Full genome sequence analysis of a 1-7-4-like PRRSV strain in Fujian Province, China

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ABSTRACT

30 PRRS virus (PRRSV) has undergone rapid evolution and resulted in immense economic losses in

31 worldwide. In the present study, a PRRSV strain named FJ0908 causing high abortion rate (25%)

32 and mortality (40%) was detected in a swine herd in China. To determine if a new PRRSV

33 genotype had emerged, we characterized the genetic characteristics of FJ0908. Phylogenetic

34 analysis indicated that FJ0908 was related to 1-7-4-like strains circulating in United States since

35 2014. Furthermore, the ORF5 sequence restriction fragment length polymorphism (RFLP) pattern

36 of FJ0908 was 1-7-4. Additionally, FJ0908 had a 100 aa deletion (aa329–428) within nsp2, as

37 compared to VR-2332, and the deletion pattern was consistent with most of 1-7-4 PRRSVs.

38 Collectively, the data of this study contribute to the understanding of 1-7-4-like PRRSV molecular

39 epidemiology in China.

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INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is a global viral swine disease and causing severe economic loss in the global pig industry (*Neumann et al., 2005; Zhou and Yang 2010; Holtkamp et al., 2013; Gao et al., 2017*). PRRS virus (PRRSV), the causative agent of PRRS, is a small enveloped virus with positive-sense single-stranded RNA virus belonging to the Arteriviridae family in the Nidovirales order (*Benfield et al., 1992; Meulenberg et al., 2000*).

The PRRSV genome is about 15 kb in length and contains ten open reading frames (ORFs), ORF1a, 1b, 2a, 2b, 3, 4, 5a, 5, 6 and 7. ORF1a and ORF1b encode at least 16 non-structural proteins (nsps) (Nsp1α, Nsp1β, Nsp2-6, Nsp2TF, Nsp2N, Nsp7a, Nsp7b and Nsp8-12), while ORF2-ORF7 encode viral structural proteins: GP2a, E (2b), GP3, GP4, GP5a, GP5, M, and N (*Meulenberg et al., 2000; Wu et al., 2005; Firth et al., 2010; Fang and Snijder 2010; Fang et al., 2012*).

PRRSV is characterized by extensive genetic variation. Based on global PRRSV classification systems, type 2 PRRSV strains in China can be classified into lineage 1 (NADC30-like), lineage 3 (QYYZ-like), lineage 5.1 (VR2332-like), and lineage 8.7 (JXA1-like) (*Gao et al., 2017; Liu et al., 2017b; Guo et al., 2018*). Fujian Province, one of the largest livestock trading areas in China, herd movements across provinces and national borders are also common. Hence, multiple PRRSV types co-exist in swine herds. Recently, PRRSV isolates of the ORF5 restriction fragment length polymorphism (RFLP) 1-7-4 viruses emerged in America, causing dramatic abortion "storms" in the sow herd (*Alkhamis et al., 2016; van Geelen et al., 2018*). Here, we report a genetic and phylogenetic analysis of PRRSV isolate FJ0908 belong to the ORF5 1-7-4 viruses in Fujian Province, China.

MATERIALS AND METHODS

Clinical case

77 In September 2018, severe reproductive and respiratory disease was observed in piglets in a farm.

78 The affected pigs had respiratory distress, and high abortion rate (25%) and mortality (40%).

79 **Strain identification and nucleotide sequencing**

80 PRRSV infection was confirmed by Real-time RT-PCR testing of the serum of affected pigs

81 according to the manufacturer's instructions. The ORF5 sequence RFLP pattern was inferred

82 according to Wesley et al. The complete genomic sequences of FJ0908 were amplified as described

83 previously (*Zhang et al., 2018*). The PCR products were purified and cloned into pGEM-T Easy

84 according to the manufacturer's instructions (Promega, Madison, WI, USA) and three recombinant

85 clones of every fragment were sequenced by Ruibo Life Technologies Corporation (Beijing,

86 China).

87 **Complete genomic sequence analysis**

88 Fifty-four representative type 2PRRSV sequences in GenBank were utilized in phylogenetic

89 analyses (Table 1). Multiplex sequence alignments were performed using CLUSTAL X (version

90 1.83) and the phylogenetic relationships were assessed by MEGA 6.0 as described *(Liu et al.,*

91 *2017b)*. The ORF5 sequences were classified according to the global PRRSV classification

92 systems (*Shi et al., 2010*).

93   Recombination events were detected using Simplot v 3.5.1 and the boot scanning analysis

94 was performed with a 200-bp window, sliding along the genome alignments with a step size of 20

95 bp.

