MINISTRY OF EDUCATION AND TRAINING

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ISOLATION AND SELECTION OF POTENTIAL PARVOVIRUS STRAINS FOR VACCINE PRODUCTION AGAINST PORCINE MUMMIFICATION

Major: Biotechnology

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DOCTORAL DISSERTATION SUMMARY

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INTRODUCTION

1. Rationale of the Study

In Vietnam, no institution has yet produced or commercialized vaccines against reproductive failure in pigs caused by porcine parvovirus (PPV). All vaccines currently in use for the prevention of PPV-associated reproductive disorders are imported. These imported vaccines are often costly and may not be fully compatible with PPV strains circulating in Vietnam. Furthermore, reliance on imported vaccines places a financial burden on the national budget. For these reasons, the Ministry of Agriculture and Rural Development has identified the development of PPV vaccines for pigs as an urgent priority for the swine industry.

The dissertation entitled "*Isolation and Selection of Potential Parvovirus Strains for Vaccine Production against Porcine Mummification*" aims to isolate PPV strains with high virulence, genetic and biological stability, and strong immunogenicity. These strains will serve as the foundation for the development of PPV vaccines, thereby enabling proactive prevention and control of reproductive failure in pigs in line with the Government's strategic direction.

2. Research Objectives

2.1. General Objectives

To isolate, characterize, and establish a library of potential PPV strains for use in research and development of vaccines against porcine fetal mummification.

2.2. Specific Objectives

1) To isolate PPV strains from field samples.

2) To determine the viral type and analyze the VP2 gene of the isolated strains.

3) To characterize the biological properties, stability, and immunogenicity of the PPV strains.

3. Research Contents

1) Isolation of parvovirus strains causing fetal mummification from representative samples across various regions in Vietnam

2) Identification and classification of the isolated parvovirus strains

3) Genetic relationship analysis of the isolated strains

4) Characterization of the biological properties of the parvovirus strains

5) Evaluation of the stability, virulence, and immunogenicity of selected viral strains

4. Scientific and Practical Significance of the Study

4.1. Scientific Significance

This study represents the successful isolation of PPV strains in Vietnam using the continuous PK-15 cell line. The research provides valuable insights into both the biological and genetic characteristics of PPV strains isolated in Vietnam, thereby contributing to a more comprehensive understanding of the PPV strains currently circulating in the country. Furthermore, two PPV strains with stable biological and genetic traits across multiple passages, high virulence, and strong immunogenicity were identified. These strains represent potential candidates for the development of vaccines against reproductive failure in pigs.

4.2. Practical Significance

The isolation and selection of PPV strains with high virulence, genetic and biological stability, and strong immunogenicity provide a foundation for the development of vaccines against reproductive failure in pigs. This will contribute to securing domestic vaccine supply, enhancing disease prevention, and supporting national strategies for epidemic control. The availability of locally produced vaccines, at lower cost and with more reliable supply compared to imported products, will empower farmers to implement effective preventive measures, improve livestock productivity, reduce production risks, and increase economic returns.

CHAPTER I

LITERATURE REVIEW

1.1. Overview of the Study Subject

1.1.1. Overview of Porcine Parvovirus-Induced Reproductive Failure

PPV is is a major etiological agent of reproductive disorders in swine, classically manifested as SMEDI syndrome (stillbirth, mummification, embryonic death, and infertility). Pregnant sows are the principal susceptible host, and transplacental transmission plays a critical role in fetal outcomes. The gestational stage at infection determines disease severity: exposure during days 10–30 often causes embryonic death or resorption; infection between days 30–70 results in fetal mummification; whereas infection after day 70 rarely induces fetal mortality due to the development of fetal immunocompetence (Cartwright & Huck, 1967).

PPV enters herds via contaminated feed, water, feces, semen, or genital secretions. Viral replication primarily targets endothelial and mesenchymal cells of the chorionic membrane, leading to placental tissue destruction, circulatory impairment, and fetal demise (Cotmore & Tattersall, 2014). Clinically, infection manifests as delayed or absent estrus, reduced litter size, fetal mummification, abortion, stillbirth, and decreased neonatal viability. Gross lesions include fetal growth retardation, congestion, edema, hemorrhage, and mummification (Mengeling et al., 1975; Liu, 2022).

The economic impact is substantial. Internationally, outbreaks may cause the loss of hundreds to thousands of piglets per farm, equating to thousands to tens of thousands of USD in losses (Morrison & Joo, 1984). In Vietnam, PPV remains an important cause of reduced conception rates, fetal mortality, and pseudopregnancy, with national losses potentially reaching millions of fetuses annually (Võ Văn Ninh, 2003).

1.1.2. Overview of Parvovirus

Porcine parvovirus belongs to the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Protoparvovirus*, species *Ungulate protoparvovirus*, and comprises types PPV1–PPV7 (Streck et al., 2015). PPV1 is the classical and most clinically relevant type, associated with SMEDI syndrome, whereas PPV2–PPV7—classified within *Tetraparvovirus*, *Copiparvovirus*, and *Chaphamaparvovirus*—display greater evolutionary diversity and uncertain pathogenic roles. These emerging types are

frequently detected as co-infections alongside PCV2 or PRRSV (Streck et al., 2015; Wang et al., 2010).

The PPV genome is a linear, single-stranded DNA (≈5 kb), non-enveloped, with an icosahedral capsid. It contains two major open reading frames: ORF1 encoding the nonstructural proteins NS1–NS3 involved in viral replication and transcriptional regulation, and ORF2 encoding the capsid proteins VP1–VP3 (Streck & Truyen, 2020). Viral entry relies on interactions with surface glycoproteins and sialic acid residues and occurs via clathrin-mediated endocytosis, macropinocytosis, or other pathways. Following nuclear transport, the ssDNA is converted into a dsDNA intermediate to support replication (Boisvert et al., 2010). PPV replicates efficiently in porcine cell lines such as PK-15, ST, and SLC, inducing mitochondrial injury, cytochrome C release, and activation of intrinsic apoptosis, resulting in characteristic cytopathic changes. The severity and pattern of tissue lesions are strain- and cell-type specific (Zhang et al., 2015; Xu et al., 2022)

1.2. Overview of the Study Sites

Swine production represents a cornerstone of Vietnam's livestock sector, contributing over 60% of the total pork output and approximately 26% of the agricultural GDP (Department of Animal Husbandry, 2024). The provinces of Thai Binh, Thanh Hoa, Binh Dinh, Dak Lak, Dong Nai, and Binh Duong were selected as study sites due to their large pig populations, well-developed industrial farms, and established facilities for breeding high-quality pigs. These provinces span the northern, central, and southern regions of the country, encompassing diverse ecological conditions. This selection enables a comprehensive assessment of porcine parvovirus circulation across a wide geographical range and facilitates the identification of viral strains with potential applicability under the epidemiological conditions of Vietnam.

