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Analysis of genetic variation of porcine reproductive and respiratory syndrome virus (PRRSV) isolates in Central China

Can LIU[1,2], Yibao NING[1)]*, Binrui XU[2)], Wenzhi GONG[1)] and Dongdong ZHANG[1)]

[1)]*Department of Inspection Technology Research, China Institute of Veterinary Drug Control, Beijing 100081, China*
[2)]*College of Veterinary Medicine, China Agricultural University, Beijing 100193, China*

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ABSTRACT. Porcine reproductive and respiratory syndrome virus (PRRSV) is an epidemic etiology in pigs of all ages causing reproductive failure and respiratory manifestation. PRRSV has been circulating in Chinese pig farms for almost 20 years. The aim of the present study was to fully understand the extent of the genetic diversity and molecular characteristics of PRRSVs in Central China. A strain of PRRSV isolated from a recent outbreak farm in Hunan province in Central China, designated HUN-2014, was sequenced and analyzed with 39 other PRRSVs from 1998 to 2014 in Central China. Comparative results of genomic sequences revealed that all 40 PRRSVs belonged to the North American genotype (NA genotype) and shared 88.8–99.0% homology. Phylogenetic analysis showed three subgenotypes, namely conventional PRRSV (C-PRRSV), specially mutant PRRSV (S-PRRSV) and highly pathogenic PRRSV (HP-PRRSV), in all 40 PRRSVs. Moreover, comparative analysis of amino acid (AA) sequences of NSP2, GP3, GP5 and ORF5a revealed the main evolution trend of PRRSVs in Central China from 1998 to 2014, which was from C-PRRSV to HP-PRRSV, accompanied by different evolving directions to S-PRRSV. In conclusion, both the major evolutionary trend and special features of genetic variation should be emphasized as theoretical basis for development of new vaccines and control strategies for PRRS.

KEY WORDS: analysis of mutation, Central China, epidemic, molecular characteristics, porcine reproductive and respiratory syndrome virus (PRRSV)

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Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure in sows and respiratory dyspnea in piglets and is responsible for tremendous financial losses worldwide [29]. The whole genome length of PRRSV is 14.9–15.5 kb, and it consists of at least 10 open reading frames (ORFs): ORF1a and ORF1b, encoding nonstructural proteins (NSPs), and ORF2–7, encoding structural proteins (SPs) [8]. PRRSV falls into two genotypes, the European genotype (EU genotype) and North American genotype (NA genotype), which share almost 60% homology at the nucleotide level, and the NA genotype was reported to be more virulent than the EU genotype in previous studies [6, 7, 15].

It has been two decades since the first Chinese PRRSV was isolated in 1995 [29], and enormous damage has been caused by PRRSV in that time, especially the outbreak of highly pathogenic PRRSV (HP-PRRSV) in 2007–2008 [23, 31] and reemergence in 2009–2010 [10, 19]. PRRSV is still one of the pandemic diseases in the Chinese pig-breeding industry [25]. In this study, we sequenced the complete genome sequence of the new PRRSV HUN-2014 strain, which was isolated in Hunan province, Central China, in February 2014, and then performed a comparative analysis with 39 other genome sequences of PRRSVs of Central China from 1998 to 2014, to investigate the evolutionary diversity of PRRSV in Central China and to better understand the epidemic characteristics of this virus.

*CORRESPONDENCE TO: NING, Y., Department of Inspection Technology Research, China Institute of Veterinary Drug Contro, No. 8 Zhongguancun South Street, Beijing, 100081 China.
e-mail: ningyibao@163.com

MATERIALS AND METHODS

Ethics statement: All animal experiments were approved by the Animal Care and Use Committee of the China Institute of Veterinary Drug Control (IVDC); we followed the guidelines of the IVDC Animal Care and Use Committee in handling the experimental animals during this study.

Clinical samples: Lungs and lymph nodes were collected from suspected pigs in Hunan province, Central China, in February 2014. All of these pigs displayed typical signs of HP-PRRS, including high fever, labored breathing, pyrexia, lethargy and anorexia. Clinical tissues were homogenized for RNA extraction and virus isolation, and the remaining samples were kept at −80°C until use.

