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Research Article

Study on the Antibody Level Differences of PED IgG and IgA, TGE IgG and PoR IgG After Immunization with Different Porcine Viral Diarrhea Vaccine Combinations

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Abstract

To prevent porcine viral diarrhea, a vaccine combination that can provide good antibody levels needs to be determined. In this study, we screened 30 pregnant sows divided into six experimental groups, namely, five immunized groups and one control group, to investigate the antibody level differences of different vaccine combinations on porcine epidemic diarrhea (PED), porcine transmissible gastroenteritis (TGE), porcine rotavirus (PoR) IgG, and PED IgA. The antibody level was detected by ELISA. Results showed that the antibody levels of PED and TGE IgG in serum and PED IgA in breast milk of the "PT+PT*" vaccine combination group were higher than those of the other groups, and vaccine combination including "PTR" could stimulate the sows to produce PoR IgG antibody. These findings revealed that the vaccine combination of "PT+PT*" is optimal for preventing porcine viral diarrhea, and "PTR+PT*" may be an alternative option in areas under PoRV infection risk. This study suggested that pig farms should select suitable immunization on the basis of the local epidemic situation of porcine viral diarrhea.

Keywords: *Antibody level differences, Porcine epidemic diarrhea IgG and IgA, Porcine transmissible gastroenteritis IgG, Porcine rotavirus IgG, Vaccine combination, ELISA detection*

Farklı Domuz Viral Diyare Aşı Kombinasyonları İle İmmunizasyon Sonrası PED IgG ve IgA, TGE IgG ve PoR IgG Antikor Seviyelerindeki Farklılıkların Araştırılması

Öz

Domuz viral diyareyi önlemek için iyi antikor seviyesi sağlayan bir aşı kombinasyonunu belirlemeye ihtiyaç bulunmaktadır. Bu çalışmada, farklı aşı kombinasyonlarının domuz epidemik diyare, domuz bulaşıcı gastroenteritisi ve domuz rotavirus IgG seviyeleri ile domuz epidemik diyare IgA seviyesine etkilerini araştırmak amacıyla 30 gebe domuz 5'i immunize grup 1'i kontrol olmak üzere altı gruba ayrıldı. Antikor seviyeleri ELISA ile belirlendi. Elde edilen sonuçlar, domuz epidemik diyare ve domuz bulaşıcı gastroenteritisi serum IgG seviyeleri ile meme sütünde domuz epidemik diyare IgA seviyesinin "PT+PT*" aşı kombinasyonu grubunda diğerlerinden daha yüksek olduğunu ve "PTR"yi içeren aşı kombinasyonunun domuzları domuz rotavirus IgG antikoru üretmek üzere stimüle ettiğini gösterdi. Bu sonuçlar "PT+PT*" aşı kombinasyonunun domuz viral diyareyi önlemede en iyi olduğunu ve "PTR+PT*"in domuz rotavirus enfeksiyon riski bulunan bölgelerde bir alternatif olabileceğini gösterdi. Çalışma sonucunda domuz viral diyarenin bölgesel epidemik durumuna göre domuz çiftliklerinin immunizasyon seçiminde bulunması önerilir.

Anahtar sözcükler: *Antikor seviyesi farklılığı, Domuz epidemik diyare IgG ve IgA, Domuz Bulaşıcı Gastroenteritisi IgG, Domuz rotavirus IgG, Aşı kombinasyonu, ELISA tespiti*

INTRODUCTION

Porcine epidemic diarrhea virus (PEDV), porcine transmissible gastroenteritis virus (TGEV), and porcine rotavirus (PoRV) are the three main pathogens causing viral diarrhea in pigs. They can infect pigs of all ages and causes watery diarrhea, vomiting, dehydration, and gradual weight loss [1]. In recent years, porcine viral diarrhea diseases have shown mixed infections with multiple pathogens. Additionally, considering new problems, such as the epidemics of variant PEDV, the protective rate of porcine viral diarrhea vaccines has decreased. The immune protection effect of

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many immune pig farms has not reached the desirable expectation [2,3], causing huge economic losses to the pig breeding industry in China.

