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Production of recombinant goose parvovirus origin VP2 protein based on baculovirus expression system

Remziye Özbek[1]*, Mustafa Ozan Atasoy[1], Turhan Turan[1], Hakan Işidan[1], Hasan Abayli[2], Kezban Şahna[2]

[1] Department of Virology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Türkiye; [2] Department of Virology, Faculty of Veterinary Medicine, Firat University, Elazığ, Türkiye.

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Abstract

Goose parvovirus causes major economic losses in the waterfowl industry due to the high mortality. Therefore, it is essential to establish protection/control objectives in the fight against the disease. The genome of goose parvovirus consists of three structural proteins, including VP1, VP2, and VP3. The VP2 is a candidate antigen in developing vaccines and diagnostic kits. This study aimed to produce the VP2 protein from a local goose parvovirus strain that causes serious infections in geese in Türkiye using the baculovirus expression vector system. To achieve this, the *VP2* gene was first amplified by polymerase chain reaction, followed by purification and insertion into the pENTR™/TEV/D-TOPO™ entry vector. Then, the target gene in the pENTR™/TEV/D-TOPO™ vector was transferred to linear N-Term BaculoDirect™ DNA through LR recombination (site-specific recombination between attL and attR sites). The construct was transfected into *Spodoptera frugiperda* cells. To verify the production of baculoviral virions, a band of approximately 600 bp in length was obtained as a result of polymerase chain reaction amplification using external primer sets for both the *VP2* gene and expression vector. The obtained band was purified and sequenced for confirmation. In addition, to confirm the production of the recombinant protein, western blot analysis was conducted utilizing the V5 epitope located at the N-terminus of the expressed protein, resulting in the detection of a ~65.00 kDa band corresponding to the *VP2* gene. To detect protein expression in *S. frugiperda* cells infected with the recombinant baculovirus, immunofluorescence analysis was performed using the same epitope.

Introduction

Goose parvovirus (GPV) infection, first identified as Deutz disease in the 1960s, is an acute gastrointestinal disease that causes highly contagious and exudative inflammatory bowel disease, characterized by acute or subacute sepsis, especially in goslings aged 4 - 20 days.[1] This disease is characterized by anorexia, lethargy, and mortality in 30-day-old goslings, causing a serious threat to the global goose industry.[2] The GPV is a non-enveloped single-stranded DNA virus that belongs to *Dependovirus* genus in *Parvoviridae* family.[2,3] The virus has a diameter of 20.00 - 22.00 nm and an icosahedral structure composed of 32 capsomers. The entire genome consists of approximately 5.10 kb. The genome contains two main open reading frames (ORFs), which are divided into a left ORF and a right ORF. The left ORF encodes non-structural proteins (one and two) being required to regulate viral genome replication and capsid gene expression. The right ORF encodes three capsid proteins (VP1, VP2, and VP3) sharing a common region at the C-terminus of the genome.[1,4] The VP2 has an anti-receptor that can interact with the receptor on the cell surface and is the main GPV immunological functional domain, capable of triggering neutralizing antibodies in GPV-infected geese.[5]

The baculovirus expression vector system (BEVS) platform utilizes the ability of baculoviruses to infect insect cells.[6] The fundamental characteristic of this platform is to replace the non-essential gene in the baculovirus with the foreign gene of interest, use baculovirus as a foreign gene carrier, and use insect cells as the host for gene amplification and target protein expression.[7] *Autographa californica multicapsid nucleopolyhedrovirus* and *Bombyx mori nucleopolyhedrovirus* are the most frequently used baculovirus vectors, and both have been widely used for expression of eukaryotic recombinant proteins.[8] The first

***Correspondence:**

Remziye Özbek. PhD
Department of Virology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Türkiye
E-mail: remziyeozbek@cumhuriyet.edu.tr

report of foreign protein expression in insect cells in 1983 stimulated significant interest in the use of BEVS for recombinant protein production, mainly due to the high expression levels of foreign proteins and proper post-translational modifications in insect cells.[9] The BEVS has many advantages over other expression systems, such as the ability to express large proteins and simultaneous expression of multiple genes. Furthermore, proteins expressed using the BEVS are correctly folded and biologically active. The cost of protein production through the BEVS is significantly cheaper compared to the mammalian cell system.[10,11] The existence of GPV in Türkiye, the large-scale outbreaks it caused, and the significant losses it caused have been demonstrated by studies.[12-14] Therefore, the application of effective vaccines and treatment protocols is essential in the fight against the disease. There is currently an absence of vaccines developed for infections in geese in Türkiye. A vaccine against GPV (Palmivax, Boehringer Ingelheim, France) has been imported from abroad. In this context, the aim of this study was to produce a *VP2*-gene-based recombinant subunit protein for using in domestic vaccine development through BEVS.

