Research in Veterinary Science 103 (2015) 193–200

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/yrvsc

# Concurrent vaccination of pigs with type 1 and type 2 porcine reproductive and respiratory syndrome virus (PRRSV) protects against type 1 PRRSV but not against type 2 PRRSV on dually challenged pigs

Changhoon Park [1], Kyuhyung Choi [1], Jiwoon Jeong, Ikjae- Kang, Su-Jin Park, Chanhee Chae *

*College of Veterinary Medicine, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea*

ARTICLE INFO

*Article history:*
Received 22 May 2015
Received in revised form 19 October 2015
Accepted 25 October 2015

*Keywords:*
Concurrent vaccination
Interference
Porcine reproductive and respiratory syndrome virus vaccine
Porcine reproductive and respiratory syndrome virus

ABSTRACT

The objective of the present study was to evaluate the effect of concurrent vaccination of pigs with both type 1 and type 2 porcine reproductive and respiratory syndrome virus (PRRSV) vaccine against heterologous dual challenge of both genotypes and compare with single vaccination of pigs against heterologous single challenge of both genotypes. Pigs were administered both type 1 and type 2 PRRSV vaccine concurrently into separate anatomical sites at 28 days of age and inoculated intranasally with both genotypes at 63 days of age. Neutralizing antibodies (NA) were not detected in any pigs in any group (NA titer $<2$ $\log_2$) throughout the experiment. In addition, concurrent vaccination of pigs with two PRRSV genotypes had significantly lower numbers of type 1 and type 2 PRRSV-specific interferon-γ secreting cells (IFN-γ-SC) compared to vaccination of pigs with type 1 or type 2 PRRSV only. Despite the decreased induction of type 1 PRRSV-specific IFN-γ-SC, concurrent vaccination is still able to reduce type 1 PRRSV viremia whereas the decreased induction of type 2 PRRSV-specific IFN-γ-SC by concurrent vaccination correlates with lack of reduction of type 2 PRRSV viremia after dual challenge. The results of this study demonstrated that concurrent vaccination of pigs with two PRRSV genotypes is able to reduce the levels of type 1 PRRSV viremia and lung lesions but not able to reduce the levels of type 2 PRRSV viremia and lung lesions.



## 1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) has become one of the most important causes of huge economic losses in global swine production. The etiological agent, PRRS virus (PRRSV), is an enveloped, single-stranded positive sense RNA virus belonging to the order *Nidovirales*, family *Arteriviridae*, genus *Arterivirus* (Snijder et al., 2013). PRRSV is divided into type 1 (European) and type 2 (North American) genotypes based on antigenic, genetic, and pathogenic differences (Nelsen et al., 1999; Allende et al., 1999). PRRSV causes abortion, stillbirth, premature farrowing, and increased mortality in neonatal pigs. PRRSV also causes respiratory disease and increased susceptibility to secondary bacterial infection, leading to impaired growth and high mortality rate in young piglets (Zimmerman et al., 2012).

Currently, both type 1 and type 2 PRRSV are circulating in Korean farms. Co-infection with both genotypes is also prevalent (16.2%) in pig farms (Lee et al., 2010). Previous studies demonstrated lack of cross-protection by PRRSV vaccine between two genotypes (van Woensel et al., 1998; Labarque et al., 2000; Han et al., 2014). When both genotypes were detected concurrently in diagnostic cases of respiratory diseases in growing pigs (personal communication with producers), the producers raised a question if vaccination of pigs with both genotypes is necessary to control co-infection with two genotypes. Theoretically, one possible way to control co-infection of pigs with two genotypes may be the concurrent vaccination of pigs with both type 1 and type 2 PRRSV because no combined vaccine of type 1 and type 2 is commercially available yet. Therefore, the objective of the present study was to evaluate the effect of concurrent vaccination of pigs with both type 1 and type 2 PRRSV against heterologous dual challenge of type 1 and type 2 PRRSV.

## 2. Materials and methods

### 2.1. PRRSV inocula

Type 1 (SNUVR090485, pan-European subtype 1) and type 2 (SNUVR090851, lineage 1) PRRSVs were used as inocula (Han et al., 2012, 2013).

