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Short communication

Antibody level comparison after porcine epidemic diarrhea vaccination via different immunization routes

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Abstract

Porcine epidemic diarrhea (PED) is a disease extremely harmful to pig health. Intramuscular and Houhai acupoint injections are the main immunization routes to prevent and control PED. This study aimed to evaluate the efficacy of these two routes in pregnant sows based on serum IgG, IgA, and neutralizing antibody levels. PED virus (PEDV) immunoprophylaxis with live-attenuated and inactivated vaccines was administered. The vaccinations for the intramuscular injections elevated IgG and neutralizing antibody levels more than Houhai acupoint injections at most timepoints after immunization. However, the anti-PEDV IgA antibodies induced by vaccination with the two immunization routes did not differ significantly. In conclusion, intramuscular injections are better than Houhai acupoint injections for PEDV vaccination of pregnant sows.

Keywords: porcine epidemic diarrhea, IgG, IgA, neutralizing antibody

Introduction

Porcine epidemic diarrhea (PED) is an acute and highly contagious enteritis with extensive diarrhea caused by the PED virus (PEDV). Its main manifestations include vomiting, watery diarrhea, and dehydration (Jung et al. 2020). Piglets up to 7 days of age infected with PEDV exhibit a mortality rate of up to 100% (Shibata et al. 2000). China witnessed a PED epidemic in pig herds, posing a significant threat to the pig industry (Sun et al. 2016).

PED is associated with a high mortality in neonatal piglets (Shibata et al. 2000). However, owing to the immune response after PEDV vaccination, a few farms provide immunoprophylaxis to neonatal piglets to prevent and control PED. Most farms vaccinate pregnant

sows, thereby supplying sufficient maternal antibodies to neonatal piglets for passive immune protection (Lee 2015). Currently, PEDV vaccines injected intramuscularly or at Houhai acupoints are often used to prevent PED in China (Lv et al. 2016, Sun et al. 2016). The Houhai acupoint is a site for acupuncture stimulation located in the fossa between the anus and the tail base in animals. However, which immunization route is more effective remains unknown. This study aimed to evaluate the intramuscular and Houhai acupoint administration routes for PEDV vaccination of pregnant sows based on serum IgG, IgA, and neutralizing antibody levels.

Materials and Methods

Thirty sows at 60 days of gestation were randomly divided into two groups for PEDV vaccination as follows: intramuscular group, including 16 sows administered with the PEDV vaccine intramuscularly, and Houhai acupoint group, including 14 sows administered with the PEDV vaccine at Houhai acupoints. The attenuated and inactivated PEDV vaccines had been manufactured by Harvac Biotechnology, Co., Ltd., Harbin, China, and Pulike Biological Engineering Inc., Luoyang, China, respectively. The needle was placed parallel to the spine and inserted to a depth of 1.5-3.0 cm. First, a dose of the attenuated vaccine was administered 35 days before farrowing. After 21 days, a booster dose of 4 mL of the inactivated vaccine was administered. Blood samples were collected 3 days before the first dose and 20, 35, 42, and 52 days after administration of the first dose. Sera were separated by centrifugation at 5,000 rpm for 10 min and preserved at -20°C. Serum samples used in this study were obtained based on informed consent from farm owners. Collection of serum samples from the pigs was approved by the Institutional Animal Care and Use Committee (IACUC) of Yichun University (permit number JXSTUDKY2019009), and all the methods were performed in accordance with the relevant guidelines and regulations.