Materials and Methods

Extraction of goose parvovirus (GPV) DNA. For the study, the GPV isolate (*Dependoparvovirus anseriform 1* isolate Konya/19; GenBank Accession No. MW386078.1) isolated in a previous study conducted by Isidan *et al*. was used.[12] Viral DNA was extracted from virus supernatant using the QIAwave DNA Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer's instruction. The amount of DNA extract was measured using the Nano-Drop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and it was stored at – 80.00 ˚C until use.

Amplification, gel purification, and cloning of the *GPV-VP2* gene into the pENTR™/TEV/D-TOPO™ plasmid. The sequence encoding the *VP2* gene of GPV was amplified by polymerase chain reaction (PCR) using GPV DNA obtained as a template. Specific forward (5′CACCATG GCACCCGAAAAAAAAATACAG-3′) and reverse primers (5'TTACAGATTTTGAGTTAGATATCTGGTTC-3') used in PCR amplification were designed using Geneious Prime Software (version 2024.x; Biomatters Ltd., Auckland, New Zealand) conducted by Kearse *et al*.[15] The PCR amplification was performed using Q5 High-Fidelity DNA Polymerase (NEB, Ipswich, UK) according to the kit's standard procedure. The VP2 amplicon with the correct size was identified and excised from the gel using a sterile scalpel on the transilluminator. Then, the amplicon was purified from the gel according to the instructions of the GeneJET Gel Extraction Kit (Thermo Fisher Scientific).

Cloning of *VP2* gene into N-terminal BaculoDirect expression vector. The recombinant pENTR™/TEV/D-TOPO™ plasmid was cloned into the BaculoDirect™ Linear DNA (shuttle vector and expression vector) construct containing the attR motif and selection markers using LR recombination reaction, through the attL motif it contains. At this step, the LR Clonase II for BaculoDirect™ Kits (Invitrogen, Waltham, USA) was used to ligate VP2 amplicon. The recombinant pENTR™ /TEV/D-TOPO™ plasmid containing the *VP2* gene was propagated using *Escherichia coli* DH5α cells (MAX Efficiency; Thermo Fisher Scientific). The plasmid was purified using the QIAprep Spin Mini Prep Kit (Qiagen). To confirm the transfer of the *GPV-VP2* gene into the baculoviral vector, conventional PCR protocol was applied using external primer sets specific to the expression vector and *VP2* gene (polyhedrin F: 5′-AAATGATAACCATCTCGC-3′ and an internal sequencing primer GPV R: 5'- AGGCATTACCCACTCCATCG-3'). The PCR amplification was performed using Quick-Load® Taq 2.00 X Master Mix (NEB). Briefly, 2.00 µL was taken from the LR reaction and diluted 200-fold with nuclease-free water. Subsequently, 2.00 µL of this dilution was used as a template DNA in the PCR amplification. The PCR amplification was carried out with the following thermal cycling conditions: An initial denaturation at 95.00 ˚C for 2 min, followed by 40 cycles of denaturation at 94.00 ˚C for 20 sec, annealing at 50.00 ˚C for 30 sec, and extension at 68.00 ˚C for one min. Finally, a 10-min extension step was performed at 72.00 ˚C. After the reaction, positive samples were detected by 1.00% agarose gel electrophoresis using an imaging system (Quantum ST4 Vilber Lourmat, Marne-la-Vallée, France).