1.3. Overview of Research Methodology

PPV Isolation: Porcine parvovirus (PPV) predominantly localizes in the fetal liver and lungs due to its efficient replication in rapidly dividing cells, whereas maternal serum typically contains only low viral loads. Effective sampling combines sera from suspected infected sows with tissues from stillborn fetuses. Detection of positive samples is primarily conducted via PCR targeting the highly conserved NS1 gene, ensuring both

sensitivity and specificity. Prior to isolation, samples are screened to exclude co-infections with PCV2, PRRSV, CSFV, or ASFV.

PPV Cell Culture: Continuous cell lines, particularly PK-15, are employed due to their high susceptibility to PPV, capacity to maintain viral propagation over multiple passages, and ease of manipulation. Cultivation is typically performed in E-MEM supplemented with FBS, with parameters such as FBS concentration and incubation temperature optimized to maximize viral replication. Growth curves and $TCID_{50}$ titers are established to determine the optimal harvest time and standardize culture conditions.

PPV Typing and Genetic Analysis: Multiplex PCR is used to detect PPV types 1–7. Sequencing of the NS1 gene provides insights into viral origin and evolutionary relationships, while VP2 gene analysis evaluates antigenicity, transmissibility, virulence, and enables monitoring for emerging variants.

Assessment of Biological Characteristics, Virulence, and Immunogenicity: Viral growth kinetics and $TCID_{50}$ titers reflect replication efficiency. Genetic and phenotypic stability is evaluated across multiple successive passages to ensure suitability for vaccine research. Virulence is assessed in pregnant sows infected at day 40 of gestation, with necropsy performed at day 90 to observe fetal lesions and detect viral DNA via PCR. Immunogenicity is evaluated in both swine and guinea pigs using hemagglutination inhibition (HI) titers, enabling the selection of strains capable of eliciting robust immune responses as candidate vaccine strains for PPV.

1.4. Overview of PPV Research and Vaccine Development

1.4.1. PPV Prevalence and Potential for Vaccine Research

1.4.1.1. Global and Vietnamese Prevalence of PPV

Globally, seven Porcine Parvovirus types (PPV1–PPV7) have been identified. PPV1, first isolated in Germany in 1965, remains the primary causative agent of reproductive disorders in swine. Emerging types (PPV2–PPV7) have been characterized using molecular biology approaches, with PPV2 and PPV3 classified under the genus Tetraparvovirus. PPV1 is widely distributed across continents but exhibits relatively low infection rates (0.5–29%), potentially reflecting widespread vaccination. In contrast, PPV2–PPV7 are prevalent with higher incidence, particularly in Asia, the Americas, and Europe, indicating strong adaptability and potential for co-infection with other pathogens

such as PCV2 or PRRSV.

In Vietnam, PPV was first detected in 1993, causing reproductive failure in sows, with infection rates ranging from 21% to 88% depending on the region. Multiple PPV types (PPV1–PPV4) co-circulate, reflecting a complex epidemiological landscape similar to that observed in other countries. Isolation of indigenous PPV strains is therefore essential for the development of locally tailored vaccines, enhancing protective efficacy.

1.4.1.2. Application of Isolated Strains in PPV Vaccine Development

The isolation, characterization, and selection of PPV strains with defined biological and antigenic properties constitute the foundation for vaccine development. Globally, conventional PPV vaccines are primarily based on the classical PPV1 strain (NADL-2); however, the emergence of variants such as PPV-27a can compromise cross-protection. In China, several indigenous strains have been utilized to produce inactivated or subunit vaccines, including virus-like particles (VLPs), demonstrating strong immunogenicity and effective protection in sows. Use of locally isolated strains enhances antigenic compatibility, ensures a stable vaccine supply, reduces production costs, and improves regional protective efficacy.

1.4.2. Status of PPV Vaccine Production

Global Perspective: Traditional inactivated vaccines predominantly utilize PPV1 (NADL-2), eliciting protective antibody titers that decline over time. The challenge-protection model remains the gold standard for evaluating vaccine efficacy. Recombinant vaccines targeting the VP2 protein, either as VLPs or via viral vectors, confer robust and safe immune responses and facilitate scalable production. Commercial vaccines such as FarrowSure Gold, Porcilis® Parvo, Parvovax, and PPV VAC are available worldwide; however, high costs and potential antigenic mismatches limit their applicability in local contexts.

Vietnamese Context: Currently, PPV vaccines in Vietnam are exclusively imported, associated with high costs and limited antigenic compatibility. Research on the isolation and development of domestic vaccines remains limited but is critically needed to ensure an autonomous supply, reduce costs, enhance protective efficacy, and support sustainable growth in the swine industry.

CHAPTER II
MATERIALS AND METHODS

2.1. Research subjects

The research subjects were Parvovirus isolated from six provinces of Vietnam: Thai Binh, Thanh Hoa, Binh Dinh, Dak Lak, Dong Nai, and Binh Duong.

2.2. Research methods

2.2.1. Method for isolating porcine parvovirus

*** Sample collection**

The sampling procedure was conducted in compliance with the national technical regulation QCVN 01-83:2011/BNNPTNT: Animal diseases – General requirements for sampling, preservation, and transportation of clinical specimens.

*** Sample processing**

Serum samples: Serum samples were centrifuged at 3,000 rpm for 10 minutes, and the supernatant was collected and stored at –20 °C (Tuck et al., 2009).

Embryonic tissue samples: Liver and lung tissues from embryos were collected and prepared as a 10% suspension in PBS (Nguyen et al., 2022).

*** Identification of PPV-positive samples**

Samples were screened for PPV by PCR, following the method described by Lyoo et al., 2001.

*** Determination of samples eligible for virus isolation**

Samples were tested for the presence of other viruses, including PRRSV (according to TCVN 8400-21:2014), PCV (TCVN 8400-36:2015), CSFV (TCVN 8400-47:2019), and ASFV (TCVN 8400-41:2019). Only samples negative for all the above viruses were considered eligible for PPV isolation using the PK-15 continuous cell line.

*** PPV isolation method**

The isolation of PPV was carried out by culturing on PK-15 cells, as described by Kim et al., 2022.

2.2.2. Determination of parvovirus types

The isolated viral strains were typed using the PCR method of Kim Seung-Chai et al., 2022.

