Acta virologica **65**: 221 – 231, 2021 doi: 10.4149/av_2021_213

Genetic variation analysis of Type 2 porcine reproductive and respiratory syndrome virus in Guangdong Province from 2016 to 2018

Pandeng Zhao[1#], Linyang Yu[3#], Yanling Liu[3#], Leyi Zhang[3], Pengshuai Liang[3], Lei Wang[3], Huiyuan Jing[1], Li Huang[2], Changxu Song[3*], Jianguo Dong[2*]

[1]Henan University of Animal Husbandry and Economy, Zhengzhou 450046, P. R. China; [2]School of Animal Husbandry and Medical Engineering, Xinyang Agriculture and Forestry University, Xinyang 464000, P. R. China; [3]College of Animal Science & National Engineering Center for Swine Breeding Industry, South China Agriculture University, Guangzhou 510642, P. R. China

Received July 14, 2020; revised November 30, 2020; accepted April 22, 2021

Summary. – Porcine reproductive and respiratory syndrome virus (PRRSV) is a notable threat to the pig industry. Long-term epidemiological investigations and genetic variation analyses of PRRSV isolates benefit PRRSV prevention and control. In our study, 43 PRRSV strains were successfully isolated from the lungs of sick pigs, and the genetic variations of these isolates were analyzed. Phylogenetic analysis showed that the isolates belonged to PRRSV2 and that lineage 8 (8.7) subgroup III strains remained the dominant type circulating in South China. In addition, sequence alignment analysis identified many novel deletions and mutations in the Nsp2 and GP5 genes. Furthermore, phylogenetic analysis showed that highly frequent recombination events of PRRSV between different lineages might occur in Guangdong Province. These results may help to elucidate the epidemiology and genetic variation of PRRSV isolates in Guangdong Province.

Keywords: GP5; Nsp2; phylogenetic analysis; sequence alignment; porcine reproductive and respiratory syndrome virus (PRRSV)

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important diseases in the swine industry and leads to considerable economic losses each year. PRRS was first reported in America in 1987 and later was described in Europe and Asia (Wensvoort *et al*., 1991; Baron *et al*., 1992; Kuwahara *et al*., 1994). The causative agent of PRRS is porcine reproductive and respiratory syndrome virus (PRRSV), which was first isolated in 1991 in the Netherlands and 1992 in the USA, and the isolated strains were named Lelystad Virus (the European prototypic strain) and ATCC VR-2332 (the North American prototypic strain), respectively (Collins *et al*., 1992; Wensvoort *et al*., 1992). PRRSV can be classified into two genotypes: the European genotype (Type 1) and the North American genotype (Type 2). Although the strains of the two types cause the same clinical symptoms, their genomes only share 50–60% nucleotide homology (Darwich *et al*., 2011).

PRRSV is a single-strand positive-sense RNA virus and belongs to the family of Arteriviridae with approximately 15 kilobases (kb) of genome length (Cavanagh, 1997; Allende *et al*., 1999). The genome of PRRSV includes at least 12 open reading frames (ORFs): ORF1a, ORF1b, ORF2a, ORF2b, ORF3-4, ORF5a, and ORF5-7 (Conzelmann *et al*., 1993; Li *et al*., 2015; Lunney *et al*., 2016). ORF1a and ORF1b encode two polyproteins, which are further cleaved into 16 viral nonstructural proteins (nsps). Other ORFs encode

*Corresponding authors. E-mail: dongjianguo213@163.com (Jianguo Dong), cxsong2004@163.com (Changxu Song); phone: +86 13829723528. #Pandeng Zhao, Linyang Yu, Yanling Liu contributed equally to this work.

Abbreviations: PRRS = porcine reproductive and respiratory syndrome; PRRSV = PRRS virus; LP-PRRSV = low-pathogenic; HP-PRRSV = highly pathogenic PRRSV; MLV = modified live vaccine; TM = transmembrane; PNE = primary neutralizing epitope (PNE)

the following structural proteins: GP2a, E, GP3, GP4, GP5, M, N and ORF5a (Snijder *et al*., 1994; den Boon *et al*., 1995; van Dinten *et al*., 1996; Wassenaar *et al*., 1997; Allende *et al*., 1999; Fang *et al*., 2012). Among these proteins, Nsp2 and GP5 possess higher variability and are usually used for phylogenetic analyses.

Since the first PRRSV strain CH-1a was isolated in China in 1996, Type 2 PRRSV has been circulating and has become the predominant strain in the field (An *et al*., 2007). Because of extensive genetic diversity, the PRRSV strains in China have been divided into four different lineages (lineage 1, lineage 3, lineage 5/sublineage 5.1, lineage 8/sublineage 8.7) based on the comprehensive analysis of the ORF5 gene sequence (Shi *et al*., 2010; Guo *et al*., 2018). Lineage 1 includes NADC30-like strains and has been observed in many areas of China since 2013 (Zhou *et al*., 2015; Li *et al*., 2016). Lineage 3 includes QYYZ-like strains that have been circulating in Southern China since 2010 (Wenhui *et al*., 2012; Lu *et al*., 2015). Lineage 5 (5.1) includes VR-2332-like strains and emerged as early as 1996 but lineage 5 (5.1) has not become the predominant strain in China (An *et al*., 2007). Lineage 8 (8.7) includes low pathogenic PRRSV (LP-PRRSV) strains (CH-1a-like) prevalent before 2006 and highly pathogenic PRRSV (HP-PRRSV) strains (JXA1-like) prevalent after 2006 (Tian *et al*., 2007).

PRRSV has been evolving by the mutation and recombination of different lineages/sublineages (Liu *et al*., 2017; Li *et al*., 2017; Zhao *et al*., 2015; Zhao *et al*., 2017). South China is an important area for pig breeding in China, and because of the damp weather, diseases are likely to occur. The overuse of a cell-attenuated modified live vaccine (MLV) to prevent PRRS increases the immune selective pressure in pigs and accelerates PRRSV variation. To fully understand the genetic evolution of PRRSV in this area, in this paper, we analyzed the prevalence and genetic diversity of PRRSV in South China to characterize PRRSV prevalence and disease control strategies.

Table 1. Information of the representative strains

No.	Strain	Area	Time	Acc. No.
1	Leystad virus	Netherlands	1993	M96262
2	CH-1a	Beijing, China	1996	AY032626
3	BJ-4	Beijing, China	1996	AF331831
4	HB-1(sh)/2002	Hebei, China	2002	AY150312
5	VR-2332	America	2003	AY150564
6	HB-2(sh)/2002	Hebei, China	2004	AY262352
7	NB-04	Zhejiang,China	2004	FJ536165
8	Resp PRRS MLV	America	2005	AF066183
9	SHB	Guangdong, China	2005	EU864232
10	JXA1	Jiangxi, China	2006	EF112445
11	JXwn06	Jiangxi, China	2006	EF641008
12	HB-1/3.9	Hebei,China	2007	EU360130
13	HuN4	Hunan, China	2007	EF635006
14	CH-1R	Heilongjiang, China	2008	EU807840
15	JXA1-P80	Guangdong, China	2008	FJ548853
16	NADC30	America	2008	JN654459
17	BJ0706	Beijing,China	2007	GQ351601
18	GM2	Guangdong, China	2011	JN662424
19	QYYZ	Guangdong, China	2011	JQ308798
20	JL580	Jilin, China	2013	KR706343
21	HENAN-HEB	Henan, China	2014	KJ143621
22	CHsx1401	Beijing, China	2015	KP861625

The strain designation, area, isolated time, Acc. No. of representative strains were listed in Table 1.