Transfection of *Spodoptera frugiperda* (Sf9) cells and production of recombinant baculovirus. The Sf9 insect cell line used in this study was propagated at 27.00 ˚C in 25.00 cm^2 plastic flasks with standard cap using SF900 II Serum-Free Medium (SFM; Thermo Fisher Scientific, Waltham, USA) containing 10,000 U mL^{-1} penicillin, 10.00 mg mL^{-1} streptomycin, and 25.00 µg mL^{-1} amphotericin B (Thermo Fisher Scientific). Upon reaching 90.00 - 95.00% confluency, Sf9 cells cultured in T25 flasks were distributed into 6-well plates at a density of 2.00 × 10^6 cells *per* mL in each well, in the logarithmic growth phase. The plates were incubated at 27.00 ˚C for approximately an hr to allow for cell adherence. The baculoviral vector containing the gene of interest was transfected into Sf9 cells using a liposome-based method (Cellfectin® II Reagent; Invitrogen) according to the kit protocol to generate recombinant baculovirus virions. In addition, the cells were treated with the anti-viral agent ganciclovir at a concentration of 100 µM, following the BaculoDirect™ N-Term Transfection Kit (Invitrogen) protocol, to remove non-recombinant viruses. The cells were incubated at 27.00 ˚C until evidence of viral infection was observed. Approximately 72 hr after infection, following observing the cytopathic effect in the cells, the cells were scraped with a cell scraper and transferred into

the 15.00 mL sterile Falcon tubes to prepare the P1 viral stock. Following centrifugation of the cells at 4.00 ˚C and 3,000 - 5,000 rpm for 5 min, the supernatants were transferred to 2.00 mL Eppendorf tubes. The P1 viral stocks were then stored at 4.00 ˚C protecting from light for subsequent re-passage.

Confirmation of recombinant baculovirus production using polymerase chain reaction (PCR) and sequence analysis. The PCR analysis was performed to confirm whether the *GPV-VP2* gene was intact in the baculoviral vector. First, DNA was isolated from 200 µL of supernatant from infected cells. For this purpose, the GeneAll® Exgene™ Cell SV Mini Kit (GeneAll Biotechnology, Seoul, South Korea) was utilized. Subsequently, conventional PCR amplification was performed using the same primers and protocol applied for the amplification of the *VP2* gene cassette. Amplified PCR products were sequenced using the commercial ABI 310 Genetic Analysis System (Refgen Biotechnology, Ankara, Türkiye).

Indirect immunofluorescence assay. Immunofluorescence assay was carried out, following the method of Wheatley and Wang,[16] to demonstrate protein expression in Sf9 cells infected with recombinant baculovirus containing the *GPV-VP2* gene. Protein expression was confirmed using the V5 epitope located at the N-terminus of the VP2 protein. For this purpose, the anti-V5 primary antibody (rabbit-derived anti-FLAG; Sigma-Aldrich, St. Louis, USA) and appropriately conjugated secondary antibody (Alexa Fluor™ 488 goat anti-rabbit immunoglobulin G) were employed.

Western blotting. The expected protein weight of 65.00 kDa was confirmed by western blot analysis using the Protein Molecular Weight Online Tool (www.bioinformatics.org). Infected Sf9 cell pellet stored at –80.00 ˚C, infected cell supernatant, and Sf9 cell pellet samples used for negative control were treated with radioimmunoprecipitation assay buffer (RIPA lysis and extraction buffer; Thermo Fisher Scientific) containing protease inhibitor (Pierce™ Protease Inhibitor Mini Tablets, EDTA-free, Thermo Fisher Scientific). Western blot analysis was then performed using the Bio-Rad Blotting System (Hercules, USA). The V5 epitope, located at the N-terminus of the protein, was utilized in this analysis. For this purpose, an anti-V5 tag monoclonal antibody (Ms mAb to V5 tag; Abcam, Cambridge, UK) and the corresponding horseradish peroxidase-conjugated secondary antibody (goat pAb to Ms immunoglobulin G; Abcam) were employed. Alpha-tubulin was also examined simultaneously as a western blotting control. The Ms mAb to alpha-tubulin (Abcam), and goat pAb to Ms immunoglobulin G were used.

Purification of recombinant VP2 protein. The recombinant VP2 protein was purified using the 6X His tag attached to protein. For this purpose, the protein was purified using the commercial Ni-NTA Spin Kit (Invitrogen) according to the instructions. NanoDrop spectrophotometer (Denovix, Wilmington, USA) was used to measure purified protein.

Results

Amplification of the *VP2* gene and cloning into the pENTR™/TEV/D-TOPO™ plasmid. The PCR amplification was performed using specific primers for the *VP2* gene with the *D. anseriform 1* isolate Konya/19, resulting in the amplification of the full-length *VP2* gene at the expected size of 1,761 bp. After the amplicon was cloned into the pENTR™/TEV/D-TOPO™ plasmid, it was run again in agarose gel electrophoresis and obtained a 4,371 bp band size (Figs. 1A and 1B).