Virus isolation: MARC-145 cells were propagated in Dulbecco's modified Eagle's medium (DMEM) containing 8% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, U.S.A.), 100 microgram penicillin and 100 units of streptomycin per milliliter of growth medium. Lymph node homogenates were suspended in DMEM (10% v/v) and then subjected to centrifugation. The supernatant was filtered (0.22 μm filter) and then applied to inoculate MARC-145 cells. Then, the isolated viruses were amplified at 37°C with 5% CO_2 and monitored daily for cytopathic effects (CPEs). The culture supernatants were harvested when

Table 1. Primer sets used in this study

Oligonucleotides	Sequence	Location
1F	ATGACGTATAGGTGTTGGCTCT	1–22
1R	CTTACTCTTTCAGGAAGGGTGGT	1,555–1,577
2F	AAAACACGCTCTGGTGCGACTAC	1,357–1,379
2R	GAGATGGGAAACGAGGCTGAAAAC	3,793–3,816
3F	ATGATAGTTCCGCCCGCAGATAC	3,649–3,671
3R	GGGTGACGAGACCAGCAATGTTAG	5,351–5,374
4F	TGCTTGCTGGTGTTTATGTGACTG	4,889–4,912
4R	GCCTCGGACCTTATCAACCTGTA	6,866–6,888
5F	GAGACTCACTGACGAGGACTTGGAT	6,720–6,744
5R	CAGGCGAGTTCATAAAGAAGATTGG	8,833–8,857
6F	TGTGCGAGAAAACTGGCAAACTG	8,546–8,568
6R	GCCCTGGTGATAGCAACAAGAGC	10,596–10,618
7F	TTCAACCAGATTACAGGGACAAACT	10,387–10,411
7R	ATTAGCCATTGCTGAAAATCGTG	12,592–12,614
8F	CATTGTCTCGCATTAGTGGTTTG	12,386–12,408
8R	CGATAGAGTCTGCCCTTAGTGTC	14,136–14,158
9F	CGCTGATTTGCTTTGTCATTAGG	14,053–14,075
9R	GCACGGTTCTCGCCAATTATACT	15,288–15,310

CPEs appeared in 80% of the cells and stored at −80°C as the virus stock until use.

RNA isolation and RT-PCR: Viral RNA was extracted using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) and dissolved in nuclease-free water. Reverse transcription PCR (RT-PCR) was carried out with a PrimeScript™ One Step RT-PCR Kit (Takara, Dalian, China) according to the manufacturer's instructions. Four microliter (μl) aliquots of the RNA template were added to 46 μl of the RT-PCR master mix. The cycling conditions were 94°C for 5 min; 30 cycles of denaturation (95°C for 30 sec), annealing (58°C for 30 sec) and extension (72°C for 3 min); and then a final extension at 72°C for 10 min. The primer sets (Table 1) used in RT-PCR were as reported in previous research [14].

Genome cloning and sequencing: The amplified PCR products were subjected to agarose gel electrophoresis and excised from the agarose gel for later purification, which was performed by using a E.Z.N.A. Gel Extraction Kit (OMEGA Bio-tek, Norcross, GA, U.S.A.). The PCR products were cloned into pMD18-T vector according to the manufacturer's instructions (Takara) and sequenced. For each amplified genomic region, three clones were sequenced in both directions by Life Technologies (Shanghai, China), and the results of sequencing were analyzed.

Genome and amino acid analysis: After sequencing nine fragments, each fragment was spliced together with an overlapping sequence, obtaining the complete genome sequence of HUN-2014. Every ORF and most of the derived amino acids were compared with other isolates. Phylogenetic trees were constructed with MEGA (Version 5.1) using the neighbor-joining method. Bootstrap values were calculated on 1,000 replicates of the alignment. The evolutionary trend of PRRSV in China was analyzed based on nucleotide sequences of HUN-2014 and other known isolates of Central China (Table 2). Multiple sequence alignments were generated with the DNAMAN software (Version 5.1). The nucleotide and amino acid sequence homologies of HUN-2014 with twelve other PRRSVs of Central China were assess further using the BioEdit software (Version 7.0) (Table 3). To explore the genetic variation of PRRSVs of Central China, comparison of NSP2, GP3, GP5 and ORF5a of the HUN-2014 isolate with 39 other strains of Central China were performed.

RESULTS

Analysis of full-length genomic sequences of HUN-2014: The sequence data showed that, excluding the poly (A) tail, the genomic sequence of HUN-2014 was 15,336 nucleotides (nt) in length, consisting of a 189-nt 5′UTR and a 165-nt 3′UTR, and the genome sequence of the HUN-2014 strain has been submitted to GenBank under the accession No. KP330232. HUN-2014 was identified as an HP-PRRSV of the NA genotype that possessed the genetic marker of a 1+29 AA deletion in NSP2.