Materials and Methods

Sample collection. Lung and blood samples were collected from sick pigs in Guangdong Province in South China from January 2015 to August 2018. The lung samples were homogenized and centrifuged, and the supernatants were used for virus isolation. All the samples were collected according to the animal ethics regulations of the National Engineering Center for Swine Breeding Industry (NECSBI 2015-16).

Virus isolation. Virus isolation was performed in MARC-145 cells, which were maintained in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific™), 100 mg/ml penicillin, and 100 units/ml streptomycin. MARC-145 cells seeded in 6-well cell culture plates (Corning Inc., USA) were incubated with the supernatants from the homogenized lung samples for 1 h. Then the supernatants were discarded, DMEM was added to the wells, and the cells were maintained at 37°C with 5% CO_2. The cultured cells and supernatants were harvested when the cytopathic effect (CPE) appeared in 70% of the cells, the recovered strains were passaged twice in MARC-145 cells, and the viral cultures of the third passage were used for genomic sequence analysis.

RNA extraction and RT-PCR. Total RNA was extracted from blood samples using TRIzol reagent (Life Technologies, USA) according to the manufacturer's instructions. Reverse transcription was performed in a total volume of 20 µl containing 10.5 µl of total RNA, 4 µl of 5 x reverse transcription buffer, 2 µl of deoxynucleoside triphosphate (dNTP) mixture (10 mM), 1 µl of 9-mer random primers (50 pM), 2 µl of reverse transcriptase (5 U/µl; M-MLV, Takara), and 0.5 µl of RNase inhibitor (40 U µl). The reactants were mixed gently, placed in a water bath at 42°C for 1 h, and then incubated on ice for 2 min. Primers to amplify the Nsp2 hypervariable (HV) region were as follows: forward primer, 5'-CTCCGTGGTGCAACAA-3'; reverse primer,

Table 2. Information on the Nsp2 and GP5 genes of 43 PRRSV isolates from South China

No.	Designation	Area	Region	Year	ORF5 (bp)	Acc. No. (ORF5)	Nsp2 (bp)	Acc. No. (Nsp2)
1	GDgz1-16	Guangzhou	Guangdong	2016	603	MK079309	1020	MK079288
2	GDjm1-16	Jiangmen	Guangdong	2016	603	MK079308	969	MK079254
3	GDkp1-16	Kaiping	Guangdong	2016	603	MK079300	1020	MK079286
4	GDmm1-16	Maoming	Guangdong	2016	603	MK079311	969	MK079252
5	GDmz1-16	Meizhou	Guangdong	2016	603	MK079313	969	MK079253
6	GDsg1-16	Shaoguan	Guangdong	2016	603	MK079317	969	MK079256
7	GDsg2-16	Shaoguan	Guangdong	2016	603	MK079315	969	MK079257
8	GDzj1-16	Zhanjiang	Guangdong	2016	603	MK079312	969	MK079268
9	GDzj2-16	Zhanjiang	Guangdong	2016	603	MK079314	969	MK079258
10	GDgz1-17	Guangzhou	Guangdong	2017	603	MK079297	969	MK079259
11	GDgz2-17	Guangzhou	Guangdong	2017	603	MK079328	969	MK079269
12	GDhy1-17	Heyuan	Guangdong	2017	603	MK079301	969	MK079255
13	GDhy2-17	Heyuan	Guangdong	2017	603	MK079302	960	MK079284
14	GDhz1-17	Huizhou	Guangdong	2017	603	MK079332	969	MK079263
15	GDhz2-17	Huizhou	Guangdong	2017	603	MK079333	969	MK079265
16	GDhz3-17	Huizhou	Guangdong	2017	603	MK079334	969	MK079264
17	GDjm1-17	Jiangmen	Guangdong	2017	603	MK079305	969	MK079261
18	GDkp1-17	Kaiping	Guangdong	2017	603	MK079316	1059	MK079279
19	GDkp2-17	Kaiping	Guangdong	2017	603	MK079298	1059	MK079280
20	GDsg1-17	Shaoguan	Guangdong	2017	603	MK079295	1059	MK079285
21	GDsg2-17	Shaoguan	Guangdong	2017	603	MK079296	831	MK079275
22	GDzj1-17	Zhanjiang	Guangdong	2017	603	MK079329	969	MK079266
23	GDgz1-18	Guangzhou	Guangdong	2018	603	MK079318	969	MK079262
24	GDgz2-18	Guangzhou	Guangdong	2018	603	MK079319	969	MK079260
25	GDhz1-18	Huizhou	Guangdong	2018	603	MK079330	918	MK079276
26	GDjy1-18	Jieyang	Guangdong	2018	603	MK079331	966	MK079283
27	GDmm1-18	Maoming	Guangdong	2018	603	MK079306	969	MK079270
28	GDmm2-18	Maoming	Guangdong	2018	603	MK079307	969	MK079271
29	GDmm3-18	Maoming	Guangdong	2018	603	MK079322	969	MK079272
30	GDmm4-18	Maoming	Guangdong	2018	603	MK079299	969	MK079278
31	GDqy1-18	Qingyuan	Guangdong	2018	603	MK079326	1020	MK079287
32	GDqy2-18	Qingyuan	Guangdong	2018	603	MK079327	633	MK079289
33	GDsg1-18	Shaoguan	Guangdong	2018	603	MK079324	642	MK079293
34	GDsg2-18	Shaoguan	Guangdong	2018	603	MK079335	642	MK079290
35	GDsg3-18	Shaoguan	Guangdong	2018	603	MK079337	642	MK079292
36	GDsg4-18	Shaoguan	Guangdong	2018	603	MK079336	642	MK079291
37	GDsh1-18	Sihui	Guangdong	2018	603	MK079323	609	MK079294
38	GDyj1-18	Yangjiang	Guangdong	2018	603	MK079320	1059	MK079281
39	GDyj2-18	Yangjiang	Guangdong	2018	603	MK079321	1083	MK079282
40	GDzq1-18	Zhaoqing	Guangdong	2018	603	MK079303	924	MK079273
41	GDzq2-18	Zhaoqing	Guangdong	2018	603	MK079304	924	MK079274
42	GXnn1-18	Nanning	Guangxi	2018	603	MK079325	927	MK079277
43	GXnn2-18	Nanning	Guangxi	2018	603	MK079310	969	MK079267

The designation, isolated year, Acc. No., and area of isolated strains are listed in Table 2.

