Veterinary Microbiology 146 (2010) 226–237

Contents lists available at ScienceDirect

Veterinary Microbiology

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

The genomic diversity of Chinese porcine reproductive and respiratory syndrome virus isolates from 1996 to 2009

Bin Li [a,b], Liurong Fang [a], Suyan Liu [a], Fuwei Zhao [a], Yunbo Jiang [a], Kongwang He [b], Huanchun Chen [a], Shaobo Xiao [a,*]

[a] Division of Animal Infectious Diseases, State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China
[b] Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, Jiangsu Province, China

ARTICLE INFO

Article history:
Received 12 January 2010
Received in revised form 24 April 2010
Accepted 3 May 2010

Keywords:
Porcine reproductive and respiratory syndrome virus
Genomic diversity
Chinese isolates

ABSTRACT

Since it was first reported in 1995, porcine reproductive and respiratory syndrome (PRRS) has become one of the most important swine diseases in China. A large number of field PRRSV strains have been isolated from different regions of China at different times, especially after a highly pathogenic PRRSV emerged in 2006. Previous studies based on ORF5 gene sequences revealed extensive genetic diversity among Chinese PRRSV isolates. To fully understand the extent of genetic diversity of PRRSV in China, we determined the genomic sequence of PRRSV WUH1, a highly pathogenic PRRSV isolated in late 2006. Based on the complete genomic sequences of strain WUH1 and 66 other field Chinese PRRSV strains isolated from 1996 to 2009, we further analyzed their genetic diversity. The results showed that all the tested Chinese PRRSV isolates belong to the North American genotype and can be clearly divided into four highly diverse subgenotypes. Furthermore, the analysis supported the concept that the highly pathogenic PRRSV in China emerged by gradual variation and evolution from the Chinese domestic virus. In addition, different deletions within Nsp2, deletion and potential antigenic drift within GP3, and point mutations within GP5, were extensively observed in Chinese PRRSV isolates. Interestingly, in addition to a unique discontinuous deletion of 30 aa in Nsp2, there was a 1 nucleotide deletion in both the 5′UTR and 3′UTR in nearly all highly pathogenic PRRSV isolates. These results will contribute to the elucidation of the evolutionary mechanisms of PRRSV in China.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the cause of an economically important swine disease that has been devastating the swine industry since the late 1980s (Keffaber, 1989; Wensvoort et al., 1991). PRRSV is a positive-strand RNA virus belonging to the family *Arteriviridae* (Dea et al., 2000). The PRRSV genome is approximately 15 kb and encodes nine overlapping open reading frames (ORFs) (Stadejek et al., 2002). ORF1a and ORF1b are located downstream of the 5′ untranslated region (UTR) and encode viral non-structural proteins (Nsps): Nsp1α, Nsp1β, and Nsp2–12 (Meulenberg et al., 1993; Van et al., 1996; Ropp et al., 2004). ORF2a, ORF2b, and ORF3–7 are located at the 3′ end of the genome and encode viral structural proteins GP2, E, GP3, GP4, GP5, M, and N, respectively (Stadejek et al., 2006).

There are two distinct PRRSV genotypes, which share only about 60% nucleotide identity, represented by the North American prototype VR-2332 and the European prototype Lelystad virus (LV) (Nelsen et al., 1999).

* Corresponding author at: Laboratory of Animal Virology, College of Veterinary Medicine, Huazhong Agricultural University, 1 Shi-zi-shan Street, Wuhan 430070, China. Tel.: +86 27 8728 6884; fax: +86 27 8728 2608.
E-mail address: vet@mail.hzau.edu.cn (S. Xiao).

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doi:10.1016/j.vetmic.2010.05.011

Remarkable sequence differences have been found among isolates of the same genotype, particularly in Nsp2 and ORF5 (Forsberg et al., 2002; Mateu et al., 2003).

Since it was first reported in 1995, PRRS has now become one of the most important swine diseases in mainland China. In particular, a so-called porcine high fever syndrome, caused by highly pathogenic PRRSV and characterized by high fever and high mortality in pigs of all ages, has emerged in China and affected more than 20 million pigs in 2006 (Tian et al., 2007; Tong et al., 2007). A large number of field PRRSV strains have been isolated from different regions at different times. Several previous studies based on ORF5 gene sequences have revealed that most Chinese isolates belonged to the North American type and could be divided into two major subgenotypes (An et al., 2007; Li et al., 2009b).

To more fully understand the extent of genetic diversity of Chinese PRRSV, we sequenced the genome of PRRSV WUH1, a highly pathogenic PRRSV isolated in central China during the pandemic period of porcine high fever syndrome. We also analyzed the characteristics of all available complete genomic sequences of field PRRSV strains isolated in mainland China from 1996 to 2009.