Porcine epidemic diarrhea virus is an enveloped, single-stranded, positive-sense RNA virus belonging to the order Nidovirale, the family Coronaviridae, subfamily Coronavirinae, and genus *Alphacoronavirus* [4]. Sequencing and genotyping based on the S gene is suitably used for molecular epidemiology analysis and vaccine development of PEDV [5,6]. Phylogenetic analysis of the full-length S gene inferred by a neighbor-joining method indicates that PEDV could be genetically divided into two groups, which include GI and GII. GI and GII can be further divided into subgroups Ia and Ib, and IIa and IIb [3].

Transmissible gastroenteritis virus is a member of the enteropathogenic *alpha-coronavirus* family, with a large positive-stranded RNA genome [7]. And it is currently divided into two distinct genogroups: the Miller cluster and the Purdue cluster, most of strains isolated since 2010 from China has a close relationship with the Purdue strain and is more distant evolutionarily from the Miller strains group [8]. PoRV is double-stranded RNA (dsRNA) viruses with 11 genomic segments encoding 6 structural viral proteins (VP1-VP4, VP6, VP7) and 5 or 6 nonstructural proteins, is a member of *Rotavirus* genus, within the *Reoviridae* family [9].

Vaccination has been used for many years in China, and vaccines used include inactivated and live-attenuated vaccines. There are three main commercial vaccines include: A genotype Ia strain CV777-based attenuated trivalent vaccine was licensed by the Harbin Veterinary Research Institute, Chinese Academy of Agriculture Sciences in 2014. A genotype II a strain ZJ08-based attenuated bivalent vaccine was licensed by the Beijing Dabeinong Technology Group Co., Ltd. in 2015. A genotype II b strain AJ1102-based inactivated bivalent vaccine developed by Wuhan Keqian Biology Co., Ltd. in 2016.

At present, A consensus of commercial vaccines for preventing and controlling porcine viral diarrhea has been reached regarding the immunization time of commercial vaccines, but the differences of antibody levels of different vaccine combinations have not been further studied. In this study, pregnant sows were immunized with different vaccine combinations. The blood samples and breast milk were collected at different stages, and relevant specific antibody levels were obtained to analyze the differences in antibodies between different vaccine combinations. Results of this study provided an experimental basis for viral diarrhea vaccine immunization in pig farms.

MATERIAL and METHODS

Ethical Statement

All experimental procedures involving pigs were performed in accordance with the regulations of the Administration of Affairs Concerning Experimental Animals, approved by Laboratory Animal Bioethics Committee of Institute of Animal Husbandry and Veterinary Medicine in accordance with animal ethics guidelines and approved protocols. The approval numbers of the ethics committee are IAHV-AEC-2018-0126.

Experimental Sows and Sites

A total of 30 "Landrace ×Yorkshire" gestation sows with similar gestational ages were screened for this study. The sows' feces and blood samples were collected to confirm that pregnant sows were PEDV, TGEV, and PoRV pathogen negative by RT-PCR, and serological antibody levels were consistent via ELISA before immunization. The experimental sows and sites were provided by the Farm of Fujian Academy of Agricultural Sciences.

Experimental Vaccines and Main Reagents

PEDV-TGEV-PoRV (CV777 + H + NX-G5) trivalent attenuated vaccine (PTR for short), PEDV-TGEV (ZJ08 + HB08) duple attenuated vaccine (PT for short), and PEDV-TGEV (AJ1102 + WH-1) duple inactivated vaccine (PT* for short) were employed. The PED, TGE, and PoR ELISA antibody detection kits (batch numbers: 20170526, 20170713, 20170526) were purchased from Harbin Animal Biological Products National Engineering Research Center Co., Ltd., China. The PED IgA ELISA antibody detection kit was purchased from BIONOTE Biotechnology Co., Ltd., Korea.

Experimental Sow Groups and Immunization Procedures

The 30 gestational sows were randomly divided into six experimental groups, with five gestational sows in each group. Five immunization groups and one control group were established. The sow immunization schedule is shown in *Table 1*. All sows were vaccinated by injection at Houhai acupoint twice at 40 days and 20 days before farrowing. In group A, the sows were vaccinated PTR and PT*, respectively. In group B, the sows were vaccinated PTR and PTR, respectively. In group C, the sows were vaccinated PT and PT*, respectively. In group D, the sows were vaccinated PT and PT, respectively. In group E, the sows were vaccinated PT* and PT*, respectively. And the sows in control group were injected with 4 mL sterile 0.9% NaCl.

