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SPECIALTY SECTION

This article was submitted to
Veterinary Pharmacology and
Toxicology,
a section of the journal
Frontiers in Veterinary Science

RECEIVED 10 May 2022

ACCEPTED 04 July 2022

PUBLISHED 28 July 2022

CITATION

Ho TT, Trinh VT, Tran HX, Le PTT,
Nguyen TT, Hoang HTT, Pham MD,
Conrad U, Pham NB and Chu HH
(2022) The immunogenicity of
plant-based COE-GCN4pII protein in
pigs against the highly virulent porcine
epidemic diarrhea virus strain from
genotype 2. *Front. Vet. Sci.* 9:940395.
doi: 10.3389/fvets.2022.940395

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The immunogenicity of plant-based COE-GCN4pII protein in pigs against the highly virulent porcine epidemic diarrhea virus strain from genotype 2

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Porcine epidemic diarrhea virus (PEDV) is a serious infectious causative agent in swine, especially in neonatal piglets. PEDV genotype 2 (G2) strains, particularly G2a, were the primary causes of porcine epidemic diarrhea (PED) outbreaks in Vietnam. Here, we produced a plant-based COE-26K-equivalent epitope (COE) variant from a Vietnamese highly virulent PEDV strain belonging to genotype 2a (COE/G2a) and evaluated the protective efficacy of COE/G2a-GCN4pII protein (COE/G2a-pII) in piglets against the highly virulent PEDV G2a strain following passive immunity. The 5-day-old piglets had high levels of PEDV-specific IgG antibodies, COE-IgA specific antibodies, neutralizing antibodies, and IFN- γ responses. After virulent challenge experiments, all of these piglets survived and had normal clinical symptoms, no watery diarrhea in feces, and an increase in their body weight, while all of the negative control piglets died. These results suggest that the COE/G2a-pII protein produced in plants can be developed as a promising vaccine candidate to protect piglets against PEDV G2a infection in Vietnam.

KEYWORDS

COE, GCN4-pII motif, PEDV genotype 2, plant-based vaccine, piglet protection, passive immunity

Introduction

Porcine epidemic diarrhea (PED) is an acute and highly contagious enteric disease in swine, caused by the porcine epidemic diarrhea virus (PEDV) (1). Pigs of all ages can be infected by PEDV, resulting in the rapid spread of acute and severe diarrhea, dehydration, and vomiting. Suckling piglets, especially at the age of under 7 days, are the most severely

affected with mortality reaching nearly 100% (2). Since the first outbreaks in Europe, PEDV has caused severe economic loss to the pig industry worldwide, particularly in Asian countries and the United States (1, 3, 4). In Vietnam, PED was first reported in 2009 and then continuously spread in several pig farms throughout the country, causing remarkable economic damage (5).

Porcine epidemic diarrhea virus is a single-stranded RNA virus and a member of the genus Alphacoronavirus, in the family Coronaviridae (6). PEDV can be clustered into two genogroups (G1 and G2) based on the nucleotide and amino acid sequence of Spike (S), especially the changes in amino acid sequence at the N-terminal of the S1 domain (7, 8). Each genogroup contains subgroups a and b. PEDV strains circulating in the north, central, and southern regions of Vietnam from 2015 to 2016 belonged to G1b, G2a, and G2b (4, 9). The differentiation in genetic information between the emerging PEDV strains and PEDV vaccine strains, especially at the neutralizing epitopes, results in the low effectiveness of the current vaccine in the protection of pigs against PEDV strains in the field (9–11). In Vietnam, PEDV G2 strains were found as the major disease causative agents in PED outbreaks (4). To date, subunit vaccines against Vietnamese PEDV G2 strains are not available. Therefore, the development of a novel vaccine against emerging PEDV strains, especially G2 strains, is urgent to control PED outbreaks in Vietnam (4, 9).

The CO-26K-equivalent epitope (COE) is located in amino acids 499–638 within the Spike (S) protein (12). It is a neutralizing epitope recognized by the monoclonal antibody (6). COE protein is considered an important target for developing subunit vaccines against PEDV (12, 13). The plant-based production systems, especially agroinfiltration, have been widely considered a rapid, convenient, and low-cost method to produce a large number of recombinant proteins within a few days in several plants (14, 15). There have been efforts to produce S1 or COE protein as fusion forms against PEDV in various plants such as lettuce, rice, *Nicotiana benthamiana*, *Nicotiana tabacum*, and maize using stable transformation or transient expression (13, 16–21). In a previous study, we demonstrated that plant-based COE/G1a-pII induced neutralizing antibodies in mice (22). However, the immunogenicity and protectivity of plant-based COE/G1a-pII in piglets against PEDV *via* challenge experiments have still not been evaluated.

This study aims to evaluate whether our previous concept could also be worked on another COE variant from a highly virulent PEDV strain belonging to G2 (COE/G2a-pII), which is the currently circulating strain in Vietnam. Moreover, we analyzed if a two-dose vaccination scheme with COE/G2a-pII could induce protective immune responses in piglets from virulent PEDV strains *via* challenge experiments. As expected, all piglets from sows vaccinated with purified COE/G2a-pII protein survived, while all negative control piglets died after the challenge with the highly virulent PEDV G2a strain. In addition,

COE/G2a-pII elicited high levels of neutralizing antibodies, PEDV-specific IgG antibodies, COE-specific IgA antibodies, and IFN- γ in sows and piglets.

