DOI: 10.1002/vms3.402

ORIGINAL ARTICLE

WILEY

Genetic characterization of a novel recombined porcine reproductive and respiratory syndrome virus 2 among Nadc30-like, Jxa1-like and TJ-like strains

Jun Zhao[1] | Ling Zhu[2] | Jianbo Huang[1] | Zexiao Yang[1] | Lei Xu[1] | Sirui Gu[1] | Yao Huang[1] | Rubo Zhang[1] | Xiangang Sun[1] | Yuancheng Zhou[3] | Zhiwen Xu[2]

[1]College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, China

[2]College of Veterinary Medicine, Key Laboratory of Animal Diseases and Human Health of Sichuan Province, Sichuan Agricultural University, Chengdu, China

[3]Animal Breeding and Genetics Key Laboratory of Sichuan Province, Sichuan Animal Science Academy, Chengdu, China

Correspondence
Zhiwen Xu, College of Veterinary Medicine, Key Laboratory of Animal Diseases and Human Health of Sichuan Province, Sichuan Agricultural University, No. 211, Huimin Road, Wenjiang District, Chengdu, China.
Email: abtcxzw@126.com

Funding information
The National Key Research and Development Program, Grant/Award Number: 2018YFD0500802; The Science and Technology Program of Sichuan province, Grant/Award Number: 2018JY0369; The Sichuan Veterinary Medicine and Drug Innovation Group of the China Agricultural Research System, Grant/Award Number: CARS-SVDIP

Abstract

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically devastating viral diseases in the global pig industry, including China. Recently, we successfully isolated a porcine reproductive and respiratory syndrome virus (PRRSV) from lung tissue and peripheral blood of piglets at a farm from Dujiangyan in Sichuan, China, and named it the DJY-19 strain. The full-length genome sequence of DJY-19 shared 86.8%–94.1% nucleotide similarity with NADC30-like and NADC30 PRRSV strains. We compared the open reading frame (ORF) 5 gene of DJY-19 with 34 PRRSV strains from Genbank. Phylogenetic analysis showed that DJY-19 clustered with NADC30 strains, characterized by a predicted 131-amino-acid deletion in the nonstructural protein (NSP) 2. The results of homology analysis showed that the homology between DJY-19 and NADC30 (JN654459.1) strains was the highest (95.9%), whereas homology with other domestic strains was lower (80.9%–92.6%). Furthermore, we identified four recombination breakpoints in the DJY-19 genome; they separated the DJY-19 genome into four regions. The 8106–9128 nucleotide (nt) region of DIY-19 was highly similar to the TJ strain, and the 12106–12580 nt region of DIY-19 was highly similar to the JXA1-R strain. Our findings demonstrate that DJY-19 arose from the recombination of North America NADC30 strain and TJ strain and JXA1-R in China. The application of multiple attenuated vaccine strains has led to complex recombination of PRRSV strains in China. This study provides a theoretical basis for making a more reasonable PRRS virus control and prevention strategy.

KEYWORDS

genetic evolution analysis, new recombinant porcine reproductive and respiratory syndrome virus strain, reference basis

1 | INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is an immunosuppressive disease. It has been characterized by respiratory symptoms and disorders in piglets and reproductive failure in sows. The causative pathogen of PRRS is porcine reproductive and respiratory syndrome virus (PRRSV). It's an enveloped, positive-stranded RNA virus of the Arteriviridae family (Zuo et al., 2015). It has strict host and histiocytic tropism and mainly infects monocyte lines of domestic and wild pigs, especially porcine alveolar macrophages (Baron et al., 1992). The full length of PRRSV genome is approximately 15 kilobases (kb). It contains a 5′-untranslated region (UTR) and at least 10 open reading frames (ORFs) and a 3'-UTR (Hanada et al., 2005). ORF1a and ORF1b encode at least 16 non-structural proteins (NSPs). These nsps are involved in viral replication and transcription (Snijder et al., 2001). ORF2a, ORF2b, ORF5a and ORF3–7 encode eight viral structural proteins (Belák et al., 2002). GP5 is the main viral envelope protein. It is related to virus neutralization, adsorption and cell entry. PRRSV is divided into two genotypes, including type I and type II; the ORF5 gene (which codes GP5) determines the type (Chen et al., 2006). Although the occurrence over time, genome structure and clinical symptoms for both types is similar, their nucleotide (nt) similarity is only about 60% (Thiel et al., 1993). The type II virus is prevalent in China. The probability of virus mutation producing recombinant strains is likely to increase with the use of vaccines. Recently, a recombinant PRRSV strain was found in Iowa, USA. The strain had the same gene sequence as the vaccine strain (Wang et al., 2019). The phenomenon of PRRSV recombination was not only found in type II PRRSV strains, but also in type I strains (Marton et al., 2019). PRRSV field strains recombine with vaccine strains to escape the capture of neutralizing antibodies produced after vaccination, and these recombinant and mutated strains often have strong pathogenicity, such as RFLP 1-7-4 strain found in the USA and KU-N1202, a mutated recombinant strain reconstituted with VR2332, found in the Korea (Kwon et al., 2019; van Geelen et al., 2017). This kind of reorganization is more obvious in China.

