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Characterization of three porcine reproductive and respiratory syndrome virus isolates from a single swine farm bearing strong homology to a vaccine strain

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ABSTRACT

Three porcine reproductive and respiratory syndrome viruses (PRRSV), NT1, NT2, and NT3, were isolated from three dying piglets from a single pig farm in Jiangsu Province, China. Whole genome sequencing revealed that the three isolates share the highest homology with JXA1-P80, an attenuated vaccine strain developed by serial passage of highly pathogenic PRRSV JXA1 in MARC-145 cells. More than ten amino acids residues in ORF1a, ORF1b, GP4, and GP5 that were thought to be unique to JXA1 attenuated on MARC-145 cells were each found in the corresponding locations of NT1, NT2, and NT3. In virulence assays, piglets infected with NT1, NT2, or NT3 exhibited clinical signs of disease, including high fever, anorexia, and respiratory distress, leading to the death of the majority of the piglets within two weeks. Collectively, these data indicate that NT1, NT2, and NT3 are highly pathogenic PRRSVs and they are likely to be revertants of the vaccine strain JXA1-P80.

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1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the major infectious diseases in pig farms. It first emerged in North America and Europe, followed by China in 1995. PRRS has spread rapidly and is now found worldwide (Lunney et al., 2010; Neumann et al., 2005; Zhou and Yang, 2010). The causative agent, porcine reproductive and respiratory syndrome virus (PRRSV), is an enveloped, single-stranded, positive sense RNA virus belonging to the family Arteriviridae within the order Nidovirales, which also includes equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus (SHFV) (Benfield et al., 1992; Meulenberg et al., 1994; Snijder and Meulenberg, 1998). The PRRSV genome mes (ORFs), a short 5′ untransis approximately 15 kb in length, contains at least ten open reading fralated region (UTR), and a poly-A tail at the 3′ terminus. ORF1a and ORF1b encode the replication-related polymerase proteins and are processed into at least 13 nonstructural proteins by self-cleavage. Other ORFs encode eight structural proteins (Bautista et al., 1996; den Boon et al., 1995; Firth et al., 2011; Meulenberg et al., 1997; Snijder et al., 1994; van Aken et al., 2006; van Dinten et al., 1996), and among these proteins, GP5, GP3, and Nsp2 are often used for phylogenetic analyses (Fan et al., 2014; Shi et al., 2010; Zhou et al., 2009).

PRRSV exists as two major genotypes, the European prototype (EU-type, type1), known as the Lelystad virus (LV), and the North American prototype (NA-Type, type2), VR-2332. The two genotypes share about 55–70% nucleotide and 50–80% amino acid similarity (Benfield et al., 1992; Nelsen et al., 1999; Wensvoort et al., 1992). In 2006, a highly pathogenic PRRSV (HP-PRRSV) with a discontinuous 30-amino acid deletion in the Nsp2 gene emerged in China. This virus caused high fever, high morbidity, and high mortality in pigs, leading to huge economic losses in the swine industry (Guo et al., 2012; Tian et al., 2007; Tong et al., 2007; Zhou et al., 2008). HP-PRRSV is now the dominant strain circulating in Chinese swine herds. Since 2010, HP-PRRSV-derived commercial live vaccines including JXA1 (P80), HuN4-F112 (P120), and TJM (P90) have been widely used (Han et al., 2009; Leng et al., 2012; Tian et al., 2009). Thus, multiple PRRSV types co-exist in swine herds and have caused concern about potential virus

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recombination or virulence reversion. Here, we characterize three PRRSV isolates, NT1, NT2, and NT3, isolated from three dying piglets from a single pig farm that appear to be revertants of the vaccine strain JXA1-P80.

2. Materials and methods

2.1. Clinical samples

Sera were collected from sick and dying piglets from a pig farm in Jiangsu Province, China, in 2012. The pig farm had approximately 200 sows and they did not use HP-PRRS vaccine before. At first, several litters of new born piglets emerged clinical manifestations including fever, cough and dyspnea, and later more piglets got ill, finally most diseased piglets were dead. The RNeasy Mini Kit (QIAGEN, Germany) and the RevertAid First Strand cDNA Synthesis Kit (Thermo, USA) were used for RNA extraction and cDNA reverse transcription. Sera were stored at −80 °C for virus isolation.

2.2. Virus isolation

Virus isolation was conducted as described previously (Zhu et al., 2011). The sera were passaged through a 0.22-μm filter and inoculated onto MARC-145 cell layers for 1 h at 37 °C. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) with 2% bovine serum (Gibco, USA) at 37 °C for 3–5 d. The culture supernatants were harvested when cytopathic effects appeared and were stored at −70 °C as viral stocks. Pulmonary alveolar macrophages (PAM) were obtained from 15-day-old piglets that were confirmed to be free of infection with PRRSV, porcine circovirus 2, and classical swine fever virus as described previously (de Abin et al., 2009). The isolated PAMs were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) with 10% heat-inactivated FBS, 100 U/ml penicillin, 10 μg/ml streptomycin in a 37 °C/5% CO_2 incubator for determining growth kinetics of PRRSV.

