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Comparison of the virulence of European and North American genotypes of porcine reproductive and respiratory syndrome virus in experimentally infected pigs

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ABSTRACT

The objective of this study was to compare the virulence of Korean types 1 and 2 porcine reproductive and respiratory syndrome virus (PRRSV) isolated from weaned pigs with respiratory disease. Affected pigs were within the same herd and animals infected with type 2 virus had significantly higher mean rectal temperatures than those with type 1 virus between days 2 and 9 post-inoculation ($P < 0.05$). Similarly, mean serum viral titres, expressed as tissue culture infective doses 50% ($TCID_{50}$)/mL, as well as macroscopic and microscopic pulmonary lesion scores, were significantly higher at multiple time points in pigs infected with type 2 PRRSV compared to those infected with type 1 virus. Mean numbers of PRRSV-positive cells/unit area of lungs and lymph nodes were also significantly higher in type 2 PRRSV infected pigs. This study demonstrates that type 2 PRRSV is more virulent than type 1 PRRSV in this experimental setting as reflected by the pulmonary pathology induced, the extent of virus distribution, and oral shedding of the virus.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is characterised by late-term abortion in gilts and sows and by severe respiratory diseases in neonatal and nursing pigs (Zimmerman et al., 2006). It is considered one of the most economically important infectious diseases affecting pig production globally (Neumann et al., 2005). The causative agent, PRRS virus (PRRSV), is a single-strand, positive-sense, enveloped RNA virus of the family Arteriviridae and order Nidovirales (Cavanagh, 1997), and two distinct genotypes have been identified, namely, the European (type 1), and the North American (type 2) (Meng et al., 1995).

The biological and genetic differences identified between the two genotypes have raised the possibility that the types vary in their virulence and, in particular, in their ability to cause respiratory disease (Murtaugh et al., 1995, 2010). Although studies have demonstrated that type 2 PRRSV induces more severe respiratory disease than the type 1 virus (Halbur et al., 1996; van der Linden et al., 2003; Martínez-Lobo et al., 2011), those studies used different genotypes of the virus from different continents. Recently, it has been reported that 'Korean' type 1 PRRSV induces more severe grossly visible lung lesions than Korean type 2 virus, 14 and 21 days post-inoculation (dpi) (Kim et al., 2011). However, these findings should be interpreted with some caution, as there is currently a lack of data regarding the full extent of the distribution, replication and shedding of these virus genotypes. This study was thus established to compare the pathogenicity of 'Korean' types 1 and 2 PRRSV isolated from weaned pigs with respiratory disease within the same herd. Parameters assessed included the humoral immunological response, extent of viraemia and shedding, location of viral replication, and the severity of both macroscopically and microscopically visible lesions.

Materials and methods

Animal selection

Sixty-four 3-week old pigs were purchased from a PRRSV-free herd. All pigs were negative for PRRSV and porcine circovirus type (PCV) 2, as determined by routine serology and real-time PCR carried out both prior to delivery and on arrival (Wasilk et al., 2004; Gagnon et al., 2008). The animals were housed in isolation in pens with concrete floors and with an automatic watering system. The study was approved by the Seoul National University, Institutional Animal Care and Use Committee (SNU-111201-3).

PRRSV isolates

Korean type 1 (SNUVR100057 strain) and type 2 (SNUVR090851 strain) PRRSV were isolated from lung samples from different, recently-weaned pigs in a 1000-sow herd in Chungcheung Providence in 2009 and 2010, respectively. This herd had had severe respiratory disease in recently weaned pigs aged between 4 and 8 weeks. The SNUVR100057 strain was identified as type 1 PRRSV on the basis of

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nucleotide sequences of ORFs 5 (GenBank JN411262) and 7 (GenBank JN411261). The SNUVR090851 strain was also identified as type 2 on the basis of nucleotide sequences of ORFs 5 (GenBank JN315685) and 7 (GenBank JN399072). These strains were analysed phylogenetically along with prototype PRRSV (Lelystad, VR-2332, and Lena), and nine other strains used in earlier pathogenesis studies listed in the GenBank database for ORF 5 (Fig. 1).