The China swine industry has been impaired over the last 20 years by the far-reaching financial losses caused by PRRS (Zhou & Yang, 2010). There was an outbreak of highly pathogenic PRRSV from 2007 to 2008, with high fever, high morbidity and high mortality, and a reemergence in 2009–2010 (Tian et al., 2007). This epidemic seriously impacted the Chinese swine industry. Commercial PRRS vaccines currently in clinical use in China include eight live attenuated vaccines and two inactivated vaccines. PRRS was once well-controlled with those vaccines. In 2011–2012, different subtypes and recombinant strains of PRRSV appeared in China. In 2013, Zhou Feng found variant strains HENAN-XINX (KF611905) and HENAN-HEB (KJ143621), both of which are highly homologous to NADC30, in Henan Province, and called them NADC30-like strains (Zhou et al., 2015). Since 2014, NADC30-like strains have broken out in a large area in China, and NADC30-like strains have gradually become the dominant strains in some areas. Because the parent strains of commercial vaccines are classical PRRSV and HP-PRRSV, the vaccines have a weak effect on the prevention and control of NADC30-like PRRS. As an RNA virus, PRRSV may utilize high rates of mutation as a strategy to escape host immune surveillance (Tian, 2017). In addition, abuse of the attenuated vaccine has led to a significant variation in PRRSV strains in China. The above problems make it difficult to prevent and control PRRS. To explore the genetic evolution of the complete gene sequences of PRRSV strains isolated in Sichuan, China, we obtained the complete gene sequences of a newly isolated PRRSV strain. Subsequently, we analysed its homology, genetic relationship and recombination events with reference strains.

2 | MATERIALS AND METHODS

2.1 | Sample collection and virus culture

The PRRSV-positive material came from a swine farm in Sichuan, China, which experienced symptoms of high fever and PRRS in 2019. We collected blood serum and lung tissue and used it to inoculate Meat Animal Research Center-145 cells (Marc-145 cells). We subcultured the virus for 10 generations. After freezing and thawing and centrifuging at 4,000 rpm for 15 min, the supernatant was collected and stored at −80℃.

2.2 | RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

We extracted RNA according to the manufacturer's instructions from the isolated strain supernatant using the Viral RNA Extraction Kit (Takara). We designed the primers online according to the sequences of the complete gene sequence of PRRSV logged by Genbank (the relevant primer information is shown in the supplementary file). We generated complementary DNA (cDNA) following the supplier's instructions using Superscript III reverse transcriptase (Invitrogen, Waltham, MA, USA). We then used the cDNA in PCR amplification using twelve primers (A-L) specific for the viral genes as listed in Table S1. The reaction conditions were as follows: 95°C for 5 min; 30 cycles of 95°C for 30 s, 58°C for 30 s 72°C for 1 min; and a final extension at 72°C for 7 min. Furthermore, the products were Sanger sequenced in both directions by Sangon Biotech (Shanghai) Co., Ltd.

2.3 | Genetic evolution analysis

We compared the PRRSV DJY-19 (Accession No.MT075480) complete gene sequences and Nsp2 gene sequences and ORF5 gene sequences with the gene sequences from different regions in China and abroad (see Table S2). The genetic and evolutionary relationships

analysis was done using DNAStar (version 7.1) and MEGA software (version 6.06).

2.4 | Recombination analyses

The sequences of the PRRSV were identified in this study and 26 isolated sequences from Genbank for recombination screening. RDP v4.8 was used to predict the recombination sites of sequences. The recombination events have only been considered when supported by at least three of RDP, GENECONV, MAXCHI, CHIMAERA and 3SEQ methods. Then we used SIMPLOT v3.5.1 to check the recombination signals and estimate the breakpoint locations. Furthermore, the recombination events were confirmed with a neighbour-joining phylogenetic tree.

3 | RESULTS AND DISCUSSION

We named the isolated PRRSV strain DJY-19. The homology of the complete gene sequences with other strains was between 58.6% and 94.1% (Figure 1a). The ORF5 gene sequences of 34 PRRSV reference strains were obtained from the GenBank for a homology comparison and genetic evolutionary tree analysis. The results showed that the homology of the ORF5 gene with NADC30-like strains was between 84.4% and 95.9%. The ORF5 gene of DJY-19 was in the same branch as the ORF5 gene of the foreign NADC30 strain (JN654459) from the USA (95.9% homology). It was more distantly related (84.4% homology) to the TJnh1501(KX510269) strain (see Figure 1c and Table 1). Based on the results of the phylogenetic tree, the DJY-19 strain was in the same lineage as the NADC30 strain.

We used the Nsp2 gene sequences and complete gene sequences of 24 PRRSV reference strains obtained from NCBI to perform a homology comparison and genetic evolutionary tree analysis. There was a 131-amino-acid discontinuous deletion from Nsp2 (Figure 2). The homology of the Nsp2 gene with other strains was between 50.4% (LV-Netherlands-1993-M96262) and 94.2%. The Nsp2 gene of DJY-19 was in the same branch of the foreign NADC30 strain from the USA (94.2% homology). But it was more distantly related (50.4% homology) to the LV strain (Figure 1b). The homology of the Nsp2 gene with NADC30-like strains was between 85.5% and 94.2% (Table 1).