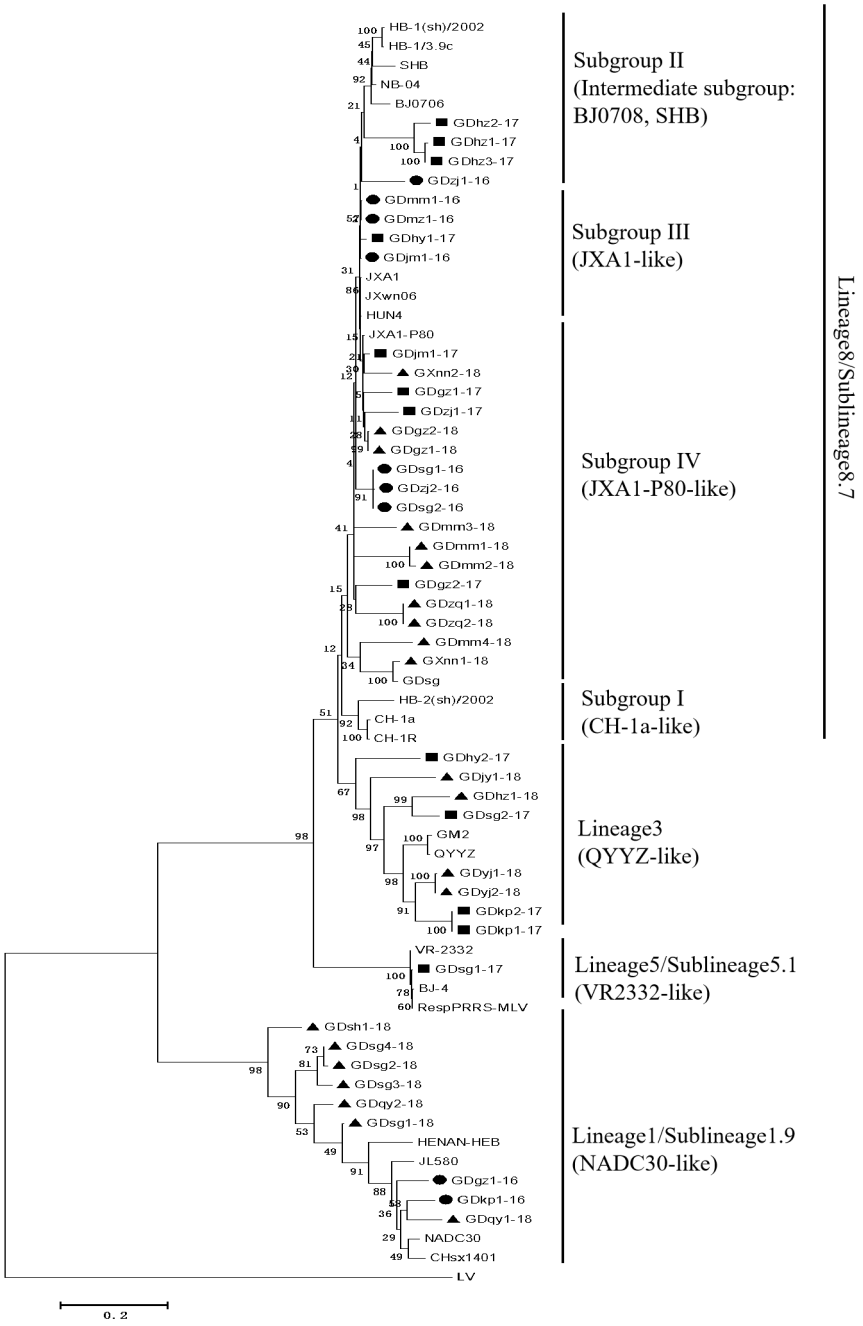

Fig. 1

Phylogenetic tree of PRRSV field isolates based on the translated amino acid sequence of the Nsp2 HV region
The different lineages are marked, and the PRRSV isolates are labeled with black solid circles.

5'-GGCTTGAGCTGAGTAT-3'; Primers to amplify the ORF5 gene were as follows: forward primer, 5'-ATGTTGGGGAAGTGCTT-3'; reverse primer, 5'-GACGACCCCATTGTT-3'. Amplification was performed using the following reaction conditions: one cycle at 94°C for 5 min; 30 cycles at 94°C for 30 s, 57°C for 45 s, and 72°C for 30 s followed by incubation at 72°C for 10 min and a final hold at 4°C. PCR products were visualized by 1% agarose gel electrophoresis and ultraviolet light.

DNA cloning and sequencing. PCR products were purified using the Wizard SV Gel and PCR Clean-Up system (Promega, USA) and then cloned into pEASY Simple Blunt vector (TransGen Tech Co., Beijing, China). Plasmids were submitted to BGI (Guangzhou, China) for sequencing, and the complete PRRSV genome sequence was obtained with the SeqMan program of DNASTAR7.0 software (DNASTAR Inc., Madison, WI, USA). The complete genome sequence was submitted to GenBank, and the accession numbers are listed in Table 2.

Sequence alignment and phylogenetic analysis. Nucleotide and deduced amino acid (aa) sequences were aligned using the MegAlign program of DNASTAR7.0 software (DNASTAR Inc., WI,

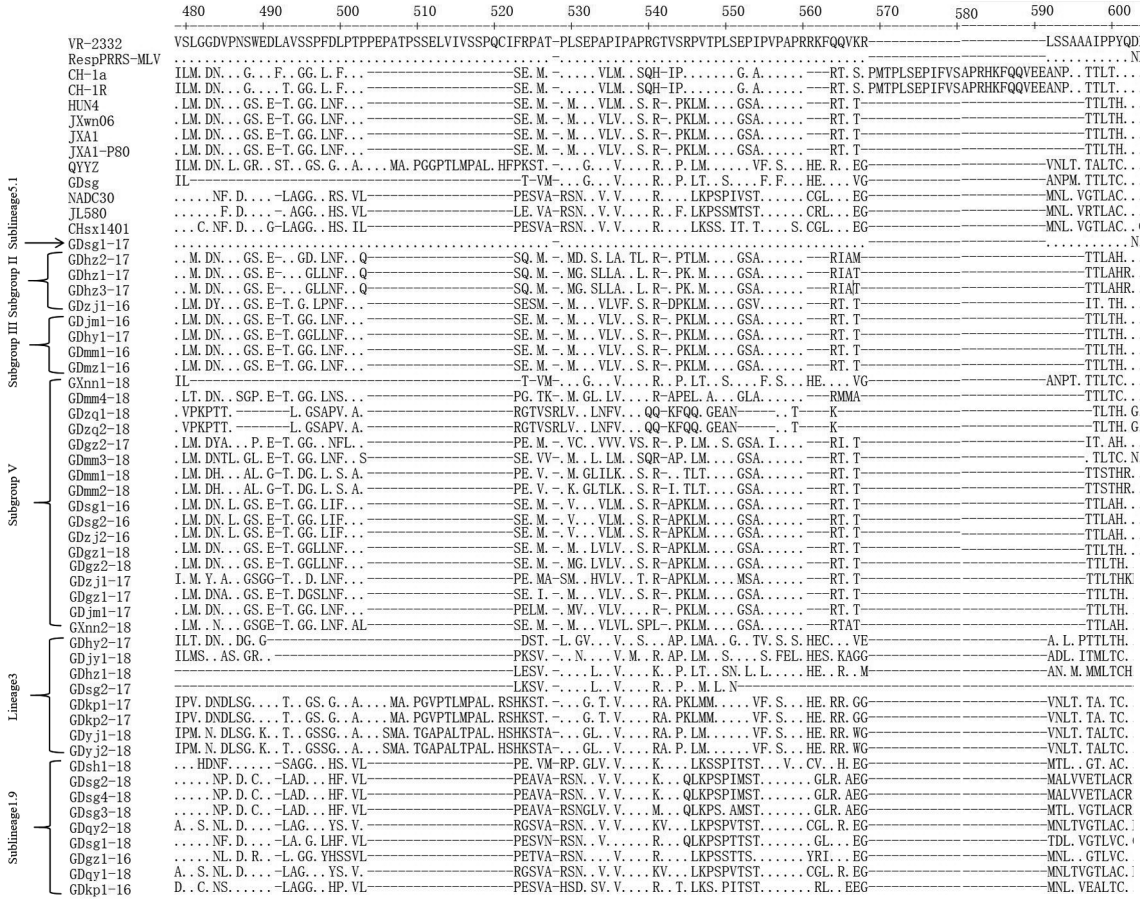

Fig. 2

Alignment of the deduced Nsp2 HV region amino acid sequences from 43 isolates

The sequences of VR-2332 (AY150564), RespPRRS-MLV (AF066183), CH-1a (AY032626), CH-1R (EU807840), JXA1 (EF112445), JXA1-P80 (FJ548853), JXwn06 (EF641008), HUN4 (EF635006), QYYZ (JQ308798), GDsg (KX621003), NADC30 (JN654459), JL580 (KR706343), and CHsx1401 (KP861625) were included for comparison. The deleted regions are highlighted by the dotted line.

