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# Generation of a DNA vaccine expressing the porcine epidemic diarrhea virus immunogen fused with cholera toxin B subunit

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## Abstract

Porcine Epidemic Diarrhea (PED), which is caused by porcine epidemic diarrhea virus (PEDV), continues to cause serious economic losses to swine industry globally. PEDV mainly infects and replicates in enterocytes; thus, a vaccine capable of inducing mucosal immune response, particularly, in the gut mucosa, is desirable. The aim of this study was to develop a PEDV vaccine with enhanced mucosal targeting. PEDVSME, an immunogen that was newly designed from spike protein of PEDV, was fused with cholera toxin B subunit (CTB) for enhancement of mucosal targeting. Both PEDVSME and CTB genes were codon-optimized and synthetically made. The gene fragment of PEDVSME-CTB was constructed using overlapping PCR. A DNA vaccine was then generated by inserting the PEDVSME-CTB gene into the mammalian expression plasmid pTH. Expression of the PEDVSME-CTB protein by DNA vaccine was studied in HEK 293A cell. Immunofluorescence staining showed a high expression of PEDVSME-CTB in transfected cells. Western blot analysis displayed a detected band at the expected size and also confirmed a high expression of the protein. Moreover, the expression level of PEDVSME-CTB was comparable to that of PEDVSME. Importantly, by using ELISA, binding of PEDVSME-CTB to GM1 ganglioside, a receptor of CTB, showed that PEDVSME-CTB could bind specifically to GM1 ganglioside. The vaccine generated in this study may be applied as a mucosal vaccine for PED.

## Introduction

Porcine epidemic diarrhea virus (PEDV) infection causes a disease called porcine epidemic diarrhea (PED) with a main symptom of yellowish diarrhea and vomiting[1] . This symptom commonly develops and eventually induces death in pig, particularly piglets, with a mortality rate of 80%-100% from watery diarrhea and dehydration[1]. Due to high mortality rate, this disease causes huge economic losses in pig industries worldwide, particularly, north America and Asian countries[2]. PEDV is an enveloped, single stranded, positive sense RNA virus[3]. It is a member of the genus *Alphacoronavirus* within the family *Coronaviridae* in the order *Nidovirales*[3]. PEDV genome is approximately 28-kb long RNA with a 5' cap and a 3' polyadenylated tail[3]. The ORFs at the 3'-proximal genome regions encode four structural proteins: spike (S) protein, membrane (M) protein, envelope (E) protein, nucleocapsid (N) protein[3].

Among the 4 structural proteins, PEDV Spike (S) protein has been considered a main target for vaccine development as it plays an important role in interacting with specific host

cell receptor during virus entry into the cell[4-5]. S protein is large in size (around 1386 amino acids) and glycosylated; thus, expression of this protein could be difficult. Often, core of epitopes (CO-26K equivalent, COE) domain, which has been identified as neutralizing epitope domain[6], is used and has become the main antigen for PEDV vaccine development. COE-based vaccines have been shown to be immunogenic and capable of inducing antibodies against PEDV[5,7]. However, antibodies induced by COE-based vaccines could not provide complete PEDV neutralization[8]. With the aim to enhance immunogenicity and efficacy of PEDV vaccine, we have recently developed a new vaccine immunogen, termed PEDVSME.

PED is considered a mucosal disease because the disease onsets in small intestines, which is caused by the binding of PEDV and its receptor on the surface of villous epithelial cells or enterocyte in intestines[3]. As an enteric disease, prevention of PED is recognized to depends mainly on the presence of secretory IgA (sIgA) antibodies in the intestinal mucosa. Thus, vaccines developed with a strategy that can enhance production of intestinal sIgA is desirable. One of the strategies that has been extensively used is targeting a vaccine antigen to mucosal sites, resulting in an induction of vaccine-specific immune responses including sIgA at the mucosal sites.

Cholera toxin B subunit (CTB) has been recognized as an effective mucosal adjuvant and widely used to enhance vaccine potency to induce mucosal immune response[9]. CTB is employed for mucosal delivery of vaccine antigens by mimicking the mechanism of *Vibrio cholerae* toxin binding to host receptor during infection[10]. When fused to vaccine antigen, CTB functions as a ligand for binding to GM1 ganglioside, which expresses in all epithelial cells of intestines[10–12]. As a result, vaccine antigen is targeted to intestinal epithelial cells and thus can be found and taken up by dendritic cell (DC) during DC surveillance in the intestinal epithelial layer[12-13]. Many studies reported that, regardless of vaccination routes, conjugating CTB to the vaccine antigens could enhance production of antigen-specific sIgA at the gut mucosa[14–16].

