



Prokaryotic Expression of GP5 Protein of *Betaarterivirus suid 2* and its Applications as Coating Antigen in Development of Indirect ELISA

Fatema Akter¹, Parimal Roychoudhury¹, Tapan Kumar Dutta¹, Sanjeev Kumar¹
Prasant Kumar Subudhi², Parthasarathi Behera², Jaganmohanarao Gali²

10.18805/IJAR.B-5591

ABSTRACT

Background: Porcine reproductive and respiratory syndrome (PRRS) is an emerging swine disease, causing huge economic losses. This virus has four immunogenic proteins out of which glycoprotein 5 (GP5) is the major immunogenic protein, followed by nucleocapsid protein (N), glycoprotein 3 (GP3) and matrix protein (M). The present study was designed to express the major immunogenic protein (GP5) of an isolate of *Betaarterivirus suid 2* in the prokaryotic system and its application as a coating antigen for ELISA.

Methods: GP5 protein was expressed in the prokaryotic system and the expression was detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. The GP5 protein was purified by nickel affinity chromatography. The purified GP5 protein was used as coating antigen in indirect enzyme-linked immunosorbent assay (ELISA) and was compared with the commercial kit PRRSV Antibodies ELISA Kit (Cat no.: E-AD-E006, Elabscience).

Result: The indirect ELISA results utilizing the present assay and the commercial kit were comparable with accurate detection of positive and negative serum samples.

Key words: *Betaarterivirus suid 2*, ELISA, Glycoprotein 5, SDS-PAGE, Western blotting.

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) is an economically very important disease causing great loss in pig husbandry worldwide. The causative agents are classified into two species *Betaarterivirus suid 1* and *2* showing only around 60% genetic homology (Nelsen *et al.*, 1999). The *Betaarterivirus suid 2* is highly prevalent in American and Asian countries with several novel strains including the highly pathogenic strain (HP-PRRSV) (Han *et al.*, 2006; Tong *et al.*, 2007). The virus genome is a single-stranded positive-sense RNA of around 15.1 - 15.5 kb with ten overlapping open reading frames (ORFs) (Choi *et al.*, 2014) and the ORF5 coded for the most immunogenic protein, GP5 (25 kDa) which has been targeted for the development of recombinant vaccines (Park *et al.*, 2016; Cui *et al.*, 2019). Recombinant GP5 protein has also been used for the development of genotype-specific diagnostics (Chen *et al.*, 2011). There is a report of development of peptide-based ELISA targeting GP5 protein of NADC30-like PRRSV from China (Sun *et al.*, 2025). The recombinant Pseudorabies virus expressing GP3 and GP5 proteins of PRRSV showed a good response as candidate vaccine (Guo *et al.*, 2025). This virus contains four immunogenic proteins out of which glycoprotein 5 (GP5) is the major immunogenic protein, followed by nucleocapsid protein (N), glycoprotein 3 (GP3) and matrix protein (M). The GP5 and N proteins are the two important targets for serological diagnosis of the PRRS. The GP5 protein has six antigenic determinants which neutralize the virus infection *in vitro* (Chen *et al.*, 2011). In India, the virus was first detected in

¹Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (Imphal), Selesih, Aizawl-796 014, Mizoram, India.

²Department of Veterinary Biochemistry, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (Imphal), Selesih, Aizawl-796 014, Mizoram, India.

Corresponding Author: Parimal Roychoudhury, Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (Imphal), Selesih, Aizawl-796 014, Mizoram, India. Email: parimal74@rediffmail.com

How to cite this article: Akter, F., Roychoudhury, P., Dutta, T.K., Kumar, S., Subudhi, P.K., Behera, P. and Gali, J. (2025). Prokaryotic Expression of GP5 Protein of *Betaarterivirus suid 2* and its Applications as Coating Antigen in Development of Indirect ELISA. *Indian Journal of Animal Research*. 1-7. doi: 10.18805/IJAR.B-5591.

Submitted: 29-03-2025 **Accepted:** 20-08-2025 **Online:** 29-09-2025

2013 from Mizoram and since then it kept on spreading with several outbreaks in different parts of the state (Rajkhawa *et al.*, 2015). In 2013 - 2014, the virus was also detected in other Northeastern states including Assam, Manipur, Meghalaya and Nagaland (Linden, 2014). The virus was not restricted to the Northeastern parts of India, within a few years it has also spread to other states of India namely Punjab, Odisha, Karnataka, Madhya Pradesh, Maharashtra, Goa, Uttar Pradesh, Telangana (Kashyap *et al.*, 2019). Without proper restriction on animal movement and a low cost sensitive sero-surveillance, it is impossible to

control such a disaster like PRRS which may take the form of a national endemic in near future. There are no indigenous commercial kits available in India for large-scale sero-surveillance of PRRS and are dependent on the International market for any sero-diagnostic kits to be imported. The present study was designed to express the GP5 protein in a prokaryotic expression system and application of the recombinant protein as coating antigen for development of indirect ELISA.

