J. Comp. Path. 2014, Vol. 150, 297–305

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EXPERIMENTALLY INDUCED DISEASE

Comparative Virulence of Reproductive Diseases Caused by Type 1 (European-like) and Type 2 (North American-like) Porcine Reproductive and Respiratory Syndrome Virus in Experimentally Infected Pregnant Gilts

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Summary

The aim of this study was to compare the virulence of type 1 and type 2 porcine reproductive and respiratory syndrome virus (PRRSV) as assessed by the level of viral replication, viral distribution and apoptosis in stillborn fetuses and live-born piglets from infected pregnant gilts. Type 1 or type 2 PRRSV was given intranasally to pregnant gilts at 3 weeks before the expected date of parturition. Regardless of virus genotype, PRRSV-infected gilts farrowed between 102 and 109 days of gestation, while control uninfected gilts carried the pregnancy to term and farrowed at 114–115 days of gestation. There were no significant differences in the mean number of virus-infected cells per unit area of tissue when type 1 and type 2 virus infections were compared between stillborn fetuses and live-born piglets. Stillborn fetuses from the type 1 PRRSV-infected pregnant gilts had a significantly higher mean number of apoptotic cells per unit area of thymus ($P = 0.013$) than those from type 2 PRRSV-infected pregnant gilts. Significant differences in virulence were not observed between types 1 and 2 PRRSV in terms of female reproductive failure, although thymic apoptosis differed in stillborn fetuses from type 1 and type 2 PRRSV-infected pregnant gilts.

Keywords: pathogenicity; porcine reproductive and respiratory syndrome virus; reproductive failure; virulence

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure in sows and respiratory disease in growing pigs (Zimmerman *et al.*, 2012). The causative agent of this syndrome is the PRRS virus (PRRSV), which was first isolated in the Netherlands in 1991 (Wensvoort *et al.*, 1991). A similar virus was also isolated in the USA at the same time (Collins *et al.*, 1992). It soon became clear that all PRRSV isolates in Europe were closely related and that all North American isolates were also closely related, though only distantly related to the European isolates (Allende *et al.*, 1999; Nelsen *et al.*, 1999; Murtaugh *et al.*, 2010). Genetic analysis has established the existence of two predominant PRRSV genotypes: type 1 (European-like) and type 2 (North American-like) (Allende *et al.*, 1999; Nelsen *et al.*, 1999). These genotypes are biologically and genetically distinct (Murtaugh *et al.*, 2010). Type 2 PRRSV has been isolated routinely on MA-104 simian cells and their subclone MARC-145, while type 1

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2013.11.205

PRRSV has been more difficult to adapt to a simian cell culture system (Murtaugh *et al.*, 2010).

The two genotypes also differ significantly in terms of their virulence in causing respiratory diseases of growing pigs; type 2 PRRSV isolates have been reported to be more virulent compared with those of type 1 (Halbur *et al.*, 1995; Martínez-Lobo *et al.*, 2011; Han *et al.*, 2012). In contrast, putative virulence differences between the two genotypes in the reproductive diseases of sows have yet to be proven experimentally, although early outbreaks of PRRS in Europe were more commonly associated with type 1 PRRSV infection of sows (van der Linden *et al.*, 2003). Hence, more work needs to be done to determine whether differences in virulence between the two genotypes exist in female reproductive diseases. The aim of this study was to compare the virulence of type 1 and type 2 PRRSV in experimentally infected pregnant gilts in terms of the sites of viral distribution and replication and apoptosis in stillborn fetuses and live-born piglets from these gilts.

Materials and Methods

Porcine Reproductive and Respiratory Syndrome Virus Isolates

Type 1 (SNUVR100058) and type 2 (SNUVR100059) PRRSV were used as inocula. The two viruses were isolated from the lung of aborted fetuses from different herds in 2010. SNUVR100058 and SNUVR100059 were identified as type 1 and type 2 PRRSV, respectively, on the basis of the nucleotide sequences of the open reading frame (ORF) 5 (GenBank accession numbers JX988617 for SNUVR100058 and JX988620 for SNUVR100059) and ORF7 (GenBank accession numbers JX988612 for SNUVR100058 and JX988615 for SNUVR100059).

Experimental Design

Twelve seronegative pregnant gilts were purchased from a PRRSV-free herd. Pregnant gilts were allocated randomly to an infected ($n = 10$) or control group ($n = 2$). Each of five pregnant gilts was inoculated intranasally with 6 ml of tissue culture fluid containing 10^4 tissue culture infective doses 50% ($TCID_{50}$)/ml of type 1 PRRSV (SNUVR100058, second passage in alveolar macrophages, group 1) or type 2 PRRSV (SNUVR100059, second passage in MARC-145 cells, group 2), respectively, at 3 weeks before the expected farrowing date. Two control pregnant gilts (group 3) were similarly exposed to uninfected cell culture supernatant. Each inoculum was instilled over a period of 4–5 min into both nostrils.

The gilts were housed separately in isolation facilities and allowed to farrow naturally, but the farrowing was attended. All live-born piglets were killed by an intravenous overdose of pentobarbital for tissue collection (lung, inguinal lymph node, heart, tonsil, thymus, liver and spleen) and evaluation. All expelled fetuses (mummified, dead and live-born) from all groups were subjected to necropsy examination and evaluated for gross lesions. Crown-to-rump measurements were used to determine the approximate time of fetal death during the gestation of mummified and dead fetuses (Ullrey *et al.*, 1965). The study was approved by the Seoul National University, Institutional Animal Care and Use Committee.

Virus Isolation

Tissue samples collected from stillborn fetuses and live-born piglets from all infected and negative control gilts were used for virus isolation. PRRSV was isolated from these organs as previously described (Cheon and Chae, 2000).

Sequence Analysis

PRRSV isolated from the fetal tissues was further analyzed for the ORF5 sequence. RNA was extracted from PRRSV-infected alveolar macrophages and MARC-145 cell lines (Cheon and Chae, 2000) and amplified from the ORF5 region by reverse transcriptase polymerase chain reaction (RT-PCR) (Oleksiewicz *et al.*, 1998). Sequencing was performed on the purified RT-PCR products of amplified ORF5.

Serology

Blood samples were collected from type 1 and type 2 PRRSV-infected and negative control gilts at 0, 7, 14 and 21 days post inoculation (dpi). Blood samples from live-born piglets at farrowing prior to feeding colostrum and thoracic fluid from stillborn fetuses were collected for serology. The serum samples and thoracic fluids were tested using the commercial PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS 2XR™, IDEXX Laboratories Inc., Westbrook, Maine, USA). Serum samples and thoracic fluids were considered positive for PRRSV antibody if the ratio between the value for the test sample and the positive control sample (S:P ratio) was >0.4 according to the manufacturer's instructions.

Quantification of Porcine Reproductive and Respiratory Syndrome Virus RNA

RNA was extracted from serum samples from PRRSV-infected and negative control gilts at 0, 7,