In this study, the goal was to develop PEDV vaccines with enhanced ability at inducing mucosal immune response. Our newly designed immunogen, PEDVSME, was fused to CTB, for redirecting the immunogen to intestinal epithelia. The immunogen was vectored by plasmid pTH, thus generating DNA vaccine. The binding ability of PEDVSME-CTB to GM1 ganglioside was investigated using GM1 ganglioside binding ELISA assay.

**Methodology:**

*Construction of immunogen gene fragments*

Nucleotide sequence of PEDVSME was codon-optimized with pig codon usage and the gene was synthetically made by IDT Singapore. Sequence of CTB obtained from GenBank accession number D30053.1 was codon-optimized and synthesized by Genscript, China. CTB fragment was added to PEDVSME fragment through overlapping PCR. The Gene fragment of PEDVSME was amplified from the PEDVSME-bearing plasmid using primers New_5'flanking-F (5′ATTCCTGCAGGAAGCTTTTCCCCGGGGCCACCATG3′) and V5-Linker-R (5′AGCAGCGGCTTCTCCGCCTCCTGTGCTGTCCAGTCCCAGCAG 3'). The CTB gene fragment was amplified from the CTB-bearing plasmid using primers V5-Linker-F (5′CTGCTGGGACTGGACAGCACAGGAGGCGGAGAAGCCGCTGCT 3′) and Stop-RE-R (5′ TAAGCCCGGGGCGGCCGCTTATCA3′). PCR -amplifications of both PEDVSME and CTB were performed using Phusion Green HS II HF Master Mix (Thermo Scientific) with the same condition as follows: initial denaturation at 98°C for 30 seconds, 10 cycles of touchdown PCR composed of denaturation at 98°C for 10 seconds, annealing at 65°C with temperature decreasing 1°C/ cycle for 15 seconds and extension at 72°C for 30 seconds, followed by 15 cycles composed of denaturation at 98°C for 10 seconds, annealing at 55°C for 15 seconds and extension at 72°C for 30 seconds **.**The reaction was ended with a final extension of 5 minutes at 72°C **.**The gene fragments of PEDVSME and CTB obtained from PCR were

then used for construction of PEDVSME-CTB using overlapping PCR. One microlitre of each PCR product was mixed together and used as templates in overlapping PCR. Overlapping PCR was carried out with primers New-5' flanking-F and Stop-RE-R using the PCR condition described above.

*Generation of DNA vaccine*

The gene fragment of PEDVSME-CTB were separated in 1% agarose gel and then gel-purified using Gel/PCR DNA Fragments Extraction Kit (TIANGEN). After digested with *Pst*I (NEB) and *Not*I (NEB), the gene fragments was ligated to plasmid pTH (kindly provided by Prof. Dr. Tomas Hanke, university of Oxford) in the presence of T4 DNA ligase (NEB). Competent *E. coli* DH5α was transformed with the ligation mixture using heat shock method and plated on LB agar containing Ampicillin (100 ng/μl). Recombinant clones were screened using rapid size screening. The presence of the gene fragment in recombinant clones was confirmed by digesting the plasmids with *Pst*I and *Not*I. Nucleotide sequence of selected clones was then confirmed by automate DNA sequencing.

*HEK 293A Cells Transfection*

Human Embryonic Kidney 293A cells (HEK 293A) were cultured in Dulbecco modified Eagle medium (DMEM, Invitrogen (supplemented with 10% Fetal Bovine Serum) FBS, GE healthcare (and 1 % Pen/Strep (GE healthcare) in 5 % $CO_2$ oven. When the cells reached 80% confluency, transfection was performed with 2 μg of the recombinant plasmids using polyethylenimine (PEI, Sigma). The transfected cells were further cultured for 48 h and then harvested for immunofluorescence staining and Western blot.