Materials and methods

Production and characterization of COE/G2a-pII protein from plants

A DNA sequence encoding for the COE/G2a region from the highly virulent NAVET/PEDV/PS6/2010 strain (NAVETCO, Vietnam) belonging to G2a was codon-optimized for expression in *N. benthamiana*, synthesized, and inserted into a cloning vector pUC57-COE (Genewiz, United States). COE/G2a was amplified and inserted in pRTRA-COE/G1a-GCN4pII-cmyc-his-KDEL (22) at sites *Bam*HI and *psp*OMI. A plant expression vector containing COE/G2a-pII was then constructed according to the previous study (22). In this study, COE/G2a-pII protein was expressed in *N. benthamiana* *via* the agroinfiltration protocol that was mentioned in the previous study (22) with a modification in the OD₆₀₀ agrobacterium suspension mixture of 0.1. After 3 days of infiltration, leaves were collected and stored at -80°C . The expression of COE/G2a-pII protein in plants was detected by SDS-PAGE and Western blot assay using anti-cmyc-antibody as the primary antibody and goat anti-mouse IgG-HRP (Invitrogen) as the secondary antibody. A signal was detected using the Amersham ECL Prime Western blotting detection reagent. The expression level of COE/G2a-pII protein was measured based on the H5N1-specific ScFv protein amount in a standard curve (23) that was conducted and analyzed using the ImageQuant TL 8.0 software (Cytiva) after the signal was detected using the AmershamTM Imager 680 machine. Leaves were subjected to COE/G2a-pII protein purification using the immobilized metal affinity chromatography (IMAC) protocol that was reported in the previous study with a modification in washing buffer containing 10 mM of imidazole (22).

The molecular weight of COE/G2a-pII protein was characterized by size exclusion chromatography (SEC) following the protocol as described earlier (24). An amount of 500 μg IMAC-purified COE/G2a-pII protein was applied onto a SuperoseTM 6 increase 10/300GL column (GE Healthcare). A high molecular weight kit (GE Healthcare) containing standard proteins with molecular weights ranging from 75 to 2,000 kDa was used to estimate the molecular weight of COE/G2a-pII protein. The presence of COE/G2a-pII protein in SEC fractions was confirmed by SDS-PAGE and Western blotting assay.

Cells and viruses

Vero cells (ATCC CCL-81) were cultured in Eagle's Minimum Essential Medium (EMEM, Gibco) supplemented

with 10% fetal bovine serum (FBS, Gibco), 50 µg/ml gentamycin at 37°C, and 5% CO₂. The NAVET/PEDV/PS6/2010 strain was isolated from piglet samples infected with PEDV and propagated on Vero cells using the protocol previously described (25). The virus in passage 5 was used for challenge experiments in piglets. There was no change in the nucleotide sequence of the S gene of the NAVET/PEDV/PS6/2010 strain in passage 5 when compared to that of the isolated PEDV strain.

Pig immunization and viral challenge experiments

The pig study was reviewed and approved by the ethical committees of the Institute of Biotechnology (IBT); Vietnam Academic of Science and Technology (VAST), Hanoi, Vietnam; and NAVETCO, Ho Chi Minh City, Vietnam, under decision number 07/2015/HĐ-NĐT. The animal protocols were performed based on the “3Rs” and the European Communities Council Directives of 24 November 1986 (86/609/EEC) guidelines for the care and use of animals. Pigs were raised by NAVETCO, Vietnam, and monitored to minimize the stress, suffering, unnecessary pain, or lasting harm in the experiments by veterinarians.

Before 2 weeks of vaccination, blood samples of pregnant sows were collected for serum neutralizing antibody assays performed at NAVETCO's laboratory. Two pregnant sows with negative results of neutralizing antibodies against PEDV were selected for the vaccination experiments. Either purified COE/G2a-pII protein or PBS was formulated extensively with in-house water in oil adjuvant (NAVETCO, Vietnam) at a ratio of 3:7. The primiparous sow (~80 days of gestation, $n = 1$) was intramuscularly immunized on the neck with either adjuvanted COE/G2a-pII protein (100 µg per dose) or PBS on days 0 and 14 post-immunization (pi). After 21 days of the second immunization, farrowing was induced. Sow blood samples were collected on days 0, 35, and 50 pi. Eight piglets born to one COE/G2a-pII-vaccinated sow and two piglets born to one PBS-vaccinated sow were suckled and kept together with their sows. Each 5-day-old piglet was challenged orally with 1 ml of the highly virulent NAVET/PEDV/PS6/2010 strain (10^3 TCID₅₀). Piglets were monitored daily for clinical signs, diarrhea, weight, and death from days 0 to 14 post-challenge (pc). Clinical scores were modified from a scoring method previously described (26). Clinical scores were defined as follows: healthy = 0; anorectic, depression, vomiting, and emaciated = 1; dead = 2. The body weight of piglets was recorded on days 0 and 10 pc. Fecal scores were evaluated as follows based on a scoring method previously described (27): normal = 0, pasty = 1, semi-solid = 2, watery diarrhea = 3. Blood samples of all the piglets were collected *via* the

marginal ear veins in serum separator tubes on days 0 and 10 pc for analysis. All blood samples were centrifuged at $3,000 \times g$ for 10 min at 4°C, and the sera were stored at -20°C until analysis.

