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Porcine epidemic diarrhea vaccine efficacy evaluation by vaccination timing and frequencies

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ABSTRACT

Porcine epidemic diarrhea (PED) virus is a causative agent of enteric disease characterized by watery diarrhea and dehydration. Because PED has high morbidity and mortality, especially in suckling piglets, it causes a great economic loss to swine farms worldwide. Although various PED vaccines have been developed and commercialized, their efficacies are still controversial. In particular, current PED vaccination protocol (vaccination at 2 and 4 weeks before farrowing) may cause stress in pregnant sows. In this study, we compared the effects of PED vaccination timing and frequency for its efficacy by measuring the PED virus-specific antibodies. We found that vaccination at early stages of pregnancy induces similar levels of serum and colostrum antibodies with those at late stages of pregnancy. As the number of vaccinations increased, the amounts of antibody in serum and colostrum, and neutralizing activities increased. Our results provide important information for establishing a more efficient PED vaccination protocol.

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1. Introduction

Porcine epidemic diarrhea (PED), which is caused by the porcine epidemic diarrhea virus (PEDV), is an enteric disease affecting pigs of all ages [1]. The disease is characterized by acute watery diarrhea, dehydration, and vomiting, with high morbidity and mortality in suckling piglets [1]. PED was first identified in the United Kingdom and Belgium in the early 1970s [2,3], and then it has emerged in numerous European and Asian countries for decades [4–7]. In 2010, variant strains of PEDV associated with high mortality emerged in China and pose a serious concern for China's swine industry [8,9]. From 2013 to 2014, outbreaks of variant PED were reported in the United States and rapidly spread across North America, resulting in high mortality in infected newborn piglets in more than 17 states [10,11]. The worldwide outbreaks of virulent PEDVs cause economic losses in the swine industry and raises the need for an effective vaccine against emergent PEDV strains.

Most vaccines in use are traditional live attenuated or killed vaccines using CV777-like strain [12,13]. In South Korea, a multiple-dose vaccination program at 2- or 3-week intervals starting before farrowing is commonly recommended in pregnant sows

[14]. Despite nationwide use of commercially available vaccines, South Korea experienced a devastating PED epidemic in 2013–2014 [15,16], suggesting that new strains and/or strategies for PED vaccines are needed. Nevertheless, the current PED vaccine strategy increases the stress on pregnant sows due to the timing of vaccination. Although most swine farms follow this vaccine strategy, its effectiveness has not been evaluated for PEDV.

In this study, we compared antibody titers on serum and colostrum from sows vaccinated at different timing and parity. Our results would provide valuable information for the development of a new PED vaccine strategy.

2. Materials and methods

2.1. Experimental design

All sows were sourced from PEDV-free swine herds, confirmed to have no antibodies against PEDV, and bled before vaccination. PED vaccination was performed through intramuscularly with inactivated PEDV vaccines (PED-CUP-B2014 strain, IMMUNIS-PED-M[®], Woogene B&G Co., Ltd.) at 2 and 4 weeks before farrowing unless otherwise indicated. For vaccination frequency experiments, 25 sows with various parity were randomly selected. Because each farm vaccinates sows with PED vaccine two times before farrowing, each parity means two vaccinations. For

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example, the 1st parity group had 2 vaccinations, and the second parity group had 4 vaccinations. For vaccination timing experiment, 10 sows were divided into two groups. Five sows were vaccinated twice at 2 and 4 weeks before farrowing (2/4 program). The other five sows were vaccinated twice at 17 and 19 weeks before farrowing (17/19 program). Colostrum was collected at the day of farrowing. Sera from sows and piglets were collected at 7 days after farrowing. All animal-related experimental protocols were approved by the Chungnam National University Institutional Animal Care and Use Committee.

2.2. Enzyme-linked immunosorbent assay

PEDV (SM98 strain) was coated in 96 wells at 4 °C overnight. The plates were washed three times with 200 μ l of phosphate buffered saline (PBS, pH 7.2) containing 0.1% Tween20, and blocked with 100 μ l blocking solution. Sera were diluted 1:200 in blocking solution, then incubated for 1 h at 37 °C. After washing, 100 μ l of horseradish peroxidase-conjugated goat anti-swine IgG or goat anti-swine IgA were added. The color was developed using 3,3',5,5'-Tetramethylbenzidine solution for 10 min at room temperature in the dark. Optical density was measured at 450 nm.

2.3. Serum neutralization (SN) test

Sera samples were inactivated at 56 °C for 30 min and stored at –20 °C. The heat-inactivated sera were serially diluted two-folds

and PEDV (200 TCID₅₀/0.1 ml) was mixed with equal volume of diluted sera. Mixture was incubated for 1 h at 37 °C. Vero cells were incubated with 0.1 ml mixture for 1 h at 37 °C. Cells then were washed three times with PBS and maintained in Minimum Essential Medium containing 5 μ g/ml trypsin for 5 days at 37 °C. SN titer expressed as the reciprocals of the highest serum dilution resulting in the inhibition of cytopathic effect.