Experimental design

Pigs were randomly allocated to two infected (1 and 2) and one control (3) groups. In group 1, 24 pigs were inoculated intranasally with 3 mL of tissue culture fluid containing 10^6 tissue culture infective doses 50% ($TCID_{50}$)/mL of Korean type 1 PRRSV (second passage in MARC-145 cells). In group 2, 24 pigs were inoculated intranasally with 3 mL of tissue culture fluid containing 10^6 $TCID_{50}$/mL of Korean type 2 PRRSV (second passage in MARC-145 cells). In group 3, 16 controls were inoculated in the same manner with uninfected cell culture supernatants. Rectal temperatures were recorded daily from 2 days prior to inoculation (−2) through to 28 dpi. Three infected animals from groups 1 and 2, along with two controls were euthanased at 1, 3, 5, 7, 10, 14, 21 and 28 dpi as previously described (Beaver et al., 2001). Tissues were collected from each pig at necropsy.

Serology

Blood samples from each pig were collected by jugular venepuncture at 0, 1, 3, 5, 7, 10, 14, 21 and 28 dpi and the sera stored at −20 °C. Serum samples were tested using the commercially available PRRSV ELISA (HerdCheck PRRS 2XR, IDEXX Laboratories), and were considered seropositive where the S/P ratio was >0.4 as per the manufacturer's instructions. Serum virus neutralisation (SVN) tests were also performed using homologous strains as previously described (Yoon et al., 1994; Jusa et al., 1996). Samples were considered positive for neutralising antibody where the titre was >2.0 (log_2) (Zuckermann et al., 2007).

Quantification of viral RNA

RNA was extracted from serum at 0, 1, 3, 5, 7, 10, 14, 21 and 28 dpi from both infected and control pigs, and real-time PCR was used to quantify PRRSV genomic cDNA copy numbers (Wasilk et al., 2004). To construct a standard curve, real-time PCR was performed in 10-fold serial dilutions (from 10^6 $TCID_{50}$/mL to 10^{-1} $TCID_{50}$/mL) of the PRRSV plasmid and in type 1 and type 2 virus cultured in MARC-145 cells (Han et al., 2011).

Virus isolation, sequence analysis and in situ hybridisation

Lungs and mediastinal lymph nodes were collected for virus isolation from all infected and control pigs as previously described (Kim et al., 1993). The PRRSV isolates from lungs and lymph nodes were further analysed for the ORF 5 sequence (Oleksiewicz et al., 1998). For type 1 PRRSV, a 354 base-pair cDNA fragment representing the 5′ region of ORF 6 and 7 was used as a probe. The forward and reverse primers were 5′-CGCTGTGACAAAGCCCGGAC-3′ (nucleotides 14482–14501) and 5′-TCGATTGCAAGCAGAGGGAG-3′ (nucleotides 14814–14835), respectively. For type 2 PRRSV, a 349 base-pair cDNA fragment representing the 5′ region of ORF 6 and 7 was used as a probe. The forward and reverse primers were 5′-TCGTTCGGCGTCCCGGCTCC-3′ (nucleotides 14775–14794) and 5′-TTGACGACAGACACAATTGC-3′ (nucleotides 15122–15141), respectively. The PCR was carried out as previously described (Kono et al., 1996). The purified product was labelled by random priming with digoxigenin-dUTP using a commercial kit (Boehringer Mannheim). In-situ hybridisation was carried out as previously described (Cheon et al., 1997).

