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Research article

Expression of recombinant porcine reproductive and respiratory syndrome virus nucleocapsid protein with antigenicity and immunogenicity study

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Abstract

Porcine respiratory and reproductive syndrome virus (PRRSV) causes significant economic losses in the swine industry worldwide. Nucleocapsid protein (NP) encoded by ORF7 is the most abundant and high immunogenicity. However, there were only few studies on recombinant NP expression with antigenicity and immunogenicity from PRRSV in Thailand. The aim of this study was to express rNP from PRRSV found in Thailand and evaluate its antigenicity and immunogenicity. ORF7 gene was amplified by RT-PCR. The PCR products were cloned into pGEM®-T Easy vector, and subcloned into pET-24a (+) expression vector and transformed into *E. coli*. The rNP properties were proved by SDS-PAGE and Western blot. The antigenicity of rNP was evaluated by indirect ELISA comparing to a commercial test kit. The immunogenicity of rNP was proved by rabbit immunization. Then, the antiserum was tested with tissues from PRRSV-infected swine by immunohistochemistry (IHC). In addition, amino acid sequence of the rNP was analyzed and compared to PRRSV reference strains. The PCR products of a whole ORF7 gene were 388 bp. The rNP size was 18 kDa. The indirect ELISA assay showed high antigenicity of the rNP with 100% sensitivity and 93.33% specificity at 0.25 O.D. cutoff value. The rNP could induce specific antibody production in the rabbit, which reacted specifically to PRRSV in tissues by IHC. The amino acid sequence of the rNP had similarities to US, HP-PRRSV and EU PRRSV at 95.93-99.19%, 96.75%, and 57.03-61.47%, respectively. Our rNP can be used for PRRSV diagnosis and test kit development.

Keywords: Antigenicity, Immunogenicity, Nucleocapsid protein, PRRSV

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INTRODUCTION

Porcine respiratory and reproductive syndrome virus (PRRSV) causes significant economic losses in the swine industry worldwide (Renken et al., 2021). Infected pigs show multisystem clinical signs based on ages. Sows are affected on reproductive system such as infertility, mummification, and late term abortion (Karniychuk and Nauwynck, 2013). Suckling pigs mostly die from respiratory system abnormalities or secondary infections. The clinical signs are high fever, nasal discharge, cyanosis on ears and distal limbs (Helke et al., 2015).

PRRSV has been classified in the genus *Arterivirus*, a member of *Arteriviridae* family in the Nidovirales order. It is generally accepted that there are 2 predominantly PRRSV genotypes (typical PRRSV); type 1 (European genotype; EU) and type 2 (North American genotype; NA); which share 60% genomic identity (Meng, 2000). In 2006, highly pathogenic PRRSV (HP-PRRSV) classified in type 2 was firstly outbreak in China and rapidly spread to Thailand (Nilubol et al., 2012), which was reported to the OIE in mid-2010. Since then, HP-PRRSV has become a dominant circulating strain in Thailand (Jantafong et al., 2015). This positive sense single-stranded enveloped RNA virus contains 15 kb genome including 11 open reading frames (ORFs) (Chaudhari and Vu, 2020). The virus particle contains three major structural proteins including envelope protein (E), membrane protein (M), and nucleocapsid protein (NP), encoded by ORFs 5, 6, and 7, respectively. One of the most abundant proteins with high antigenicity and immunogenicity is non-glycosylated 15 kDa nucleocapsid protein (Meulenberg et al., 1995, Dea et al., 2000a). Protein antigenicity is important to select antigen for antibody detection by enzyme-linked immunosorbent assay (ELISA) assay, while protein immunogenicity is important to select antigen for antibody production. The produced antibody can be used to detect antigen in tissues by immunohistochemistry (IHC) (Duraiyan et al., 2012).

HP-PRRSV strains cause severe reproductive and/or respiratory syndrome with high fever and neurological signs. However, molecular and serological diagnostic applications nowadays are mostly based on only typical strains. For example, recombinant nucleocapsid protein (rNP) from EU and NA strains were previously expressed as an antigen to detect PRRSV antibody by ELISA assay (Dea et al., 2000b, Seuberlich et al., 2002). Moreover, since the high fever syndrome with nervous sign different from typical PRRS has emerged in 2006, there were only few data investigating on its nucleocapsid protein and tissue tropism. Type of the virus used in this study was proved as the HP-PRRSV from ORF5 DNA sequencing analysis that closely related to HP-PPRSV found in Thailand (98.18-99%), Vietnam (98.17-98.33%), Laos PDR (98.83%), and China (98.17-98.67%). Moreover, *ORF7* analysis showed the consistent results that our virus was similar to HP-PRRSV, while it shared approximately 60% identity to EU strains. Thus, the virus used in this study could be another choice for diagnostic assay development as it was a good match with the circulating strains in Thailand and neighboring countries. The aim of this study was to express the rNP of HP-PRRSV and evaluate its antigenicity and immunogenicity. This research will be useful for preparing a new rNP from HP-PRRSV for serological diagnosis development and swine herd immunity status monitoring.

