G202241 Padahom 2.1 a pio G2022351 SXCDK 2.1 a pio G202241 Padahom 2.1 a pio G20223351 SXCDK 2.1 a pio G202241 Padahom 2.1 a pio G20241 Padahom 2.1 a pio G20241 Padahom 2.1 a pio G2024241 Pada	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E		CRPK 280 CKPK R. .R. .R. .R. .R. .R. .R. .R. .R. .R.	E III: E V V V V V V V V V V V V V V	SAGPVRK 290 SAGPVRK M.	A EFV 	·····	350 VALLGG	3 RYVIWI		VII L A IV. IV. IV. IV. IV. IV. IV. IV	370 TEQLA
	S12014-1 pio S12014-2 pio S12014-3 pio AF092448 Shin en 11 pio AF091661 Bresch 11 pio AF091661 Bresch 11 pio M287939 Alfort 187 11 pio PM237795 Koshv 1.1 pio KC503764 CSFV-FR15C-NG79-11 1.1 pio K4873238 Roxe 1.2 pio AK576688 NUCSPEILM 1.2 pio G202941 Padahom 2.1 a pio G202951 SXCDK 2.1 a pio AV556397 96ID 2.1 a pio KP233071 HuX232013 2.1 b pio KP233071 HuX232013 2.1 b pio X266291 HNIX-011 2.1 c pio X262391 HNIX-011 2.1 c pio KP233070 GP292013 2.1 c pio KP35678 HeN15565 pio KP35678 HeN15565 pio AF407339 CSFV 39 2.2 E2 pio	270 LDGRJ S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E		CRPK CKPK	E III E III V V V V V V V V V V V V V	SAGPVRK 290 SAGPVRK	A EFV A EIV . F. . F.	·····	350 VALLGG	3 RYVLWL		VII L A IV. IV. IV. IV. IV. IV. IV. IV	370 TEQLA P.P.P.
	SU2014-1 pio SU2014-2 pio SU2014-3 pio AF092448 Shin en 11 pio AF091661 Bresch 11 pio AF091661 Bresch 11 pio X87939 Alfort 187 11 pio DQ127910 SWH 11 pio HQ237795 Koshv 11 pio HQ237795 Koshv 11 pio HQ23707 Koshv chine Kos 11 pio KC503764 CSFV-FK15C-NG79-11 11 pio KK977607 Koshv chine Kos 11 pio KM262189 CSFV IMIVB-131 11 pio KM262189 CSFV IMIVB-131 11 pio KM262189 CSFV IMIVB-131 11 pio KM26288 RUCSFPIIM 12 pio GQ902911 Pachabon 21 a pio GQ90291 Fachabon 21 a pio GQ90291 GG729013 21 b pio Ficher 200 GF29011 21 c pio Ficher 200 GF29011 21 c pio Ficher 200 GF290 Ficher 200 22 pio Ficher 200 GF290 Ficher 200 22 pio Ficher 200 GF290 Ficher 200 22 pio Ficher 200 GF290 Ficher 200 Fich	270 LDGRJ S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E		CRPK 280 	E IIIS V.	SAGPVRK 290 SAGPVRK M.	A EFV F.		350 VALLGG	3 RYVIWI		VII L A IV. IV. IV. IV. IV. IV. IV. IV	370 TEQLA P. P. F.
