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Interaction between single-dose *Mycoplasma hyopneumoniae* and porcine reproductive and respiratory syndrome virus vaccines on dually infected pigs

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ABSTRACT

The objective of this study was to determine the effects of *Mycoplasma hyopneumoniae* and/or porcine reproductive and respiratory syndrome virus (PRRSV) vaccination on dually infected pigs. In total, 72 pigs were randomly divided into nine groups (eight pigs per group), as follows: five vaccinated and challenged groups, three non-vaccinated and challenged groups, and a negative control group. Single-dose vaccination against *M. hyopneumoniae* alone decreased the levels of PRRSV viremia and PRRSV-induced pulmonary lesions, whereas single-dose vaccination against PRRSV alone did not decrease nasal shedding of *M. hyopneumoniae* and mycoplasma-induced pulmonary lesions in the dually infected pigs. The *M. hyopneumoniae* challenge impaired the protective cell-mediated immunity induced by the PRRSV vaccine, whereas the PRRSV challenge did not impair the protective cell-mediated immunity induced by the *M. hyopneumoniae* vaccine. The present study provides swine practitioners and producers with efficient vaccination regimes; vaccination against *M. hyopneumoniae* is the first step in protecting pigs against co-infection with *M. hyopneumoniae* and PRRSV.

1. Introduction

Porcine respiratory disease complex (PRDC) is a serious respiratory disease in most pig-raising countries and is caused by multiple pathogens. Among these pathogens, *Mycoplasma hyopneumoniae* and porcine reproductive and respiratory syndrome virus (PRRSV) are two of the pathogens that are commonly isolated from pigs suffering from PRDC and are two of the major contributors to this syndrome (Van Alstine, 2012).

The relationship between *M. hyopneumoniae* and PRRSV is well known. *M. hyopneumoniae* potentiates pneumonia induced by PRRSV, whereas PRRSV does not potentiate pneumonia induced by *M. hyopneumoniae* (Thacker et al., 1999, 2000; Van Alstine et al., 1996). In addition, two-dose vaccination against *M. hyopneumoniae* decreases the potentiation of PRRSV-induced pneumonia on dually infected pigs (Thacker et al., 2000). However, previous studies were limited to determining the microbiological (viremia and nasal shedding) and immunological (interferon-γ-secreting cells [IFN-γ-SCs]) effects of PRRSV (or *M. hyopneumoniae*) infections in pigs that received only a *M. hyopneumoniae* (or PRRSV) vaccine and were then dually challenged.

Currently, a single-dose *M. hyopneumoniae* bacterin-based vaccine is widely used in pig production worldwide. Nevertheless, no studies have evaluated the effect of single-dose vaccination against *M. hyopneumoniae*, PRRSV, or both on dually infected pigs. Therefore, the objective of the present study was to determine the effects of a single-dose *M. hyopneumoniae* (or PRRSV) vaccine on dually challenged pigs based on microbiological (PRRSV viremia and nasal shedding of *M. hyopneumoniae*), immunological (anti-IgG antibodies and IFN-γ-SCs), and pathological outcomes.

2. Materials and methods

2.1. Commercial vaccines

The inactivated *M. hyopneumoniae* vaccine (RespiSure-One, Zoetis, Medison, NJ, USA) and modified live PRRSV vaccine (Fostera PRRS, Zoetis) were used in this study. The *M. hyopneumoniae* vaccine is an inactivated whole cell culture of *M. hyopneumoniae*, coupled with an oil adjuvant. The modified live PRRSV vaccine is based on a virulent US PRRS isolate (P129) attenuated using CD163 expressing cell lines. All of the vaccines that were used in this study were administered according to the manufacturer's instruction (one dose, via intramuscular route).

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Table 1
Experimental designs and results of lesion score, *Mycoplasma hyopneumoniae* (Mhp) DNA, and porcine reproductive and respiratory syndrome virus (PRRSV) antigen in different groups at 14 and 28 days post challenge (dpc).

Groups	Vaccination	Challenge	dpc	Lesion score		Mhp DNA	PRRSV antigen
				Mycoplasmal pneumonia*	PRRSV pneumonia†		
1	Mhp	Mhp	14	0.33 ± 0.55^{a}	0.17 ± 0.41^{a}	0.33 ± 0.52^{a}	0
			28	0.50 ± 0.52^{a}	0.33 ± 0.50^{a}	0.33 ± 0.52^{a}	0
2	Mhp	Mhp + PRRSV	14	0.67 ± 0.52^{ab}	2.17 ± 0.41^{c}	0.67 ± 0.52^{ab}	25.67 ± 6.25^{ab}
			28	0.65 ± 0.47^{a}	1.83 ± 0.41^{b}	1.00 ± 0.63^{a}	18.65 ± 6.12^{ab}
3	PRRSV	PRRSV	14	0.17 ± 0.41^{a}	1.33 ± 0.52^{b}	0	18.00 ± 5.02^{a}
			28	0.31 ± 0.48^{a}	0.83 ± 0.43^{a}	0	9.33 ± 5.89^{a}
4	PRRSV	Mhp + PRRSV	14	1.17 ± 0.75^{bc}	2.50 ± 0.55^{c}	1.17 ± 0.75^{b}	27.33 ± 5.75^{ab}
			28	2.17 ± 0.75^{b}	2.33 ± 0.52^{c}	2.00 ± 0.63^{b}	24.00 ± 6.16^{bc}
5	Mhp + PRRSV	Mhp + PRRSV	14	0.65 ± 0.51^{ab}	2.00 ± 0.63^{bc}	0.67 ± 0.48^{ab}	20.50 ± 3.62^{a}
			28	0.67 ± 0.52^{a}	1.83 ± 0.75^{b}	0.83 ± 0.41^{a}	13.00 ± 5.55^{a}
6	–	Mhp	14	1.19 ± 0.42^{bc}	0.50 ± 0.55^{a}	1.00 ± 0.63^{ab}	0
			28	2.00 ± 0.63^{b}	0.67 ± 0.52^{a}	1.83 ± 0.41^{b}	0
7	–	PRRSV	14	0.33 ± 0.52^{a}	2.83 ± 0.41^{c}	0	31.00 ± 5.06^{b}
			28	0.50 ± 0.55^{a}	2.00 ± 0.63^{bc}	0	17.67 ± 4.76^{ab}
8	–	Mhp + PRRSV	14	1.50 ± 0.83^{c}	3.33 ± 0.51^{c}	1.15 ± 0.38^{b}	35.00 ± 4.77^{b}
			28	2.33 ± 0.52^{b}	2.84 ± 0.43^{c}	2.17 ± 0.41^{b}	28.83 ± 4.67^{c}
9	–	–	14	0	0	0	0
			28	0	0	0	0