Collection of Serum and Breast Milk Samples from Immunized Sows

About 5 mL of blood samples were collected from each experimental group through the ear vein at 0, 21, 35, and 49 days post-first immunization. The collected blood samples were centrifuged to obtain the supernatant, which was stored at -20°C before use. Approximately 2 mL of breast milk was collected from each experimental group at 1, 3, 7, and 14 days post-delivery, centrifuged

Table 1. *Sow immunization schedule*

Groups	The First Immunization Time	Vaccine Species	Immunization Dose	The Second Immunization Time	Vaccine Species	Immunization Dose	Immunization Pathways
A	Prenatal 40d	PTR	Attenuated vaccine 1 dose	Prenatal 20d	PT*	Inactive vaccine 4 mL	Houhai acupoint injection
B	Prenatal 40d	PTR	Attenuated vaccine 1 dose	Prenatal 20d	PTR	Attenuated vaccine 1 dose	Houhai acupoint injection
C	Prenatal 40d	PT	Attenuated vaccine 1 dose	Prenatal 20d	PT*	Inactive vaccine 4 mL	Houhai acupoint injection
D	Prenatal 40d	PT	Attenuated vaccine 1 dose	Prenatal 20d	PT	Attenuated vaccine 1 dose	Houhai acupoint injection
E	Prenatal 40d	PT*	Inactive vaccine 4 mL	Prenatal 20d	PT*	Inactive vaccine 4 mL	Houhai acupoint injection
F	Prenatal 40d	0.9% NaCl	0.9% NaCl 4 mL	Prenatal 20d	0.9% NaCl	0.9% NaCl 4 mL	Houhai acupoint injection

to remove the cream to obtain whey, and stored at -20°C before use.

ELISA Antibody Detection Method

Detection of PED, TGE, and PoR IgG antibodies in serum were carried out according to the instructions of each ELISA antibody detection kit. PED IgA antibody in breast milk was detected following the kit instructions of BIONOTE Biotechnology Co., Ltd., Korea. S/P values for PED, TGE, and PoR IgG antibodies were calculated as follows: (Mean OD_{450nm} of sample - Mean OD_{450nm} of the standard negative control)/(Mean OD_{450nm} of the standard positive control - Mean OD_{450nm} of the standard negative control). The judging criteria were as follows: PED, TGE, and PoR IgG antibody S/P value ≥0.4 is positive; PED IgA antibody cut off value = 0.35 + mean OD_{450nm} of the standard negative control, mean OD_{450nm} of the sample above the cut off value is positive, and mean $OD_{450nm}$of the sample less than the cut off value is negative. The rest of the operating procedures and conditions for establishment were performed in accordance with corresponding kit instructions.

Statistics

We used SPSS16.0 and Excel 2010 for data statistics and charting. Data are presented as mean ± standard deviation (SD). Statistical significance was calculated using a one-way analysis of variance (ANOVA) that was applied for multiple comparisons between the groups. The significance was considered as significant at $P<0.05$ and highly significant at $P<0.01$.

RESULTS

The blood samples of the six groups of experimental sows were obtained at different times after immunization, and the PED IgG antibodies were detected by ELISA. The results showed *(Fig. 1A)* that the PED IgG antibody levels of sows varied at 21 days post-first immunization. The antibody levels increased with different degrees in groups A, B, C, and D and decreased in groups E and F. The PED IgG antibody levels in groups C and D were significantly different from those in group E and control group F ($P<0.05$) *(Table 2)*. Moreover, the IgG antibody levels of groups C and D were higher than those of groups A and B, but those of groups C and D were not significantly different from those of groups A and B ($P>0.05$) .

After secondary immunization, the PED IgG antibody levels of sows increased at 35 days post-first immunization in groups A, C, and E due to PT* *(Fig. 1A)*. The three groups reached the peak value, and the order of S/P value from highest to lowest was groups C, A, and E. Unfortunately, after sows were boosted by PTR in group B and PT in group D, the PED IgG antibody level of the sows decreased. Group B was significantly different from groups A ($P<0.05$), C ($P<0.01$), and E ($P<0.05$), but no significant difference was observed among A, C, and E ($P>0.05$). Thus, the immunization of secondary booster with attenuated vaccines resulted in the decrease in PED IgG antibody levels. The PED IgG antibody level of sows in the control group F showed a downward trend.

At 49 days post-first immunization, the PED IgG antibody levels of sows in all groups showed a decreasing trend. The order of S/P values from highest to lowest was C, D, A, E, B, and F. The above results showed that different vaccine combinations and PEDV vaccine strains exerted certain effects on the PED IgG antibody level in the sows.

