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Genetic characterization and pathogenicity of two recombinant PRRSV-2 strains from lineages 1, 3, 5, and 8 emerged in China

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Abstract

Background Porcine reproductive and respiratory syndrome virus (PRRSV) is a major economic threat to the global swine industry. Currently, NADC30-like PRRSV has undergone complex recombination with local Chinese strains, which has exacerbated the evolution of PRRSV. Recently, new recombinant PRRSV-2 strains from four lineages (lineages 1, 3, 5, and 8) have emerged in China. However, information on the pathogenicity of the novel isolate in China remains limited. To further our knowledge about the isolate, FJLIUY2017 and PRRSV2/CN/G8/2018 were selected to analyze their pathogenicity for piglets.

Methods The PRRSV FJLIUY2017 and PRRSV2/CN/G8/2018 strains were isolated by porcine alveolar macrophages (PAMs) and MARC-145CD^{163}. Complete genomic sequence analyses were conducted using the DNASTAR 7.0 software and the phylogenetic tree was constructed with MEGA 7.0. Recombination events were detected using RDP V4.10 and SIMPLOT software 3.5.1. Five PRRSV-free per group were inoculated with 2 mL (2×10^5 TCID50) of the FJLIUY-2017 and PRRSV2/CN/G8/2018. Clinical signs of disease were recorded daily after challenge. Blood samples were collected from all piglets on days 0, 4, 7, 11, and 14 dpi for analysis of viral load by IFA and PRRSV-specific antibody levels by ELISA kit. Lung gross and microscopic lesions of the inoculated piglets were examined by scoring system for lung lesion.

Results Full-length genome analysis revealed that FJLIUY2017 and PRRSV2/CN/G8/2018 share 89.2% identity with each other, and in particular, they had a low degree of homology (< 92%) with PRRSV sequences available in GenBank. Phylogenetic and recombination analyses revealed that the two strains were recombinant viruses from lineages 1, 3, 5.1, and 8.7 strains. Animal studies indicated that FJLIUY-2017 resulted in the typical clinical signs of PRRSV, including persistent fever, higher viremia, severe lung lesions, and 20% mortality, whereas PRRSV2/CN/G8/2018 caused moderate clinical symptoms and no mortality during the challenge period. Hyper-immune sera against the major vaccine strains JXA1-R (lineage 8) and Ingelvac PRRS MLV (Lineage 5) failed to neutralize two strains.

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Conclusions FJLIUY-2017 caused persistent fever, higher viremia, 20% mortality and exhibited higher pathogenicity in piglets compared to PRRSV2/CN/G8/2018. Our results suggest that recombination between different PRRSV-2 lineages can result in the development of PRRSV variants with increased pathogenicity.

Keywords Porcine reproductive and respiratory syndrome virus (PRRSV), Genetic characterization, Recombination, Pathogenicity

Background

Porcine reproductive and respiratory syndrome (PRRS) has resulted in severe economic losses in the swine industry since its first recognition in the United States in the late 1980s [1]. PRRS virus (PRRSV), the causative agent of PRRS, is an enveloped, single-stranded, positive-sense RNA virus belonging to the genus *Betaarterivirus*, subfamily *Variartevirinae* family *Arteriviridae* (https://talk.ictvonline.org/taxonomy). The genome of PRRSV is ~15 kb in length and contains more than 11 open reading frames (ORFs) designated as ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5a, ORF5-ORF7 and NSP2 (TF), which encode at least 14 non-structural proteins (NSP) and eight structural proteins [1, 2].

Based on the whole genome, PRRSV can be typically classified into two species *Betaarterivirus suid* 1 (former PRRSV-1, European type) and *Betaarterivirus suid* 2 (former PRRSV-2, American type), PRRSV-2 is predominant in China since its initial recognition in 1996 [3–5]. To date, based on the global PRRSV classification system, the majority of Chinese PRRSV-2 strains can be classified into four lineages/sublineages: lineage 1 (sublineage 1.8/NADC30-like), lineage 3 (QYYZ-like), lineage 5 (sublineage 5.1/VR2332-like), and lineage 8 (sublineage 8.7/JXA1-like and CH-1a-like) [3, 4, 6]. Lineage 8 mainly contains the highly pathogenic PRRSV-2 (HP-PRRSV/JXA1-like/sublineage 8.7), which emerged in China in 2006 and caused at least one million pig deaths [7]. Lineage 1, defined as NADC30-like PRRSV (sublineage 1.8) with the highest similarity to the American NADC30 strain, was imported into China from the United States in 2012 and has spread rapidly throughout China [3, 4, 8]. Lineage 3 (QYYZ-like) strains were first reported in China in 2010, while lineage 5 (VR-2332-like) strains first appeared in China in 1996 [9, 10]. Now, lineage 3 strains were mainly prevalent in southern China [3, 11, 12]. Recently, NADC34-like PRRSV belonging to sublineage 1.5 has emerged in several provinces in China since 2017 [13, 14].

Currently, multiple lineages/sublineages of PRRSV strains co-existed in Chinese swine herds, potentially facilitating virus recombination. In particular, the recombination characteristics of NADC30-like PRRSV contribute to the emergence of novel variant viruses [15–17]. Indeed, numerous studies have found that novel PRRSV variants resulting from recombination between two (sublineage 1.8 and lineage 3, sublineage 1.8 and 5.1, or sublineage 1.8 and 8.7) or three (sublineages 1.8, 5.1, and 8.7, or sublineage 1.8, 3 and lineage 8.7) lineages exhibit mild/high pathogenicity in pigs [18–26]. In the current study, two PRRSV-2 strains, FJLIUY-2017 and PRRSV2/CN/G8/2018, were isolated from Fujian province of China during 2017–2018, which are natural recombinant viruses from four lineages (lineages 1, 3, 5 and 8) of PRRSV-2 circulating in China. However, there is no information on the pathogenicity as well as growth properties of the newly-emerged PRRSV isolates; thus, we described the genome characteristics of the two new isolates while also analyzing their pathogenicity in piglets.

Methods

Cells and virus isolation

Porcine pulmonary alveolar macrophages (PAMs) were cultured in RPMI 1640 medium (Fisher Scientific, USA) with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO2. MARC-145CD^{163} cells were cultured in Gibco DMEM (Fisher Scientific, USA) with 10% FBS at 37 °C with 5% CO2 [6].

The strain FJLIUY-2017 was isolated in 2017 from a pig herd experiencing 25% abortion of sows and 25% of piglets died in Fujian Province, China [27]. The strain PRRSV2/CN/G8/2018 was isolated in 2018 from a pig herd experiencing 35% abortion of sows in Fujian Province, China. The strain PRRSV2/CN/G8/2018 was isolated and identified from lung tissue using porcine pulmonary alveolar macrophages (PAMs) and MARC-145CD^{163} cells as previously described [6, 27]. Then two PRRSV strains have been used for subsequent studies after being purified for three generations via plaque assay.

Growth curve analysis of the FJLIUY-2017 and PRRSV2/CN/G8/2018 viruses was assessed by measuring the virus titers at different time points post-infection in porcine alveolar macrophages (PAMs) and MARC-145 CD^{163} cells.

Genome sequencing and recombinant analysis

A nucleic extraction kit (Tiangen Biotech, Beijing, China) was used to extract total RNA from the cell culture and lung samples according to the manufacturer's instructions. The genome of PRRSV2/CN/G8/2018 was amplified as previously described [27]. Meanwhile, the 5′-and 3′-untranslated regions (UTR) of the PRRSV2/CN/G8/2018 genome were amplified using the SMARTer® RACE 5′/3′ kit (TaKaRa) according to the manufacturer's

instructions. The PCR products were purified using a TIANquick Midi purification kit (Tiangen Biotech, Beijing, China) and cloned into pEASY®-Blunt Simple Cloning Kit (Transgen Tiangen Biotech, Beijing, China); at least three recombinant clones were sequenced by Ruibo Life Technologies Corporation (Beijing, China).

To analyze the evolutionary relationship of the two PRRSV field strains with representative PRRSV isolates of different lineages (Supplementary Table S1), phylogenetic analysis was performed using the neighbor-joining (NJ) method in MEGA 7.0, as previously described [28] and bootstrap values were evaluated on 1000 replicates. Recombination signals were detected using seven methods (RDP, BootScan, GENECONV, Chimaera, Maxchi, SiScan, and 3Seq) within the Recombination Detection Program version 4.10 (RDP V4.10) and were further confirmed in SIMPLOT software version 3.5.1 within a 200-bp window sliding along the genome alignment (20-bp step size) [29]. To avoid false positive results, recombination events were confirmed in RDP 4.10 using at least five different methods, with the highest acceptable p-value being 0.01 [30].

