

# Complete genomic characterization of a porcine reproductive and respiratory syndrome virus isolate in Xinjiang province of China

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**Abstract** Porcine reproductive and respiratory syndrome (PRRS) is a swine disease of major economic importance that causes reproductive and respiratory problems in pigs. In the present study, one strain of porcine reproductive and respiratory syndrome virus (PRRSV) was isolated in Xinjiang province, Northwest China, designated XJu-1. The full-length genome of XJu-1 was found to be 14,987 nucleotides in length, including the poly(A) tail. Comparative analysis with the genomic sequences of type 2 isolates revealed that XJu-1 shared 87.2–99.2 % identity with these isolates, but only 60.4 % with the type 1 virus—Lelystad Virus, indicating that this new Chinese isolate was closely related to the North American PRRSV genotype. XJu-1 was a novel strain with unique deletions in NSP2 region, namely that 150-amino acid deletion in NSP2. The genomic variations of XJu-1 strain provided the basis for further studies of virulence determinants and evolution for PRRSVs.

**Keywords** PRRSV · Full-length · 150 aa deletion · China · Phylogenetic analysis

## Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important diseases of swine worldwide and characterized by severe reproductive failure in pregnant sows and respiratory diseases in young pigs [1]. PRRS emerged nearly simultaneously on two different continents, with its initial

recognition in North America in 1987 [2], followed by Europe in 1990 [3, 4].

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped virus belonging to the family Arteriviridae containing a positive-strand RNA genome. PRRSV strains were divided into the European type (Lelystad virus, LV) (type 1) and the North American type (ATCC-VR2332) (type 2) with approximately 60 % identity at the genome level [5–7]. The genome of PRRSV is about 15 kb in length and contains eight open reading frames (ORF1a, ORF1b, ORF2–7), 5′ nontranslated region (UTR), and 3′ UTR [8]. ORF1a and ORF1b encode viral replicase polyproteins, which are predicted to be cleaved into 13 nonstructural proteins (NSPs), including NSP1a, NSP1b, and NSP2 to NSP12 [9], while ORF2a, ORF2b, and ORFs 3–7 encode the viral structural proteins GP2, E, GP3, GP4, GP5, M, and N, respectively [10]. Among the nonstructural proteins of PRRSV, Nsp2, the largest part of the cleavage product of the replicative protein possesses a chymotrypsin-like cysteine protease domain [11]. The biological functions of PRRSV Nsp2 are currently unclear, although the Nsp2 protein of PRRSV has an organization similar to that of the Nsp2 counterpart of EAV, and EAV Nsp2 has been considered to assemble the replicative double-membrane vesicles in collaboration with Nsp3 [12].

In this paper, the genome of XJu-1, isolated in Xinjiang province, northwest China, in 2014 was sequenced, and we analyzed the sequences with other strains of the North American and European genotypes.

## Materials and methods

### Clinical samples

Lungs and lymph nodes were collected from suspected piglets in Xinjiang province of China in 2012. All of these

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**Table 1** Primers used in this study

Fragment	Primer	Sequence	Location
1	A1	5'-ATGACGTATAGGTGTTGGCTC-3'	1–21
	A2	5'-TGTTTTCTTGTCTCTTTC-3'	1,343–1,362
2	B1	5'-ATTTCCGGTTTGGCAGTCA-3'	1,306–1,325
	B2	5'-CTACCCGCGTGGACACCACC-3'	2,607–2,627
3	C1	5'-AAAGACCAGATGGAGGAGGA-3'	2,500–2,519
	C2	5'-TTCTCAAACCTTGGGACCCTGT-3'	3,746–3,767
4	D1	5'-CTGCTGTGAGTTTGACTCGC-3'	3,692–3,712
	D2	5'-TCACTGAACACCGTCAATGT-3'	5,191–5,210
5	E1	5'-GACATTCATCATTACACCAGT-3'	5,020–5,040
	E2	5'-TGCCTCAACATGAGGAATGC-3'	6,423–6,443
6	F1	5'-TGACGAGGACTTGGATTCCTT-3'	6,369–6,390
	F2	5'-GTGGTGTACCCAGGGCTTC-3'	8,311–8,330
7	G1	5'-GCGAGAAAACCTGGCAAACCTGT-3'	8,189–8,209
	G2	5'-GGTGCCGGCTATATGGTGGGC-3'	10,686–10,706
8	H1	5'-CAACCCAGAACAATGAAA-3'	10,606–10,623
	H2	5'-TCAAATGTAAACCATAGTGAT-3'	12,151–12,171
9	I1	5'-GAGACTTGTAATAATTTGG-3'	12,091–12,109
	I2	5'-TGTACCAGATATACCAACTT-3'	13,749–13,768
10	J1	5'-TGCATGTCCTGGCGCTAC-3'	13,728–13,745
	J2	5'-GCCGCATTTAAATTTTTTTTTTTTTT-3'	poly(A)

