Isolation and Sequence Analysis of Highly Pathogenic Porcine reproductive and respiratory syndrome virus from Swine Herds in the Jilin Province of China

Feng-Xue Wang · Li Guo · Yan-Ling Yang ·
Ni Song · Li-Zhi Chen · Shi-Peng Cheng ·
Yong-Jun Wen

Received: 6 October 2012 / Accepted: 29 December 2012 / Published online: 12 January 2013
© Indian Virological Society 2013

Abstract The aim of the present study was to determine the causative agent of infected swines in the Jilin province of China and assess its genetic characteristics. Virus was isolated from tissues suspected of being infected by porcine reproductive and respiratory syndrome virus (PRRSV) and inoculated onto MARC-145 cells. Virus detection was carried out by RT-PCR, immunofluorescence, electron microscopy and sequencing. The results showed that the isolate was the North American genotype PRRSV, termed the JL-04/12 strain, with a 15,320 bp genome. The homology of the amino acid sequences in two nonstructural proteins and GP2 to GP5, between strains JL-04/12 and HUN4, ranged from 97.2 to 99.3 %. However, JL-04/12 GP6 and N protein were identical in HP-PRRSV JXA1 and HUN4. JL-04/12 was characterized by two discontinuous deletions in Nsp2. We speculate that the isolate is a variant of highly pathogenic porcine reproductive and respiratory syndrome derived from strains in 2006–2008. Altogether, these results indicate that highly pathogenic porcine reproductive and respiratory syndrome virus still exists in the Jilin province of China.

Keywords Porcine reproductive and respiratory syndrome virus · High pathogenicity · Isolation · Sequencing analysis · Nonstructural protein 2 · China

Porcine reproductive and respiratory syndrome (PRRS) was first reported in the United States and Europe in the early 1990s [4, 9] and the first isolate was reported from China in 1996 [3]. PRRS has had a devastating effect on the swine industry, causing tremendous economic losses worldwide [1, 5]. The responsible pathogen was identified as porcine reproductive and respiratory syndrome virus (PRRSV). PRRSV is a positive-sense single-stranded RNA virus belonging to the family *Arteriviridae*. The genome of PRRSV is ~15 kb in length. The genome consists of a methyl-capped 5' end, ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5, ORF6, ORF7 and a polyadenylated (Poly A) 3' end [6, 7]. ORF1a and ORF1b encode two large polyproteins (nonstructural proteins, Nsps), replicase and polymerase, respectively. At least 14 Nsps are generated as a result of serial cleavages of the two polyproteins expressed from ORF1a and ORF1b.

Porcine reproductive and respiratory syndrome virus can be divided into distinct European and North American genotypes based on nucleotide sequence comparisons [2]. In early 2006, the first epidemic of highly pathogenic PRRSV (HP-PRRSV) was noticed in pigs in the central region of China [8]. Subsequently, many HP-PRRSV isolates were reported from most of the provinces in China [11]. However, to date, there have been no isolates reported in the Jilin province. In this paper, we report a strain of HP-PRRSV isolated from the lymph node of one pig suspected of having PRRS, and we determined its biology and genomic characterization.

The tissues (derived from lungs, liver, spleens, and lymph nodes) were collected from animals suspected of being infected by PRRSV from the Jilin province and then processed. The tissues were first homogenized, 20 % (w/v), in 10 % (w/v) sterile phosphate-buffered saline and filtered through 0.22-mm membrane filters. Virus isolation was

F.-X. Wang (✉) · L. Guo · Y.-L. Yang · N. Song ·
L.-Z. Chen · S.-P. Cheng · Y.-J. Wen
State Key Laboratory for Molecular Biology of Special
Economic Animals, Institute of Special Economic Animal
and Plant Sciences, Chinese Academy of Agricultural Sciences
CAAS, No. 4899 Juye Street, Jilin 130112,
People's Republic of China
e-mail: wangfx_vet@126.com