* Corresponding author.
*E-mail address:* swine@snu.ac.kr (C. Chae).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.rvsc.2015.10.011
0034-5288/

### 2.2. Experimental design

A total of 72 colostrum-fed, cross-bred, conventional piglets were purchased at 14 days of age from a commercial PRRSV-free farm. All piglets were negative for PRRSV according to commercially PRRSV enzyme-linked immunosorbent assay (ELISA; HerdCheck PRRS X3 Ab test, IDEXX Laboratories Inc). All piglets were negative for type 1 and type 2 PRRSV viremia by real-time polymerase chain reaction (PCR) as previously described (Wasilk et al., 2004).

Pigs were divided into 9 groups (8 pigs/group) using the random number generation function (Excel, Microsoft Corporation) (Table 1). Porcilis PRRS (MSD Animal Health, Lot No. D353A07) was used as type 1 PRRSV vaccine (Vac1) and Fostera PRRS (Zoetis, Lot No. A405013B) was used as type 2 PRRSV vaccine (Vac2) according to the manufacturer's recommendations. Pigs in Vac1-2/Ch1-2, Vac1-2/Ch1, and Vac1-2/Ch2 were immunized intramuscularly with both Vac1 (left side of the neck, 2.0 mL) and Vac2 (right side of the neck, 2.0 mL) at the same time, respectively, at 28 days of age. Pigs in Vac1/Ch1 were immunized intramuscularly with Vac1 (2.0 mL) while pigs in Vac2/Ch2 were immunized intramuscularly with Vac2 (2.0 mL) at 28 days of age.

Type 1 PRRSV inoculum consisted of SNUVR090485, which was propagated on alveolar macrophages to a titer of $10^5$ 50% tissue culture infective doses ($TCID_{50}$)/mL. Type 2 PRRSV inoculums consisted of SNUVR090851, which was propagated on MARC-145 cells to a titer of $10^5$ $TCID_{50}$/mL. At 63 days of age (0 day post-challenge, dpc), the pigs in Vac1-2/Ch1-2 and UnVac/Ch1-2 were inoculated intranasally with 3 mL of each type 1 and type 2 PRRSV inoculum. The pigs in Vac1-2/Ch1, Vac1/Ch1, and UnVac/Ch1 were inoculated intranasally with 3 mL of type 1 PRRSV inoculum. The pigs inVac1-2/Ch2, Vac2/Ch2, and UnVac/Ch2 were inoculated intranasally with 3 mL of type 2 PRRSV inoculum. The pigs in UnVac/UnCh served as negative controls and were neither vaccinated nor challenged.

The pigs in each group were housed in separate experimental rooms equipped with biosecurity to avoid possible transmission of the pathogen between groups throughout the experiment in the research facility. Following PRRSV inoculation, the physical condition of the pig was monitored daily and their rectal temperatures were taken. Blood samples were collected at −35, −28, −21, −14, −7, 0, 3, 7, 10, and 14 dpc. All pigs were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 14 dpc as previously described (Beaver et al., 2001). All of the methods were previously approved by the Seoul National University Institutional Animal Care and Use, and Ethics Committee (SNU-130,219-11B, date of approval 13 February 2013).

### 2.3. Clinical observation

Clinical respiratory signs were recorded daily using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing) (Halbur et al., 1995). Observers were blinded to vaccination and challenge status. Rectal temperatures were recorded daily at the same time by same personnel.

### 2.4. Serology

The serum samples were tested using the commercially available PRRSV ELISA (HerdCheck PRRS X3 Ab test, IDEXX Laboratories Inc). Serum samples were considered positive for PRRSV antibody if the S/P ratio was greater than 0.4, according to the manufacturer's instructions. Virus neutralization tests were also performed with type 1 (SNUVR090485) and type 2 (SNUVR090851) PRRSV, as previously described (Yoon et al., 1994). Serum samples were considered to be positive for neutralizing antibodies (NA) if the titer was greater than 2.0 ($log_2$) (Zuckermann et al., 2007).