On the basis of PED IgG antibody growth and decline after immunization *(Fig. 2A)*, we found that the PED IgG antibody level showed a change law of "rise, rise, and decline" in groups that used the vaccine combination of "attenuated vaccine + inactivated vaccine" (groups A and C). In groups that used the vaccine combination of "attenuated vaccine + attenuated vaccine" (groups B and D), the PED IgG antibody level showed a change law of "rise, decline, and decline". With the vaccine combination of "inactivated vaccine + inactivated vaccine" (group E), the PED IgG antibody level showed a change law of "decline,

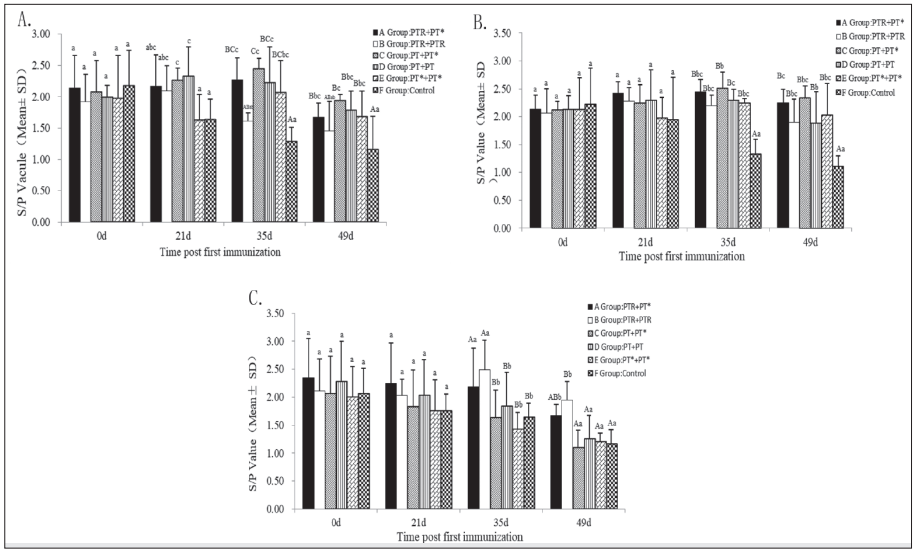

Fig 1. Specific IgG antibody responses in sows serum. The sows (n=5 per group) were vaccinated with five vaccine combinations or sterile 0.9% Nacl. Serum samples were collected at 0, 21, 35, and 49 days post-first immunization. ELISA antibody titers were measured by an indirect ELISA. Each bar represents mean + SD. Statistical significance was calculated using a one-way analysis of variance (ANOVA). Different uppercase letters on the S/P value bars indicate that the difference is extremely significant (P<0.01), and different lowercase letters indicate significant difference (P<0.05). (A) PED, (B) TGE, (C) PoR