2.4. Statistical analysis

Data are presented as mean \pm standard deviation (SD). Statistical significance was calculated using the Holm-Sidak multiple Student's *t*-test procedure. A *P* value of <0.05 was considered statistically significant.

3. Results

3.1. The effect of PED vaccination timing on its efficacy

For testing vaccination at early stages of pregnancy is sufficient to induce immune responses, sows were vaccinated at 17 and 19 weeks before farrowing, and then immune responses were compared to those acquired from 2/4 vaccination program. The PEDV-specific IgG levels in sow sera were similar between 2/4 and 17/19 groups (Fig. 1A). Although mean value of IgG levels between the two groups was similar, variation in OD value was higher in sow sera collected from 2/4 group. The IgG level in

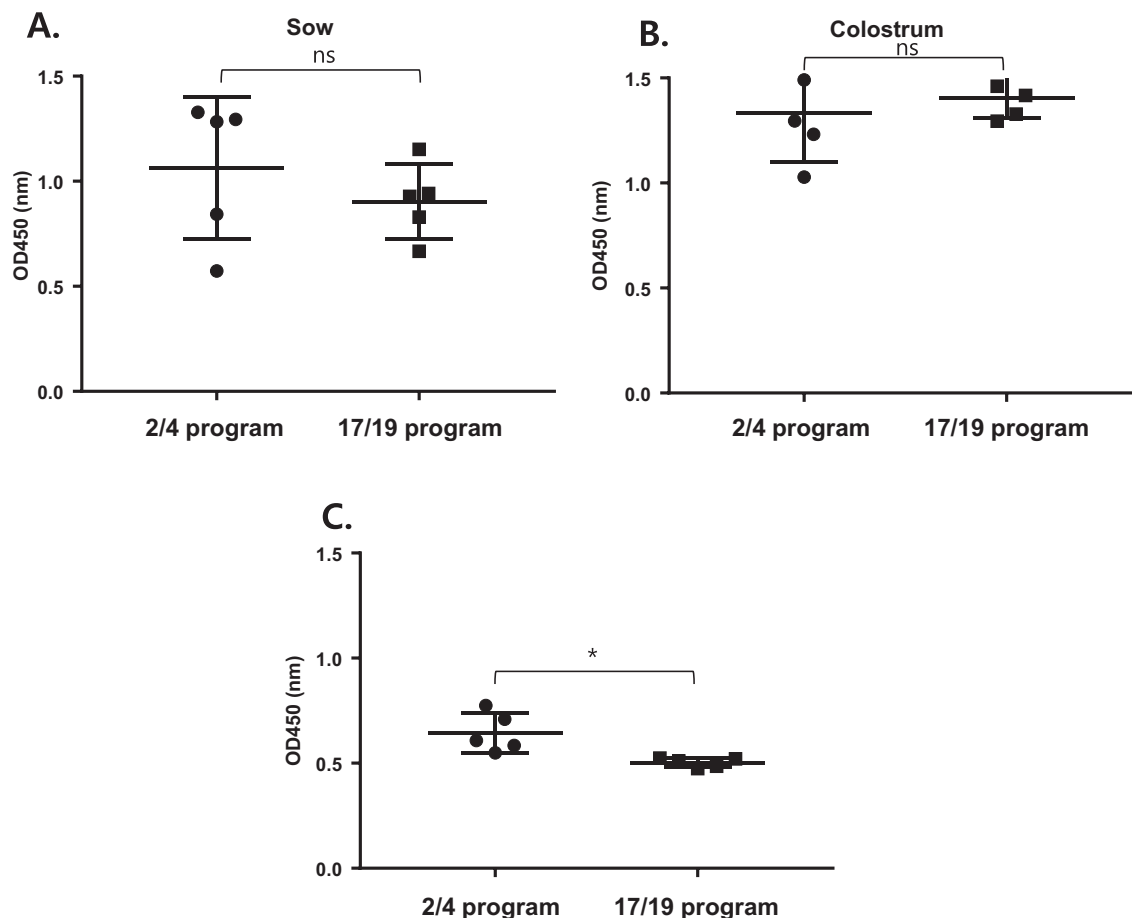


Fig. 1. The effect of vaccination timing on production of PEDV-specific antibodies. The pregnant sows were vaccinated with commercial PEDV vaccines twice at 2 and 4 weeks (2/4 program) or at 17 and 19 weeks (17/19 program) prior to farrowing. Serum samples of sows (A) and piglets (C) were collected at 7 days after farrowing. Colostrum (B) samples were collected at the day of farrowing. PEDV specific IgG antibodies in serum and colostrum were measured by ELISA. Error bars present SD from the mean (n = 5). Statistical significance was assessed by student's *t*-test. **P* < 0.05; ns, not significant.

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