*Immunofluorescence staining*

Transfected HEK 293A cells grown on 12-well plates were fixed with 3.7% formaldehyde for 10 min and washed once with PBS. The cell membrane was permeabilized with 90% cold methanol for 5 min at 4 °C. Cells were then washed once with PBS and blocked with 2% FBS/PBS for 1 h at room temperature. The 2% FBS/PBS solution was replaced with primary antibody, mouse anti-V5 (Ebioscience) diluted in 2% FBS/PBS. Following a 2-h incubation at room temperature, the cells were washed twice with PBS, followed by addition of Alexafluor 594-conjugated anti-mouse IgG diluted in 2% FBS/PBS. After an incubation for 1 h at room temperature, the cells were washed twice with PBS and the nucleus was stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma) at a final concentration of 2 μM in PBS and incubated at room temperature for 10 min. The cells were washed once with PBS. Lastly, fluorescence images were obtained under an inverted fluorescence microscope (IX71, Olympus).

*Western blot analysis*

The transfected HEK 293A cells grown on 12-well plate were harvested using 150 μl of cold lysis buffer, RIPA (25 mM Tris-HCl [pH 7.6], 150 mM NaCl, 5 mM EDTA, 1% NP-40, 0.1% SDS), followed by addition of 50 μl of 4x SDS loading buffer (277.8 mM Tris-HCl [pH 6.8], 44.4% (v/v)glycerol, 4.4% SDS,0.02% bromophenol blue).Whole cell lysates were subsequently analyzed by 10% SDS-polyacrylamide gel electrophoresis (PAGE). Proteins from the gel were transferred to nitrocellulose membrane (Bio-Rad) using semi-dry blot transfer equipment (Bio-Rad). The membrane was blocked with 5% skim milk in PBST (PBS buffer with 0.1% Tween) with agitation for 1 h. Next, primary antibody, mouse anti-V5 mAb (Ebioscience) diluted in 5% skim milk/PBST, was added and incubated for 2 h at room temperature. The membrane was washed 3 times with PBST and then incubated with secondary antibody, HRP-conjugated anti-mouse IgG diluted in 5% skim milk/PBST for 1 h 30 min at room temperature. The membrane was washed 3 times with PBST and then subjected to

chemiluminescence substrate (WESTAR SUPERNOVA, Cyanagen) by incubating for 5 min. Finally, the membrane was exposed and analyzed using Alliance MINI HD9 AUTO Western Blot Imaging System (Uvitec Mini HD9, BioSPX).

*GM1 Ganglioside Binding Assay*

Transfected HEK 293A cells were lysed and collected with ice-cold RIPA without SDS )25 mM Tris-HCl [pH 7.6], 150 mM NaCl, 5 mM EDTA, 1% NP-40(. After incubation on ice for 1 h, cell lysates were centrifuged, and supernatant was kept and used in the assay. Unlike other samples, CTB was produced in *E. coli* by IPTG induction and the cell lysate was extracted using CellLytic B (Sigma). ELISA plate was coated with 100 µl of 3 µg/ml GM1 ganglioside (Sigma) diluted in bicarbonate buffer )15 mM Na2CO3, 25 mM NaHCO3, pH 9.6(. The plate was covered with plastic wrap and incubated at 4˚C overnight. The wells were washed thrice with PBST buffer )PBS plus 0.05% Tween 20(, blocked by 150 µl/well of filtrated 1% fish gelatin (Ajax Finechem) in PBS, and incubated at room temperature for 2 h. After four rounds of washing with PBST buffer, the wells were loaded with the cell lysates extracted, followed by incubation for 2 h at 37˚C .The plate was washed three times with PBST buffer and then incubated with 50 µl per well of either mouse anti-V5 or mouse anti-His antibodies (Ebioscience) diluted in PBST buffer for 1 h at 37˚C .The plate was washed thrice with PBST buffer and loaded with 50 µl/well of HRP-conjugated rabbit anti-mouse IgG (Abcam) for 1 h at 37˚C and washed four times with PBST buffer .The plates were developed with the addition of 100 µl/well of the TMB substrates (1-Step™ Ultra TMB-ELISA Substrate Solution, Thermo Scientific) for 30 min at room temperature in the dark. 2M Sulfuric acid (100 ul/well) was added into each well and absorbance at wavelength 450 nm was measured using an ELISA reader (Multiskan™ FC Microplate Photometer, Thermo Scientific) .

**Results**

*Design of the vaccine immunogens*

In this study, we aimed to develop a vaccine capable of enhancing production of PEDV-specific immune response, particularly, sIgA, in the gut mucosa. To achieve the goal, Cholera toxin beta subunit (CTB), a mucosal vaccine adjuvant[10,12], was added to the C-terminus of our newly designed immunogen, PEDVSME. Thus, this modified immunogen is termed PEDVSME-CTB. To allow separation of the two protein and thus maintain their function, Alpha-helical linker (GGGEAAAKGGG)[15-16] was added between PEDVSME and CTB (Fig. 1).