Anti-PEDV IgA and IgG antibody test kits were purchased from IDEXX Laboratories, Inc. (Westbrook, Maine, USA), and Combetter Biological, Inc. (Changsha, China), respectively. All anti-PEDV antibody tests were performed according to manufacturer's instructions. In brief, validation of anti-PEDV IgA antibody results required the mean optical density (OD) of the positive control to be ≥0.55 and mean OD of the negative control to be ≤0.10. The sample OD results were transformed into S/P values as follows: [(sample OD − mean OD negative control)/mean OD positive control − mean OD negative control)]. An S/P value ≥0.50 was determined as a positive anti-PEDV IgA antibody test. Moreover, validation of anti-PEDV IgG antibody kit results required the mean OD of the positive control to be ≥0.50 and mean OD of the negative control to be ≤0.10. The sample OD results were transformed into S/P values as aforementioned. An S/P value ≥0.20 was determined as a positive anti-PEDV IgG antibody test. Neutralizing serum antibody titers were measured with the microtiter method. First, the 50% tissue culture infectious dose ($TCID_{50}$) of the PEDV strain (isolated and preserved at the Preventive Veterinary Laboratory of Hunan Agricultural University) was determined. The serum to be tested and the PEDV-positive and PEDV-negative control sera were inactivated at 56°C for 30 min and diluted with two-fold series. A volume of 50 μL of serum with different dilutions was equally mixed with the PEDV containing the virus titer at 200 $TCID_{50}$/0.1 mL. The mixtures were incubated at 37°C for 1 h and then with Vero cell monolayers. Incubation at 37°C with 5% CO_2 was continued for 5 days. Cytopathic conditions were observed and recorded daily and serum neutralizing (SN) antibody titers were calculated with the Reed–Muench method. The SN antibody titer was expressed as the reciprocal of the highest serum dilution showing the inhibition of cytopathic effects. A titer of ≥20 was recorded as a positive anti-PEDV neutralizing antibody test (Brown et al. 2023).

An independent *t*-test was used to assess the antibody data using SPSS software (version 19.0; IBM Inc., Chicago, IL, USA). The results were considered significant at $p<0.05$.

Results and Discussion

The average S/P values for the anti-PEDV IgG antibodies before the intramuscular and Houhai acupoint injections were 4.70 and 4.26, respectively, showing no significant difference ($p>0.05$). However, 20, 35, 42, and 52 days after administration of the first dose, the average S/P value for the anti-PEDV IgG antibody in the intramuscular injection group was significantly higher than that in the Houhai acupoint injection group (Fig. 1a, $p<0.05$).

Before vaccination, the average S/P values for the anti-PEDV IgA antibody in the intramuscular and Houhai acupoint injection groups were 1.96 and 2.02, respectively, showing no significant difference ($p>0.05$). The anti-PEDV IgA antibody levels in both groups had significantly increased 20 and 35 days after vaccination (Fig. 1b, $p<0.05$). However, 20, 35, 42, and 52 days after administration of the first dose, the average S/P values

