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Short communication

Whole genome characterization of a novel porcine reproductive and respiratory syndrome virus 1 isolate: Genetic evidence for recombination between Amervac vaccine and circulating strains in mainland China

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ABSTRACT

Genotype 1 porcine reproductive and respiratory syndrome virus (PRRSV 1) have been continuously isolated in China in recent years. Complete genome sequences of these isolates are important to investigate the prevalence and evolution of Chinese PRRSV 1. Herein, we describe the isolation of a novel PRRSV 1 isolate, denominated HLJB1, in the Heilongjiang province of China. Complete genome sequencing of HLJB1 showed that it shares 90.66% and 58.21% nucleotide identities with PRRSV 1 and 2 prototypic strains Lelystad virus and ATCC VR-2332, respectively. HLJB1 has a unique 5-amino-acid insertion in nsp2, which has never been described in other PRRSV 1 isolates. Whole genome-based phylogenetic analysis revealed that all Chinese PRRSV 1 isolates are clustered in pan-European subtype 1 and can be divided into four subgroups. HLJB1 resides in the subgroup of BJEU06-1-like isolates but is also closely related to the Amervac-like isolates. Additionally, recombination analyses suggested that HLJB1 is a recombinant from the Amervac vaccine and the BJEU06-1 isolate. To our best knowledge, our results provide the first genetic evidence for recombination between Amervac vaccine and circulating strains. These findings are also beneficial for studying the origin and evolution of PRRSV 1 in China.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the most economically important swine virus worldwide. It is an enveloped, positive-sense single-strand RNA virus belonging to the family of *Arteriviridae* in the order of *Nidovirales* (Lunney et al., 2016). PRRSV genome is about 15 kb in length, containing a 5′ cap structure, 5′ untranslated region (UTR), at least 10 open reading frames (ORFs), 3′ UTR and a 3′ poly (A) tail. ORFs 1a and 1b encode at least 16 non-structural proteins (nsp1α, nsp1β, nsp2N, nsp2TF, nsp2-6, nsp7α, nsp7β, nsp8-12) that play critical roles in viral replication and transcription, while ORFs 2-7 encode eight structural proteins (GP2a, E, GP3, GP4, GP5, GP5a, M and N) to constitute an infectious virion (Fang et al., 2012; Firth et al., 2011; Johnson et al., 2011).

Due to the distinct genetic and antigenic diversity, PRRSV isolates are divided into two genotypes according to the taxonomy of 2016 International Committee on Taxonomy of Viruses (ICTV): PRRSV 1 and PRRSV 2. Although PRRS 2 viruses, including highly pathogenic PRRSV (HP-PRRSV) isolates and NADC30-like isolates, are predominant in China (Tian et al., 2007; Zhao et al., 2015; Zhou et al., 2015a), PRRSV 1 isolates also exist in China for more than ten years (Chen et al., 2011). Furthermore, increased numbers of PRRS 1 viruses have been isolated in China in recent years and no PRRS 1 MLV vaccine has been licensed in China yet, which cause more attention for PRRS 1 control in China (Li et al., 2014; Liu et al., 2017b; Wang et al., 2016; Zhou et al., 2013; Zhou et al., 2015b). Noticeably, recombination events between two PRRSV 1 MLV strains (Amervac (VP-046 Bis) and Porcilis (DV)) and between the Porcilis vaccine and a diverse field strain have been reported (Frossard et al., 2013; Renson et al., 2017). Here we describe the identification of a novel PRRSV 1 recombinant between Amervac vaccine and a PRRSV 1 circulating isolate in China.

2. Materials and methods

During the routine investigation of PRRSV prevalence in August 2014, a new PRRSV 1 was detected by using PRRSV differential RT-PCR assays (Chen et al., 2009) in the serum of a diseased pig from Heilongjiang province of China, which showed fever, reddened conjunctiva, and respiratory symptoms. No PRRSV 1 vaccine was used in this pig farm previously. The virus was isolated using primary porcine alveolar macrophages (PAMs) as we previously described (Chen et al., 2011). Briefly, the serum was inoculated on PAMs. The inoculated cells

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Table 1
Detailed complete genome comparison of HLJB1 to representative PRRSV 1 strains.

