Microbial Pathogenesis 112 (2017) 295–302

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Cloning and molecular characterization of the ORF5 gene from a PRRSV-SN strain from Southwest China

Xi-Wen Chen [a, b, *], Lian Li [a, c], Miao Yin [a], Qian Wang [a, c], Wen-Tao Luo [a, c], Ying Ma [a, b], Zhong-Hui Pu [a, b], Jie-Long Zhou [c]

[a] Institute of Applied Animal Technology, Mianyang Normal University, Mianyang, Sichuan, 621000, China
[b] Research Center of Ecological Agriculture and Animal Husbandry in Northwest Sichuan, Mianyang, Sichuan, 621000, China
[c] College of Life Science, Southwest Forestry University, Kunming, Yunnan, 650224, China

ARTICLE INFO

Article history:
Received 6 February 2017
Received in revised form
23 August 2017
Accepted 11 September 2017
Available online 29 September 2017

Keywords:
Porcine reproductive and respiratory syndrome virus (PRRSV)
ORF5
Molecular characterization
Southwest China

ABSTRACT

To monitor the genetic variation of PRRSV, the ORF5 gene of the PRRSV-SN strain found in Suining City, Sichuan Province, was cloned and sequenced. The results showed that the PRRSV-SN strain was a highly pathogenic PRRSV (HP-PRRSV) variant strain with the North American (NA) genotype. Homology analysis showed that the ORF5 gene of the PRRSV-SN isolate shared 89.4% (86.5%) nucleotide (amino acid) sequence similarity with the North American strain VR-2332, 98.8% (96%) similarity with JXA1, and 63.8% (57.7%) similarity with the European type representative strain Lelystad virus. Phylogenetic analysis showed that PRRSV-SN belongs to the NA genotype and has the same subtype as other highly pathogenic PRRSV strains. Amino acid sequence analysis showed that compared with the VR2332 strain, PRRSV-SN has different degrees of variation in the signal peptide, transmembrane region (TM), primary neutralizing epitope (PNE), non-neutral epitopes and N-glycosylation sites. Antigenicity analysis showed that the PRRSV-SN ORF5 gene products and JXA1 have similar antigenic characteristics, and the antigenic epitopes are mainly located in aa30-39, aa50-60, aa128-141, aa146-155 and aa161-183 regions. In contrast, the antigenic characteristics of PRRSV-SN are quite different from those of the VR2332 strain. The main differences were that the PRRSV-SN strain was significantly narrower than the VR2332 strain in the aa30-39 and the aa50-60 regions but was significantly wider in the aa136-141 region. The results of this study showed that the epidemic strains that cause PRRSV outbreaks in the farm are still mainly JXA1 variants, but due to the more frequent use of live vaccine immunizations, the genes of the PRRSV epidemic strain still show constant variation. Vaccination with live PRRSV should be reduced, and surveillance of PRRSV strains should be enhanced.

1. Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is a global epidemic in terms of porcine infectious diseases. It was first reported in the United States in 1987 and soon afterwards in Europe, and it caused an unprecedented “abortion crisis” in the United States, where the country's pig industry suffered catastrophic economic losses [1,2]. PRRS has been known as mystery swine disease (MSD) and blue ear disease (blue-eared pig disease) and has spread to countries around the world. It is currently one of the diseases causing serious economic losses in the global pig industry [3–7]. The pathogen causing PRRS is named porcine reproductive and respiratory syndrome virus (PRRSV). Wensvoort et al. isolated the virus for the first time in the Netherlands [3]. Later, it was isolated in the United States, Canada, Europea, China and Japan [7–9]. The virus is a single, positive-stranded RNA virus with eight reading frames. Because its genomic structure and mechanism of transcription are similar to those of the arterial virus, in 1995, the International Classification Committee for Viruses classified PRRSV as belonging to the family Arteriviridae and genus Arterivirus. The major pathogen causing swine fever in China in 2006 was a PRRSV variant (highly pathogenic PRRSV, HP-PRRSV), which was associated with high morbidity and mortality and the onset of death in pigs [10,11].

The genome of PRRSV is approximately 15 kb in length and

* Corresponding author. Institute of Applied Animal Technology, Mianyang Normal University, Mianyang, Sichuan, 621000, China.
E-mail address: xwch05@163.com (X.-W. Chen).

https://doi.org/10.1016/j.micpath.2017.09.011
0882-4010/

contains at least 9 open reading frames (ORFs). ORF1a and ORF1b constitute almost two-thirds of the genome and encode the nonstructural proteins (Nsps) necessary for viral replication. ORF2a, ORF2b and ORF3 to ORF7 encode the viral structural proteins GP2, E, GP3, GP4 and GP5 [12,13]. The ORF5 gene is one of the most variable regions of the PRRSV genome, and ORF5 encodes the GP5 glycoprotein, which is 26 kDa in size and consists of six antigenic determinants. GP5 is an important immunogenic protein of PRRSV and is associated with virus neutralization. The primary neutralizing epitope of GP5 has been localized to the N-terminal extracellular domain of the protein [14,15]. In addition, GP5 is associated with M proteins through the formation of heterodimers, which play an important role in virus attachment and macrophage internalization [16]. Genetic variation in GP5 has been reported in a number of studies from different regions and countries [17–20]. GP5 mutation directly led to decreased rates of cross-protection by the PRRSV vaccine and has brought greater challenges to the prevention and control of the disease [21–24].