USA) to determine sequence homology. A phylogenetic tree was constructed using MEGA5.2 software with the neighbor-joining method; bootstrap values were calculated for 1000 replicates for alignment with multiple sequences of representative PRRSV sequences available in GenBank (Table 2).

Nucleotide sequence accession numbers. The PRRSV nucleotide sequences reported in this paper were submitted to GenBank. The GenBank accession numbers for the Nsp2 HV genes are MK079295 to KU997091, and for the ORF5 genes, they are MK079252 to MK079335 (Table 2).

Results

Phylogenetic analysis of Nsp2 gene sequences

Forty-three PRRSV strains were successfully isolated from the lungs of sick pigs, and the Nsp2 and GP5 genes of isolates were further amplified and sequenced. Based on the global PRRSV classification system, the hypervariable region (HR) sequences of the Nsp2 gene of the isolates from this study and representative PRRSV HR sequences of the Nsp2 gene previously deposited in the database (Table 1), was used to construct the phylogenetic trees using the neighbor-joining method. The results showed that the reference strains and the 43 isolates from this study could be divided into four lineages: lineage 1, lineage 3, lineage 5 (5.1) and lineage 8 (8.7) (Fig. 1). The VR2332-like/BJ-4-like strains belonged to sublineage 5.1, which also included GDsg1-17, GDsg2-17 and GDgz1-17. The sublineage 8.7 could be further divided into four subgroups. The representative strain CH-1a and its cell-attenuated MLV strain CH-1R belonged to subgroup I, and the representative strains BJ0706, HB-1-3.9 and SHB belonged to subgroup II (intermediate subgroup). There were no isolates belonging to subgroup I or subgroup II. There were 20 strains

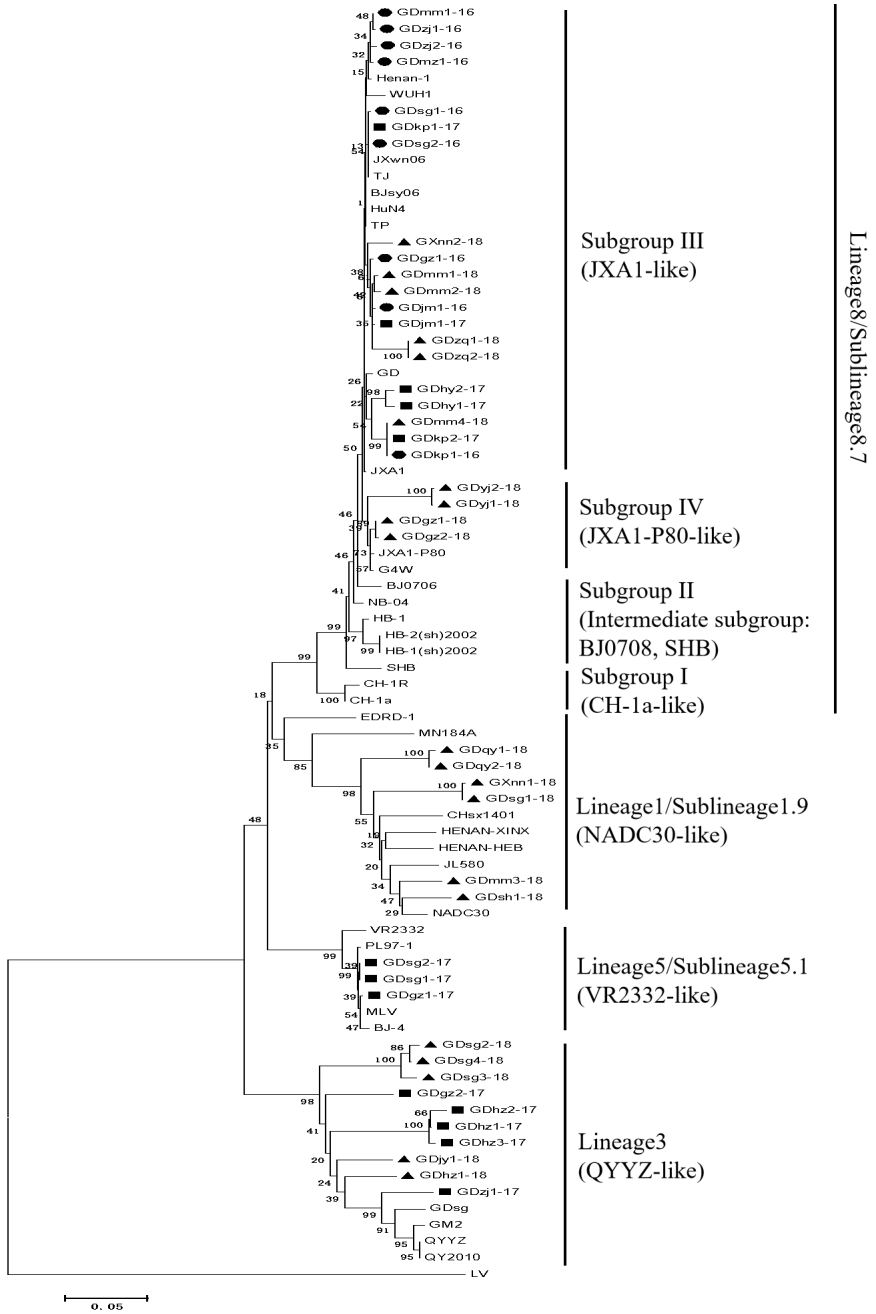

Fig. 3

Phylogenetic tree of PRRSV field isolates based on the translated amino acid sequence of ORF5

The different subgroups are marked, and the PRRSV field isolates are labeled with black solid circles.

belonging to subgroup III that shared close identity with the representative strains JXA1, GD, JXwn06 and Henan-1. GDyj1-18, GDyj2-18, GDgz1-18 and GDgz2-18 belonged to subgroup V, which also included the cell-attenuated MLV strain of JXA1, JXA1-P80. Ten isolates belonged to lineage 3, which contained the representative strains QYYZ and GDsg. The other 6 isolates belonged to sublineage 1.9 and shared high identity with the NADC30-like strains.

Sequence alignment and analysis of Nsp2 gene sequences

The Nsp2 aa sequences of all 43 positive samples were analyzed with the representative strains. Sequence alignments indicated that aa sequence identities among the 43 isolates ranged from 31.3–68.1%, with 31.30–99.2%, 29.2–89.5% and 30.7–98.9% aa similarity among the representative strains VR-2332, CH-1a, and JXA1, respectively.

