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EXPERIMENTALLY INDUCED DISEASE

Comparison of the Pathogenesis of Single or Dual Infections with Type 1 and Type 2 Porcine Reproductive and Respiratory Syndrome Virus

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Summary

The aim of this study was to compare the pathogenicity of single or dual infections with type 1 and type 2 porcine reproductive and respiratory syndrome virus (PRRSV) in pigs. Pigs were inoculated intranasally with type 1 or type 2 PRRSV or both viruses together. Pigs infected with type 1 and type 2 PRRSV together had significantly ($P<0.05$) fewer genomic copies of type 1 PRRSV than did pigs infected with type 1 PRRSV alone. Pigs infected with type 2 PRRSV alone or type 1 and type 2 PRRSV together had significantly ($P<0.05$) higher gross and microscopical lung lesion scores than did pigs infected with type 1 PRRSV alone. Pigs infected with type 2 PRRSV alone or type 1 and type 2 PRRSV together had significantly ($P<0.05$) higher scores for PRRSV-positive cells in the lung than did pigs infected with type 1 PRRSV alone. Pigs infected with type 1 PRRSV alone had significantly ($P<0.05$) higher scores for type 1 PRRSV-positive cells in the lung than did pigs infected with both types of PRRSV together. Pigs infected with both types of PRRSV together developed similar clinical disease and lesions as pigs infected with type 2 PRRSV alone. Significant differences in virulence were not observed between pigs infected with type 2 PRRSV alone and pigs infected with both types of PRRSV together in terms of viraemia, lung lesion score and virus distribution within lung lesions.

Keywords: co-infection; pig; porcine reproductive and respiratory syndrome virus

Introduction

Porcine reproductive and respiratory syndrome (PRRS) has been recognized as one of the most economically important diseases in the swine industry, causing both reproductive failure in pregnant sows (late-term abortions and stillbirths) and respiratory disease (pneumonia) in nursery and grower/finishing pigs (Zimmerman *et al.*, 2012). The PRRS virus (PRRSV) is a linear positive-sense, single-stranded RNA virus that is a member of the genus *Arterivirus*, the family *Arteriviridae* and the order *Nidovirales* (Cavanagh, 1997). The two main genotypes have only a 60–70% nucleotide homology; the type 1 (European) and type 2 (North American) genotypes are clearly genetically distinguished (Allende *et al.*, 1999; Nelsen *et al.*, 1999). Type 2 PRRSV is more virulent than type 1 PRRSV in terms of its ability to cause respiratory diseases (Halbur *et al.*, 1995; Martínez-Lobo *et al.*, 2011; Han *et al.*, 2012).

In the 2-year period 2013–2014, lung tissues from 500 growing pigs were examined at the Department of Veterinary Pathology, Seoul National University, and both type 1 and type 2 PRRSV was detected in 73 cases. However, the severity of respiratory disease in pigs co-infected with type 1 and type 2 PRRSV has not been compared experimentally with that of pigs with a single infection. The aim of this study

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was therefore to compare the pathogenicity of dual infection (type 1 and type 2 PRRSV) versus single infection (type 1 or type 2 PRRSV), as measured by virus distribution, sites of viral replication and gross and microscopical lesions in infected pigs.

Materials and Methods

Porcine Reproductive and Respiratory Syndrome Virus Isolates

Type 1 (SNUVR090485, pan-European subtype 1) and type 2 (SNUVR090851, lineage 1) PRRSVs were used as inocula. The type 1 SNUVR090485 virus was isolated from lung samples from an aborted fetus in southwestern Gyeonggi Province in 2009 (Han *et al.*, 2012). The type 2 SNUVR090851 virus was isolated from lung samples from different newly weaned pigs in Chungcheung Providence in 2010 (Han *et al.*, 2013a). The type 1 SNUVR090485 and type 2 SNUVR090851 viruses used in this study were analyzed phylogenetically by comparison with the prototypes of PRRSV (Lelystad, Lena and VR2332) based on open reading frame (ORF) 5 (Fig. 1).