MATERIALS AND METHODS

Place and time of the work

The work was carried out in the Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram. The work on the clone preparation and confirmation was carried out during 2020 (March to July) and the indirect ELISA part was done in 2024 (November to December).

GP5 gene amplification and cloning in expression vector

The virus isolate used for the full-length GP5 gene amplification in the present study was obtained from the repository of the Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Mizoram (GenBank accession number MN928985) (Aker *et al.*, 2021). Expression of GP5 protein was performed using Expresso Rhamnose SUMO Cloning and Expression System kit (Cat. No. 49013-1, Lucigen) and *E. coli* 10G competent cells provided with the kit was used for transformation and expression. Virus isolated in Porcine Alveolar Macrophage cells (PAM), maintained in the department (Aker *et al.*, 2021), was used for RNA extraction. RNA extraction was carried out from the virus isolated using the QIAamp Viral RNA extraction kit (Cat. No. 52904 QIAGEN) as per the manufacturer's protocol. The cDNA was synthesized using the Maxima H Minus First Strand kit (Cat. No. K1651, Thermo Scientific) as per the manufacturer's protocol. The full-length GP5 gene was amplified and cloned in a TA cloning vector and sequenced by outsourcing. The annotated sequence was submitted to GenBank for accession number (MN928985). Using the same cDNA full-length GP5 gene was amplified using polylinker primer pair (Table 1) for prokaryotic cloning and expression purpose. For this, the full-length GP5 gene was amplified by using *Pfu* DNA polymerase and GP5 specific primers with polylinker (vector adaptor sequence) to have 18 nucleotides homology of the vector sequence. A reaction volume of 100 µl was prepared by using 1 µl of 2 U/µl *Pfu* DNA polymerase enzyme (Cat. No. EP0572, Thermo Scientific) and GP5 was amplified at an annealing temperature of 56.1°C. The PCR product was run in 1.5% agarose gel electrophoresis and the UV unexposed product was purified from the gel by using GeneJET Gel Extraction Kit (Cat. No. K069, Thermo Scientific) as per the manufacturer's protocol. The purified product was

quantified by spectrophotometer (Thermo Scientific Multiskan GO, Thermo Fisher Scientific) and the purified GP5 was cloned into the pRham N-His SUMO Kan vector and transformed to *E. coli* 10G competent cells by heat-shock method which was plated on LB agar containing kanamycin (30 µg/ml) and incubated for 18 hours at 37°C. Positive clone detection was performed by orientation PCR at an annealing temperature of 55°C utilizing the primers provided with the kit, SUMO Forward Primer and pETite Reverse Primer (50 pmol/ µl).

GP5 protein expression and detection

One of the positive clones was used for induction of GP5 protein expression. For this, the transformed *E. coli* 10G bacteria were grown in three tubes each in 10 ml LB kanamycin broth. The tubes were incubated at 37°C at 120 rpm till the optical density value reached 0.6 and one tube was shifted at 4°C for uninduced control. D-glucose at a final concentration of 0.05% and L-rhamnose at a final concentration of 0.2% were added with the culture of the other two tubes for early autoinduction. Cells were incubated at 37°C at 120 rpm for 4 hours and 8 hours. After induction, 5 ml of culture media from each of the two tubes were separated and stored for purification. The rest of the 5 ml cultures were used for lysate preparation. For that induced and uninduced control cells were harvested by centrifugation at 10,000 × g for 10 min and pellets were washed twice with PBS and finally, the pellets were resuspended in 1X NRSB (non-reducing sample buffer). The samples were sonicated using an ultrasonicator (Ultrasonic Processor, UP100H, hielscher). The sonicated samples were incubated for 15 min at room temperature, then boiled for 5 min and then incubated at room temperature for 15 min followed by centrifugation at 13,000 × g for 15 min and the supernatants were separated to use for SDS-PAGE (Sodium dodecyl sulfate-polyacrylamide gel electrophoresis). SDS-PAGE analysis was carried out using 12% resolving gel and 4% stacking gel. Protein samples of both 4 hours and 8 hours induction, as well as uninduced control lysates, were run in the gel along with a standard protein marker (Cat. No. MBT092, Himedia). The electrophoresis was run for 3 hours at a constant 70 volts. After completion of SDS-PAGE, the gel was stained with Coomassie Brilliant Blue R-250 solution for 1 hour followed by destaining for overnight.

Purification of GP5 protein

Purification of the GP5 protein was performed using the His-Spin Protein Miniprep kit (Cat. No. P2001, The Epigenetics Company, Zymo Research) as per the manufacturer's protocol as the targeted protein contains a 6× histidine-tag. For this, the 5 ml cultures of each 4 hours and 8 hours induction were used for harvesting the cells pellet by centrifugation at 10,000 × g for 10 min. Then cells were washed and resuspended in 1 ml His-Binding Buffer. Cells were then sonicated using the ultrasonicator and centrifuged at 13,000 × g for 5 min. Supernatants were

used for purification of the 6× histidine tagged GP5 protein and were eluted with 150 µl elution buffer. Further purified proteins were quantified by Bradford dye assay (BDA) using bovine serum albumin (BSA) as standard protein. The purified proteins were run in the SDS-PAGE gel for checking the purity.