96 **RESULTS**

97 **Complete genomic sequence analysis**

98 The genomes of FJ0908 (GenBank accession no. MK202794) was 15112 nt in length, excluding

99 the poly (A) tail at the 3′end, 300 nt shorter than the genome of the prototypic VR2332. Genome

100 alignments revealed that FJ0908 shared 83.6% identity with JXA1, 84.7% with VR2332, 82.2 %

101 with QYYZ, 86.3% with NADC30, 97.3–97.6% with 1-7-4 PRRSV family (IA/2014/NADC34,

102 IA/2015/NADC35) and 98.7% with LNWK130 (Table 2).

103   The results also showed that 5′-UTR, ORF1a, ORF1b, ORFs 2-7 and the 3′-UTR of FJ0908

104   shared 97.2–98.2% nucleotide homology with 1-7-4 PRRSV family (IA/2014/NADC34,

105   IA/2015/NADC35), which was higher than the homology shared with other representative strains,

106   indicating that FJ0908 strain belonged to 1-7-4 PRRSV. ORF1a and ORF1b encode 16 nsps of

107   PRRSV, Nsp1β and Nsp2 are the most variable protein products among these nsps (Table 2). ORFs

108   2 to 7 encode the PRRSV structural proteins, among these structural proteins, GP2, GP3, GP4,

109   GP5a and GP5 exhibited the most variance (Table 2).

110   **Amino acid analysis of Nsp2**

111   Nsp2 contains different deletions and insertions, as compared to VR2332 and is the most variable

112   protein in PRRSV genome (*Fang et al., 2011; Li et al., 2011*). Strikingly, the nsp2 gene of the

113   FJ0908 was 2640 nt in length and encoded 880 aa, with a 100 aa deletion (aa329–428) within nsp2,

114   as compared to VR2332, and the deletion pattern was consistent with most of 1-7-4 PRRSVs.

115   **Antigenic analysis of GP2-GP5**

116   The antigenic regions (ARs) and glycosylation sites within the GP2, GP3, GP4, and GP5 proteins

117   of FJ0908 were predicted and compared to OH/2014/ISU-7 IA/2014/ISU-8, IA/2014/NADC34,

118   IA/2015/NADC35, IA/2015/NADC36, LNWK96, LNWK130, NV-21, NV-25, VR2332, CH-1a,

119   JXA1 and NADC30.

120   In GP2, two antigenic regions (AR 41−55 and AR123−135) were confirmed in type 2 PRRSV

121   (*de Lima et al., 2006*). The predicted AR at aa 41−55 were highly conserved and no aa substitution

122   was detected in AR123−135. In GP3, four predicted antigenic regions (AR32−46, AR 51−105, AR

123   111−125, and AR 137−159) were proven (*de Lima et al., 2006; Zhou et al., 2006; Wang et al.,*

124   *2014*). The AR comprising aa32−46, aa51−105, aa111−125, and aa137−159 of FJ0908 was most

125   similar to 1-7-4 representative PRRSVs including LNWK130, but differed from VR2332, CH-1a,

126   JXA1 and NADC30. GP4 has one predicted AR at aa51−65 (*de Lima et al., 2006*). FJ0908 had 1-2

127   aa substitutions as compared to the 1-7-4 representative PRRSVs, but had 2-6 aa substitutions as

128   compared to VR2332, CH-1a, JXA1 and NADC30 and LNWK96. Additionally, no aa substitution

129   was detected in AR51−65 between FJ0908 and LNWK130. The putative glycosylation sites in

130 GP2-4 were completely conserved among the investigated strains, except for GP2 of isolate

131 IA/2015/NADC36 that had one substitution $N^{184}D$.

132   GP5 is the major envelope protein encoded by ORF5 and is the most variable PRRSV protein.

133 Sequences alignments of GP5 revealed that FJ0908 shares 87.0%, 86.0-86.5%, 85.5%, 88.0-90.5%

134 and 97.5% amino acid identity with VR-2332-like (VR2332 and BJ-4), JXA1-like (JXA1 and

135 HuN4), QYYZ-like (FJFS and QYYZ), NADC30-like strains (NADC30 and ISU30), and 1-7-4-

136 like (IA/2014/NADC34, IA/2015/NADC35), respectively (Table 2). Furthermore, the restriction

137 sites analysis showed that the ORF5 RFLP of FJ0908 has the same 1-7-4 pattern [1 (MluI = 0

138 sites), 7 (HindII =nt 88, 219, 360), 4 (SacII = nt 24, 555)] (Figure S1).

