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Interaction of porcine circovirus type 2 and *Mycoplasma hyopneumoniae* vaccines on dually infected pigs

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ABSTRACT

The objective of this study was to determine the effects of porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* vaccinations on disease severity in an experimental PCV2-*M. hyopneumoniae* dual challenge model. Vaccine effectiveness was evaluated using microbiological (PCV2 viremia and *M. hyopneumoniae* nasal shedding), immunological (neutralizing antibodies and interferon-γ-secreting cells), and pathological (gross lung lesions, histopathologic pulmonary and lymphoid lesions, and the presence of PCV2 antigen and *M. hyopneumoniae* DNA within the lesions) evaluations. Although *M. hyopneumoniae* potentiates the severity of PCV2-associated lesions and lesion-associated PCV2 antigen in dually challenged pigs, vaccination against *M. hyopneumoniae* alone did not reduce PCV2 viremia, PCV2-induced lesions, or PCV2 antigen in dually challenged pigs. In addition, vaccination against PCV2 did not reduce the nasal shedding of *M. hyopneumoniae*, the *M. hyopneumoniae*-induced pulmonary lesions or the lesion-associated *M. hyopneumoniae* DNA in dually challenged pigs. Dual challenge with PCV2 and *M. hyopneumoniae* did not interfere with the induction of active immunity induced by a previous single vaccination for either PCV2 or *M. hyopneumoniae*. The results of this study demonstrated that (i) vaccination against *M. hyopneumoniae* alone did not decrease the potentiation of PCV2-induced lesions by *M. hyopneumoniae* and (ii) vaccination against PCV2 alone decreased the potentiation of PCV2-induced lesions by *M. hyopneumoniae* in dually challenged pigs.

1. Introduction

Porcine circovirus type 2 (PCV2) is the primary etiological agent for several diseases and syndromes, which are collectively referred to as porcine circovirus-associated disease (PCVAD) [1]. Among these conditions, postweaning multisystemic wasting syndrome (PMWS) and porcine respiratory disease complex (PRDC) are the most important. *Mycoplasma hyopneumoniae* is the primary pathogen causing enzootic pneumonia, which is characterized by a dry, non-productive cough, reduced growth rate and poor feed conversion efficiency [2]. Co-infection with PCV2 and *M. hyopneumoniae* plays a primary role in the PRDC and continues to have a major economic impact on the global swine industry [3].

Several studies based on experimental dual infection have been conducted to better understand the interaction between PCV2 and *M. hyopneumoniae* [4–6]. In a sequential challenge model, *M. hyopneumoniae* potentiated the severity of PCV2-associated lung and lymphoid lesions, and increased the incidence of PMWS in pigs that were first inoculated with *M. hyopneumoniae* and then inoculated with PCV2 2 weeks later [4]. In contrast, in a concurrent infection model, pigs that were inoculated with both *M. hyopneumoniae* and PCV2 at 6 weeks of age did not produce the synergistic clinical outcomes observed when using the sequential challenge model [5].

Since dual infection of pigs with *M. hyopneumoniae* and PCV2 results in increased severity of PCV2-induced lesions and incidence of PMWS using the sequential challenge model [4], one possible way to minimize the effect of the *M. hyopneumoniae*-associated enhancement of PCV2 replication may be the use of a *M. hyopneumoniae*-based vaccine. Surprisingly, however, it have been reported that *M. hyopneumoniae* vaccination alone actually increased the incidence of PMWS under experimental and field conditions [7,8]. These unexpected results make difficult to understand the interaction between *M. hyopneumoniae* vaccination and incidence of PMWS. Hence, it is necessary to conduct experimental studies to elucidate the effects of a single vaccination for either PCV2 or *M. hyopneumoniae* on dually infected pigs. Currently, commercial PCV2 and *M. hyopneumoniae* vaccines are widely used in swine production worldwide. Therefore, the objective of this

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Table 1
Study design with vaccination and challenge statuses of *Mycoplasma hyopneumoniae* and PCV2[a].

Group	Vaccination		Challenge	
	M. hyopneumoniae (−42 dpc)	PCV2 (−28 dpc)	*M. hyopneumoniae* (−14 dpc)	PCV2 (0 dpc)
1	+	–	+	–
2	+	–	+	+
3	–	+	–	+
4	–	+	+	+
5	+	+	+	+
6	–	–	+	–
7	–	–	–	+
8	–	–	+	+
9	+	–	–	–
10	–	+	–	–
11	–	–	–	–

[a] There were eight animals in each groups, and necropsy was performed at 28 days post challenge (dpc) in all cases.

study was to determine the effects of single PCV2 and/or *M. hyopneumoniae* vaccinations on pigs in an experimental PCV2 and *M. hyopneumoniae* dual challenge model.