Confirmation of LR recombination by PCR. To confirm whether the *VP2* gene was successfully transferred to BaculoDirect™ Linear DNA, PCR amplification was performed using external primer sets targeting both the *VP2* gene (GPV reverse primer) and expression vector (polyhedrin forward primer). This yielded a band of approximately 600 bp (Fig. 1C).

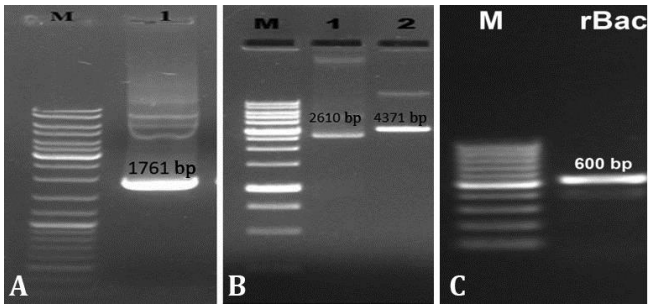

Fig. 1. A) The *GPV-VP2* gene amplification. M: 100 bp DNA ladder (GeneRuler); 1: 1761 bp polymerase chain reaction amplicon using GPV-VP2 primers. **B)** Recombinant pENTR™/TEV/D-TOPO™ plasmid. M: GeneON BioScience 10.00 kb DNA ladder; 1: Non-recombined pENTR™/TEV/D-TOPO™ plasmid; 2: Recombinant pENTR™/TEV/D-TOPO™ plasmid. **C)** Polymerase chain reaction result/gel electrophoresis for confirmation of recombinant baculovirus. M: 100 bp DNA ladder (GeneRuler); rBac: Recombinant baculovirus.

Production of recombinant baculovirus in Sf9 cells. The Sf9 cells transfected with recombinant baculovirus were followed up daily under a microscope for cytopathic effect. Cell death was observed after an average of 72 hr. In other infected cells, the nuclei filled the cell cytoplasm, resulting in a notable increase in cell diameter of 25.00 to 50.00% (Fig. 2).

Confirmation of recombinant baculovirus production in Sf9 by immunofluorescence assay (IFA). According to the analysis results using anti-V5 primary antibody and corresponding labeled secondary antibody, green fluorescence was observed in infected cells under a fluorescence microscope (Fig. 3).

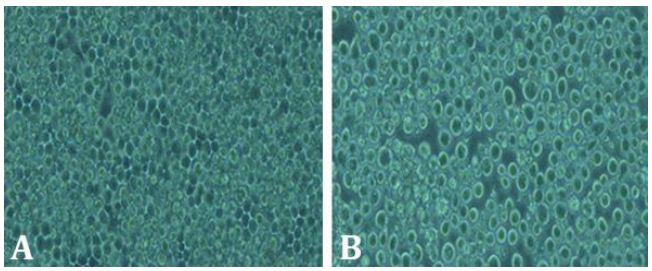

Fig. 2. Cytopathic effect of *Spodoptera frugiperda* transfected with recombinant baculovirus. **A)** Non-transfected cell (control) and **B)** Transfected cell, (20× for both sections).

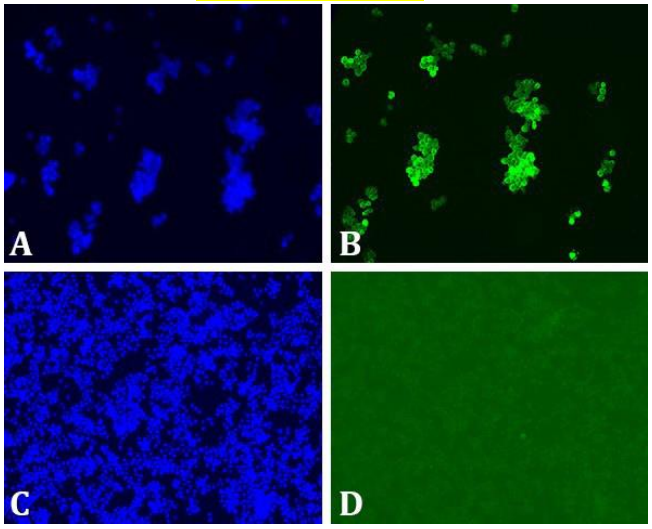

Fig. 3. Confirmation of recombinant baculovirus by immunofluorescence assay. **A** and **B)** Infected *Spodoptera frugiperda* cells; **C** and **D)** Uninfected *S. frugiperda* cells, (20× for all sections).