We detected all recombination events in RDP and confirmed them by SIMPLOT and phylogenetic trees. We identified two recombination breakpoints in the sequence alignment (Figure 3a). The RDP4 graph indicated that the DIY-19 was highly similar to that of TJ in the 8106–9128 nt region. This stretch contains partial Nsp9-coding regions. We also found that DJY-19 was highly familiar with JXA1-R strain in the 12106–12580 nt region, which contains partial Nsp12-coding regions. The phylogenetic tree showed that the position 8106–9128 nt of DJY-19 clustered with TJ strain (Figure 3b), but the 1–8105 and 9129–15021 nt regions with reference to the NADC30 strain (Figure 3c). The DJY-19 clustered with JXA1-R at position 12106–12580 nt (Figure 3d), but the 1–12105 and 12580–15021 regions with reference to the NADC30 strain (Figure 3e). In addition, we found out by asking that this farm introduced 1,000 new piglets in April 2019, and the PRRSV vaccine used for swine immunization is a TJM attenuated vaccine strain. This evidence further supports the possible recombination between the attenuated vaccine and NADC30-like PRRSV.

In 2013, two PRRSV NADC30-like strains, HENAN-XINX (KF611905) and HENAN-HEB (KJ143621), were isolated from Henan province, China. HENAN-XINX recombined with classical PRRSV, including VR-2332. HENAN-HEB recombined with HP-PRRSV, including JXA1. In 2014, JL580 (KR706343) was isolated from Jilin province, China; it resulted from recombination

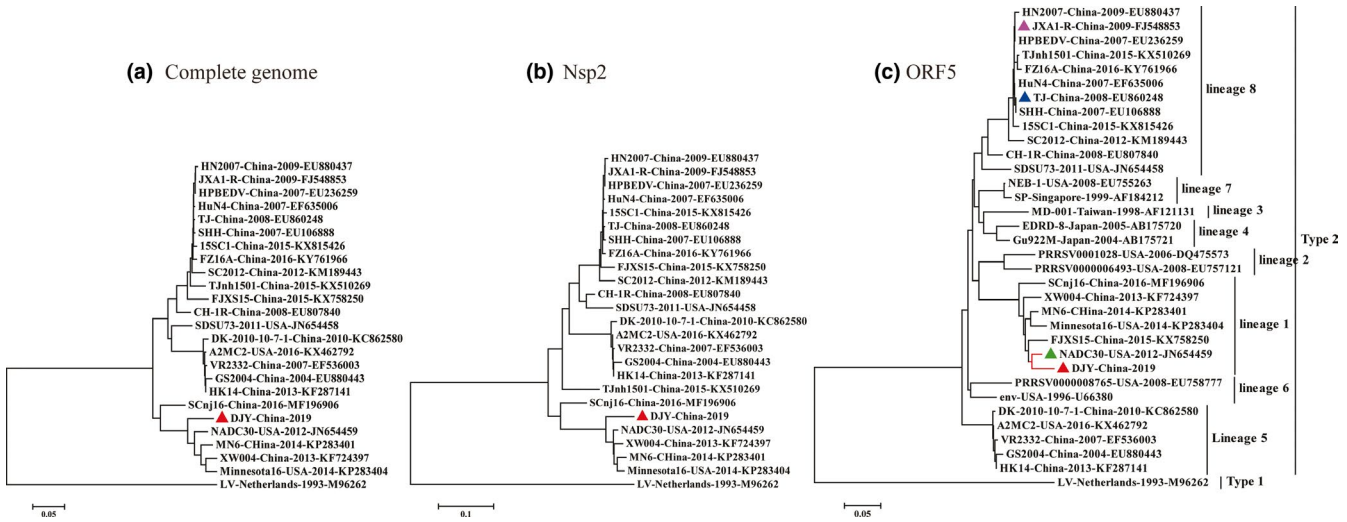

FIGURE 1 Neighbour-joining tree analysis of the complete genome, Nsp2 and ORF5 genes derived from the DJY-19 strain in comparison with other reference strains. We constructed the trees using the number of differences model with 1,000 bootstrap replicates. (a) Phylogenetic analysis based on the complete genome. (b) Phylogenetic analysis based on the Nsp2 gene. (c) Phylogenetic analysis based on the ORF5 gene

TABLE 1 Nucleotide identity and amino acid of DJY-19 compared with NADC30 and other representative PRRSV strains