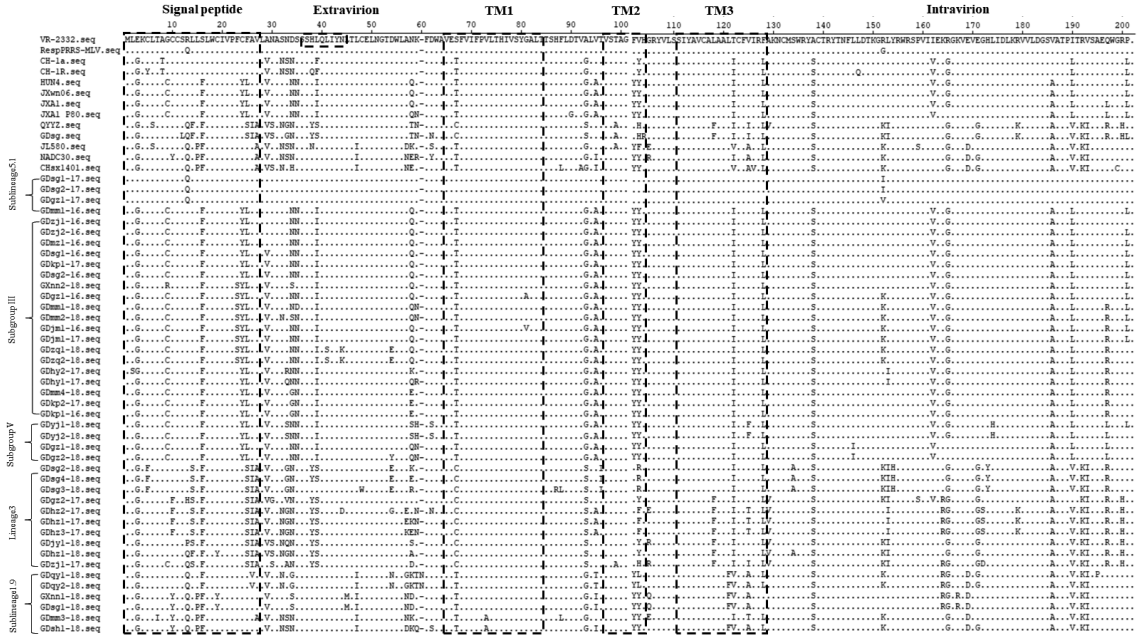

Fig. 4

Alignment of the translated GP5 amino acid sequences of the 43 field isolates

The sequences of VR-2332 (AY150564), RespPRRS-MLV (AF066183), CH-1a (AY032626), CH-1R (EU807840), JXA1 (EF112445), JXA1-P80 (FJ548853), JXwn06 (EF641008), HUN4 (EF635006), QYYZ (JQ308798), GDsg (KX621003), NADC30 (JN654459), JL580 (KR706343), and CHsx1401 (KP861625) were included for comparison. Functional domains, including the signal peptide, extravirion, transmembrane region (TM), and intravirion are marked with dashed boxes. The primary neutralizing epitope (PNE) is also marked with a dashed box.

Compared with QYYZ and NADC30, they shared 29.4–83.2% and 34.7–83.6% aa similarity, respectively. The aa identity with the Type 1 representative strain LV was only 13.8–17.3%. These results indicate that the Nsp2 gene sequences of isolates belonged to Type 2. As shown in Fig. 2, many samples had extensive mutations in the Nsp2 HV region. Compared with VR-2332 and CH-1a strains, 22 samples (51.16%) had the representative deletions of discontinuous 30 aa (aa 492 and 568–596), which is a characteristic of HP-PRRSV. In these 22 samples, GDzq1-18 and GDzq2-18 had another 15 aa deletions (aa 487–493, 552–556 and 565–567). Sixteen of the 43 isolates had continuous 24 aa (aa 568–591) deletions; in these 16 samples, GDhy2-17 had another 12 aa deletions (aa 491 and 493–503), GDjy1-18 had another 11 aa deletions (aa 493-503), 4 samples (GDkp1-17, GDkp2-17, GDyj1-18 and GDyj2-18) had similar deletions as QYYZ strain, and the others had the same deletion as the NADC30-like strains JL580 and CHsx1401. In addition to these different types of aa deletions in the 43 samples, GDsg1-17 had 23 aa deletions (aa 568–590). GXnn1-18 had 68 discontinuous aa deletions (aa 481–523, 525 and 568–592) and had the same deletions as the QYYZ-like strain GDsg. GDhz1-18 had more extensive deletions at aa positions 479–522 and 568-591. Except for deletions at aa 479–522, GDsg2-17 had more extensive deletions at aa 552–603.

Phylogenetic analysis of GP5 gene sequences

Based on the global PRRSV classification system, the GP5 gene of the isolates from this study and representative PRRSV GP5 sequences previously deposited in the database, phylogenetic trees were constructed using the neighbor-joining method. As shown in Fig. 3, the reference strains and 43 isolates from this study could be divided into four lineages: lineage 1 (1.9), lineage 3, lineage 5 (5.1) and lineage 8 (8.7). The VR2332-like/BJ-4-like strains belonged to lineage 5 (5.1), which also included GDsg1-17, GDsg2-17 and GDgz-17. Lineage 8 (8.7) could be further divided into four subgroups. The representative strain CH-1a and its cell-attenuated MLV strain CH-1R belonged to subgroup I, and the representative strains BJ0706, HB-1-3.9 and SHB belonged to subgroup II (intermediate subgroup). There were no isolates belonging to subgroup I or subgroup II. Twenty isolates belonged to subgroup III and shared closed identity with the representative strains JXA1, GD, JXwn06 and Henan-1. GDyj1-18, GDyj2-18, GDgz1-18 and GDgz2-18 isolates belonged to subgroup V, which also included the cell-attenuated MLV strains JXA1, JXA1-P80. Ten positive samples of isolates belonged to lineage 3, which contained the representative strains QYYZ and GDsg. The remaining 6 positive samples of

isolates belonged to sublineage 1 (1.9), and shared high identity with the NADC30-like strains.

Sequence alignment and analysis of GP5 gene

The GP5 nucleotide and aa sequences of all 43 positive samples were analyzed with the representative strains. GDqy1-18 and GDqy2-18 had 1 asparagine (N) aa insertion at position 60 of GP5. Sequence alignments indicated that aa sequence identities among the 43 isolates ranged from 70.6–100% and had 71.1–99.0%, 72.6–93.5% and 71.6–99.0% aa similarity with the representative strains VR-2332, CH-1a, and JXA1, respectively. Compared with QYYZ and NADC30 strains, they shared 73.6–94.5% and 69.7–95.5% aa similarity, respectively. As shown in Fig. 4, the putative signal peptide, extravirion and introvirion regions of the isolates had more aa substitutions, and the TM1, TM2 and TM3 regions were relatively conserved.

The primary neutralizing epitope (PNE) at aa 37–44 of GP5 and epitope V27LVN are important in immune responsiveness of host. Compared with the VR2332 strain, all isolates in subgroup III and subgroup V had an L39I mutation. Except for GDsg3-18, all the other isolates in lineage 3 had H38Y and L39S mutations. Furthermore, GDqz1-18 and GDqz2-18 had L41S and N44K mutations, and GDhz2-17 had an N34D mutation. All isolates of lineage 3 had a V27A mutation. Except for GDmm1-16, GDzj1-16, GDzj2-16, GDmz1-16 and GDzj1-17, all the other strains in subgroup III, subgroup V, lineage 3 and lineage 1 (1.9) had an A29V mutation. In addition, GDgz2-17 had an N30G mutation, and GDjy1-18, GDhz1-18 and GDzj1-17 had an N30S mutation.