Western blot analysis

Western blotting was performed by using a semi-dry horizontal trans-blotter (AE 6675, ATTO) following the manufacturer's protocol. The 12% resolving gel and 4% stacking gel were prepared and purified 6× His tagged SUMO GP5 proteins both 4 hours and 8 hours induction were used in duplicate for SDS-PAGE, one half was used for staining and another half was used for trans blotting. After completion of SDS-PAGE, half of the gel was used for sandwich preparation using absorbent papers 3 numbers from the bottom, on top of that nitrocellulose membrane and then SDS-PAGE gel and on top 3 absorbent papers. All the membranes were equilibrated in the transfer buffer for around 5 min. The trans-blotter was run at a voltage of 24 volts for 3 hours. After completion of the transfer, the nitrocellulose membrane (NCM) was blocked by using a blocking buffer (0.5% bovine serum albumin, 5% skim milk powder, 0.2% Tween-20 in PBS) at 4°C overnight. After discarding the blocking buffer the next day, the NCM was washed thoroughly with PBS-T. The commercial PRRSV-GP5 polyclonal antibody raised in rabbit (Cat. No. bs-4504R, Bioss Antibodies, USA) was used as the primary antibody at 1:500 dilution and incubated at room temperature for 1 hour. After washing 3 times with PBS-T, NCM was treated with secondary antibody conjugate (Cat. No. G×4501PC2R, Peroxidase conjugate Goat-anti Rabbit IgG) at 1:500 dilution and incubated at room temperature for 30 min. After washing with PBS-T, DAB substrate with nickel (Ref. no. SK-4100, Vector Laboratories) was used as chromogen and incubated at room temperature in dark for 15 min. After incubation, the NCM was washed with distilled water and dried at room temperature.

Indirect ELISA

Application of the recombinant GP5 (rGP5) protein as coating antigen was explored for indirect ELISA. Ten positive and 10 negative pig serum samples which were tested by Porcine Reproductive and Respiratory Syndrome virus Antibodies ELISA Kit (Cat no.: E-AD-E006, Elabscience) were used for indirect ELISA. MaxiSorp Nunc ELISA plate (Cat. No.: 442404, Thermo Scientific) was coated with recombinant GP5 protein @ 200 ng/well and 500 ng/well diluted in coating buffer (0.05 M Carbonate bicarbonate buffer, pH 9.6) and incubated overnight at 4°C. After overnight coating, the plate was washed with wash buffer (PBS-T, PBS with 0.05% Tween-20) and then blocking was performed by blocking buffer (3% BSA in PBS) and incubated for 1 hour at 37°C. The positive and negative sera at 1:10 dilution were added and incubated for 1 hour at 37°C. Anti-Pig IgG (whole molecule)-Peroxidase antibody produced in rabbit (Cat. no.: A5670, Sigma Aldrich) at a

dilution of 1:2500 was used as secondary antibody conjugate and incubated for 45 min at 37°C. After each step, the plate was washed three times with PBS-T. As substrate, the TMB (3,3',5,5'-tetramethylbenzidine) (Cat. No.: T0440, Sigma) was used and incubated for 15 min in dark at room temperature and the reaction was stopped by 1M sulfuric acid. Absorbance was taken at 450 nm wavelength keeping substrate control as zero.

RESULTS AND DISCUSSION

GP5 gene amplification and cloning in expression vector

The full-length GP5 gene (603 bp) was amplified by vector adaptor primers and an expected band of ~621 bp was observed when analyzed in agarose gel electrophoresis (Fig 1). The gel purified GP5 PCR product was cloned in pRham N-His SUMO Kan expression vector and transformed in *E. coli* 10 G cells. The transformed clones were verified for positive transformation by orientation PCR, where an expected band of ~753 bp was observed as the primers amplify 150 bp of the vector along with the insert.

Expression and purification of GP5 protein

Expression of GP5 protein in *E. coli* 10 G cells was carried out when the OD value reached 0.6 using 0.05% of D-glucose and 0.2% of L-rhamnose solution for 4 hours and 8 hours incubation. Bacterial pellet at 0 hour, 4 hours and 8 hours from a volume of 5 ml culture was processed for detection of the expressed protein. A clear expression of the protein was observed in the SDS-PAGE gel at around 44 kDa molecular weight size in the 4 hours and 8 hours samples when compared with a standard protein marker, whereas 0 hour pellet protein expression could not be detected (Fig 2). This is the expected size of the target

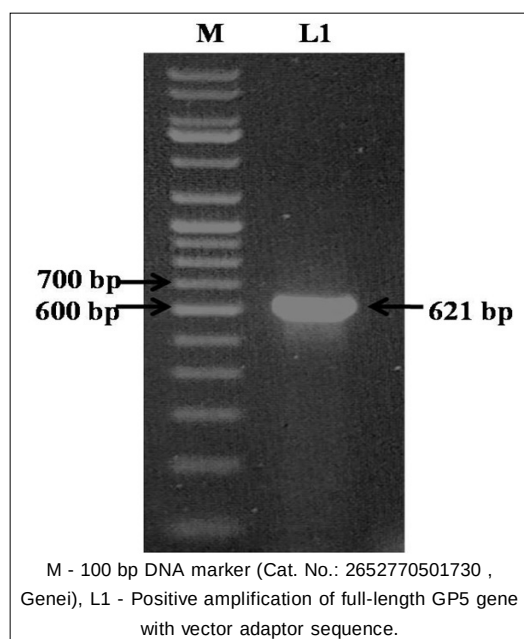


Fig 1: Agarose gel analysis of full-length GP5 gene amplification.