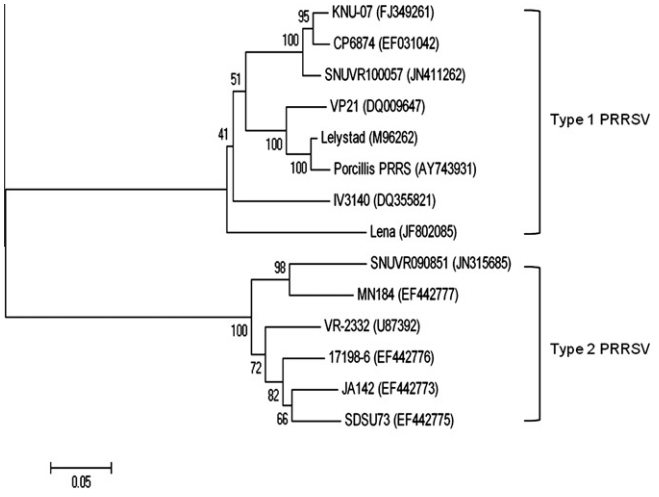

Fig. 1. Phylogenetic analysis of ORF 5 of the porcine reproductive and respiratory syndrome virus (PRRSV) genome. An unrooted neighbour-joining tree was constructed from aligned nucleic acid sequences of several PRRSVs found in GenBank.

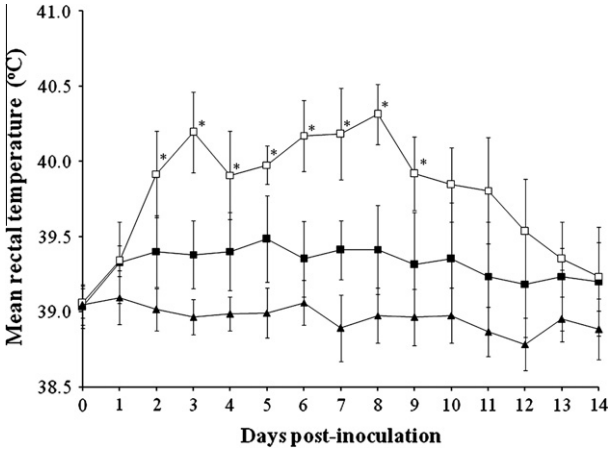

Fig. 2. Mean rectal body temperature of pigs experimentally infected with Korean type 1 (■) and 2 (□) porcine reproductive respiratory syndrome virus (PRRSV), and negative control pigs (▲). A significant difference between types 1- and 2-infected pigs was detected at each time point. *$P < 0.05$.

Lesion morphometry

Morphometric analysis of macroscopically and microscopically visible pulmonary lesions was performed as previously described (Halbur et al., 1995). To obtain quantitative data, histological slides containing lesions were analysed using the NIH Image J 1.43 m Program.[1] For each histoslide of lung and lymph node, ten fields were randomly selected, and the number of positive cells/unit area (0.95 mm^2) counted (Halbur et al., 1996). Mean values were then calculated.

Statistical analysis

The Student's *t* test for paired samples was used to estimate differences at each time point. Pearson correlation analysis was used to detect any relationship between two parameters. *P* values <0.05 were considered statistically significant.

Results

Clinical signs

There were minimal signs of respiratory disease in both type 1- and type 2-infected pigs. In the case of type 1-infection, two pigs developed mild, transient dyspnoea, and five had moderately laboured abdominal respiration 5–10 dpi. By 14 dpi, all pigs had recovered. Similarly for the type 2-infected pigs, four had moderate transient dyspnoea, and five exhibited severely laboured abdominal respiration 5–10 dpi. By 14 dpi, all pigs had recovered. Pigs in the control group remained normal throughout. The mean rectal temperature was significantly lower in the controls than in animals infected with: type 1 virus at 4 dpi ($P = 0.040$), 5 dpi ($P = 0.005$), and 7 dpi ($P = 0.034$); type 2 virus between 2 and 9 dpi ($P < 0.001$), at 10 dpi ($P = 0.002$), and 11 dpi ($P = 0.017$). The mean rectal temperature was significantly higher in type 2- than in type 1-infected pigs between 2 and 9 dpi ($P < 0.05$) (Fig. 2).

Serology

Antibodies were detected in all pigs at 10 dpi. Based on the S/P ratio, the titre rose significantly between 7 and 14 dpi ($P < 0.05$) in

[1] See: http://rsb.info.nih.gov/ij.