MATERIALS AND METHODS

Ethics statement

Rabbits in this experiment were conducted in accordance with the guiding principles for the care and use of research animals, and the protocol was approved by the Committee on the Ethics of Animal Experiments of the National Institute of Animal Health, Thailand (Project approval number EA-0012/63(R)).

Sample preparation

Organs including brain, lymph nodes, lung, saliva gland, spleen, and kidney from a swine in Ubon Ratchathani province, Thailand showing HP-PRRSV clinical signs and confirmed as PRRSV positive by real-time RT-PCR (Kleiboeker et al., 2005) were provided by Veterinary Research and Development Center (VRDC), Lower Northeastern Region, Thailand. A negative lung sample was selected from a case confirmed PRRSV negative by real-time RT-PCR at National Institute of Animal Health, Thailand. The tissue samples were cut to 1 cm dimension and stored at -80°C or in 10% formalin. Thirty swine sera were provided by VRDC, Upper Northern Region, Thailand. One mL of each serum sample was stored at -20°C until use.

ORF7 amplification

Approximately 200 mg of the lungs were used to perform viral RNA extraction. TRIzol® plus RNA Purification kit (Thermo Fisher Scientific Inc, USA) was used according to the manufacturer's instructions. Subsequently, the total RNA was eluted in 30 µl RNase free water and immediately reverse transcribed to cDNA using random hexamer primers and Superscript™ III Reverse Transcriptase (Invitrogen™, USA). The primers were designed based on in silico analysis according to sequence of PRRSV complete genome published in the GenBank (NC_001961.1). The cDNA was used as the template to amplify a full-length *ORF7* gene with specific primers 5'-GCAGAATTCATGCCAAATAACAACGGCAAGCAG-3'(forward) and 5'-CATGCGGCCGCCGCTGAGGGTGATGCTGTGAC-3'(reverse) containing restriction sites (underline) of EcoRI and NotI, respectively. The PCR was performed in a 25 µl total volume, which contains 1X PCR buffer without Mg, 0.2 mM dNTP, 1.5 mM MgCl2, 0.2 µM of each primer, 5 µl of DNA template, 1 unit of Platinum™ Tag DNA polymerase (Invitrogen™, USA). The PCR cycle was done 1 cycle of denaturation at 94 ºC for 2 min, 35 cycles of 94 ºC for 30 s, 55 ºC for 30 s and 72 ºC for 1 min, and 1 cycle of final extension at 72 ºC for 7 min. The PCR products were run on 1% agarose gel electrophoresis and visualized under UV illumination.

Cloning and protein expression

The PCR products were purified by Wizard® SV Gel and PCR Clean-Up System (Promega, USA) according to the manufacturer's instructions. The amplified product of *ORF7* was ligated into pGEM®-T Easy Vector (Promega®, USA) according to manufacturer's instruction. DH5α Competent Cells (Invitrogen™, USA) were transformed with the vectors containing

ORF7. Positive clones were identified based on blue-white screening and were double digested with *EcoR*I and *Not*I enzymes to release the *ORF7* gene. Next, the clones were subcloned into pET-24a (+) expression vector (Novagen®, Germany). An inserted DNA was positioned downstream and in frame with a sequence encoded the C-terminal histidine-tag fusion protein. A chosen colony of BL21 (DE3) competent *E. coli* (Invitrogen™, USA) containing pET-24a (+) with *ORF7* was cultivated in 500 ml LB-medium supplemented with kanamycin at 37 ºC with shaking at 180 rpm for 3 h the *E. coli* cultures reached an A600 of O.D. 0.6. The expression of recombinant protein was then induced by isopropyl-β-D-thiogalactoside (IPTG) to a final concentration of 1 mM at 25 °C with shaking at 120 rpm for 16 h. The cells were pelleted at 3,000 rpm for 15 min and were resuspended in Tris lysis buffer (100 mM NaH2PO4, 10 mM Tris-Cl pH 8.0, 8M urea) with 50 mg/ml lysozyme and protease inhibitor (Amresco®, USA). The cells were disrupted by sonication on ice and centrifuged at 10,000 rpm at 4 ºC for 30 min to collect the supernatant containing recombinant nucleocapsid protein.