Animal challenge experiment

Fifteen four-week-old piglets confirmed to be free of PRRSV, PRV, PCV2 and CSFV were purchased from a commercial pig farm (Supplementary Table S2, Table S3). All animals were randomly divided into three groups ($n = 5$), designated as control, FJLIUY-2017-inoculated group, and PRRSV2/CN/G8/2018-inoculated group. Each group was housed separately in different isolation rooms with feed and water provided ad libitum. Each piglet in the two infection groups (FJLIUY-2017 and PRRSV2/CN/G8/2018) was inoculated with 2 mL of virus containing 2×10^5 TCID50, 1 mL by the intranasal route and 1 mL by intramuscular route. Each piglet in the control group was mock-injected with the same dose of DMEM.

After inoculation, rectal temperatures were recorded twice daily from − 2 days post-inoculation (dpi), and the threshold of fever was set at 40.0 °C. Clinical signs of disease were recorded daily for each piglet according to a scoring system based on a previous study [31]. Blood samples were collected from all piglets on days 0, 4, 7, 11, and 14 dpi for analysis of viral load and PRRSV-specific antibody levels. Meanwhile, all piglets in this study were weighed at 0 and 14 dpi, and the average daily weight gain (ADWG) was calculated. All animals were euthanized at 14 dpi for pathological examination. At death, individual lungs were collected and lung lesions were scored as previously described [31, 32].

Virus Titration and serology

The viral titers of PRRSV in sera from experimentally infected piglets were analyzed through immunofluorescence assay (IFA) as previously described [33] and then calculated as described by Reed and Muench (1938) [34] and expressed as TCID50/mL. Briefly, a 10-fold serial dilution of each serum sample was inoculated on MARC-145 cells in 96-well plates and incubated for 90 min at 37 ℃. The cells were then washed with PBS and cultured in DMEM supplemented with 5% fetal bovine serum (FBS) for an additional 48 h. The virus titers were determined by indirect immunofluorescence assay (IFA) with anti-N protein monoclonal antibody (MAb) 1AC7 (INGENASA, Spain).

PRRSV-specific antibodies in serum samples collected from all piglets were analyzed by the commercial PRRSV antibody ELISA kit (IDEXX Laboratories Inc., Westbrook, ME, USA) according to the manufacturer's instructions. Samples were considered positive for the presence of PRRSV antibodies when their S/P is > 0.4.

Pathological examination

Lung gross pathological examination was performed immediately when the piglets died in the experiment. At 14 dpi of the experiment, the piglets were euthanized using deep anaesthesia by intravenous injection with an overdose of sodium pentobarbital (100 mg/kg body weight), followed by exsanguination [35]. All euthanasias in this study followed Chinese Laboratory animal-Guidelines for euthanasia. Gross lesions of the lung were scored by estimating the percentage of the lung surface affected as previously described [32]. Lung tissues of piglets were collected at necropsy, fixed in 10% buffered formalin, and then processed by routine histopathological procedures. Sections of 5 μm were stained with haematoxylin and eosin (HE), and microscopic lesions were evaluated blindly and scored (from 0 to 4) to analyze the severity of interstitial pneumonia as previously described [32].

The distribution of PRRSV antigen in the lung samples was detected by immunohistochemistry (IHC) staining with the monoclonal antibody 1AC7 (Ingenasa, Madrid, Spain) specific for PRRSV N protein based on a previously published procedure [33]. The number of positive cells per section of lung taken from each piglet was evaluated through a ranked score of 0–4 as follows: 0 (no PRRSV-antigen cells), 1 (1–10 positive cells), 2 (11–30 positive cells), 3 (31–100 positive cells), 4 (= or > 100 positive cells) [36].

Virus cross-neutralization assay

Seven live attenuated PRRSV-2 vaccines, including classical PRRSV-derived live vaccines (Ingelvac PRRS MLV, CH-1R, and R98) and HP-PRRSV-derived live vaccines (JXA1-R, HuN4-F112, TJM-F92, and GDr180) are

commercially available in China. In this study, two representative MLV vaccines, Ingelvac PRRS MLV and JXA1-R (MLV-derived HP-PRRSV), were selected to use for cross-neutralization assays. Hyper-immune sera (#1–3 and #5–7) were collected from pigs vaccinated twice with two doses of JXA1-R and Ingelvac PRRS® MLV vaccine, respectively, and used for cross-neutralization assays as previously described [37]. Neutralizing antibody (NA) titers against FJLIUY-2017 and PRRSV2/CN/G8/2018 were calculated according to the Reed-Muench method [34]. Serum samples were considered positive in neutralization against two strains if a neutralization titer was ≥ 8.

Statistical analysis

The data are expressed as the mean ± standard deviations (SD). Data were evaluated with GraphPad Prism software 6.0 (San Diego, CA, USA). The significance of differences among groups was determined by one-way or two-way analysis of variance. Results were considered statistically significant if $p < 0.05$.

Results

Virus isolation

FJLIUY-2017 and PRRSV2/CN/G8/2018 were isolated in PAM and MARC-145CD^{163} cells. The typical CPE was observed at 48 h post-infection in MARC-145CD^{163} cells (Supplementary Figure S1). Multi-step growth curve showed that two viruses produced high titers in both MARC-145 CD^{163} cells and PAM cells, but FJLIUY-2017 exhibited a faster and higher replication rate than PRRSV2/CN/G8/2018 from 36 to 72 h (Supplementary Figure S2).

Complete genomic analysis of PRRSV2/CN/G8/2018

The genome of PRRSV2/CN/G8/2018 (GenBank No. OQ357725) was 14,944 nt in length, excluding the poly (A) tails. BLASTn analysis of the PRRSV2/CN/G8/2018 revealed that it had low nucleotide similarity (< 92%) to the closest available PRRSV sequences in the GenBank database and was classified as NADC30-like PRRSV based on the whole genome.

Genome alignments revealed that the PRRSV2/CN/G8/2018 isolate shared 91.4%, 83.9%, 86.2%, 85.3%, and 89.2% homology with NADC30 (lineage 1), QYYZ (lineage 3), VR2332 (lineage 5), JXA1(lineage 8), and FJLIUY-2017, respectively (Table 1). ORF1a and ORF1b of PRRSV2/CN/G8/2018 shared high nucleotide homology with the NADC30 strain (90.5%/95.5%), whereas ORF2a-7 shared high identity with those of VR-2332 (91.2%) (Table 1). In addition, PRRSV2/CN/G8/2018 and FJLIUY-2017 had extensive nucleotide sequence differences in every region of the genome (86.6-93.1%) (Table 1). In particular, PRRSV2/CN/G8/2018 has a characteristic 131-amino acid deletion in the NSP2-coding

Table 1 Nucleotide and deduced amino acid identities of PRRSV2/CN/G8/2018 compared with the reference strains of PRRSV

Region	Pairwise % Identity to PRRSV2/CN/G8/2018 (nt/aa)											
	NADC30 (Lineage 1)		QYYZ (Lineage 3)		VR2332 (Lineage 5)		JXA1(Lineage 8)		FJLIUY-2017		LV	
	nt%	aa%	nt%	aa%	nt%	aa%	nt%	aa%	nt%	aa%	nt%	aa%
Full-length	91.4	/	83.9	/	86.2	/	85.3	/	89.2	/	60.7	/
5′ UTR	97.4	/	89.5	/	91.5	/	90.5	/	91.6	/	63.0	/
ORF1a	90.5	90.2	80.1	80.4	83.0	82.6	83.2	83.9	87.8	88.3	55.6	49.0
ORF1b	95.5	98.1	85.1	94.2	87.3	95.1	86.3	95.6	90.1	96.5	64.1	69.4
ORF2a-7	87.1	/	90.3	/	91.2	/	88.2	/	90.9		66.3	/
ORF2a	86.4	86.8	93.8	92.2	91.3	89.5	90.3	87.5	92.2	90.2	65.1	62.2
ORF2b	90.5	91.9	95.0	95.9	92.8	93.2	92.3	91.9	95.5	97.3	72.3	73.2
ORF3	83.7	82.0	87.1	84.6	95.2	93.7	88.4	85.9	89.8	87.4	64.7	66.4
ORF4	89.0	88.3	84.9	85.4	92.7	96.6	86.2	87.2	90.7	88.8	68.3	70.2
ORF5a	84.4	78.7	93.6	92.3	82.1	78.8	79.4	70.2	92.2	87.2	59.1	47.7
ORF5	84.1	84.6	90.7	93.5	82.6	80.5	83.1	81.1	89.9	90.5	63.1	55.6
ORF6	89.5	93.1	95.2	97.7	94.3	96.6	92.4	96.6	93.1	96.0	70.9	80.3
ORF7	95.4	96.0	88.4	91.1	93.8	93.5	90.1	90.3	86.6	90.2	66.8	62.8
3′ UTR	98.0	/	88.5	/	92.6	/	90.5	/	93.2	/	73.6	/

region, region, which is identical to the NADC30. Meanwhile, PRRSV2/CN/G8/2018 has an additional 25-aa deletion at the position aa471-495 of NADC30 (Fig. 1A).