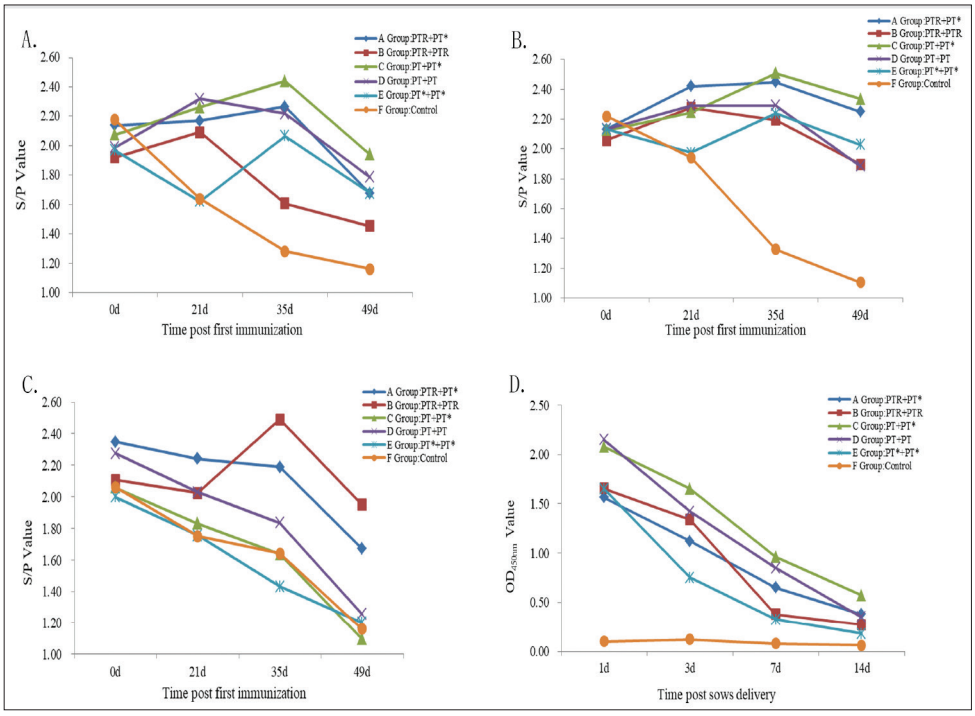

Fig 2. Growth and decline of specific antibody in sows after vaccinated with five vaccine combinations or sterile 0.9% Nacl. (A) PED-specific IgG antibodies in serum. (B) TGE-specific IgG antibodies in serum. (C) PoR-specific IgG antibodies in serum. (D) PED-specific IgA antibodies in breast milk

Table 2. *The detection results of PED IgG antibody in different vaccine combinations*

Groups	S/P Value (Mean±SD)/Time			
	1d	21d	35d	49d
A	2.14±0.52^{a}	2.17±0.50^{abc}	2.27±0.35^{BCc}	1.68±0.22^{Bbc}
B	1.92±0.44^{a}	2.09±0.40^{abc}	1.61±0.14^{ABab}	1.45±0.47^{ABab}
C	2.08±0.50^{a}	2.26±0.19^{c}	2.44±0.17^{Cc}	1.94±0.10^{Bc}
D	1.99±0.20^{a}	2.32±0.48^{c}	2.22±0.57^{BCc}	1.79±0.30^{Bbc}
E	1.97±0.69^{a}	1.62±0.41^{a}	2.07±0.51^{BCbc}	1.68±0.41^{Bbc}
F	2.18±0.56^{a}	1.64±0.33^{a}	1.28±0.24^{Aa}	1.66±0.53^{Aa}

Different uppercase letters on the S/P or OD450nm value columns indicate that the difference is extremely significant (P<0.01), and different lowercase letters indicate significant difference (P<0.05)

Table 3. *The detection results of TGE IgG antibody in different vaccine combinations*

Groups	S/P Value (Mean±SD)/Time			
	1d	21d	35d	49d
A	2.13±0.25^{a}	2.42±0.21^{a}	2.45±0.22^{Bbc}	2.25±0.24^{Bc}
B	2.06±0.44^{a}	2.28±0.24^{a}	2.19±0.19^{Bbc}	1.90±0.41^{Bbc}
C	2.12±0.15^{a}	2.24±0.33^{a}	2.51±0.29^{Bb}	2.33±0.22^{Bbc}
D	2.13±0.25^{a}	2.29±0.55^{a}	2.29±0.20^{Bc}	1.88±0.57^{Bb}
E	2.13±0.56^{a}	1.98±0.37^{a}	2.32±0.09^{Bbc}	2.03±0.56^{Bbc}
F	2.22±0.65^{a}	1.94±0.76^{a}	1.33±0.26^{Aa}	1.11±0.19^{Aa}

Different uppercase letters on the S/P or OD450nm value columns indicate that the difference is extremely significant (P<0.01), and different lowercase letters indicate significant difference (P<0.05)

rise, and decline" whereas the control group (group F) showed a trend of "decline, decline, and decline". These results indicated that the vaccine combination of "attenuated vaccine + inactivated vaccine" showed better immune effects than the other combinations, and group C demonstrated improved PED IgG maternal antibody for suckling piglets.

The TGE IgG antibody detection results showed that the TGE IgG antibodies levels in groups A, B, C, and D increased at 21 days post-first immunization *(Fig. 1B)*, and the order of S/P values from highest to lowest was A, D, B, and C. Inversely, the TGE IgG antibody levels in groups E and F decreased. However, the difference among six experimental groups was not significant (P>0.05) *(Table 3)*.