Analysis on homology of PRRSV in Central China: Comparative analysis of the whole genome sequence and amino acid sequences analysis suggested that all 40 PRRSVs of Central China belonged to the NA genotype and shared 88.8–99.0% homology (Table 2); In addition, we found that more conserved proteins of PRRSV in Central China were NSP1a, NSP4, NSP6, NSP8, NSP12 and M, whereas less conserved proteins were NSP1b, NSP2, GP3, GP5 and ORF5a (Table 3).

Phylogenetic analysis: Phylogenetic trees were built by applying the nucleotide sequences of the whole genome sequences and amino acid sequences of NSP2, GP3 and GP5 (Fig. 1). Phylogenetic analysis revealed that the 40 PRRSVs of Central China could be categorized into three subgenotypes, namely C-PRRSV, the reference strain of which was HN1, which was isolated between 1996 and 2005 [29]; S-PRRSV, reference strains of which were Em2007 and HZ-31, which possessed specific features of mutation

Table 2. PRRSV strains used in this study

No.	Isolate	Identity (%)[a)]	Province	Year	Access no.	No.	Isolate	Identity (%)[a)]	Province	Year	Access no.
1	S1	89.1	Henan	1998	DQ459471	21	09HEN2	97.7	Henan	2009	JF268680
2	HN1	88.8	Henan	2002	AY457635	22	09HUN1	97.6	Hunan	2009	JF268673
3	HuN	98.4	Hunan	2006	EF517962	23	09HUN2	98.0	Hunan	2009	JF268674
4	HN-HW	98.7	Hunan	2006	FJ797690	24	WUH4	99.0	Hubei	2011	JQ326271
5	HUN4	98.9	Hunan	2006	EF635006	25	HZ-31	95.2	Hubei	2012	KC445138
6	HUB1	98.6	Hubei	2006	EF075945	26	HeNan-A1	98.5	Henan	2013	KJ002451
7	HUB2	98.7	Hubei	2006	EF112446	27	HeNan-A2	96.6	Henan	2013	KJ002452
8	WUH1	98.1	Hubei	2006	EU187484	28	Henan-A3	96.9	Henan	2013	KJ019330
9	WUH2	97.7	Hubei	2006	EU678352	29	Henan-A4	97.0	Henan	2013	KJ534539
10	Henan-1	98.6	Henan	2007	EU200962	30	Henan-A5	97.0	Henan	2013	KJ534540
11	07HEN	98.6	Henan	2007	FJ393457	31	Henan-A6	97.2	Henan	2013	KJ534541
12	HN2007	98.5	Henan	2007	EU880437	32	Henan-A7	97.5	Henan	2013	KJ534542
13	Em2007	93.1	Hubei	2007	EU262603	33	Henan-A8	97.5	Henan	2013	KJ534543
14	08HuN	98.6	Hunan	2008	GU169411	34	Henan-A9	96.2	Henan	2013	KJ546412
15	WUH3	98.6	Hubei	2008	HM853673	35	Henan-A10	97.2	Henan	2013	KJ609516
16	09HUB1	97.9	Hubei	2009	JF268682	36	Henan-A11	96.2	Henan	2013	KJ609517
17	09HUB2	97.9	Hubei	2009	JF268683	37	Henan-A12	97.2	Henan	2014	KJ819934
18	09HUB5	97.9	Hubei	2009	GU168568	38	Henan-A13	97.0	Henan	2014	KJ819935
19	09HUB7	97.6	Hubei	2009	GU168567	39	Henan-A14	96.7	Henan	2014	KJ819936
20	09HEN1	97.7	Henan	2009	JF268684	40	HUN-2014	100	Hunan	2014	KP330232

a) Identity with PRRSV HUN-2014 strain.

Table 3 Detailed comparison of the full-length genomes of HUN-2014 with 12 reference strains of PRRSV of Central China