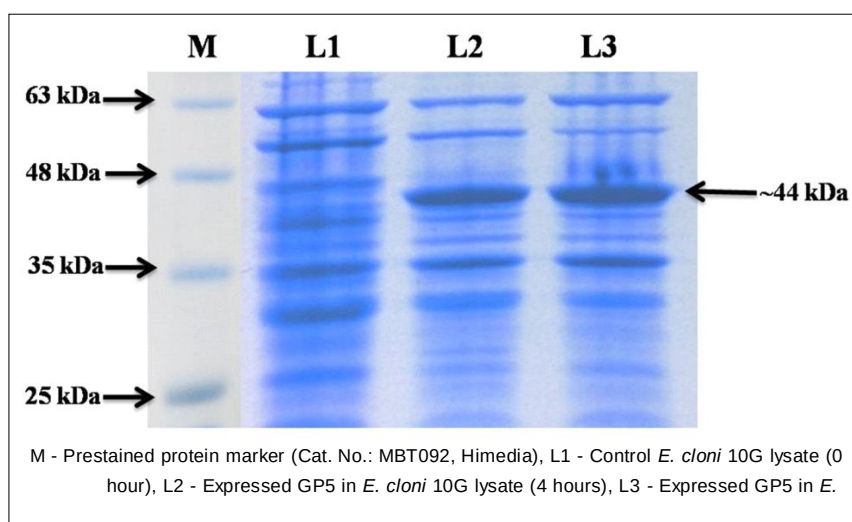


Fig 2: SDS-PAGE analysis of expressed GP5 protein.

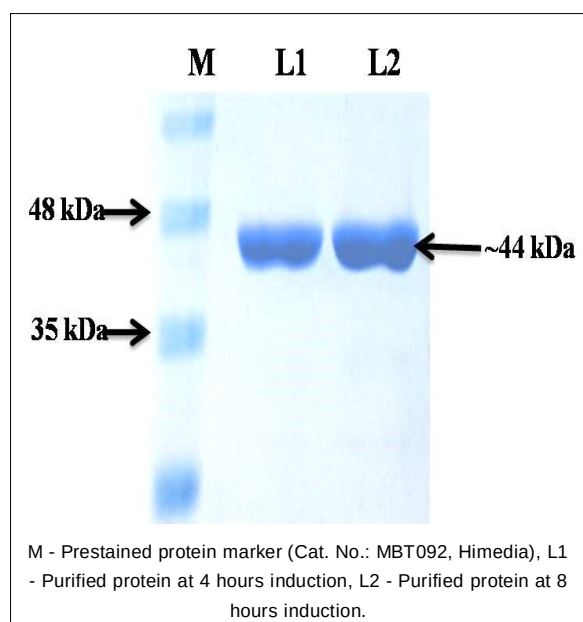


Fig 3: SDS-PAGE analysis of purified GP5 protein.

protein because the protein is fused with 6× histidine and SUMO tag. The protein was purified by using the nickel affinity chromatography and the purified fused protein was quantified by Bradford dye assay (BDA) and a yield of 80.6 µg/µl was detected at 4 hours induction, however, purified protein from 8 hours induction in cells was found to be 61.8 µg/µl. In the present study, the 4 hours induction was found to be optimum time for GP5 expression in prokaryotic system. The purified protein was again run in the SDS-PAGE and a single purified band of ~44 kDa was observed in the both 4 hours and 8 hours induction (Fig 3).

Western blotting

The western blotting experiment was performed to confirm the GP5 protein expression in the present system. The

pRham N-His SUMO Kan vector provides a 6× histidine tag for purification of the protein. The expressed protein was purified using the Ni-NTA affinity chromatography. Initially, around 500 µg of protein was run in SDS-PAGE in duplicate along with the standard protein marker, one for western blotting and the other for SDS-PAGE detection purposes. The gel separated protein was blotted to a nitrocellulose membrane (NCM). The NCM was developed with immunostaining of the protein using the commercial polyclonal GP5 antibody raised in rabbit. The 44 kDa GP5 protein was detected by western blotting (Fig 4).