Region	Length[a]	Similarity to HLJB1 (%)					
		LV	Porcilis	Amervac	HKEU16	BJEU06-1	NMEU09-1
Nucleotides (nt)							
5′UTR	221	96.38	–[b]	97.74	94.12	95.02	94.57
ORF1a	7206	88.88	86.04	89.98	84.25	86.79	81.56
ORF1b	4392	92.05	91.80	92.78	88.64	90.00	86.86
ORFs 2-7	3189	92.29	91.66	93.23	88.02	88.87	86.45
3′UTR	114	94.74	–	97.37	95.61	92.98	95.61
Complete	15,113	90.66	88.35	91.64	86.51	88.31	84.39
Proteins (aa)							
Nsp1α	180	92.78	91.67	92.22	87.78	88.89	93.33
Nsp1β	205	80.98	77.56	79.02	78.54	85.37	74.63
Nsp2N	736	80.57	72.96	83.83	70.92	76.36	67.93
Nsp2TF	905	81.99	75.58	84.86	73.92	78.45	72.04
Nsp2	1083	84.95	79.96	87.90	78.02	81.90	76.64
Nsp3	230	94.78	93.48	96.96	94.35	93.48	93.91
Nsp4	203	95.57	95.57	98.52	92.12	92.12	86.70
Nsp5	170	95.88	95.88	95.88	92.35	95.29	88.82
Nsp6	16	100	100	100	93.75	100	100
Nsp7α	149	95.30	94.63	95.30	95.30	96.64	94.63
Nsp7β	120	93.33	94.17	93.33	94.17	95.83	90.00
Nsp8	45	93.33	93.33	91.11	91.11	95.56	93.33
Nsp9	685	97.66	97.66	98.10	96.64	97.08	95.91
Nsp10	442	97.29	97.29	97.51	93.89	96.38	93.67
Nsp11	224	98.21	97.77	98.21	95.98	97.32	95.98
Nsp12	152	96.05	95.39	96.05	94.08	93.42	92.11
GP2a	249	91.57	90.36	89.56	88.35	93.98	86.75
E	70	92.86	92.86	92.86	92.86	95.71	95.71
GP3	265	90.57	89.81	89.06	81.89	86.04	80.75
GP4	183	89.07	89.07	91.80	83.61	85.79	83.06
GP5	201	92.54	92.54	93.03	93.53	90.55	87.56
GP5a	43	95.35	93.02	93.02	93.02	90.70	95.35
M	173	97.11	97.69	97.69	94.22	94.80	94.22
N	128	93.75	93.75	93.75	89.84	87.50	91.41

[a] The length of each fragment/protein in HLJB1 genome. The cleavage products were determined based on previous description (Fang and Snijder, 2010; Fang et al., 2012; Lunney et al., 2016).
[b] "–" indicates that the complete sequences of 5′UTR and 3′UTR of Porcilis vaccine strain are not available.

were maintained at 37 °C in a 5% CO_2 atmosphere and monitored daily for cytopathic effects (CPE). Approximately 70% CPE was reached at 72 h post-inoculation and the resultant virus, denominated HLJB1, was harvested and stored at − 80 °C until use.

Total RNA was extracted from the cell culture using an RNeasy Mini Kit (Qiagen, Germany). The whole genome sequence was amplified by 16 pairs of primers producing 16 overlapped fragments that spanned the entire genome (Chen et al., 2011). The amplicons were purified with an E.Z.N.A. Gel Extraction Kit (Omega, USA) and cloned into pEASY-T1 Vector (Transgen, China). At least three recombinant clones were sequenced with an ABI Automatic DNA Sequencer (Invitrogen, China). The DNAMAN software (http://www.lynnon.com/) was used to align and assemble the obtained sequences to generate the complete genome of HLJB1, which has been deposited in the GenBank database with the accession number of KT224385.

A total of 70 PRRSV genomes, including all 67 available PRRSV 1 genomes and 3 representative PRRSV 2 genomes, were obtained from the GenBank database (Table S1). Multiplex sequence alignments were generated using ClustalX 2.0 (Larkin et al., 2007). Whole genome-based phylogenetic analysis was performed using MEGA 6.06 program (Tamura et al., 2013). Phylogenetic tree was constructed from aligned complete genomes using the neighbor-joining method as previously reported (Yu et al., 2012). The robustness of the phylogenetic tree was evaluated by bootstrapping using 1000 replicates.

The multiple alignment of 67 PRRSV 1 genomes was submitted to screen homologous recombination events by using recombination detection program 4 (RDP4) (Martin et al., 2015). Seven methods embedded in RDP4 software package, including RDP, GENECONV, BootScan, Maxchi, Chimaera, SiScan, and 3Seq, were utilized to detect recombination events and breakpoints. The default settings were used for all methods and the highest acceptable P value cut-off was set at 0.05 (Chen et al., 2013). The detected recombination events were further confirmed by SimPlot 3.5.1 (Lole et al., 1999).

3. Results and discussion

The complete genome of HLJB1 was 15,113 nucleotides (nt) in length, excluding the poly (A) tail. HLJB1 shared 90.66% nucleotide identity with the prototypic PRRSV 1 Lelystad virus (LV), but only 58.21% with the representative PRRSV 2 ATCC VR-2332, indicating that HLJB1 belongs to PRRSV 1. When compared with 67 available PRRSV 1 genomes from GenBank, HLJB1 shared the highest similarity with Amervac vaccine strain (91.64% nucleotide identity) (Table 1).

Each fragment of HLJB1 genome was compared with six representative strains (Table 1). Nsp2N shows to be the most variable nsp of HLJB1 (Fang and Snijder, 2010; Fang et al., 2012) (Table 1). The alignment of nsp2N from all 17 Chinese PRRSV 1 isolates and the representative LV and Amervac vaccine strain showed that HLJB1 contains a unique 5-amino-acid insertion at 32–36 positions in nsp2N (Fig. 1A), which has never been described previously. The influence of the insertion requires further investigation, which at least may serve as a genetic marker of the novel isolate. GP3 and GP4 are the most variable structural proteins of HLJB1 when comparing with other PRRSV 1 strains (Table 1), which contain a hypervariable region in the overlapping region of GP3 and GP4. Deletions in the overlapping region were commonly found in Chinese PRRSV 1 isolates, such as HKEU16, BJEU06-1 and NMEU09-1 isolates. However, no deletion was detected in this and any other variable regions of HLJB1 genome (Fig. 1B). GP5

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