Confirmation of *VP2* gene expression. According to the results of western blot analysis using V5 epitope located at the N-terminus of the produced protein, bands of expected sizes were obtained for the *VP2* gene (~65.00 kDa) and simultaneously studied alpha-tubulin control (50.00 kDa; Fig. 4).

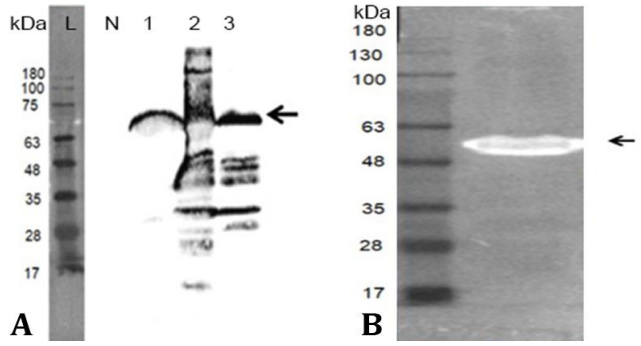

Fig. 4. A) Confirmation of *VP2* gene expression by western blotting. L: Protein pre-stained ladder (Abcam, Cambridge, UK); N: Negative control; 1: Supernatant of infected cell (P5); 2: Infected cell (P1); 3: Infected cell (P5). **B)** Confirmation of alpha-tubulin expression in *Spodoptera frugiperda* cells by western blot.

Sequence modelling of recombinant baculovirus. Sequence modelling of recombinant baculovirus was performed using Geneious Prime Software conducted by Kearse *et al*.[15] For this purpose, the protein sequence of the *VP2* gene used in the study (*D. anseriform 1* isolate Konya/19; GenBank Accession Code: QUX80129.1) obtained from a previous study was transferred to the baculoviral shuttle vector backbone (Fig. 5).

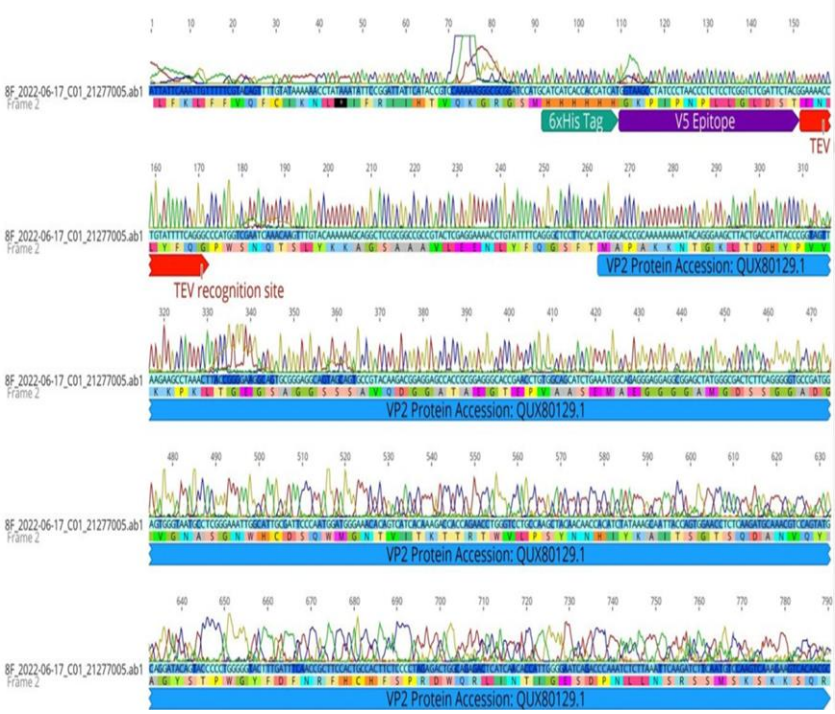

Fig. 5. Sequence modeling of recombinant baculovirus (rBac-VP2).