	CH-1R		HuN4		JXA1-R		NADC30		SCnj16		SDSU73		TJ		TJnh1501		VR2332	
Genomic region	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**
Complete genmone	83.1		83		83		94.1		86.5		83.2		83		86.8		83.3	
ORF1α	76.3	61.3	76.7	61.3	76.6	61	94.1	86.7	79.8	66.5	76.4	61.9	76.6	61.2	84.2	69.7	77.5	62.8
NSP1α	89.3	95	87.6	95	87	95	95.9	96.7	86.9	91.1	89.4	94.4	87.2	95	87.6	94.4	89.4	94.4
NSP1β	78.2	72.4	78.5	74.4	78.2	73.9	92	90.1	77	74.4	77.8	71.9	78.5	74.4	78.3	74.4	80	75.9
NSP2	68.4	57.2	70.1	59.4	70.2	59.5	93.4	89	75.8	68.1	68.4	57.5	70	59.5	85.5	75.2	70	60
NSP3	84.5	90	83	90.4	83	90	93.9	97.4	86.7	93.5	84.1	90.4	82.9	90	82.5	90.4	85.7	91.7
NSP4	83.8	91.7	83.2	91.7	83.5	91.7	94.9	97.1	82.5	90.7	84.5	92.6	83.3	91.7	83.7	91.7	84.2	91.7
NSP5	91.6	93.5	89.6	93.5	88.8	93.5	95.7	97.6	88	92.4	93.5	97.1	89.4	93.5	89.4	93.5	89.8	93.5
NSP6	90.7	100	90.7	100	90.7	100	96.3	100	88.9	100	90.7	100	90.7	100	90.7	100	90.7	100
NSP7	83.4	85.7	82.5	84.9	82.2	84.9	94.9	93.1	81.1	83.8	83	86.5	82.2	84.2	82.2	84.9	85.7	88.4
NSP8	86.7	93.3	87.4	93.3	85.9	93.3	94.1	93.3	88.1	95.6	84.4	91.1	86.7	93.3	86.7	93.3	86.7	93.3
ORF1β	90.3	95.8	90.6	96.5	90.8	96.4	92.6	96.8	92.6	96.5	90.2	96.5	90.6	96.6	90.6	96.4	88.1	95.3
NSP9	90	72.6	90.7	73.5	91	74.2	92.5	82.6	93	79.6	90	73.5	90.5	72.9	91	74.2	88.5	72.2
NSP10	84.6	92.3	83.5	93.4	83.7	93	95	96.4	93.3	96.1	85.6	93.2	83.5	93	83.4	93.2	84.1	93.4
NSP11	95.1	98.7	95.8	98.2	95.7	98.2	93.3	96.9	89.5	96.4	94.3	98.2	96	98.7	95.4	97.8	90.4	95.1
NSP12	94.1	96.7	95.6	99.3	95.9	99.3	91.3	95.4	90.2	94.8	93	98	95.9	99.3	96.1	99.3	90.4	94.8
GP2	88.2	89.1	88.6	88.7	88.6	89.1	95.2	93	92.1	90.3	87.9	88.3	88.8	89.1	88.7	89.1	87.7	87.5
GP3	81.2	80.8	81.2	80.4	81.2	80	92.3	91.4	89.3	85.5	82.1	81.6	81.2	80.4	81.4	80.8	81.4	80.8
GP4	86	86.6	85.7	87.7	84.7	85.5	95.9	93.9	91.8	88.3	87.7	87.2	85.5	87.7	85.7	87.7	87.9	87.2
GP5	85.4	84.6	84.7	85.1	84.6	84.6	95.9	96.5	93.5	93	85.1	85.1	84.7	85.1	84.4	84.1	86.1	84.6
M	87.2	88.7	88.2	87.1	88.2	87.1	96.8	92.7	95	91.9	87.6	87.9	88	87.1	88.2	86.3	89.1	90.3
N	89.5	89.5	88.4	87.9	88.4	87.9	95.7	93.5	93.8	92.7	90.1	88.7	88.4	87.9	87.9	87.1	91.1	91.1
8106–9128 nt	95.8		97.6		97.7		87.4		96.4		94.3		97.8		97.4		90.9	
12106–12580 nt	96.1		98.9		98.9		89.5		96.2		93.4		98.9		98.9		91.1	