139   GP5 has six ARs (AR1–15, AR27–35, AR37–51, AR149–156, AR166–181, and AR192–

140 200) (*de Lima et al., 2006; Zhouet al., 2009*). In GP5, the N-terminus ARs (AR1-15 and AR27-

141 35) were very variable among all strains and only three aa substitutions (K^4N, $Q^{13}R$ and $L^{15}P$) were

142 found in the two antigenic regions between FJ0908 and LNWK130, whereas the other four ARs

143 (AR 37–51, AR 149–156 , AR 166–181, and AR 192–200) were conserved. GP5 had differential

144 predicted N-glycosylation among PRRSV strains. FJ0908 possessed five predicted sites (N32,

145 N44, N51, N57 and N59). Although N-glycosylation at N57 was not a novel finding, N57 was

146 detected in most of the 1-7-4 sequences. The results also revealed that FJ0908 had the same N-

147 glycosylation pattern as LNWK96 and LNWK130.

148 **Phylogenetic analysis**

149 PRRSV ORF5 is the most variable and has been used as a marker of PRRSV genetic variability.

150 Based on global PRRSV classification systems, type 2 PRRSV was divided into nine monophyletic

151 lineages (1-9) and lineage1 was further classified into 9 sublineages (1.1-1.9) (*Shi et al., 2010*;

152 Guo *et al., 2017*). The ORF5-based phylogenetic tree showed that FJ0908, as well as 1-7-4 isolates

153 including LNWK130, were clustered in sublineage 1.5 (Fig. 1A). Whole genome phylogenetic

154 analysis also indicated that FJ0908 was most closely related to a genetic cluster in1-7-4-like

155 lineage 1 (Fig. 1B).

156 **Recombination analysis**

157   To test for possible recombinant events within FJ0908 strain, we performed recombinant detection

158   using SimPlot v3.5.1 software. From the similarity plot, two recombination breakpoints within the

159   FJ0908 genome were identified, which were located in Nsp1 (nt 760 and nt 1300) (Fig. 2A). To

160   further confirm the putative recombination events, phylogenetic trees for each of the sequence

161   regions identified during the analysis were generated, we identified two recombination breakpoints

162   located in nsp1 (nt 760 and nt 1300) (Fig. 2B, Fig. 2C). The two breakpoints separated the genome

163   of FJ0908 into 3 regions. For FJ0908, the region between the breakpoints (nt 760–1300) is closely

164   related to ISU30 strain, the two regions between the breakpoints (nt 1–759 and nt 1301–15534)

165   are closely related to IA/2014/NADC34. Collectively, the above results suggested that FJ0908

166   derived from recombination between IA/2014/NADC34 and ISU30 (Fig. 2). Moreover,

167   LNWK130 strain (1-7-4-like PRRSV) firstly identified in Liaoning Province, China was also

168   deriving from the recombination of 1-7-4 isolates and ISU30 (*Zhang et al., 2018*). However, the

169   recombination pattern was different between LNWK130 and FJ0908. For LNWK130, the two

170   breakpoints (nt 760–1300 and nt 1–759) are closely related to ISU30 strain, one breakpoints (nt

171   1301–15534) is closely related to IA/2014/NADC34. Additionally, sequences alignments of the

172   recombination region (nt 1-759) revealed that FJ0908 shares 98.8%, 94.8% and 92.6% nucleotide

173   identity with IA/2014/NADC34, ISU30 and NADC30, respectively, in contrast, LNWK130 shares

174   95.8%, 95.9% and 93.1% nucleotide identity with IA/2014/NADC34, ISU30, and NADC30,

175   respectively. Sequences alignments of the recombination region (nt 760-1300) revealed that

176   FJ0908 shares 92.8%, 94.3% and 82.0% nucleotide identity with IA/2014/NADC34, ISU30 and

177   NADC30, respectively, similarly, LNWK130 shares 89.4%, 93.0% and 80.2% nucleotide identity

178   with IA/2014/NADC34, ISU30, and NADC30, respectively. For all of the nsp sequences and

179   structural proteins, the most variable regions were found in nsp1β, nsp3, nsp5, nsp6, nsp8, GP3

180   and GP5 between FJ0908 and LNWK130.

181   **DISCUSSION**

182   PRRSV causes major economic losses in swine industry since 1990s. Notably, PRRSV continues

183   to expand its genetic diversity. According to Shi et al., 2010, type 2 PRRSV was classified into

184   nine monophyletic lineages based on ORF5 and extensive genetic variation exists among strains

185   within each lineage. It is hard to define the PRRSV homologous, heterologous virus and

186   pathogenic biotype only focused on single gene analysis. To classify and infer the likely

187   pathogenic biotype, RFLP patterns of ORF5 for type 2 strains is standard approach for

188   veterinarians (*Wesley et al., 1998*). The RFLP pattern 1-7-4 emerged in the US and has become

189   prevalent since 2014, this nomenclature has been associated with severe disease in herds leading

190   to significant economic losses (*van Geelen et al., 2018*). In the present study, FJ0908 was isolated

191   in a farm with high abortion rate and mortality in sows, the restriction sites analysis revealed that

192   the ORF5 RFLP of FJ0908 has the 1-7-4 pattern.