*Construction of the immunogen genes and generation of DNA vaccines*

The genes encoding the PEDVSME and CTB proteins were codon-optimized with pig's most frequently used codons and then synthetically made. To generate gene fragment PEDVSME-CTB, DNA fragments of PEDVSME-linker and CTB were PCR-amplified and then linked together by overlapping PCR (Fig. 2A). Nucleotide sequence encoding Alpha-helical linker was included into the construct by primers used in the PCR. PCR amplification showed the products of PEDVSME-linker and CTB at the expected size of 969 and 387 bp, respectively. Overlapping PCR between fragments PEDVSME-linker and CTB produced a few DNA fragments including the expected product of 1314 bp, which is predicted to be PEDVSME-CTB fragment (Fig. 2B).

Plasmid pTH[18], a mammalian expression vector, was then used for generation of DNA vaccine. The gene fragment was ligated into plasmid pTH at *Pst*I and *Not*I sites. Following the transformation of *E.coli* DH5α with ligation mixture, *E.coli* clones that carry recombinant

plasmid were screened using rapid size screening. A few clones were observed to contain insert gene (Fig. 3A) and 2 recombinant clones were selected for plasmid extraction. The presence of the PEDVSME-CTB gene fragment in recombinant plasmids was confirmed by digestion with *Pst*I and *Not*I. The digested product at the expected size of 1314 bp was observed (Fig. 3B). The nucleotide sequence of both clones was then confirmed by automate DNA sequencing. The DNA sequencing results demonstrated that nucleotide sequences of both clones showed 100% identity to the designed sequence.

