Sequencing *p72* gene of field strain of African swine fever virus (ASFV) in Vietnam and generation of enhanced immunogenic fusion protein G-p72 potentially expressed as a recombinant antigen in ASFV subunit vaccine

Mai N. Tran[1], Hoang M. Nguyen[1], Loc T. Le[1], Hue T. Doan[1], Mi M. T. Nguyen[1], Binh T. Nguyen[2], & Phat X. Dinh[1*]

[1]Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City, Vietnam

[2]Biotechnology Center of Ho Chi Minh City, Ho Chi Minh City, Vietnam

ARTICLE INFO

Research Paper

Received: May 30, 2023
Revised: June 06, 2023
Accepted: June 17, 2023

Keywords

Gene fusion
G-VSV
Protein expression
p72-ASFV

***Corresponding author**

Dinh Xuan Phat
Email:
dinhxuanphat@hcmuaf.edu.vn

ABSTRACT

Protein *p72* is the major surface protein of African swine fever virus (ASFV), which is immunogenic and can prime the host to elicit a protective immune response, while G protein is the surface glycoprotein of vesicular stomatitis virus (VSV), which is well-known to be a strong antigen to stimulate an effective humoral immunity. The aim of this study was to sequence full-length *p72* gene of a field strain of ASFV causing typical ASF in Dong Nai province in 2020 and fuse this *p72* gene with VSV *G* gene to generate a recombinant fusion gene G-p72 that could simultaneously express both proteins and stimulate a better host immune response than *p72* expression alone. The sequence of the gene showed 99.59% nucleotide sequence similarity to an ASFV isolate from China. The PCR was employed to produce the recombinant G-p72 gene, which was cloned into plasmid pET28a, followed by transformation into *E. coli* BL21 (DE3) for protein expression. The G-p72 expression was induced at 37°C and 28°C for 6 and 16 h, respectively. The expression showed that G-p72 was observed at 28°C for 16 h. In summary, the full length *p72* gene of a field strain of ASFV was successfully sequenced and expressed as the recombinant G-p72 protein in *E. coli* BL21 (DE3). The expression level of the G-p72 fusion should be optimized and the immunogenicity of the recombinant protein should be examined in futher studies.

Cited as: Tran, M. N., Nguyen, H. M., Le, L. T., Doan, H. T., Nguyen, M. M. T., Nguyen, B. T., & Dinh, P. X. (2023). Sequencing *p72* gene of field strain of African swine fever virus (ASFV) in Vietnam and generation of enhanced immunogenic fusion protein G-p72 potentially expressed as a recombinant antigen in ASFV subunit vaccine. *The Journal of Agriculture and Development* 22(6), 32-41.

1. Introduction

African swine fever (ASF) is a lethal viral disease of swine that leads to a very high mortality in domestic and wild pigs. ASF cases were first recorded in Kenya in 1921 and then spread to some sub-Saharan African countries, Central and Eastern Europe, and Asia which were causing significant damage in global economic losses (Costard et al., 2009; Urbano & Ferreira, 2020). In Vietnam, the ASF outbreak started in February 2019 (Nguyen et al., 2022). There have been many efforts to develop vaccines against this disease so far. Unfortunately, the current vaccines have not been successful (Ramirez-Medina et al., 2022). Presently, early detection and depopulation of pig farms are the primary methods for decreasing the virus' spread.

The ASF is caused by the African swine fever virus (ASFV), a large-double strand DNA virus genome is 170-190 kbp in length and also the only member of the Asfarviridae family, genus Asfivirus (Alonso et al., 2018; Miao et al., 2023). The most important structural element of the virion is the protein *p72*, which is the primary capsid protein and accounts for approximately 31 - 33% of the total mass of the virion (Alonso et al., 2018; Revilla et al., 2018; Miao et al., 2023). The protein *p72* is considered to be the primary and highly immunogenic antigen in infected pigs (Kollnberger et al., 2002; Miao et al., 2023). Additionally, studies using monoclonal antibodies demonstrated that *p72* contains a variety of neutralizing epitopes that could be utilized to develop vaccines or other control strategies against virulent ASFV isolates (Yin et al., 2022; Miao et al., 2023).