In this study, the lung, lymph nodes and blood were collected from specimens at a pig farm with suspected cases of PRRSV infection in Suining, southwestern China. After identification of the pathogen by RT-PCR, the entire ORF5 gene was cloned and sequenced, and molecular characterization was performed. The results of this study not only provide an understanding of the genetic variation of PRRSV genes in swine infection in Southwest China but also provide a scientific reference for PRRSV prevention, control and vaccine screening.

2. Materials and methods

2.1. Sample collection

The samples were collected from a swine farm in Suining City, Sichuan Province, southwest China, in 2015. Lung, lymph node and blood samples were collected from pigs with high fever and that had had abortions, then frozen at -80 °C.

2.2. Primer design and synthesis

The primers P1 and P2(P1:5′-CAAAGAYCAGATGGAGGAG-3′,P2:5′-ATRATGGCTTGAGCTGAG-3′), which amplify the partial sequence of the Nsp2 gene, can distinguish between the classical strain and the HP-PRRSV strain (Expected length of amplification, the classical strain 768 bp, the HP-PRRSV strain 678 bp). The primers P3 and P4 (P3: 5′-GTTTACCCAACGCTCCTTA-3′, P4: 5′-ACTGGCGTGTAGGTAATGG-3′) were able to amplify the ORF5 whole gene sequence and the expected amplification length was 801 bp. All primers were designed using the Primer 5.0 software according to the sequence of HP-PRRSV JXA1 in GenBank (GenBank accession: EF112445.1). Primers were synthesized by Sangon Biotech (Shanghai) Co. Ltd. and the purity level was verified using PAGE.

2.3. RNA extraction and RT-PCR

Viral RNA was extracted from tissue and serum samples using the MiniBEST Viral RNA/DNA Extraction Kit Ver.5.0 (Takara Co. Dalian, China) according to the manufacturer's instructions. Virus cDNA was constructed by reverse transcription using random hexanucleotide primers and PrimeScript™ 1st Strand cDNA Synthesis Kit (Takara Co. Dalian, China) following the supplier's instructions. The PCR reactions were done in a total volume of 50 μl containing 3 μl of the extracted cDNA,4 μl dNTP (2.5 mM), 5 μl 10 × Buffer (TakaRa), 5 μl $MgCl_2$, 0.5 μl Taq polymerase (TakaRa), and 2 μl of each of the PRRSV-specific primers described above. The PCR was performed using the following cycling parameters: 95 °C 5 min; 40 cycles of 94 °C 30 s, 56 °C 30 s, 72 °C 1 min; and final extension at 72 °C for 10 min. After completion of the reaction, the PCR products were observed using 1% agarose gel electrophoresis under ultraviolet light.

2.4. Cloning and sequencing

The amplified ORF5 fragment was recovered using a DNA Purification Kit protocol (Takara Co. Dalian, China). The fragment was ligated into the pMD18-T vector overnight at 4 °C and transformed into DH5α competent cells. Positive plasmids were sequenced by Sangon Biotech (Shanghai) Co. Ltd.

2.5. Sequence alignment and phylogenetic analysis

The obtained PRRSV ORF5 gene was compared with that of the PRRSV strains registered in GenBank (Table 1). Phylogenetic trees were constructed with MEGA 6.0 analysis software using the neighbour-joining method, and sequence alignments were performed using Clustal W.

Table 1
Information of the representative PRRSV strains downloaded from GenBank database.

Name	Country	Isolation year	Accession no.
NMEU09-1	China	2009	GU047345
BJEU06-1	China	2009	GU047344
HENAN-XINX	China	2013	KF611905
JL580	China	2013	KR706343.1
SC2012	China	2014	KM189443
SCwhn09CD	China	2011	JN836553
SCwhn14DY	China	2014	KT819203.1
CH-1a	China	2001	AY032626
HB-1 (sh)	China	2002	AY150312
HB-2 (sh)	China	2003	AY262352
GZ106	China	2014	KJ541663
GZ1101	China	2013	KF771273
HLJB1	China	2013	KT351740.1
NT0801	China	2010	HQ315836
JXA1	China	2006	EF112445
HUN4	China	2007	EF635006
TJ	China	2008	EU860248
ZP-1	China	2010	HM016159
AH0701	China	2010	GU461292
CHsx1401	China	2014	KP861625.1
DC	China	2011	JF748718
FJYR	China	2015	KT804696.1
FJZH	China	2015	KP998478.1
GDQY2	China	2010	GU454850
GX1002	China	2012	JQ955658
HeNan-A9	China	2014	KJ546412
HENZZ-8	China	2016	KU950375.1
HNP5	China	2014	KT445876.1
HPBEDV	China	2007	EU236259
HUN-2014	China	2014	KP330232.1
NT2	China	2014	KP179403.1
NT3	China	2014	KP179404.1
SD16	China	2012	JX087437
SDA3	China	2012	JX878380
Shanxi-6	China	2014	KJ855518
XJu-1	China	2013	KF815525
YN-1	China	2014	KJ747052
DK-2010-10-1-2	Denmark	2013	KC862579
PL97-1	Korea	2004	AY585241
Lelystad Virus	Netherlands	2000	M96262.2
SP	Singapore	1999	AF184212.1
NADC30	USA	2011	JN654459
ATCC VR-2332	USA	1993	PRU87392.3
JA142	USA	2003	AY424271
OH155-2015	USA	2015	KR534894.1