2.2.3. Method for determining the genetic relationships of parvovirus strains

The NS1 gene fragment was amplified using the primer pair PPVF and PPVR, as described by Lyoo et al., 2001. The VP2 gene fragment was amplified using PCR

according to the method of Xu et al., 2013. The amplified DNA was sequenced at First BASE Laboratories (Singapore). Sequence data were analyzed using BLAST against the NCBI GenBank database. Phylogenetic trees were constructed based on nucleotide sequences of the NS1 gene from the isolated PPV strains, referenced with selected sequences from GenBank, using MEGA6 software with the Neighbor-Joining method (Saitou and Nei, 1987). Bootstrap analysis with 1,000 replicates was performed to assess the reliability of the phylogenetic tree (Felsenstein, 1985).

2.2.4. Methods for determining selected viral characteristics

*** Determination of $TCID_{50}$ titer**

The TCID50 was determined using the method of Reed and Muench (1938).

*** Determination of optimal culture conditions**

The culture conditions evaluated included temperatures of 33°C, 37°C, and 39°C, and fetal bovine serum (FBS) concentrations of 2%, 3%, and 5%. Parvovirus was inoculated onto PK-15 cells at a multiplicity of infection (MOI) of 0.01. The virus was then cultured under the respective conditions for 7 days. Every 24 hours, 200 µl of viral suspension was collected, followed by DNA extraction and quantification of viral load using real-time PCR.

*** Determination of viral growth curve**

Viral strains were inoculated onto PK-15 cells at an MOI of 0.01. The viruses were cultured in E-MEM supplemented with 5% FBS and 1% penicillin–streptomycin at 37°C with 5% CO_2. Samples were collected every 24 hours for 7 days post-inoculation. The viral load at each time point was determined by real-time PCR, as described by Miao et al., 2009.

2.2.5. Assessment of the Potential Use of PPV in Vaccine Research and Development

*** Determination of stability in selected biological and genetic characteristics**

Viral samples were serially passaged for 15 generations. At passages 1, 5, 10, and 15, evaluations were conducted on growth kinetics, $TCID_{50}$ titers, cytopathic effects, and sequence analysis of the NS1 and VP2 genes.

*** Assessment of virulence**

The virulence of parvovirus strains was determined following the description of Zeeuw et al., 2007.

*** Assessment of immunogenicity**

Evaluation of immunogenicity in guinea pigs: The immunogenicity in guinea pigs was assessed according to the method described by Ma et al., 2011.

Evaluation of immunogenicity in pigs: The immunogenicity of parvovirus strains was evaluated in pregnant sows following the protocol described by Foerster Tessa et al., 2016.

2.3. Data processing methods

All experimental data were analyzed using Minitab 18 software. The prevalence of Parvovirus among different groups or locations was compared using the Chi-square (χ^2) test. Quantitative data, including viral replication efficiency, $TCID_{50}$ titers, and antibody responses measured by HI and ELISA, were evaluated using a General Linear Model (GLM) to assess the effects of experimental factors. When significant differences were detected, Tukey's post hoc test was applied to differentiate between groups at a significance level of $p < 0.05$. Results are presented as mean ± standard deviation (Mean ± SD).

2.4. Animal ethics

All animal experiments conducted in this study were approved by the Animal Ethics Committee of Nong Lam University, Ho Chi Minh City, under certificate number NLU-230313, issued on May 24, 2023.

CHAPTER III
RESULTS AND DISCUSSION

3.1. Research Results

3.1.1. Isolation of Porcine Parvovirus

3.1.1.1. Identification of PPV-Positive Samples

In this study, a total of 392 specimens, comprising 360 serum samples and 32 fetal tissues collected from six provinces representing different ecological regions of Vietnam, were screened by PCR. Thirty-two samples tested positive for PPV (8.16%), yielding a specific and reliable PCR product of approximately 330 bp. The positivity rate among provinces ranged from 4.76% to 10.0% (Table 3.1), with no statistically significant difference observed ($\chi^2 = 1.81$; $p = 0.875$). These findings indicate that PPV is widely circulating at a low prevalence without forming localized outbreaks, reflecting an endemic and stable pattern in Vietnamese swine populations.

Table 3.1. Detection of PPV-Positive Samples

Province	Serum	Fetal Tissue	Total	Serum Positive	Serum (%)	Fetal Positive	Fetal (%)	Total Positive	Total (%)
Thai Binh	60	0	60	5/60	8.33	0/0	0	5/60	8.33
Thanh Hoa	60	3	63	3/60	5.0	0/3	0	3/63	4.76
Binh Dinh	60	0	60	4/60	6.67	0/0	0	4/60	6.67
Dak Lak	60	9	69	5/60	8.33	1/9	11.11	6/69	8.70
Dong Nai	60	10	70	5/60	8.33	2/10	20.0	7/70	10.0
Binh Duong	60	10	70	7/60	11.67	0/10	0	7/70	10.0
Total	360	32	392	29/360	8.06	3/32	9.38	32/392	8.16

3.1.1.2. Screening of samples eligible for isolation

All 32 PPV-positive samples tested negative for CSFV, PRRSV, PCV2, and ASFV, thereby meeting the criteria for subsequent PPV isolation procedures

3.1.1.3. PPV Cultivation Results

- **Observation of cytopathic effects (CPE)**

The observed CPE revealed that within 24 hours post-infection, the virus induced cellular alterations, including irregular cell morphology, nuclear condensation, and granulation (Figure 3.1). The virus subsequently replicated and caused progressive cell

destruction. By day 5 post-infection, extensive cellular damage was evident, resulting in the formation of clear areas on the surface of the culture flask (Figure 3.2)

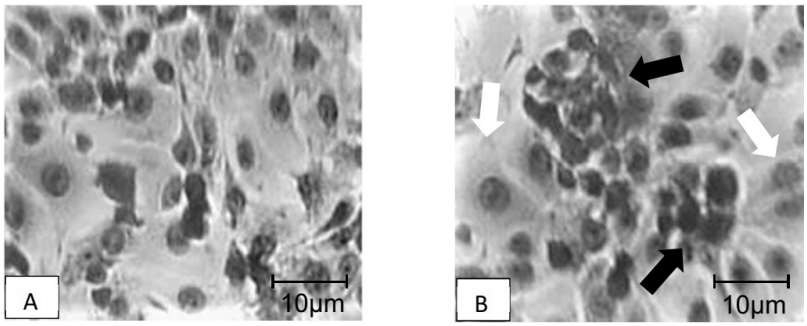

Figure 3.1. PK-15 cells 24 hours post-infection with PPV

Magnification: 100X under inverted microscope

(A) Uninfected PK-15 cells, serving as the negative control.