ELISA

PEDV-specific IgG antibodies in the sera of sows and piglets were assessed using a commercial ELISA kit (INgezim PEDV 11.PED.K.1/5, Eurofins INGENASA) according to the manufacturer's recommendation. The S/P values were calculated using the formula: [(test sample value - negative control value) / (positive control value - negative control values)]. Samples were defined as positive for PEDV-specific IgG if the S/P value is > 0.35. COE-specific IgA antibodies in sow and piglet sera were analyzed using an indirect ELISA using SEC-purified COE/G2a-pII protein (1 µg/ml) as antigen according to the protocol described previously (22).

Virus neutralizing antibody test

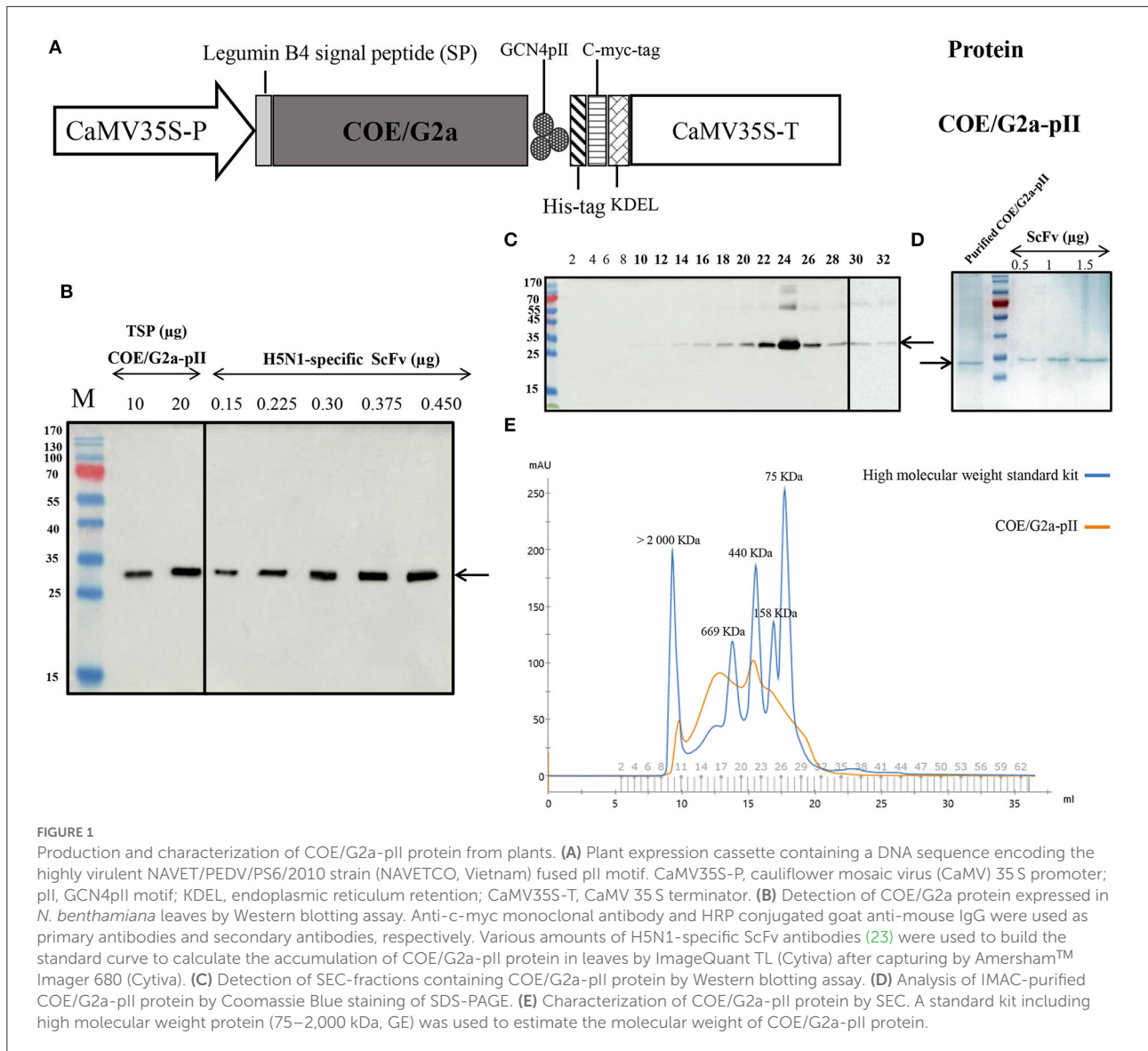
The presence of neutralizing antibodies in porcine sera was evaluated using a virus-neutralizing assay according to the protocol previously described (28). PEDV strain NAVET/PEDV/PS6/2010 (200 TCID₅₀/0.1 ml) was performed for the assay. The virus neutralization titer (VN titer) was determined as the highest serum dilution that inhibited the cytopathic effect.

Cytokine assay

The level of interferon-gamma (IFN-γ) in the sera of sows and piglets was detected using a commercial ELISA kit (Porcine IFN-γ ELISA Basis kit, Matech) according to the manufacturer's recommendation. Porcine sera with a dilution of 1:20 were used for the cytokine assay. The concentration of IFN-γ in diluted sera (IFN-γ DL) was calculated based on the standard curve of recombinant porcine IFN-γ standard with the range of 16–1,600 pg/ml. The final concentration of IFN-γ in pig sera was calculated using the formula: IFN-γ DL × 20.

Statistical analysis

All statistical analyses were performed in the Sigma Plot software. The differences between the two groups were compared and shown as mean ± standard deviation (SD) using a Mann-Whitney U test. The significant difference was determined if the *p*-value was <0.05.