Indirect ELISA

Indirect ELISA was performed using recombinant GP5 as coating antigen at the concentration of 200 ng/well and 500 ng/well. Average OD_{450} of 10 known negative serum samples, (tested through the commercial kit) was taken as cut off value in the present assay. Cut off value considered in the assay was 0.21 ± 0.049 (200ng/well coating) and 0.21 ± 0.052 . The average OD_{450} values of positive serum samples and negative serum samples were calculated and presented graphically (Fig 5). At 200 ng/well rGP5 protein-coated indirect ELISA, the average positive sample OD_{450} value is 0.587 and the average negative sample OD_{450} value is 0.195. Whereas at 500 ng/well coated indirect ELISA, the average positive sample OD_{450} value is 0.653 and the average negative sample OD_{450} value is 0.253. The same samples when analyzed using the commercial kit (Cat no.: E-AD-E006, Elabscience) the average positive sample OD_{450} is 0.643 and average negative sample OD_{450} is 0.105 and the difference between positive and negative values is 0.538. The cut-off value for positive sample was ≥ 0.38 and for negative sample < 0.2 for the kit and suspicious result was set for the OD_{450} of 0.2 - 0.38.

The expected size of GP5 protein is 25 kDa, using pRham N-His SUMO Kan vector the expressed protein is usually fused with SUMO tag increasing the expressed protein size (Namvar *et al.*, 2018; Tiwari *et al.*, 2020). In the

present study, we considered the clear expression of the protein at 44 kDa molecular weight as the fused GP5 protein. The expression of various proteins by using the pRham N-His SUMO Kan vector is reported by many authors. SUMO enhances the expression and solubility of the target protein resulting in an efficient expression and purification of the protein (Malakhov *et al.*, 2004). SUMO is an efficient expression system for the expression of relatively difficult proteins. Expression of toxic protein using pRham N-His SUMO Kan vector in *Escherichia coli* was reported earlier (Giacalone *et al.*, 2006). SUMO tag has also been used for enhanced expression of target protein in the eukaryotic system (Liu *et al.*, 2008). There is also a report on the expression of cytotoxic protein of *Luffa cylindrica* plant by using the SUMO tag vector for effective expression (Namvar

et al., 2018). There is a report of expression of twin-arginine translocation D (tatD) family deoxyribonuclease of *Clostridium chauvoei* using pRham N-His SUMO Kan vector (Tiwari *et al.*, 2020). There is report of construction of pET-SUMO vector for expression of *sry* gene of goat to increase the protein expression level (Wang *et al.*, 2019). There are many reports of GP5 expression in prokaryotic systems like pET30a vector to express 18 kDa of partial GP5 devoid of signal peptide and transmembrane sequences (Ren *et al.*, 2010); pGEX-6p-1 vector to express N-terminal GST tag 26 kDa of GP5 (Chen *et al.*, 2011); pGEM His-tag to express ~25 kDa GP5 (Liu *et al.*, 2013); pGEM-aroA-kan vector to express the GP5 of 27 kDa (Park *et al.*, 2016). There are also some reports of expression of fused GP5 protein in various prokaryotic expression systems like pCold I vector for expression of 67 kDa fused GP5 and FliB protein (Xiong *et al.*, 2015); pGEX4T1 vector to express fused GP5 and M protein of 42.6 kDa (Roques *et al.*, 2013). Detection of expressed protein by western blotting using a specific antibody is the usual way to authenticate the expression of a protein in a heterologous system (Park *et al.*, 2016; Akash *et al.*, 2025). Use of monoclonal antibody increases the specificity in comparison to polyclonal antibody (Bastos *et al.*, 2002; Jogi *et al.*, 2025). Using recombinant GP5 glycoprotein as coating antigen for indirect ELISA for PRRSV antibody detection has been published earlier (Chen *et al.*, 2011; Wang *et al.*, 2016). The recombinant GP5 protein for coating the ELISA plate was explored earlier. The authors have coated at various concentration of rGP5 including 0.8 µg/well, 0.4 µg/well, 0.2 µg/well, 0.1 µg/well and 0.05 µg/well and out of these 0.2 µg/well with 1:40 dilution of the serum samples was found to be optimum for viral antibody detection (Chen *et al.*, 2011). In another study, the optimum recombinant GP5 protein concentration using checkerboard titration was explored and it was found to be 2.5 µg/ml was the optimum concentration for a serum dilution of 1:800. They have

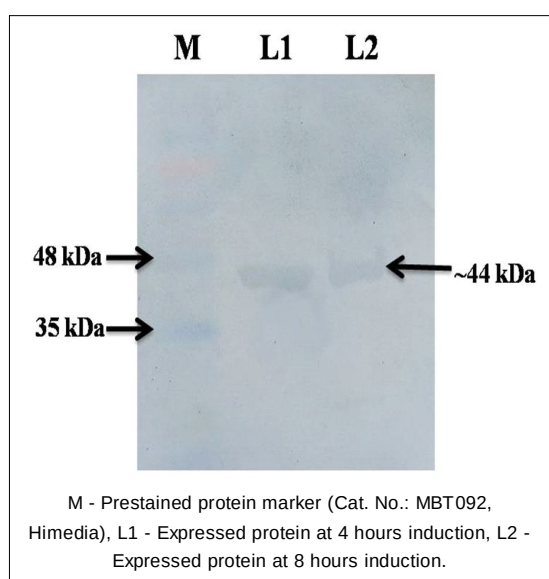


Fig 4: Western blotting of purified GP5 protein in nitrocellulose membrane.

