Genomic characterization of emergent pseudorabies virus in China reveals marked sequence divergence: Evidence for the existence of two major genotypes

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ABSTRACT

Recently pseudorabies outbreaks have occurred in many vaccinated farms in China. To identify genetic characteristics of pseudorabies virus (PRV) strains, we obtained the genomic sequences of PRV strains HeN1 and JS, which were compared to 4 PRV genomes and 729 partial gene sequences. PRV strains isolated in China showed marked sequence divergence compared to European and American strains. Phylogenetic analysis revealed that for the first time PRV can be divided into 2 distinct clusters, with Chinese strains being genotype II and PRVs isolated from other countries being genotype I. Restriction fragment length polymorphism analysis confirmed differences between HeN1 and Bartha strains, as did the presence of unique insertion/deletion polymorphisms and microsatellites. This divergence between the two genotypes may have been generated from long-term, independent evolution, which could also explain the low efficacy of the Bartha vaccine in protecting pigs infected with genotype II PRV.

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Introduction

Pseudorabies (PR), also known as Aujeszky's disease, causes significant economic losses to the swine industry and has been intensively investigated by researchers across the globe. PR is characterized by reproductive losses in sows and respiratory and nervous disorders and high mortality in piglets (Lee and Wilson, 1979). Pseudorabies virus (PRV), the causative agent of PR, is taxonomically classified in the *Herpesviridae* family, the *Alphaherpesvirinae* subfamily, and the *Varicellovirus* genus (Pellett et al., 2011). Although PRV can infect most mammals except for humans and other higher primates (Mettenleiter, 2008), pigs are the only known natural reservoir of PRV; hence the name suid herpesvirus 1 (SuHV-1).

PRV infection has spread throughout the world and has recently become the focus of worldwide eradication programs. As a result of the vaccination and DIVA (differentiating infected from vaccinated animals) strategy, PRV is nearly eradicated from domestic pigs in several countries, mostly in Europe, the United States, and New Zealand, with only the occurrence of sporadic outbreaks (Hahn et al., 2010; MacDiarmid, 2000; Muller et al., 2003). China is responsible for 46% of global pork production, making it the largest producer of pork products in the world. In 2008, the number of slaughtered pigs and penned pigs was 610 million and 460 million, respectively (Wan, 2011). The earliest report of a PRV outbreak in China occurred in 1947, and since this time the virus has spread throughout the country (Tong and Chen, 1999). Since the 1990s, more than 80% of pigs in China have been vaccinated with the Bartha-K61 vaccine. Despite these efforts, in late 2011, a PR outbreak occurred in northern and eastern China in Bartha-K61 vaccinated farms, characterized by neurological symptoms and high mortality in newborn piglets (An et al., 2013). Subsequent reports indicated that the magnitude of the outbreaks have continued to rise in China (Wu et al., 2013; Yu et al., 2014). Previously we isolated a circulating PRV strain (HeN1) from a Bartha-K61 vaccinated pig farm and confirmed that Bartha-K61

vaccine did not provide effective protection against PRV HeN1 infection (An et al., 2013). We also found that inoculating 35-day-old piglets with HeN1 at a dose of $1 \times 10^{6.0}$ TCID$_{50}$ per piglet showed 100% lethality (unpublished data).

Restriction fragment length polymorphism (RFLP) analysis and a modified multiplex PCR method coupled with RFLP analysis were successively developed and used to differentiate the genotypes of PRV (De-Giuli et al., 2002; Paul et al., 1982; Takayama et al., 1996).

Among the numerous restriction endonucleases tested, *Bam*H I has been most widely used for RFLP analysis, as shown in several reports mainly studying strains isolated from Europe, Japan, Argentina, and Brazil (Muller et al., 2010; Nishimori et al., 1987; Piatti et al., 2001; Serena et al., 2011). In a previous study, PRV isolates from European and American countries were divided into 4 major types and several subtypes by RFLP analysis, but Chinese strains were not included (Herrmann et al., 1984). However, RFLP

Fig. 1. Genomic organization of the HeN1 PRV strain and comparison with the conserved regions within the Kaplan, Becker, Bartha, TJ, and JS strains. (A) ORFs (horizontal bars in gray color) together with internal and terminal repeats (horizontal bars in orange color) are depicted along the genome. (B) Plots showing sequence conservation among PRV HeN1, Kaplan, Becker, Bartha, TJ, and JS. Gene conservation was determined from a multiple sequence alignment, and the conservation score between any 2 genomes is plotted in a sliding 100 bp window. (C) SSRs are represented as they occurred along the PRV HeN1 genome, including minisatellites (repeat unit ≥ 10 bp), microsatellites (repeat unit < 10 bp), and homopolymers (minimum length 6). (D) A phylogenetic tree based on whole-genome multiple sequence alignment that reflects the divergence observed between PRV HeN1, Kaplan, Becker, Bartha, TJ, and JS. Bootstrap values are shown at node points.