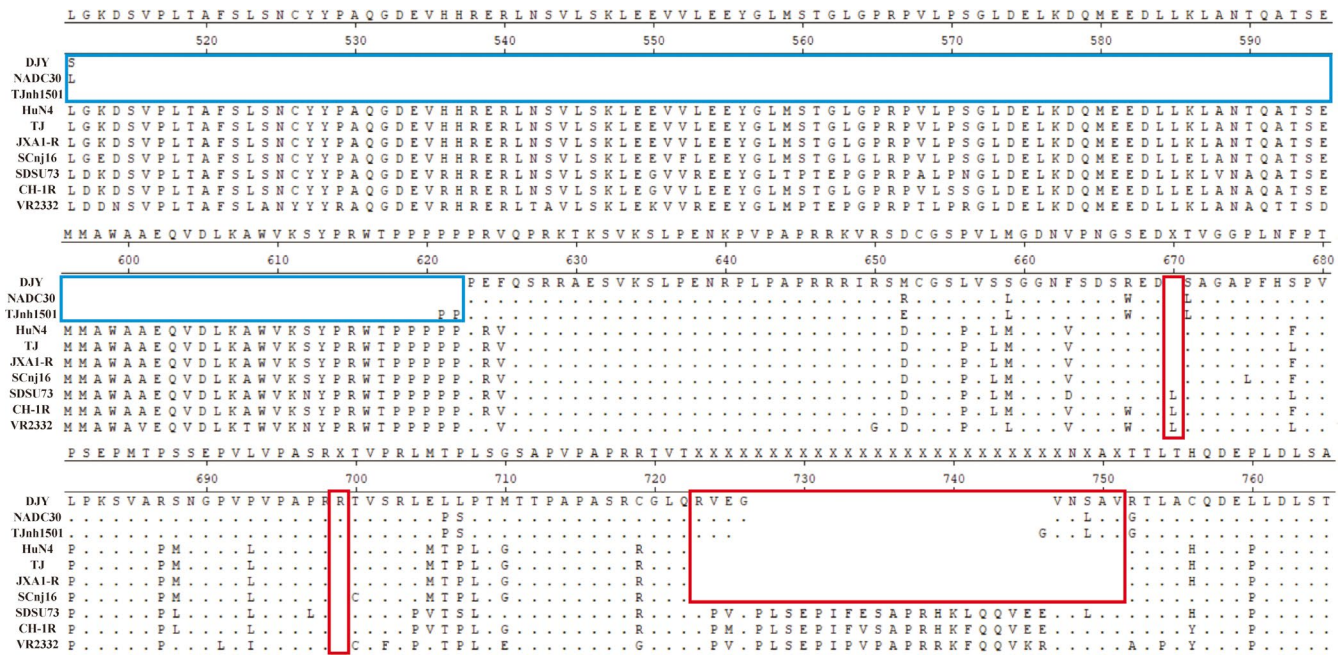

FIGURE 2 Analysis and comparison of the deduced amino acid sequences of Nsp2 variation region between porcine reproductive and respiratory syndrome virus (PRRSV) DJY-19 strain and nine other reference strains by DNAstar

with HP-PRRSV, such as 09NEN1. The Chsx1401 (KP861625) and HNyc15 (KT945018) strains reported in 2015 recombined with VR-2332/CH1a. Researchers performed an epidemiological survey on PRRSV in 16 provinces of China. Recombination analyses showed that nine of 28 isolates and one isolate from other laboratories were potentially complicated recombinants between the vaccine JXA1-R-like strains and the predominant circulating strains (Zhao et al., 2007). FJXS15 (KX758250) reported in 2016 recombined with JXA1-R. XJzx1-2015 (KX689233) was reported in 2016 recombined with QYYZ (JQ308798). In 2018, Zhou et al. reported that five NADC30 PRRSV recombinant strains (SCnj16, SCcd16, SCN17, SCcd17 and SCya17) were isolated from Sichuan in China, and all of them recombined with JXA1-like, VR-2332-like and QYYZ-like in single or multiple fragments. The results showed that the variation in PRRSV prevalent in Sichuan was significant (Zhou, Kang, et al., 2017; Zhou, Yang, et al., 2017; Zhou, Kang, Xie, et al., 2018; Zhou, Kang, Zhang, et al., 2018). The hotspots for recombination of NADC30-like PRRSV with other virus strains occurred in both non-structural protein regions, including nsp1a, nsp2–9 and nsp11, and structural protein regions, such as ORF2-7 (Zhao et al., 2007). In this study, the NADC30-like PRRSV DJY-19 strain was isolated from a vaccinated pig farm in the Sichuan province. Recombination analysis showed that the DJY-19 strain was a recombinant PRRSV. Furthermore, the DJY-19 8106–9128 nt gene fragment was closely related to TJ PRRSV, and 12106–12580 nt gene fragment was closely related to JXA1-R PRRSV. The remaining fragments were similar to NADC30 PRRSV. Therefore, our findings indicate that the parental virus of DJY-19 is NADC30 PRRSV. We found that the recombinant segment (nt 8106–9128) of DJY-19 displayed high homology with TJ (approximately 97.8%), while the recombinant segment (nt 12106–12580) of DJY-19 displayed high homology with JXA1-R, TJ, HuN4 and TJnh1501 (approximately 98.9%). These data indicate that the DJY-19 is a recombinant MLV-evolved virus. Therefore, the efficacy and safety of HP-PRRSV MLV vaccines deserve attention. Sichuan is a big pig raising Province in China. PRRSV MLV vaccines were widely used and there were abuse cases of MLV. The recombination of the PRRSV has caused great pressure to prevent and control PRRSV at the present time.

At present, many scholars have done a lot of research on traditional PRRSV MLV vaccines against recombinant wild strains. Due to immune evasion strategies and the antigenic heterogeneity of the virus, current commercial PRRSV vaccines (killed-virus and modified-live vaccines) are of unsatisfactory efficacy, especially against heterologous infection (Hu & Zhang, 2014; Pileri & Mateu, 2016). The protective effect of the vaccine on the field strains was evaluated by the indexes of viraemia, time of high fever and virus elimination. Previous studies have shown that the autogenous inactivated PRRSV vaccines can enhance the neutralization antibody level in pigs after immunizing pigs with the same type of PRRSV commercial vaccines (Geldhof et al., 2013). Piglets were vaccinated with typeIPRRSV MLV vaccine and challenged with typeII field PRRSV strain. The results showed that non-neutralizing antibodies induced by vaccination prior to challenge might play a key role in protecting against field PRRSV infection, especially in the early time course (Ko et al., 2016). The duration of viraemia and fever was reduced after the pigs immunized with typeIIPRRSV MLV vaccines and infected non-mutated typeIIfield PRRSV (Lager et al., 2014). The protective effect of commercial vaccines on the mutant field PRRSV strains was very poor. The results show that some commercial MLV vaccines (JXA1-R, TJM-F92, VR2332 and R98) can only provide partial protective efficacy in the vaccinated piglets upon virus (HNjz15,