	S12014-1 pio S12014-2 pio S12014-3 pio AF092448 Shin en 11 pio AF091661 Bresch 11 pio AF091661 Bresch 11 pio Q127910 SWH 11 pio PM237795 Koshv 11 pio KC503764 CSFV-PK15C-NG79-11 11 pio KC977607 Koshv chne Kos 11 pio RM262189 CSFV JRUVB-131 11 pio KC972388 Roxec 12 pio AV578688 RUCSPILM 12 pio GQ902941 Padabon 21 a pio GQ902941 Padabon 21 a pio GQ902941 Padabon 21 a pio GQ923951 SCDK 21 a pio AV554397 961D 2.1 a pio GU522383 SX12006 21 b pio GU522700 HEEZ 21 b pio AV367767 GW202 2 1	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E	LGPMP	CRPK 280 CKPK -	E IIIS V.	SAGPVRK 290 SAGPVRK M. 	AEFV - F. -		350 VALLGG	3 RYVIWI		VII L IV. IV. IV. IV. IV. IV. IV.	370 TEQLA P. P. F.
2.1a-2.1d isolates	S12014-1 pio S12014-2 pio S12014-3 pio AF092448 Shin en 11 pio AF091507 HCIV 1.1 pio AF091507 HCIV 1.1 pio AF091507 HCIV 1.1 pio D127910 SWH 1.1 pio H237795 Koshv 1.1 pio H237795 Koshv 1.1 pio H237795 Koshv 1.1 pio H2360231 CSFV-6Z-2009 1.1 pio KC503764 CSFV-PK15C-NG79-11 1.1 pio KC503764 CSFV-PK15C-NG79-11 1.1 pio KC977607 Koshv chne Kos 1.1 pio KM262189 CSFV JRUVB-131 1.1 pio KC972388 Roxe: 1.2 pio AX578688 RUCSPFUM 1.2 pio G2923951 SXCDK 2.1 a pio AX578688 RUCSPFUM 1.2 pio G2923951 SXCDK 2.1 a pio AX554397 961D 2.1 a pio G1552790 HEIZ 2.1 b pio KP233071 HuN232013 2.1 b pio AX367767 GW202 2.1 b pio J226291 HNLY-2011 2.1 c pio J226291 HNLY-2011 2.1 c pio KP233070 GEF292013 2.1 c pio J226291 HNLY-2012 2.1 d pio KU13935 CSFV SUDKIAI-290 2.2 pio KC556758 HeN1505 pio KC55153 CSFV NUDKIAI-290 2.2 pio KC551533 CSFV 9.2 E 2 pio K266020 Sp01 2.3 pio G2233731 Borken 2.3 pio HQ148061 HRNoxska 2.3 pio	270 LDGRI S.E. S.E. S.E. S.E. S.E. S.E. S.E. S.E		CRPK 280 CKPK R. .R. .R. .R. .R. .R. .R. .R. .R. .R.	E IIIS V.	SAGPVRK 290 SAGPVRK M. 	A EFV 	·····	350 VALLGG	3 RYVLWL		VII L IV. IV. IV. IV. IV. IV. IV.	370 TEQLA P.P.P. F.
2.1a-2.1d isolates	S12014-1 pio S12014-2 pio S12014-3 pio AF092448 Shin en 11 pio AF091661 Bresch 11 pio AF091661 Bresch 11 pio Q127910 SWH 11 pio PM237795 Koshv 11 pio KC503764 CSFV-PK15C-NG79-11 11 pio KC977607 Koshv chne Kos 11 pio RM262189 CSFV JRUVB-131 11 pio KC972388 Roxec 12 pio AV578688 RUCSPILM 12 pio GQ902941 Padabon 21 a pio GQ902941 Padabon 21 a pio GQ902941 Padabon 21 a pio GQ923951 SCDK 21 a pio AV554397 961D 2.1 a pio GU522383 SX12006 21 b pio GU522700 HEEZ 21 b pio AV367767 GW202 2 1	270 LDGRJ S.E.	LGPMP	CRPK CKPK CKPK CKPK CKPK R .R	E IIIS V	SAGPVRK 290 SAGPVRK M. 	A EFV A EIV A EIV - F - F - F - F - F - F - F - F	·····	350 VALLGG	3 RYVLWL		VII L IV. IV. IV. IV. IV. IV. IV. IV	370 TEQLA

