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Genetic diversity and phylogenetic analysis of porcine reproductive and respiratory syndrome virus isolates in East China

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) has been reported to have evolved at a high evolutionary rate and the extensive genetic variation. In this study, 44 PRRSV positive cases obtained from different provinces of China were sequenced and analyzed. Comparative analysis of partial isolates based on nsp2 sequences revealed that highly pathogenic PRRSV were the dominant viruses in China from 2008 to 2010 and some novel strains with an extra deletion of 19 aa. Phylogenetic analysis based on the GP5 genes showed that the PRRSV isolates from 1996 to 2010 had a great variation and the North American genotype was further divided into six subgenotypes. No apparent relationship between the heterogeneity and the geographic origin of isolates was observed. The 44 isolates and 29 representative strains were divided into six subgenotypes. Further analysis of the GP5 protein suggested that these strains of subgenotypes I, II and III exhibited variations in the primary neutralizing epitope and almost all isolates of subgenotypes II and III had more N-linked glycosylation sites. In addition, some mutations which could mirror the viral evolution and adaptation were also observed in this study. All these results might be useful to study the genetic variation and genetic relatedness among PRRSV strains in China.

1. Introduction

Porcine reproductive and respiratory syndrome was first described in 1987 (Keffaber, 1989) in the United States and identified in 1991 in the Netherlands (Wensvoort et al., 1991). Now, it is worldwide and the most economically significant viral disease affecting the swine industry (Neumann et al., 2005). The disease causes severe reproductive disorders in gilts and sows and respiratory syndrome in pigs of all ages (Keffaber, 1989; Bilodeau et al., 1991; Collins et al., 1992).

PRRSV, the causative agent of PRRS, is a small enveloped, single-stranded, nonsegmented and positive sense RNA virus, which belongs to the order *Nidovirales*, family *Arteriviridae* (Cavanagh, 1997; Benfield et al., 1992; Meulenberg et al., 1993). PRRSV genome is about 15 kb in size, which comprises at least ten mostly overlapping open reading frames (ORFs) (Conzelmann et al., 1993; Meulenberg et al., 1993; Stadejek et al., 2002). ORF1a and ORF1b comprise two-third of the whole genome, encode non-structural proteins which responsible for the replication (Snijder and Meulenberg, 1998; Snijer et al., 2001; Allende et al., 1999). Nsp2, the largest replicative protein of PRRSV, is the highly variable gene of PRRSV and has experienced the deletion, insertion and point mutations (Yuan et al., 2001; Fang et al., 2004; Gao et al., 2004; Han et al., 2006; Shen et al., 2000; Tian et al., 2007). Nsp2 is so diverse as to be the ideal marker for monitoring genetic variation (Music and Carl, 2010; Zhou et al., 2009a,b). ORF2a and ORFs 3-4 encode three N-glycosylated minor envelope proteins GP2a, GP3 and GP4. ORF2b and ORF6 encode the nonglycosilated membrane proteins (GP2b, M) (Wu et al., 2001) and ORF7 encodes the nucleocapside protein N which is the immunodominant protein. The major envelope glycoprotein (GP5) which forms a heterodimer with M is encoded by ORF5 (Mardassi et al., 1996). GP5 is the most variable structural gene of PRRSV, which contains the immunologically domains correlated with virus neutralization and it is crucial for virus infectivity (Pizadeh et al., 1998;Wissink et al., 2003; Plagemann, 2004).

PRRSV is further divided into two major genotypes, the North American type (NA, type 2) and the European type (EU, type 1), between which show a highly antigenical and genetical divergence (Nelsen et al., 1999; Meng, 2000; Key et al., 2001; Batista et al., 2002). Based on the highest degree of genetic diversity, the sequence of ORF5 has been determined to investigate the genetic diversity and used for diagnostic identification of PRRSV (Kapur et al., 1996; Andreyev et al., 1997; Dee et al., 2001; Cha et al.,

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Table 1
Forward and reverse primers used for detection and amplification the whole sequences of Nsp2 and ORF5 according to the genome sequences of PRRSV (accession number:EU708726). Prefixes: F, forward PCR primer; R, reverse PCR.

Name	Sequence (5′–3′)	Use
STF	ATGACGTATAGGTGTTGGC	RT-PCR
SR2949	CTGGTGCGTCAGCGTTGTTGTC	RT-PCR
SF2230	GCGAATCAGACAACCGAACAACC	RT-PCR
SR6670	ACACCCCTTCCCTCAACTTTCCCTC	RT-PCR
SFORF5	GTTTACCCAACGCTCCTTA	RT-PCR
SRORF5	ACTGGCGTGTAGGTAATGG	RT-PCR

2004). Lots of studies on the genetic variation of GP5 indicated that the genetic divergences within one type have increased in different countries (Stadejek et al., 2006; Cha et al., 2006; Indik et al., 2000; An et al., 2007; Kim et al., 2010) which may affect the vaccination efficacy. In addition, the presence of vaccine virus-like sequences also increase the difficulty of controlling PRRSV.