Purification of recombinant nucleocapsid protein (rNP)

Ni-NTA agarose beads (QIAGEN®, Germany) was used to purify the rNP according to the manufacturer's instructions. The bead-bound protein was washed with washing buffer (50 mM imidazole, 100 mM NaH_2PO_4, 10 mM Tris-Cl pH 8.0, 8 M urea) and eluted with elution buffer (300 mM imidazole, 100 mM NaH_2PO_4, 10 mM Tris-Cl pH 8.0). The concentration of recombinant protein was determined by DC protein assay kit at 750 nm (Biorad®, USA) using bovine serum albumin (BSA) as a standard.

SDS-PAGE and Western blot

The rNP purity was analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The rNP was mixed with Laemmli sample buffer and heated at 70 °C for 10 min. The protein mixture was subsequently size-separated on a 10% polyacrylamide gel in Tris-glycine buffer and stained with Coomassie brilliant blue. For Western blot, separated proteins were transferred onto a PVDF membrane (Immunoblot®, USA) by setting electrotransfer at constant voltage 15V for 16 h. The membrane was blocked with 5% skim milk in PBS at room temperature for 1 h. The membrane was incubated with 1:1,000 HisProbeTM-HRP conjugated (Invitrogen™, USA) in PBST (PBS with 1% NaCl and 0.05 % Tween® 20) with 3% skim milk. The membrane was washed, and the reaction was then made visible with 3,3'-diaminobenzidine (DAB) substrate solution (Bio-Rad Laboratories, Inc., USA).

Finding antigenicity by indirect ELISA (iELISA)

Antigenicity of the rNP is evaluated by iELISA which was firstly optimized by checkerboard titration. The rNP was two-fold serially diluted from the highest concentration at 100 µg/ml (10 µg/well or 1:10) to the lowest concentration at 0.098 µg/ml (0.0098 µg/well or 1:10240) in coating buffer (50 mM carbonate-bicarbonate, 8 M urea, pH 9.6). Then, 100 µl of rNP solution was subjected to coat in 96-well flat-bottom microtiter plates (Nunc™, Denmark) and incubated at 4 ºC overnight. The coated plates were washed

three times with PBST and blocked with 5% skim milk in PBS at 37 ºC for 1 h. The plates were washed and incubated with 100 µl of the pooled sera at 37 ºC for 1 h. The positive and negative sera were from the field that was tested positive or negative by IDEXX PRRS X3 Ab Test (IDEXX Laboratories, USA). The serum samples were diluted two-fold serially from 1:10 to 1:640. After washing, the plates were incubated with optimized secondary antibody of HRP-conjugated rabbit-anti pig IgG (Invitrogen™, USA) in the blocking solution at 37 ºC for 1 h. Following the washing step, the color was developed by 100 µl of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution and the reaction was stopped by sulfuric acid. Then, the O.D. was read at 450 nm using a MultiskanTM microplate reader (Thermo Scientific™, USA). The O.D. values were plotted against the antigen and serum dilutions to determine an optimal condition. To estimate diagnostic sensitivity and specificity of our rNP iELISA assay, 15 positive and 15 negative swine sera confirmed by the commercial ELISA kit, IDEXX PRR X3 Ab Test (IDEXX Laboratories, USA) were used in this study. All experiments were repeated in triplicate. The receiver operating characteristic (ROC) curve implemented in MedCalc software Version 16.8.4 (MedCalc Software Ltd., Belgium) was used to determine a cutoff value. The alpha level was set at 0.05 and confidence interval (CI) was 95% for calculation.

Finding immunogenicity by IHC using rabbit immunization

To determine whether rNP could induce specific antibody response, a rabbit was injected subcutaneously with 1 ml of 150 µg/ml purified rNP with incomplete Freund's adjuvant for 4 times at 2 weeks interval. The rabbit antiserum was evaluated for the presence of rNP specific IgG by immunohistochemistry (IHC) using swine tissue samples from HP-PRRS infection and non-infection. The IHC procedure was modified from a previous study (Chantamaneechote et al., 2000). Briefly, the tissue samples such as brain, lung, salivary gland, and kidney were fixed in 10% neutral formalin for 24 h, and then processed to embedded in paraffin blocks. The 3 µm-thick tissue sections were mounted on positive charge glass slides (Thermo Scientific, USA). The slides were deparaffinized through heating in 60 °C for 1 h and rehydrated with xylene and a series of different ethanol concentrations (100%, 95%, 80%, and 70%, respectively) for 5 min each. Then, endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol for 20 min. Antigen retrieval was performed by using heat induces epitopes for 5 min at 100 ºC in citrate buffer solution (pH 6.0), then washed in a solution of TBS + 1% Tween (TBST) for 5 min. The slides were equilibrated in PBS (pH 7.4) and non-specific antigens were blocked by 1% normal bovine serum for 1 h. The antiserum was diluted at 1:500 and incubated with the slides at 4 ºC overnight. Then, the slides were rinsed in PBS 3 times for 5 min each. A couple drops of HRP labelled polymer anti-rabbit (Dako, USA) were added and incubated for 10 min at RT. After washing, DAB substrate (Dako, USA) was applied and incubated at for 2 min. Subsequently, the slides were counterstained with hematoxylin. The sections were dehydrated and mounted. The histopathologic examination and detection of PRRSV by IHC were observed using a light microscope.