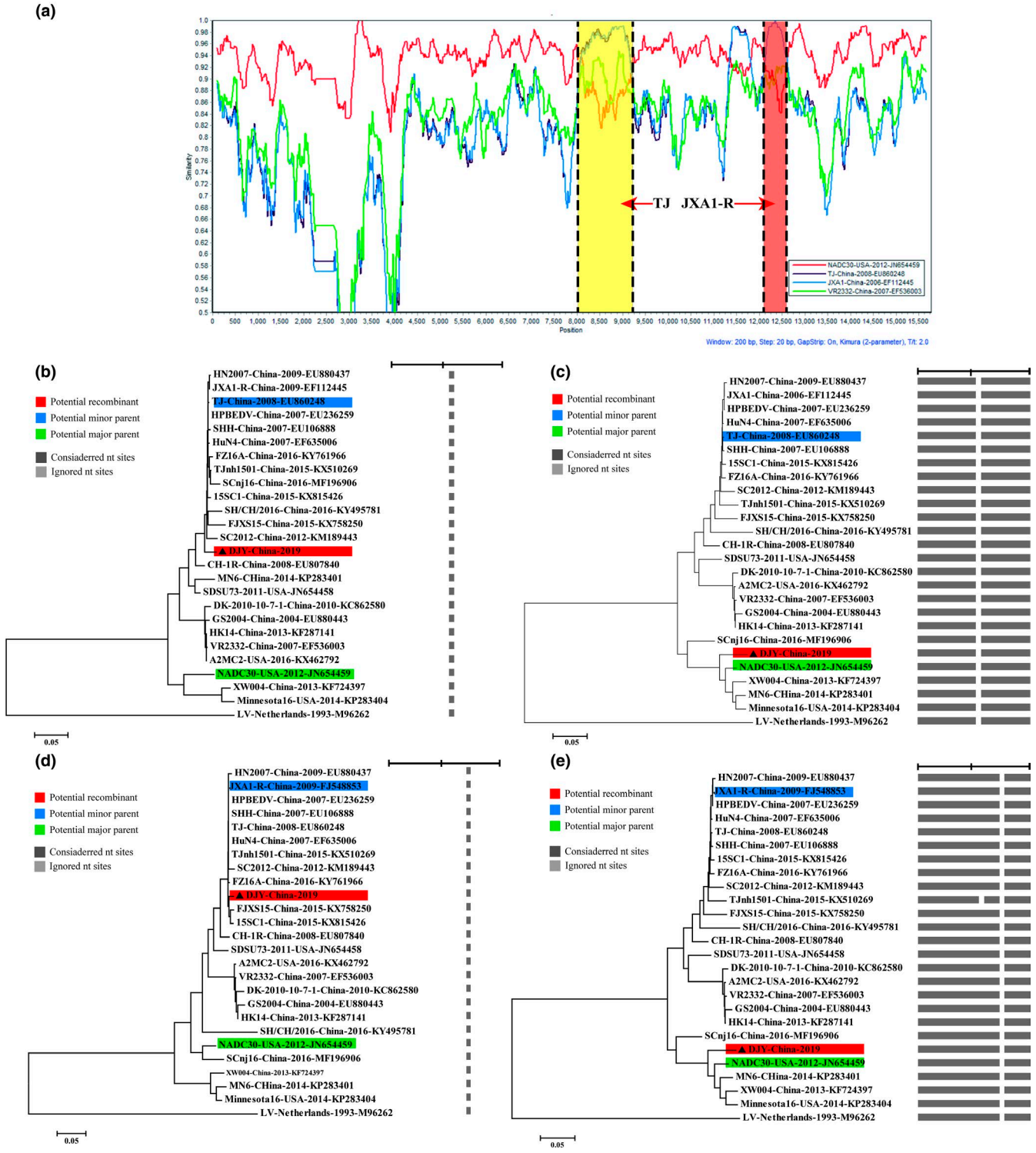

FIGURE 3 SimPlots for all putative recombinants analysed in this study. (a) We calculated recombination analysis with Simplot 3.5.1 software. We used the complete DJY-19 genome as the query sequence. Recombination breakpoints are shown with black dotted lines, and the locations are shown at the bottom. (b) Phylogenetic analysis based on the 8106–9128 nucleotide (nt) region. (c) Phylogenetic analysis based on the 1–8105 and 9129–15021 nt regions. (d) Phylogenetic analysis based on the 12106–12580 nt region. (e) Phylogenetic analysis based on the 1–12105 and 12580–15021 nt regions

CHsx1401, FJZ03, FJ1402) challenges (Lager et al., 2014; Zhou, Kang, et al., 2017). In summary, the emergence of new PRRSV recombinant strains exerts great pressure on the prevention and control of PRRSV.