2. Materials and methods

2.1. Virus and cells

MARC-145 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units of penicillin, and 20 units of streptomycin, and maintained in a 37 °C humidified chamber with 5% CO_2. PRRSV strain WUH1 was isolated from the brain of a pig with the "high fever" syndrome in China in late 2006. Briefly, brain tissues were homogenized with DMEM, freeze-thawed three times and centrifuged at 10,000 × *g* for 10 min. The supernatant was passed through a 0.22 μm filter and transferred onto MARC-145 cell monolayers. The cells were incubated at 37 °C for 5 days and examined for cytopathic effect (CPE) daily. The presence of propagation was confirmed by RT-PCR using primers (5′-TAGGTGACTTAGAGGCACAGT-3′ and 5′-TAAATATGCCAAATAACAAC-3′), which amplify the ORF7 gene of PRRSV. After three passages, virus was purified by plaque cloning three times. The purified virus was designated WUH1 and the 50% tissue culture infectious dose ($TCID_{50}$) was determined.

2.2. Primer design

Oligonucleotide primers, which amplify the WUH1 genome, were designed based on sequence information available on GenBank for PRRSV strains VR-2332 and JXA1. These primers, together with the lengths, sequence compositions, and relative positions of the regions amplified within the WUH1 genome are given in Table 1.

2.3. Preparation of RNA and RT-PCR

Virus RNA was extracted from infected cells with the QIAamp viral RNA kit (Qiagen). Virus cDNA was constructed by reverse transcription, which used random hexanucleotide primers and SuperScript II reverse transcriptase (Gibco BRL) following the supplier's instructions. The cDNA was used as a template in subsequent PCR reactions in a final volume of 50 μl, including 25 pmol of each PRRSV-specific primer described above, 1× Pfu polymerase buffer, 200 μM of dNTP, and 2.5 units of Pfu DNA polymerase (Stratagene).

Table 1
Oligonucleotide primer sequences utilized to generate cDNA clones encompassing the full-length genome of the WUH1 virus.

Oligonucleotides	Sequence[a]	Location[b]
PRRS01F	5′-atgacgtataggtgttggctc-3′	1–21
PRRS01R	5′-caaggcaggcaggatcaaatccg-3′	1821–1843
PRRS02F	5′-cttgaatgtgttcagggctgttgt-3′	1753–1776
PRRS02R	5′-gagtcgatgatggcttgagctga-3′	3160–3182
PRRS03F	5′-atcacacgcccaaaatactcagc-3′	3142–3164
PRRS03R	5′-agtcaagcatttgattgaagccgac-3′	5677–5701
PRRS04F	5′-actgccgcacatgtccttacg-3′	5632–5652
PRRS04R	5′-gccaagacggagcgcccatcagtc-3′	8174–9197
PRRS05F	5′-aatacaaggtttggagacatacctta-3′	8079–8104
PRRS05R	5′-ttcccagcaccgggtgggccgat-3′	9969–9991
PRRS06F	5′-aagagatcaacatggtcgctgtcg-3′	9916–9939
PRRS06R	5′-aggcctaaagttggttcaatgaca-3′	11,951–11,974
PRRS07F	5′-aggactgggaggattacaatgatgc-3′	11,851–11,875
PRRS07R	5′-cttgacgtgttggacgtagctg-3′	13,546–13,567
PRRS08F	5′-tttggcaatgtgtcaggcatcgtggc-3′	13,502–13,527
PRRS08R	5′-gcggccgctttttttttttttt-3′	Poly (A) tail

[a] Primers were designed based on sequence information available for VR-2332 and JXA1.
[b] Location corresponds to position within the WUH1 genome.

2.4. Amplification and cloning of 5′ terminal sequences

To obtain and analyze the 5′ end of PRRSV genome segments, 5′ Rapid Amplification of cDNA End (RACE) was performed (Frohman et al., 1988), using the RACE System (Version 2, Life Technologies, USA). The specific primer 5′-GGTCGTTGACAAGTTGGTCATCTACCGGTTTATCCTCGGA-3′ was used as a reverse primer to synthesize first-strand cDNA. Nested PCR was used to amplify the 5′UTR. The internal primers were: Int-F 5′-GGAATGCACGTGGCAACGTCCA-3′ and Int-R 5′-CTGCTGGCTTTCTGCGATCTTT-3′. Nested specific primers were: Out-F 5′-ACATGGTTAAAGGGGTGGAGA G-3′ and Out-R 5′-GTCGCTGGTACCCCATTGTCGG-3′.

2.5. DNA sequencing and analysis

The PCR products were purified using a QIAquick gel extraction kit (QIAquick) and cloned into pMD18-T vector (TaKaRa), and sequenced. For each amplified genomic region, three clones from each of the three independent amplifications were sequenced in both directions, thus the sequence of each region was determined nine times.

2.6. The collection of genomic sequences of all the Chinese isolates and sequence analysis

In addition to the PRRSV WUH1, we used all 66 PRRSV genome sequences from mainland China from 1996 to 2009 that were deposited in GenBank. The origin and GenBank accession number of each PRRSV isolate is listed