Amino acid sequence analysis

Five *E. coli* positive clones were cultured in 5 ml LB media, and plasmids containing pGEM®-T Easy vectors harboring *ORF7* were extracted according to manufacturer's instruction (Promega®, USA). The plasmids were sent to a local company for DNA sequencing (Macrogen, Korea). Then, the amino acid sequence of the rNP was translated by Expasy website (http://web.expasy.org/translate/) and the protein was predicted by Smart BLAST (NCBI). To evaluate relationships among reference strains, the protein was compared to 14 references available in GenBank database under the accession numbers indicated in Tabel 1. Identities were defined by ClustalW Multiple alignment with BLOSUM62 scoring matrix using MEGA 7 software. Linear epitopes of the rNP were also predicted by IEDB website.

RESULTS

Expression of rNP in *E. coli*

The approximately 388 bp of *ORF7* gene was successfully amplified by the designed primers (Figure 1). The positive clones of pGEM®-T Easy vector harboring the *ORF7* gene were confirmed by restriction enzyme digestion (Figure 2). SDS-PAGE showed that rNP was successfully expressed in *E. coli* BL21 using pET-24a (+) vector with the protein size at 18 kDa (Figure 3A). The concentration of the expressed rNP was about 1.5 mg/mL. The purified rNP-His-tag showed specific reaction to HisProbe™-HRP conjugated demonstrated by Western blot (Figure 3B).

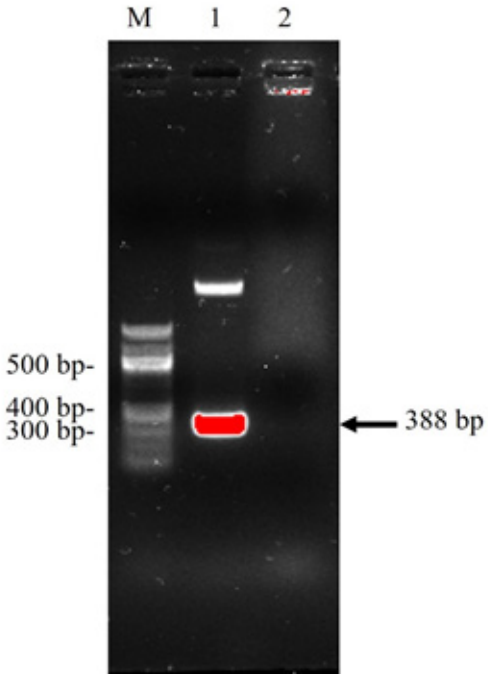

Figure 1 Amplification of *ORF7* gene by RT-PCR analyzed in 1% agarose gel stained with fluorescent dye (GeneDireX, Taiwan). Lane M = 100 bp DNA marker (BioLabs, USA), Lane 1 = 388 bp PCR product, Lane 2 = negative control.

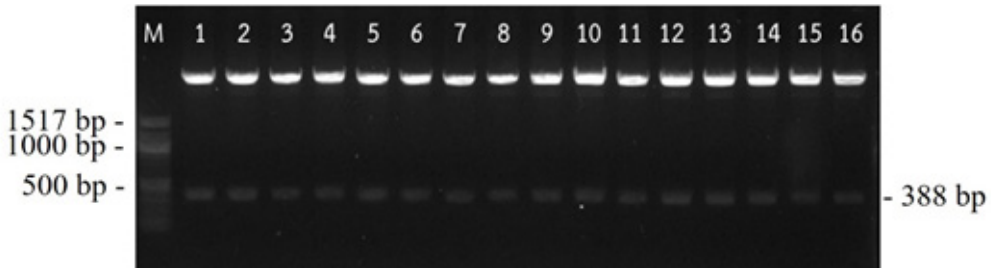

Figure 2 Restriction enzyme digestions of pGEM®-T Easy vectors analyzed in 1% agarose gel stained with fluorescent dye (GeneDireX, Taiwan). Lane M = 100 bp DNA marker (BioLabs, USA), Lane 1-16 = 388 bp PCR product of selected positive clones.

