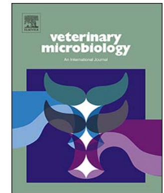
Veterinary Microbiology 208 (2017) 150–158

Contents lists available at ScienceDirect

Veterinary Microbiology

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



# A novel recombinant porcine reproductive and respiratory syndrome virus with significant variation in cell adaption and pathogenicity

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ARTICLE INFO

*Keywords:*
Porcine reproductive and respiratory syndrome virus
Recombination
ORF5a
GP2
Cell adaption
Pathogenicity

ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is an important pathogen that causes huge economic losses to the swine industry worldwide. In this study, a type 2 PRRSV strain was isolated from primary porcine alveolar macrophage cells and designated as GD1404. Interestingly, this strain was unable to grow in MARC-145 cells. Analysis of the full-length genome sequence revealed that strain GD1404 was an inter-subgenotype recombinant of strains QYYZ and JXA1. The C-terminus of the GP2 protein of strain GD1404 had an amino acid deletion. Also, the ORF5a protein had 51 codons, five more than most other highly pathogenic (HP-PRRSV) strains. Phylogenetic analysis based on ORF5 gene sequences showed that strain GD1404 and five others isolated in China formed a new subgenotype represented by strain QYYZ. Challenge experiments with piglets showed that the GD1404 and HP-PRRSV BB0907 strains caused similar rates of mortality and interstitial pneumonia. However, strain GD1404 infection resulted in lower viremia and viral loads in the lungs, as compared with strain BB0907. The results of this study provide evidence of the circulation of type 2 PRRSV QYYZ-like strains in China with variations in cell adaption and pathogenic abilities.

## 1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an economically important disease of swine worldwide that is caused by the PRRS virus (PRRSV) of the family *Arteriviridae* and genus *Arterivirus* (Fan et al., 2014; Lunney et al., 2016; Zhang et al., 2016a,b,c). PRRSV is divided into two genotypes, European (type 1) and North American (type 2), with Lelystad and VR-2332 as prototypical strains, respectively (Allende et al., 2000; Nelsen et al., 1999). The viruses of these two genotypes are further divided into different subgroups by phylogenetic analysis (Fan et al., 2014).

PRRSV has very restricted cell tropism in vitro. Porcine alveolar macrophages (PAMs) are the primary target cells of this virus in vivo and primary cultures can be used to isolate PRRSV in vitro (Zhang et al., 2016a,b,c). Two non-porcine immortalized cell lines, MARC-145 and CL2621 (subclones of the MA104 monkey kidney cell line), are routinely used for in vitro propagation of PRRSV (Provost et al., 2012). Type 1 (European type) PRRSV is mainly isolated from PAMs, while type 2 (North American type) can be directly isolated from MARC-145 cells (Albina and Buffereau, 1993).

The full genome of PRRSV is about 15 kb in length and contains at least 11 open reading frames (ORFs). Nearly 75% of the viral genome consists of ORF1a and ORF1b, which encode the polyproteins pp1a and pp1ab, respectively. These two large polyproteins are processed into at least 14 nonstructural proteins (NSPs) (Kimman et al., 2009). Among the NSPs, NSP2 is one of the most variable. A discontinuous 30-amino acid (aa) deletion in the NSP2 region has been considered as a molecular characteristic of highly pathogenic PRRSV (HP-PRRSV) strains in China since 2006 (Li et al., 2007; Zhou et al., 2009, 2008). The 3′ end of the viral genome encodes eight structural proteins: GP2, E, GP3, GP4, GP5, ORF5a, M, and N (Dokland, 2010; Leng et al., 2014). GP5 is one of the most variable structural proteins of PRRSV. Antibodies against GP5 can protect piglets from PRRS viremia and the development of associated lesions (Barfoed et al., 2004; Kimman et al., 2009). Another structural protein, ORF5a, is encoded in an alternate ORF upstream from ORF5. A previous study showed that ORF5a is essential for virus viability (Sun et al., 2013), while another revealed that ORF5a and GP5 co-evolved through a fine balance of purifying codon usage to maintain a conserved RQ-rich motif in the ORF5a protein, while eliciting a variable N-linked glycosylation sites (NGSs) in the alternative GP5 reading frame (Robinson et al., 2013). Three minor structural proteins, GP2, GP3, and GP4, form the multimeric complex GP2-GP3-GP4, which

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http://dx.doi.org/10.1016/j.vetmic.2017.07.028
Received 20 June 2017; Received in revised form 24 July 2017; Accepted 26 July 2017
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is critical for virus entry. The GP2 protein, which is less heterogeneous than GP3 and GP5, consists of a predicted N-terminal signal sequence, an ectodomain, a single transmembrane (TM) helix, and a 20-residue endodomain.