Phylogenetic trees were constructed based on the ORF3 gene, ORF5 gene, and full-length genome using the sequences of 30 representative PRRSV isolates of different lineages including PRRSV-2 isolates ($n = 29$) and PRRSV-1 isolate ($n = 1$) (Supplementary Table S1). The results showed that PRRSV2/CN/G8/2018 clustered in lineage 1 of PRRSV-2 containing NADC30 and Chinese NADC30-like strains based on the full-length genome (Fig. 1B), however, PRRSV2/CN/G8/2018 clustered as lineage 5 of PRRSV-2 containing VR-2332 strain based on the ORF3 gene (Fig. 1C). In addition, PRRSV2/CN/G8/2018 was more closely related to lineage 3 PRRSV strains based on the ORF5 gene (Fig. 1D). These results indicate that PRRSV2/CN/G8/2018 are recombinant viruses. The genomic characteristics and phylogenetic analysis of the FJLIUY-2017 isolate are described in detail in a previous study [27].

Recombination analysis

Recombination analysis of the full-length genome was performed using RDP4.10 (Table 2), and SIMPLOT 3.51 software (Fig. 2). The results from molecular software strongly supported that PRRSV2/CN/G8/2018 is a natural recombinant virus from four lineage viruses with NADC30-like virus serving as the major parental virus while JXA1-like, VR2332-like and QYYZ-like as the minor parental viruses (Table 2). Additionally, the multiple crossovers were further confirmed by Bootscan analysis in the SIMPLOT 3.5.1 software (Fig. 2). Six recombination breakpoints were identified in the genome of PRRSV2/CN/G8/2018 at nt 694, nt 2000, nt 12,317, nt 13,614, nt 13,933 and nt 15,112, which were located in the NSP1, NSP2, NSP12, ORF4, ORF5 and ORF7 regions, respectively (Fig. 2; Table 2). Recombinant analysis of the FJLIUY-2017 isolate is described in detail in a previous study [27].

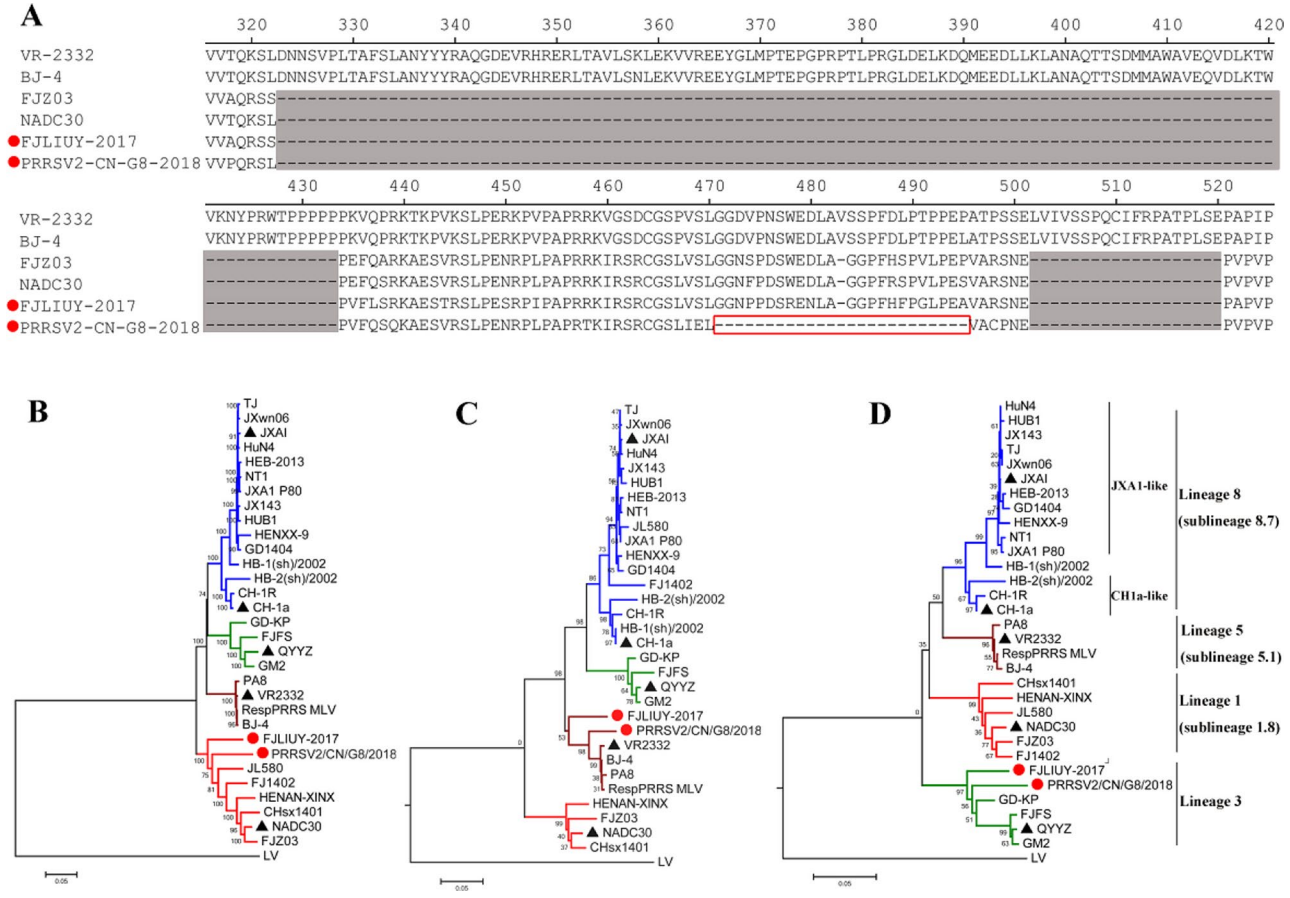

Fig. 1 Alignment of the NSP2 amino acids sequences and phylogenetic analysis of both strains with representative strains. (**A**) Alignment of the amino acid sequence of Nsp2. The discontinuous deletions highlighted in grey regions show the deletion signature of NADC30-like PRRSVs. Additional 25-aa deletion in PRRSV2/CN/G8/2018 is marked in the red box. (**B**-**D**) Phylogenetic trees based on the ORF3 (**B**), ORF5 (**C**), and complete genome of FJLIUY-2017 and PRRSV2/CN/G8/2018 (**D**) with the reference PRRSV strains. The strains in this study were highlighted with a red circle (●). The representative strains from different lineages were labeled with black triangles (▲)

Table 2 Recombination breakpoints identified in PRRSV2/CN/G8/2018 and associated parental strains

Isolate	Breakpoint position in alignment		Major Parent	Minor Parent	*P*-value						
	Beginning	Ending			RDP	GENECONV	Bootscan	MaxChi	Chimaera	SiScan	3Seq
PRRSV2/CN/G8/2018	694	2000	NADC30	JXA1	2.810×10^{-107}	2.693×10^{-93}	2.124×10^{-106}	2.491×10^{-34}	1.023×10^{-23}	8.159×10^{-37}	1.942×10^{-14}
	12,317	13,614	NADC30	VR-2332	3.222×10^{-54}	6.137×10^{-36}	4.256×10^{-53}	1.694×10^{-22}	1.031×10^{-24}	9.501×10^{-21}	3.885×10^{-15}
	13,933	15,112	NADC30	QYYZ	4.634×10^{-42}	1.463×10^{-5}	2.053×10^{-36}	2.741×10^{-15}	1.070×10^{-8}	4.580×10^{-10}	1.094×10^{-8}