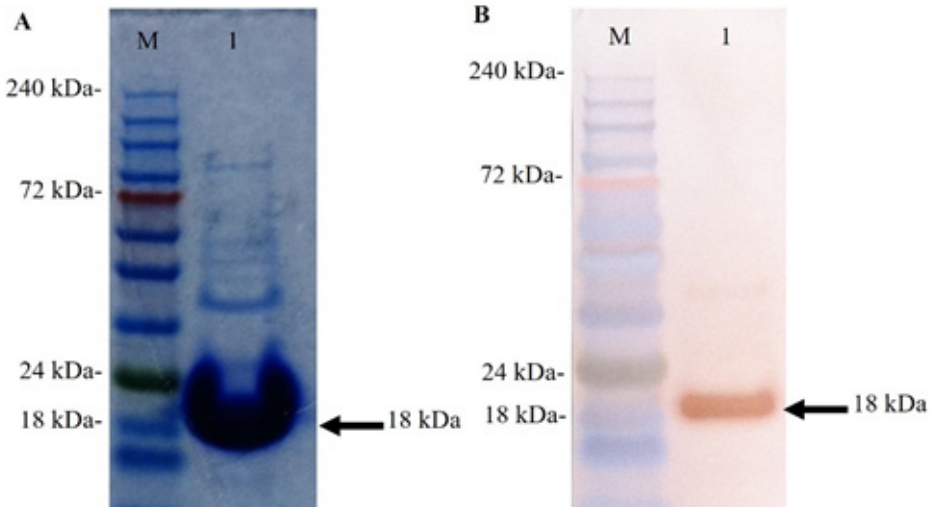

Figure 3 (A) SDS PAGE and (B) Western blot of the rNP-His-tag expressed in *E. coli* BL21. Lane M = protein marker (Bio-Helix, Taiwan); Lane 1 = purified rNP (18 kDa).

Antigenicity and immunogenicity of rNP

An iELISA was developed to evaluate the antigenicity of the expressed rNP. Regarding the ELISA optimization, the optimal rNP concentration was 12.5 µg/mL or 1.25 µg/well, the optimal swine serum dilution was 1:80 (Figure 4A), and the optimal conjugate was 1:10,000 (data not shown). The preliminary ELISA assay demonstrated that all 15 positive swine serum samples bound to the purified rNP indicating its high antigenicity with 100% sensitivity, while 14 from 15 negative swine serum samples showed negative indicating 93.33% specificity at 0.25 cutoff (Figure 4B). The IHC results showed that the immunized rabbit sera reacted specifically to PRRSV in the infected tissues including brain, lung, trachea, salivary gland, and kidney of the naturally infected pigs. The reaction was not observed in lung negative control (Figure 5).

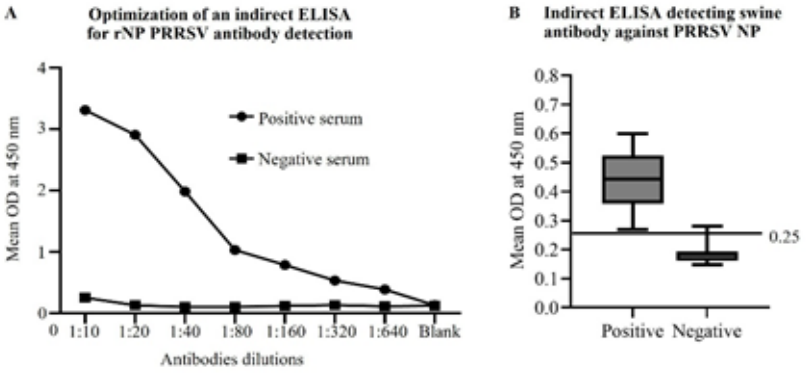

Figure 4 Indirect ELISA optimization and cutoff value evaluation. (A) The optimum concentration of rNP was tested with two-fold serial dilutions of positive and negative swine sera. (B) Detection of swine antibody against PRRSV nucleoprotein by iELISA in positive and negative sample from IDEXX PRRS X3 Ab Test. The boxplot illustrates the distribution of mean O.D. values from 15 positive and 15 negative swine sera at 0.25 cutoff value.