PRRSV is genetically heterogeneous and the recombination between different isolates is an important mechanism in the evolution of this and other viruses. In this study, by using PAMs, the novel recombinant PRRSV strain GD1404 was isolated from piglets in Guangdong province, China. GD1404, which is an inter-subgenotypic recombinant of the HP-PRRSV JXA1 and QYYZ strains, and five other strains form a new subgenotype represented by the Chinese isolate QYYZ. Moreover, strain GD1404 has unique characteristics in cell adaption and pathogenicity in swine.

## 2. Materials and methods

### 2.1. Sample collection and virus isolation

Lung samples were obtained from pigs raised on farm in Guangdong province, China, in 2014. The piglets displayed significant clinical signs of PRRS, including labored breathing, pyrexia, lethargy, and anorexia. The reverse transcriptase chain reaction (RT-PCR) analysis showed the samples were definitely PRRSV positive.

PAMs were obtained as previously described (Wensvoort et al., 1991; Zhang et al., 2009). PRRSV-positive tissues were homogenized in phosphate-buffered saline (PBS) and subjected to three freeze-thaw cycles. After centrifugation, the supernatant was filtrated through a 0.22-μm filter before inoculation. After inoculation, the cells were maintained in Roswell Park Memorial Institute 1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum and 1% antibiotic–antimycotic solution under a humidified 5% $CO_2$ atmosphere at 37 °C. When 70% of the cells displayed cytopathic effects (CPEs), the cell cultures were harvested and stored at −70 °C. The isolated virus was designated GD1404. After another two passages in PAMs, the obtained virus was used for further complete genome sequencing and in animal experiments.

### 2.2. Genome cloning and sequencing

Total RNA was extracted from strain GD1404 using the RNA Isolation Kit (Omega Bio-Tek, Inc., Norcross, GA, USA). An 8-μL aliquot of the extracted RNA was used for reverse transcription with the SuperScript III First-Strand Synthesis Kit (Invitrogen, Carlsbad, CA, USA). After reverse transcription, the cDNA was used as a template for the following PCR analysis.

The whole genome of strain GD1404 was divided into seven overlapping fragments. The overlapping fragments were amplified by PCR using the primers provided in Table 1 and LA Taq polymerase (TaKaRa Biotechnology Co., Ltd., Dalian, China) under the following thermal cycling conditions: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 3 min, with a final extension at 72 °C for 5 min. After agarose gel electrophoresis, the PCR products were purified with the Biospin Gel Extraction Kit (BioFlux, Beijing, China) and then cloned into the pMD19-T vector (TaKaRa Biotechnology Co., Ltd.). Three clones were sequenced by a commercial service provider (Genscript Co., Ltd., Nanjing City, Jiangsu Province, China).

### 2.3. Nucleotide (nt) and aa sequence analyses

The overlapping sequences of the PCR products were combined to obtain the full-length genomic sequence of strain GD1404. BLAST analysis was used to compare the nt and aa sequences of strain GD1404 with those of the reference PRRSV strains (Table 2). Phylogenetic trees of the full-length genome, ORF5, ORF5a and NSP2 nt sequences were generated with MEGA 6.06 software (http://www.megasoftware.net/) using the distance-based neighbor-joining method. The bootstrap values were calculated based on 500 replicates and the evolutionary distances were computed using the Jukes–Cantor method. Comparisons of aa sequences were conducted using BioEdit 7.0.9.0 software (http://www.mbio.ncsu.edu/BioEdit/page2.html). Potential NGSs were analyzed using NetNGlyc version 1.0 software (http://www.cbs.dtu.dk/services/NetNGlyc/).