piglets displayed typical symptoms of PRRS, including labored breathing, pyrexia, and anorexia with mortality of 8 %. Clinical tissues were homogenized for RNA extraction and virus isolation, and the remaining samples were kept at  $-80^{\circ}\text{C}$ .

#### Virus isolation

Virus isolation from the tissue homogenates was performed in Marc-145 cells maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS; Thermo), 100 mg penicillin, and 100 units of streptomycin per ml of growth medium. The XJu-1 strain was recovered from clarified homogenates of lung and lymph nodes of suspected piglets with fever. Then, the isolated virus was amplified in Marc-145 cells. The inoculated cells were maintained at  $37^{\circ}\text{C}$  with 5 %  $\text{CO}_2$  and monitored daily for cytopathic effects (CPE). The culture supernatants were harvested when CPE appeared in 70 % of the cells and stored at  $-80^{\circ}\text{C}$  as the virus stock until use.

#### Primers design

In order to determine the full-length genomic sequence of XJu-1, primers were first selected based on published known sequences of the North American prototype PRRSV strain (VR-2332; GenBank Accession No. AY150564).

Other Chinese isolates available in NCBI were used as references. The primers used for the XJu-1 genome sequencing are given in Table 1.

#### Viral RNA extraction and RT-PCR

Total RNA was extracted using TRIzol reagent (Sangon, China) following the manufacture's protocol. The reverse transcription and polymerase chain reaction mixture consists of 3  $\mu\text{l}$  total RNA, 25  $\mu\text{l}$  2  $\times$  Reaction Mix, 5  $\mu\text{l}$  dNTPs, 3  $\mu\text{l}$  primers, 1  $\mu\text{l}$  RT/Platinum Taq, and 13  $\mu\text{l}$  RNase Free  $\text{ddH}_2\text{O}$ . The cycling condition was 1 cycle of Reverse transcription ( $60^{\circ}\text{C}$  for 30 min,  $94^{\circ}\text{C}$  for 2 min), followed by 30 cycles of polymerase chain reaction including denaturation ( $94^{\circ}\text{C}$  for 15 s), annealing ( $58^{\circ}\text{C}$  for 30 s), and extension ( $68^{\circ}\text{C}$  for 4 m), followed by the final extension at  $68^{\circ}\text{C}$  for 5 min. The reverse transcription and polymerase chain reaction was conducted using SuperScript III One-Step RT-PCR Platinum Taq HiFi (Invitrogen, US).

#### Genome cloning and sequencing

The PCR products were extracted from agarose gel using an DNA Gel Extraction Kit (Sangon, China) as manufacturer's recommendation and cloned into pMD-18T vector (TaKaRa, Japan). The clones were sequenced.