Vesicular stomatitis virus (VSV) belongs to the Vesiculovirus genus of the family Rhabdoviridae and order of Mononegavirales, and is a zoonotic pathogen (Liu et al., 2021). This virus is an unsegmented, negative-stranded RNA virus, 180 nm long and 65 nm wide, with a single-stranded approximately 11 kb in size (Rozo-Lopez et al., 2018; Velazquez-Salinas et al., 2020). The VSV genome encoded five structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), large protein polymerase (L), and glycoprotein (G). These proteins were structural protein and constituted the bullet-shaped virions of the rhabdovirus (Liu et al., 2021). Glycoprotein G was the first component of the virus to attach to the host cells and was the major immunogen of VSV. Multiple research demonstrated that the recombinant G protein effectively stimulated immune responses (Falkensammer et al., 2009; Brakel et al., 2022). Additionally, Cobleigh et al. (2010) demonstrated that VSV virus vector was able to induce strong protective cell-mediated and humoral responses. Nowadays, the applications of G glycoprotein from VSV are diverse. It could be used for the creation of prosthetic vesicles that transport several therapeutic agents, such as antibodies and DNA directly into cells, as well as being used for anti-cancer treatments (Scher & Schnell, 2020). As a foreign protein in various constructs of recombinant antigens for vaccine development, VSV G has been successfully applied for a number of pathogens including Influenza virus, Hepatitis B virus (HBV), Ebola virus, Hepatitis C virus, etc. (Ezelle et al., 2002; Schwartz et al., 2007; Crozier et al., 2022). Our long-term research was to examine the potential of producing a recombinant antigen that could effectively trigger an immune response against ASFV. In this initial study, we focused on sequencing the entire *p72* gene from an ASFV field strain and fusing this *p72* gene with VSV *G* gene to create a recombinant gene that could express ASFV-*p72* and VSV-G proteins together in one frame. The successfully expressed recombinant protein G-p72 was expected to have

better immunogenicity and could be applied in vaccine development against ASFV compared to *p72* expression alone.

2. Materials and methods

2.1. Sequences of ASFV-p72 and VSV-G

For sequences of ASFV-*p72*, the positive blood samples for ASFV were collected from a field sample of infected ASFV in Dong Nai province in 2020 and confirmed by Realtime PCR. WizPrepTM Viral DNA/RNA Mini Kit was used to collect the pathogen's viral DNA. The *p72* gene of the ASFV was intended to be amplified with a product size of 1,941 bp using the primers GAAGTGCCTTTTGTACTTAGCCTTT-3', Tm = 61.61°C" and p72-R primer TTACTTTCCAAGTCGGTTCATCTCTATG-3', Tm = 61.05°C". This amplicon was sequenced and used for subsequent works. The sequences of VSV-*G* gene used for PCR amplification was obtained from a plasmid containing full-length genome of a wild type VSV Indiana strain which was published previously (Dinh et al., 2012).

2.2. Primers for PCR

The specific primers were designed for fusion of the two genes. The primer sequences of ASFV *p72* gene and VSV *G* gene were based on reference sequences MN793051.1 and J02428.1, respectively (Figure 1). EcoRI and NotI were added for cloning purpose (underlined) and the stop codon of VSV-*G* gene was removed (Table 1).

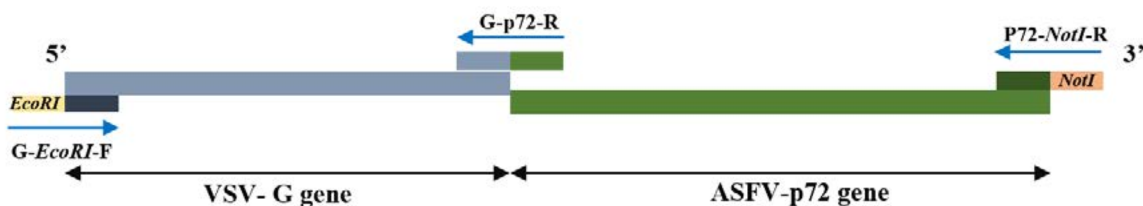

Figure 1. Diagram of designed primers to fuse VSV-*G* gene and ASFV-*p72* gene.

