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Short Communication

Re-emerging of porcine respiratory and reproductive syndrome virus (lineage 3) and increased pathogenicity after genomic recombination with vaccine variant

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ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) was first reported in China since late 1995 and several variants were further reported in subsequence years, causing huge economic losses to the Chinese swine industry. To date, three major lineages (lineage 3, 5.1 and 8.7) of Type 2 PRRSV were reported in China based on our global genotyping. The present study provides the epidemiology of the PRRSV in South China based on the isolates collected during 2009–2012, indicating three lineages (lineage 3, 5.1 and 8.7) of Type 2 PRRSV were still circulating in this area. Our phylogenetic reconstruction indicated that lineage 3 re-emerged in 2010 formed a huge cluster with closely related to the 2004 isolates from Hong Kong. Furthermore, the inter-lineage genomic recombination between MLV vaccine strain (lineage 5) and a recently re-emerged lineage 3 virus (QYYZ) has also been found in a farm practicing MLV vaccination. Our *in vivo* experiment comparing the pathogenicity and clinical presentations among currently isolated viruses indicated that pigs infected with recombinant lineage 3 virus (GM2) showed persistent higher fever compared to pigs infected by its wild counterpart (QYYZ). This study enhanced our understanding on potential importance of the recombination of PRRSV along with their evolution.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a major swine disease causing significant economic losses in the global swine industry (Neumann et al., 2005; Zhou and Yang, 2010). The causative agent, PRRS virus (PRRSV) is a small enveloped, positive single-stranded RNA virus belonged to the family *Arteriviridae* under the order *Nidovirales* (Cavanagh, 1997). The 5′-capped and 3′-polyadenylated genome of PRRSV is about 15.4 kb in length and contains at least 10 ORFs (Johnson et al., 2011). The ORF1a and ORF1b comprise approximate 75% of the viral genome. At least 14 non-structural proteins (nsp) are generated by the proteolytic processing of pp1a and pp1ab

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encoded by ORF1a and ORF1b, of which involve in processing of the viral polyproteins, genome replication, and transcription. The ORF2a, ORF2b, ORF3–7 encode seven viral structure proteins (Lunney et al., 2010; Meulenberg, 2000). An additional novel structural protein encoded by ORF5a was identified to be importance for PRRSV replication (Johnson et al., 2011). Regarding genetic diversity, PRRSV is mainly classified into European type (Type 1) and American type (Type 2) with approximately 60% of genome sequence homology (Meng et al., 1995; Nelsen et al., 1999), and up to 20% of genetic variation within each genotype (Han et al., 2006).

The prodigious genetic diversity of PRRSV had been reported in the field, perhaps related to the viral recombination and mutation (Shi et al., 2010a). The first outbreak of PRRS in China was documented in 1995 and the epidemic strain named CH-1a was isolated in 1996. Since then, PRRS becomes one of the most severe viral diseases in Chinese swine industry. In May 2006, the highly pathogenic PRRSV with a unique discontinuous deletion of 30 amino acids in nsp2 emerged in China and affected more than 20 million pigs (Zhou & Yang, 2010). Based on the global genotyping, three lineages (lineage 3, 5.1 and 8.7) of Type 2 PRRSV were reported in China (Shi et al., 2010b). Among these three lineages, only lineage 8.7 was the most significant variant due to their clinical severity to the hosts. Although lineage 3 viruses were previously reported in China, Hong Kong and Taiwan, no isolation record is found in China after 2005. This may be due to clinically less important of this lineage in the file. Here, we present the re-emerging of the lineage 3 viruses in Southern China during 2010. Subsequently, we identified inter-lineage genomic recombination between MLV vaccine virus (lineage 5) and recently re-emerged lineage 3 virus in a farm practicing MLV vaccination. We also performed *in vivo* experiment to compare the pathogenicity and clinical presentations of this inter-lineage recombinant virus and compared with their parental viruses (MLV, and QYYZ) as well as the high fever virus (QY1) isolated from Southern China.

2. Materials and methods

2.1. Sample collection and virus isolation

Serum and tissue samples of pigs were collected from the farm with 2.2% mortality rate in young pigs. Virus was initially isolated from the positive sera on Marc-145 cells as described by Zhu (Zhu et al., 2011). The details of the isolated viruses used in the *in vivo* experimentations were shown in Table 1. The QY1 strain was isolated from the high fever outbreak in 2007, and other two strains QYYZ and GM2 were newly isolated from Guangdong province of China during 2010–2011.

2.2. PRRSV genome sequencing and recombination analysis

To avoid the genetic drift happened during virus isolation process, total viral RNA was extracted from serum and infected cell culture separately with TRIzoL reagent (Invitrogen, US) according to the manufacturer's instructions. Both the reverse transcription and the polymerase chain reaction (RT-PCR) were conducted using Prime Script One Step RT-PCR Kit (TaKaRa, Japan). To sequence the full-length genomes of GM2 and QYYZ strains, primers were first selected based on the known nucleotide sequence of strain VR-2332 described by Zhu et al. (2011) and two alternate primers pairs synthesized based on newly obtained GM2 sequences were employed (Table 2). A 3′-full RACE kit and RNA PCR Kit (AMV) Ver. 3.0 (TaKaRa, Japan) were used to amplify the 3′ UTR of these strains according to instructions of the manufacturer.

PCR products were purified and clone into pMD19-T vector (TaKaRa), at least three clones were sent for sequencing (BGI Ltd) in both directions for each fragment. Sequences were assembled and analyzed by using the DNAStar lasergene v.7.2. Multiple-sequence alignments were generated with ClustalX (Jeanmougin et al., 1998). Recombination events were detected using the Recombination Detection Program (RDP4) v.4.1.3 (Martin et al., 2010). Putative recombination breakpoints were identified

Table 1
Virus used in this study.

	Virus name		
	QY1	QYYZ	GM2
Isolation date	2007	2010	2011
Accession	JN387271	JQ308798	JN662424
Clinical signs	High fever and high mortality	Poor growth performance and diarrhea	Poor growth performance and herds with about 2.2% mortality
Cell passage times	7	3	3

Table 2
Additional primers used in genome sequence.

Fragment	Primer	Sequence (5′–3′)	Position
1	F4389	5′-GCGGTATCTGGCAGTTTATGCTTAGGCT-3′	4389–6450
	R6450	5′-AGACGAAGAACCCCACGGCAACTAAT-3′	
2	F12842	5′-CTTCTTGTACTCTGTTCGTAGTG-3′	12,842–13,880
	R13880	5′-TTCAGACAGCCAACAGAATAGC-3′	