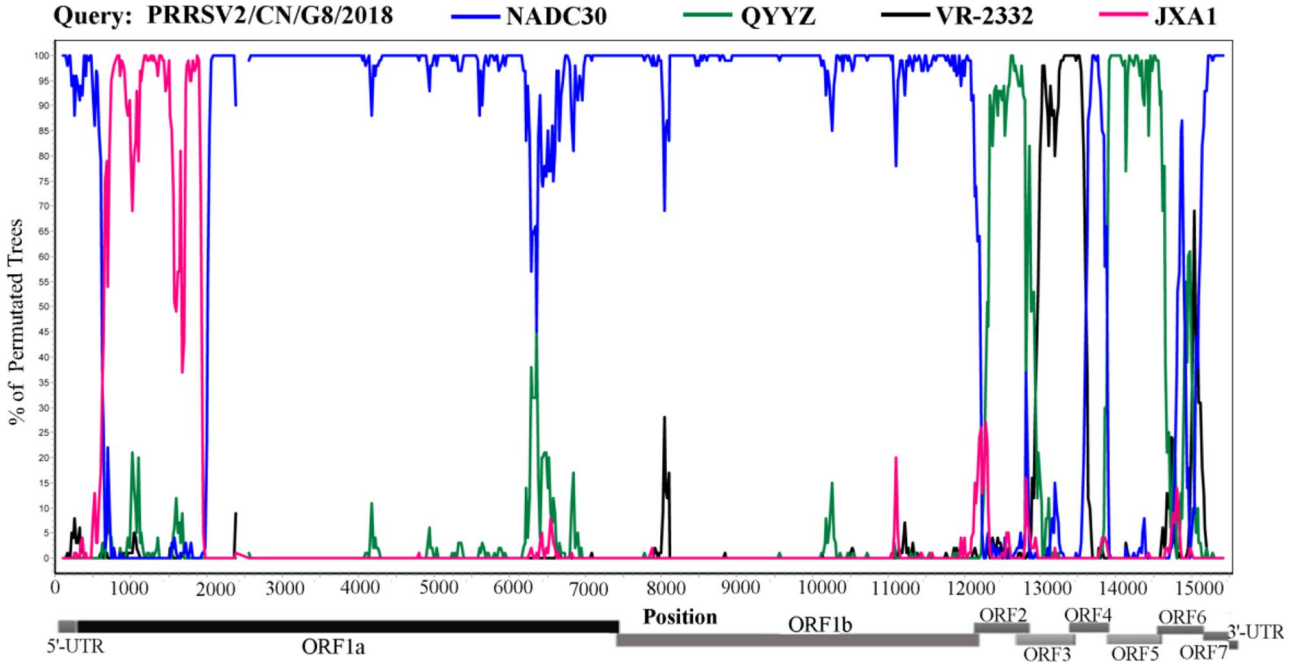

Fig. 2 The recombination regions in the PRRSV2/CN/G8/2018 genome were further confirmed using SimPlot 3.5.1 software. For the Bootscan analysis, the y-axis shows the percentage of permutated trees using a sliding window of 200 bases and a step size of 20 bases, while the X-axis showed the position of PRRSV genome

Clinical signs

The group of piglets infected with FJLIUY-2017 displayed more severe symptoms compared to the PRRSV2/CN/G8/2018 group

After PRRSV challenge, the piglets infected with FJLIUY-2017 developed a fever (40.3 ∘C) at 2 dpi, and hovered over 40.5 °C from day 4 to 12 dpi with a peak of 41.2 °C at 7 dpi. The temperatures of PRRSV2/CN/G8/2018-inoculated piglets developed fever at 2 dpi and hovered over 40 °C at 2–6 dpi and 9–13 dpi, with a peak (40.6 °C) at 11 dpi (Fig. 3A). Piglets inoculated with FJLIUY-2017 had significantly higher temperatures at 6, 7, 8, 9, and 14 dpi compared to the PRRSV2/CN/G8/2018 group ($p<0.05$) (Fig. 3A). Piglets infected with FJLIUY-2017 developed more severe clinical signs, including cough, lethargy, dyspepsia, and shivering from 3 dpi to 14 dpi, with significantly higher clinical signs scores than the PRRSV2/CN/G8/2018 -inoculated group with moderate clinical signs from 7 to 14 dpi ($p<0.05$) (Fig. 3B).

The body weights of the piglets were recorded at 0, 7, and 14 dpi. As shown in Fig. 3C, the ADWG of the PRRSV2/CN/G8/2018 and control groups was significantly higher than that of the FJLIUY-2017 group at 14 dpi ($p<0.05$). Notably, one piglet died at 8 dpi in the FJLIUY-2017 group, whereas all animals in the PRRSV2/CN/G8/2018-inoculated and control groups survived throughout the experiment (Fig. 3D). In contrast, the negative control group maintained a normal rectal temperature and behavior throughout the trial.

The FJLIUY-2017-inoculated group induced higher levels of viral loads and humoral immune response than the PRRSV2/CN/G8/2018-inoculated group

Blood samples were collected at 0, 4, 7, 11, and 14 dpi in every PRRSV- inoculated group to detect viremia and PRRSV-specific antibodies in piglets. As illustrated in

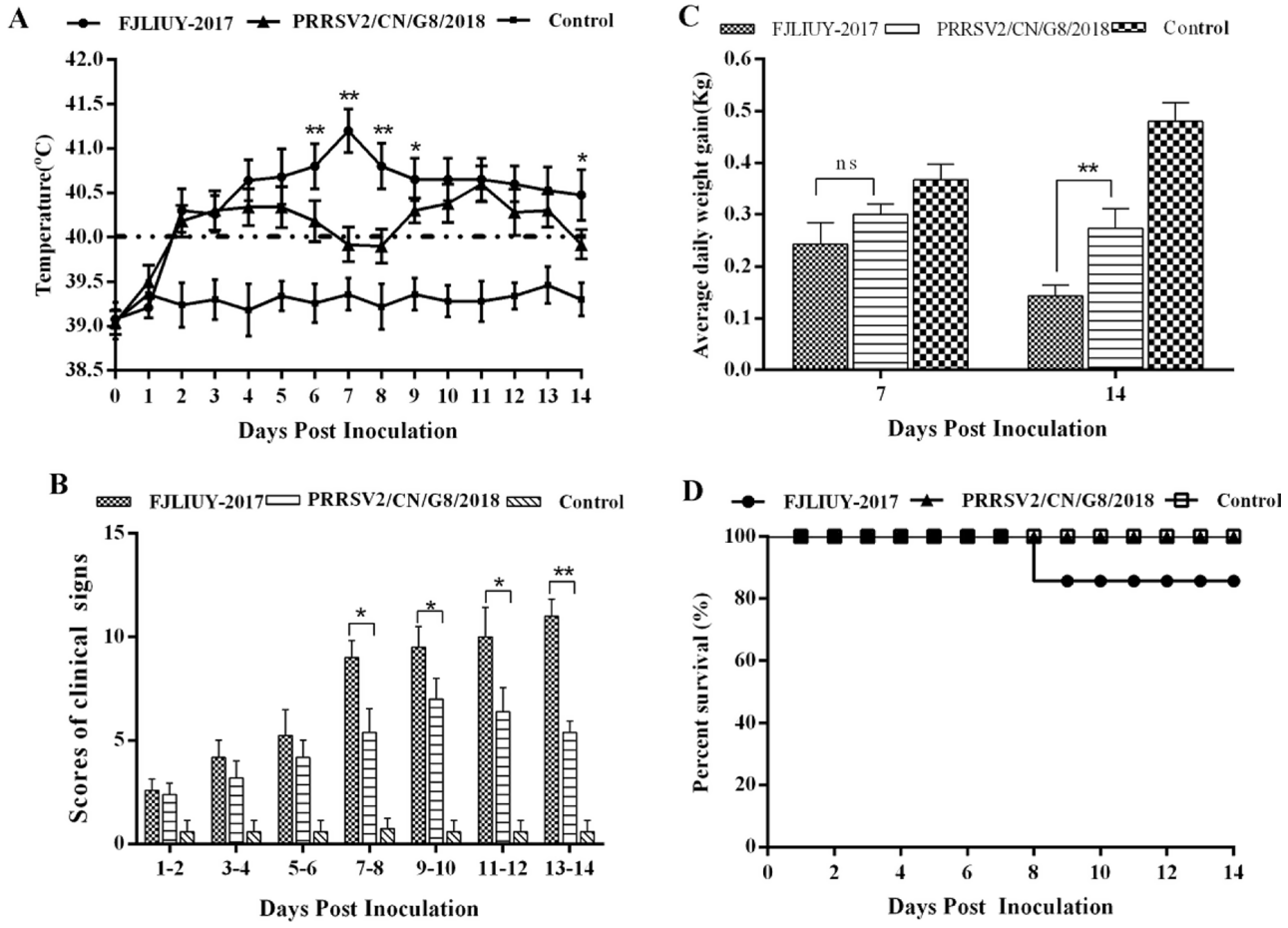

Fig. 3 The rectal temperatures, clinical scores, and the mortality and weight gain of piglets in different groups during the study. (**A**) Rectal temperatures of piglets infected with FJLIUY-2017, PRRSV2/CN/G8/2018, and DMEM. The threshold of fever was set at 40.0 °C. (**B**) The scores of clinical signs of piglets in each group. (**C**) Average daily weight gain (ADWG) of the piglet during each week of the challenge study. (**D**) The survival curves of piglets in each group. Data are the mean ± SD (error bars) of every group. Asterisk indicates significant differences between the FJLIUY-2017-inoculated group and PRRSV2/CN/G8/2018-inoculated group (*$p < 0.05$; **$p < 0.01$)

Fig. 4A, the virus titers in both infected groups started to increase at 4 dpi, reaching their peaks ($10^{6.4}$ TCID50/mL) at 7 dpi in the FJLIUY-2017 group, and at 11 dpi ($10^{5.2}$ TCID50/mL) in the PRRSV2/CN/G8/2018 group (Fig. 4A). Compared with the PRRSV2/CN/G8/2018-inoculated group, the virus titers of the FJLIUY-2017-inoculated group were significantly higher at DPIs 4, 7, and 11. No viremia was detected in the serum samples of control piglets (Fig. 4A).