Table 1. Primers were used for PCR

Primer's name	Sequence 5' --> 3'	Tm
G-EcoRI-F	atatatGAATTCATGAAGTGCCTTTTGTACTTAGCCTTT	61.61
G-p72-R	CAAAAAGCTCCTCCTGATGCCATCTTTCCAAGTCG-GTTCATCTCTATGT	61.44
P72-NotI-R	atatatGCGGCCGCTTAGGTACTGTAACGCAGCACAG	60.61

2.3. PCR condition

In order to fuse the two genes, the PCR was performed in two steps. The PCR-1 using G-EcoRI-F and G-p72-R amplified *G*-gene of VSV. In PCR-2, the products in PCR-1 were used as the forward primer, in combination with p72-NotI-R primer as the reverse primer to amplify the *p72* gene. As a result, VSV-G and ASFV- *p72* gene were linked together in one open reading frame with EcoRI and NotI restriction sites at 5' and 3' ends of the fused gene, respectively.

The PCRs were performed with 10 μL of DreamTaq Mastermix 2X (Cat# K1081, ThermoFisher), 1 μL of each primer pair (with the initial concentration of primers being 10 μM), 1 μL template, and DEPC (Cat# K1081, ThermoFisher) - treated water was added to a total volume of 20 μL. The PCR conditions for both two-steps were established with initial denaturation at 96°C for 4 min and followed by 35 cycles with denaturation at 96°C for 1 min, annealing at 60°C for 60 sec, and extension at 72°C for 2 min, and final extension at 72°C for 7 min. After that, the amplicons were analyzed by electrophoresis in a 1% (w/v) agarose gel in 0.5X Tris-Acetate-EDTA (TAE) (Cat#B49, ThermoFisher) containing Midori Green Advance DNA stain (Cat#MG04, Nippon Genetics). The DNA marker 1Kb plus (Cat#10787018, Invitrogen) was included in each DNA gel electrophoresis to indicate the PCR product size and analyzed for experiments under UV light.

2.4. Cloning and transformation

After electrophoresis, the fused gene G-p72 was recovered from low-temperature agarose gel following manufacturer's procedure of WizPrep™ Viral DNA/RNA Mini Kit (V2) (Cat#NAPK059388WIZ, Wizbio solutions, Korea). The recovered fused gene was then inserted vector pET28a (Novagen, Germany). The successful insertion was examined by PCR using primers binding to T7 promoter and T7 terminator flanking the inserted gene. The pET28a containing the G-p72 gene was then transformed into *E. coli* DH10β-capable cells (NEB, England) by heat shock method. The transformed *E. coli* DH10β cells were spread onto LB agar plates at 37°C containing 50 ng/mL kanamycin, then incubated for 16 - 18 h at 37°C. Plasmid DNA from transformed cells was purified using GeneJET Plasmid Miniprep Kit (Cat#K0503, Thermo Scientific) according to the manufacturer's protocol. The plasmid DNA constructs were sequenced to reconfirm the fidelity of the fused G-p72 gene. The sequence data were analyzed by Bioedit (version 7.2), MEGA software (version 11), and BLAST tools before gene expression experiments.

2.5. Recombinant protein expression

In the next step, the purified plasmid pET28a containing the G-p72 gene was transformed into *E. coli* BL21 (DE3) cells by heat shock method. The pure selected colony was grown in LB broth medium until the OD reached 0.6 - 0.8. Then, 0.4 mM IPTG (Cat#15529019) was added for inducing protein expression. The expression experiments were carried out at two conditions: 37°C for 6 h and 28°C for 16 h. *E. coli* BL21 containing empty vector of pET28a was used as the negative control. The recombinant protein expression was analyzed by 12.5% SDS-PAGE gel containing Coomassie Brilliant Blue G-250 staining (Cat#20279, Thermo Scientific).

3. Results

3.1. PCR generating G-p72 gene

The amplicon of 1,981 bp (including 1,941 bp of *p72* gene together with restriction sites

and extra nucleotide designed for cloning) was successfully amplified (Figure 2A) and the nucleotide sequence was determined via Sanger sequencing method. Alignment of the sequencing result of the *p72* gene shared 99.59% and 98.7% identity of nucleotide and amino acid sequence compared to the the Chinese reference strain (MN793051.1) and it belonged to genotype II of ASFV. Similarly, the *G* gene of VSV was also amplified successfully from DNA plasmid to produce a product of 1,568 bp (Figure 2B).