[a,b,c] Different letters (a, b, and c) indicate significant ($P < 0.05$) difference among groups.
* Mycoplasmal pneumonia = peribronchiolar and perivascular lymphoid tissue hyperplasia.
† PRRSV pneumonia = interstitial pneumonia.

2.2. Animals

A total of 72 colostrum-fed, cross-bred, conventional piglets were weaned and purchased at six days of age from a PRRSV-free commercial farm. They were all negative for porcine circovirus type 2 (PCV2), PRRSV, swine influenza virus and *M. hyopneumoniae* according to routine serological testing. *M. hyopneumoniae* and PRRSV were not detected in the nasal and serum samples, respectively, by the real-time polymerase chain reaction (PCR) (Dubosson et al., 2004; Wasilk et al., 2004). Individual piglets from seven days of age were uniquely identified by their ear notches.

2.3. Experimental design

In total, 72 pigs were randomly divided into nine groups (eight pigs per group), as follows: five vaccinated challenged (VC) groups, three unvaccinated challenged (UVC) groups, and one unvaccinated unchallenged group (Table 1). At seven days of age (–42 days post challenge, dpc), the pigs in Groups 1, 2, and 5 were injected intramuscularly in the right side of the neck with 2.0 ml of the *M. hyopneumoniae* vaccine. At 21 days of age (–28 dpc), the pigs in Groups 3, 4, and 5 were injected intramuscularly in the left side of the neck with 2.0 ml of the PRRSV vaccine. An equal volume of phosphate-buffered saline (PBS) (2.0 ml) was injected in the same anatomical location in the positive (Groups 6, 7, and 8) and negative (Group 9) control pigs at 7 and 21 days of age.

At 49 days of age (0 dpc), pigs in some of the VC (Groups 1, 2, 4, and 5) and UVC (Groups 6 and 8) groups were intratracheally administered a 10-ml dose of a 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 in the morning, as previously described (Kim et al., 2011). In the afternoon of the same day, the pigs in some of the VC (Groups 2, 3, 4, and 5) and UVC (Groups 7 and 8) groups were intranasally administered a 2-ml dose of PRRSV (strain SNUVR090851; 5th passage in MARC-145 cells) containing 1.2×10^5 tissue culture infective dose of 50% ($TCID_{50}$)/ml (Table 1).

Blood samples and nasal swabs were collected at –42, –28, 0, 7, 14, 21, and 28 dpc. The pigs from each group were sedated by intravenous injection of sodium pentobarbital and then euthanized by electrocution at 14 and 28 dpc. 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 *M. hyopneumoniae* DNA in nasal swabs

DNA was extracted from the nasal swabs using the QIAamp DNA Mini Kit (QIAGEN Ltd, Crawley, UK). The DNA extracts were used to quantify the *M. hyopneumoniae* genomic DNA copy numbers by real-time PCR as previously described (Dubosson et al., 2004). Real-time PCR was performed with primers based on the putative ABC transporter (GenBank no. U02537) (Dubosson et al., 2004).

2.5. Quantification of PRRSV RNA

RNA was extracted from serum samples and nasal swabs to quantify North American PRRSV genomic cDNA copy numbers, as previously described (Wasilk et al., 2004).

2.6. Serology

The serum samples were tested using the commercially available *M. hyopneumoniae* and PRRSV enzyme-linked immunosorbent assay (ELISA; IDEXX M.hyo Ab TEST and PRRS X3 Ab Test, IDEXX Laboratories Inc., Westbrook, ME, USA). Serum samples were considered positive for *M. hyopneumoniae* and PRRSV antibody if the S/P ratio was greater than 0.4 according to the manufacturer's instructions.

2.7. Preparation of *M. hyopneumoniae* antigen

M. hyopneumoniae antigen was prepared as previously described (Bandrick et al., 2008). Briefly, *M. hyopneumoniae* (strain SNU98703) was cultured in Friis' medium. When the pH of the culture reached 6 or lower, the organisms were harvested by continuous-flow centrifugation at 70,000*g*. The harvested *M. hyopneumoniae* was resuspended in Tris-sodium chloride (TN) buffer (pH 7.2 to 7.4) in 1/100 of the original volume of the culture and washed three times by centrifugation, each with the same proportion of TN buffer. The washed *M. hyopneumoniae* was inactivated by one freeze-thaw cycle and then by sonic disruption