Sera were detected for specific antibodies against the N protein of PRRSV using a commercial ELISA kit. All of the piglets challenged with FJLIUY-2017 had seroconverted at 7 dpi, while the piglets in PRRSV2/CN/G8/2018-inoculated groups were seropositive at 11 dpi (Fig. 4B). Meanwhile, antibody levels in the FJLIUY-2017-inoculated group were significantly higher than those in the PRRSV2/CN/G8/2018- inoculated group at 7, 11 and 14 days (Fig. 4B). All animals in the control group were negative for PRRSV-specific antibodies during the experiment.

Piglets in the FJLIUY-2017-inoculated group demonstrated more severe interstitial pneumonia than those in the PRRSV2/CN/G8/2018-inoculated group

At necropsy, lung tissues of the pigs infected with the two viral strains were characterized by interstitial pneumonia and pulmonary consolidation, whereas no lung gross lesions were observed in the control group (Fig. 5A-C). Compared with the PRRSV2/CN/G8/2018 group, the FJLIUY-2017-inoculated group had higher gross lung lesion scores with significant difference ($p < 0.05$) (Fig. 6A). In addition, histopathological examination showed FJLIUY-2017-inoculated piglets presented more severe lung lesions with lung structures dimly discernible, severe interstitial pneumonia, and alveolar walls markedly thickened compared with those in the PRRSV2/CN/G8/2018-inoculated group (Fig. 5D-E).

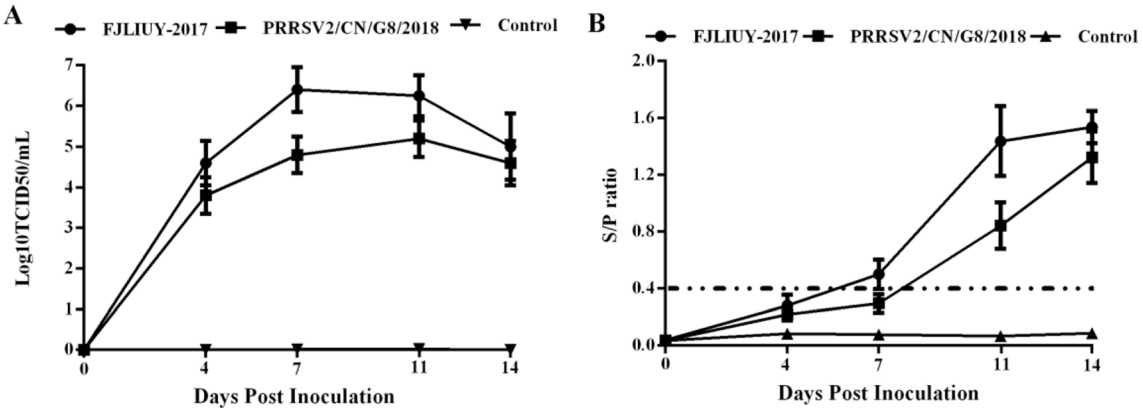

Fig. 4 Virus load and PRRSV-special antibody levels of the infected piglets during the challenge experiment. (**A**) The dynamics of virus titers in sera were determined by the IFA-microtitration infectivity assay. Serum samples were collected at 0, 4, 7, 11, and 14 dpi and virus titers are expressed as 50% of the tissue culture infective dose per mL (TCID50/mL) of the sample. (**D**) PRRSV-specific N protein antibody levels in sera after challenge. Serum samples were collected at 0, 4, 7, 11, and 14 dpi and the threshold for seroconversion was set at a sample-to-positive (s/p) ratio of 0.4. sterisk indicates significant differences between the FJLIUY-2017-inoculated group and PRRSV2/CN/G8/2018-inoculated group (*$p < 0.05$)

FJLIUY-2017-inoculated piglets had statistically higher microscopic lung lesion scores than PRRSV2/CN/G8/2018-infected group ($p < 0.05$) (Fig. 6B). Negative controls did not show any obvious microscopic pathological lesions (Fig. 5F).

PRRSV antigens in the lungs of piglets were examined by immunohistochemistry (IHC). The PRRSV-positive signals could be seen in the macrophages, epithelial cells, and within the alveolar wall cells in the lung tissue of all challenged groups (Fig. 5G-H), but the FJLIUY-2017-inoculated group had significantly higher scores of IHC than the PRRSV2/CN/G8/2018-inoculated group ($p < 0.05$) (Fig. 6C). No PRRSV-positive antigens were observed in the lungs of the control group (Fig. 5I).

Virus cross-neutralization assay of FJLIUY-2017 and PRRSV2/CN/G8/2018

Hyper-immune sera (#1–3) generated from JXA1-R (lineage 8) and hyper-immune sera (#5–7) generated from Ingelvac PRRS MLV (Lineage 5) were used for viral cross-neutralization against FJLIUY-2017 and PRRSV2/CN/G8/2018. Six hyper-immune sera presented high NA titers for FJZH (JXA1-R-like PRRSV, GenBank accession number: KP998478) and PRRSV2/CN/101,805/2018 (RespPRRS MLV-like PRRSV, GenBank accession number: MT416548) (1:16 – 1:32), however, all these hyper-immune sera showed low NA titers for two strains (< 1:8) and failed to neutralize FJLIUY-2017 and PRRSV2/CN/G8/2018 (Supplementary Figure S3).

Discussion

Since it first emerged in China in 1995, PRRSV has spread widely, causing several outbreaks over the past two decades. Despite the widespread use of many domestic and imported commercial modified live virus (MLV) vaccines, PRRS continues to plague the Chinese swine industry. To date, four distinct PRRSV-2 lineages (lineages 1, 3, 5, and 8) have been identified in Chinese swine herds, promoting PRRSV recombination between different lineages [3, 4, 6]. Recombination and mutation are important mechanisms for PRRSV evolution that contribute to the continuous generation of novel strains with altered pathogenicity in pigs [15, 17, 38–42]. In recent years, various recombinant PRRSV strains from two (sublineage 1.8 and lineage 3, sublineage 1.8 and 5.1, or sublineage 1.8 and 8.7) or three (sublineage 1.8, 5.1, and 8.7, or sublineage 1.8, 3, and lineage 8.7) lineages have exhibited varying degrees of pathogenicity in piglets and have also caused significant financial losses to swine herds [18–26]. However, no information is available on the pathogenicity of recombinant viruses derived from the four lineages of PRRSV-2. In this study, we describe two recombinant PRRSV-2 viruses from four different lineages and use piglets to assess the pathogenicity of the virus.

Since its initial identification in China in 2012, NADC30-like PRRSV has undergone complex recombination with local Chinese strains, aggravating PRRSV evolution and making prevention and control of PRRS more difficult. Although two strains in this study are likely a product of complex genomic recombination between lineage 1, 3, 5, and 8 strains, FJLIUY-2017 and PRRSV2/CN/G8/2018 differed extensively in all regions of the genome. Previous studies demonstrated that QYYZ-like PRRSV strains (e.g. QYYZ and GM2), VR2332-like strains (VR-2332 and BJ-4), NADC30 and NADC30-like isolates (e.g. FJZ03, HNjz15, and CHsx1401) exhibited low or moderate pathogenicity in animal experiments [9, 10, 21, 33, 43–45]. However, recombinant PRRSVs isolated in recent years have shown increased pathogenicity