Y.-J. Wen
e-mail: yongjunwen@126.com

Fig. 1 Identification of PRRSV. **a** MARC-145 cells were infected with strain JL-04/12 at a MOI of 0.01. Following 48 h of incubation, cells were immunostained by IFA using a monoclonal antibody specific for the N protein of PRRSV. **b** Electron micrographs of virus isolates in cell culture fluid harvested from infected cells. **c** Phylogenetic trees of the nucleotide sequences for Nsp2 genes of the Chinese

isolate JL-04/12 and related reference viruses. Alignments of porcine reproductive and respiratory syndrome virus sequences were generated using the ClustalW program. *Black triangles* indicate the virus isolate that was isolated in this study. Two main subgroups of PRRSV isolates (North American and European) are indicated for Nsp2 genes

attempted on all samples using Marc-145 cells, and isolates were passaged and identified by indirect immunofluorescence assay (IFA) using a monoclonal antibody against the N protein of PRRSV (SDOW17, kindly provided by Dr. Yuan Shi-shan). For assessing morphological characteristics of the viral isolate, cell culture supernatants containing the virus were examined by electron microscopy. Total RNA was extracted from 200 μ L supernatant using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. PRRSV RNA was detected by RT-PCR and sequenced. RT-PCR was performed using a pair of primers corresponding to ORF7 of PRRSV (ORF7-F 5'CG TGTGGGTGGCAGAA3', ORF7-L 5'TTAGAGGCACA GTGTAATCAG3').

Porcine reproductive and respiratory syndrome virus RNA was detected in the lungs of 10/68 (14.7 %), livers of 8/68 (11.8 %), spleens of 7/68 (10.3 %), and lymph nodes of 10/68 (14.7 %) suspected pigs. Strain JL-04/12 was successfully isolated from one PRRSV RNA-positive sample (lymph node). Virus isolation was detected by IFA and green fluorescence was visible in the virus-infected cells (Fig. 1a).

Morphologically, JL-04/12 was found to be an enveloped virus with a size of $\sim$ 45–55 nm in diameter. As shown in Fig. 1b, the virion contains an icosahedral core. Overall, JL-04/12 resembled members of the family *Arteriviridae*. Its entire genome was obtained and submitted to GenBank (Accession No. JX177644). The amino acid sequences containing the complete Non-structural protein 2 (Nsp2) (3,602 bp) was used to group the isolates. Sequence analysis confirmed it to be the North American genotype PRRSV. The full-length of the genome was 15,320 bp, not including the polyA tail. JL-04/12 sequences shared 99.1 % nucleotide identity with HP-PRRSV JXA1, and HUN4 sequences, while the nucleotide homology between JL-04/12 and classic isolates CH-1a and VR-2332 were 89.5 and 94.9 %, respectively. The homologies of the amino acid sequences in two nonstructural proteins and GP2 to GP4, between JL-04/12 and HUN4, were $\sim$ 97.2–99.3 %, and that of GP5 between JL-04/12 and JXA1 was 98.5 %. However, GP6 and N protein of JL-04/12 were identical to HP-PRRSV JXA1, TJ and HUN4 (100 % homology). Discontinuous deletions of 30 amino acids were found in JL-04/12 Nsp2. When

Table 1 PRRSV isolates mentioned in this study

Isolate name	Origin	Isolation or report date	Accession no.
JL-04/12	Jilin, China	2012	JX177644
TJ	Tianjin, China	2008	EF635006
JXA1	Jiangxi, China	2007	EF112445
HUN4	Hunan, China	2007	EF635006
CH-1a	Haerbin, China	1998	AY032626
VR-2332	USA	1993	U87392

comparing JL-04/12 with HP-PRRSV JXA1 and HUN4, the homology of Nsp2 was 97.8 % for both. Phylogenetic analysis showed that JL-04/12 belonged to the HP-PRRSV cluster. In other words, the isolate was a variant of the highly pathogenic porcine reproductive and respiratory syndrome virus, which derived from strains in 2006–2008. The origin and GenBank No. are shown in Table 1.

PRRS is an epidemic disease in swineherds of China. Since its emergence, the highly pathogenic PRRS has caused enormous economic losses [8]. With improved preventative measures and new vaccines, the disease is controlled in most areas. However, some areas still have occasional morbidity. Although numerous cases of highly pathogenic PRRS have been reported in China, virus isolates have been reported only in several provinces. The present study is the first to report HP-PRRSV isolated from pigs in the Jilin province of China.

