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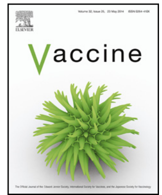

Efficacy of a new bivalent vaccine of porcine circovirus type 2 and *Mycoplasma hyopneumoniae* (Fostera™ PCV MH) under experimental conditions

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ABSTRACT

The objective of this study was to evaluate the efficacy of a new bivalent vaccine (Fostera™ PCV MH, Zoetis) of porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* in growing pigs under experimental conditions. A total of 80 pigs were randomly divided into 8 groups (10 pigs per group). The pigs were administered the bivalent vaccine intramuscularly as a 2.0 mL dose at 21 days of age based on the manufacturer's instructions. Three weeks after vaccination, the pigs were inoculated with either PCV2 (intranasal route) or *M. hyopneumoniae* (intratracheal route) or both. Regardless of the type of inoculation, vaccinated pigs after challenge exhibited effective reduction of clinical signs, PCV2 viremia levels and mycoplasma nasal shedding, and lung and lymphoid lesion when compared to unvaccinated challenged pigs. Vaccinated challenged pigs had significantly higher ($P < 0.05$) levels of PCV2-specific neutralizing antibodies, and numbers of PCV2-and *M. hyopneumoniae*-specific interferon-γ secreting cells compared to unvaccinated challenged pigs. This study demonstrates that the bivalent vaccine is able to protect pigs against either PCV2 or *M. hyopneumoniae* infection or both based on clinical, microbiological, immunological, and pathological evaluation.

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1. Introduction

Dual infection of porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* is a common causative agent of post-weaning multisystemic wasting syndrome (PMWS) and porcine respiratory disease complex (PRDC) [1–4]. PMWS and PRDC are complex diseases with a huge economic impact on the global pork industry [1,5]. PMWS is a clinical manifestation of porcine circovirus-associated disease (PCVAD), and is primarily characterized by chronic wasting and severely impaired weight gain in pigs between 6 to 11 weeks of age [3,4]. PRDC is characterized clinically by a decrease in feeding efficiency, anorexia, slow growth, lethargy, fever, dyspnea and an antibiotic treatment-resistant cough in growing and finishing pigs typically between 16 and 22 weeks of age [1,5].

Vaccination and medication along with health management has been widely used to control PMWS and PRDC in most Asian countries. In recent years, the use of antibiotics has been limited due to increased risk of antimicrobial resistance and residue in carcasses [6,7] while vaccination has increasingly been used in a strategic manner to control these diseases. In the field, both PCV2 and *M. hyopneumoniae* vaccines are usually administered to pigs at the same time (3 weeks of age) and 55% of growing pigs typically receive both vaccines (http://www.kahpha.or.kr). This trend increases the demand for an efficacious single dose bivalent vaccine of PCV2 and *M. hyopneumoniae*. Advantages of a single dose bivalent vaccine are reduced stress to the animals as well as decreased labor for the farm workers.

Recently a new bivalent vaccine for PCV2 and *M. hyopneumoniae* (Fostera™ PCV MH, Zoetis, Florham Park, NJ) was introduced into the international market to protect pigs against PCV2 and *M. hyopneumoniae* infection (http://www.zoetisus.com/products/pork/fostera_pcv_mh/documents/Fostera_PCV-MH_Safety_TB_4page_final.pdf). The objective of this study was to evaluate the efficacy of a new single dose bivalent vaccine of PCV2 and *M. hyopneumoniae* in pigs experimentally infected with either PCV2 or *M. hyopneumoniae* or both based on clinical, microbiological, immunological, and pathological outcomes.

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2. Materials and methods

2.1. Animals

A total of 80 colostrum-fed, cross-bred, conventional piglets were weaned and purchased at 18 days of age from a porcine reproductive and respiratory syndrome virus (PRRSV)- and *M. hyopneumoniae*-free commercial farm based on serological testing of the breeding herd, and long term clinical and slaughter history. At 21 days of age, pigs were negative for PCV2, PRRSV, and *M. hyopneumoniae* according to routine serological testing. Sera samples were negative for PCV2 and PRRSV and nasal swabs were negative for *M. hyopneumoniae* when tested by real-time polymerase chain reaction (PCR) [8–10].

2.2. Experimental design

A total of 80 pigs were divided into 8 groups (10 pigs per group) using random number generation function (Excel, Microsoft Corporation, Redmond, WA) (Table 1). The pigs in Vac/PCV2-Mhp, Vac/PCV2, Vac/Mhp, and Vac/UnCh were administered Fostera™ PCV MH (Zoetis; Lot No.1405582B) intramuscularly as a 2.0 mL dose at 21 days of age based on the manufacturer's instructions. The pigs in UnVac/PCV2-Mhp, UnVac/PCV2, UnVac/Mhp, and UnVac/UnCh were administered an equal volume of phosphate buffered saline (PBS, 0.01 M, pH 7.4, 2.0 mL) at 21 days of age.