Results

Production and characterization of COE/G2a-pII

To construct the expression cassette harboring COE/G2a-pII, we replaced the DNA sequence encoding COE from the PEDV DR13 strain (G1a) with that encoding COE/G2a from the highly virulent NAVET/PEDV/PS6/2010 strain (Figure 1A). The expression of COE/G2a-pII protein in *N. benthamiana* leaves was successfully detected by a Western blotting test using anti-cmyc-antibody as the primary antibody (Figure 1B). The accumulation of COE/G2a-pII protein in leaves was calculated based on the standard curve of the H5N1-specific ScFv protein (23). COE/G2a-pII protein accumulated in plant leaves at

approximately 118 mg/kg fresh leaves, which accounted for 2.01% of total soluble protein (TSP). COE/G2a-pII protein was further purified by IMAC, and then the resulting product was further purified and characterized by SEC (Figures 1C,D). The predicted molecular weight of COE/G2a-pII monomer is approximately 26 kDa; however, N-glycosylation sites in COE protein may influence the PAGE separation resulting in the increase in band size of COE/G2a-pII protein in SDS-PAGE and Western blot of approximately 35 kDa (Figure 1D). The molecular weight of COE/G2a-pII was calculated and characterized by SEC based on the standard kit containing high molecular weight proteins (75 to >2,000 kDa). COE/G2a-pII protein was presented in SEC fractions 10–32 corresponding to the molecular weight of 75 to >2,000 kDa (Figure 1E). COE/G2a-pII protein was mostly detected in SEC fractions 22–26 corresponding to

the molecular weight of approximately 440 kDa. Therefore, COE/G2a-pII is a mixture of multimer forms with a wide range in molecular weight.

Humoral immune responses and cytokine responses in sows

The immunogenicity of IMAC-purified COE/G2a-pII protein was evaluated in pregnant sows. The immunization scheme in pregnant sows is presented in Figure 2A. PEDV-specific IgG responses were detected in the serum of the sow vaccinated with COE/G2a-pII protein with an S/P value of 2.49 on day 35 pi (Figure 2B). There was no increase in PEDV-specific IgG responses in this inoculated sow on day 50 pi compared to that on day 35 pi. In contrast, no PEDV-specific IgG antibody was found in the sow immunized with PBS. The pregnant sow vaccinated with COE/G2a-pII protein developed COE-specific IgA antibodies in serum on day 35 pi with an OD450 mean value of 3.8. Similar to the PEDV-specific IgG responses, the COE-specific IgA antibodies did not increase in the serum of this vaccinated sow on day 50 pi. In contrast, COE-specific IgA antibodies were not detected in the serum of the sow immunized with PBS (Figure 2C). The presence of neutralizing antibodies against PEDV in sow sera was assessed by virus-neutralizing antibody assay. On day 35 pi, the VN titer of 32 was observed in the sow vaccinated with COE/G2a-pII protein. However, the neutralizing antibody response of the COE/G2a-pII-immunized sow was decreased on day 50 pi (Figure 2D). The IFN- γ were found at a high concentration of 932.3 ± 145.2 pg/ml in serum of the sow injected with COE/G2a-pII protein on day 35 pi. Notably, there was a 2-fold increase in IFN- γ level (1796.1 ± 8.6 pg/ml) in this sow on day 50 pi (Figure 2E). IFN- γ was not found in the negative control sow vaccinated with PBS. These data indicate that the COE/G2a-pII protein induces strong humoral immune responses and cytokine responses in the pregnant sow.

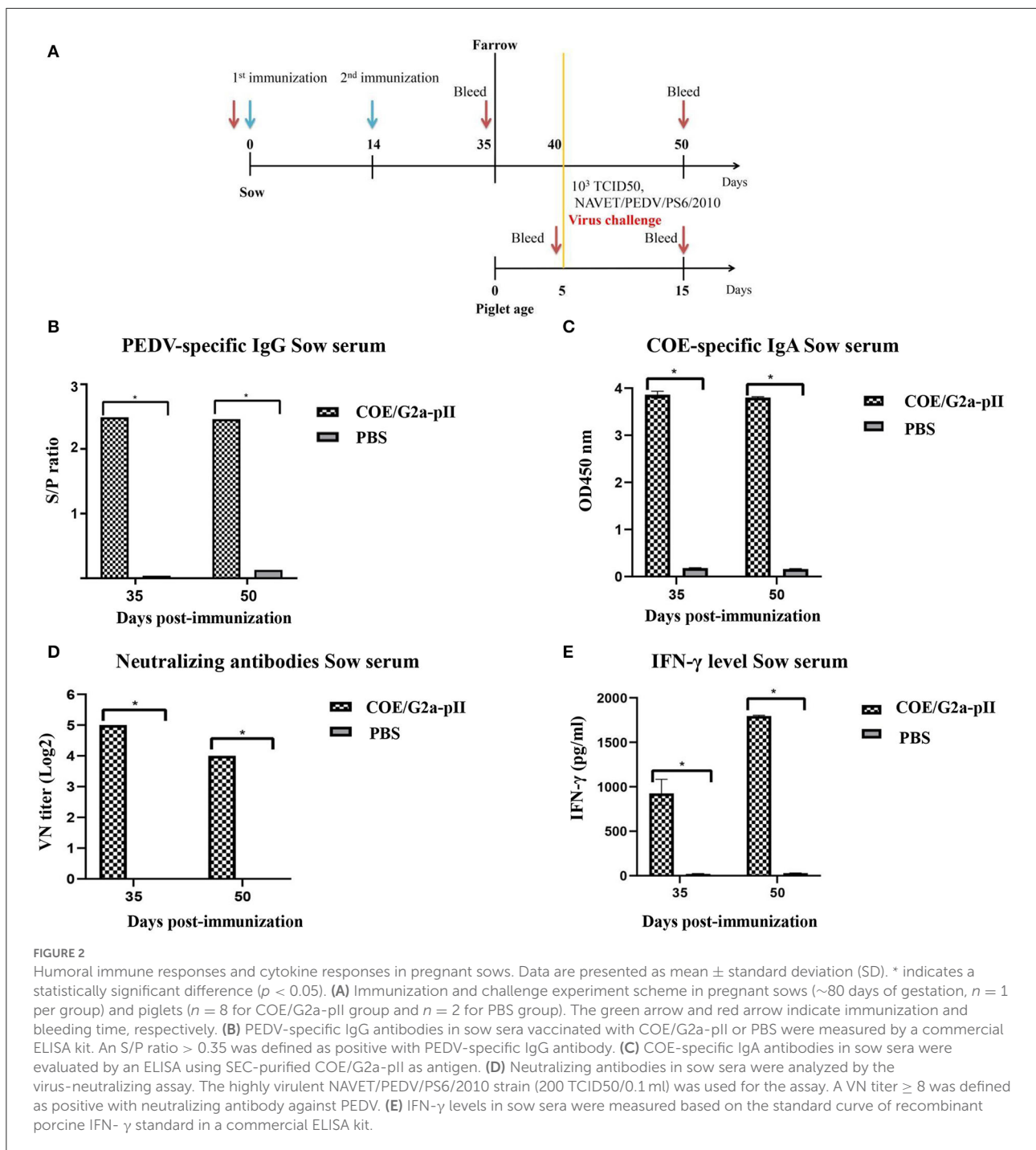
Humoral immune responses and cytokine responses in piglets