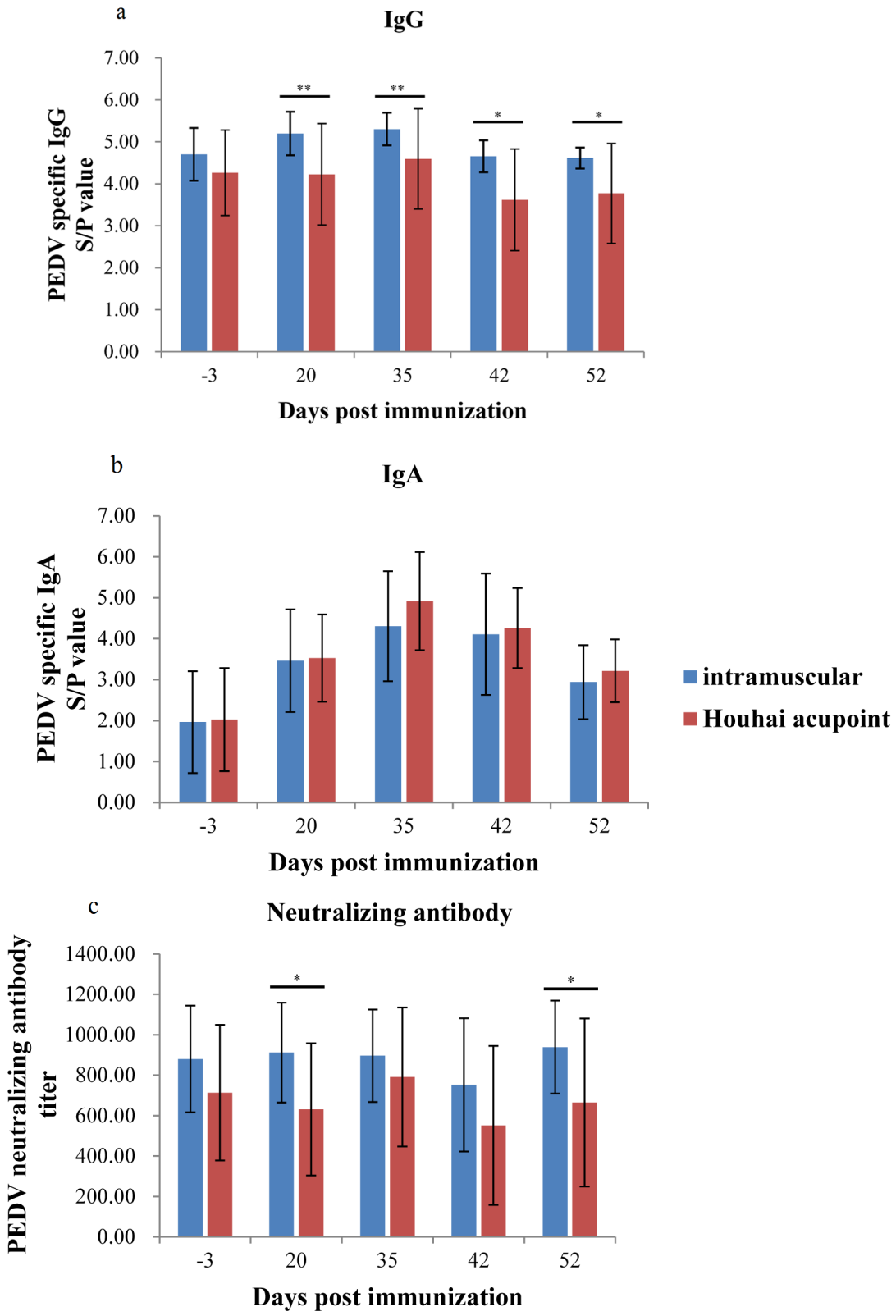

Fig. 1. Antibody responses in sows after intramuscular and Houhai acupoint injections of the Porcine epidemic diarrhea virus vaccine. Pregnant sows were injected with a dose of the attenuated vaccine 35 days before farrowing intramuscularly or at Houhai acupoints, and booster-immunized with 4 mL of the inactivated vaccine 21 days after the primary immunization. PEDV-specific IgG (a), IgA (b), and neutralizing antibodies (c) were tested. Significance was determined by the independent-samples t-test. * $p<0.05$; ** $p<0.01$ (intramuscular vs. Houhai acupoint)

for the anti-PEDV IgA antibodies in the two groups were not significantly different (Fig. 1b, $p>0.05$).

Before vaccination, the average anti-PEDV neutralizing antibody titers in the intramuscular and Houhai acupoint injection groups were 880 and 713.14, respectively, showing no significant difference ($p>0.05$). However, 20 and 52 days after administration of the first dose, the average anti-PEDV-neutralizing antibody titer in the intramuscular injection group was significantly higher than that in the Houhai acupoint injection group (Fig. 1c; $p<0.05$). Although the anti-PEDV-neutralizing antibody titer in the intramuscular injection group was higher than that in the Houhai acupoint injection group 35 and 42 days after the administration of first dose, the antibody titers in the two groups were not significantly different (Fig. 1c, $p>0.05$).

Although intramuscular vaccination is the most common form of immunization, immunization with vaccines at the Houhai acupoint enhances the immune response (Jin et al. 2020, Xu and Hu 2021). Immunization with the PEDV vaccine at the intramuscular and Houhai acupoints has been carried out to control PED in some pig farms in China (Lv et al. 2016). In the present study, serum IgG and neutralizing antibody levels were higher in the intramuscular injection group than in the Houhai injection group. Moreover, the antibody levels were significantly different between the two groups at most timepoints after immunization. These results suggest that intramuscular injections of the PEDV vaccine may be better than Houhai acupoint injections, which is inconsistent with a recent study on antibody production of PEDV vaccination at different anatomical sites, demonstrating higher antibody levels in the Houhai acupoint injection group (Jin et al. 2020). This inconsistency is possibly related to the immunization response of different animals and vaccination strategies.

Mucosal immunization also plays an important role in the prevention and control of PED (Langel et al. 2019, Hsueh et al. 2020, Krishna et al. 2020). We observed that the serum IgA antibody levels in the Houhai acupoint injection group were higher than those in the intramuscular injection group, indicating that the Houhai acupoint injection was superior in inducing mucosal immunity. However, the antibody levels were not significantly different between the two groups. In addition, considering the small area of the Houhai acupoint and high operation requirements, the absorption of the PEDV vaccine is poor and leads to immune failure. Therefore, we recommend intramuscular vaccination against PEDV based on the immune procedures described in the present study.

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