**Table 2** PRRSV strains used in this study

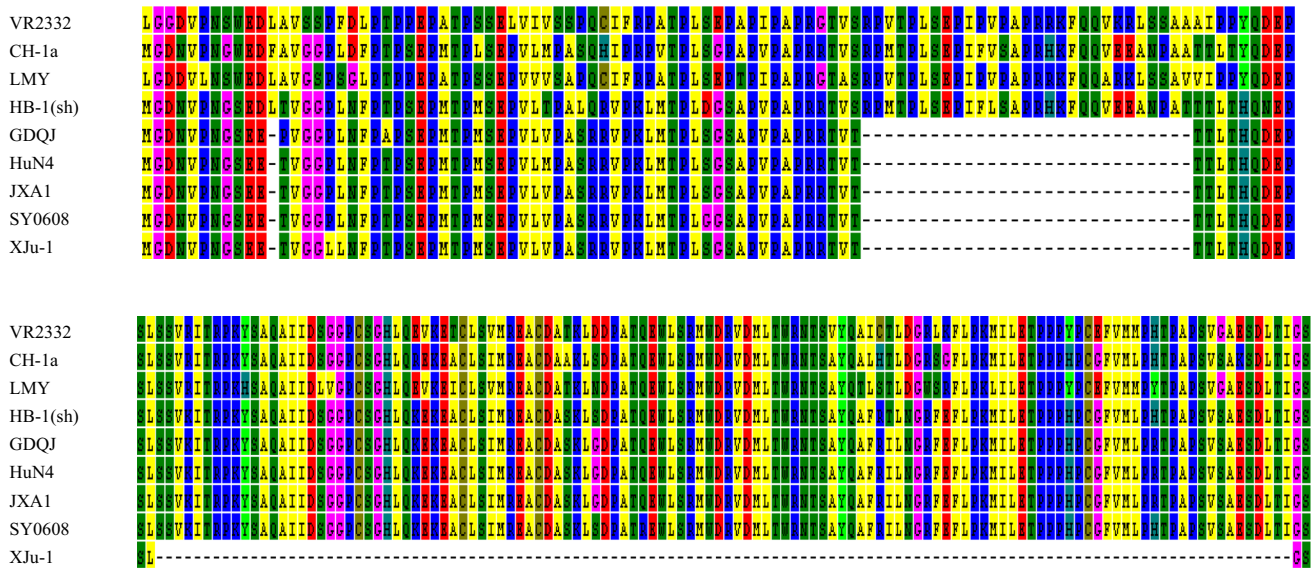
Strain	Country	Accession no.
XH-GD	China	EU624117
BJ	China	EU825723
BJ-4	China	AF331831
CC-1	China	EF153486
CH-1a	China	AY032626
SY0608	China	EU144079
S1	China	DQ459471
GD	China	EU109503
GDQJ	China	GQ374441
GDQY2	China	GU454850
HB-1(sh)	China	AY150312
HB-2(sh)	China	AY262352
Henan-1	China	EU200962
HN1	China	AY457635
HuN	China	EF517962
HuN4	China	EF635006
JXA1	China	EF112445
HKEU16	China, Hong Kong	EU076704
VR-2332	USA	U87392
MU184C	USA	EF488739
NVSL	USA	AY545985
P129	USA	AF494042
16244B	USA	AF046869
EuroPRRSV	USA	AY366525
LMY	South Korea	DQ473474
PL97-1	South Korea	AY585241
KNU-07	South Korea	FJ349261
Lelystad	Netherlands	M96262
PA8	Canada	AF176348
SP	Singapore	AF184212
EDRD-1	Japan	AB288356
01NP1.2	Thailand	DQ056373
XJu-1	Chian	KF815525

Genome and phylogenetic analysis

The complete genome sequences of XJu-1, every ORF and most of the deduced aa sequences were used for comparison with other representative PRRSV strains (Table 2) using Clustal W method and DNASTar. Phylogenetic analyses were conducted using MEGA5, based on full-length genome, ORF5, and ORF7 nucleotide sequences to determine the evolutionary trend of PRRSV.