Fig. 4. Amino acid sequence alignments of E2 genes of the 3 new CSFV isolates and 33 reference isolates. The special mutation positions of these isolates are indicated by red boxes (\square) and described in detail in the text

Several sub-genotypes, including 1.1, 2.1, 2.2, and 2.3, exist in mainland China; especially the subgenotype 2.1 has long been predominant (7). In the present study, the genomic sequences of the three CSFV isolates were compared against one another and with other representative CSFV isolates (Tables 2 and 3). The results indicated that the three isolates had high homology among each other. Additionally, they shared the highest nucleotide homology with sub-genotype 2.1b; they also showed high homology with the new sub-genotype 2.1d. Importantly, it was also demonstrated that the 5'UTRs of the three isolates were the most conserved regions in the genomes. NS3, NS4A, and NS4B proteins were more conserved than other regions of the CSFV genome.

Phylogenetic analysis has become a universally accepted classification criterion of CSFV isolates based on the 5'UTR (150 nt) and the E2 (190 and 1119 nt) and NS5B (409 nt) coding sequences (16, 20, 21). However, the complete genome sequence may provide a more credible basis for classification (15). In the present study, two phylogenic trees were constructed based on the complete genome sequences and the full-length E2 sequences, respectively (Fig. 1). Both trees showed that all CSFV isolates could be grouped into three clusters and several topology types. The three clusters were groups 1, 2, and 3, with topologies 1.1, 1.2, 1.3, 1.4, 2.1, 2.2, 2.3, and 3.4. The 2.1 isolates were further grouped into 2.1a, 2.1b, 2.1c, and 2.1d. All three new isolates belonged to 2.1b, which was consistent with the result of nucleotide homology alignment between the new isolates and representative CSFV isolates.

The 5'UTR of CSFV plays important roles in regulating initial translation of the pre-polyprotein and genome replication (10, 15). In addition, the studies on the 5'UTR mainly focused on the 3' end 2/3 region and the IRES of the 5'UTR (6, 15). Sequence alignment showed that the 5'UTRs of the three new isolates had different nucleotide substitutions or deletions at positions 44 and 357-358 compared with other CSFV isolates (Fig. 2). The substitutions or deletions affect the structural characteristics of the 5'UTRs and require experimental confirmation. However, CSFV virulence may vary according to the number and shape of the pseudoknot loop in the secondary structure of 5'UTR and its positional direction in three-dimensional space (6, 15). Thus, the new isolates may have different virulence characteristics.

The 3'UTR is the region with the most variation in the CSFV genome. Previous study reported that the poly-T deletion in the 3'UTR is characteristic of CSFV virulent isolates, which suggests a direct relationship between the poly-T deletion region and viral virulence (15). Later study confirmed that the poly-T insertion in the 3'UTR was important for the attenuation of CSFV (29). However, the insertion is not believed to be a marker for virulence (1, 31). In the present study, we found that all three new isolates had no poly-T insertion region, whereas several other strains, including HCLV, CSFV-PK15C-NG79-11, CSFV/IVRI/VB-131, RUCSFPLUM, and Rovac (AF091507, KC503764, KM262189, AY578688, and KJ873238, respectively) had the insertion region (Fig. 3). Recently, a unique poly-T tract was discovered in the 3'UTR of the CSFV Pinar del Rio strain compared with other CSFV isolates (3). Whether this novel insertion affects the pathogenicity of the virus Additionally, many remains unknown. studies demonstrated that NS3, NS5A, and NS5B of CSFV can interact with 3'UTR to regulate viral RNA synthesis and replication (2, 14, 25). In addition, the 3'UTRs of the new isolates and most sub-genotype 2.1 isolates had two discontinuous nucleotide deletions compared with those of 1.1 isolates (Fig. 3). Whether the nucleotide deletions affect the interactions with other proteins requires further research.

The E2 is the most antigenic protein of CSFV which is involved in virus neutralisation (24). Four antigenic domains, A (86-176 amino acids), B (1-83 amino acids), C (1-110 amino acids), and D (86-110 amino acids), have been mapped on E2 (27). Domain A has been subdivided into A1, A2, and A3 (27). In the present study, we found several unique amino acid substitutions for the three new isolates in these domains and in other regions (Fig. 4). However, the influence of these substitutions on the structure and function of E2 requires further study. In addition, the six cysteines at positions 4, 48, 103, 129, 139, and 167, which were essential for binding by monoclonal antibodies of the four domains, showed no variation in the E2 proteins of the three isolates (27). Furthermore, the potential N-glycosylation sites in the E2 proteins of the three isolates were consistent with previous isolates.