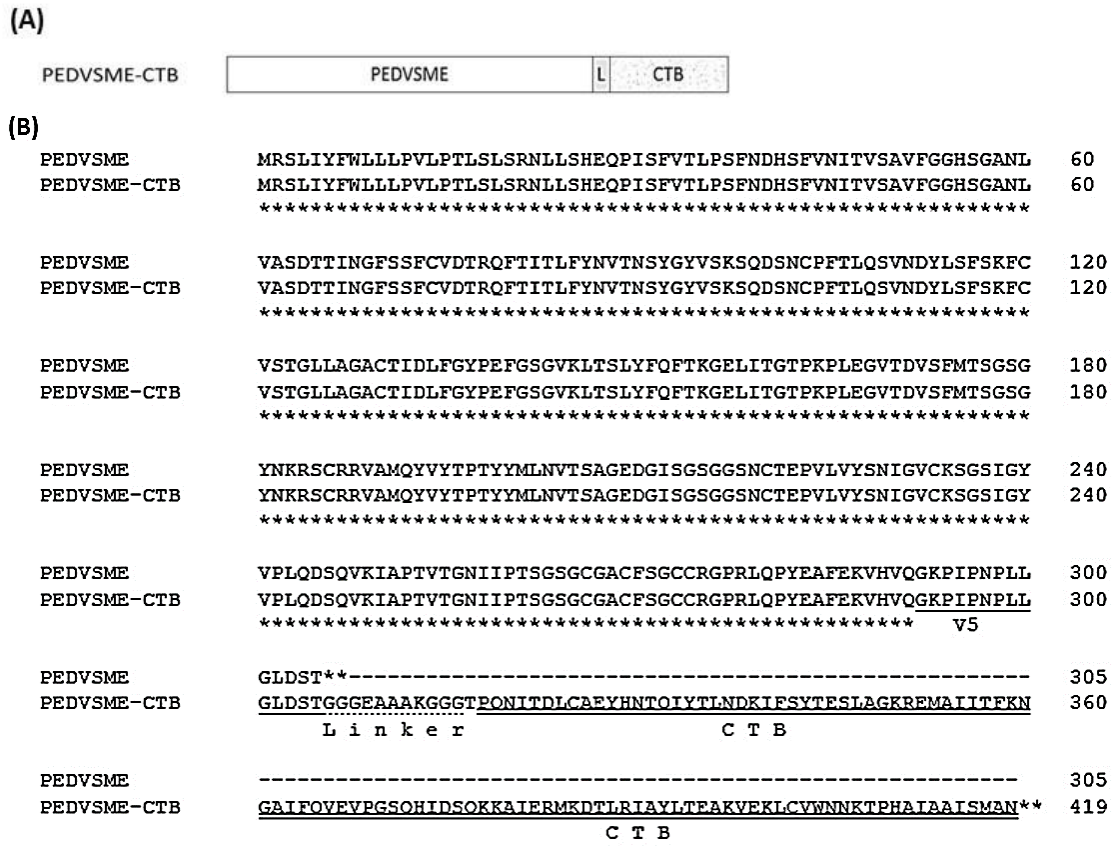


**Figure 1.** Schematic representation and amino acid sequence of PEDVSME-CTB. **(A)** Schematic representation of PEDVSME-CTB gene fragment, L represents linker. **(B)** Amino acid sequence alignment of PEDVSME and PEDVSME-CTB. V5 tag is underlined; linker is indicated with dotted line; CTB sequence is double underlined.

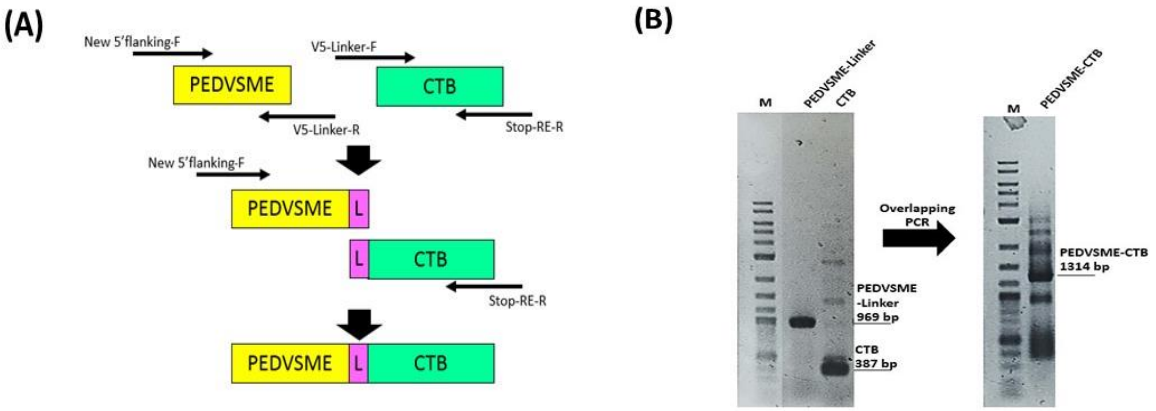


**Figure 2.** Generation of the PEDVSME-CTB gene fragment. **(A)** Schematic diagram of PCR amplification PEDVSME-CTB. Primers used for amplifications are indicated. **(B)** PCR product of PEDVSME-CTB. M represents 2-Log DNA ladder.

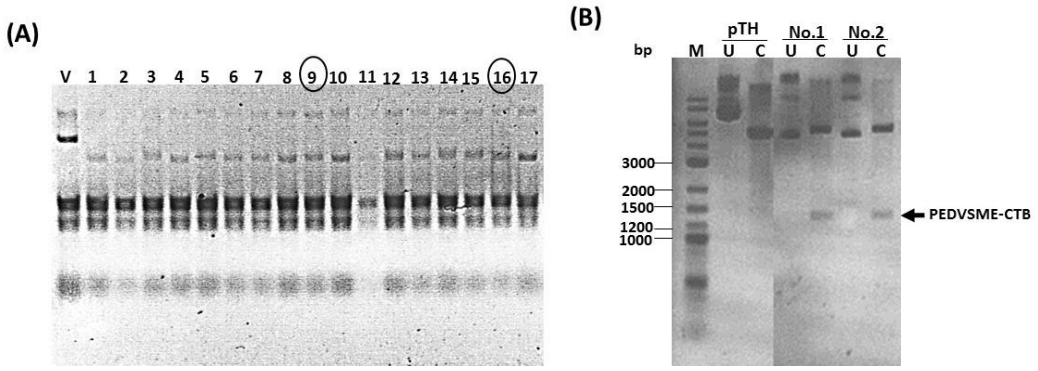


**Figure 3**. Screening and confirmation of the pTH.PEDVSME-CTB clones. **(A)** screening of recombinant clones using rapid size screening. Number represents clone number. **(B)** Presence of PEDVSME-CTB gene in recombinant clones. The presence of the PEDVSME-CTB genes in plasmid pTH was confirmed using restriction enzyme digestion *Pst*I and *Not*I. M represents 2-Log DNA ladder.