Since June 2006, a highly pathogenic PRRSV (HP-PRRSV) with a discontinuous deletion of 30-amino-acid has been emerging in China (Tian et al., 2007; An et al., 2007; Zhou et al., 2009a,b; Zhang et al., 2008; Lv et al., 2008; Tong et al., 2007). Shortly after, the HP-PRRSV has been described in neighboring countries including Vietnam (Feng et al., 2008; Normile, 2008), Russia (Kukushkin et al., 2008), and the Philippines (Normile, 2009), causing disastrous economic losses to the swine industry. Recently, some reports indicated that there are six subgenotype isolates of PRRSV in China and HP-PRRSV is the dominant virus during 2006–2008 (Zhou et al., 2009a,b). The aim of this work is to detect and evaluate the genetic characteristics and diversity of PRRSV isolates in China. We analyzed the nsp2 and ORF5 gene sequences of 44 PRRSV isolates spreading over 2008–2010. By comparing their nsp2, ORF5 sequences with other PRRSV isolates, we also explore the genetic relationships among different isolates in China.

2. Materials and methods

2.1. Field isolates

A total of 370 samples (serum, semen, lung, kidney and lymph node) from clinical pigs, were collected from a number of farms in different parts of China from 2008 to 2010. All samples experienced the RNA extraction and the remaining samples were stored at −70 °C. RNAs were kept at −70 °C or used immediately for the present work.

2.2. RT-PCR and nucleotide sequencing

For reverse transcription, 2 μl of the anchored primer SRA (5 pmol) were added to 10.5 of RNA and incubated at 65 °C for 5 min. Then the mixture was chilled on ice and thereafter added to a 7.5 μl mixture containing 5× reaction buffer, RNase inhibitor, 10 mM of each dNTP and 200u of Revert™AidM-MuLV Reverse Transcriptase (Fermentas, EU). Then the mixture was incubated 60 min at 42 °C and terminated this reaction by heating at 70 °C for 10 min. We designed a "diagnostic" primer (Table 1) pair to amplify the partial fragment of nsp2 including the discontinuous deletion of 30-amino-acid which is the gene marker of HP-PRRSV and to be used for detecting PRRSV. Otherwise, this primer pair can also differentiate the classical PRRSV and the present popular HP-PRRSV strain. The positive samples were further subject to amplifying the nsp2 and ORF5 gene by using two specific primers (Table 1). The RT-PCR amplicons were gel-purified (Axygen) and cloned into PMD-18T vector (Takara), and the clones were screened by electrophoresis in 1% agarose and restriction enzyme mapping and sequenced using the corresponding PCR primers.

2.3. Analysis of the sequence data

The nsp2 and ORF5 sequences (Table 2) we obtained were edited with the lasergene sequence analysis software (DNAStar 7.0, USA) and independently analyzed by comparing with other sequences (Table 3) available in GenBank including the EU-type strains lelystad, the NA-type stains VR-2332, the MN184C, the RespPRRSMLV and other published China strains. Multiple sequence alignment was done using the ClustalX 1.83 and DNAStar 7.0 and phylogenetic analyses were carried out in MEGA 4.1.

Table 2
PRRSV cases sequenced in this study from Republic of China. Each isolate was named according to the origin and collection year.

No.	Isolate	(Accession no.)		No.	Isolate	(Accession no.)	
		Nsp2 (aa)	ORF5 (aa)			Nsp2 (aa)	ORF5 (aa)
1	ZJ0807	950 (KF562328)	201 (KF562306)	23	JS1007	950 (KF562322)	201 (KF562295)
2	ZJ0908	950 (KF562329)	201 (KF562309)	24	JS1006		201 (KF562294)
3	ZJ1005		201 (KF562311)	25	SD0907	950 (KF562325)	201 (KF562301)
4	AH0810	950 (KF562313)	201 (KF562269)	26	SHH0905		201(KF562304)
5	AH0812		201 (KF562270)	27	AH1006	931 (KF562314)	201 (KF562275)
6	JS0905		201 (KF562282)	28	SD1004	950 (KF562326)	201 (KF562302)
7	JS0904	950 (KF562317)	201 (KF562281)	29	AH1005		201 (KF562274)
8	JS0906		201 (KF562283)	30	SHH0907	950 (KF562327)	201 (KF562305)
9	JS0812	950 (KF62316)	201 (KF562280)	31	JX1004		201 (KF562299)
10	AH0905		201 (KF562271)	32	FJ0911	950 (KF562315)	201 (KF562277)
11	AH0807	950 (KF562312)	201 (KF562268)	33	AH0911		201 (KF562273)
12	JS0808	950 (KF562324)	201 (KF562279)	34	AH0910		201 (KF562272)
13	JS0907		201 (KF562284)	35	JS1001	950 (KF562319)	201 (KF562290)
14	JS0908		201 (KF562285)	36	SD1006		201 (KF562303)
15	JS1002	931(KF562320)	201 (KF562291)	37	ZJ0905		201 (KF562307)
16	JS1003		201 (KF562292)	38	JS1005	931 (KF562321)	201 (KF562293)
17	JS0912	931 (KF562318)	201 (KF562289)	39	AH1011		201 (KF562276)
18	JS0909		201 (KF562286)	40	ZJ1004		201 (KF562310)
19	JS0910		201 (KF562287)	41	SD0905		201 (KF562300)
20	JS0911		201 (KF562288)	42	ZJ0907		201 (KF562308)
21	JX0907		201 (KF562297)	43	JSzz1008		201 (KF562296)
22	JX0909	950 (KF562323)	201 (KF562298)	44	JS0806		201 (KF562278)