### 2.5. Quantification of PRRSV RNA

RNA was extracted from serum samples to quantify PRRSV genomic cDNA copy numbers, as previously described (Wasilk et al., 2004). Real-time PCR for the type 1 and type 2 PRRSV were performed to quantify PRRSV genomic cDNA copy (Wasilk et al., 2004). Real-time PCR for the vaccine virus was also performed to quantify PRRSV genomic cDNA copy (Park et al., 2014; Kim et al., 2015). Each of type 1 (SNUVR090485) and type 2 (SNUVR090851) PRRSV was used as positive control and nuclease-free $H_2O$ was used as negative control. A standard curve for each of primers was obtained. The PCR products for the two vaccine virus (Porcilis PRRS vaccine virus, 166 base pair and Fostera PRRS vaccine virus, 129 base pair) and the two challenge virus (type 1 PRRSV, 184 base pair and type 2 PRRSV, 187 base pair) were cloned with the TOPcloner Blunt kit (Enzynomics, Daejeon, Korea) and propagated in DH5α competent cells (Enzynomics, Daejeon, Korea) according to the manufacturer's instructions. Plasmid DNA was purified with a plasmid purification kit (iNtRONBiotechnology, Sungnam, Gyeonggi-Do, Korea) and quantified using a spectrophotometer (Park et al., 2014; Kim et al., 2015).

### 2.6. Enzyme-linked immunospot (ELISPOT) assay

The numbers of PRRSV-specific interferon-γ secreting cells (IFN-γ-SC) was determined in peripheral blood mononuclear cells (PBMC) by ELISPOT (MABTECH, Mariemont, OH) according to the manufacturer's instructions. Briefly, $5 \times 10^5$ PBMC was plated in 96-well microplate precoated with swine specific IFN-γ antibody (10 μg/mL, MABTECH). Cells were stimulated with either type 1 (SNUVR090485) or type 2 (SNUVR090851) PRRSV at multiplicity of infection (MOI) of 0.01 as the recall antigen for 20 h incubation at 37 °C in a 5% $CO_2$ atmosphere. Unstimulated cells and phytohemagglutinin (10 μg/mL)-stimulated cells were used as negative and positive controls, respectively (Meier

**Table 1**
Experimental design and results of lesion score and porcine reproductive and respiratory syndrome virus (PRRSV) RNA within lung lesion at 14 days post-challenge.

| Groups | PRRSV | | Lung lesion score | | PRRSV-positive cells within lung lesion | |
|---|---|---|---|---|---|---|
| | Vaccination (28 days) | Challenge (63 days) | Macroscopic | Microscopic | Type 1 | Type 2 |
| Vac1-2/Ch1-2 | Types 1 & 2 | Types 1 & 2 | $38.7 \pm 14.5^{a}$ | $2.53 \pm 0.9^{a}$ | $0.25 \pm 0.2^{b}$ | $1.71 \pm 0.7^{a}$ |
| Vac1-2/Ch1 | Types 1 & 2 | Type 1 | $20 \pm 7.5^{b}$ | $1.37 \pm 0.5^{b,c}$ | $0.31 \pm 0.2^{b}$ | $0^{c}$ |
| Vac1-2/Ch2 | Types 1 & 2 | Type 2 | $33.7 \pm 15.9^{a,b}$ | $2.42 \pm 0.4^{a}$ | $0^{b}$ | $1.77 \pm 0.5^{a}$ |
| Vac1/Ch1 | Type 1 | Type 1 | $8.1 \pm 6.5^{c}$ | $1.01 \pm 0.5^{c}$ | $0.15 \pm 0.1^{b}$ | $0^{c}$ |
| Vac2/Ch2 | Type 2 | Type 2 | $7.5 \pm 8.8^{c}$ | $1.53 \pm 0.4^{b,c}$ | $0^{b}$ | $0.97 \pm 0.4^{b}$ |
| UnVac/Ch1-2 | – | Types 1 & 2 | $41.2 \pm 14.5^{a}$ | $2.47 \pm 0.6^{a}$ | $0.06 \pm 0.1^{b}$ | $1.82 \pm 0.6^{a}$ |
| UnVac/Ch1 | – | Type 1 | $12.5 \pm 8.8^{b,c}$ | $1.86 \pm 0.5^{b}$ | $1.16 \pm 0.5^{a}$ | $0^{c}$ |
| UnVac/Ch2 | – | Type 2 | $21.2 \pm 8.3^{b}$ | $2.63 \pm 0.4^{a}$ | $0^{b}$ | $1.93 \pm 0.6^{a}$ |
| UnVac/UnCh | – | – | $1.2 \pm 3.5^{c}$ | $0.05 \pm 0.1^{d}$ | $0^{b}$ | $0^{c}$ |

[a,b,c]Different letters (a, b, and c) indicate significant ($P < 0.05$) difference among groups.