*Protein expression of PEDVSME-CTB in HEK 293A cell*

To investigate the expression of the PEDVSME-CTB protein by DNA vaccine, indirect immunofluorescence assay and western blot analysis were performed. HEK 293A cells were transfected with 2 µg of pTH.PEDVSME-CTB. In addition, the cells were also transfected with pTH.PEDVSME and empty pTH for comparation. By detecting V5 tag located at the C-terminus of PEDVSME with anti-V5 mAb, immunofluorescence staining showed a strong fluorescence signal in HEK 293A cells transfected with pTH.PEDVSME-CTB (Fig. 4A). The expression of PEDVSME-CTB was comparable to that of PEDVSME. As expected, HEK 293A cells transfected with parental vector pTH did not show the fluorescence.

When studied by Western blot and using anti-V5 mAb for detection, expected protein band of 46 kDa was observed in the PEDVSME-CTB sample, while a smaller band at 36 kDa was detected in the PEDVSME sample (Fig. 4B). As expected, no protein could be detected in protein lysate of HEK 293A cells transfected with empty pTH. In addition, the protein aggregation was also found in the PEDVSME and PEDVSME-CTB samples. As clear and thick bands were observed with the exposure time of only 1 second, this confirmed a high level of PEDVSME-CTB expression, which is consistent to the immunofluorescence staining result. Moreover, the protein expression level of PEDVSME-CTB detected by Western blot was

comparable to that of PEDVSME. This result indicates that addition of CTB into the PEDVSME does not affect the expression of the PEDVSME immunogen.

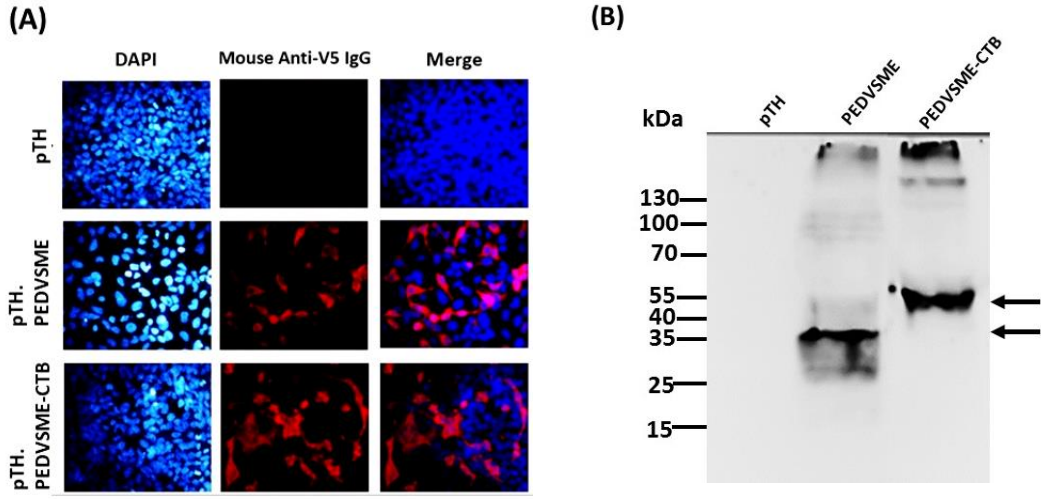


**Figure 4.** Expression of the PEDVSME and PEDVSME-CTB proteins. HEK 293A cells were transfected with plasmids pTH, pTH.PEDVSME and pTH.PEDVSME-CTB and protein expression was detected 3 days post-transfection. **(A)** Immunofluorescence staining for detection of the immunogens in transfected cells. Transfected HEK 293A cells were stained with anti-V5 antibody, followed by Alexafluor-conjugated anti-mouse IgG antibody (red). Blue indicates DAPI staining of nucleus. **(B)** Detection of the immunogens by Western blot analysis. The immunogens on membrane were detected using anti-V5 antibody. The image was obtained by exposing the membrane for 1 second in the Western Blot Imaging System.