HUN-2014	HN1	HUN4	HUB1	Em2007	09HUB2	09HEN2	HZ-31	Henan-A4	Henan-A5	Henan-A8	Henan-A10	Henan-A11
	% identity to HUN-2014											
Nucleotides (length)												
5′ UTR (189)	88.2	99.5	99.5	94.7	98.4	98.4	98.4	98.9	98.4	98.9	98.9	99.5
ORF1a (7,422)	86.5	98.8	98.4	92.7	97.6	97.4	96.5	96.8	96.2	96.8	97.9	95.7
ORF1b (4,385)	91.1	99.0	99.1	94.0	98.4	97.9	97.8	97.4	97.5	98.4	98.3	97.4
ORF2-7 (3,188)	90.9	98.7	98.5	92.8	98.1	98.1	88.4	96.5	98.2	97.8	93.7	95.5
3′ UTR (165)	93.3	98.7	97.3	91.3	98.0	97.3	96.0	96.7	96.7	96.7	97.3	98.7
Complete (15,336)	88.8	98.9	98.6	93.1	97.9	97.7	95.2	97.0	97.0	97.5	97.2	96.2
Amino acids (length)												
NSP1a (166)	95.8	100	100	97.0	100	100	98.8	100	100	99.4	99.4	99.4
NSP1b (217)	82.9	98.6	97.7	87.1	96.3	97.2	92.2	94.9	96.8	98.2	97.7	97.2
NSP2 (950)	75.7	97.7	97.2	91.8	89.9	95.4	93.7	93.9	93.8	92.6	95.5	92.0
NSP3 (446)	94.4	99.8	99.3	96.4	99.6	99.1	98.7	98.2	98.9	98.0	99.6	98.7
NSP4 (204)	94.1	100	99.0	96.1	100	100	99.5	99.0	98.0	99.0	100	99.0
NSP5 (170)	92.4	98.8	98.2	93.5	98.2	98.2	94.7	97.1	96.5	97.6	98.2	97.1
NSP6 (16)	93.8	100	100	100	93.8	100	100	93.8	100	100	100	100
NSP7 (259)	88.8	98.8	98.8	90.7	98.8	98.8	98.1	97.7	97.7	98.1	96.5	97.7
NSP8 (45)	97.8	100	100	100	100	100	97.8	97.8	97.8	100	100	97.8
NSP9 (643)	97.5	99.1	99.5	97.7	98.9	98.9	98.9	98.1	98.4	98.4	98.9	98.1
NSP10 (441)	96.4	99.8	99.5	96.8	99.5	99.8	99.3	98.6	99.3	99.5	99.8	99.5
NSP11 (223)	94.6	99.1	99.6	95.5	99.1	99.6	99.1	98.2	99.1	98.2	99.1	98.7
NSP12 (153)	94.8	100	99.3	97.4	100	98.0	99.3	100	100	100	98.0	97.4
GP2 (256)	91.4	97.3	97.7	92.2	97.3	96.5	86.7	94.9	97.7	97.7	93.1	93.5
E (73)	93.2	98.6	98.6	93.2	98.6	98.6	90.4	98.6	98.6	97.3	97.3	97.3
GP3 (255)	85.4	96.9	96.5	88.6	96.9	96.9	83.5	93.3	97.2	96.1	90.6	90.9
GP4 (178)	88.8	97.2	96.6	94.4	96.6	96.6	87.1	95.5	96.6	95.5	96.6	96.6
ORF5a (47)	76.1	93.5	93.5	80.4	93.5	93.5	71.7	89.1	93.5	91.3	89.1	89.1
GP5 (200)	86.0	97.0	97.0	91.5	96.0	96.0	83.5	93.5	95.0	96.0	90.0	94.0
M (174)	97.7	100	100	96.6	100	100	94.8	99.4	100	99.4	98.3	100
N (123)	91.9	97.6	97.6	89.4	96.7	97.6	93.5	97.6	95.9	95.1	95.1	96.7