193   Comparison to PRRS sequences in GenBank indicated FJ0908 belonged to 1-7-4-like

194   PRRSV. The genomic regions with the highest variation were found in Nsp1β, Nsp2, ORF2,

195   ORF3, ORF4, ORF5a and ORF5, the lowest variation were found in Nsp1α, Nsp8–12, and ORF6

196   (Table 2). FJ0908 had 100aa deletions within Nsp2 (corresponding to position 328–427 of VR2332

197   nsp2), as compared to the reference strain VR2332, and the deletion pattern was consistent with

198   1-7-4 viruses.

199   The pathogenesis of PRRSV has been linked to the N-glycosylation motifs at certain sites of

200   GP2-GP5 by acting as a glycan shield against minimizing the viral neutralizing antibody response

201   (*Wissink et al., 2004; Ansari et al., 2006; Faaberg et al., 2006; Das et al., 2011; Delisle et al.,*

202   *2012; Wei et al., 2012*). Many reports have also suggested that N-glycosylation motifs in GP5 of

203   PRRSV is important for viral infectivity and viral immune evasion *(Wissink et al., 2004; Ansari*

204   *et al., 2006; Jianget al., 2007; Delisle et al., 2012; Wei et al., 2012)*. In the present study, GP5

205   contained five predicted N-glycan motifs in FJ0908: N32, N44, N51, N57 and N59. More

206   interestingly, the Chinese strains LNWK96, LNWK130 and FJ0908 have an additional N-glycan

207   at 59 compared to 1-7-4 isolates in the United States.

208   Recombination may play an important mechanism in generating genetic diversity in PRRSV

209   (*Liu et al., 2011; Murtaugh et al., 2010*). Most of the 1-7-4 PRRSV isolates may be most

210   potentially derived from different recombination patterns occurring among the local strains in the

211 United States (van Geelen et al., 2018). Recently, 1-7-4-like PRRSV strains, LNWK130 isolated

212 in Liaoning Province, China was reported to originate from recombination events between

213 IA/2014/NADC34 and ISU30. Currently, recombination events involving NADC30-like PRRSV

214 strains and other PRRSV strains frequently occurred in China (*Zhao et al., 2015; Zhang et al.,*

215 *2016; Bian et al., 2017; Liu et al., 2017a; Liu et al., 2017c; Zhao et al., 2017;Wang et al., 2018;*

216 *Zhou et al., 2018; Liu et al., 2019*). To test for possible recombinant events within FJ0908 strain,

217 we performed recombinant detection using SimPlot v3.5.1 software. Recombination analysis

218 performed with the available full-length genome sequences revealed FJ0908 maybe originate from

219 recombination events between IA/2014/NADC34 and ISU30. Although FJ0908 and LNWK130

220 maybe drive from recombination events between IA/2014/NADC34 and ISU30, the recombination

221 pattern of two strains were different. Two recombination breakpoints were identified in nsp1 (nt

222 760 and nt 1300) in FJ0908 strain, whereas one recombination breakpoint in nsp2 (nt 1480) in

223 LNWK130, suggesting the ancestor of FJ0908 and LNWK130 were most probably transported

224 from different region of United States.

225   In conclusion, 1-7-4-like PRRSV was also detected in Fujian Province of China besides

226 Liaoning Province. Therefore, effective strategy should be taken to control 1-7-4-like PRRSV and

227 to monitor herd movements.

228 **CONCLUSION**

229   In summary, we thoroughly analyzed a new sublineage of PRRSV strain FJ0908 isolated

230 from Fujian Province, China on the basis of a comprehensive study with the full-length genome.

231 These novel sublineage 1.5 virus is closely related to the ORF5 RFLP 1-7-4 strains. Phylogenetic

232 and molecular evolutionary analyses indicated that FJ0908 originated from a natural

233 recombination event between IA/2014/NADC34 and ISU30. Our data enhance our understanding

234 of the PRRSV evolution in China.

235 **Data Availability**

236 The following information was supplied regarding data availability.

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372

373   **Figure legends**

374   **Figure. 1 Phylogenetic tree based on the ORF5 genes (A) and full length (B) of the FJ0908**

375   **and reference viruses.** Reliability of the tree was assessed by bootstrap analysis of 1000

376   replications. Our representative isolate FJ0908 were marked with the red triangle (▲). Lineage 1

377   PRRSVs are divided into nine sublineages.

378

379   **Figure. 2 Characterization of the supported recombinant events between FJL0908 and**

380   **representative PRRSV lineages.** (A) Similarity plot and bootscan analyses of FJ0908 by SimPlot.

