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Characterization of two newly emerged isolates of porcine reproductive and respiratory syndrome virus from Northeast China in 2013

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ABSTRACT

A newly emerged porcine reproductive and respiratory syndrome virus (PRRSV) that has caused severe reproductive losses in sows appeared in some regions of China in 2013. To explore the biology of this new PRRSV and understand more fully genetic diversity in PRRSV isolates from China, the complete genome of the two 2013 Chinese isolates, designated HLJA1 and HLJB1, were analyzed. Genomic sequence analysis showed that HLJA1 and HLJB1 shared 88.6–98.3% nucleotide identity with genotype 2 (North American type, NA-type) isolates, but only 61.1% with the genotype 1 (European type, EU-type) isolate of Lelystad virus, indicating that both these isolates belong to the NA-type PRRSV genotype. Phylogenetic analysis showed that the NA-type PRRSV isolates formed three subgroups (1, 2 and 3); representatives of these subgroups are VR-2332, CH-1a and HUN4, respectively. HLJA1 and HLJB1 belong to subgroup 2. Analysis of NSP2 revealed that HLJA1 has a 48-amino acid deletion at positions 473–480 and 482–521, unlike other HP-PRRSV isolates, while HLJB1 has only a 1-amino acid deletion at position 481 compared with CH-1a. Interestingly, HLJA1 replicated in PAM cells but not in MARC-145 cells, whereas HLJB1 replicated in both cell types. The neutralizing antibody titer of pig hyperimmune sera against HUN4 was significantly higher than that of HLJA1 or HLJB1. Additionally, genetic variability in GP5 and GP3 proteins and in the novel ORF5a protein was evident. In addition to elucidating the genetic relationships between PRRSV isolates, our results suggest that Chinese PRRSV will remain a pandemic virus.

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1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is currently one of the most economically important diseases affecting the swine industry worldwide (Neumann et al., 2005). The pathogenic PRRS virus (PRRSV), which causes reproductive failure in sows and respiratory disease in pigs of all ages, emerged in North America and Europe almost simultaneously between 1980 and 1990 (Benfield et al., 1992; Wensvoort et al., 1991). PRRSVs, which are divided mainly into the genotype 1 (European type, EU-type) and genotype 2 (North American type, NA-type), share only about 60% nucleotide sequence identity with each other. Representative strains of the two distinct genotypes are Lelystad virus (LV) and VR-2332 (Benfield et al., 1992; Wensvoort et al., 1991). PRRSV is an enveloped single-stranded positive-sense RNA virus belonging to the Arteriviridae family within the Arterivirus genus. The family also contains equine arteritis virus (EAV), lactate dehydrogenase-elevating virus (LDV) of mice and simian hemorrhagic fever virus (SHFV) (Cavanagh, 1997). The PRRSV genome is approximately 15 kb and contains at least nine open reading frames (ORFs), a 5′ nontranslated region (UTR) and a 3′UTR (Meulenberg et al., 1997). ORF1a and ORF1b encode 13 nonstructural proteins (NSPs) involved in viral replicase polyproteins, while ORF2a, ORF2b, and ORFs 3–7 encode the viral structural proteins, GP2, E, GP3, GP4, GP5, M, and N, respectively (Meulenberg et al., 1997). Recently, a novel ORF overlapping the GP5 coding sequence was discovered (Firth et al., 2011; Johnson et al., 2011).

In June 2006, a highly pathogenic PRRSV (HP-PRRSV) was reported as the main cause of porcine high-fever disease associated with high mortality in China (Tong et al., 2007). Currently, the virus is endemic in China and Southeast Asian countries (An et al., 2011). A 30-amino acid deletion in the NSP2 region of the virus has been used for molecular characterization of HP-PRRSV strains in China since 2006. To further explore the epidemic status of PRRS and genetic diversity in PRRSV, we describe herein the complete genomic sequence and genetic relationships with other PRRSV isolates of two Chinese PRRSV isolates that emerged in 2013. Additionally, we examined the ability of the virus to replicate in porcine alveolar macrophage (PAM) and MARC-145 cells and examined antigenic differences affecting neutralizing antibody reactivity of the two isolates. The amino acid sequences of NSP2, GP5, GP3 and ORF5a of the two isolates were fully analyzed.

2. Materials and methods

2.1. Clinical samples

Clinical samples including lung, lymph nodes, and sera were collected from suspected cases of PRRSV in pigs from different pig farms in the Heilongjiang province of Northeast China in 2013. Clinical tissues were homogenized in Dulbecco's modified Eagle's medium (DMEM; Gibco) using TissueLyser II (Qiagen, Germany) for RNA extraction and virus isolation, and the remaining samples were kept at −70 °C.

2.2. Virus isolation

Virus isolation was conducted as described previously (Zhu et al., 2010). The tissue homogenates and sera were centrifuged at 10,000 × *g* for 15 min. The suspension was passed through a 0.22-μm filter and transferred to MARC-145 and PAM cells. The cells were then incubated at 37 °C for 3–5 days and monitored daily for cytopathic effects (CPE). The culture supernatants were harvested when CPE appeared and were stored thereafter at −70 °C as viral stocks.

2.3. Indirect fluorescent antibody assay (IFA)

IFAs were conducted as previously described (Leng et al., 2012). Viral antigens were prepared by inoculating MARC-145 or PAM cells with PRRSV HUN4 and the newly isolated PRRSV isolates. A specific monoclonal antibody against the PRRSV M protein (prepared by our laboratory) and goat anti-mouse IgG antibody conjugated with FITC (Sigma, USA) were used as the primary and second antibodies, respectively.

2.4. Virus cross-neutralization assay

Viral cross-neutralization assays were performed as described previously (Leng et al., 2012). Briefly, sera were diluted using a twofold serial dilution technique in RPMI-1640 medium (Gibco). A 100-μL aliquot of each diluted sample was mixed with an equal volume of each virus at a rate of 10^3 tissue culture infective doses $(TCID)_{50}$/mL. Mixtures were incubated for 1 h at 37 °C and then each mixture was inoculated into PAM cells prepared in 96-well Plates 7 h earlier. Thereafter, the cells were incubated at 37 °C for 5 days. The presence of virus-infected cells in each well was determined by IFA. The neutralizing antibody (NA) titers of the sera against the different PRRSV isolates were calculated using the Reed–Muench method (Reed and Muench, 1938).

2.5. Primer design and RT-PCR

Tissue homogenates and sera samples were prepared for reverse transcription PCR (RT-PCR) using an RNA extraction QIAamp viral RNA Mini Kit (Qiagen). RT-PCR was carried out with the One Step RT-PCR kit (Qiagen) according to the manufacturer's instructions. Eight-microliter aliquots of the RNA template were added to 42 μL of the RT-PCR master mix. PCR was performed with the primer sets shown in Table 1. RT-PCR conditions comprised 95 °C for 5 min, 30 cycles of denaturation (95 °C for 30 s), annealing (56 °C for 30 s), and extension (72 °C for 3 min), followed by a final extension at 72 °C for 10 min. According to results from the RT-PCR assays and viral isolation attempts, we selected two representative samples from two independent pig farms to determine the full-length genomic sequences of PRRSV. The two PRRSV isolates were designated HLJA1 and HLJB1. Primers were designed based on the published known sequence of HP-PRRSV HuN4 (GenBank accession no. EF635006).