*GM1 Ganglioside Binding*

As immunogen PEDVSME-CTB was designed for gut-mucosa targeting by binding to GM1 ganglioside present on intestinal epithelia, binding activity between PEDVSME-CTB and GM1 ganglioside was investigated using GM1 Ganglioside binding ELISA assay. The PEDVSME-CTB protein lysate extracted from HEK 293A transfected with pTH.PEDVSME-CTB was added into ELISA plate coated with GM1 ganglioside. Protein lysate extracted from *E. coli* expressing CTB (E.coli-CTB) was included in the assay as a positive control, while protein lysates PEDVSME and pTH extracted from HEK 293A transfected with pTH.PEDVSME and empty pTH, respectively, were included as a negative control. Concentration of protein lysates were determined using Bradford protein assay. Protein lysates were 2-fold diluted ranging from 12.5-0.4 µg/50 µl, except E.coli-CTB, ranging from 6.25-0.4 µg/50 µl due to the limitation of original protein concentration. Diluted protein lysates were added into the GM1 ganglioside coated plate (50 µl/well). The PEDVSME-CTB and PEDVSME proteins bound to GM1 ganglioside were detected using anti-V5 antibody, while GM1-bound E.coli-CTB was detected with anti-His antibody.

As a negative control, OD450 readouts of pTH sample were constant at all dilutions. Compared to all other samples, while PEDVSME-CTB showed the highest binding affinity to GM1 ganglioside. As expected, OD450 readouts of PEDVSME-CTB sample were increased when the concentrations of protein lysate increased (Fig. 5), indicating that the binding between PEDVSME-CTB to GM1 ganglioside was specific. Importantly, although increased OD450 readouts were observed in increased concentration of PEDVSME, the readouts obtained from PEDVSME-CTB were markedly higher than those of PEDVSME at all concentrations. This result, again, confirmed the specific binding between PEDVSME-CTB and GM1 ganglioside.

Surprisingly, OD450 readouts of E.coli-CTB, which was used as a positive control, was considerably lower than those of PEDVSME-CTB.

Altogether, this result indicates that PEDVSME-CTB has biological function in binding to GM1 ganglioside, a natural receptor for cholera toxin entry into mucosal epithelial cells. Thus, it is potentially that linking CTB to the immunogen PEDVSME will enhance an induction of PEDV-mucosal immune response, particularly, at the gut mucosa.

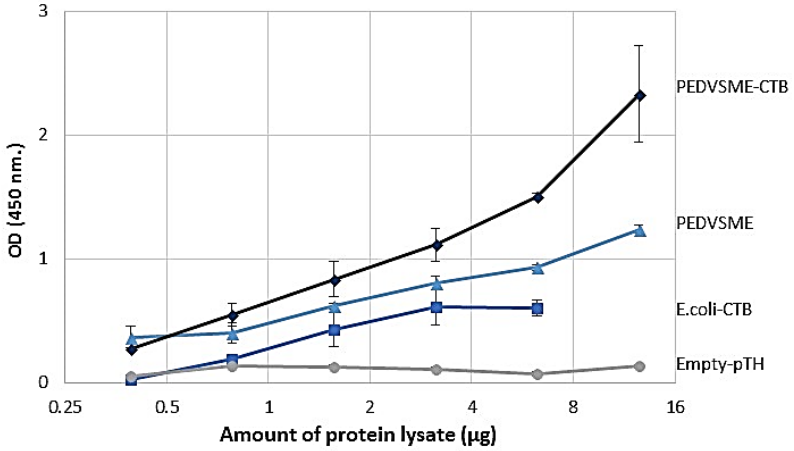


**Figure 5**. GM1 Ganglioside binding ELISA assay. Protein lysates extracted from transfected HEK 293A cell were 2-fold diluted and tested for GM1 ganglioside binding using anti-V5 mAb and ELISA assay. Absorbance at wavelength 450 nm (OD450) was determined. The OD450 reads at different dilutions of PEDVSME-CTB (♦), PEDVSME (Δ), E.coli-CTB (■), and pTH (●) are shown.

## Discussion

Porcine epidemic diarrhea is globally contagious and epidemic disease in porcine industry[19]. A PEDV vaccine capable of providing protective immune response against PEDV is highly required. PEDV uses intestinal enterocytes for primary infection as well as the main site for virus replication[3]; thus, protection against PEDV requires the establishment of PEDV-specific immune response in the intestinal mucosa. Frequently, sIgA in the gut mucosa is emphasized as the most required agent for effective protection against PEDV[20-21].

In PEDV vaccine development, the efficiency of the PEDV vaccine has been though that it depends largely on the access of the immunogen to mucosal inductive site, particularly, gut-mucosa. It has been shown that oral vaccination could stimulate the production of intestinal sIgA capable of neutralizing PEDV, while vaccination using parenteral routes (e.g., i.m., i.d. and s.c.) generally failed to induce mucosal immunity[1-2,22].