381   The y-axis indicates the percentage similarity between the parental sequences and the query

382   sequence. Phylogenetic trees based on major parental regions (nt1-759 and nt 1301-15534) (B)

383   and the minor parental region (nt760-1300) (C). The major parental group (1-7-4 viruses, reference

384   strain IA/2014/NADC34) is shown in red, while the minor parental groups (reference strain

385   ISU30) are shown in blue, respectively.

Figure 1

Phylogenetic tree based on the ORF5 genes (A) and full length (B) of the FJ0908 and reference viruses.

Reliability of the tree was assessed by bootstrap analysis of 1000 replications. Our representative isolate FJ0908 were marked with the red triangle (▲). Lineage 1 PRRSVs are divided into nine sublineages.

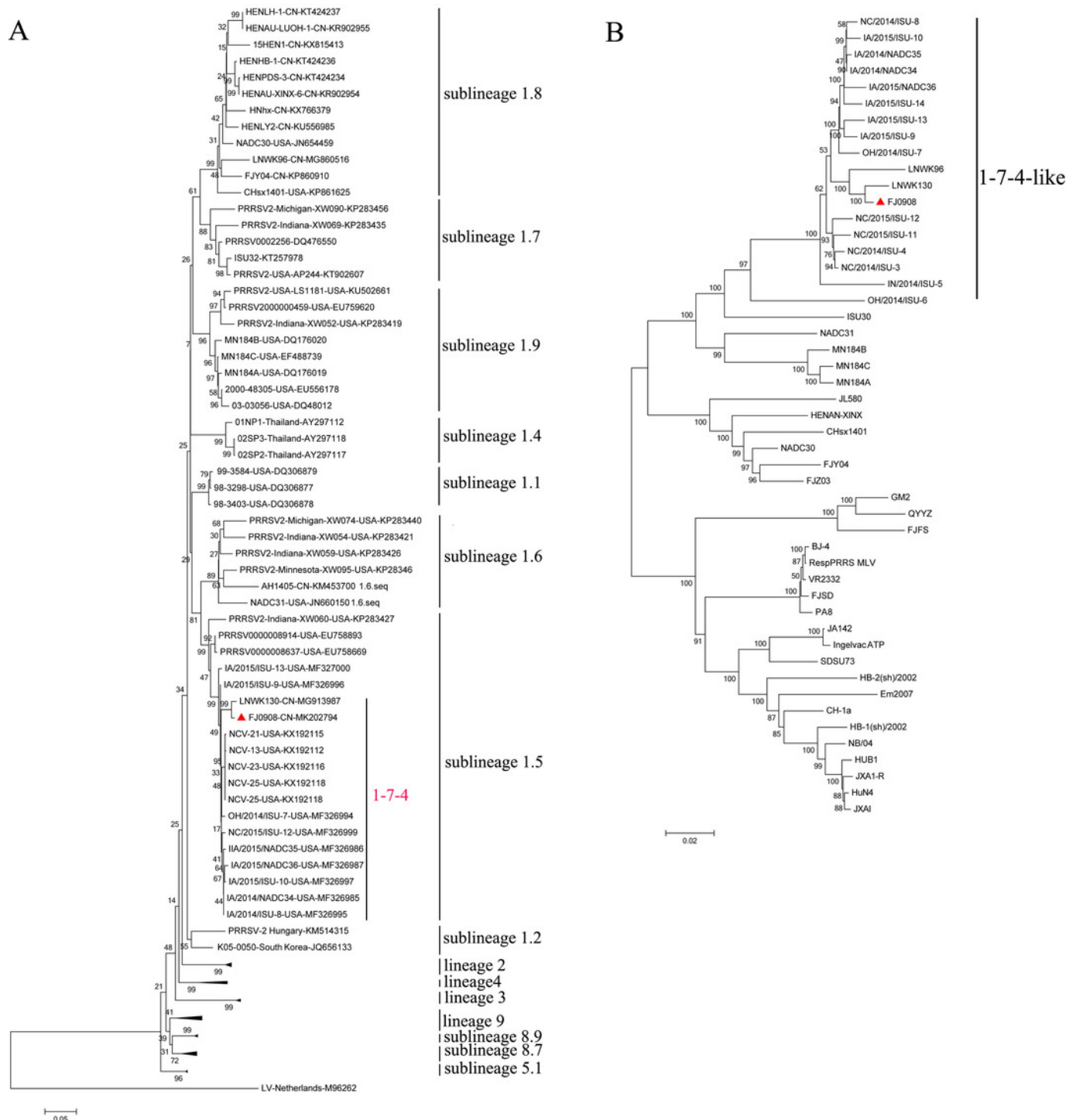



Figure 2

Characterization of the supported recombinant events between FJL0908 and representative PRRSV lineages.

(A) Similarity plot and bootscan analyses of FJ0908 by SimPlot. The y-axis indicates the percentage similarity between the parental sequences and the query sequence. Phylogenetic trees based on major parental regions (nt 1-760 and nt 1301-15534) (B) and the minor parental region (nt 760-1300) (C). The major parental group (1-7-4 viruses, reference strain IA/2014/NADC34) is shown in red, while the minor parental groups (reference strain ISU30) are shown in blue, respectively.