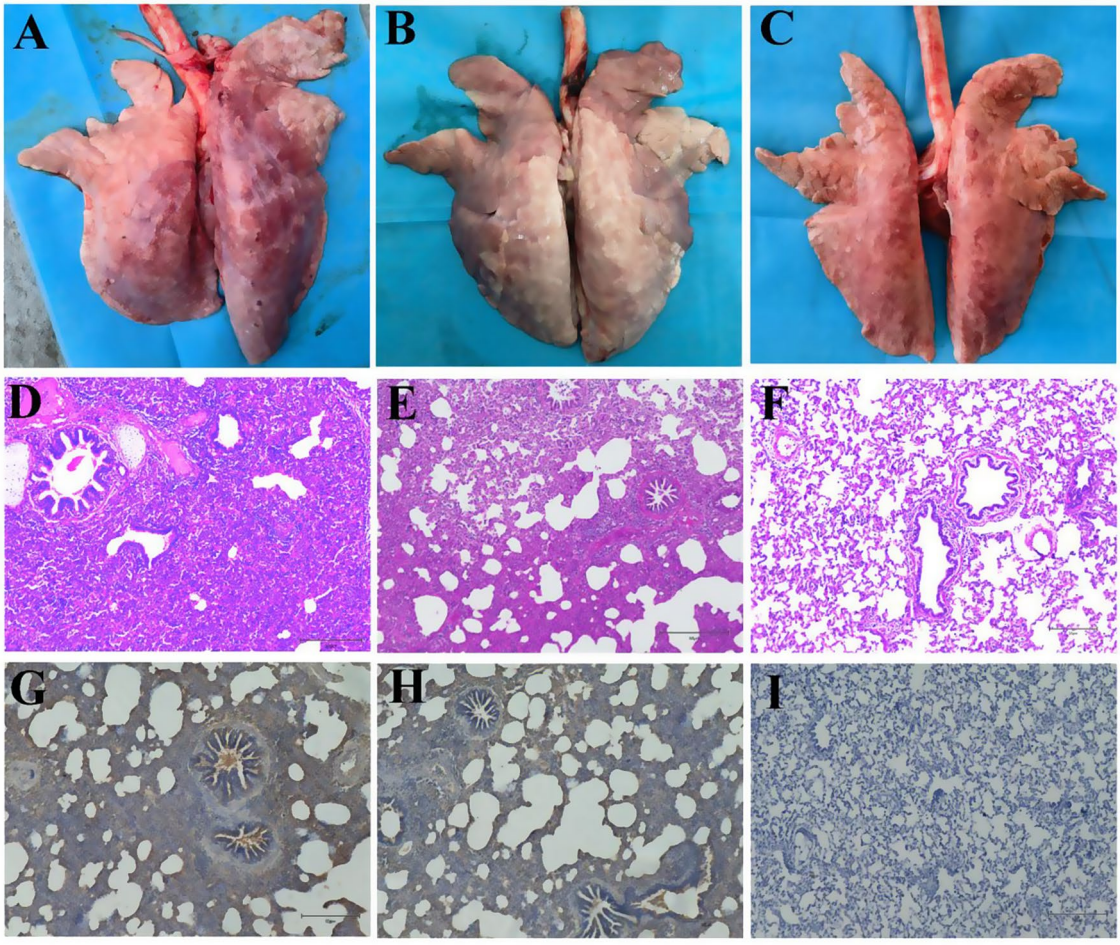

Fig. 5 Gross pathology, microscopic lung lesions and lung IHE examination of the inoculated piglets. (**A**) Severe interstitial pneumonia with consolidation was observed in FJLIUY-2017-challenged piglets. (**B**) Moderate interstitial pneumonia with consolidation were observed in PRRSV2/CN/G8/2018-challenged piglets. (**C**) No obvious gross lung lesions were observed in control group. Microscopic lesions in the lungs of piglets inoculated with strains FJLIUY-2017 (**D**) and PRRSV2/CN/G8/2018 (**E**) showed significant lung lesions characterized by interstitial pneumonia compared to piglets inoculated with DMEM (**F**). Lung IHE examination in the inoculated piglets, PRRSV-positive signals in bronchial epithelial cells and macrophages of lung could be detected from piglets challenged with PRRSV strains FJLIUY-2017 (**G**) and PRRSV2/CN/G8/2018 (**H**), whereas no positive staining cells were detected in the control group (**I**)

in pigs. For instance, virulent strains JL580, 14LY01-FJ, 14LY02-FJ, 15LY01-FJ, and 15LY02-FJ, resulting from recombination between NADC30-like and JXA1-like PRRSV exhibited clinical symptoms in piglets similar to those caused by highly pathogenic PRRSV (HP-PRRSV) [21, 23]. In addition, the strains GD1404, and FJNP2017, which were derived from QYYZ-like PRRSV recombined with JXA1-like PRRSV, were highly pathogenic to piglets and caused 20–40% mortality [11, 46]. The triple recombinant viruses SD17-38 (a recombinant isolate from lineages 1, 5, and 8), JS18-3 and GZgy17 (recombinant isolates from lineages 1, 3, and 8) caused 20–40% mortality in piglets, similar to HP-PRRSV [18, 19, 25]. The NADC34-like (lineage 1.5) and QYYZ-like (lineage 3) recombinant strain TJnh2021 shows higher pathogenicity in piglets compared to other lineage 1.5 strains [47]. Similarly, PRRSV/CN/FJGD01/2021, a product of recombination of NADC30-like, NADC34-like and JXA1-like viruses, displayed higher pathogenicity than the NADC30-like and NADC34-like strains [16]. Of note, several commercially available PRRS modified live virus (MLV) vaccines, such as Ingelvac PRRS MLV and certain HP-PRRS MLV vaccines, have been reported to undergo recombination with field strains. These recombinant strains exhibit increased virulence, raising significant concerns regarding their safety and efficacy in disease control. For instance, the TJnh1501 strain and FJXS15 strains, which recombined with the low pathogenicity strains HP-PRRSV MLV (JXA1-P80) and NADC30-like PRRSV, exhibit moderate to high virulence for pigs [21, 48]. Similarly, the GDsg strain, a recombinant of JXA1-P80 MLV and QYYZ, has higher pathogenicity than

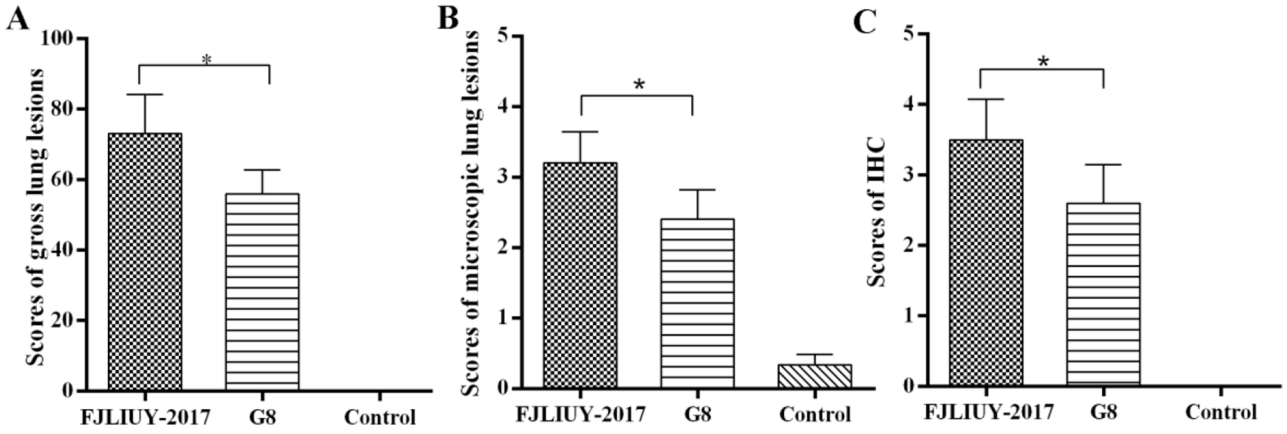

Fig. 6 Scores of gross lung lesions, microscopic lung lesions and IHE of the infected piglets. (**A**) The mean scores of the gross lesions of the lungs in each group. Gross lesions were scored based on the percentage of lung area affected. (**B**) The mean scores of the microscopic lung lesions in each group. (**C**) Scores of IHC of piglets inoculated with PRRSV. Data are expressed as the Mean ± SD (error bars) of each group. Asterisk indicates significant differences between the FJLIUY-2017- inoculated group and PRRSV2/CN/G8/2018-inoculated group (*$p < 0.05$). Note: G8 denotes PRRSV2/CN/G8/2018

the QYYZ strain [49]. Additionally, the SCN17 strain, a recombinant of RespPRRS MLV, NADC30-like PRRSV, and JXA1-like PRRSV, has been shown to be a moderately virulent isolate [24]. In the current study, FJLIUY-2017 exhibits higher pathogenicity than PRRSV2/CN/G8/2018 in *vivo*: FJLIUY-2017 resulted in the typical clinical signs of PRRSV, including persistent fever, higher viremia, severe lung lesions, and 20% mortality, whereas PRRSV2/CN/G8/2018 caused moderate clinical symptoms and no mortality during the challenge period. Taken together, this study combined with the recent report described above suggest that recombination among distinct lineages of PRRSV-2 may lead to the generation of novel strains with enhanced pathogenicity. In addition, a retrospective survey revealed that recombinant viruses from four strains (1, 3, 5, and 8) were detected in four pig farms in Fujian Province between 2017 and 2021 (Supplementary Figure S4). Given the complex nature of the virus strains and the potential serious threat to pig health, the affected pig farms implemented a PRRS eradication program, and these PRRSV have not been detected in these pig farms since 2022. Meanwhile, we carried out a detailed analysis of the PRRSV sequences in the NCBI database and combined them with the results of previous research, showed that the main recombinant lineages were L1 + L3, L1 + L8 or L1 + L3 + L8 in China [50–51].