Although oral vaccination tends to be an effective protocol for induction of intestinal sIgA, it usually requires higher dose with multiple immunization and the level of response is rather low[20,23]. To present, intramuscular (i.m.) injection is still a preferred and practical means of vaccine administration in pig. Thus, a vaccine that can effectively induce PEDV-specific sIgA in the intestine although given by parenteral routes is needed. There are several strategies to enhance the capability of the vaccine at inducing mucosal immune responses. One of the most frequently used methods is by fusing Cholera toxin beta subunit (CTB) to vaccine antigens. Previous studies have shown that the antigen fused with CTB could induce higher mucosal sIgA[14-15,24-25].

In this study, we linked CTB to our newly designed immunogen, PEDVSME, for targeting the immunogen to intestinal epithelia. As previously reported that CTB allows the immunogen to access to the mucosal system under the gut barrier by binding to GM1 ganglioside present on intestinal epithelia[10], we thus expected that CTB should redirect our immunogen to the intestine for immune induction. We generated DNA vaccine by inserting

our immunogen gene into DNA plasmid pTH as a vaccine vector. This is because DNA vaccine is considered to be safe, easy to produce, and capable of inducing both humoral and cellular immune responses that are solely directed against such vaccine antigen[26]

Detected by Immunofluorescence staining and Western blot, PEDVSME-CTB was highly expressed by DNA vaccine in HEK 293A cells and the expression level was comparable to that of PEDVSME. The high expression of PEDVSME-CTB may be facilitated by several factors. First, the entire fragment of the PEDVSME-CTB gene was codon-optimized with pig's most frequently used codons. It has been evidenced in many studies that codon optimization could enhance protein expression from such gene, compared to original codon sequence[27–29]. Second, addition of Kozak sequence to the 5' end of start codon could enhance translation as it has been previously shown[30]. Third, the PEDVSME-CTB gene in plasmid pTH was expressed by a cassette of enhancer/immediate-early CMV promoter/intron A, which has been shown to efficiently express transgene in plasmid pTH [18,31]. Additionally, the linker added between PEDVSME and CTB may contribute to the high expression of the immunogen by separating the two proteins and thus maintaining structure and function of each protein. The advantage of linker for enhancing expression of fusion protein has been evidenced in many studies such as the co-expression of CagA of *Helicobacter pylori* and CgeA spore coat protein in *Bacillus subtilis*[32], and co-expression of Granulocyte Colony-Stimulating Factor and Transferrin Fusion protein in transfected HEK293 cells[33].

When compared to the negative control and PEDVSME, PEDVSME-CTB exhibited higher reads of OD450 in GM1 ganglioside binding assay, which indicated a specific binding to GM1 ganglioside, a CTB receptor. However, the positive control, E.coli-CTB, showed lower OD450 reads. This can be due to several factors. First, the CTB protein was expressed in *E. coli* system; as a result, the expression and protein characteristics can be different from PEDVSME-CTB expressed in mammalian cell. We used protein lysates for the assay; thus, each protein lysate may not contain the same amount of target protein. Second, PEDVSME-CTB is tagged with V5 tag while E.coli-CTB is tagged with His tag; thus, detection of the protein in ELISA plate was done with different antibodies. It is possible that binding affinity of anti-His antibody to its target protein is lower than that of anti-V5 antibody as we previously observed in other works.

**Conclusion**

With the aim to develop a mucosal vaccine against PEDV, we have designed and constructed an immunogen PEDVSME-CTB, by which CTB is linked to the PEDVSME immunogen. The PEDVSME-CTB was vectored by plasmid pTH, a DNA vector and thus called DNA vaccine. PEDVSME-CTB was successfully expressed in a high level by DNA vaccine in mammalian cell. GM1 ganglioside binding assay exhibited binding capability of the PEDVSME-CTB to GM1 ganglioside.

However, further work to study immunogenicity and efficacy in animal model needs to be done. The immunogen can also be delivered by other vaccine delivery systems such as adenovirus and modified vaccinia Ankara virus (MVA), which have been proven to be an efficient system for vaccine delivery and induction of immune system. Moreover, different vaccine vectors bearing the same antigen could be used for heterologous prime/boost immunization that has been shown as an effective protocol of vaccination.

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