A

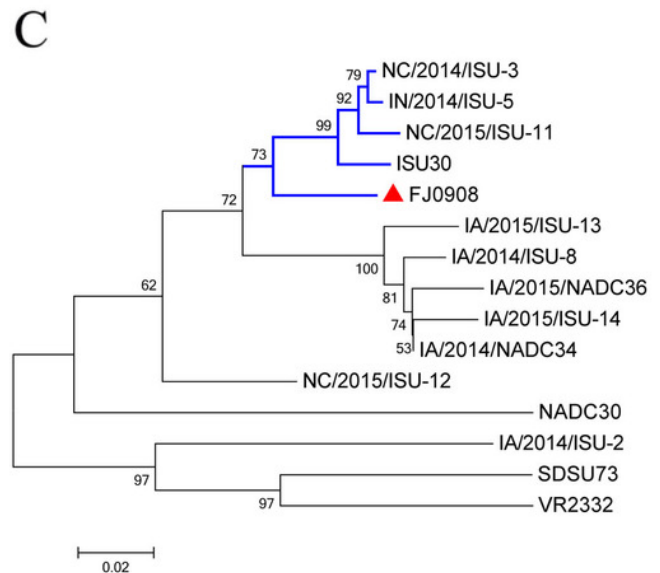
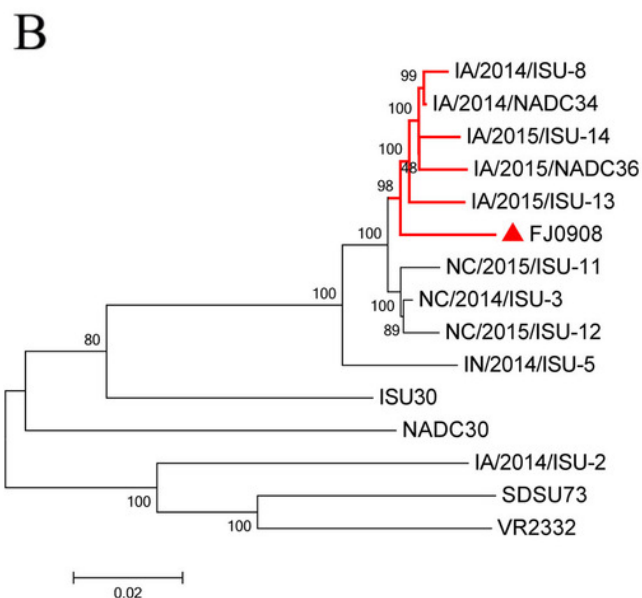
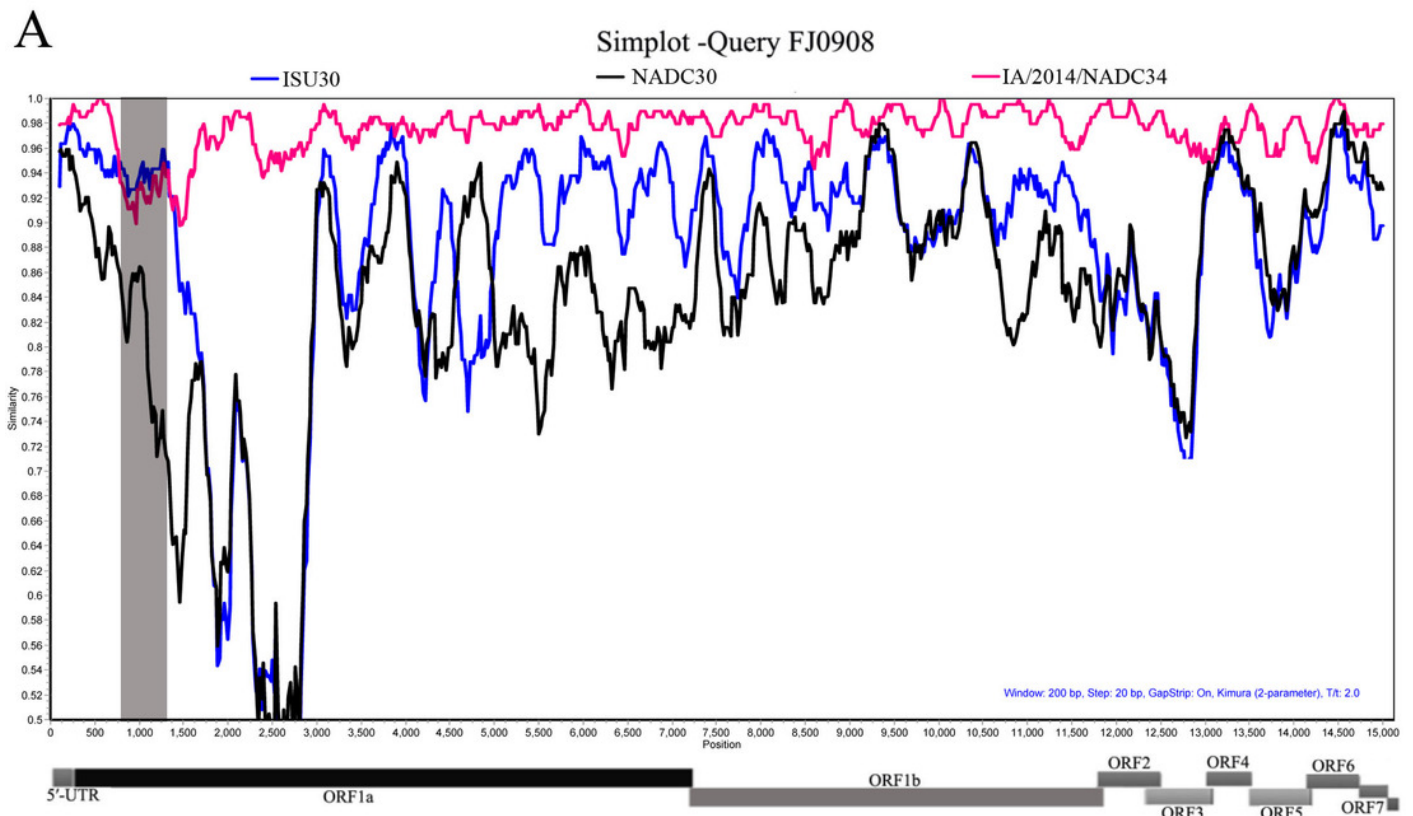
Simplot -Query FJ0908