At 35 days post-first immunization, the results of TGE IgG antibody levels showed that the groups that used PT* booster immunization for the second immunization (groups A, C, and E) had higher TGE IgG antibody levels than groups B and D, which used PTR or PT, respectively. The order of peak S/P values of TGE IgG antibody in each group from highest to lowest was C, A, E, D, and B. The difference between groups C and D was significant (P<0.05), whereas groups A and C showed no significant difference (P>0.05).

The antibody levels of TGE IgG decreased in all groups at 49 days post-first immunization. The above results indicated that the difference in the antibody level of TGE IgG between the groups was mainly caused by the combination of attenuated vaccine or inactivated vaccine.

After immunization with different vaccine combinations, the results of the growth and decline law of TGE IgG antibody levels showed that the groups showed a trend of "rise, rise, and decline" *(Fig. 2B)*, which used the "attenuated vaccine + inactivated vaccine" combination (groups A and C). In the groups that used the combination of "attenuated vaccine + attenuated vaccine" (groups B and D), the growth and decline of TGE IgG antibody levels showed a law of "rise, fall (or plateau), and decline". Moreover, the TGE IgG antibody level of group E that used "inactivated vaccine + inactivated vaccine" showed a law of "decline, rise, and decline" but a decreasing trend in control group F. These results showed some differences in the growth and decline law of TGE IgG antibody among vaccine combinations, and the vaccine combination of groups A and C was suitable in practice. However, the difference between groups A and C was not significant (P>0.05), thereby indicating that the TGE IgG antibody level was not significantly correlated with the TGEV vaccine strain. Concurrently, the results showed that the second booster immunization could reduce the TGE IgG antibody levels of sows by using the "attenuated vaccine + attenuated vaccine" combination.

The PoR IgG antibody detection results showed that the PoR IgG antibody levels of sows in each group decreased to some extent at 21 days post-first immunization *(Fig. 1C)*. In the groups that used the triplex attenuated vaccine containing PoRV (groups A and B), the PoR IgG antibody levels of the sows decreased to a lesser extent than in the other groups. The antibody level of the sows in group F decreased the most, and the decline in the antibody level of the sows in groups A and B did not significantly differ (P>0.05) *(Table 4)*.

The PoR IgG antibody levels of the sows in groups C, D, E, and F remained decrease at 35 days post-first immunization but peaked in group B, which showed an extremely significant difference (P<0.01) from groups C, D, E, and F. The PoR IgG antibody levels of the sows in group A decreased, similar to those in other groups (groups C, D, E, and F) at 49 days post-first immunization. However, the reduction in group A was lower (P>0.05) than that in group B and significantly different compared with that in groups C, D, E, and F (P<0.05). The PoR IgG antibody level of group B was highest compared with those of group A and other groups. Thus, the triplex attenuated vaccine that included PoRV had a certain immunizing effect in stimulating the production of PoR IgG antibodies in the sows.

After immunization with different vaccine combinations, the PoR IgG antibody levels in the group immunized with the "PTR+PTR" vaccine combination (group B) demonstrated a change trend of "decline, rise, and fall" *(Fig. 2C)*. In the other immune combinations (groups A, C, D, and E), the

Table 4. *The detection results of PoR IgG antibody in different vaccine combinations*

Groups	S/P Value (Mean± SD)/Time			
	1d	21d	35d	49d
A	2.35±0.71^{a}	2.24±0.72^{a}	2.19±0.69^{Aa}	1.67±0.20$^{A\,Bb}$
B	2.11±0.57^{a}	2.02±0.29^{a}	2.49±0.53^{Aa}	1.95±0.33^{Bb}
C	2.06±0.68^{a}	1.83±0.65^{a}	1.64±0.49^{Bb}	1.10±0.31^{Aa}
D	2.27±0.72^{a}	2.03±0.64^{a}	1.83±0.61^{Bb}	1.25±0.42^{Aa}
E	2.00±0.55^{a}	1.75±0.56^{a}	1.43±0.30^{Bb}	1.20±0.16^{Aa}
F	2.06±0.45^{a}	1.75±0.30^{a}	1.64±0.25^{Bb}	1.16±0.26^{Aa}

Different uppercase letters on the S/P or OD450nm value columns indicate that the difference is extremely significant (P<0.01), and different lowercase letters indicate significant difference (P<0.05)

Table 5. *The detection results of PED IgA antibody in different vaccine combinations*