**Table 3** Detailed comparison of the full-length genome of the XJu-1 isolate to other type 1 and type 2 isolates

XJu-1 %	Identity to XJu-1					
	HuN4	Sy0608	LMY	EDRD-1	VR-2332	LV
Nucleotide (length)						
5'UTR (189)	99.5	99.5	90.5	92.6	91.5	62.8
ORF1a (7,062)	99.2	99.1	85.8	84.2	87.6	55.6
ORF1b (4,383)	99.2	99.2	89.3	89.6	91.1	63.4
ORF2 (771)	99.4	99.7	91.3	91.7	93.4	65.3
ORF3 (765)	99.0	99.3	88.0	87.6	89.4	65.4
ORF4 (537)	99.3	99.6	89.0	89.9	90.1	66.5
ORF5 (603)	99.3	98.8	87.1	89.4	88.7	63.8
ORF6 (525)	100.0	100.0	94.7	91.8	95.0	69.5
ORF7 (372)	99.5	99.2	91.7	91.1	93.5	68.1
3'UTR (150)	100.0	99.3	88.9	91.5	94.1	72.6
Complete (14,987)	99.2	99.1	88.4	87.2	89.6	60.4
Amino acid (length)						
NSP1 $\alpha$ (180)	100.0	100.0	95.6	95.6	96.1	66.9
NSP1 $\beta$ (203)	99.0	98.5	82.3	78.3	83.7	41.7
NSP2 (830)	97.1	97.1	74.1	72.7	75.7	29.2
NSP3 (446)	99.1	99.3	93.5	91.5	94.4	58.7
NSP4 (204)	100.0	100.0	94.6	94.1	94.6	62.6
NSP5 (170)	100.0	99.4	92.9	92.4	92.4	71.8
NSP6 (16)	100.0	100.0	93.8	93.8	93.8	75.0
NSP7 (259)	100.0	100.0	88.4	87.6	90.0	46.3
NSP8 (45)	100.0	100.0	100.0	97.8	100.0	68.2
NSP9 (643)	99.1	99.2	96.9	97.0	97.5	75.0
NSP10 (441)	99.5	99.3	95.7	95.5	95.9	64.1
NSP11 (223)	99.1	99.1	95.1	95.5	94.2	77.0
NSP12 (153)	100.0	100.0	92.8	93.5	95.4	42.8
ORF2a/GP2 (257)	99.2	99.6	91.4	89.9	92.6	62.0
ORF2b/E (74)	100.0	100.0	94.6	93.2	90.5	71.8
ORF3/GP3 (255)	98.8	99.2	88.6	85.5	99.2	57.1
ORF4/GP4 (179)	97.8	98.9	88.3	93.3	90.5	71.5
ORF5/GP5 (201)	98.0	97.5	85.1	88.1	86.6	57.4
ORF6/M (175)	100.0	100.0	97.1	96.0	97.1	80.5
ORF7/N (124)	99.2	98.4	93.5	92.7	94.4	62.0



**Fig. 1** Alignment of amino acids sequence of NSP2 of PRRSV, where the discontinuous 150 aa deletions are indicated by “-”

## Results

### Genomic comparison between XJu-1 and other PRRSV strains

The sequence data showed that, including the poly(A) tail, the genomic sequence of XJu-1 was 14,987 nucleotides (nt) in length, has a 189-nt 5'UTR, and a 150-nt 3'UTR. The complete nucleotide sequence of XJu-1 was further compared to other PRRSV isolates, including one north American type strain (VR-2332), one European type strain (LV), one Japanese strain (EDRD-1), one South Korea strain (LMY), and two Chinese isolates (HuN4 and SY0608) (Table 3). Full-length sequence analysis showed that XJu-1 shared high-level similarity with PRRSV strains from the North American type, exhibiting 87.2–99.2 % identity at the nucleotide level, especially to the strains isolated in China, which exhibited a higher identity that 99.2 and 99.1 % with HuN4 and SY0608, respectively (Table 3). By contrast, a subsequent comparison analysis with European type strain LV showed a low homology of 60.4 % (Table 3). The nucleotide sequence identity varied with regard to individual ORFs, of which ORF1a shared the lowest identity with type 2 strains, ranging from 84.2 to 99.2 %, whereas ORF1b, ORF2, ORF3, ORF4, ORF5, ORF6, and ORF7 were comparatively conserved with identities of 89.3–99.2, 91.3–99.7, 87.6–99.3, 89.0–99.6, 87.1–99.3, 91.8–100.0, and 91.1–99.5 % (Table 3), the highest identity existed in ORF6.