— ISU30   — NADC30   — IA/2014/NADC34

Window: 200 bp, Step: 20 bp, GapStrip: On, Kimura (2-parameter), T/t: 2.0

5'-UTR   ORF1a   ORF1b   ORF2   ORF3   ORF4   ORF5   ORF6   ORF7

B

C

PRRSV strains used in this study.

1   Table 1 PRRSV strains used in this study.

No.	Name	GenBank accession no.	Origin	No.	Name	GenBank accession no.	Origin
1	FJ0908	MK202794	China	29	SDSU73	JN654458	U.S.A
2	FJZ03	KP860909	China	30	NADC31	JN660150	U.S.A
3	FJY04	KP860910	China	31	NB/04	FJ536165	China
4	CH-1a	AY032626	China	32	BJ-4	AF331831	China
5	JA142	AY424271	U.S.A	33	MN184A	DQ176019	U.S.A
6	VR-2332	U87392	U.S.A	34	MN184B	DQ176020	U.S.A
7	JXA1-R	R FJ548855	China	35	HENNAN-XINX	KF611905	China
8	JXA1	EF112445	China	36	NADC30	JN654459	U.S.A
9	FJFS	KP998476	China	37	JL580	KR706343	China
10	PA8	AF176348	Canadia	38	CHsx1401	KP861625	China
11	Em2007	EU262603	China	39	ISU30	KT257977	U.S.A
12	GM2	JN662424	China	40	MN184C	EF488739	U.S.A
13	QYYZ	JQ308798	China	41	NC/2014/ISU-3	MF326990	U.S.A
14	HB-1(sh)/2002	AY150312	China	42	LV	M96262	Netherlands
15	HB-2(sh)/2002	AY262352	China	43	NC/2014/ISU-4	MF326991	U.S.A
16	HUN4	EF635006	China	44	IN/2014/ISU-5	MF326992	U.S.A
17	OH/2014/ISU-6	MF326993	U.S.A	45	Ingelvac ATP	DQ988080	U.S.A
18	NC/2015/ISU-11	MF326998	U.S.A	46	RespPRRS MLV	AF066183	U.S.A
19.	IA/2015/ISU-13	MF327000	U.S.A	47	NC/2015/ISU-12	MF326999	U.S.A
20	LNWK130	MG913987	China	48	IA/2015/NADC35	MF326986	U.S.A
21	NCV-13	KX192112	U.S.A	49	IA/2015/NADC36	MF326987	U.S.A
22	NCV-23	KX192116	U.S.A	50	IA/2015/ISU-10	MF326997	U.S.A
23	NCV-25	KX192118	U.S.A	51	IA/2014/NADC34	MF326985	U.S.A
24	OH/2014/ISU-7	MF326994	U.S.A	52	LNWK96-CN	MG860516	China
25	IA/2014/ISU-8	MF326995	U.S.A	53	NCV-21	KX192115	U.S.A
26	IA/2015/NADC36	MF326987	U.S.A	54	IA/2015/ISU-9	MF326996	U.S.A
27	FJSD	KP998474	China	55	HUB1	EF075945	China
28	IA/2014/ISU-2	MF326989	U.S.A	56	IA/2015/ISU-14	MF327001	U.S.A

Detailed comparison of the full-length genomes of FJ0908 to other PRRSV reference strains.