(B) PK-15 cells 24 hours post-infection with parvovirus. The white arrow indicates normal cells, the black arrow highlights virus-infected cells exhibiting nuclear clumping and morphological alterations.

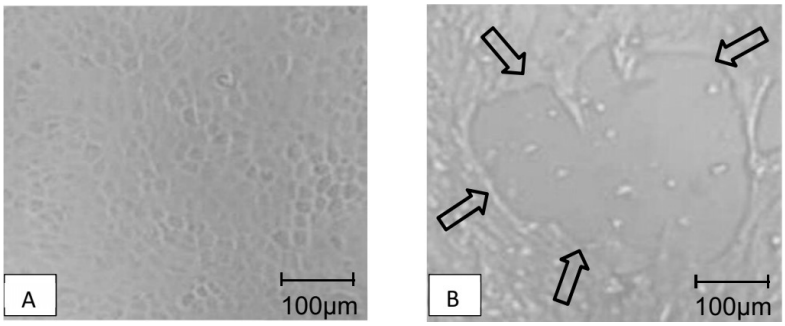

Figure 3.2. Cytopathic effects on PK-15 cells 5 days post-infection with PPV

Magnification: 10X under inverted microscope

(A) PK-15 cells remained uninfected after five days, serving as the negative control.

(B) PK-15 cells exhibiting cytopathic effects following five days of parvovirus infection. Arrows indicate virus-infected cells that have undergone lysis, leading to the formation of gaps on the surface of the culture flask.

All 32 PPV strains in this study exhibited cytopathic effects on PK-15 cells following infection.

❖ **Viral Propagation Kinetics During Cultivation**

Real-time PCR analysis demonstrated that all 32 PPV isolates replicated efficiently and stably in PK-15 cells, exhibiting a significant increase in viral copy number over three consecutive passages (P1–P3, $p < 0.001$). The $\log_{10}$ viral copy number increased from 2.73–5.13 at P1 to 4.53–7.00 at P3, indicating robust adaptation and sustained replication capacity. Tukey's post-hoc analysis identified isolates VC5 and TX7 as

achieving the highest viral loads, accompanied by pronounced cytopathic effects, reflecting rapid replication, genetic stability, and strong potential as master seed strains for studies on virulence, immunogenicity, and PPV vaccine development.

3.1.2. Parvovirus typing

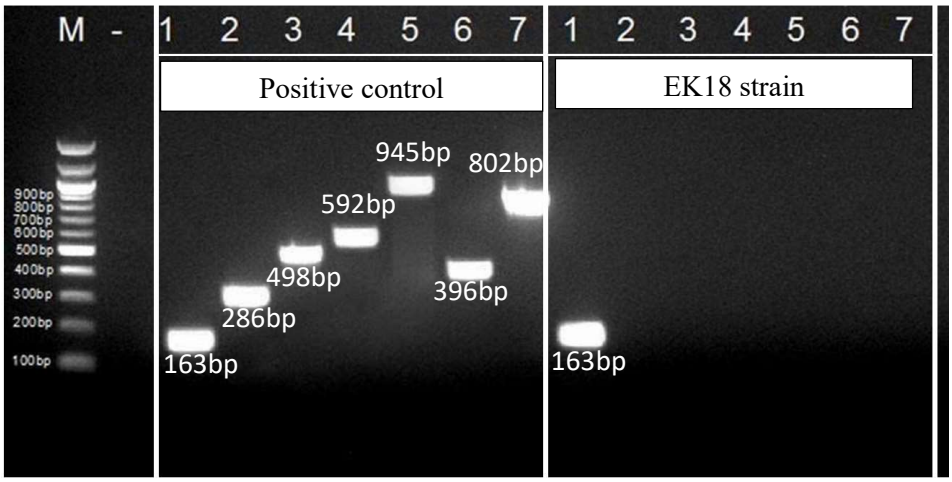

Figure 3.3. Typing of EK18 virus strain by PCR

M: 100 bp plus DNA ladder (Gold Biotechnology, USA); – : Negative control (PCR sample without template DNA); Positive controls 1–7: PCR samples with positive DNA from type 1 to type 7 (Parvovirus DNA provided by Kangwon University, Republic of Korea); EK18 virus strain 1–7: PCR samples with DNA of EK18 virus strain tested for type 1 to type 7.

The typing of PPV strains was conducted through analysis of PCR products on agarose gels. As illustrated in Figure 3.3, all positive control samples for the seven virus types exhibited distinct, well-defined DNA bands, with no nonspecific amplification, and band sizes fully consistent with the descriptions by Kim et al. (2022). The EK18 sample displayed a DNA band only when amplified with primers specific for PPV1, while no amplification products were detected with primers specific for PPV2 through PPV7. All 32 virus isolates in this study were identified as PPV1, with detailed results provided in Appendix 7.

3.1.3. Genetic Relationship Analysis of Parvovirus

3.1.3.1. Genetic Relationship Analysis Based on the NS1 Gene Sequence

Following the isolation of PPV strains on PK-15 cells, their genetic relationships were analyzed based on the NS1 gene sequence, a highly conserved gene with significant value in Parvovirus classification. The analysis revealed that all 32 isolates exhibited 100% identity in the NS1 sequence. Two representative strains, VC5 and TX7, were further compared with international reference strains. The PCR products were approximately 330 bp in size, sharp, and free of nonspecific bands. The nucleotide

sequences of VC5 and TX7 demonstrated high similarity (≥99%) to PPV1 strains from China and Brazil, with particularly high identity of 99.70% to PPV1-0225-L-SD (China, 2022).

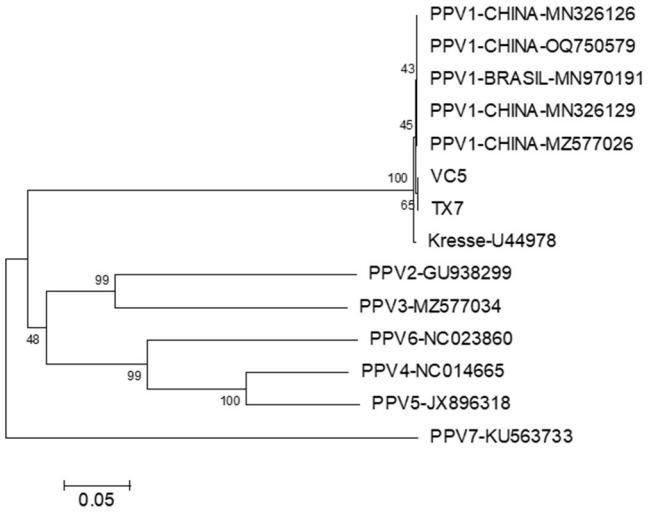

Figure 3.4. Phylogenetic Tree Based on NS1 Gene Sequence

The phylogenetic tree constructed from the NS1 gene sequence positioned VC5 and TX7 within the same cluster as international PPV1 strains, with a bootstrap value of 100, confirming the close evolutionary relationship and genetic stability of the PPV1 group. Other PPV types (PPV2–PPV7) formed distinct branches with considerable genetic distances, reflecting differences in genome structure and biological characteristics. Two outgroup viruses were included as distant controls to define evolutionary boundaries, thereby reinforcing the reliability of the analysis.