To generate fused gene *G-p72* encoding two proteins (*G* and *p72*) simultaneously, primers G-EcoRI-F and G-p72-R were used in PCR-1 to amplify *G* gene, followed by PCR-2 using the product of PCR-1 as forward primer together with *P72-NotI-R* as reverse primer. The product of the fusion *G-p72* gene was 3,481 bp in size (Figure 2C).

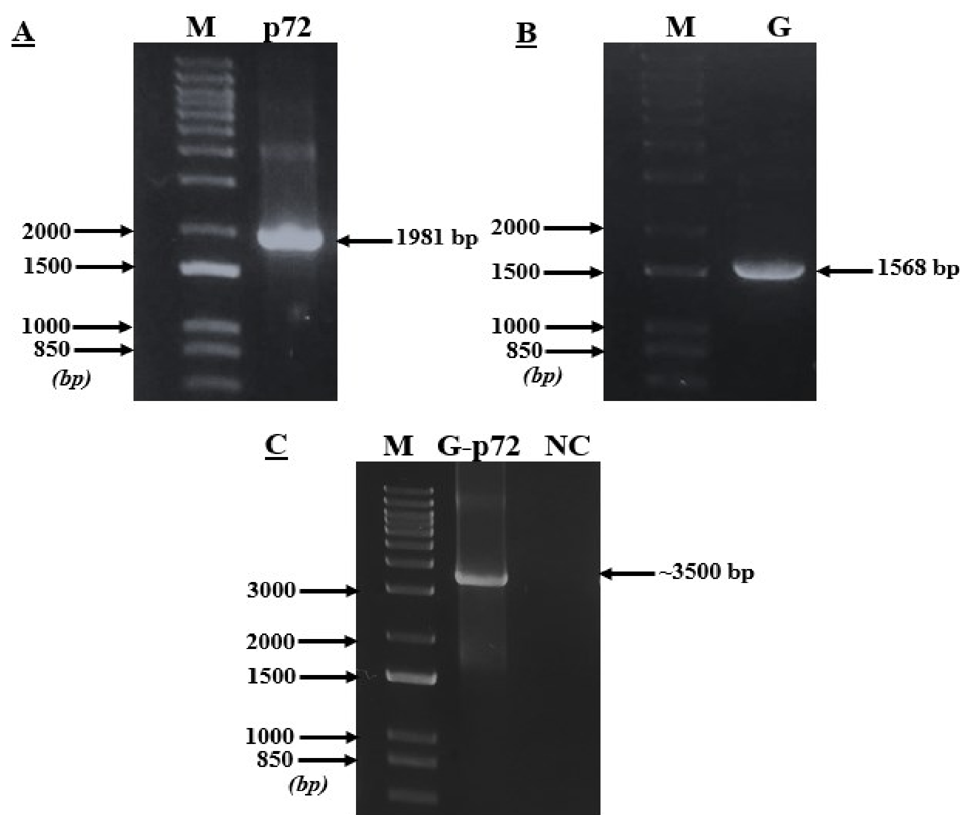

Figure 2. PCR amplification. Lane M: DNA ladder 1Kb plus; (A). *p72* gene of African swine fever virus; (B). G gene of vesicular stomatitis virus; (C). Fused product of *G-p72* gene. NC: Negative control with pure water.

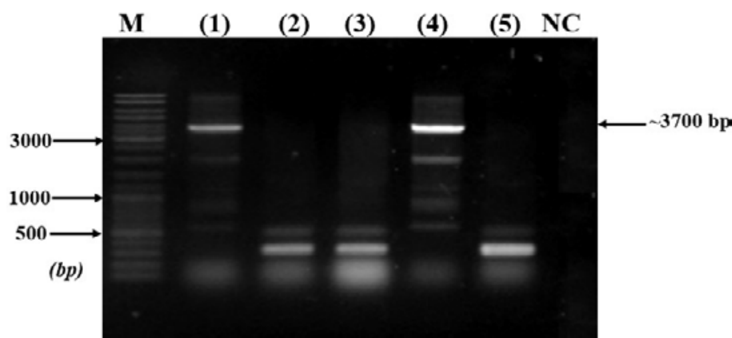

Figure 3. Colony-PCR results of colonies with primer pair T7: Lane M: DNA ladder 1Kb plus; Lane 1-5: Pure white colonies; Lane (NC) Negative control with pure water.