1 Table2 Detailed comparison of the full-length genomes of FJ0908 to other PRRSV reference strains.

	VR2332	BJ-4	JXA1	HuN4	FJFS	QYYZ	NADC30	ISU30	LNWK96	IA/2014/NADC34	IA/2015/NADC35	LNWK130
	Sublineage 5.1		Sublineage 8.7		Lineage 3			Sublineage 1.8			Sublineage 1.5	
	Pairwise % Identity to FJ0908 (nt/aa)											
Nucleotides												
Complete genome	84.7	84.6	83.6	83.6	82.2	82.2	86.3	89.4	96.2	97.6	97.5	98.7
5' UTR	93.6	93.6	92.5	92.5	91.0	91.5	95.7	92.9	96.3	97.9	97.3	97.9
ORF1a	82.0	81.9	80.6	80.7	78.6	78.6	82.8	87.8	96.3	97.2	97.2	98.0
ORF1b	86.6	86.6	86.2	86.2	85.8	85.6	88.8	86.2	98.2	98.2	98.1	99.2
ORF2-7	87.2	87.1	85.9	86.0	84.9	84.9	89.4	88.3	93.1	97.6	97.2	99.6
3' UTR	92.7	92.7	90.0	90.1	86.1	88.7	95.4	91.5	95.9	98.0	98.0	98.0
nt 1-760	90.0	89.9	88.1	88.4	86.7	89.1	92.6	94.8	95.4	98.8	98.6	97.1
nt 760-1300	80.4	80.4	80.6	80.6	78.3	77.2	82.0	94.3	93.9	92.8	9.26	94.4
Amino acids												
NSP1α	94.6	95.2	97.0	97.0	93.4	96.4	95.8	96.4	97.0	98.8	98.8	98.2
NSP1β	78.8	78.8	77.4	77.9	78.3	77.0	79.7	91.7	94.0	91.7	91.7	94.5
NSP2	67.0	67.0	66.5	66.7	63.3	64.6	71.4	72.1	92.7	94.5	94.7	96.4
NSP3	91.3	91.3	89.9	90.1	87.7	87.7	91.7	91.9	97.5	97.8	97.5	98.7
NSP4	93.1	93.1	95.1	95.1	92.6	93.1	92.6	96.6	98.5	99.5	99.5	99.0
NSP5	88.8	88.8	89.4	89.4	90.0	89.4	87.6	94.7	97.6	98.8	98.8	98.8
NSP6	87.5	87.5	93.8	93.8	93.8	93.8	87.5	87.5	93.8	93.8	93.8	93.8
NSP7	90.3	89.2	87.3	87.3	86.5	84.6	89.2	95.4	98.8	98.8	98.8	99.2
NSP8	93.3	93.3	93.3	93.3	88.9	93.3	95.6	100	100	100	100	97.8
NSP9	95.8	95.5	96.1	95.8	93.9	94.8	95.8	97.2	98.3	98.9	98.9	99.1
NSP10	95.0	95.0	94.6	95.2	92.3	94.1	98.4	98.2	99.8	99.8	99.5	99.5
NSP11	95.1	95.5	96.0	96.0	94.6	94.6	95.1	94.6	100	99.6	99.6	100
NSP12	90.3	90.2	90.9	90.9	92.2	91.5	89.0	98.0	99.3	98.7	98.7	100
ORF2a/GP2	85.5	86.3	85.9	85.5	80.1	82.8	83.6	84.0	97.7	98.4	96.9	99.6
ORF2b/E	87.8	89.0	86.3	86.3	80.8	91.8	90.4	90.4	97.3	100	98.6	98.6

ORF3/GP3	80.7	80.7	79.9	79.5	80.7	79.9	81.5	81.9	89.4	94.5	93.3	99.2
ORF4/GP4	86.0	84.8	86.5	88.8	88.8	86.5	94.4	92.1	91.6	94.4	94.4	99.4
ORF5/GP5	87.0	87.0	86.0	86.5	85.5	85.5	90.5	88.0	90.0	97.5	97.5	98.5
ORF5a	91.3	91.3	84.8	84.8	84.8	87.0	95.7	93.5	95.7	100	100	97.8
ORF6/M	95.4	94.8	93.7	93.7	95.4	94.8	94.8	93.7	95.4	97.7	97.7	100
ORF7/N	91.1	90.2	90.2	90.2	87.8	87.8	95.9	93.5	9.19	96.7	95.1	99.2