In recent years, the reports on whole-genome analysis of CSFV have been limited. In the present study, the complete genomes of the three new CSFV isolates were fully analysed. We found that all the isolates belonged to the genetic subgroup 2.1b. Furthermore, some genomic variations were found in the UTRs and E2. These data indicate that sub-genotype 2.1b exhibits a trend for wide variation.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The research and the article were financed with the funds of the National Natural Science Foundation of China (No. 31502097), the National Key R and D Programme (2016YFD0500100), the Scientific and Technological Project of Henan Province (182102110240), the Foundation of Nanyang Normal University (No. 15082), and the Key Programme Foundation of Higher Education of Educational Commission of Henan Province (No. 15A230026).

Animal Rights Statement: The collection of clinical samples was approved by the Animal Ethics Committee

of School of Harbin Veterinary Research Institute of the Chinese Academy of Agricultural Sciences and performed in accordance with animal ethics guidelines and approved protocols.

References

- Bjorklund H.V., Stadejek T., Vilcek S., Belak S.: Molecular characterization of the 3' noncoding region of classical swine fever virus vaccine strains. Virus Genes 1998, 16, 307–312.
- Chen Y., Xiao J., Xiao J., Sheng C., Wang J., Jia L., Zhi Y., Li G., Chen J., Xiao M.: Classical swine fever virus NS5A regulates viral RNA replication through binding to NS5B and 3'UTR. Virology 2012, 432, 376–388.
- Coronado L., Liniger M., Munoz-Gonzalez S., Postel A., Perez L.J., Perez-Simo M., Perera C.L., Frias-Lepoureau M.T., Rosell R., Grundhoff A., Indenbirken D., Alawi M., Fischer N., Becher P., Ruggli N., Ganges L.: Novel poly-uridine insertion in the 3'UTR and E2 amino acid substitutions in a low virulent classical swine fever virus. Vet Microbiol 2017, 201, 103–112.
- Deng M.C., Huang C.C., Huang T.S., Chang C.Y., Lin Y.J., Chien M.S., Jong M.H.: Phylogenetic analysis of classical swine fever virus isolated from Taiwan. Vet Microbiol 2005, 106, 187–193.
- Edwards S., Fukusho A., Lefevre P.C., Lipowski A., Pejsak Z., Roehe P., Westergaard J.: Classical swine fever: the global situation. Vet Microbiol 2000, 73, 103–119.
- Fletcher S.P., Jackson R.J.: Pestivirus internal ribosome entry site (IRES) structure and function: elements in the 5' untranslated region important for IRES function. J Virol 2002, 76, 5024–5033.
- Gong W., Wu J., Lu Z., Zhang L., Qin S., Chen F., Peng Z., Wang Q., Ma L., Bai A., Guo H., Shi J., Tu C.: Genetic diversity of subgenotype 2.1 isolates of classical swine fever virus. Infect Genet Evol 2016, 41, 218–226.
- He C.Q., Ding N.Z., Chen J.G., Li Y.L.: Evidence of natural recombination in classical swine fever virus. Virus Res 2007, 126, 179–185.
- Horzinek M.C.: Pestiviruses--taxonomic perspectives. Arch Virol Suppl 1991, 3, 1–5.
- Hsu W.L., Chen C.L., Huang S.W., Wu C.C., Chen I.H., Nadar M., Su Y.P., Tsai C.H.: The untranslated regions of classic swine fever virus RNA trigger apoptosis. PLoS One 2014, 9, e88863.
- Jiang D.L., Gong W.J., Li R.C., Liu G.H., Hu Y.F., Ge M., Wang S.Q., Yu X.L., Tu C.: Phylogenetic analysis using E2 gene of classical swine fever virus reveals a new subgenotype in China. Infect Genet Evol 2013, 17, 231–238.
- Kumar S., Stecher G., Tamura K.: MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016, 33, 1870–1874.
- Leng C.L., Zhang H.L., Kan Y.C., Yao L.G., Li M.L., Zhai H.Y., Li Z., Liu C.X., Shi H.F., Ji J., Qiu R., Tian Z.J.: Characterisation of newly emerged isolates of classical swine fever virus in China, 2014–2015. J Vet Res 2017, 61, 1–9.
- Li S., Feng S., Wang J.H., He W.R., Qin H.Y., Dong H., Li L.F., Yu S.X., Li Y., Qiu H.J.: eEF1A interacts with the NS5A protein and inhibits the growth of classical swine fever virus. Viruses 2015, 7, 4563–4581.