ORF1a is associated with the primary determinants of virulence of PRRSV, in particular, Nsp9 and Nsp10 are linked to the lethal virulence of HP-PRRSV [31]. In addition, a previous study suggests that the amino acids at positions 519 and 544 in Nsp9 are involved in the replicative efficiency and increased virulence of HP-PRRSV [52]. In the present study, three non-structural protein regions (Nsp1 to Nsp2, Nsp6 to 9, and Nsp11 to Nsp12) of FJLIUY-2017 were provided by HP-PRRSVs, whereas only one (Nsp1 to Nsp2) of PRRSV2/CN/G8/2018 was provided by HP-PRRSVs. Interestingly, FJLIUY-2017 possesses a serine (S) at position 519 and a threonine (T) at position 544 in NSP9, which were consistent with HP-PRRSVs. In contrast, PRRSV2/CN/G8/2018 features a serine and an alanine (A) at these two positions, which is distinct from HP-PRRSVs but consistent with NADC30 or NADC30-like PRRSV. Recombinant strains GZgy17 and SCya18, both classified within lineage 3, exhibited similar recombination patterns but differed markedly in pathogenicity, with GZgy17 causing 20% mortality in piglets, whereas SCya18 did not cause any deaths [25]. Similarly, FJNP2017 and GDZS2016, which shared 95.2% nucleotide homology and were classified within lineage 8.7, exhibited different pathogenicity, with FJNP2017 being more virulent in pigs [11]. Analysis of the Nsp9 sequences revealed that GZgy17 and FJNP2017 possess identical amino acids at positions 519 (S) and 544 (T) with HP-PRRSV, whereas SCya18 (with S^{519} and A^{544}) and GDZS2016 (with T^{519} and T^{544}) shared only one position with HP-PRRSV (Supplementary Figure S5). In addition, the triple recombinant SD17-38, classified within lineage 1 but with S^{519} and T^{544} in Nsp9 (Supplementary Figure S5), exhibited similar pathogenicity to the HP-PRRSV, resulting in 100% morbidity and 40% mortality in piglets [18]. Lineage 3 recombinant strain GDsg, with S^{519} and T^{544} in Nsp9, exhibited higher pathogenicity than the parental strain QYYZ with T^{519} and A^{544} in Nsp9 [49]. In support of these findings, recombinant PRRSVs generated by swapping Nsp9 between L1 (NADC30-like) and L8 (HP-PRRSV) backbones revealed that L1-based recombinants exhibited higher replication capacity in porcine primary alveolar macrophages than L8-based recombinants [50]. Taken together with the above results, this may partly explain why the pathogenicity of the two strains in this study differed in the animal experiment, despite the similarity of their recombination patterns.

Conclusions

The two isolates had similar recombination patterns, but FJLIUY-2017 was more virulent in piglets than PRRSV2/CN/G8/2018. The different pathogenicity of the two recombinant viruses may be attributed to the different components provided by HP-PRRSV. Our study suggests that novel PRRSV variants evolved through recombination among distinct lineages of PRRSV-2 and the risk of these variants should be emphasized with much attention.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04779-9.

Supplementary Material 1

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Author contributions

Chunhua Wei: performed the experiments, analyzed the data and wrote the draft manuscript. Chen Liu, Guangsong Chen, Yuan Yang, Jiarui Li and Huijuan Dan: performed the experiments and analyzed the data. Ailing Dai, Cuiqin Huang and Manlin Luo: analyzed the data and wrote the draft manuscript. Jiankui Liu: designed the experiments, revised the manuscript.

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Data availability

The data used to support the findings of this study are included within the article. The PRRSV nucleotide sequence data of this study were submitted to GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) under accession number MG011718 and OQ357725.

Declarations

Ethics approval and consent to participate

In this study, piglets' clinical samples used had obtained a written consent from farm owners. The animal experiments were conducted based on the Animal Ethics Procedures and Guidelines of the People's Republic of China. Animal use and animal trials in this study were approved by the Longyan University animal ethics committee (permit no. LY2022004X). The authors declare that all methods of experiments were carried out in strict accordance with the approved guidelines. In addition, informed consent was obtained from farm owners.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Lunney JK, Fang Y, Ladinig A, Chen N, Li Y, Rowland B, et al. Porcine reproductive and respiratory syndrome virus (PRRSV): pathogenesis and interaction with the immune system. Annu Rev Anim Biosci. 2016;4:129–54.
2. Li Y, Tas A, Sun Z, Snijder EJ, Fang Y. Proteolytic processing of the Porcine reproductive and respiratory syndrome virus replicase. Virus Res. 2015;202:48–59.
3. Gao JC, Xiong JY, Ye C, Chang XB, Guo JC, Jiang CG, et al. Genotypic and geographical distribution of Porcine reproductive and respiratory syndrome viruses in Mainland China in 1996–2016. Vet Microbiol. 2017;208:164–72.
4. Guo Z, Chen XX, Li R, Qiao S, Zhang G. The prevalent status and genetic diversity of Porcine reproductive and respiratory syndrome virus in China: a molecular epidemiological perspective. Virol J. 2018;15:2.
5. Kuhn JH, Lauck M, Bailey AL, Shchetinin AM, Vishnevskaya TV, Bào Y, et al. Reorganization and expansion of the nidoviral family arteriviridae. Arch Virol. 2016;161(3):755–68.
6. Liu J, Xu Y, Lin Z, Fan J, Dai A, Deng X, et al. Epidemiology investigation of PRRSV discharged by faecal and genetic variation of ORF5. Transbound Emerg Dis. 2021;68(4):2334–44.
7. Tian K, Yu X, Zhao T, Feng Y, Cao Z, Wang C, et al. Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. PLoS ONE. 2007;2(6):e526.
8. Zhou L, Wang Z, Ding Y, Ge X, Guo X, Yang H. NADC30-like strain of Porcine reproductive and respiratory syndrome virus, China. Emerg Infect Dis. 2015;21(12):2256–7.
9. Li H, Yang H. Infection of Porcine reproductive and respiratory syndrome virus suppresses the antibody response to classical swine fever virus vaccination. Vet Microbiol. 2003;95:295–301.
10. Lu WH, Tun HM, Sun BL, Mo J, Zhou QF, Deng YX, et al. Re-emerging of Porcine respiratory and reproductive syndrome virus (lineage 3) and increased pathogenicity after genomic recombination with vaccine variant. Vet Microbiol. 2015;175(2–4):332–40.
11. Sun YK, Li Q, Yu ZQ, Han XL, Wei YF, Ji CH, et al. Emergence of novel recombination lineage 3 of Porcine reproductive and respiratory syndrome viruses in Southern China. Transbound Emerg Dis. 2019;66(1):578–87.
12. Xu H, Xiang L, Tang YD, Li C, Zhao J, Gong B, et al. Genome-Wide characterization of QYYZ-Like PRRSV during 2018–2021. Front Vet Sci. 2022;9:945381.
13. Liu J, Wei C, Lin Z, Xia W, Ma Y, Dai A, Yang X. Full genome sequence analysis of a 1-7-4-like PRRSV strain in Fujian Province, China. PeerJ. 2019;7:e7859.
14. Zhang HL, Zhang WL, Xiang LR, Leng CL, Tian ZJ, Tang YD, et al. Emergence of novel Porcine reproductive and respiratory syndrome viruses (ORF5 RFLP 1-7-4 viruses) in China. Vet Microbiol. 2018;222:105–8.
15. Jiang Y, Li G, Yu L, Li L, Zhang Y, Zhou Y, et al. Genetic diversity of Porcine reproductive and respiratory syndrome virus (PRRSV) from 1996 to 2017 in China. Front Microbiol. 2020;11:618.
16. Liu J, Liu C, Xu Y, Yang Y, Li J, Dai A, et al. Molecular characteristics and pathogenicity of a novel Recombinant Porcine reproductive and respiratory syndrome virus strain from NADC30-, NADC34-, and JXA1-Like strains that emerged in China. Microbiol Spectr. 2022;10(6):e0266722.
17. Yu F, Yan Y, Shi M, Liu HZ, Zhang HL, Yang YB, et al. Phylogenetics, genomic recombination, and NSP2 polymorphic patterns of Porcine reproductive and respiratory syndrome virus in China and the united States in 2014–2018. J Virol. 2020;94(6):e01813–19.
18. Chen N, Ye M, Li S, Huang Y, Zhou R, Yu X, et al. Emergence of a novel highly pathogenic Recombinant virus from three lineages of Porcine reproductive and respiratory syndrome virus 2 in China 2017. Transbound Emerg Dis. 2018;65(6):1775–85.
19. Han G, Lei K, Xu H, He F. Genetic characterization of a novel Recombinant PRRSV2 from lineage 8, 1 and 3 in China with significant variation in replication efficiency and cytopathic effects. Transbound Emerg Dis. 2020;67(4):1574–84.