Three weeks after vaccination (0 days post challenge, dpc; 42 days of age), the pigs in Vac/PCV2-Mhp (vaccinated with Fostera™ PCV MH and challenged with PCV2 and *M. hyopneumoniae*) and UnVac/PCV2-Mhp (unvaccinated and challenged with PCV2 and *M. hyopneumoniae*) were inoculated with PCV2b (strain SNUVR000463, GenBank no. KF871068) and *M. hyopneumoniae* (strain SNU98703). Co-infection with PCV2b and *M. hyopneumoniae* induced severe pneumonia in lungs and lymphoid depletion in the lymph node in infected pigs [11]. For inoculation, a 5 h interval was chosen after PCV2 inoculation before inoculating with *M. hyopneumoniae* to avoid mixture of two pathogens which may decrease infectivity. Pigs were intranasally administered a 3 mL inoculation of PCV2b containing 1.2×10^5 50% tissue culture infective dose ($TCID_{50}$)/mL. Five hours after PCV2 inoculation, pigs were anesthetized with a mixture of 2.2 mg/kg xylazine hydrochloride (Rumpon, Bayer) and 2.2 mg/kg tiletamine hydrochloride and 2.2 mg/kg zolazepam hydrochloride (Zoletil 50, Virbac) by intramuscular injection, and were inoculated intratracheally with 7 mL of *M. hyopneumoniae* culture medium containing 10^7 color changing units (CCU)/ml as previously described [12,13]. The pigs in Vac/PCV2 and UnVac/PCV2 were inoculated with PCV2. The pigs in Vac/Mhp and UnVac/Mhp were inoculated with *M. hyopneumoniae*. The pigs in UnVac/UnCh served as the negative control group.

The pigs in each group were randomly assigned into 2 rooms (5 pigs per room) and housed separately within the same airspace, ventilation, and temperature controlled facility. Blood and nasal swabs were collected at −28, −21, −7, 0, 7, 10, 14, and 21 dpc. All 80 pigs were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 21 dpc as previously described [14]. Tissues were collected from each pig at necropsy. Tissues were fixed for 24 h in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. All of the methods were previously approved by the Seoul National University Institutional Animal Care and Use, and Ethics Committee (SNU-150313-3).

Table 1
Study design with vaccination and challenge status of porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae*.

Group	Vaccination (21 days of age)	Challenge (42 days of age)	
		PCV2	*M. hyopneumoniae*
Vac/PCV2-Mhp	+	+	+
Vac/PCV2	+	+	–
Vac/Mhp	+	–	+
Vac/UnCh	+	–	–
UnVac/PCV2-Mhp	–	+	+
UnVac/PCV2	–	+	–
UnVac/Mhp	–	–	+
UnVac/UnCh	–	–	–

2.3. Clinical observation

The pigs were monitored daily for clinical signs and scored weekly from 0 (normal), 1 (rough haircoat), 2 (rough haircoat and dyspnea), 4 (severe dyspnea and abdominal breathing), and 6 (death) as previously described [15]. Observers were blinded to vaccination and challenge status.

2.4. Quantification of PCV2 DNA in blood

DNA was extracted from serum samples using the commercial kit (QIAamp DNA Mini Kit, QIAGEN, Valencia, CA) to quantify PCV2 genomic DNA copy numbers by real-time PCR [16]. To construct a standard curve, real-time PCR was performed in quadruplicate in two different assays: (i) 10-fold serial dilutions of the PCV2 plasmid were used as the standard, with concentrations ranging from 10^{10} to 10^2 copies/mL, and (ii) 10-fold serial dilutions of PCV2b cultured in PCV1-free PK-15 cells were used at concentrations ranging from $10^{4.5}$ $TCID_{50}$/mL to $10^{-3.5}$ $TCID_{50}$/mL. The PCV2 plasmid was prepared as described previously [16]. Culture supernatants of PCV1-free PK-15 cells were used as negative control.

2.5. Quantification of M. hyopneumoniae DNA in nasal swabs

DNA was extracted from nasal swabs using the commercial kit (QIAamp DNA Mini Kit, QIAGEN) to quantify the *M. hyopneumoniae* genomic DNA copy numbers by real-time PCR with primers based on multidrug resistance protein homolog genes (GenBank no. U02537) [10]. To construct a standard curve, real-time PCR was performed in quadruplicate in 10-fold serial dilution of chromosomal DNA from *M. hyopneumoniaes* strain SNU98703, with concentrations ranging from 10 ng/μL to 1 fg/μL. One fetogram of chromosomal DNA from *M. hyopneumoniae* is considered to be approximately one genome equivalent [17]. A negative control was included in each run using double distilled water as the template.

2.6. Serology

The serum samples were tested for antibodies to PCV2 and *M. hyopneumoniae* using the commercial PCV2 ELISA (Synbiotics, Lyon, France) and *M. hyopneumoniae* ELISA (IDEXX, Westbrook, ME). Serum virus neutralization (SVN) test for PCV2 was performed as previously described [18].

2.7. Enzyme-linked immunospot assay

PCV2 and *M. hyopneumoniae* antigens were prepared as previously described [19,20]. The numbers of PCV2- and *M. hyopneumoniae*-specific interferon-γ secreting cells (IFN-γ-SC) were determined in peripheral blood mononuclear cells (PBMC) as previously described [21,22].

2.8. Pathology

Macroscopic lung lesions, and microscopic lung and lymphoid lesions were scored and analyzed morphometrically as previously described [2,23].

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