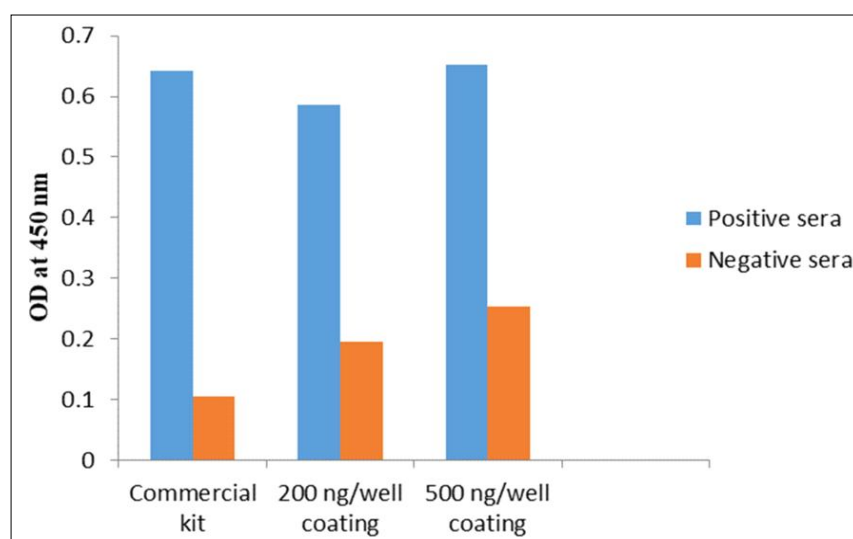


Fig 5: Graphical representation of average OD₄₅₀ of serum samples tested by indirect ELISA using rGP5 as coating antigen.

reported that there were no cross-reactions of GP5 ELISA with other swine pathogens including PCV2, CSFV, JEV, PRV and PPV (Wang *et al.*, 2016). In a recent study by Sun and co-workers developed a peptide-based ELISA targeting GP5 protein and found good co-relation with the commercial kit (IDEXX PRRSV X3 Ab ELISA) for detection of neutralizing antibody for NADC30-like PRRSV (Sun *et al.*, 2025). As the nucleocapsid (N) protein is also another major immunogenic protein, there are also some works claiming the use of recombinant nucleocapsid (N) protein as a coating antigen for the detection of PRRS antibodies (Kashyap *et al.*, 2020; Ma *et al.*, 2016), where they have optimized the coating concentration as 1.24 µg/ml and 0.2 µg/well respectively. There is also work on the use of fused nucleocapsid protein and GP5 glycoprotein as coating antigen for the detection of PRRS infection (Chen *et al.*, 2013). The authors have constructed a recombinant fused protein of nucleocapsid protein and C-terminal 78 amino acid of GP5 protein (rN5c). An optimum concentration of 100 ng/well of rN5c protein was used for coating in indirect ELISA with a serum dilution of 1:100.

CONCLUSION

In the present study, we established that the GP5 protein of *Betaarterivirus suid 2* can be expressed in the prokaryotic system and the expressed protein retains its antigenicity. Expressed protein can be used for coating ELISA plates for the detection of specific antibodies against *Betaarterivirus suid 2*. Recombinant protein antigen coated plates could detect the positive and negative serum samples within the acceptable range of indirect ELISA.

ACKNOWLEDGEMENT

Authors are thankful to the Dean, College of Veterinary Sciences and Animal Husbandry and Vice-Chancellor, Central Agricultural University for providing the necessary facility to complete the research studies. Help and support received from DBT-ADMAC project (DBT-NER/LIVS/11/2012) is duly acknowledged.

Funding

This research work was funded by the project ADMAC (DBT-NER/LIVS/11/2012).

Ethical approval

Not applicable.

Consent to participate

All the authors have given their consent for participation of the research work.

Consent for publication

All authors give their consent to publish this research paper in your esteemed journal.

Conflict of interest

Authors declare that they have no conflict of interest.