2.3. Genome cloning and sequencing

RT-PCR was performed using primers based on the HP-PRRSV strain HuN4 (accession number EF635006) as previously described (Zhou et al., 2008). The amplified PCR products were purified using a Gel Extraction Kit (OMEGA, USA), cloned into the pMD18-T vector (TaKaRa, and sequenced (Sangon Biotech, Shanghai, China).

2.4. Viral growth properties and kinetics

The isolated viruses NT1, NT2 and NT3 were passaged (F2–F3) in MARC-145 cells. Viruses were harvested from the supernatant when 80% of cells developed CPE, followed by removal of cell debris by centrifugation at 10,000 × *g* for 10 min. The third viral passage was used for sequencing, growth analyses, and *in vivo* studies. For the growth kinetics, 1×10^6 MARC-145 cells or PAM cells were cultured in 6-well plates and infected at a multiplicity of infection (MOI) of 0.01, followed by incubation at 37 °C for 1 h. Cells were washed and incubated in RPMI-1640 with 2% heat-inactivated FBS, 100 U/ml penicillin, 10 μg/ml streptomycin at 37 °C for another 96 h. The virus-infected supernatants were collected every 12 h, until 96 h post infection, and viral titers were determined and expressed as $TCID_{50}$ per milliliter in MARC-145 cells as described (Zhang et al., 2011).

2.5. Sequence alignments and phylogenetic analysis

The complete genome sequences of NT1, NT2, and NT3 were aligned after sequencing. Each ORF was annotated based upon the PRRSV VR2332 sequence (accession number U87392) and compared with other PRRSV isolates (Table 1) by using ClustalW in Lasergene software (DNASTAR Inc., Madison). Phylogenetic trees were constructed using MEGA software (version 4.1) using the neighbor-joining method and the reliability of the tree was assessed by bootstrap analysis of 1000 replications, and the bootstrap values below 60 were removed.

Table 1
Information of the representative PRRSV strains.

No.	Name	Country	Accession no.	No.	Name	Country	Accession no.
1	LV	Netherlands	M9626	28	LN	China	EU109502
2	VR2332	USA	U87392	29	CG	China	EU864231
3	BJ-4	China	AF331831	30	07BJ	China	FJ393456
4	Ch-1a	China	AY032626	31	07HEBTJ	China	FJ393458
5	Ch-1R	China	EU807840	32	07HEN	China	FJ393457
6	HB-1(sh)-2002	China	AY150312	33	07QN	China	FJ394029
7	HB-2(sh)-2002	China	AY262352	34	10-10HEB-3	China	JQ663553
8	CC-1	China	EF153486	35	10-10GX-2	China	JQ663559
9	HuN4	China	EF635006	36	JX	China	JX317649
10	HuN4-F112	China	/	37	JXA1	China	EF112445
11	TJ	China	EU864232	38	JXA1-P10[a]	China	FJ548854
12	Em2007	China	EU262603	39	JXA1-P15[a]	China	FJ548855
13	GD	China	EU825724	40	JXA1-P45[a]	China	FJ548851
14	HEB1	China	EF112447	41	JXA1-P70[a]	China	FJ548852
15	HUB1	China	EF075945	42	JXA1-P80[b]	China	FJ548853
16	HUB2	China	EF112446	43	JXA1-P100[a]	China	KC422725
17	JX143	China	EU708726	44	JXA1-P110[a]	China	KC422726
18	JXwn06	China	EF641008	45	JXA1-P120[a]	China	KC422727
19	NM1	China	EU860249	46	JXA1-P130[a]	China	KC422728
20	SHH	China	EU106888	47	JXA1-P140[a]	China	KC422729
21	JN-HS	China	HM016158	48	JXA1-P150[a]	China	KC422730
22	JSYX	China	EU939312	49	JXA1-P160[a]	China	KC422731
23	TP	China	EU864233	50	JXA1-P170[a]	China	JQ804986
24	BJ	China	EU825723	51	NT1	China	KP179402
25	Henan-1	China	EU200962	52	NT2	China	KP179403
26	XH-GD	China	EU624117	53	NT3	China	KP179404
27	jiangxi-3	China	EU200961				

[a] Derivatives of in vitro passaged highly pathogenic PRRSV JXA1 in MARC-145 cells.
[b] A live attenuated vaccine strain of highly pathogenic PRRSV JXA1.

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