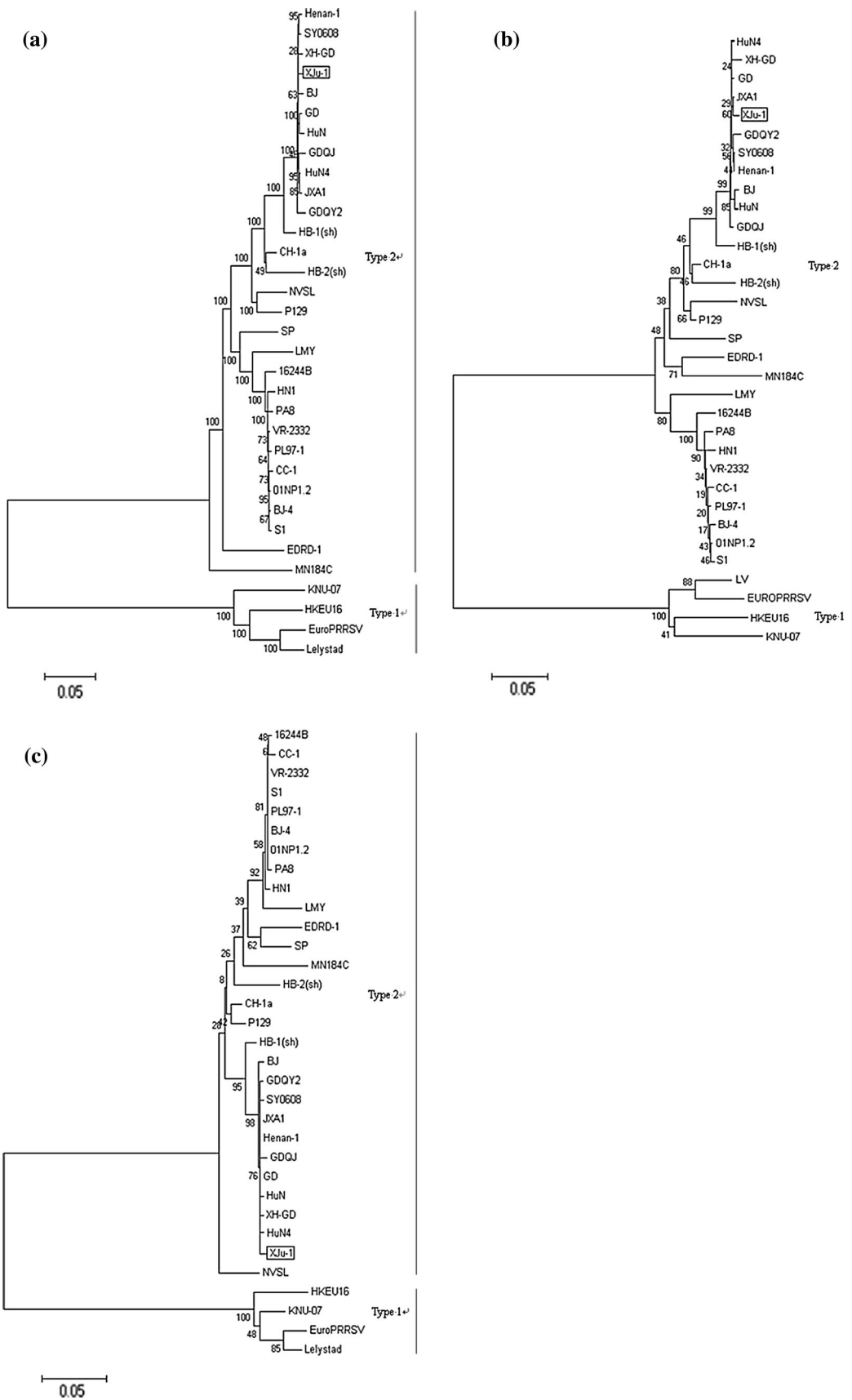
The 189-nt 5'UTR of XJu-1 had a 91.5 % nucleotide identity with the type 2 strain VR-2332, and 90.5–99.5 % with other type 2 strains, but only 62.8 % identity with the LV strain. The 3'UTR of XJu-1 was determined to be 150-nt long,

followed by a 27-nt poly(A) tail. The sequence comparison of 3'UTR reveals that XJu-1 displayed 88.9–100.0 % identity with other type 2 isolates and 72.6 % with LV strain.

The XJu-1 ORF1a encodes a 2354 amino acid polyprotein, and this polyprotein is predicted to be cleaved into nine small final products composed of NSP1 $\alpha$ , NSP1 $\beta$ , and NSP2–NSP8. NSP2 of XJu-1 displayed the lowest identity (72.7–97.1 %) to type 2 strains, shared 97.1 % amino acid identity both with HuN4 and Sy0608, shared 72.7, 74.1, and 75.7 % with EDRD-1, Lmy, and VR-2332, respectively, but only 29.2 % with LV (Table 3). Although a unique noncontiguous 30-amino acid deletion in NSP2 has been reported [13, 14], multiple alignment based on NSP2 deduced aa sequence showed that XJu-1 has three discontinuous 150 aa deletion in NSP2 region with deletion size consisting of 1, 29, and 120 amino acids, corresponding to the amino acid positions in PRRSV strain VR-2332 NSP2 of 481, 533–561, and 628–747, respectively (Fig. 1), this is the first finding of additional 120 amino acid deletion in this coding region (Fig. 1). Consequently, probably due to the deletions, NSP2 of XJu-1 shared the lowest identity with type 2 isolates (Table 3).