3.1.3.2. Genetic Relationship Analysis Based on the VP2 Gene Sequence

The VP2 gene, encoding the major capsid protein of PPV, plays a critical role in genetic classification and the assessment of evolutionary relationships. PCR successfully amplified a ~1.7 kb fragment, yielding specific products without any nonspecific bands. Sequence analysis of VP2 from the 32 PPV isolates revealed complete identity (100%), indicating a high level of genetic stability among strains from diverse geographic regions. Six representative strains, each from a different province, were selected and deposited in NCBI (OR263486–OR263491).

The phylogenetic tree constructed from the VP2 sequences demonstrated clear separation among PPV types (PPV1–PPV7) with high bootstrap support, confirming the robustness of the clustering model. All six Vietnamese isolates clustered within PPV1, forming a distinct branch closely related to international PPV1 strains. These findings

indicate that PPV1 circulating in Vietnam exhibits high genetic homogeneity, with no significant divergence observed in the VP2 gene. Vietnamese strains are closely related to those circulating in China, consistent with regional epidemiological trends. The absence of evidence for multiple distinct evolutionary lineages within the Vietnamese PPV1 population suggests that the risk of genetic variation compromising current vaccine efficacy remains low.

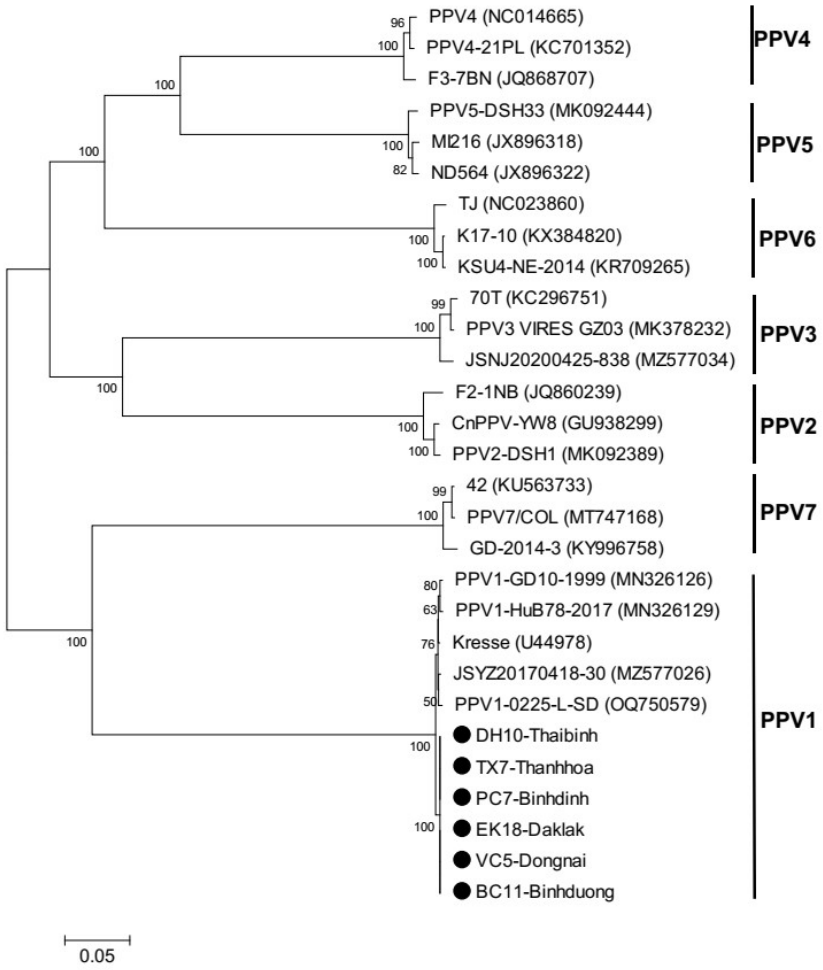

Figure 3.5. Phylogenetic Tree Based on VP2 Gene Sequence

3.1.4. Determination of Selected Biological Characteristics of Parvovirus Strains

3.1.4.1. Growth Curve Analysis of PPV on PK-15 Cells

The growth curves of the 32 PPV isolates on PK-15 cells demonstrated that viral replication increased sharply from day 1 to day 4 post-infection, reaching a peak on day 5 and maintaining a stable level through day 7. Based on these observations, day 5 was determined as the optimal time for virus harvest under standardized culture conditions

(MOI = 0.01; E-MEM supplemented with 5% FBS and 1% Penicillin–Streptomycin; incubation at 37°C with 5% CO_2).

3.1.4.2. Determination of $TCID_{50}$

Table 3.3. Determination of $TCID_{50}$ of PPV isolates

Location	Sample	Mean $TCID_{50}$/1ml (log10) ± SD (n = 3)	Tukey's mean comparison group* (95% CI, n = 3)
Thai Binh	DH2	5.1 ± 0.1	D
	DH4	4.6 ± 0.1	E
	DH11	5.4 ± 0.1	C
	DH15	4.5 ± 0.0	E
Thanh Hoa	KX24	6.1 ± 0.1	A, B
	TS8	4.5 ± 0.0	E
	TX7	**6.3** ± 0.0	**A**
	TX13	4.6 ± 0.1	E
Binh Dinh	PM21	5.1 ± 0.1	D
	PM27	4.5 ± 0.0	E
	PC7	5.5 ± 0.1	C
	PC15	4.5 ± 0.0	E
Dak Lak	EK18	4.5 ± 0.0	E
	EK20	4.5 ± 0.0	E
	EK27	5.5 ± 0.1	C
	EK31	4.6 ± 0.1	E
	MD22	4.6 ± 0.1	E
	MD28	4.6 ± 0.1	E
Dong Nai	**VC5**	**6.3** ± 0.0	**A**
	VC10	6 ± 0.1	B
	VC15	4.5 ± 0.0	E
	VC32	5.1 ± 0.1	D
	LT23	4.5 ± 0.0	E
	LT32	4.5 ± 0.0	E
	LT36	5 ± 0.0	D
Binh Duong	DA6	4.6 ± 0.1	E
	DA12	5.5 ± 0.1	C
	DA27	4.5 ± 0.0	E
	BC2	5 ± 0.0	D
	BC4	4.5 ± 0.0	E