Discussion

Goose parvovirus has caused significant economic losses in the waterfowl industry, spreading across Europe, Asia, and America since 1956.[17] Many studies have been published regarding the selection of GPV capsid proteins as antigen targets that can trigger both cellular and humoral immune responses. In the study conducted by Ju *et al*.,[18] it was found that recombinant proteins elicited an immunoreactive response with specific antibodies, and the neutralizing activities of the antibodies against VP2 and VP3 were higher than the response elicited against VP1. Another study conducted by Tu *et al*.,[5] demonstrated that the cellular immune reactivity of VP2 and VP3 was stronger than that of VP1. Lee *et al*.,[19] formulated recombinant parvovirus VP2 (rVP2) with different adjuvants and investigated the immune responses following vaccination in ducklings. The results demonstrated that the adaptive immune response induced by the rVP2 protein was capable of protecting the ducklings from GPV infection. Similarly, studies by Shang *et al.,*[20] and Zhang *et al.,*[21] reported that virus-like particles (VLPs) consisting of N-GPV VP2 protein produced through BEVS protected ducks against N-GPV infection. Considering these literatures, the recombinant VP2 protein produced from a local strain in Türkiye in this study can be regarded as a potential antigen candidate capable of strongly inducing an immune response in geese. However, to determine whether the produced protein has potential to be used for vaccination, animal experiments must also be conducted.

Recombinant expression systems have become widely used in developing many drugs, therapies, and vaccines. Recent advancements in recombinant genetic engineering techniques have resulted in significant progress in the development of new vaccines in modern medicine.[22] There are studies reporting the expression of the *GPV-VP2* gene using prokaryotic systems. In the study by Fan *et al*.,[23] the structural *VP2* gene was expressed in *E. coli*. In another study by Liu *et al*.,[24] the *VP2* gene was expressed using *Lactobacillus plantarum* NC8 vector, producing a recombinant protein with an approximate molecular weight of 70.00 kDa. Producing the full-length *VP2* gene recombinant protein of GPV, which carries an immuno-dominant region, is only possible through the use of certain systems, such as eukaryotic expression systems.[25] Therefore, an insect cell-based eukaryotic system with many important advantages for the expression of the *VP2* gene has been preferred. The BEVS has become one of the most commonly used protein expression methodologies for various biotechnological applications. Previous studies on GPV using such a system have shown that when the genes are expressed, parvovirus capsid proteins self-assemble to form VLPs, and in most cases, the resulting recombinant particles are antigenically and immuno-logically similar to the native parvovirus virions.[26,27]

It has been reported that the yield of recombinant proteins produced in insect cells is almost 30.00% higher than that in *E. coli*, yeast, or mammalian cells.[28] The BEVS platform is a versatile technology that is useful for the production of many products. It possesses the ability to produce recombinant proteins at high yields and carry out specific post-translational modifications, thereby maintaining, to a certain extent, the biological activity of the original proteins. The BEVS platform has become an established production platform for the production of viral vaccines and gene therapy vectors. However, other platforms may be more suitable for the production of certain proteins. For example, small proteins that do not require post-translational modification can be produced in *E. coli* at low cost, quickly, and efficiently. Yeasts, such as *Saccharomyces cerevisiae*, can also produce proteins at low cost, in high yields, and with some post-translational modifications.[6,9]

Baculoviruses have become a popular system for the production of recombinant proteins in eukaryotic cells.[25] This platform has been widely used for the formation of VLPs of various parvoviral structural proteins, including human parvovirus B19,[29] porcine parvovirus,[30,31] human bocavirus,[32] canine parvovirus,[33-35] feline parvovirus,[36] and bovine parvovirus.[37] Previous studies on GPV[18,20,27,38] have similarly utilized this platform, particularly for the development of VLP-based vaccines.

In this study, the gene encoding the capsid protein VP2 of *D. anseriform 1* isolate Konya/19 was cloned into a baculovirus expression system and successfully expressed in insect cells. The recombinant protein, with an average molecular weight of approximately 65.00 kDa, was validated by western blot analysis. Additionally, it was found that the protein reacted with an immuno-fluorescently labeled specific antibody and localized in the nuclei of Sf9 cells.