Discussion

Since the outbreak of HP-PRRSV in large areas of China in 2006, HP-PRRSV has been a serious threat to the Chinese swine industry (Tian *et al*., 2007; Zhou *et al*., 2014). From 2007 to 2012, HP-PRRSV was the predominant strain in the field and the major cause of PRRS outbreaks (Li *et al*., 2011; Liu *et al*., 2013; Zhou *et al*., 2014). However, since then new QYYZ-like (lineage 3) and NADC30-like (lineage 1) strains emerged in 2010 and 2013, respectively (Wenhui *et al*., 2012; Lu *et al*., 2015; Zhou *et al*., 2015; Li *et al*., 2016). Many reports showed that PRRSV strains emerged by different forms of recombination between members of lineages/sublineages (Lu *et al*., 2015; Zhao *et al*., 2015; Li *et al*., 2017; Zhang *et al*., 2017; Dong *et al*., 2018; Liu *et al*., 2018; Wang *et al*., 2018). To date, PRRSV is still a considerable problem in China's pig industry.

South China is the main pig breeding area. To fully understand the genetic variation of PRRSV in the area, 43 PRRSV strains were successfully isolated from positive samples collected from the lungs of sick pigs. The Nsp2 HV region and GP5 gene of isolates were amplified and sequenced. Genetic variations in the Nsp2 HV region and GP5 genes were analyzed by phylogenetic tree construction and sequence alignment. The 30 discontinuous deletions in the Nsp2 coding region are main characteristics of HP-PRRSV (Li *et al*., 2007). From 2016 to 2018, most isolates (51.16%) had 30 discontinuous aa deletions in the Nsp2 coding region. To control the HP-PRRSV strains, MLVs that were attenuated from HP-PRRSV by passaging in cells and had 30 discontinuous deletions in the Nsp2 coding region were extensively used, which increased the prevalence of the vaccine strain in China. In our study, we found that subgroup III (JXA1-like) was epidemic in 2016 in South China; however, from 2017 to 2018, subgroup IV (JXA1-P80-like) became predominant, implying that the MLV was overused.

The Nsp2 gene often has naturally deletions in the HV region and is used for molecular epidemiology research (Han *et al*., 2006). In addition to the 30 aa discontinuous deletions, more different types of deletions were found in the Nsp2 region. In 2010, the QYYZ-like strain was first reported in Guangdong (Wenhui *et al*., 2012). Subsequently, in 2015, an NADC30-like strain emerged in Jilin and Shanxi. These two strains also had more extensive deletions in the Nsp2 region. In our study, 10 strains had the same deletion and high similarity to the NADC30-like strains JL580 and CHsx1401. These results were consistent with previous reports (Zhao *et al*., 2015; Liang *et al*., 2018). Eight isolates had high similarity to the QYYZ-like strains QYYZ and GDsg. However, different from the Nsp2 deletion in the QYYZ-like strain, in these 8 isolates, GDhz1-18 had more extensive deletions at aa positions 479–522 and 568–591. GDsg2-17 had more extensive deletions at aa 552–603. These results suggest that PRRSV had undergone a sizable mutation (Han *et al*., 2017). It has been reported that the deletion and mutation of Nsp2 aa might elicit a strong antibody response against PRRSV and affect the viral titer (Han *et al*., 2007; Wang *et al*., 2013). The effect of deletion and mutation of Nsp2 aa on the antibody response and virus titer, leading to the prevalence of PRRSV, should be researched further in the future.

PRRSV GP5 is a variable structural protein that is often used for analyzing genetic mutations in the virus (Cha *et al*., 2004). A phylogenetic tree was constructed based on the GP5 gene. Data indicated that all 43 isolates belonged to Type 2 PRRSV. The percentages of lineage 1, lineage 3, subgroup III and subgroup IV were 13.95%, 23.25%, 46.51% and 9.30%, respectively. These data indicated that the HP-PRRSV strain was still predominant in South China, which was similar with Liang *et al*.'s conclusion (Liang *et al*., 2018) but different from Sun *et al*.'s results, which

showed that the prevalence of lineage 3 was 35.68%, which was higher that our data. The reason might be the varying duration of the survey.

Recent studies have revealed that PRRSV has undergone recombination between different lineages (Sun *et al*., 2018; Zhou *et al*., 2018). Compared with the phylogenetic tree of GP5 genes, we further found that some isolates belonged to different lineages when the phylogenetic tree was constructed based on Nsp2 genes. In 2016, 8 isolates might have undergone recombination between subgroup III and subgroup II, which belonged to lineage 8 (8.7). Moreover, in 2017, 11 isolates might have undergone recombination between lineage 3 and lineage 8 (8.7). In 2018, our results showed that recombination between lineage 1 (NADC30-like) and lineage 3 (QYYZ-like) might have existed. These results further imply that highly frequent recombination of PRRSV between different lineages existed in South China, which will increase the difficulty of PRRV control among Chinese scientists and culturists. Whether complex genome recombination existed between more than two lineages and the pathogenicity of recombinant strains will be researched in the future.

The epitopes V27LVN and PNE of GP5 play vital roles in immune responsiveness of the host (Li *et al*., 2009). Compared with VR2332 strain, all isolates of lineage 3 had a V27A mutation. Except for GDmm1-16, GDzj1-16, GDzj2-16, GDmz1-16 and GDzj1-17, all the other strains in subgroup III, subgroup V, lineage 3 and sublineage 1.9 had the A29V mutation. Except for GDsg3-18, all the other isolated strains in lineage 3 had H38Y and L39S mutations. In recent years, lineage 3 strains have been becoming epidemic and have higher virulence for pigs in South China (Dong *et al*., 2017; Gao *et al*., 2017). Gao *et al*. indicated that lineage 1 and lineage 3 strains have spread rapidly in recent years (Gao *et al*., 2017). Our study also found that lineage 1 and lineage 3 strains have been epidemic since 2016, and the number of infected pigs has significantly increased in recent years. Therefore, these results implied that the aa substitutions of PNE and V27LVN might be major reasons for PRRSV to escape immune defenses and virus neutralization, resulting in cell-attenuated live vaccine strains to be useless.

In conclusion, at present, lineage 8 subgroup III strains are still the dominant type circulating in South China. However, lineage 1 (1.9) and lineage 3 strains have become epidemic, and more attention should be paid to them. In addition, many novel deletions and mutations have been found in the Nsp2 and GP5 genes. Highly frequent recombination of PRRSV between different lineages might exist in South China. Therefore, it is necessary to research the influence of deletions/mutations and recombination events on the pathogenicity of the isolates further and to develop effective means for PRRSV prevention and control.

Acknowledgments. This work was supported by National Key Research and Development Program of China (2018YFD0501102), National Key Technologies R&D Program (2015BAD12B02-5), Key Technologies Research and Development Program of Henan Province (202102110095), Natural Science Foundation of Henan (202300410187), Henan Science and Technology Project(212102110364), Youth Backbone Training Program of Colleges and Universities in Henan Province (Jiaogao [2020] No. 354), Key and Cultivation Discipline (preventive veterinary medicine) of 2017 Xinyang Agriculture and Forestry University (ZDXK201702) and National Natural Sciences Foundation of China (32002265).

