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Dynamics and evolution of highly pathogenic porcine reproductive and respiratory syndrome virus following its introduction into a herd concurrently infected with both types 1 and 2

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ABSTRACT

Since its first emergence in Thailand in late 2010, highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) has caused sporadic outbreaks on Thai swine farms. The objective of this study was to investigate the dynamics and evolution of PRRSV in a herd experiencing an HP-PRRSV outbreak. Following its introduction, HP-PRRSV caused severe outbreaks and subsequently established persistent infection in the herd, resulting in the emergence of a novel cluster of type 2 (North American, NA) isolates. HP-PRRSV co-existed with type 1 (European, EU) isolates without influencing their development. In contrast, HP-PRRSV influenced the evolution of the type 2 (NA) isolates by increasing diversity through the addition of a novel cluster and influencing the evolution of other viral clusters previously existing in the herd. Recombination between the endemic and emerging isolates was observed. The recombinants, however, disappeared and were not able to survive in the herd. The results of this study suggest that the introduction of HP-PRRSV to a herd results in an increased diversity of genetically related isolates and persistent HP-PRRSV infection.

1. Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes a disease characterized by reproductive disorders in sows, including abortion, reduced numbers of weaned pigs, increased numbers of stillborn pigs, mummified fetuses and weak pigs, as well as respiratory disorders in nursery to finishing pigs. PRRSV, an enveloped, positive-sense single-stranded RNA virus belonging to the *Arteriviridae* family, order *Nidovirales*, is the causative agent of the syndrome. The PRRSV genome consists of ten open reading frames (ORFs), including ORFs 1 to 7 (Meulenberg et al., 1997). ORF1a and ORF1b encode non-structural proteins (Nsp) that comprise approximately 80% of the entire genome of PRRSV. The other 6 ORFs (ORFs 2–7) encode six structural proteins. ORFs 2 through 5 of PRRSV encode glycoproteins (GP) 2 through 5, which are glycosylated proteins located in the viral envelope. Two additional structural proteins, E and ORF5a, have also been discovered (Johnson et al., 2011; Wu et al., 2001). ORF5 of PRRSV is the most variable region (Murtaugh et al., 1998), playing important roles in pathogenesis, including host cell entry and apoptosis (Sur et al., 1998), and it contains a neutralizing epitope (Gonin et al., 1999; Plagemann et al., 2002; Weiland et al., 1999; Wissink et al., 2003).

Two distinct genotypes of PRRSV, type 1 (or European, EU) and type 2 (or North American, NA), have been identified, and their genetics are markedly different. The two genotypes of PRRSV have been described as evolving independently on each continent (Nelsen et al., 1999). However, over the past few years, the co-existence of the two genotypes has been increasingly reported in several countries, including China and Korea (Chen et al., 2011; Kim et al., 2010; Lee et al., 2010; Nilubol et al., 2013b; Thanawongnuwech et al., 2004). Concurrent infection with both PRRSV genotypes has long been observed in Thai swine herds (Nilubol et al., 2013b; Thanawongnuwech et al., 2004). Both genotypes co-exist in the Thai swine population without influencing each other's development. At present, both genotypes have been continuously and separately evolving, resulting in the development of their own clusters separate from other Asian PRRSVs (Nilubol et al., 2013b).

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Since June 2006, highly pathogenic PRRSV (HP-PRRSV), a PRRSV variant with a unique hallmark of a discontinuous deletion of 30 aa in the Nsp2 region of its genome, has emerged in China (Tian et al., 2007). Following its emergence, the variant became dominant and caused sporadic outbreaks in China (Hu et al., 2009). Later, this PRRSV variant continuously spread and caused high pig mortality in many countries, including Vietnam, Lao PDR and Thailand (Feng et al., 2008; Ni et al., 2012; Nilubol et al., 2012). In addition to concurrent infection with both PRRSV genotypes, in early 2010, several Thai swine herds experienced severe outbreaks caused by HP-PRRSV (Nilubol et al., 2012). The disease spread throughout the country upon its introduction, and at present, it has developed into an endemic pathogen causing sporadic outbreaks in several herds. The co-existence of both PRRSV genotypes in conjunction with the introduction of HP-PRRSV into the country has raised questions concerning clinical severity, genetic diversity, and control methods. Studies involving genomic analysis are urgently needed to fully understand the molecular evolution of PRRSV following the introduction of HP-PRRSV into a herd in which both genotypes of PRRSV concurrently exist. Therefore, the objective of the present study was to investigate the dynamics and evolution of PRRSV in a herd experiencing an outbreak of HP-PRRSV, especially those of the ORF5 gene, which plays an important role in avoiding immune recognition by employing several mechanisms such as the addition of N-linked glycosylation at positions between the decoy and primary neutralizing epitopes. The results reported herein could provide an understanding of the genetic evolution of PRRSV in the herd following the introduction of novel isolate.