As a result, in this study, the expression of VP2 protein in insect cell lines was successfully carried out using the BEVS, a highly advanced and specific platform. These results indicate that further studies are required to determine whether the VP2 protein could be a reliable, useful, and effective vaccine for preventing GPV.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Huo X, Chen Y, Zhu J, et al. Evolution, genetic recombination, and phylogeography of goose parvovirus. Comp Immunol Microbiol Infect Dis 2023; 102: 102079. doi: 10.1016/j.cimid.2023.102079.
2. Wang J, Duan J, Meng X, et al. Cloning of the genome of a goose parvovirus vaccine strain SYG61v and rescue of infectious virions from recombinant plasmid in embryonated goose eggs. J Virol Methods 2014; 200: 41-46.
3. Zadori Z, Erdei J, Nagy J, et al. Characteristics of the genome of goose parvovirus. Avian Pathol 1994; 23(2): 359-364.
4. Tarasiuk K, Holec-Gąsior L, Ferra B, et al. The development of an indirect ELISA for the detection of goose parvovirus antibodies using specific VP3 subunits as the coating antigen. BMC Vet Res 2019; 15(1): 274. doi: 10.1186/s12917-019-2027-1.
5. Tu M, Liu P, Liu F, et al. Construction of expression vectors of capsid proteins from goose parvovirus and investigation of the immunogenicity. Acta Virol 2018; 62(4): 415-423.
6. Felberbaum RS. The baculovirus expression vector system: a commercial manufacturing platform for viral vaccines and gene therapy vectors. Biotechnol J 2015; 10(5): 702-714.
7. Hong Q, Liu J, Wei Y, et al. Application of baculovirus expression vector system (BEVS) in vaccine development. Vaccines (Basel) 2023; 11(7): 1218. doi: 10.3390/vaccines11071218.
8. Tsai CH, Chuang YC, Lu YH, et al. Carbohydrate metabolism is a determinant for the host specificity of baculovirus infections. iScience 2021; 25(1): 103648. doi: 10.1016/j.isci.2021.103648.
9. Liu F, Wu X, Li L, et al. Use of baculovirus expression system for generation of virus-like particles: successes and challenges, Protein Expr Purif 2013; 90 (2): 104-116.
10. Fath-Goodin A, Kroemer J, Martin S, et al. Polydnavirus genes that enhance the baculovirus expression vector system. Adv Virus Res 2006; 68: 75-90.
11. Adeniyi AA, Lua LH. Protein expression in the baculovirus-insect cell expression system. In: Gerrard J, Domigan L (Eds). Protein nanotechnology: methods in molecular biology. 1st ed. New York, USA: Humana Press 2020; 17-37.
12. Isidan H, Turan T, Atasoy MO, et al. Molecular analysis of goose parvovirus field strains from a Derzsy's disease outbreak reveals local European-associated variants. Arch Virol 2021; 166(7): 1931-1942.
13. Kardoğan Ö, Müştak HK, Müştak İB. The first detection and characterization of goose parvovirus (GPV) in Turkey. Trop Anim Health Prod 2020; 53(1): 36. doi: 10.1007/s11250-020-02463-8.
14. Bulut O, Dik I, Aslım HP, et al. Molecular characterization of goose parvovirus in geese of Turkey. Pesq Vet Bras 2021; 41: 3. doi: 10.1590/1678-5150-pvb-6903.
15. Kearse M, Moir R, Wilson A, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 2012; 28(12): 1647-1649.
16. Wheatley SP, Wang YL. Indirect immunofluorescence microscopy in cultured cells. Methods Cell Biol 1998; 57: 313-332.
17. Yan YQ, Jin LB, Wang Y, et al. Goose parvovirus and the protein NS1 induce apoptosis through the AIF-mitochondrial pathway in goose embryo fibroblasts. Res Vet Sci 2021; 137: 68-76.
18. Ju H, Wei N, Wang Q, et al. Goose parvovirus structural proteins expressed by recombinant baculoviruses self-assemble into virus-like particles with strong immunogenicity in goose. Biochem Biophys Res Commun 2011; 409(1): 131-136.
19. Lee JW, Lin YM, Liu CH, et al. Passive and protective immunity in ducklings elicited by a parvovirus subunit vaccine with CpG adjuvant. Taiwan Vet J 2016; 42(2): 75-80.
20. Shang Y, Ma Y, Tang S, et al. Virus-like particles based on the novel goose parvovirus (NGPV) VP2 protein protect ducks against NGPV challenge. Vaccines (Basel) 2023; 11(12): 1768. doi: 10.3390/vaccines11121768.
21. Zhang Q, Sun Y, Sun Y, et al. Expression of VP2 protein of novel goose parvovirus in baculovirus and evaluation of its immune effect. Microb Pathog 2024; 195: 106751. doi: 10.1016/j.micpath.2024.106751.
22. Duan Z, Xu H, Ji X, et al. Recombinant Newcastle disease virus-vectored vaccines against human and animal infectious diseases. Future Microbiol 2015; 10(8): 1307-1323.
23. Fan JH, Zuo YZ, Yang Z, et al. The development of an indirect ELISA for the detection of antibodies to goose parvovirus in blood serum. Lett Appl Microbiol 2013; 57(1): 26-32.
24. Liu YY, Yang WT, Shi SH, et al. Immunogenicity of recombinant Lactobacillus plantarum NC8 expressing goose parvovirus VP2 gene in BALB/c mice. J Vet Sci 2017; 18(2): 159-167.
25. Murphy CI, Piwnica-Worms H. Overview of the baculovirus expression system. Curr Protoc Neurosci 2000; Chapter 4: Unit 4.18. doi: 10.1002/0471142301.ns0418s10.
26. Le Gall-Reculé G, Jestin V, Chagnaud P, et al. Expression of muscovy duck parvovirus capsid proteins (VP2 and VP3) in a baculovirus expression system and demonstration of immunity induced by the recombinant proteins. J Gen Virol 1996; 77(Pt 9): 2159-2163.