Since colostrum/milk antibodies from vaccinated mother sows are regularly transferred to piglets *via* suckling, passive immunity to the piglets was assessed by ELISA, virus-neutralizing antibody assay, and cytokine assay. On day 0 pc, PEDV-specific IgG antibodies were detected in the 5-day-old piglets born to sows vaccinated with COE/G2a-pII. The increase in PEDV-specific IgG antibody level was found in piglet sera on day 10 pc (Figure 3A). In addition, the sera of these piglets had high COE-specific IgA antibody responses on day 0 pc. However, there was a slight decrease in COE-specific IgA antibody levels

in these piglets on day 10 pc (Figure 3B). Neutralizing antibodies against the highly virulent NAVET/PEDV/PS6/2010 strain were detected in piglets born to sows immunized with COE/G2a-pII protein on day 0 pc. Notably, VN titers found in these piglets on day 10 pc were more than 2-fold higher than those in piglets on day 0 pc (Figure 3C). High levels of IFN- γ were detected in sera of these piglets on day 0 pc with a concentration of 824.5 ± 157.68 pg/ml. Notably, there was a strong increase in IFN- γ levels in piglets born to sows inoculated with COE/G2a-pII protein on day 10 pc (Figure 3D). In contrast, no PEDV-specific IgG, COE-specific IgA, virus-neutralizing antibodies, and cytokine responses were observed in piglets born to sows vaccinated with PBS. These results demonstrate that there was a passive immunity transfer of PEDV-specific IgG, COE-specific IgA, virus-neutralizing antibodies, and IFN- γ responses from immunized pregnant sows to its piglets *via* sow colostrum/milk.