Groups	OD450nm (Mean±SD/Time			
	1d	3d	7d	14d
A	1.56±0.33^{Bb}	1.12±0.30^{BCc}	0.65±0.21^{Cc}	0.38±0.15^{BCbc}
B	1.65±0.39^{Bbc}	1.34±0.31^{Bcd}	0.38±0.11^{Bb}	0.27±0.18^{ABb}
C	2.08±0.13^{Bcde}	1.65±0.28^{Bd}	0.96±0.16^{Dde}	0.57±0.17^{Cc}
D	2.15±0.49^{Be}	1.42±0.29^{Bcd}	0.85±0.15^{CDd}	0.35±0.18^{BCb}
E	1.65±0.40^{Bbcd}	0.75±0.30^{Cb}	0.33±0.14^{ABb}	0.18±0.14^{ABab}
F	0.10±0.05^{Aa}	0.12±0.05^{Aa}	0.08±0.06^{Aa}	0.06±0.04^{Aa}

Different uppercase letters on the S/P or OD450nm value columns indicate that the difference is extremely significant (P<0.01), and different lowercase letters indicate significant difference (P<0.05)

PoR IgG antibody levels showed a "decreasing" trend, and the declining degree in each test group differed. Moreover, the decline in the PoR IgG antibody levels of the "PTR+PT*" vaccine combination (group A) was lower than those of the other test groups (groups C, D, and E). The PoR IgG antibody growth and decline trends showed that the level of PoR IgG antibody produced in the sows that used the "PTR + PTR" vaccine combination (group B) was superior to that in the sows with the "PTR + PT*" vaccine combination (group A) and other vaccine combinations.

The specific PED IgA antibody detection results showed that IgA antibody was positive in all experimental groups at 1-3 days post-delivery, except control group F *(Fig. 2D)*. As expected, the PED IgA antibodies showed a downward trend at 1-14 days post-delivery in all the experimental groups, but they changed differently in experimental groups. In particular, the PED IgA antibody in groups A and D turned negative at 14 days post-delivery, whereas that in group B and E turned negative at 7 days post-delivery. Fortunately, the PED IgA antibody remained positive at 14 days post-delivery in group C.

Moreover, group C showed significant different form group F (P<0.01) *(Table 5)*, and from groups A (P<0.05) at 1 days after post-delivery *(Table 5)*. As the days increase after delivery, more groups showed significant different from group C. The groups E and F had extremely significant different (P<0.01) from group C at 3 days after post-delivery, and the groups A, B, E and F (P<0.01) showed extremely significant different from group C at 7 days after post-delivery. At 14 days post-delivery, group C also showed significant different from group D (P<0.05) and groups B, E and F (P<0.01). The results abovementioned revealed that group C, which was immunized with PT+PT*, exhibited more long-lasting PED IgA antibody than the other groups.

DISCUSSION

In recent years, an outbreak of porcine virus diarrhea created an epidemic in much of China [10]. The disease is one of the main causes of growth retardation and high mortality in suckling piglets, causing huge economic losses in the pig industry [11]. Therefore, the change trends of IgG antibody in the serum and IgA antibody levels in the breast milk of sows should be understood, and effective vaccine combinations are necessary to immunize sows for the prevention and control of porcine viral diarrhea in pig farms.

IgG drawn in the serum of sows mainly exists in the colostrum, from which suckling piglets obtain passive immunoprotection by sucking. This result indicates that IgG in serum plays an important role in the immune protection of suckling piglets [12,13]. By monitoring the PED

IgG antibodies in serum, we found that the IgG antibody levels continued to increase in groups A and C after immunization at 0-35 days, and their S/P values were higher than those in other groups. Therefore, the vaccine combination of groups A and C may be an alternative for sows to enhance antibody protection and prevent the suckling piglets from PEDV infection. Moreover, the regeneration of intestinal epithelial cells in suckling piglets is slow, and the development of mucosal immune system is imperfect, so hosts cannot produce effective mucosal immune responses due to the inoculated vaccines. Thus, IgA antibody in breast milk plays the most important role to protect suckling pigs from PEDV infection [14]. Therefore, the level of IgA antibodies in breast milk is important for the immunity of suckling piglets [15,16]. The results of this study showed that the IgA antibodies in the different vaccine combinations presented varying growth and decline rules. The PEDV IgA antibody level started to decrease to the negative level at 7 days post-delivery *(Fig. 2D, Table 5)*. Moreover, group C showed significant different with A, B, E and F ($P<0.05$, $P<0.01$) from 1 to 7 days after post-delivery *(Table 4)*. At 14 days post-delivery, except for group C, the antibody levels in the other groups dropped to a negative level, which was consistent with findings of previous report [17]. The results of PEDV IgA antibody monitoring revealed that the vaccine combination of "PT+PT*" led to longer positive antibody levels compared with the other vaccine combinations. Thus, the vaccine combination of "PT+PT*" (group C) demonstrated good effects in stimulating the production of specific PED antibodies in sows, and this result may be related to vaccine type and strain.