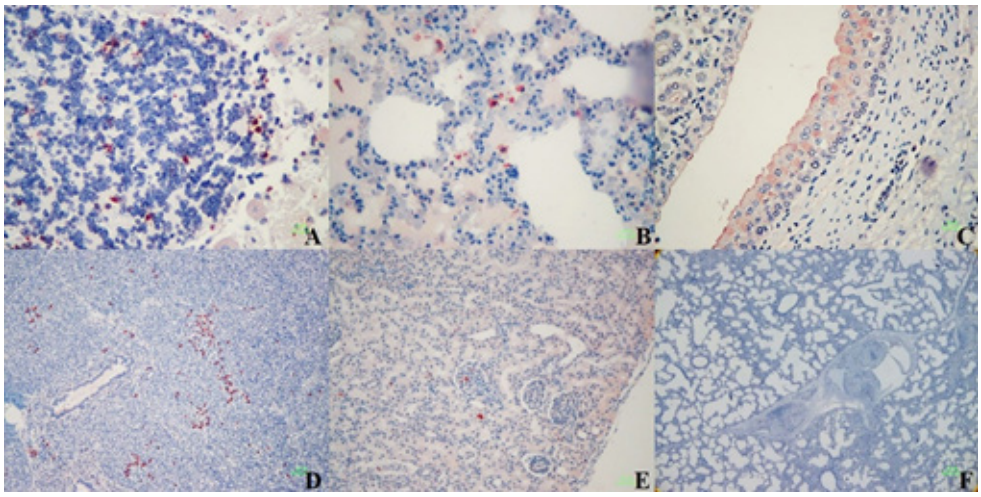

Figure 5 IHC for PRRSV in tissue stained with H&E. The red-brown color represents positive PRRSV. (A) cerebellum; (B) bronchiolar epithelium; (C) trachea; (D) salivary gland; (E) kidney; (F) lung negative control. All images were obtained at a magnification of 200x.

Analysis of amino acid sequence encoded by *ORF7* gene

The amino acid sequence of the rNP encoded by *ORF7* gene was translated to 128 aa (Figure 6A). A phylogenetic tree was built with 14 reference nucleocapsid proteins under the accession numbers indicated in Table 1. The tree was divided into two groups; NA and EU. The rNP in this study was in the NA group and closely related to HP-PRRSV (Figure 6B). Multiple sequence alignment of the rNP comparing to 14 reference PRRSV strains illustrated that the protein was in a full-length relationship with nucleocapsid proteins from at least 14 PRRSV isolates, indicating that our product was literally nucleocapsid protein of PRRSV. The rNP contained four sites of predicted linear epitopes at 1-18 aa, 34-64 aa, 84-93 aa, and 119-123 aa (Figure 7). Our rNP had identities with US, HP-PRRSV, and EU strains at 95.93-99.19%, 96.75%, and 57.03-61.47%, respectively (Table 1).

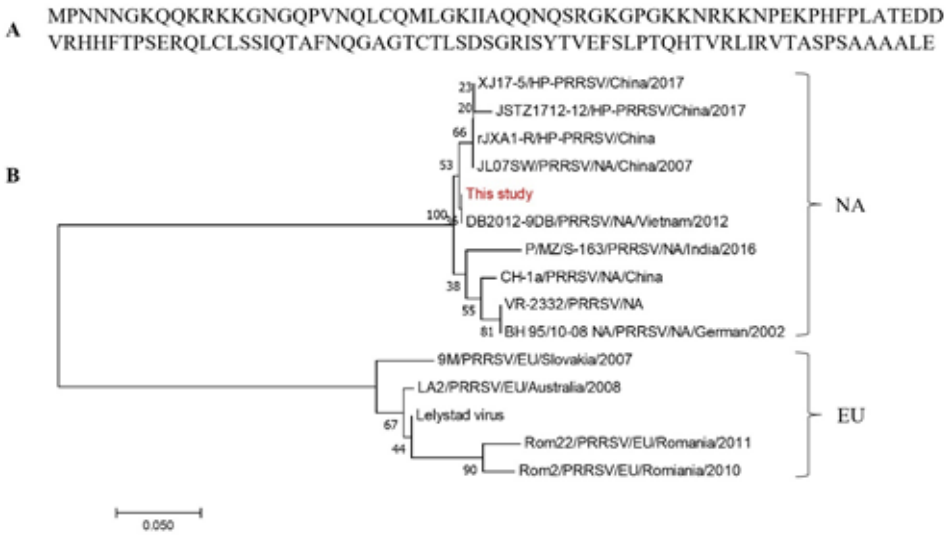

Figure 6 Analysis of amino acid sequence of the rNP. (A) Amino acid sequence of 128 aa translated by Expasy website. (B) Neighbor-Joining tree of PRRSV nucleocapsid protein. Phylogenetic analysis showed that the protein in this study (red) was nucleocapsid of PRRSV by comparing with 14 reference sequences.