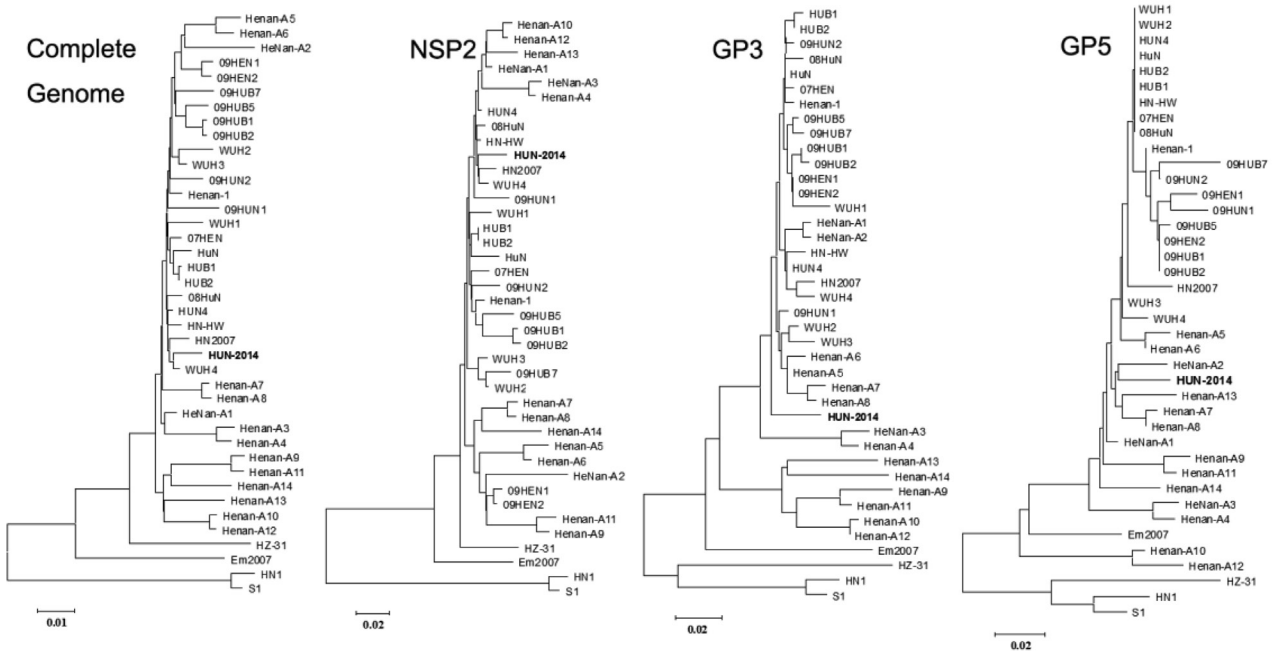

Fig. 1. Phylogenetic trees of the 40 PRRSV isolates of Central China based on the complete genomic sequences and amino acids sequences of NSP2, GP3 and GP5. Four unrooted neighbor-joining trees were constructed from the aligned complete genomic sequences and amino acids sequences of NSP2, GP3, GP5 and ORF5a of the 40 PRRSVs of Central China by the distance-based neighbor-joining method using the MEGA software (Version 5.1). Bootstrap values were calculated on 1,000 replicates of the alignment.

and was considered to be strains of the virus generated by recombination between HP-PRRSV and vaccine for C-PRRSV [11, 18]; and HP-PRRSV, reference strains of which were WUH4 and HUN-2014, which were all isolated after 2006 and contain the consistent gene marker of a 1+29 AA deletion in NPS2 [23, 31].

Amino acid analysis of NSP2: NSP2 is the most variable protein in the viral genome of PRRSV and is a multifunctional protein participating in modulation of the host inflammatory response [2], and it is closely related to the replication ability of PRRSV [24]. By comparing the NSP2 amino acid sequences of the 40 PRRSVs of Central China, we found 75.7–97.7% identity between HUN-2014 and the 39 other PRRSVs of Central China (Table 3). Moreover, the genetic marker of a 1+29 AA deletion could be seen in all the 38 PRRSVs isolated since 2006 (denoted with red boxes in Fig. 2), but five of them exhibited special mutations in NSP2, such as a discontinuous 37+2+29 deletion in Em2007; 30+29 deletion in 09HUB1, 09HUB2 and HZ-31; and 49+29 deletion in Henan-A14 (denoted with blue boxes in Fig. 2).

Amino acid analysis of GP3: GP3 has been regarded as a less conserved structural protein PRRSV that interacts with GP2 and GP4, forming multiprotein complexes that are believed to be crucial for the assembly of infectious PRRSV [1]. After comparing the GP3 amino acid sequences of the 40 PRRSVs of Central China, we found 83.5–97.2% identity between HUN-2014 and the 39 other PRRSVs of Central China (Table 3). Previous studies showed that three antigenic epitopes of GP3, ^{67}YEPGRSLW^{74} (denoted with a red box in Fig. 3), ^{74}WCRIGHDRCGED^{85} (denoted with a red box in Fig. 3) and ^{87}HDELGFMV^{94} (denoted with a blue box in Fig. 3), were well conserved among most of the NA-type isolates, whereas the epitope ^{59}TRQAAAEILE^{68} (denoted with a blue box in Fig. 3) differed in some NA-genotype strains [1, 30]. In this study, most of the HP-PRRSVs were found to have relatively consistent mutations (T^{64}A, Y^{67}L/F, R^{71}K, L^{73}F, Y^{79}H, E^{83}S & D^{85}N/E) in three of the abovementioned epitopes and four sporadic mutations in the relatively conserved epitope (Fig. 3).