ACKNOWLEDGEMENTS

This research was funded by the National Key Research and Development Program (2018YFD0500802), the Science and Technology Program of Sichuan province (2018JY0369) and the

Sichuan Veterinary Medicine and Drug Innovation Group of the China Agricultural Research System (CARS-SVDIP).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

Jun Zhao: Data curation; Formal analysis; Writing-original draft; Writing-review & editing. **Ling Zhu:** Conceptualization; Resources. **Jianbo Huang:** Data curation. **Zexiao Yang:** Methodology. **Lei Xu:** Investigation. **Sirui Gu:** Investigation. **Yao Huang:** Writing-review & editing. **Rubo Zhang:** Writing-review & editing. **Xiangang Sun:** Writing-review & editing. **Yuancheng Zhou:** Conceptualization. **Zhiwen Xu:** Conceptualization; Project administration; Resources.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.402.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available in the NCBI repository (No.MT075480).The data of genetic evolution analysis and design of primers used to support the findings of this study are included within the supplementary information file(s).

ORCID

Jun Zhao https://orcid.org/0000-0002-5804-6816

REFERENCES

Baron, T., Albina, E., Leforban, Y., Madec, F., Guilmoto, H., Duran, J. P., & Vannier, P. (1992). Report on the first outbreaks of the porcine reproductive and respiratory syndrome (PRRS) in France. *Diagnosis and Viral Isolation*, *23*(2), 161.

Chen, J., Liu, T., Zhu, C.-G., Jin, Y.-F., & Zhang, Y.-Z. (2006). Genetic variation of Chinese PRRSV strains based on ORF5 sequence. *Biochemical Genetics*, *44*(9–10), 421–431. https://doi.org/10.1007/s10528-006-9039-9

Geldhof, M. F., Van Breedam, W., De Jong, E., Lopez Rodriguez, A., Karniychuk, U. U., Vanhee, M., Van Doorsselaere, J., Maes, D., & Nauwynck, H. J. (2013). Antibody response and maternal immunity upon boosting PRRSV-immune sows with experimental farm-specific and commercial PRRSV vaccines. *Veterinary Microbiology*, *167*(3–4), 260–271. https://doi.org/10.1016/j.vetmic.2013.08.017

Hanada, K., Suzuki, Y., Nakane, T., Hirose, O., & Gojobori, T. (2005). The origin and evolution of porcine reproductive and respiratory syndrome viruses. *Molecular Biology & Evolution*, *22*(4), 1024–1031. https://doi.org/10.1093/molbev/msi089

Hu, J., & Zhang, C. (2014). Porcine reproductive and respiratory syndrome virus vaccines: Current status and strategies to a universal vaccine. *Transboundary & Emerging Diseases*, *61*, 109–120. https://doi.org/10.1111/tbed.12016

Ko, S.-S., Seo, S.-W., Sunwoo, S.-Y., Yoo, S. J., Kim, M.-H., & Lyoo, Y. S. (2016). Efficacy of commercial genotype 1 porcine reproductive and respiratory syndrome virus (PRRSV) vaccine against field isolate of genotype 2 PRRSV. *Veterinary Immunology & Immunopathology*, *172*, 43–49. https://doi.org/10.1016/j.vetimm.2016.02.016

Kwon, T., Yoo, S. J., Park, J. W., Kang, S. C., Park, C.-K., & Lyoo, Y. S. (2019). Genomic characteristics and pathogenicity of natural recombinant porcine reproductive and respiratory syndrome virus 2 harboring genes of a Korean field strain and VR-2332-like strain. *Virology*, *530*, 89–98. https://doi.org/10.1016/j.virol.2019.01.030

Lager, K. M., Schlink, S. N., Brockmeier, S. L., Miller, L. C., Henningson, J. N., Kappes, M. A., Kehrli, M. E., Loving, C. L., Guo, B., Swenson, S. L., Yang, H.-C., & Faaberg, K. S. (2014). Efficacy of Type 2 PRRSV vaccine against Chinese and Vietnamese HP-PRRSV challenge in pigs. *Vaccine*, *32*(48), 6457–6462. https://doi.org/10.1016/j.vaccine.2014.09.046

Marton, S., Szalay, D., Kecskeméti, S., Forró, B., Olasz, F., Zádori, Z., Szabó, I., Molnár, T., Bányai, K., & Bálint, Á. (2019). Coding-complete sequence of a vaccine-derived recombinant porcine reproductive and respiratory syndrome virus strain isolated in Hungary. *Archives of Virology*, *164*, 2605–2608. https://doi.org/10.1007/s00705-019-04338-2

Pileri, E., & Mateu, E. (2016). Review on the transmission porcine reproductive and respiratory syndrome virus between pigs and farms and impact on vaccination. *Veterinary Research*, *47*(1), 108. https://doi.org/10.1186/s13567-016-0391-4

Snijder, E. J., van Tol, H., Roos, N., & Pedersen, K. W. (2001). Non-structural proteins 2 and 3 interact to modify host cell membranes during the formation of the arterivirus replication complex. *Journal of General Virology*, *82*(5), 985–994. https://doi.org/10.1099/0022-1317-82-5-985