**Table 1**
Primers used for amplification of the genome of GD1404 isolate.

| Fragment | Sequence of PCR primers | Position in genome | Length of PCR products |
|---|---|---|---|
| A | 5′-ATGACGTATAGGTGTTGGCTCT-3′<br>5′-CACAGTTCTACGCGGTGCAGGAACT-3′ | 1-2928 | 2928bp |
| B | 5′-ACCTTTGAGTGGGTCGGCACCAGTT-3′<br>5′-TGTAACGCCATCGAGCAGGCAACAT-3′ | 2883-4886 | 2004bp |
| C | 5′-GGTGTAAAAAGTGCTGGGGATCTTG-3′<br>5′-CAAGTACAAAATTATGGCAAGGAGG-3′ | 4277-6567 | 2291bp |
| D | 5′-GTCGCAGATCTTGCGGTAACTCAAG-3′<br>5′-CTTAAATTTGTTTTTCCCGAGGGCG-3′ | 6415-8741 | 2327bp |
| E | 5′-CGCCCTCGGGAAAAACAAATTTAAGG-3′<br>5′-CATTGTTCTGGGTTGTCACCACGG-3′ | 8717-10980 | 2264bp |
| F | 5′-GCTAAACTCCCGGCAGAACTTGCAC-3′<br>5′-GCGAACGCCTGAGAAACCACGAAAC-3′ | 10923-13215 | 2293bp |
| G | 5′-GCAATTGGTTTCACCTGGAATGGCTGC-3′<br>5′-TTTTTAATTACGGCCGCATGGTTCTCGC-3′ | 13132-15326 | 2195bp |

**Table 2**
PRRSV strains used in this study.

| No. | Name | Country | Year | Accession number |
|---|---|---|---|---|
| 1 | GD1404 | China | 2015 | MF124329 |
| 2 | ZJ1503 | China | 2015 | KT961381 |
| 3 | HN1506 | China | 2015 | KT961406 |
| 4 | JX1411 | China | 2015 | KT961391 |
| 5 | JL580 | China | 2015 | KR706343 |
| 6 | BB0907 | China | 2013 | HQ315835 |
| 7 | YD | China | 2012 | JF748717 |
| 8 | SH1211 | China | 2012 | KF678434 |
| 9 | NADC30 | USA | 2012 | JN654459 |
| 10 | LMY | South Korea | 2012 | DQ473474 |
| 11 | TJ | China | 2012 | EU860248 |
| 12 | WUH4 | China | 2011 | JQ326271 |
| 13 | QYYZ | China | 2011 | JQ308798 |
| 14 | Em2007 | China | 2011 | EU262603 |
| 15 | 10-10GX-1 | China | 2010 | JQ663558 |
| 16 | 10-10HEB-1 | China | 2010 | JQ663551 |
| 17 | JXwn06 | China | 2009 | EF641008 |
| 18 | CH-1R | China | 2008 | EU807840 |
| 19 | YN9 | China | 2008 | GU232738 |
| 20 | HuN | China | 2007 | EF517962 |
| 21 | SY0608 | China | 2007 | EU144079 |
| 22 | 07NM | China | 2007 | FJ393456 |
| 23 | BJ | China | 2007 | EU825723 |
| 24 | GD | China | 2007 | EU109503 |
| 25 | MN184C | USA | 2007 | EF488739 |
| 26 | HUN4 | China | 2007 | EF635006 |
| 27 | HEB1 | China | 2006 | EF112447 |
| 28 | JXA1 | China | 2006 | EF112445 |
| 29 | HUB2 | China | 2006 | EF112446 |
| 30 | Ingelvac ATP | USA | 2006 | DQ988080 |
| 31 | Prime Pac | USA | 2006 | DQ779791 |
| 32 | HUB1 | China | 2006 | EF075945 |
| 33 | HB-2(sh)/2002 | China | 2004 | AY262352 |
| 34 | HB-1(sh)/2002 | China | 2002 | AY150312 |
| 35 | BJ-4 | China | 2000 | AF331831 |
| 36 | EuroPRRSV | USA | 1999 | AY366525 |
| 37 | CH-1a | China | 1996 | AY032626 |
| 38 | RespPRRS MLV | USA | 1994 | AF066183 |
| 39 | VR-2332 | USA | 1992 | AY150564 |
| 40 | Lelystad virus | Europe | 1991 | M96262 |
| 41 | ATCC VR-2332 | USA | 1990 | U87392 |

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