Location	Sample	Mean $TCID_{50}$/1ml (log10) ± SD (n = 3)	Tukey's mean comparison group* (95% CI, n = 3)
	BC11	6 ± 0.1	B
	BC15	4.5 ± 0.0	E

** p-value* < 0.001

The determination of $TCID_{50}$ titers revealed that the PPV strains exhibited varying levels of replication, with log$TCID_{50}$/mL values ranging from 4.5 to 6.3. Statistical analysis confirmed significant differences among the isolates ($F = 222.24$; $p < 0.001$), and subsequent Tukey's post hoc test grouped the strains according to their corresponding titers. Notably, strains VC5 and TX7 achieved the highest titers (6.3 log$TCID_{50}$/mL), surpassing both the intermediate group (5.0–5.5 log$TCID_{50}$/mL) and the low-titer group (4.5–4.6 log$TCID_{50}$/mL).

3.1.4.3. Determination of Optimal Culture Conditions

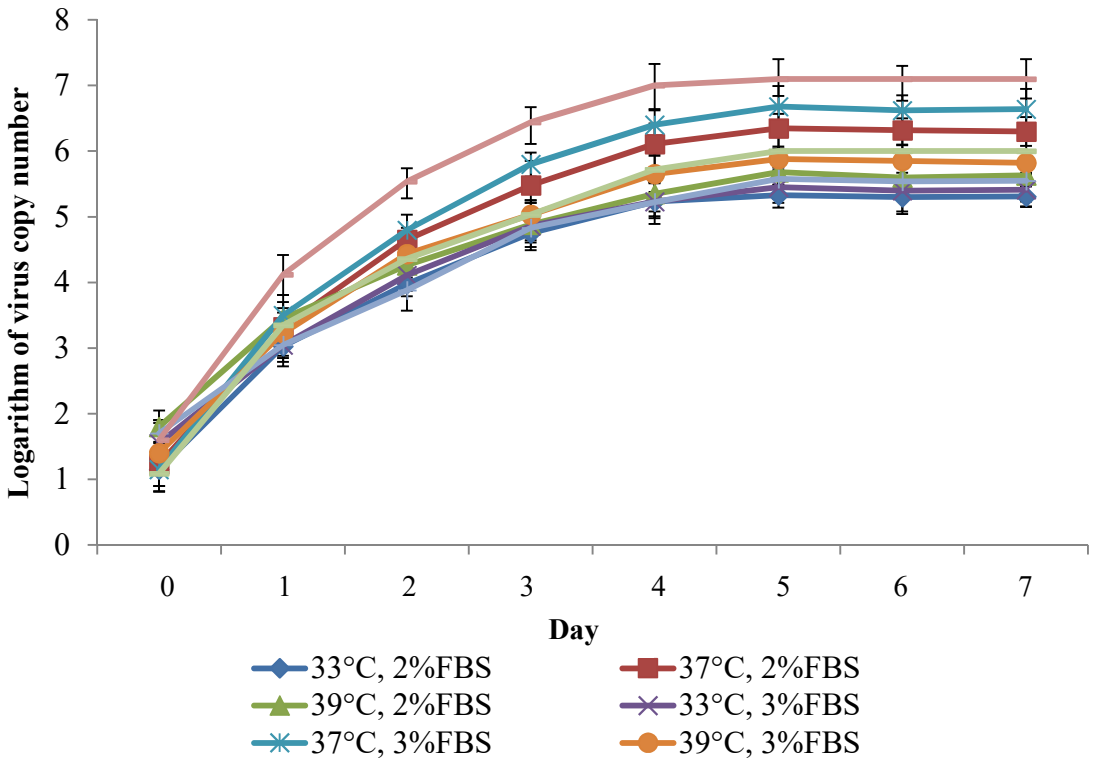

Figure 3.6. Assessment of Optimal Culture Conditions

Quantification of viral copy numbers by real-time PCR, combined with generalized linear model analysis, demonstrated that both temperature and FBS concentration significantly influenced PPV replication in PK-15 cells, with a notable interaction between the two factors ($P < 0.001$). Among them, temperature exerted the stronger

effect. A temperature of 37°C yielded the highest replication efficiency, significantly outperforming 33°C and 39°C. Likewise, 5% FBS supported markedly higher viral proliferation than 2% or 3%.

The optimal combination of culture conditions was identified as 37°C with 5% FBS, which produced the highest viral copy numbers. In contrast, other combinations—particularly 33°C with 2% FBS—resulted in the lowest replication levels.

3.1.5. Evaluation of the Stability, Virulence, and Immunogenicity of Strains VC5 and TX7

3.1.5.1. Assessment of the Stability of VC5 and TX7

a) Evaluation of Biological Stability

Cytopathic characteristics in cell culture: Observation of cytopathic effects (CPE) revealed that both VC5 and TX7 consistently induced stable CPE after 15 serial passages. The CPE were characterized by granulation, marked alterations in cell morphology, and extensive cell destruction, resulting in large clear areas on the culture surface.

Growth profiles across serial passages: The growth kinetics of TX7 and VC5 were highly consistent across passages, showing rapid viral replication from day 1 to day 4, peaking on day 5, and remaining stable through day 7. Viral copy numbers did not differ significantly among passages, indicating that both strains maintained stable replication capacity after 15 passages.

$TCID_{50}$ titers across passages: Determination of $TCID_{50}$ demonstrated that viral titers remained stable over 15 serial passages, consistently ranging from 6.3 to 6.5 $logTCID_{50}/mL$.

b) Evaluation of Genetic Stability

Stability of the NS1 gene: Sequence comparison of the NS1 gene across 15 passages showed no detectable changes, indicating complete conservation of the NS1 region.

Stability of the VP2 gene: Similarly, no sequence variation in the VP2 gene was observed for either VC5 or TX7 throughout 15 passages.

Taken together, these findings demonstrate that strains VC5 and TX7 exhibit stable biological and genetic characteristics across multiple passages. These attributes support their suitability as candidate strains for the development of PPV vaccines.