Amino acid analysis of GP5: GP5 is the most heterogeneous structural protein of PRRSV [5], and it could induce IFN-β production in host cells and played a significant role during viral attachment and internalization [4, 13]. After comparing the GP5 amino acid sequences of the 40 PRRSVs of Central China, 83.5–97.0% identity was found between HUN-2014 and the 39 other PRRSVs of Central China (Table 3). A previous study recognized that GP5 and ORF5a coevolved through a fine balance of purifying codon usage to maintain a conserved RQ-rich motif in the ORF5a protein while eliciting a variable N-linked glycosylation motif (^{30}NASNDS^{35}) (denoted with a blue box in Fig. 4) in the alternative GP5 reading frame [16]. In this work, four relatively identical mutations (A^{29}V, D^{34}N/S, S^{35}N and L^{39}I) were seen from C-PRRSV to most of the HP-PRRSVs in the decoy epitope (denoted with a red box in Fig. 4), the abovementioned N-linked glycosylation motif and primary neutralizing epitope (PNE) (denoted with a yellow box in Fig. 4), yet particular mutations observed in S-PRRSV as

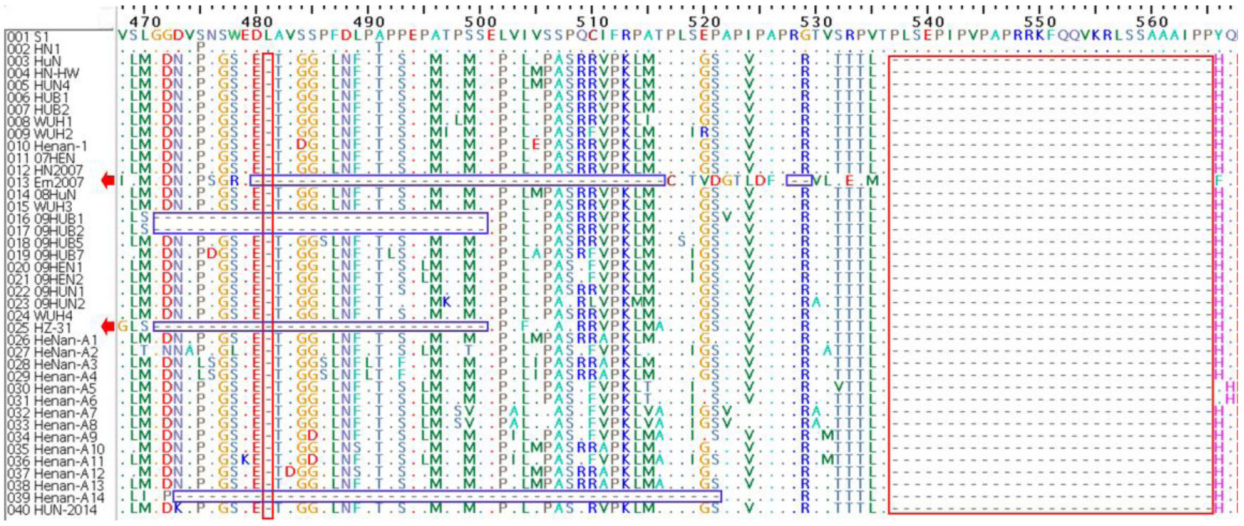

Fig. 2. Amino acid sequence alignments of the partial NSP2 gene of the 40 PRRSVs of Central China. The discontinuous 1+29 AA deletion regions are indicated by red boxes, and special deletions are indicated by blue boxes.