- Li X., Xu Z., He Y., Yao Q., Zhang K., Jin M., Chen H., Qian P.: Genome comparison of a novel classical swine fever virus isolated in China in 2004 with other CSFV strains. Virus Genes 2006, 33, 133–142.
- Lowings P., Ibata G., Needham J., Paton D.: Classical swine fever virus diversity and evolution. J Gen Virol 1996, 77, 1311–1321.
- Luo Y., Li S., Sun Y., Qiu H.J.: Classical swine fever in China: a minireview. Vet Microbiol 2014, 172, 1–6.
- Meyers G., Thiel H.J.: Molecular characterization of pestiviruses. Adv Virus Res 1996, 47, 53–118.
- Moennig V., Plagemann P.G.: The pestiviruses. Adv Virus Res 1992, 41, 53–98.
- Pan C.H., Jong M.H., Huang T.S., Liu H.F., Lin S.Y., Lai S.S.: Phylogenetic analysis of classical swine fever virus in Taiwan. Arch Virol 2005, 150, 1101–1119.
- Paton D.J., McGoldrick A., Greiser-Wilke I., Parchariyanon S., Song J.Y., Liou P.P., Stadejek T., Lowings J.P., Bjorklund H., Belak S.: Genetic typing of classical swine fever virus. Vet Microbiol 2000, 73, 137–157.
- Postel A., Schmeiser S., Bernau J., Meindl-Boehmer A., Pridotkas G., Dirbakova Z., Mojzis M., Becher P.: Improved strategy for phylogenetic analysis of classical swine fever virus based on fulllength E2 encoding sequences. Vet Res 2012, 43, 1–15.
- Postel A., Schmeiser S., Perera C.L., Rodriguez L.J., Frias-Lepoureau M.T., Becher P.: Classical swine fever virus isolates from Cuba form a new subgenotype 1.4. Vet Microbiol 2013, 161, 334–338.
- 24. Risatti G.R., Borca M.V., Kutish G.F., Lu Z., Holinka L.G., French R.A., Tulman E.R., Rock D.L.: The E2 glycoprotein of classical swine fever virus is a virulence determinant in swine. J Virol 2005, 79, 3787–3796.
- 25. Sheng C., Chen Y., Xiao J, Xiao J., Wang J., Li G., Chen J., Xiao M.: Classical swine fever virus NS5A protein interacts with 3'-untranslated region and regulates viral RNA synthesis. Virus Res 2012, 163, 636–643.
- 26. Tu C., Lu Z., Li H., Yu X., Liu X., Li Y., Zhang H., Yin Z.: Phylogenetic comparison of classical swine fever virus in China. Virus Res 2001, 81, 29–37.
- van Rijn P.A., Miedema G.K., Wensvoort G., van Gennip H.G., Moormann R.J.: Antigenic structure of envelope glycoprotein E1 of hog cholera virus. J Virol 1994, 68, 3934–3942.
- Vilcek S., Belak S.: Organization and diversity of the 3'-noncoding region of classical swine fever virus genome. Virus Genes 1997, 15, 181–186.
- Wang Y., Wang Q., Lu X., Zhang C., Fan X., Pan Z., Xu L., Wen G., Ning Y., Tang F., Xia Y.: 12-nt insertion in 3' untranslated region leads to attenuation of classic swine fever virus and protects host against lethal challenge. Virology 2008, 374, 390–398.
- 30. Zhang H., Leng C., Feng L., Zhai H., Chen J., Liu C., Bai Y., Ye C., Peng J., An T., Kan Y., Cai X., Tian Z., Tong G.: A new subgenotype 2.1d isolates of classical swine fever virus in China, 2014. Infect Genet Evol 2015, 34, 94–105.
- 31. Zhou W., Gao S., Podgorska K., Stadejek T., Qiu H.J., Yin H., Drew T., Liu L.: Rovac is the possible ancestor of the Russian lapinized vaccines LK-VNIVViM and CS strains but not the Chinese strain (C-strain) vaccine against classical swine fever. Vaccine 2014, 32, 6639–6642.