2. Materials and methods

2.1. Commercial vaccines

The inactivated chimeric PCV1-2 vaccine (Fostera PCV, Zoetis, Madison, NJ, USA) and the inactivated *M. hyopneumoniae* vaccine (RespiSure-One, Zoetis) were used in this study. Vaccines were administered according to the manufacturer's instructions (1 dose via the intramuscular route).

2.2. Animals

A total of 88 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 breeding herd, and long term clinical and slaughter history. Pigs were all negative for PCV2, PRRSV, and *M. hyopneumoniae* according to routine serological testing. PCV2 and PRRSV were not detected in the sera samples by the real-time polymerase chain reaction (PCR) [9,10]. *M. hyopneumoniae* was not detected in the nasal swab samples by real-time PCR [11].

2.3. Experimental design

A total of 72 pigs were randomly divided into 11 groups (8 pigs per group): 5 vaccinated challenged (VC), 3 unvaccinated challenged (UVC), 2 vaccinated unchallenged (VUC), and 1 unvaccinated unchallenged group (Table 1). At 7 days of age (−42 days post challenge (dpc)), pigs in groups 1, 2, 5, and 9 were injected intramuscularly in the right side of the neck with 2.0 mL of the *M. hyopneumoniae* vaccine (RespiSure-One, Zoetis). At 21 days of age (−28 dpc), pigs in groups 3, 4, 5, and 10 were injected intramuscularly in the left side of the neck with 2.0 mL of the PCV2 vaccine (Fostera PCV, Zoetis). An equal volume of phosphate buffered saline (PBS) (2.0 mL) was injected in the same anatomic location in the positive and negative control pigs (groups 6–8, and 11) at 7 and 21 days of age.

At 35 days of age (−14 dpc), pigs in the VC (groups 1, 2, 4, and 5) and UVC (groups 6 and 8) were intratracheally administered with a 10 mL dose of frozen lung homogenate of *M. hyopneumoniae* strain SNU98703 (1:100 dilution in Friis medium) at a final concentration of 10^4–10^5 color changing units (CCU)/mL as previously described [12]. At 49 days of age (0 dpc), pigs in the VC (groups 2–5) and UVC (groups 7 and 8) groups were intranasally administered with a 3 mL dose of PCV2b (strain SNUVR000463 (GenBank no. KF871068), 5th passage) containing 1.2×10^5 50% tissue culture infective dose ($TCID_{50}$)/mL (Table 1).

Blood samples and nasal swabs were collected at −42, −28, −14, 0, 7, 14, 21, and 28 dpc. Pigs from each group were sedated by an intravenous injection of sodium pentobarbital and then euthanized by electrocution at 28 dpc as previously described [13]. Tissues were collected from each pig at necropsy. All of the methods were approved by the Seoul National University Institutional Animal Care and Use Committee.

2.4. Quantification of PCV2 DNA in blood

DNA was extracted from serum samples using the QIAamp DNA Mini Kit (QIAGEN Ltd, Crawley, UK) to quantify PCV2 genomic DNA copy numbers by real-time PCR [8].

2.5. Quantification of M. hyopneumoniae DNA in nasal swabs

DNA was extracted from nasal swabs using the QIAamp DNA Mini Kit (QIAGEN Ltd, Crawley, UK) to quantify the *M. hyopneumoniae* genomic DNA copy numbers by real-time PCR with primers based on the putative ABC transporter [11].

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 Laboratories Inc, Westbrook, ME, USA). Serum virus neutralization (SVN) test for PCV2 was performed as previously described [14].

2.7. Enzyme-linked immunospot assay

PCV2 and *M. hyopneumoniae* antigens were prepared as previously described [15,16]. The numbers of PCV2- and *M. hyopneumoniae*-specific interferon-γ-secreting cells (IFN-γ-SCs) were determined in peripheral blood mononuclear cells (PBMCs) as previously described [17,18].

2.8. In situ hybridization and immunohistochemistry

In situ hybridization for *M. hyopneumoniae* and immunohistochemistry for PCV2 was performed as previously described [15,19,20].

2.9. Gross lung lesion scores

The total extent of gross lung lesions was estimated and calculated as previously described [21]. The frequency distribution of the lung lesion scores for each lung lobe was calculated by treatment. The percentage of total lung with lesions was calculated using the following formula: $100 \times ((0.10 \times \text{left cranial}) + (0.1 \times \text{left middle}) + (0.25 \times \text{left caudal}) + (0.10 \times \text{right cranial}) + (0.10 \times \text{right middle}) + (0.25 \times \text{right caudal}) + (0.10 \times \text{accessory}))$.

2.10. Morphometric analyses

For the morphometric analyses of the microscopic pulmonary and lymph lesion scores, tissue sections were blindly examined by two veterinary pathologists (Seo and Chae) [4,22]. The morphometric analyses of in situ hybridization for *M. hyopneumoniae*