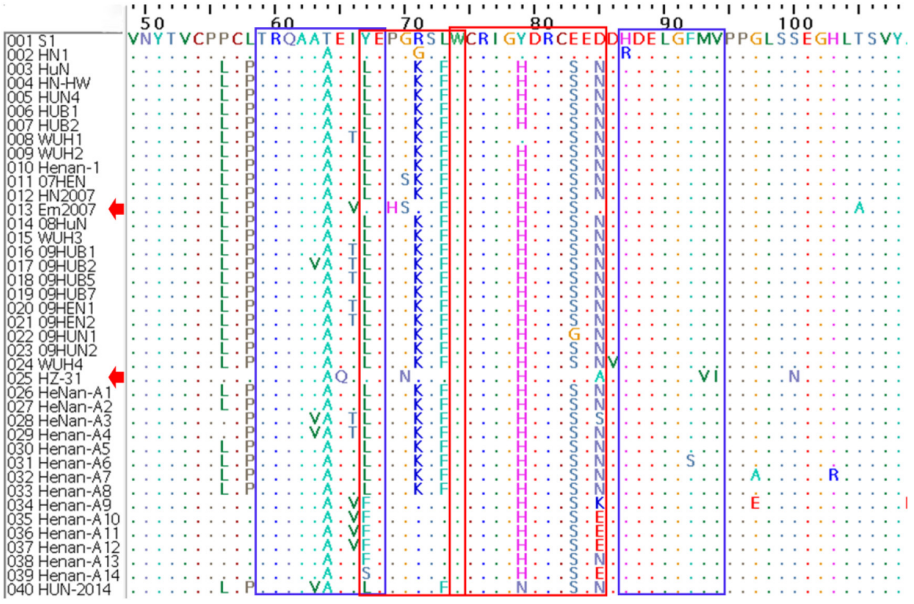

Fig. 3. Amino acid sequence alignments of the partial GP3 gene of the 40 PRRSVs of Central China. The two epitopes, ^{67}YEPGRSLW^{74} and ^{74}WCRIGHDRCGED^{85}, are denoted by red boxes, and the two epitopes, ^{87}HDELGFMV^{94} and ^{59}TRQAAAEILE^{68}, are denoted by blue boxes.

Em2007 and HZ-31.

Amino acid analysis of ORF5a: ORF5a is the newest identified structural protein of PRRSV (identified in 2011) and is encoded by an alternative ORF5a that is present in all Arteriviruses [3, 9]. The homology of ORF5a in the 40 PRRSVs of Central China is 71.7–93.5%. Previous reports suggested that ORF5a was essential for virus viability and infectivity [17, 20], whereas another study reported that the ORF5a antibody response is neither neutralizing nor protective against PRRSV [22]. The ORF5a protein possessed two cysteines at positions 29 and 30 that are highly conserved among NA genotype PRRSVs, and a previous study revealed that replacement of cysteine with glycine at position 30 caused the ORF5a protein to interact non-covalently with itself, which may account for the lethal phenotype of mutants carrying substitution of cysteine to glycine at position 30 [21]. No mutation existed at position 30 in this study (denoted with a red box in Fig. 5); additionally, the mutation R37Q/K was observed in the abovementioned RQ-rich motif [16] in most PRRSVs (denoted with a blue box in Fig. 5).

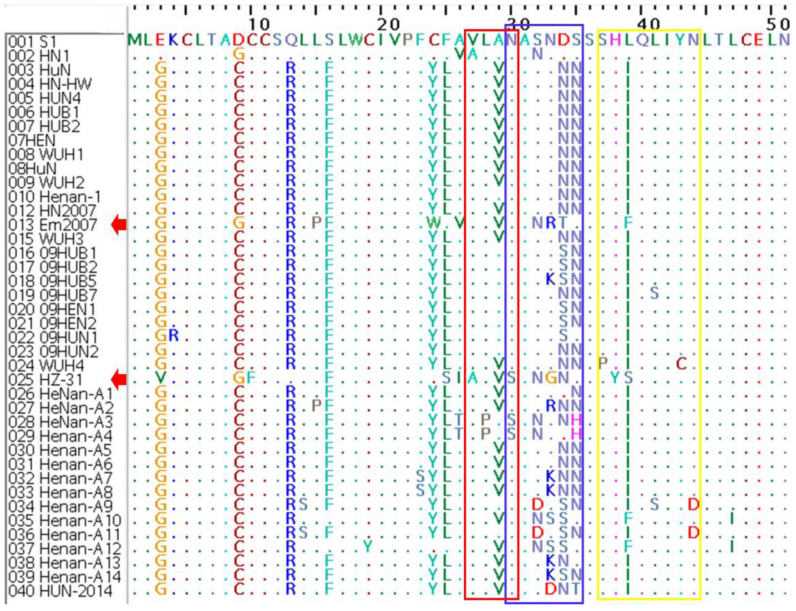

Fig. 4. Amino acid sequence alignments of the partial GP5 gene of the 40 PRRSVs of Central China. The decoy epitope (^{27}V/ALVN^{30}) is denoted with a red box, the primary neutralizing epitope (PNE) (^{37}SHL/IQLIYNL^{45}) is denoted with a yellow box, and the variable N-linked glycosylation motif (^{30}NASNDS^{35}) is denoted with a blue box.