3.2. Expression of the recombinant protein G-p72

The PCR product of G-p72 was purified, inserted in plasmid pET28a, and transformed into *E. coli* DH10β. The *E. coli* cells that received the plasmid were selected by white-blue system. The pure white colonies were collected and examined by PCR using primers specific for T7 promoter and T7 terminator in pET28a plasmid, with the amplicon of approximately 3,700 bp to determine the successfully transformed colonies (Figure 3). The fusion gene G-p72 in the plasmid pET28a was successfully sequenced to reconfirm the nucleotide fidety and the correct direction of the inserted gene compared to the promoter direction in the pET28a which is critical for successful expression (Figure 4).

The recombinant pET28a containing G-p72 was transformed into BL21 (DE3) for evaluating the protein expression under two conditions: 37°C for 6 h and 28°C for 16 h. The results showed that the G-p72 protein (130 kDa) was detected at 28°C for 16 h (Figure 4). Further experiments should be done to improve the expression level of recombinant G-p72 protein from *E. coli* BL21 and the expressed protein should be confirmed specifically by Western blotting.

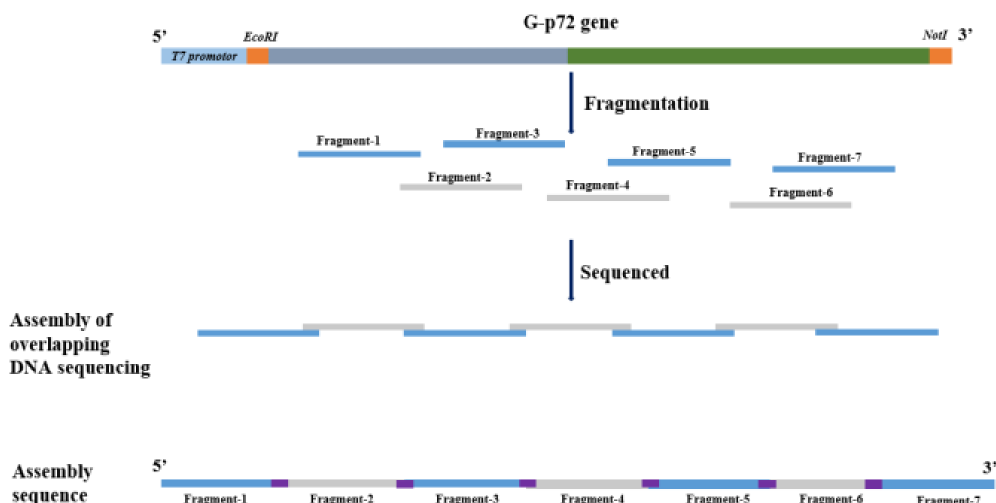

Figure 4. Principle for sequencing G-p72 gene.

4. Discussions

African swine fever (ASF) is a viral disease with a high fatality in domestic pigs and wild boars. The ASF has greatly challenged pig-raising countries and negatively impacted the regional and national trade of pork products. The development of safe and effective ASF vaccines is urgently required for the control of ASF outbreaks. So far, the viral surface protein *p72* is known to be responsible for membrane fusion and viral entry as well as being the primary target for a protective immune response. Therefore, in order to create a possibly better immunogen, we combined the G gene from VSV with the *p72* gene from ASFV, expressed the recombinant protein, and utilized it as an antigen for a subunit vaccination against ASFV.