3.1.5.2. Assessment of the Virulence of Strains VC5 and TX7

Hemagglutination inhibition (HI) titers in pregnant sows fluctuated markedly over time and differed significantly among groups, with the effects of group, time, and group ×

time all reaching strong statistical significance ($p < 0.001$). Specific antibodies were first detected on day 7 post-infection, with mean titers of 4.67 log_2 (VC5) and 5.33 log_2 (TX7), whereas the control group remained at 0, indicating early viral replication and robust stimulation of the humoral immune response by both strains.

Titers peaked on day 21, reaching 10.67 log_2 for VC5 and 11.33 log_2 for TX7. Tukey's post hoc analysis indicated that TX7 elicited the strongest response, followed by VC5, while the control group produced no detectable antibodies. By day 49, titers declined modestly yet remained high (VC5: 9.67 log_2; TX7: 10.0 log_2).

Necropsy findings on day 90 revealed distinct differences in virulence: in the control group, 100% of fetuses were healthy, whereas all fetuses in the infected groups were nonviable. The rates of mummified fetuses were 80% in the VC5 group and 71% in the TX7 group, displaying characteristic features of PPV infection, including desiccation, dark-brown discoloration, and dehydration. The proportion of PPV-positive fetuses reached 88–90% in the infected groups, while all fetuses in the control group were negative.

3.1.5.3. Evaluation of the Immunogenicity of Strains VC5 and TX7

a) Immunogenicity in Guinea Pigs

The immunogenic potential of the PPV strains VC5 and TX7 was evaluated in guinea pigs ($n = 5$ per group) at a vaccine dose equivalent to one-quarter of that used in pigs, with the aim of identifying promising viral candidates for vaccine development. HI titers differed significantly among groups, numbers of doses, and sampling times ($p < 0.001$). After the first dose, antibody responses increased only modestly, ranging from 0.47 to 1.47 log_2; the mean titer in the single-dose group was 0.91 log_2 (Group B), markedly lower than that of the two-dose group (3.53 log_2, Group A).

Following booster immunization, HI titers increased substantially: immediately before the second dose, titers reached 3.6 log_2 (TX7) and 3.2 log_2 (VC5), subsequently rising to 6.2–7.6 log_2 (TX7) and 5.6–7.4 log_2 (VC5) on days 14–21, corresponding to peak immune responses. Throughout the experiment, no clinical abnormalities or local injection-site reactions were observed in any animals, confirming the safety of both strains in an alternative animal model.

b) Immunogenicity in Pigs

HI titers in pigs increased progressively with the number of vaccine doses and over time. After the first immunization, mean HI titers reached 1.44 log_2 (VC5) and 1.56 log_2

(TX7), rising sharply after the booster and peaking on day 21 at 7.33 $\log_2$ (VC5) and 7.67 $\log_2$ (TX7), while the control group consistently remained at 0. Statistical analysis confirmed significant effects of treatment group, dose number, and sampling time ($p < 0.001$).

Upon challenge, fetal survival rates were 18/22 (81.82%) in the VC5 group and 18/21 (82.35%) in the TX7 group, whereas all fetuses in the control group were nonviable, displaying typical PPV-associated lesions: mummification in 75% of fetuses, characterized by dryness, firmness, dark-brown discoloration, and reduced size. PPV was detected in 90% of fetuses in the control group, compared with only 2/22 (9.09%) and 2/21 (9.52%) in the VC5 and TX7 groups, respectively.

ELISA results further supported the development of strong humoral responses. After the first dose, PI values increased but remained below the positivity threshold; following the booster, PI exceeded 50% and reached peak levels on day 21 (TX7: 72.48%; VC5: 71.51%). Statistical analysis revealed significant effects of treatment group, dosage, and sampling time ($p < 0.001$); booster immunization generated significantly higher PI values than the primary dose (36.74 vs. 17.62; $p < 0.05$), and day 21 yielded the highest PI values (41.94; $p < 0.05$). No significant difference was observed between TX7 and VC5.

Collectively, these findings demonstrate that VC5 and TX7 exhibit high virulence and strong immunogenic potential. Both strains represent promising candidates for further vaccine development.

3.2. Discussion

3.2.1. Prevalence of PPV in Vietnam

The present study determined that the prevalence of PPV in breeding sows in Vietnam was 8.16%, with relatively uniform distribution across regions and no apparent influence of geographical factors. This prevalence is markedly lower than reports from the United States, Canada, India, and Argentina, as well as earlier studies conducted in Vietnam. Such discrepancies are primarily attributable to differences in sampling populations, husbandry conditions, and sample types. Specifically, the current investigation focused exclusively on commercial breeding sows and included serum samples, whereas many international and domestic studies predominantly examined aborted fetuses or herds with concurrent PCV2 infection—an important factor known to increase PPV detection rates.

All PPV-positive samples were identified as genotype PPV1, which is consistent with reports from multiple countries and previous Vietnamese studies on herds exhibiting reproductive disorders. The absence of newly emerging genotypes (PPV2–PPV7), contrary to findings from slaughterhouse-based investigations or farms with co-infections, highlights the precision in selecting the appropriate target population (breeding sows with reproductive abnormalities) and underscores the high reliability of the sampling and diagnostic procedures. These procedures were performed under strictly aseptic conditions, thereby minimizing the risk of contamination or concurrent infection and ensuring the accuracy of genotype identification.

3.2.2. Biological and Genetic Characteristics of the Isolates

Analysis of the NS1 gene revealed that the Vietnamese PPV1 isolates shared a high nucleotide identity (99.3–99.7%) with PPV1-0225-L-SD and PPV1-GD10-1999 from China, indicating a close phylogenetic relationship and strong genetic conservation within the East Asian region. The VP2 gene sequences of the isolates were classified as "Kresse-like," showing high similarity to the Kresse strain (Canada, 1996). Strains within this group are known for their robust immunogenicity and effective cross-neutralization, highlighting their potential utility in the development of next-generation vaccines.

On PK-15 cells, the PPV1 isolates induced characteristic cytopathic effects, including cell deformation, nuclear condensation, and cytoplasmic inclusion bodies, confirming their strong infectivity and replication competence. Replication kinetics demonstrated that all 32 isolates exhibited rapid viral amplification from days 1–4, reached peak titers on day 5, and maintained these levels through day 7 at an MOI of 0.01. The infectious titers ranged from 4.5 to 6.3 $\log TCID_{50}$/mL, with VC5 and TX7 achieving 6.3 $\log TCID_{50}$/mL—comparable to or exceeding those of PPV strains used in international vaccine studies (Van den Born et al., 2020; Zeew et al., 2007; Foerster et al., 2016).

Optimal culture conditions were identified as 37°C with 5% FBS, consistent with previous observations (Zhang et al., 2015; Wang et al., 2024). These conditions ensured efficient viral replication, biological stability, and high reliability in assessing virulence and immunogenicity.