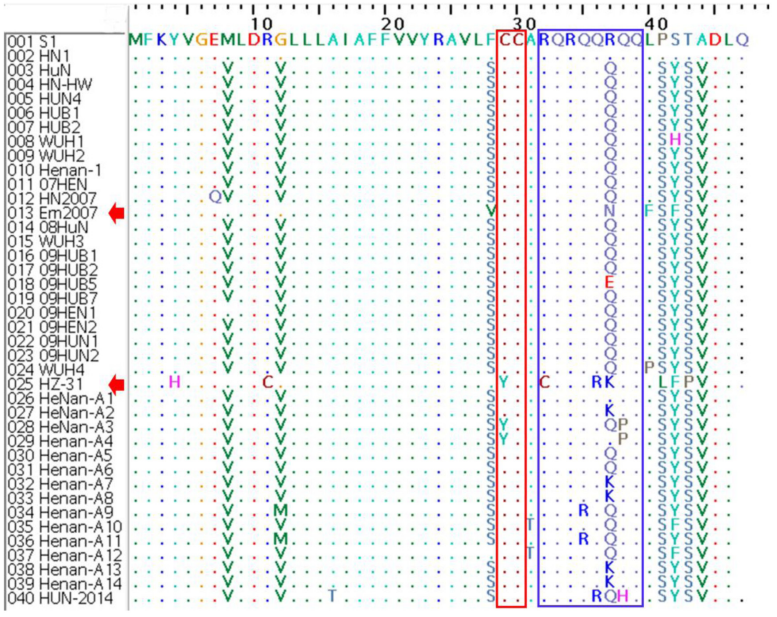

Fig. 5. Amino acid sequence alignments of the partial ORF5a gene of the 40 PRRSVs of Central China. The region of highly conserved cysteines is denoted with a red box, and the RQ-rich motif is denoted with a blue box.

DISCUSSION

Central China is composed of the three provinces, Henan, Hubei and Hunan, that account for almost 23% of the national pig-breeding industry [26], so work related to surveillance and prevention of major porcine epidemic diseases in Central China is essential for the whole pig industry, especially for pandemic disease like PRRS [12, 29]. PRRSV is a vital pathogen that has caused titanic losses for the global swine industry since its first occurrence in the 1980s. The

first Chinese PRRSV strain was isolated in 1995, namely C-PRRSV, and the reference strains, HN1 and S1, were isolated between 1996 and 2005 [12]. The first outbreak of HP-PRRSV occurred in 2006, and it resulted in higher morbidity and mortality than C-PRRSV; this HP-PRRSV possessed the genetic marker of a discontinuous 1+29 AA deletion in NSP2, although the 1+29 AA deletion has subsequently been verified as not related to the high virulence of HP-PRRSV [28]. HP-PRRSV reemerged in 2009 with the same pathogenicity but limited mutations [27, 31], and PRRS has frequently been reported since then [8, 9], which reminds us that the work of monitoring and taking precautions against PRRSV should not be slackened.

On the basis of the whole genome sequences of PRRSV HUN-2014 and the 39 other PRRSVs of Central China from 1998 to 2014, we performed a phylogenetic analysis and a comparative analysis with regard to NSP2, GP3, GP5 and ORF5a. The results demonstrated that these 40 PRRSVs of Central China could be divided into three branches, namely C-PRRSV, S-PRRSV and HP-PRRSV. Since C-PRRSV possessed significantly lower virulence than HP-PRRSV, we tried to seek the characteristics of variation between these 40 PRRSVs, and three particular features were discovered based on Fig. 2 to Fig. 5: (1) abundant concurrent mutations evolved from C-PRRSV to HP-PRRSV, (2) there were diverse mutations at one position from C-PRRSV to S-PRRSV/HP-PRRSV, and (3) there were extraordinary mutations in S-PRRSV but HP-PRRSV. Given this, we speculated that C-PRRSV evolved sophisticated mechanisms to subvert the host defense system by encoding proteins that target key components of the immune signaling pathways in S-PRRSV/HP-PRRSV.

Furthermore, there seems to be numerous concurrent mutations in some particular areas between C-PRRSV and HP-PRRSV, such as the region of 179–782 AA in NSP2, which is associated with the virulence of PRRSV [2]; three conserved epitopes in GP3 that are related to the antigenicity of PRRSV [1, 30]; the decoy epitope and PNE in GP5; and the RQ-rich motif in ORF5a [16], and some of these mutations may be correlated with the virulence or other ability of PRRSV.

To sum up, the genetic diversity of PRRSV should be recognized as a serious issue, and the major variation in PRRSVs should be emphasized, and taken into account along with the crucial changes in phenotype and applied to formulating of preventive for PRRSV.

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