REFERENCES

- Akash, J.S., Vinde, P., Bedekar, M.K., Rajendran, K.V., Tripathi, G., Krishna, G. (2025). Cloning and expression of recombinant chondroitin AC lyase protein of *flavobacterium columnare* in a prokaryotic expression system. *Indian Journal of Animal Research*. **59**(5): 777-784. doi: 10.18805/IJAR.B-5538.
- Akter, F., Roychoudhury, P., Dutta, T.K., Subudhi, P.K., Kumar, S., Gali, J.M., Behera, P., Singh, Y.D. (2021). Isolation and molecular characterization of GP5 glycoprotein gene of *Betaarterivirus suid 2* from Mizoram, India. *Virus Disease*. **32**: 1-9. <https://doi.org/10.1007/s13337-021-00735-x>.
- Bastos, R.G., Dellagostin, O.A., Barletta, R.G., Doster, A.R., Nelson, E., Osorio, F.A. (2002). Construction and immunogenicity of recombinant Mycobacterium bovis BCG expressing GP5 and M protein of porcine reproductive respiratory syndrome virus. *Vaccine*. **21**: 21-29. [https://doi.org/10.1016/s0264-410x\(02\)00443-7](https://doi.org/10.1016/s0264-410x(02)00443-7).
- Chen, C., Fan, W., Jia, X., Li, J., Bi, Y., Liu, W. (2013). Development of a recombinant N-Gp5c fusion protein-based ELISA for detection of antibodies to porcine reproductive and respiratory syndrome virus. *Journal of Virological Methods*. **189**: 213-220.
- Chen, Y., Tian, H., He, J.H., Wu, J.Y., Shang, Y., Liu, X. (2011). Indirect ELISA with Recombinant GP5 for detecting antibodies to porcine reproductive and respiratory syndrome virus. *Virologica Sinica*. **26**: 61-66. <https://doi.org/10.1007/s12250-011-3154-9>.
- Choi, H.W., Nam, E., Lee, Y.J., Noh, Y.H., Lee, S.C., Yoon, I.J., Kim, H.S., Kang, S.Y., Choi, Y.K., Lee, C. (2014). Genomic analysis and pathogenic characteristics of Type 2 porcine reproductive and respiratory syndrome virus nsp2 deletion strains isolated in Korea. *Veterinary Microbiology*. **170**: 232-245. <http://doi.org/10.1016/j.vetmic.2014.02.027>.
- Cui, J., O'Connell, C.M., Costa, A., Pan, Y., Smyth, J.A., Verardi, P.H., Burgess, D.J., Kruininjen, H.J.V., Germandia, A.E. (2019). A PRRSV GP5-Mosaic vaccine: Protection of pigs from challenge and ex vivo detection of IFNγ responses against several genotypes 2 strains. *PLoS One*. **14**: e0208801-15. <https://doi.org/10.1371/journal.pone.0208801>.
- Giacalone, M.J., Gentile, A.M., Lovitt, B.T., Berkley, N.L., Gunderson, C.W., Surber, M.W. (2006). Toxic protein expression in *Escherichia coli* using a rhamnose-based tightly regulated and tunable promoter system. *Biotechniques*. **40**: 355-364. <https://doi.org/10.2144/000112112>.
- Guo, R., Li, H., Li, J., Qv, J., Ren, G., Zhang, X., Nasir, S., Zhang, J., Luo, C., Zeshan, B., Zhou, Y., Xie, H., Wang, X. (2025). Recombinant PRV expressing GP3 and GP5 of PRRSV provides effective protection against coinfection with PRV and PRRSV. *Transboundary and Emerging Diseases*. **2025**: 4612568. <https://doi.org/10.1155/tbed/4612568>.
- Han, J., Wang, K., Faaberg, K.S. (2006). Complete genome analysis of RFLP 184 isolates of porcine reproductive and respiratory syndrome virus. *Virus Research*. **122**: 175-182. <https://doi.org/10.1016/j.virusres.2006.06.003>.
- Jogi, J., Nayak, A., Rai, A., Shakya, R.P., Gangil, R., Bordoloi, S., Lade, A., Himani, K. (2025). Cloning and expression studies of the major outer membrane protein (*OmpH*) gene of *Pasteurella multocida* P52 in Prokaryotic Vector. *Indian Journal of Animal Research*. **59**(6): 979-984. doi: 10.18805/IJAR.B-4887.