COE/G2a-PII protein protects piglets against the highly virulent pedv G2a strain

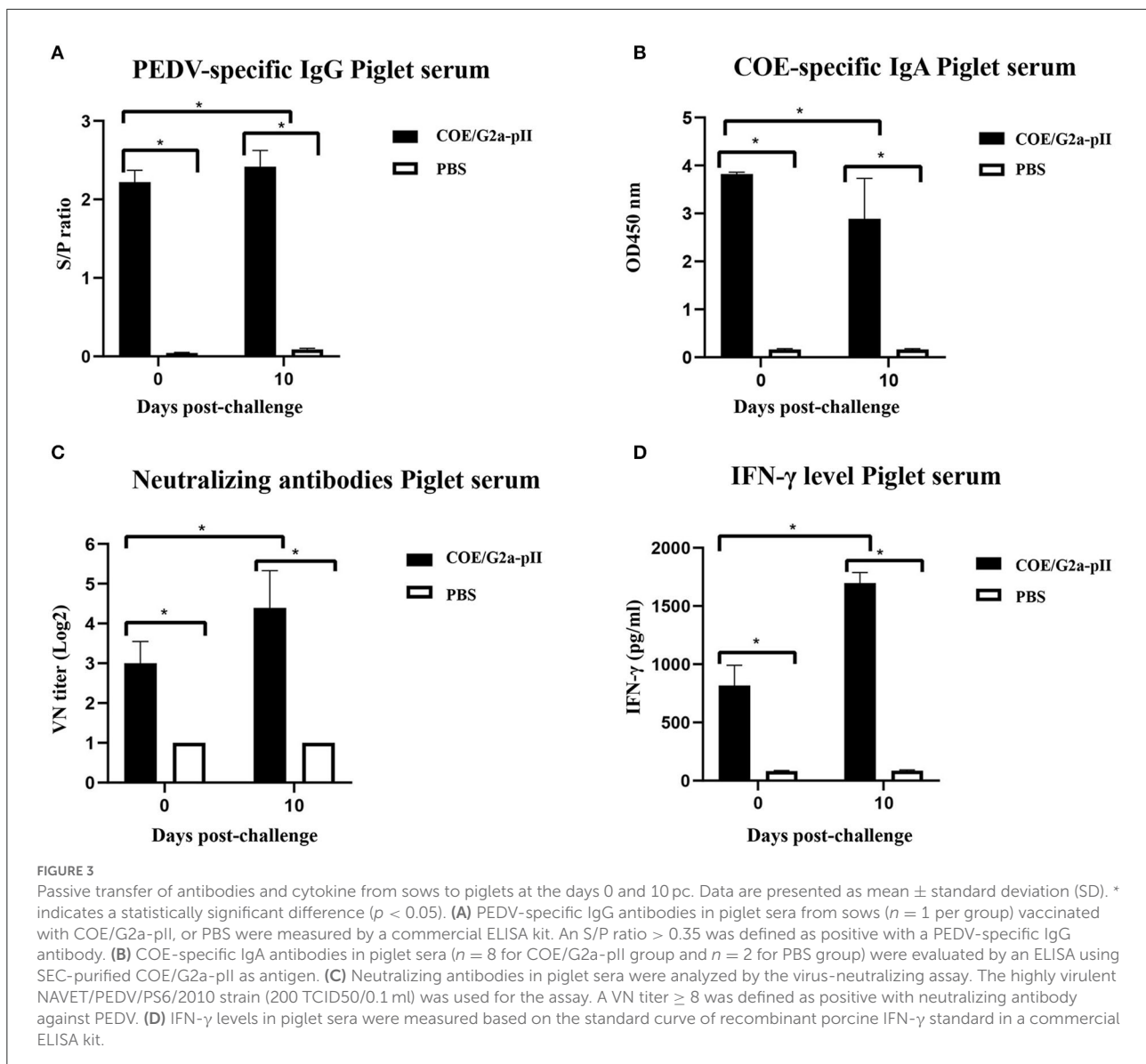
After 1 day of the challenge, all piglets born to sows vaccinated with COE/G2a-pII protein had pasty and semisolid feces (fecal scores of 1–2). However, some piglets in this group had normal feces with scores of 0 starting on day 4 pc. All piglets in this group returned to fecal scores of 0 on day 6 pc. In contrast, piglets delivered from sows immunized with PBS had semisolid feces with scores of 2 on day 1 pc. The feces of these piglets were watery, with scores of 3 beginning at day 2 pc to the later (Figure 4A). Before the challenge, there was no significant difference in the body weight of the two piglet groups. After 10 days of the challenge, there was a 2-fold increase in the body weight of piglets delivered from sow inoculated COE/G2a-pII. In contrast, two piglets delivered from negative sow control had a strong decrease in body weight after the challenge compared to that before the challenge (Figure 4B). All piglets born to sows vaccinated with COE/G2a-pII protein had daily normal clinical scores of around 0 and survived after the challenge (Figures 4C,D). In contrast, the clinical signs of piglets delivered from sows immunized with PBS were around 1 (anorectic, depression, vomiting, and emaciated) beginning at day 2 pc, and the clinical scores of this group increased to 1.5 on day 4 pc (Figure 4C). On day 4 pc, one piglet born to the sow vaccinated with PBS died. Another piglet in this group died on day 14 pc (Figure 4D). Therefore, all piglets in the negative control group died after the viral challenge. The cause of the piglet deaths in the negative group was PEDV infections, verified *via* gross lesions in the small intestine of deceased piglets (data not shown). Taken together, these data indicate that passive immunity provided by a pregnant sow vaccinated with COE/G2a-pII protein could induce protective immune responses in its piglets against a highly virulent PEDV G2a strain.



Discussion

To the best of our knowledge, this is the first study to evaluate the protective efficacy of a plant COE-pII-based subunit vaccine candidate against a highly virulent PEDV G2a in piglets following the passive immunity. Previous studies indicate that the current commercial PEDV vaccine, which contains G1a