27. Chen Z, Li C, Zhu Y, et al. Immunogenicity of virus-like particles containing modified goose parvovirus VP2 protein. Virus Res 2012; 169(1): 306-309.
28. Masoomi Dezfooli S, Tan WS, Tey BT, et al. Expression and purification of the matrix protein of Nipah virus in baculovirus insect cell system. Biotechnol Prog 2016; 32(1): 171-177.
29. Brown CS, Van Lent JW, Vlak JM, et al. Assembly of empty capsids by using baculovirus recombinants expressing human parvovirus B19 structural proteins. J Virol 1991; 65(5): 2702-2706.
30. Maranga L, Brazão TF, Carrondo MJ. Virus-like particle production at low multiplicities of infection with the baculovirus insect cell system. Biotechnol Bioeng 2003; 84(2): 245-253.
31. Antonis AF, Bruschke CJ, Rueda P, et al. A novel recombinant virus-like particle vaccine for prevention of porcine parvovirus-induced reproductive failure. Vaccine 2006; 24(26): 5481-5490.
32. Cecchini S, Negrete A, Virag T, et al. Evidence of prior exposure to human bocavirus as determined by a retrospective serological study of 404 serum samples from adults in the United States. Clin Vaccine Immunol 2009; 16(5): 597-604.
33. Elia G, Desario C, Pezzoni G, et al. Recombinant ELISA using baculovirus-expressed VP2 for detection of antibodies against canine parvovirus. J Virol Methods 2012; 184(1-2): 98-102.
34. Jin H, Xia X, Liu B, et al. High-yield production of canine parvovirus virus-like particles in a baculovirus expression system. Arch Virol 2016; 161(3): 705-710.
35. Hashemzadeh MS, Gharari N. Biosynthesis of a VLP-type nanocarrier specific to cancer cells using the BEVS expression system for targeted drug delivery. J Genet Eng Biotechnol 2023; 21(1): 20. doi: 10.1186/s43141-023-00479-9.
36. Jiao C, Zhang H, Liu W, et al. Construction and immunogenicity of virus-like particles of feline parvovirus from the tiger. Viruses 2020; 12(3): 315. doi: 10.3390/v12030315.
37. Wubshet AK, Li GX, Li Q, et al. Stability and integrity of self-assembled bovine parvovirus virus‑like particles (BPV‑VLPs) of VP2 and combination of VP1VP2 assisted by baculovirus-insect cell expression: a potential logistical platform for vaccine deployment. Virol J 2024; 21(1): 87. doi: 10.1186/s12985-024-02322-0.
38. Wang Q, Ju H, Li Y, et al. Development and evaluation of a competitive ELISA using a monoclonal antibody for antibody detection after goose parvovirus virus-like particles (VLPs) and vaccine immunization in goose sera. J Virol Methods 2014; 209: 69-75.