Nowadays, developing vaccines against ASFV is urgently necessary and thus different types of vaccines have been evaluated including live-attenuated vaccines, subunit vaccines, and DNA vaccines. Live attenuated vaccines harbor some limitations such as safety concerns and side effects in the vaccinated animals (Revilla et al., 2018). Besides, Blome et al. (2014) demonstrated that inactivated ASFV did not provide the protective ability, even when various adjuvants were used. The subunit vaccines have been considered as alternative ways to set the light to vaccine development for ASFV. Andrés et al. (2001) demonstrated the possible combination of pE120R and *p72* which could be incorporated into the virus particle when expressed in cells. The viral proteins p30, p54, p72, A104R, B602L, NP419L, and K205R have also been studied regarding their immunogenicity and vaccine application. Neutralizing epitopes found inside p72, p54, and p30 have been reported and designed for the development of ASFV vaccines and diagnostic techniques (Gaudreault & Richt, 2019; Cadenas-Fernández et al., 2020; Goatley et al., 2020).

Previously, Cobleigh et al. (2010) showed that using VSV was as a vector vaccine, it could induce strong protective T cell and antibody responses. Attenuated VSV vector or VSV G protein were widely applied in vaccine research (Chattopadhyay et al., 2013; van den Pol et al., 2017; Crozier et al., 2022). With the aim of developing a subunit vaccine that could mount a better immunity against field strain of ASFV, full-length *p72* gene from a virulent ASFV infected pig was isolated and used to design a contruct that could express *p72* protein of ASFV together with G protein of VSV in a monocistronic mRNA in hope that G protein of VSV could serve as a natural adjuvant to stimulate a bettern immune response compared to expression of *p72* protein alone. Eventually, the G-p72 coding gene was sucessfully constructed and expressed the recombinant protein from *E. coli* BL21 (DE3) at 28ºC for 16 h. The expressive condition was consistent to the report by Sidoruk et al. (2011) in which the 1 kb gene was expressed well at 28°C compared to 37°C after 17 h.

5. Conclusions

In summary, the full-length *p72* gene of a field strain of ASFV circulating in Vietnam was sucessfully sequenced and generated G-p72 fusion gene that was carried by pET28a plasmid in *E. coli* BL21 (DE3). The G-p72 was expressed at 28ºC for 16 h. Further research should be done to increase expression level and examine the potential use of the fused protein G-p72 as an immunogenic antigen.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

The authors would like to thank Nong Lam University Ho Chi Minh City for funding this study under the project CS-SV22-KHSH-01.

References

Alonso, C., Borca, M., Dixon, L., Revilla, Y., Rodriguez, F., Escribano, J. M., & ICTV Report Consortium. (2018). ICTV virus taxonomy profile: Asfarviridae. *Journal of General Virology* 99(5), 613-614. https://doi.org/10.1099/jgv.0.001049.

Andrés, G., García-Escudero, R., Viñuela, E., Salas, M. L., & Rodríguez, J. M. (2001). African swine fever virus structural protein pE120R is essential for virus transport from assembly sites to plasma membrane but not for infectivity. *Journal of Virology* 75(15), 6758-6768. https://doi.org/10.1128/jvi.75.15.6758-6768.2001.

Blome, S., Gabriel, C., & Beer, M. (2014). Modern adjuvants do not enhance the efficacy of an inactivated African swine fever virus vaccine preparation. *Vaccine* 32(31), 3879-3882. https://doi.org/10.1016/j.vaccine.2014.05.051.

Brakel, K. A., Ma, Y., Binjawadagi, R., Harder, O., Watts, M., Li, J., Binjawadagi, B., & Niewiesk, S. (2022). Codon-optimization of the respiratory syncytial virus (RSV) G protein expressed in a vesicular stomatitis virus (VSV) vector improves immune responses in a cotton rat model. *Virology* 575, 101-110. https://doi.org/10.1016/j.virol.2022.08.017.

Cadenas-Fernández, E., Sánchez-Vizcaíno, J. M., Kosowska, A., Rivera, B., Mayoral-Alegre, F., Rodríguez-Bertos, A., Yao, J., Bray, J. B., Lokhandwala, S., Mwangi, W., & Barasona, J. A. (2020). Adenovirus-vectored African swine fever virus antigens cocktail is not protective against virulent Arm07 isolate in Eurasian wild boar. *Pathogens* 9(3), 171. https://doi.org/10.3390/pathogens9030171.