- Kashyap, S.P., Hiremath, J., Subramanyam, V., Patil, S.S., Roy, P., Hemadri, D. (2019). PCR-based baseline survey indicates widespread prevalence of porcine reproductive and respiratory syndrome (PRRS) in India. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases*. **40**: 98-101. <https://dx.doi.org/10.14202%2Fvetworld.2020.2587-2595>.
- Kashyap, S.P., Hiremath, J., Vinutha, S., Patil, S.S., Suresh, K.P., Roy, P., Hemadri, D. (2020). Development of recombinant nucleocapsid protein-based indirect enzyme linked immunosorbent assay for sero-survey of porcine reproductive and respiratory syndrome. *Veterinary World*. **13(12)**: 2587-2595.
- Linden, J. (2014). India reports 23 outbreaks of PRRS. Published in The Pig Site. <https://www.thepigsite.com/news/2014/10/india-reports-23-outbreaks-of-prrs-1>. Accessed 16 March 2022.
- Liu, C., Liu, C.J., Yuan, X.G., Zhang, C. (2013). Purification and characterization of recombinant envelope protein GP5 of porcine reproductive and respiratory syndrome virus from *E. coli*. *Journal of Chromatography A*. **1304**: 133-137. <https://doi.org/10.1016/j.chroma.2013.06.079>.
- Liu, L., Spurrier, J., Butt, T.R., Strickler, J.E. (2008). Enhanced protein expression in the Baculovirus/ insect cell system using engineered SUMO fusions. *Protein Expression and Purification*. **62**: 21-28. <https://doi.org/10.1016/j.pep.2008.07.010>.
- Ma, X., Qin, Y., Ding, Y., Liu, Y., Pejsak, Z., Szczotka-Bochniarz, A., Ou, Y., Stipkovits, L., Szathmary, S., Ma, B., Jia, H., Wang, J., Zhang, Y., Zhang, J. (2016). Development of an indirect elisa to detect antibodies against porcine reproductive and respiratory syndrome virus nucleocapsid protein in gansu China. *Journal of Virology and Antiviral Research*. **5**: 2. <https://doi.org/10.4172/2324-8955.1000154>.
- Malakhov, M.P., Mattern, M.R., Malakhova, O.A., Drinker, M., Weeks, S.D., Butt, T.R. (2004). SUMO fusions and SUMO-specific protease for efficient expression and purification of proteins. *Journal of Structural and Functional Genomics*. **5**: 75-86. <https://doi.org/10.1023/B:JSFG.0000029237.70316.52>.
- Namvar, S., Barkhordari, F., Raigani, M., Jahandar, H., Nematollahi, L., Davami, F. (2018). Cloning and soluble expression of mature α -luffin from *Luffa cylindrica* in *E. coli* using SUMO fusion protein. *Turkish Journal of Biology*. **42**: 23-32. <https://dx.doi.org/10.3906%2Fbiy-1708-12>.
- Nelsen, C.J., Murtaugh, M.P., Faaberg, K.S. (1999). Porcine reproductive and respiratory syndrome virus comparison: Divergent evolution on two continents. *Journal of Virology*. **73**: 270-280. <https://doi.org/10.1128/JVI.73.1.270-280.1999>.
- Park, S.I., Seo, J.Y., Kim, T.J. (2016). Heterologous expression of porcine reproductive and respiratory syndrome virus glycoprotein 5 in *Bordetella bronchiseptica* aroA mutant. *Journal of Veterinary Medical Science*. **78**: 1625-1629. <https://doi.org/10.1292/jvms.15-0687>.
- Rajkhowa, T.K., Mohanarao, G.J., Gogoi, A., Hahnar, L., Isaac, L. (2015). Porcine reproductive and respiratory syndrome virus (PRRSV) from the first outbreak of India shows close relationship with the highly pathogenic variant of China. *Veterinary Quarterly*. **4**: 186-193. <https://doi.org/10.1080/01652176.2015.1066043>.
- Ren, X., Wang, M., Yin, J., Ren, Y., Li, G. (2010). Heterologous expression of fused genes encoding the glycoprotein 5 from PRRSV: A way for producing functional protein in prokaryotic microorganism. *Journal of Biotechnology*. **147**: 130-135. <https://dx.doi.org/10.1016%2Fj.jbiotec.2010.03.013>.
- Roques, E., Girard, A., Louis, M.C.S., Massie, B., Gagnon, C.A., Lessard, M., Archambault, D. (2013). Immunogenic and protective properties of GP5 and M structural proteins of porcine reproductive and respiratory syndrome virus expressed from replicating but non-disseminating adenovectors. *Veterinary Research*. **44**: 17-29. <https://doi.org/10.1186/1297-9716-44-17>.
- Sun, S., Zhang, K., Zhang, J., Zhang, P., He, P., Deng, D., Jiang, S., Zheng, W., Chen, N., Bai, J., et al. (2025). A novel peptide-based enzyme-linked immunosorbent assay (ELISA) for detection of neutralizing antibodies against NADC30-like PRRSV GP5 protein. *International Journal of Molecular Sciences*. **26**: 2619. <https://doi.org/10.3390/ijms26062619>.
- Tiwari, A., Dangi, S.K., Thomas, P., Nagaleekar, V.K. (2020). Cloning and expression of twin-arginine translocation D family deoxyribonuclease of *clostridium chauvoei*. *Indian Journal of Animal Research*. **54(7)**: 864-868. doi: 10.18805/ijar.B-3855.
- Tong, G.Z., Zhou, Y.J., Hao, X.F., Tian, Z.J., An, T.Q., Qiu, H.J. (2007). Highly pathogenic porcine reproductive and respiratory syndrome, China. *Emerging Infectious Disease*. **13**: 1434-1435. <https://doi.org/10.3201/eid1309.070399>.
- Wang, X., E, G-X., Cheng, S-Z., Ni, W-W., Ma, Y-H., Chu, M-X., Huang, Y-F. (2019). Construction and expression of a prokaryotic expression vector for the goat sry gene. *Indian Journal of Animal Research*. **53(6)**: 731-735. doi: 10.18805/ijar.B-963.
- Wang, Y., Guo, J., Qiao, S., Li, Q., Yang, J., Jin, Q., Zhang, G. (2016). GP5 protein-based ELISA for the detection of PRRSV antibodies. *Polish Journal of Veterinary Sciences*. **19**: 495-501. <https://doi.org/10.1515/pjvs-2016-0062>.
- Xiong, D., Song, L., Zhai, X., Geng, S., Pan, Z., Jiao, X. (2015). A porcine reproductive and respiratory syndrome virus (PRRSV) vaccine candidate based on the fusion protein of PRRSV glycoprotein 5 and the Toll-like Receptor-5 agonist *Salmonella Typhimurium* FljB. *BMC Veterinary Research*. **11**: 121-129. <https://doi.org/10.1186/s12917-015-0439-0>.