2. Materials and methods

2.1. Herd information

The study was conducted in a 1700-sow swine herd. The herd was selected based on the availability of sequence information, as the chosen herd was previously studied for the evolution of PRRSV following the introduction of a modified live PRRSV vaccine (MLV) (Nilubol et al., 2013a). In brief, the studied herd was maintained in a one-site farrow-to-finish production. There were six buildings designated for breeding, gestation, and farrowing for the breeding herd. Half of each building was designated for breeding and gestating activities, and the other half was designated for farrowing activity. The farrowing facilities operated all-in/all-out on a weekly basis, and there was 1 week of downtime between transitions. Four nursery facilities were adjacent to the breeding facilities, and each building was divided into two halves. Each half of the building operated all-in/all-out by week. Nursery pigs were moved at approximately 9 weeks of age to finishing facilities located 30 m away.

All sows were artificially inseminated on site using PRRSV-negative semen. PRRSV-free boar studs were housed at a location 5 km away from the breeding herd. Semen was PCR tested prior to insemination. Replacement gilts were mainly internally produced and housed with nursery and finishing pigs. These replacements were moved to a gilt developing unit located adjacent to the finishing facilities at 18 weeks of age and introduced to the breeding herd at 32–33 weeks of age.

Both types of PRRSV were detected in the studied herd. PRRSV control was conducted through the acclimatization of replacement gilts with culled multiparous sows and MLV vaccination against the type 2 (NA) genotype (Ingelvac PRRS MLV, Boehringer Ingelheim, St. Joseph, MO, USA). The vaccination protocol included vaccination of all sows in the herd every third month starting February 2010. Piglets were vaccinated intramuscularly once at 7–10 days of age. Replacement gilts were vaccinated three times at 20, 24 and 28 weeks of age. During the previous 3 years, the studied herd had no history of external gilt introduction. However, prior to the HP-PRRSV outbreak, grandparent gilts from an external source were imported into the studied herd to improve its genetic program. The gilts from the external source were housed with internally produced gilts. Soon after their introduction, signs of high fever and redness of the skin and ears were observed in internally produced gilts, and similar signs in conjunction with abortion and sow mortality were later observed in the breeding herd. The sick gilts and sows were examined, and HP-PRRSV was isolated.

2.2. Experimental design

The study was conducted from December 2010 to June 2013. Serum samples were randomly and cross-sectionally collected nine consecutive times (December 2010, March 2011, June 2011, September 2011, December 2011, March 2012, June 2012, December 2012, and June 2013). At each sampling time, 5 blood samples were collected from each of four population groups, including replacement gilts, suckling pigs, nursery pigs, and finishing pigs. Sera were separated and assayed for the presence of viruses by polymerase chain reaction (PCR).

2.3. PCR and sequencing

Total RNA was extracted from serum samples using Nucleospin® RNAVirus (Macherey-Nagel Inc., PA, USA) in accordance with the manufacturer's instructions. cDNA was synthesized from the extracted RNA using M-MuLV Reverse Transcriptase (New England BioLabs Inc., MA, USA). PCR amplification was performed on the cDNA. To amplify ORF5 of types 1 and 2 progeny viruses, previously reported primers (Nilubol et al., 2013a) were used, and PCR amplification was performed using GoTaq® Green Master Mix (Promega, WI, USA). Amplified PCR products were purified using a PCR purification kit (Macherey-Nagel, Germany). Sequence reactions were performed at Biobasic Inc. (Ontario, Canada) using an ABI Prism 3730XL DNA sequencer.

2.4. Sequence analyses

Identical ORF5 sequences were identified and removed. The non-redundant nucleotide and deduced amino acid sequences were aligned using the CLUSTAL W method (Thompson et al., 1994) implemented in MEGA v.6.06 (Tamura et al., 2013). To investigate the genetic relationship of these progeny viruses, phylogenetic analyses of the type 1 (EU) and 2 (NA) isolates were constructed separately. Each phylogenetic tree was constructed based on the non-redundant ORF5 sequences of PRRSV isolates collected 6 months prior to and up to 24 months after the HP-PRRSV outbreak. In addition, the type 1 (EU) prototype virus, Lelystad Virus (LV; accession number M96262), and the type 1 (EU) MLV (Porcilis PRRSV; MSD, Netherlands, accession number AY743931) were included in the analysis of the type 1 (EU) isolates. The type 2 (NA) prototype virus (VR-2332; accession number AY150564), its derived vaccine virus (Ingelvac PRRS MLV; Boehringer Ingelheim, USA, accession number AF066183) and HP-PRRSV reference isolates including JXA1 (accession number EF112445), HUN4 (accession number EF635006), WUH1 (accession number EU187484), and HUB2 (accession number EF112446) were incorporated into the phylogenetic analysis of the type 2 (NA) isolates. Neighbor-joining trees were also constructed from the aligned nucleotide sequences using MEGA v.6.06. A bootstrap resampling (1000 replicates) was used to assess the reliability of individual nodes for each phylogenetic tree. Multiple sequence alignments of the amino acid sequences were displayed using BioEdit. Pairwise sequence