Chattopadhyay, A., Wang, E., Seymour, R., Weaver, S. C., & Rose, J. K. (2013). A chimeric vesiculo/alphavirus is an effective alphavirus vaccine. *Journal of Virology* 87(1), 395-402. https://doi.org/10.1128/jvi.01860-12.

Cobleigh, M. A., Buonocore, L., Uprichard, S. L., Rose, J. K., & Robek, M. D. (2010). A vesicular stomatitis virus-based hepatitis B virus vaccine vector provides protection against challenge in a single dose. *Journal of Virology* 84(15), 7513-7522. https://doi.org/10.1128/jvi.00200-10.

Costard, S., Wieland, B., De Glanville, W., Jori, F., Rowlands, R., Vosloo, W., Roger, F., Rfeiffer, D. U., & Dixon, L. K. (2009). African swine fever: how can global spread be prevented?. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364(1530), 2683-2696. https://doi.org/10.1098/rstb.2009.0098.

Crozier, I., Britson, K. A., Wolfe, D. N., Klena, J. D., Hensley, L. E., Lee, J. S., Wolfraim, L. A., Taylor, K. L. Higgs, E. S., Montgomery, J. M., & Martins, K. A. (2022). The evolution of medical countermeasures for Ebola virus disease: lessons learned and next steps. *Vaccines* 10(8), 1213.

Dinh, P. X., Panda, D., Das, P. B., Das, S. C., Das, A., & Pattnaik, A. K. (2012). A single amino acid change resulting in loss of fluorescence of eGFP in a viral fusion protein confers fitness and growth advantage to the recombinant vesicular stomatitis virus. *Virology* 432(2), 460-469. https://doi.org/10.1016/j.virol.2012.07.004.

Ezelle, H. J., Markovic, D., & Barber, G. N. (2002). Generation of hepatitis C virus-like particles by use of a recombinant vesicular stomatitis virus vector. *Journal of Virology* 76(23), 12325-12334. https://doi.org/10.1128/JVI.76.23.12325-12334.2002.

Falkensammer, B., Rubner, B., Hiltgartner, A., Wilflingseder, D., Hennig, C. S., Kuate, S., Überla, K., Norley, S., Strasak, A., & Stoiber, H. (2009). Role of complement and antibodies in controlling infection with pathogenic simian immunodeficiency virus (SIV) in macaques vaccinated with replication-deficient viral vectors. *Retrovirology* 6, 1-12. https://doi.org/10.1186/1742-4690-6-60.

Gaudreault, N. N., & Richt, J. A. (2019). Subunit vaccine approaches for African swine fever virus. *Vaccines* 7(2), 56. https://doi.org/10.3390/vaccines7020056.

Goatley, L. C., Reis, A. L., Portugal, R., Goldswain, H., Shimmon, G. L., Hargreaves, Z., Ho, C., Montoya, M., Sánchez-Cordón, P. J., Taylor, G., Dixon, L. K., & Netherton, C. L. (2020). A pool of eight virally vectored African swine fever antigens protect pigs against fatal disease. *Vaccines* 8(2) 234. https://doi.org/10.3390/vaccines8020234.

Kollnberger, S. D., Gutierrez-Castañeda, B., Foster-Cuevas, M., Corteyn, A., & Parkhouse, R. M. E. (2002). Identification of the principal serological immunodeterminants of African swine fever virus by screening a virus cDNA library with antibody. *Journal of General Virology* 83(6), 1331-1342. https://doi.org/10.1099/0022-1317-83-6-1331.

Liu, G., Cao, W., Salawudeen, A., Zhu, W., Emeterio, K., Safronetz, D., & Banadyga, L. (2021). Vesicular stomatitis virus: from agricultural pathogen to vaccine vector. *Pathogens* 10(9), 1092. https://doi.org/10.3390/pathogens10091092.

Miao, C., Yang, S., Shao, J., Zhou, G., Ma, Y., Wen, S., Hou, Z., Peng, D., Guo, H., Liu, Wei., & Chang, H. (2023). Identification of *p72* epitopes of African swine fever virus and preliminary application. *Frontiers in Microbiology* 14, 1126794. https://doi.org/10.3389/fmicb.2023.1126794.