References

Allende R, Lewis TL, Lu Z, Rock, D.L., Kutish, G.F., Ali, A., Doster, A.R., Osorio, F.A., 1999, North American and European porcine reproductive and respiratory syndrome viruses differ in non-structural protein coding regions. J. Gen. Virol. 80 (2), 307–315.

An TQ, Zhou YJ, Liu GQ, Tian ZJ, Li J, Qiu HJ, Tong GZ (2007): Genetic diversity and phylogenetic analysis of glycoprotein 5 of PRRSV isolates in mainland China from 1996 to 2006: coexistence of two NA-subgenotypes with great diversity. Vet. Microbiol. 123, 43–52.

Baron T, Albina E, Leforban Y, Madec F, Guilmoto H, Plana DJ, Vannier P (1992): Report on the first outbreaks of the porcine reproductive and respiratory syndrome (PRRS) in France. Diagnosis and viral isolation. Ann. Rech. Vet. 23, 161–166.

Cavanagh D (1997): Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch. Virol. 142, 629–633.

Cha SH, Chang CC, Yoon KJ (2004): Instability of the restriction fragment length polymorphism pattern of open reading frame 5 of porcine reproductive and respiratory syndrome virus during sequential pig-to-pig passages. J. Clin. Microbiol. 42, 4462–4467. https://doi.org/10.1128/JCM.42.10.4462-4467.2004

Collins JE, Benfield DA, Christianson WT, Harris L, Hennings JC et al. (1992): Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs. J. Vet. Diagn. Invest. 4, 117–126. https://doi.org/10.1177/104063879200400201

Conzelmann KK, Visser N, Van Woensel P, Thiel HJ (1993): Molecular characterization of porcine reproductive and respiratory syndrome virus, a member of the arterivirus group. Virology 193, 329–339. https://doi.org/10.1006/viro.1993.1129

Darwich L, Gimeno M, Sibila M, Diaz I, de la Torre E, Dotti S, Kuzemtseva L, Martin M, Pujols J, Mateu E (2011): Genetic and immunobiological diversities of porcine reproductive and respiratory syndrome genotype I strains. Vet. Microbiol. 150, 49–62. https://doi.org/10.1016/j.vetmic.2011.01.008

den Boon JA, Faaberg KS, Meulenberg JJ, Wassenaar AL, Plagemann PG, Gorbalenya AE, Snijder EJ (1995): Processing and evolution of the N-terminal region of the arterivirus replicase ORF1a protein: identification of two papainlike cysteine proteases. J. Virol. 69, 4500–4505. https://doi.org/10.1128/JVI.69.7.4500-4505.1995

Dong J, Wang Y, Yu L, Zhang P, Liu X, Zhang L, Liu Y, Liang P, Wang L, Song C (2017): Pathogenicity of a newly emerged recombined porcine reproductive and respiratory syndrome virus strain (subgenotype III) in China. Vet. Microbiol. 210, 162–166. https://doi.org/10.1016/j.vetmic.2017.09.018

Dong JG, Yu LY, Wang PP, Zhang LY, Liu YL, Liang PS, Song CX (2018): A new recombined porcine reproductive and respiratory syndrome virus virulent strain in China. J. Vet. Sci. 19, 89–98. https://doi.org/10.4142/jvs.2018.19.1.89

Fang Y, Treffers EE, Li Y, Tas A, Sun Z, van der Meer Y, de Ru AH, van Veelen PA, Atkins JF, Snijder EJ, Firth AE (2012): Efficient -2 frameshifting by mammalian ribosomes to synthesize an additional arterivirus protein. Proc. Natl. Acad. Sci. USA 109, E2920–E2928. https://doi.org/10.1073/pnas.1211145109

Gao JC, Xiong JY, Ye C, Chang XB, Guo JC, Jiang CG, Zhang GH, Tian ZJ, Cai XH, Tong GZ, An TQ (2017): Genotypic and geographical distribution of porcine reproductive and respiratory syndrome viruses in mainland China in 1996–2016. Vet. Microbiol. 208, 164–172.

Guo Z, Chen XX, Li R, Qiao S, Zhang G (2018): The prevalent status and genetic diversity of porcine reproductive and respiratory syndrome virus in China: a molecular epidemiological perspective. Virol. J. 15, 2.

Han J, Liu G, Wang Y, Faaberg KS (2007). Identification of nonessential regions of the Nsp2 replicase protein of porcine reproductive and respiratory syndrome virus strain VR-2332 for replication in cell culture. J. Virol. 81, 9878–9890. https://doi.org/10.1128/JVI.00562-07

Han J, Wang Y, Faaberg KS (2006): Complete genome analysis of RFLP 184 isolates of porcine reproductive and respiratory syndrome virus. Virus Res. 122, 175–182. https://doi.org/10.1016/j.virusres.2006.06.003

Han J, Zhou L, Ge X, Guo X, Yang H (2017): Pathogenesis and control of the Chinese highly pathogenic porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 209, 30–47.

Kuwahara H, Nunoya T, Tajima M, Kato A, Samejima T (1994): An outbreak of porcine reproductive and respiratory syndrome in Japan. J. Vet. Med. Sci. 56, 901–909. https://doi.org/10.1292/jvms.56.901

Li B, Fang L, Guo X, Gao J, Song T, Bi J, He K, Chen H, Xiao S (2011): Epidemiology and evolutionary characteristics of the porcine reproductive and respiratory syndrome virus in China between 2006 and 2010. J. Clin. Microbiol. 49, 3175–3183.

Li B, Xiao S, Wang Y, Xu S, Jiang Y, Chen H, Fang L (2009): Immunogenicity of the highly pathogenic porcine reproductive and respiratory syndrome virus GP5 protein encoded by a synthetic ORF5 gene. Vaccine 27, 1957–1963. https://doi.org/10.1016/j.vaccine.2009.01.098

Li C, Zhuang J, Wang J, Han L, Sun Z, Xiao Y, Ji G, Li Y, Tan F, Li X, Tian K (2016): Outbreak Investigation of NADC30-Like PRRSV in South-East China. Transbound. Emerg. Dis. 63, 474–479.

Li X, Bao H, Wang Y, Tian K (2017): Widespread of NADC30-like PRRSV in China: Another Pandora's box for Chinese pig industry as the outbreak of highly pathogenic PRRSV in 2006? Infect. Genet. Evol. 49, 12–13. https://doi.org/10.1016/j.meegid.2016.12.021

Li Y, Tas A, Sun Z, Snijder EJ, Fang Y (2015): Proteolytic processing of the porcine reproductive and respiratory syndrome virus replicase. Virus Res. 202, 48–59. https://doi.org/10.1016/j.virusres.2014.12.027

Li Y, Wang X, Bo K, Wang X, Tang B, Yang B, Jiang W, Jiang P (2007): Emergence of a highly pathogenic porcine reproductive and respiratory syndrome virus in the Mid-Eastern region of China. Vet. J. 174, 577–584. https://doi.org/10.1016/j.tvjl.2007.07.032

Liang W, Zhao T, Peng Z, Sun Y, Stratton CW, Zhou D, Tang X, Tian Y, Chen H, Wu B (2018): Epidemiological and genetic characteristics of porcine reproductive and respiratory syndrome virus circulating in central and South China in 2016. Acta Trop. 190, 83–91. https://doi.org/10.1016/j.actatropica.2018.11.004

Liu JK, Wei CH, Yang XY, Hou XL, Dai AL, Li XH, Wei MK, Pan XZ (2013): Genetic diversity and evolutionary characterization of Chinese porcine reproductive and respiratory syndrome viruses based on Nsp2 and ORF5. Arch. Virol. 158, 1811–1816.