ORFs 2a to 7 occupy the 3' one-third of the genome and encode the PRRSV structural proteins. Nucleotide sequence comparison of this region indicated that XJu-1 shared 87.1–100 % identity with the type 2 strains, only shared 63.8–69.5 % identity with LV strain. Comparison of the amino acid sequence of the XJu-1 structural proteins with

**Fig. 2** Phylogenetic analysis based on nucleotide sequences of the full-length genome (a), ORF5 (b), or ORF7 (c) of 33 fully sequenced PRRSV strains



other isolates revealed that XJu-1 displayed 86.6–99.2 % identity to VR-2332, 97.5–100 % identity to the Chinese strains, and 85.1–97.1 % to the strains isolated in South Korea, Japan. In contrast, the LV strain only shared 57.1–80.5 % identity with XJu-1 (Table 3).

### Phylogenetic analysis

Phylogenetic trees were produced based on the nucleotide sequences of full-length genome, ORF5, and ORF7 nucleotide sequences as shown in Fig. 2a–c, respectively. Phylogenetic analysis showed that the PRRSV divided into two major branches, all phylogenetic trees exhibited that XJu-1 strain belonged to PRRSV type 2. Besides, the result indicated a high similarity to the virus strains isolated in China (Fig. 2).

### Discussion

PRRS is one of the pandemic diseases in major pig producing areas. In China, PRRS was first reported in 1996. As mid-July of 2007, the disease has been reported in 20 provinces, and more than 20,000 pigs died from the disease in a 2-weeks period, which has caused huge economic losses to the pig industry [15]. In our study, we determined the complete nucleotide sequence of XJu-1 and compared with other representative PRRSV strains isolated in China and other countries include type 1 and type 2 isolates. Phylogenetic analysis showed that a high degree of genetic homology was observed among most of the Chinese isolates.

NSP2 has become one of the vital regions for monitoring the evolution of PRRSV and for molecular epidemiology research on PRRSV [16]. Because NSP2 gene exhibits the highest genetic diversity in the viral genome, which can tolerate insertions, deletions, and mutations [17]. The polymorphisms of considerable sizes, including a 108-base insertion [18] and various 3–333 base deletions [13, 14, 17, 19–23]. For example, an unparalleled large-scale PRRS outbreak in China in 2006 was caused by highly virulent PRRSV strain which contains a noncontiguous 30-amino acid deletion in the NSP2 coding region. However, recent study showed that such deletion was not related with the virulence of PRRSV [24]. Interestingly, our study observed the first finding of an additional 120-amino acid deletion in this region, and it is the longest deletion (150aa) in NSP2 so far. This novel deletion within NSP2 of XJu-1 was genetically quite distinct and consequently caused the high level of identity to other type 2 isolates at the amino acid level. However, whether such deletion is related with the virulence depends on further

study, because the virulence of PRRSV is considered to be associated with multiple factors.

GP3 is the most glycosylated protein with seven NGSs that have been well preserved among PRRSV strains. In our study, alignment analysis of ORF3 deduced aa sequences indicates two aa substitution ( $F^{143} \rightarrow L^{143}$ ,  $T^{225} \rightarrow A^{225}$ ) in XJu-1. However, these changes did not result in new emerged NGS.

GP5 is a glycosylated transmembrane protein, responsible for the attachment of the virus to the host cell and contains important immunological domains associated with virus neutralization [25]. Comparative studies have showed that the GP5 is the most heterogeneous structural protein, and most of the amino acid substitutions observed in GP5 are distributed in the highly variable region between amino acids 26 and 39 [10]. However, in GP5 amino acid sequence of XJu-1 compared with PRRSV strains in China, three mutations ( $F^{23} \rightarrow S^{23}$ ,  $G^{80} \rightarrow V^{80}$ ,  $R^{151} \rightarrow K^{151}$ ) are observed.

The ORF7, encoding nucleocapsid (N) protein, is most consistent in nucleotide sequence between different strains of PRRSV [26], and has been presumed as the most useful marker for revealing the genetic relationships of PRRSV [27]. Alignment analysis of ORF7 deduced aa sequences indicates one aa substitution ( $E^{51} \rightarrow G^{51}$ ) in XJu-1.

In summary, the full genome of the type 2 PRRSV strain XJu-1 was sequenced and analyzed. The results showed that the genome of XJu-1 shared a high level of similarity in comparison to china isolates. Interestingly, XJu-1 with 150 aa deletion in NSP2 region is the longest deletion (150aa) in NSP2 so far, whether such deletion is related with the virulence depends on further study.

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