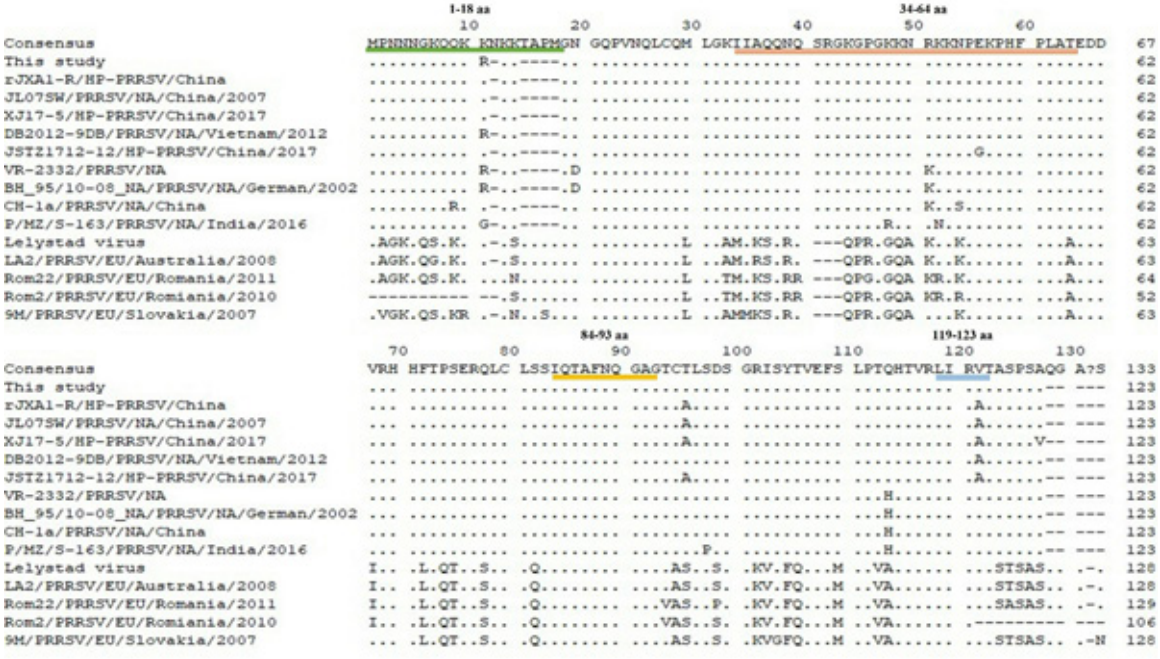

Figure 7 ClustalW Multiple alignment using BLOSUM 62 scoring Matrix with 1,000 bootstraps showed sequence alignment between our predicted rNP and 14 reference sequences of PRRSV nucleocapsid protein. The four underlines were predicted linear epitopes at 4 sites on the protein sequence (http://tools.iedb.org/main/bcell/), dashes (-) represented gaps in the sequence of NP relative to its counterparts, dots (.) represented the agreement to the consensus.

Table 1 The details of reference PRRSV used for *ORF7* amino acid analysis

No.	Accession	Isolation	Country	Genotype	% Identity to protein in this study
1	MN636823	This study	Thailand	NA/HP-PRRSV	
2	QJD21996.1	rJXA1-R/HP-PRRSV/China	China	NA/HP-PRRSV	97.56
3	AHK09725.1	JL07SW/PRRSV/NA/China/2007	China	NA	97.56
4	QFR38104.1	XJ17-5/HP-PRRSV/China/2017	China	NA/HP-PRRSV	96.75
5	AJD85784.1	DB2012-9DB/PRRSV/NA/Vietnam/2012	Vietnam	NA	99.19
6	QFR38114.1	JSTZ1712-12/HP-PRRSV/China/2017	China	NA/HP-PRRSV	96.75
7	AY150564.1	VR-2332/PRRSV/NA	USA	NA	97.56
8	AET99122.1	BH_95/10-08_NA/PRRSV/NA/German/2002	Germany	NA	97.56
9	AY032626.1	CH-1a/PRRSV/NA/China	China	NA	95.93
10	AXT99806.1	P/MZ/S-163/PRRSV/NA/India/2016	India	NA	95.93
11	M96262.2	Lelystad virus	Netherlands	EU	59.84
12	AGT28447.1	LA2/PRRSV/EU/Australia/2008	Austria	EU	59.84
13	AFP95883.1	Rom22/PRRSV/EU/Romania/2011	Romania	EU	57.03
14	ADW95394.1	Rom2/PRRSV/EU/Romiania/2010	Romania	EU	61.47
15	AGT28427.1	9M/PRRSV/EU/Slovakia/2007	Slovakia	EU	59.06