Experimental Design

Sixty-six pigs purchased from a PRRSV-free herd were used at the age of 2 weeks. The pigs were negative for PRRSV and porcine circovirus type 2 (PCV2) according to routine serological testing performed prior to delivery and again on arrival. In addition, type 1 and 2 PRRSV and PCV2 were not detected in serum samples from any pigs used in this study by real-time polymerase chain reaction (PCR) (Wasilk *et al.*, 2004; Gagnon *et al.*, 2008) prior to delivery and again on arrival.

Pigs were allocated to three infected groups ($n = 18$ pigs each) and one negative control group ($n = 12$ pigs) using the random number generation function (Excel, Microsoft Corporation, Redmond, Washington, USA). At 3 weeks of age, the pigs were inoculated intranasally with either 3 ml of type 1 PRRSV inoculum (10^5 50% tissue culture infective doses [$TCID_{50}$]/ml of SNUVR090485, second passage in alveolar macrophages), 3 ml of type 2 PRRSV inoculum (10^5 $TCID_{50}$/ml of SNUVR090851, second passage in MARC-145 cells), a mixture of equal volumes (3 ml) of each of the type 1 and type 2 PRRSV inocula (10^5 $TCID_{50}$/ml of SNUVR090485, second passage in alveolar macrophages and 10^5 $TCID_{50}$/ml of SNUVR090851, second passage in MARC-145 cells) or 3 ml of sham inoculum (uninfected cell culture supernatant). Rectal temperatures were recorded daily from 2 days post inoculation (dpi) to 28 dpi. Three infected and two control pigs from each group were sedated by an intravenous injection of sodium pentobarbital and killed at 3, 7, 10, 14, 21 and 28 dpi as previously described (Beaver *et al.*, 2001). Tissues were collected from each pig at the time of necropsy examination. Experimental methods were approved by the Seoul University Institutional Animal Care and Use and Ethics Committee.

Serology

Blood samples from each pig were collected by jugular venipuncture at 0, 3, 7, 10, 14, 21 and 28 dpi and the sera were stored at −20°C. The serum samples were tested using the commercially available PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS X3 Ab, IDEXX Laboratories Inc., Westbrook, Maine, USA). Serum samples were considered positive for PRRSV antibody if the sample/positive (S/P) ratio was greater than 0.4 according to the manufacturer's instructions. Serum virus neutralization (SVN) tests were also performed and samples were considered positive for PRRSV-neutralizing antibody if the titre was >2.0 (log_2) (Zuckermann *et al.*, 2007).

Quantification of Porcine Reproductive and Respiratory Syndrome Virus RNA

RNA was extracted from serum samples from PRRSV-infected pigs and negative controls at 0, 3, 7, 10, 14, 21 and 28 dpi. Real-time PCR was used to quantify type 1 and type 2 PRRSV genomic cDNA copy number (Wasilk *et al.*, 2004). The real-

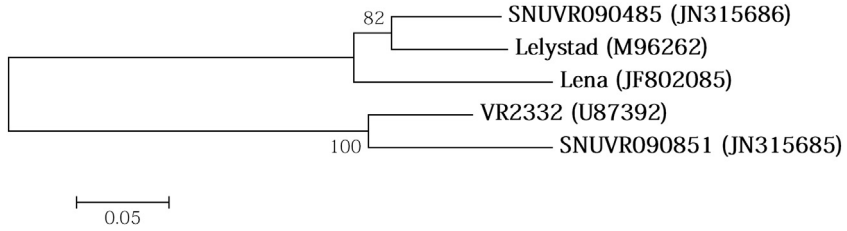

Fig. 1. Phylogenetic analysis of ORF5 from type 1 and type 2 PRRSV strains. An unrooted neighbour-joining tree was constructed from aligned nucleic acid sequences.