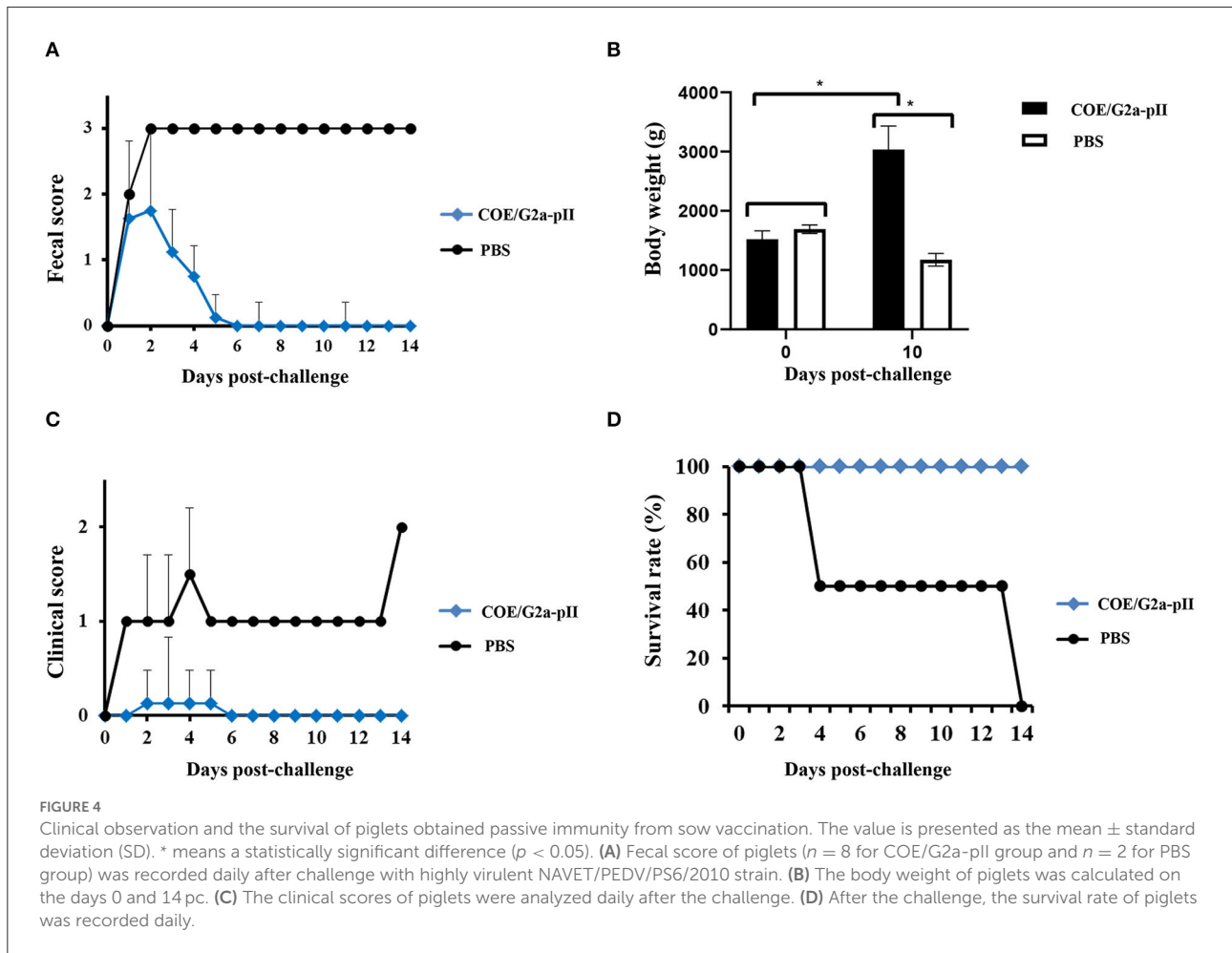
PEDV strains, may induce low protective immune responses against PEDV strains in the field (9–11). When collecting all DNA sequences encoding the full S protein of Vietnamese PEDV strains published on NCBI, we obtained 63 nucleotide sequences of full S genes. After building the phylogenetic tree based on full S gene sequences of PEDV PS6, 63 Vietnamese strains, and 62 PEDV strains in the world, then comparing the genotype



cluster of PEDV strains to previous publications, the Vietnamese PEDV G2a, G2b, and G1b strains were clustered into 35/64 sequences (54.68%), 23/64 sequences (35.93%), 8/64 sequences (12.5%), respectively (data not shown). This finding is also in agreement with the results of previous studies that PEDV strains belonging to the G2 group were detected in most of the PED outbreaks in Vietnam (4). In addition, we also found that PEDV G2a strains were the dominant agents in PED outbreaks in Vietnam. Therefore, the goal of this study was to assess whether immunization with plant-based COE/G2a-pII protein would elicit protective responses in piglets born to immunized sows against highly virulent PEDV G2a strains isolated in Vietnam.

COE/G2a-pII protein was successfully expressed at a high level in plants *via* agroinfiltration. When compared to previous studies, the expression level of COE/G2a-pII protein in *N.*

benthamiana is higher than that of COE proteins in previous publications (13, 17, 21, 29). However, the expression level of COE/G2a-pII protein is lower than that of COE/G1a-pII protein in our previous study (234 mg/kg fresh leaves, 4% TSP). This might be explained by the difference in nucleotide sequence and amino acid sequence of the two COE variants. When compared to the native sequence of COE/G1a-pII, the identity in the native nucleotide sequence and amino acid of COE/G2a-pII was 95.7 and 96.4%, respectively (Supplementary Tables 1, 2). After a commercial codon optimization for expression in *N. benthamiana*, the similarity in the nucleotide sequence of two optimized COE variants was 75.5% (Supplementary Table 3). COE/G2a-pII protein is a mixture of multimer forms with a molecular weight of approximately 440 kDa. The size of COE/G2a-pII protein is quite different when compared to our



previous publication that used native PAGE to estimate the size of COE/G1a-pII protein (22). This can be explained by the difference in the capacity of each method used to calculate the size of the protein. When compared to SEC, native PAGE has limitations in the separation of high molecular weight protein. Since high mortality caused by PEDV infection occurs mostly in neonatal piglets at the age of under 1 week, an ideal commercial vaccine against PEDV infection should immunize sows to facilitate lactogenic immunity to be transferred to neonatal piglets following suckling. Results presented here indicate that 100% of 5-days-old-piglets born to COE/G2a-pII-vaccinated sows survived after the challenge with a highly virulent PEDV strain belonging to the G2a group. All piglets born to a PBS-vaccinated sow died. The survival rate obtained in piglets of the PBS group agreed with previous studies in which very high mortality (up to 80%–100%) in suckling piglets infected with PEDV at the age of under 7 days (3, 30). The zero mortality rate in piglets born to COE/G2a-pII-vaccinated sow after the challenge can be partly explained by the strong PEDV-specific IgG, COE-specific IgA, neutralizing antibodies, and IFN- γ responses in sow and transferred passively to piglets. The level