20. Liu JK, Zhou X, Zhai JQ, Li B, Wei CH, Dai AL, et al. Emergence of a novel highly pathogenic Porcine reproductive and respiratory syndrome virus in China. Transbound Emerg Dis. 2017;64(6):2059–74.
21. Liu J, Zhou X, Zhai J, Wei C, Dai A, Yang X, et al. Recombination in JXA1-R vaccine and NADC30-like strain of Porcine reproductive and respiratory syndrome viruses. Vet Microbiol. 2017;204:110–20.
22. Wang HM, Liu YG, Tang YD, Liu TX, Zheng LL, Wang TY, et al. A natural Recombinant PRRSV between HP-PRRSV JXA1-like and NADC30-like strains. Transbound Emerg Dis. 2018;65(4):1078–86.
23. Zhao K, Ye C, Chang XB, Jiang CG, Wang SJ, Cai XH, et al. Importation and recombination are responsible for the latest emergence of highly pathogenic Porcine reproductive and respiratory syndrome virus in China. J Virol. 2015;89(20):10712–6.
24. Zhou L, Kang R, Yu J, Xie B, Chen C, Li X, et al. Genetic characterization and pathogenicity of a novel recombined Porcine reproductive and respiratory syndrome virus 2 among Nadc30-Like, Jxa1-Like, and Mlv-Like strains. Viruses. 2018;10(10):551.
25. Zhou L, Kang R, Zhang Y, Yu J, Xie B, Chen C, Li X, et al. Emergence of two novel Recombinant Porcine reproductive and respiratory syndrome viruses 2 (lineage 3) in Southwestern China. Vet Microbiol. 2019;232:30–41.
26. Zhou L, Kang R, Zhang Y, Ding M, Xie B, Tian Y, et al. 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. 2018;10(6):328.
27. Liu J, Wei C, Lin Z, Fan J, Xia W, Dai A, Yang X. Recombination in lineage 1, 3, 5 and 8 of Porcine reproductive and respiratory syndrome viruses in China. Infect Genet Evol. 2019;68:119–26.
28. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30(12):2725–9.
29. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, et al. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol. 1999;73:152–60.
30. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B. RDP4: detection and analysis of recombination patterns in virus genomes. Virus Evol. 2015;1:vev003.
31. Li Y, Zhou L, Zhang J, Ge X, Zhou R, Zheng H, et al. Nsp9 and Nsp10 contribute to the fatal virulence of highly pathogenic Porcine reproductive and respiratory syndrome virus emerging in China. PLoS Pathog. 2014;10:e1004216.
32. Halbur PG, Paul PS, Frey ML, Landgraf J, Eernisse K, Meng XJ, et al. Comparison of the pathogenicity of two US Porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. Vet Pathol. 1995;32:648–60.
33. Zhou L, Yang B, Xu L, Jin H, Ge X, Guo X, et al. Efficacy evaluation of three modified-live virus vaccines against a strain of Porcine reproductive and respiratory syndrome virus NADC30-like. Vet Microbiol. 2017;207:108–16.
34. Reed LJ, Muench H. A simple method of estimating 50% endpoints. Am J Hyg. 1938;27:493–7.
35. Chen XX, Zhou X, Guo T, Qiao S, Guo Z, Li R, et al. Efficacy of a live attenuated highly pathogenic PRRSV vaccine against a NADC30-like strain challenge: implications for ADE of PRRSV. BMC Vet Res. 2021;17:260.
36. Halbur PG, Paul PS, Frey ML, Landgraf J, Eernisse K, Meng XJ. Comparison of the antigen distribution of two US Porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. Vet Pathol. 1996;33:159–70.
37. Leng CL, An TQ, Chen JZ, Gong DQ, Peng JM, Yang YQ, et al. Highly pathogenic Porcine reproductive and respiratory syndrome virus GP5 B antigenic region is not a neutralizing antigenic region. Vet Microbiol. 2012;159(3–4):273–81.
38. Martín-Valls GE, Kvisgaard LK, Tello M, Darwich L, Cortey M, Burgara-Estrella AJ, et al. Analysis of ORF5 and full-length genome sequences of Porcine reproductive and respiratory syndrome virus isolates of genotypes 1 and 2 retrieved worldwide provides evidence that recombination is a common phenomenon and May produce mosaic isolates. J Virol. 2014;88(6):3170–81.
39. Mengeling WL. The potential role of genetic recombination in the evolution of new strains of Porcine reproductive and respiratory syndrome virus (PRRSV). J Swine Health Prod. 2002;10:273–5.
40. Murtaugh MP, Stadejek T, Abrahante JE, Lam TT, Leung FC. The ever-expanding diversity of Porcine reproductive and respiratory syndrome virus. Virus Res. 2010;154(1–2):18–30.
41. Murtaugh MP, Yuan S, Faaberg KS. Appearance of novel PRRSV isolates by recombination in the natural environment. Adv Exp Med Biol. 2001;494:31–6.
42. van Vugt J, Storgaard T, Oleksiewicz MB, Bøtne A. High frequency RNA recombination in Porcine reproductive and respiratory syndrome virus occurs preferentially between parental sequences with high similarity. J Gen Virol. 2001;82:2615–20.
43. Brockmeier SL, Loving CL, Vorwald AC, Kehrli ME Jr, Baker RB, Nicholson TL, et al. Genomic sequence and virulence comparison of four type 2 Porcine reproductive and respiratory syndrome virus strains. Virus Res. 2012;169:212–21.
44. Guo B, Lager KM, Henningson JN, Miller LC, Schlink SN, Kappes MA, et al. Experimental infection of united States swine with a Chinese highly pathogenic strain of Porcine reproductive and respiratory syndrome virus. Virology. 2013;435(2):372–84.
45. Li C, Zhuang J, Wang J, Han L, Sun Z, Xiao Y, et al. Outbreak investigation of NADC30-Like PRRSV in South-East China. Transbound Emerg Dis. 2016;63(5):474–9.
46. Zhang Q, Bai J, Hou H, Song Z, Zhao Y, Jiang P. A novel Recombinant Porcine reproductive and respiratory syndrome virus with significant variation in cell adaption and pathogenicity. Vet Microbiol. 2017;208:150–8.
47. Sun YF, Liu Y, Yang J, Li WZ, Yu XX, Wang SY, et al. Recombination between NADC34-like and QYYZ-like strain of Porcine reproductive and respiratory syndrome virus with high pathogenicity for piglets in China. Transbound Emerg Dis. 2022;69(5):e3202–7.
48. Bian T, Sun Y, Hao M, Zhou L, Ge X, Guo X, et al. A Recombinant type 2 Porcine reproductive and respiratory syndrome virus between NADC30-like and a MLV-like: genetic characterization and pathogenicity for piglets. Infect Genet Evol. 2017;54:279–86.
49. Dong J, Wang Y, Yu L, Zhang P, Liu X, Zhang L, et al. Pathogenicity of a newly emerged recombined Porcine reproductive and respiratory syndrome virus strain (subgenotype III) in China. Vet Microbiol. 2017;210:162–6.
50. Cui X, Xia D, Huang X, Sun Y, Shi M, Zhang J, et al. Analysis of Recombinant characteristics based on 949 PRRSV-2 genomic sequences obtained from 1991 to 2021 shows that viral multiplication ability contributes to dominant recombination. Microbiol Spectr. 2022;10(5):e0293422.
51. Yuan N, Yang Z, Lv F, Dou L, Li X, Zhao B, et al. Molecular epidemiology and genetic evolution of Porcine reproductive and respiratory syndrome virus in Northern China during 2021–2023. Viruses. 2025;17(1):85.
52. Zhao K, Gao JC, Xiong JY, Guo JC, Yang YB, Jiang CG, et al. Two residues in NSP9 contribute to the enhanced replication and pathogenicity of highly pathogenic Porcine reproductive and respiratory syndrome virus. J Virol. 2018;92(7):e02209–17.

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