PRRSV is difficult to isolate using subcultured cells. In fact, the European PRRSV was the first PRRSV virus to be isolated using not subcultured cells, but primary porcine pulmonary macrophages [9]. In 1996, Guo et al. [3] reported the first isolation of PRRSV on porcine alveolar macrophages and Marc-145 cells in China. In the present study, PRRSV JL-04/12 was more efficiently isolated from a MARC-145 cell line. The morphology and gene characteristics of the PRRSV JL-04/12 strain were similar to those of HP-PRRSV that have emerged since 2006. The virus titer of passage 6 was $10^{6.25}$ /mL of TCID₅₀. Compared with JXA1 and HUN4, JL-04/12 ORF1a and ORF2-5 were variable, while ORF6 and ORF7 contained no changes, indicating that highly pathogenic strains evolved to have variation in ORF1a and ORF2-5. PRRSV is an RNA virus with a high rate of genetic variation, and Nsp2 is a highly variable gene of PRRSV [10]. HP-PRRSV, which has recently emerged, is also an Nsp2 variant strain. Variation of the Nsp2 gene in PRRSV evolution may play a prominent role in pathogenesis. The PRRSV JL-04/12 strain in the present study, like other prevalent HP-PRRSV, has a discontinuous 30 amino acid deletion in Nsp2. Irregular base and amino acid mutations were scattered in the whole genome. Though the genes and amino acids of

the PRRSV JL-04/12 strain are similar with other HP-PRRSV, the pathogenicity of the virus in animals needs to be investigated further.

In summary, the present study indicates that highly pathogenic porcine reproductive and respiratory syndrome virus still exists in the Jilin province of China to date. Thus, HP-PRRSV is still a potential health risk to swineherds.

Acknowledgments This study was supported by the grant of National “Twelfth Five-Year” High Technology Research and Development Program (2011AA10A213), innovation team for special economic animal diseases prevention and control research in Jilin province (No. 20121823), and standard of epidemic diseases in wild animals and tracking study (OIE 2012).

References

1. Albina E. Epidemiology of porcine reproductive and respiratory syndrome (PRRS): an overview. *Vet Microbiol.* 1997;55:309–16.
2. Allende R, Lewis TL, Lu Z, Rock DL, Kutish GF, Ali A, Doster AR, Osorio FA. North American and European porcine reproductive and respiratory syndrome viruses differ in non-structural protein coding regions. *J Gen Virol.* 1999;80(Pt 2):307–15.
3. Guo B-Q, Chen Z-S, Liu W-X, Cui Y-Z. Isolation and identification of porcine reproductive and respiratory syndrome (PRRS) virus from aborted fetuses suspected of PRRS. *Chin Poult Infect Dis.* 1996;1–5.
4. Keffaber K. Reproductive failure of unknown etiology. *Am Assoc Swine Pract Newslett.* 1989;1:1–10.
5. Neumann EJ, Kliebenstein JB, Johnson CD, Mabry JW, Bush EJ, Seitzinger AH, Green AL, Zimmerman JJ. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. *J Am Vet Med Assoc.* 2005;227:385–92.
6. Ropp SL, Wees CE, Fang Y, Nelson EA, Rossow KD, Bien M, Arndt B, Preszler S, Steen P, Christopher-Hennings J, Collins JE, Benfield DA, Faaborg KS. Characterization of emerging European-like porcine reproductive and respiratory syndrome virus isolates in the United States. *J Virol.* 2004;78:3684–703.
7. Snijder EJ, Meulenbergh JJ. The molecular biology of arteriviruses. *J Gen Virol.* 1998;79(Pt 5):961–79.
8. Tian K, Yu X, Zhao T, Feng Y, Cao Z, Wang C, Hu Y, Chen X, Hu D, Tian X, Liu D, Zhang S, Deng X, Ding Y, Yang L, Zhang Y, Xiao H, Qiao M, Wang B, Hou L, Wang X, Yang X, Kang L, Sun M, Jin P, Wang S, Kitamura Y, Yan J, Gao GF. Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. *PLoS ONE.* 2007;2(6):e526.
9. Wensvoort G, Terpstra C, Pol JM, ter Laak EA, Bloemraad M, de Kluyver EP, Kragten C, van Buiten L, den Besten A, Wagenaar F, et al. Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *Vet Q.* 1991;13:121–30.
10. Yoshii M, Kaku Y, Murakami Y, Shimizu M, Kato K, Ikeda H. Genetic variation and geographic distribution of porcine reproductive and respiratory syndrome virus in Japan. *Arch Virol.* 2005;150:2313–24.
11. Zhou Z, Ni J, Cao Z, Han X, Xia Y, Zi Z, Ning K, Liu Q, Cai L, Qiu P, Deng X, Hu D, Zhang Q, Fan Y, Wu J, Wang L, Zhang M, Yu X, Zhai X, Tian K. The epidemic status and genetic diversity of 14 highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) isolates from China in 2009. *Vet Microbiol.* 2011;150:257–69.