of neutralizing antibody responses induced in the COE/G2a-pII-vaccinated sow and its piglets was quite similar to those elicited in sows vaccinated with the inactivated PEDV and their offsprings (31). The decrease in the level of humoral immune responses in the serum of sows vaccinated with COE/G2a-pII protein on day 50 pi might be explained by the transportation of antibodies from the blood to the milk of the sow. The IgA, IgG, and neutralizing antibody response in the serum of piglets on day 10 pc was quite similar to those in the serum of sow on day 50 pi. These findings agreed with those in a previous study in which the level of IgG and IgA in piglets mimicked those in sow sera and colostrums (32). In addition, the humoral immune responses induced in sows and piglets could change at the different time points of sampling. Further study should evaluate humoral immune response in sow and piglet sera at several time points.

Cytokines delivered from maternal responses may play important roles in generating the newborn immune response. IFN- γ is a crucial cytokine that is involved in both innate and adaptive immune responses. It has a critical role in the activation of macrophages and the induction of T helper lymphocyte

differentiation regarding the Th1, consequent aiding cellular responses and enhancing the protective immune response against pathogen infections (33). Therefore, the evaluation of IFN- γ amount is favored to partially characterize the immune response (34). Notably, the level of IFN- γ in sow (day 35 pi) and its offspring (day 0 pc) were detected at a very high concentration of 932.3 ± 145.2 pg/ml and 824.5 ± 157.68 pg/ml, respectively. When compared to the previous studies, the concentration of IFN- γ in sows vaccinated with COE/G2a-pII at farrowing was over 5-fold higher than that in sows vaccinated with killed-PEDV vaccine plus flagellin protein (Vac201-FliC) (31) and over 10-fold higher than that in sows vaccinated with inactivated PEDV harboring IgG-Fc (35). The level of IFN- γ in sow (day 50 pi) and its offspring (day 10 pc) were 2-fold increased in comparison with those before the challenge.

In this study, the amount of COE/G2a-pII protein used to vaccinate sow was 100 μ g per dose, which was 4-fold lower than that used in a previous study (36). The pregnant sow in the previous study was vaccinated three times with polyphosphazene in a triple adjuvant combination (TriAdj) with adjuvanted S1 protein (400 μ g) (36). After the viral challenge, the survival rate of piglets delivered from the S1-vaccinated sow in the previous study (36) was 87.5%, which was also lower than the survival rate of our piglets born to COE/G2a-pII sow (100%). The lower required dose of plant-based COE/G2a-pII protein for vaccination resulting in the higher protective efficacy against the highly virulent PEDV G2a can be explained by the formation of multimer forms with high molecular weight in the COE/G2a-pII protein. These results indicate that COE/G2a-pII protein produced in plants could be a potential vaccine candidate to prevent PEDV infection. However, our study still has limitations. The number of sows and piglets used in this study was not large as African swine fever outbreaks in Vietnam seriously affected the pig industry when the animal experiment was performed. The results we achieved prompted us to plan further studies involving many more sows and piglets to validate these data. Moreover, the amount of COE/G2a-pII protein per dose and the effect of different adjuvants that improve immune responses will be addressed in further experiments. In addition, the presence or absence of the PEDV in pig feces after the challenge should also be analyzed.

Conclusion

The COE/G2a-pII protein produced in plants induced strong humoral immune responses and IFN- γ responses in the pregnant sow. The sow's immune responses were passively transferred to their piglets following suckling. All piglets born to COE/G2a-pII-vaccinated sows survived and had normal clinical signs after the challenge with the highly virulent PEDV G2a strain. These results suggest that the COE/G2a-pII protein is a potential vaccine candidate to prevent PEDV G2a infection of

swine in Vietnam, and these preliminary but important data are the basis for performing more extended studies to develop a plant-based COE/G2a-pII vaccine.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Institute of Biotechnology (IBT), Vietnam Academic of Science and Technology (VAST), Hanoi, Vietnam, and NAVETCO, Ho Chi Minh City, Vietnam under decision number 07/2015/HĐ-NĐT.

Author contributions

HC, NP, and TH designed the study. TH and VT constructed vectors and performed ELISA IgA and IFN- γ . VT and TN performed transient expression. TH, VT, and TN purified protein. HT and PL performed pig immunization and challenge experiments, performed the virus-neutralizing antibody assay, ELISA IgG, and collected the clinical signs of piglets. TH performed the calculations and data analysis and wrote the manuscript. HC, NP, UC, MP, and HH revised the manuscript. HC and NP hold all the responsibilities related to this manuscript. All authors have read and approved the final manuscript.

Funding

This study was financed by the Ministry of Science and Technology through the project: Development of novel nanoconjugates from nanodiamonds and spike protein of porcine epidemic diarrhea virus toward applications in nanovaccine (code: NĐT/TW/21/09). This study is a part of the thesis results of Ph.D. student TH at GUST, VAST, Hanoi, Vietnam. TH was funded by Vingroup JSC and supported by the Master, Ph.D. Scholarship Programme of Vingroup Innovation Foundation (VINIF), Institute of Big Data (code: VINIF.2021.TS.066).

Acknowledgments

We would like to thank Dr. Hoang Trong Phan and Nhung Hong Nguyen for supporting the vector construction experiment.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fvets.2022.940395/full#supplementary-material>

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