Nguyen, V. T., Cho, K. H., Mai, N. T. A., Park, J. Y., Trinh, T. B. N., Jang, M. K., Nguyen, T. T. H., Vu, X. D., Nguyen, V. D.., Ambagala, A., Kim, Y., & Le, V. P. (2022). Multiple variants of African swine fever virus circulating in Vietnam. *Archives of Virology* 167(4), 1137-1140. https://doi.org/10.1007/s00705-022-05363-4.

Ramirez-Medina, E., Vuono, E., Silva, E., Rai, A., Valladares, A., Pruitt, S., Espinoza, N., Velazquez-Salinas, L., Borca, M., Gladue, D. P., Borca, M. V., & Gladue, D. P. (2022). Evaluation of the deletion of MGF110-5L-6L on swine virulence from the pandemic strain of African swine fever virus and use as a DIVA marker in vaccine candidate ASFV-G-ΔI177L. *Journal of Virology* 96(14), e00597-22. https://doi.org/10.1128/jvi.00597-22.

Revilla, Y., Pérez-Núñez, D., & Richt, J. A. (2018). African swine fever virus biology and vaccine approaches. *Advances in Virus Research* 100, 41-74. https://doi.org/10.1016/bs.aivir.2017.10.002.

Rozo-Lopez, P., Drolet, B. S., & Londoño-Renteria, B. (2018). Vesicular stomatitis virus transmission: a comparison of incriminated vectors. *Insects* 9(4). 190. https://doi.org/10.3390/insects9040190.

Scher, G., & Schnell, M. J. (2020). Rhabdoviruses as vectors for vaccines and therapeutics. *Current Opinion in Virology* 44, 169-182. https://doi.org/10.1016/j.coviro.2020.09.003.

Schwartz, J. A., Buonocore, L., Roberts, A., Suguitan Jr, A., Kobasa, D., Kobinger, G., Feldmann, H., Subbarao, K., & Rose, J. K. (2007). Vesicular stomatitis virus vectors expressing avian influenza H5 HA induce cross-neutralizing antibodies and long-term protection. *Virology* 366(1), 166-173. https://doi.org/10.1016/j.virol.2007.04.021.

Sidoruk, K. V., Pokrovsky, V. S., Borisova, A. A., Omeljanuk, N. M., Aleksandrova, S. S., Pokrovskaya, M. V., Gladilina, J. A., Bogush,

V. G., & Sokolov, N. N. (2011). Creation of a producent, optimization of expression, and purification of recombinant Yersinia pseudotuberculosis L-asparaginase. *Bulletin of Experimental Biology and Medicine* 152, 219-223. https://doi.org/10.1007/s10517-011-1493-7.

Urbano, A. C., & Ferreira, F. (2020). Role of the DNA-binding protein pA104R in ASFV genome packaging and as a novel target for vaccine and drug development. *Vaccines* 8(4), 585. https://doi.org/10.3390/vaccines8040585.

van den Pol, A. N., Mao, G., Chattopadhyay, A., Rose, J. K., & Davis, J. N. (2017). Chikungunya, influenza, Nipah, and Semliki Forest chimeric viruses with vesicular stomatitis virus: actions in the brain. *Journal of Virology* 91(6), 10-1128. https://doi.org/10.1128/jvi.02154-16.

Velazquez-Salinas, L., Pauszek, S. J., Holinka, L. G., Gladue, D. P., Rekant, S. I., Bishop, E. A., Stenfeldt, C., Verdugo-Rodriguez,. A., Borca, M. V., Arzt, J., & Rodriguez, L. L. (2020). A single amino acid substitution in the matrix protein (M51R) of vesicular stomatitis New Jersey virus impairs replication in cultured porcine macrophages and results in significant attenuation in pigs. *Frontiers in Microbiology* 11, 1123. https://doi.org/10.3389/fmicb.2020.01123.

Yin, D., Geng, R., Shao, H., Ye, J., Qian, K., Chen, H., & Qin, A. (2022). Identification of novel linear epitopes in *p72* protein of African swine fever virus recognized by monoclonal antibodies. *Frontiers in Microbiology* 13, 1055820. https://doi.org/10.3389/fmicb.2022.1055820.