DISCUSSION

In Thailand, HP-PRRSV was the main circulating strain for several years and become endemic in swine farms since it was introduced into the country (Jantafong et al., 2015, Poonsuk et al., 2016, Sangpratum et al., 2019). The virus caused more severe clinical signs along with high morbidity and mortality rates. In general, the disease can be controlled by farm biosecurity, vaccination, and gilt management. However, a recent study found an alternative antiviral compound of the turmeric extraction that inhibited NA PRRSV replication in MARC-145 cells (Anantikulchai et al., 2017). In terms of the virus evolution, a recent study found that 2 novel genotype 1 groups were emerged, while the evolution of the genotype 2 isolates was not found (Tripipat et al., 2021). Thus, using the rNP of HP-PRRSV found in Thailand was a good promising reagent for PRRS diagnosis.

The properties of the produced rNP were almost the same as previous studies (Seuberlich et al., 2002, Kashyap et al., 2020). As NP of PRRSV is non-glycosylated, rNP could be expressed in *E. coli* system with enough high yield to perform ELISA assay (Dea et al., 2000b, Theveethivarak et al., 2007). In contrast, although M protein is non-glycosylated, recombinant M protein could not be expressed enough for ELISA test (Frölichová et al., 2017). The molecular weight of NP was 15 kDa (Dea et al., 2000b) and 6x histidine was about 3 kDa resulting in 18 kDa of the rNP. After denaturation, the rNP could be detected and reacted specifically to HisProbe™-HRP conjugated indicating that the protein structure was linear epitopes.

Regarding the antigenicity, our iELISA assay performance was more sensitive but less specific than a previous study of rNP competitive ELISA that showed 79.80% sensitivity and 98.70% specificity in comparison with IDEXX PRRS X3 Ab test (Dea et al., 2000b). It was likely due to the monoclonal antibody used in that study provided higher specificity. Moreover, our in-house iELISA showed more sensitivity and specificity than another of rNP iELISA

assay development with 82.61% sensitivity and 76.18% specificity (Kashyap et al., 2020). Comparing to IDEXX PRRS X3 Ab test that had 98.8% sensitivity and 99.9% specificity, our iELISA had less specificity possibly due to our in-house plates were coated by only one-genotype antigen, while the commercial plates were coated by two-genotype antigen. Furthermore, theses distinct performances might be due to differences of the purification methods, affinity between antibody and antigen, and the number of samples. However, our rNP iELISA should be further validated with more samples.

With respect to the immunogenicity, the purified rNP was able to induce specific immune response in the rabbit, indicating its high immunogenicity which was consistent with previous studies (Dea et al., 2000a, Seuberlich et al., 2002). The virus antigen was detected in the brain tissue correlated to the clinical signs of seizure from highly pathogenic PRRSV infection as expected. This finding was consistent with a previous study (Dong et al., 2017). Moreover, the virus was also localized in respiratory tract, salivary gland, and kidney, indicating the tissue tropism and routes of transmission via nasal and oral secretions as well as urine which were concordant with previous studies (Wills et al., 1997, Rowland et al., 2003, Trang et al., 2014).

As for the amino acid analysis, the presence of four predicted linear epitopes in our rNP suggested that the antigen could react with polyclonal antibodies and relevance for T cells in terms of MHC molecule binding (Goding, 1996). Close relationship of our protein to the US and Chinese strains could be implied that the rNP antigen was able to detect antibodies against these two strains by iELISA assay. However, as the EU strains have distinct serological properties from US and Chinese ones (Murtaugh et al., 1995), it might be unable to use our rNP for EU strains diagnosis.

CONCLUSION

In conclusion, the rNP encoded by *ORF7* was successfully expressed from PRRSV in Thailand and significantly enabled to differentiate positive and negative serum. This indicated its high antigenicity which could be able to utilize for serological diagnosis of PRRSV infection in the country. Immunization with rNP could induce specific rNP antibody, indicating its high immunogenicity. This rabbit polyclonal antibody could be used in IHC diagnosis and investigation of tissue tropisms of the emerging PRRSV. This rNP could be used as a potential reagent for PRRSV diagnosis and test kit development. Further study should validate the iELISA with large number of serum samples to ultimately replace the expensive commercial test kits.

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AUTHOR CONTRIBUTIONS

N. Suwankitwat, J. Ratthanophart designed and performed the experiments and wrote the manuscript; and W. Starrat, P. Sagumpung performed the histopathology.

CONFLICT OF INTEREST

The authors declare no competing financial interests.

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