Liu JK, Zhou X, Zhai JQ, Li B, Wei CH, Dai AL, Yang XY, Luo ML (2017): Emergence of a novel highly pathogenic porcine reproductive and respiratory syndrome virus in China. Transbound. Emerg. Dis. 64, 2059–2074.

Liu Y, Li J, Yang J, Zeng H, Guo L, Ren S, Sun W, Chen Z, Cong X, Shi J, Chen L, Du Y, Li J, Wang J, Wu J, Yu J (2018): Emergence of Different Recombinant Porcine Reproductive and Respiratory Syndrome Viruses, China. Sci. Rep. 8, 4118. https://doi.org/10.1038/s41598-018-25625-z

Lu WH, Tun HM, Sun BL, Mo J, Zhou QF, Deng YX, Xie QM, Bi YZ, Leung FC, Ma JY (2015): Re-emerging of porcine respiratory and reproductive syndrome virus (lineage 3) and increased pathogenicity after genomic recombination with vaccine variant. Vet. Microbiol. 175, 332–340.

Lunney JK, Fang Y, Ladinig A, Chen N, Li Y, Rowland B, Renukaradhya GJ (2016): Porcine Reproductive and Respiratory Syndrome Virus (PRRSV): Pathogenesis and Interaction with the Immune System. Annu. Rev. Anim. Biosci. 4, 129–154. https://doi.org/10.1146/annurev-animal-022114-111025

Shi M, Lam TT, Hon CC, Hui RK, Faaberg KS, Wennblom T, Murtaugh MP, Stadejek T, Leung FC (2010): Molecular epidemiology of PRRSV: a phylogenetic perspective. Virus Res. 154, 7–17. https://doi.org/10.1016/j.virusres.2010.08.014

Snijder EJ, Wassenaar AL, Spaan WJ (1994): Proteolytic processing of the replicase ORF1a protein of equine arteritis virus. J. Virol. 68, 5755–5764. https://doi.org/10.1128/JVI.68.9.5755-5764.1994

Sun YK, Li Q, Yu ZQ, Han XL, Wei YF, Ji CH, Lu G, Ma CQ, Zhang GH, Wang H (2018): Emergence of novel recombination lineage 3 of porcine reproductive and respiratory syndrome viruses in Southern China. Transbound. Emerg. Dis. 66(1), 578–587. https://doi.org/10.1111/tbed.13067

Tian K, Yu X, Zhao T, Feng Y, Cao Z, Wang C, Hu Y, Chen X, Hu D, Tian X, Liu D, Zhang S, Deng X, Ding Y, Yang L, Zhang Y, Xiao H, Qiao M, Wang B, Hou L, Wang X, Yang X, Kang L, Sun M, Jin P, Wang S, Kitamura Y, Yan J, Gao GF (2007): Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. PLOS One 2, e526. https://doi.org/10.1371/journal.pone.0000526

van Dinten LC, Wassenaar AL, Gorbalenya AE, Spaan WJ, Snijder EJ (1996): Processing of the equine arteritis virus replicase ORF1b protein: identification of cleavage products containing the putative viral polymerase and helicase domains. J. Virol. 70, 6625–6633. https://doi.org/10.1128/JVI.70.10.6625-6633.1996

Wang LJ, Wan B, Guo Z, Qiao S, Li R, Xie S, Chen XX, Zhang G (2018): Genomic analysis of a recombinant NADC30-like porcine reproductive and respiratory syndrome virus in china. Virus Genes 54, 86–97. https://doi.org/10.1007/s11262-017-1516-1

Wang X, Sun L, Li Y, Lin T, Gao F, Yao H, He K, Tong G, Wei Z, Yuan S (2013): Development of a differentiable virus via a spontaneous deletion in the Nsp2 region associated with cell adaptation of porcine reproductive and respiratory syndrome virus. Virus Res. 171, 150–160. https://doi.org/10.1016/j.virusres.2012.11.006

Wassenaar AL, Spaan WJ, Gorbalenya AE, Snijder EJ (1997): Alternative proteolytic processing of the arterivirus replicase ORF1a polyprotein: evidence that Nsp2 acts as a cofactor for the NSP4 serine protease. J. Virol. 71, 9313–9322. https://doi.org/10.1128/JVI.71.12.9313-9322.1997

Wenhui L, Zhongyan W, Guanqun Z, Zhili L, JingYun M, Qingmei X, Baoli S, Yingzuo B (2012): Complete genome sequence of a novel variant porcine reproductive and respiratory syndrome virus (PRRSV) strain: evidence for recombination between vaccine and wild-type PRRSV strains. J. Virol. 86, 9543. https://doi.org/10.1128/JVI.01341-12

Wensvoort G, de Kluyver EP, Pol JM, Wagenaar F, Moormann RJ, Hulst MM, Bloemraad R, den Besten A, Zetstra T, Terpstra C (1992): Lelystad virus, the cause of porcine epidemic abortion and respiratory syndrome: a review of mystery swine disease research at Lelystad. Vet. Microbiol. 33, 185–193. https://doi.org/10.1016/0378-1135(92)90046-V

Wensvoort G, Terpstra C, Pol JM, ter Laak EA, Bloemraad M, et al. (1991): Mystery swine disease in The Netherlands: the isolation of Lelystad virus. Vet. Q. 13, 121–130.

Zhang Q, Bai J, Hou H, Song Z, Zhao Y, Jiang P (2017): A novel recombinant porcine reproductive and respiratory syndrome virus with significant variation in cell adaption and pathogenicity. Vet. Microbiol. 208, 150–158. https://doi.org/10.1016/j.vetmic.2017.07.028

Zhao H, Han Q, Zhang L, Zhang Z, Wu Y, Shen H, Jiang P (2017): Emergence of mosaic recombinant strains potentially associated with vaccine JXA1-R and predominant circulating strains of porcine reproductive and respiratory syndrome virus in different provinces of China. Virol. J. 14, 67. https://doi.org/10.1186/s12985-017-0735-3

Zhao K, Ye C, Chang XB, Jiang CG, Wang SJ, Cai XH, Tong GZ, Tian ZJ, Shi M, An TQ (2015): Importation and Recombination Are Responsible for the Latest Emergence of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus in China. J. Virol. 89, 10712–10716. https://doi.org/10.1128/JVI.01446-15

Zhou L, Kang R, Zhang Y, Ding M, Xie B, Tian Y, Wu X, Zuo L, Yang X, Wang H (2018): Whole Genome Analysis of Two Novel Type 2 Porcine Reproductive and Respiratory Syndrome Viruses with Complex Genome Recombination between Lineage 8, 3, and 1 Strains Identified in Southwestern China. Viruses 10. https://doi.org/10.3390/v10060328

Zhou L, Wang Z, Ding Y, Ge X, Guo X, Yang H (2015): NADC30-like Strain of Porcine Reproductive and Respiratory Syndrome Virus, China. Emerg. Infect. Dis. 21, 2256–2257.

Zhou L, Yang X, Tian Y, Yin S, Geng G, Ge X, Guo X, Yang H (2014): Genetic diversity analysis of genotype 2 porcine reproductive and respiratory syndrome viruses emerging in recent years in China. Biomed. Res. Int. 2014, 748068. https://doi.org/10.1155/2014/748068