Stadejek, T., Stankevicius, A., Storgaard, T., Oleksiewicz, M. B., Belák, S., Drew, T. W., & Pejsak, Z. (2002). Identification of radically different variants of porcine reproductive and respiratory syndrome virus in Eastern Europe: Towards a common ancestor for European and American viruses. *Journal of General Virology*, *83*(8), 1861–1873. https://doi.org/10.1099/0022-1317-83-8-1861

Thiel, H. J., Meyers, G., Stark, R., Tautz, N., Rümenapf, T., Unger, G., & Conzelmann, K. K. (1993). Molecular characterization of positive-strand RNA viruses: Pestiviruses and the porcine reproductive and respiratory syndrome virus (PRRSV). *Archives of Virology. Supplementum*, *7*, 41–52.

Tian, K. (2017). NADC30-like porcine reproductive and respiratory syndrome in China. *The Open Virology Journal*, *11*(1), 59–65. https://doi.org/10.2174/1874357901711010059

Tian, K., Yu, X., Zhao, T., Feng, Y., Cao, Z., Wang, C., Hu, Y., Chen, X., Hu, D., Tian, X., Liu, D. I., Zhang, S., Deng, X., Ding, Y., Yang, L. U., Zhang, Y., Xiao, H., Qiao, M., Wang, B., … Gao, G. F. (2007). Emergence of fatal PRRSV variants: Unparalleled outbreaks of atypical prrs in china and molecular dissection of the unique hallmark. *PLoS One*, *2*(6), e526. https://doi.org/10.1371/journal.pone.0000526

van Geelen, A. G. M., Anderson, T. K., Lager, K. M., Das, P. B., Otis, N. J., Montiel, N. A., Miller, L. C., Kulshreshtha, V., Buckley, A. C., Brockmeier, S. L., Zhang, J., Gauger, P. C., Harmon, K. M., & Faaberg, K. S. (2017). Porcine reproductive and respiratory disease virus: Evolution and recombination yields distinct ORF5 RFLP 1–7-4 viruses with individual pathogenicity. *Virology*, *513*, 168–179. https://doi.org/10.1016/j.virol.2017.10.002

Wang, A., Chen, Q. I., Wang, L., Madson, D., Harmon, K., Gauger, P., Zhang, J., & Li, G. (2019). Recombination between vaccine and field strains of porcine reproductive and respiratory syndrome virus. *Emerging Infectious Diseases*, *25*(12), 2335–2337. https://doi.org/10.3201/eid2512.191111

Zhao, H., Han, Q., Zhang, L., Zhang, Z., Wu, Y., Shen, H., & Jiang, P. (2007). 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. *Virology Journal*, *14*(1), 67. https://doi.org/10.1186/s12985-017-0735-3

Zhou, F., Zhao, J., Chen, L. U., Chang, H.-T., Li, Y.-T., Liu, H.-Y., Wang, C.-Q., & Yang, X. (2015). Complete genome sequence of a novel porcine reproductive and respiratory syndrome virus that emerged in China. *Genome Announcements*, *3*(4), e00702–e00715. https://doi.org/10.1128/genomeA.00702-15

Zhou, L., Kang, R., Ji, G., Tian, Y., Ge, M., Xie, B. O., Yang, X., & Wang, H. (2017). Molecular characterization and recombination analysis of porcine reproductive and respiratory syndrome virus emerged in southwestern China during 2012–2016. *Virus Genes*, *54*(1), 98–110. https://doi.org/10.1007/s11262-017-1519-y

Zhou, L., Kang, R., Xie, B. O., Tian, Y., Wu, X., Lv, X., Yang, X., & Wang, H. (2018). Identification of a novel recombinant type 2 porcine reproductive and respiratory syndrome virus in China. *Viruses*, *10*(4), 151. https://doi.org/10.3390/v10040151

Zhou, L., Kang, R., Zhang, Y. I., Ding, M., Xie, B. O., 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*(6), 328. https://doi.org/10.3390/v10060328

Zhou, L., Yang, B., Xu, L., Jin, H., Ge, X., Guo, X., Han, J., & Yang, H. (2017). Efficacy evaluation of three modified-live virus vaccines against a strain of porcine reproductive and respiratory syndrome virus NADC30-like. *Veterinary Microbiology*, *207*, 108–116. https://doi.org/10.1016/j.vetmic.2017.05.031

Zhou, L., & Yang, H. C. (2010). Porcine reproductive and respiratory syndrome in China. *Virus Research*, *154*(1–2), https://doi.org/10.1016/j.virusres.2010.07.016

Zuo, Y., Yuan, W. Z., & Sun, J. G. (2015). Complete genomic characterization of porcine reproductive and respiratory syndrome virus strain HB-XL. *Genes*, *6*(3), 672–684. https://doi.org/10.3390/genes6030672

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Zhao J, Zhu L, Huang J, et al. Genetic characterization of a novel recombined porcine reproductive and respiratory syndrome virus 2 among Nadc30-like, Jxa1-like and TJ-like strains. *Vet Med Sci.* 2021;7:697–704. https://doi.org/10.1002/vms3.402