Previous studies have shown that single attenuated or inactivated vaccine inoculation is far less effective than the alternate use of attenuated and inactivated vaccines [17]. In general, attenuated vaccines can elicit cellular immune responses in a short time period after inoculation, but their disadvantages include low antibody levels and rapid reduction. Moreover, inactivated vaccines are generally an oil emulsion adjuvant vaccine, which functions as an antigen reservoir and can stimulate the host to continuously produce antibodies. In this study, groups A and C adopted the alternate use of attenuated and inactivated vaccines get better results than that in groups B, D, and E, whose inoculated single attenuated or inactivated vaccine. These vaccines play a good role in immunity precisely because of the combination of attenuated vaccine and inactivated vaccine. In addition, the PEDV gene sequence has shown certain variations in recent years, and its genetic distance indicated that the current PEDV variant strain is far from the classical strain used to develop vaccines [18,19]. In this study, the vaccine strain CV777 in groups A and B is belong genotype GIa, the other strain ZJ08 in groups C and D and AJ1102 in groups C and E were belong genotype GIIa and IIb, respectively. Results of this study demonstrated the alternate use of genotype GIIa strain ZJ08-based attenuated bivalent vaccine and genotype GIIb strain AJ1102-based inactivated bivalent vaccine (group C) produced best immune antibody levels. Therefore, difference vaccine strain may be an important factor that influences vaccine immune antibody levels.

The TGE IgG antibody monitoring results showed that the vaccine combination in groups A and C could stimulate the sows to produce higher immune antibody levels compare with groups B, D, and E. The Miller cluster TGEV vaccine strain H in groups A and C is distant from the Purdue cluster strain WH-1 or HB08 in other experiment groups. But statistical analyses of group A showed no significant difference with groups B, C, and E, also no difference showed in group C with groups A, B, and E. Moreover, groups A and C employed an alternate pattern of attenuated and inactivated vaccines, indicating that the combination of vaccine types was the main factor that affected the production of specific TGE IgG antibodies in the sows, less related to the vaccine strain. It may have a certain relationship with the genetic conservation of TGEV in gene evolution [8,20,21], but further experiments are need to study. PoRV infection is highly common in pig herds and has a high positive rate of serology, but PoRV was the least frequent viral agent detected in the diarrheal samples [22,23]. Moreover, single infection with PoRV occurred in only 0.4% of the population in a previous report in China, whereas most cases involved mixed infections [24]. Therefore, PoRV may be a follow-up agent for PEDV or various diarrheal viruses. Thus, on the whole, prevention and control of PEDV has become particularly important. In this study, we found that the PTR attenuated vaccine could stimulate the sows to produce specific IgG antibody after 15-20 days of immunization, and it can be an alternative option in areas under PoRV infection risk.

The level of IgA antibody in breast milk of sows directly affects the acquired protective antibody effects of suckling piglets, but its detection has a certain time lag and cannot be widely used in practice. However, a previous study found that IgG antibody levels in serum are positively correlated with IgA antibody levels in breast milk [25]. Similarly, we found that the level of PEDV IgA antibody in the sows at 7 days post-delivery was correlated with the level of PEDV IgG in the serum of the corresponding vaccine combination sows at 49 days post-first immunization. This finding suggested that the IgG antibody level in serum may be a reference indicator for IgA antibodies in breast milk. Clinically, the immune antibody level could be monitored by detecting IgG antibody levels in serum, but additional clinical sample data are needed for further validation and analysis.

In conclusion, this report is the first on the special antibody differences of different vaccine combinations that are often used to prevent porcine viral diarrhea in China. Our findings proved that the vaccine combination of "PT+PT*" is optimal for preventing the disease without PoRV infection risk, and "PTR+PT*" may be an alternative option for cases with PoRV infection risk.

Conflict of Interests

The authors declare no competing financial interests.

Acknowledgments

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