Collectively, the findings demonstrate that Vietnamese PPV1 isolates are genetically stable, replicate efficiently in PK-15 cells, reach maximal replication on day 5, and exhibit optimal adaptation at 37°C with 5% FBS. Notably, VC5 and TX7 showed high

$TCID_{50}$ titers, stable biological characteristics, and close genetic relatedness to international vaccine strains, underscoring their strong potential as candidate seed strains for PPV vaccine development in Vietnam.

3.2.3. Potential Application of VC5 and TX7 in the Development of PPV Vaccines

The PPV strains VC5 and TX7 exhibited stable biological characteristics in PK-15 cells, maintaining typical cytopathic effects—including cellular deformation, nuclear condensation, and cytoplasmic inclusions—across 15 serial passages, with infectious titers reaching 6.3–6.5 log $TCID_{50}$/mL. Sequencing of the NS1 and VP2 genes revealed no mutations after 15 passages, confirming their genetic stability and compliance with international criteria for vaccine seed strains.

Challenge experiments conducted on pregnant sows at day 40 of gestation demonstrated uniformly fatal outcomes in all fetuses of the infected groups, with 71% (TX7) and 80% (VC5) exhibiting classical mummification. Viral DNA was detected in 88–90% of fetal samples. When compared with internationally recognized reference strains (PPV-27a, PPV-IDT, PPV-NADL-2), VC5 and TX7 displayed high virulence and characteristic pathological features, supporting their suitability for the development of potent inactivated vaccines.

Both strains induced strong immune responses in guinea pigs and pregnant sows. In sows, following two immunizations, HI titers peaked on day 21 at 7.33 log_2 (VC5) and 7.67 log_2 (TX7), with ELISA PI values exceeding 70%, surpassing the accepted threshold for protective immunity. In guinea pigs, booster immunization generated HI titers of 7.4–7.6 log_2, demonstrating robust secondary immune responses and validating their utility as an alternative model for vaccine strain screening.

Protective efficacy following challenge was high: fetal survival rates reached 81.82% (VC5) and 85.71% (TX7), whereas all fetuses in the control group died and 90% tested positive for PPV. These outcomes were comparable to, or exceeded, those reported for several commercial PPV vaccines and international studies (van den Born et al., 2020; Kiss et al., 2020; Noguera et al., 2021), underscoring the strong potential of VC5 and TX7 as seed strains for inactivated PPV vaccine production in Vietnam.

Taken together, VC5 and TX7 fulfill all essential criteria regarding biological and genetic stability, virulence, and the ability to induce strong immune responses, positioning them as highly promising candidates for the development of PPV vaccines targeting reproductive disorders caused by porcine parvovirus.

CONCLUSION AND RECOMMENDATIONS

1. Conclusions

This study provides comprehensive epidemiological, genetic, and biological data on PPV circulating in Vietnam and successfully identifies two promising indigenous strains for vaccine development. The major findings are as follows:

- Virus isolation**:** Thirty-two PPV strains were successfully isolated from clinical samples collected across six major provinces. All isolates produced characteristic CPE in PK-15 cells and were confirmed by PCR.
- Virus typing: All 32 isolates were classified as PPV1, with no detection of emerging genotypes (PPV2–PPV7), confirming PPV1 as the predominant circulating genotype in Vietnam.
- Genetic relationships: NS1 and VP2 sequences were highly conserved (100% identity among isolates). Phylogenetic analysis of VP2 revealed close clustering with the Kresse strain (Canada) and PPV1-0225-L-SD (China), indicating a stable evolutionary origin within the Asia–Pacific region.
- Biological characteristics: Optimal virus propagation was achieved in E-MEM supplemented with 5% FBS at 37°C with 5% CO_2. Viral replication increased rapidly from days 1–4 and peaked at 4.5–6.3 log $TCID_{50}$/mL on day 5. The VC5 and TX7 strains exhibited rapid replication kinetics, high and stable titers, and were selected for in-depth analyses.
- Stability, virulence, and immunogenicity: VC5 and TX7 remained genetically stable for ≥15 passages, with no detectable mutations in NS1 or VP2. In pregnant sows, both strains caused 100% fetal mortality, with 71–80% of fetuses showing typical SMEDI lesions. Following two doses of inactivated vaccine, HI titers reached 7.3–7.6 $\log_2$ in both guinea pigs and sows. Challenge experiments demonstrated high protective efficacy, with fetal survival rates of 81.82–85.71%, whereas all fetuses in the control group died and 90% tested positive for PPV.

Overall, VC5 and TX7 represent genetically stable local strains with characteristic virulence and strong immunogenicity, fulfilling key criteria for use as seed strains in the development of inactivated PPV vaccines in Vietnam.

2. Recommendations

To further refine the research framework and support the application of VC5 and TX7 in vaccine development, the following steps are recommended:

- Advanced characterization of viral properties: Conduct ultrastructural analysis using TEM, perform whole-genome sequencing, model the three-dimensional structure of VP2, and evaluate the effects of potential mutations on protein conformation and immunogenicity.
- Optimization of vaccine seed evaluation protocols: Determine optimal storage conditions and long-term viral stability; assess antibody persistence and booster schedules; evaluate different adjuvant formulations; and compare immune responses and protective efficacy with commercial PPV vaccines.

Implementing these activities will enhance the scientific evidence base, enable a comprehensive assessment of the selected strains, and support the development of safe, effective PPV vaccines tailored to the needs of Vietnam's swine industry.

NOVEL CONTRIBUTIONS OF THE DISSERTATION

The dissertation provides several novel findings that contribute to the field of parvovirus isolation and the selection of potential strains for vaccine development, as follows:

1. The study successfully isolated Porcine Parvovirus strains currently circulating in Vietnam.

2. The research systematically identified both the biological and genetic characteristics of PPV strains isolated in Vietnam, thereby providing a comprehensive understanding of the currently circulating strains in the country.

3. Two PPV strains with stable biological and genetic characteristics over multiple passages, high virulence, and strong immunogenicity were successfully selected. These strains constitute promising candidates for the development of vaccines against porcine mummification. Importantly, no vaccine against porcine mummification is produced or commercialized domestically in Vietnam— all available porcine parvovirus vaccines are imported—the outcomes of this study establish a scientific foundation for the development of an indigenous PPV vaccine, addressing a critical gap in veterinary disease prevention and control in Vietnam.

LIST OF PUBLICATIONS

1) TT Hang Trinh, V. Tan Do, V. Khien Do, and Hung Vu-Khac, (2024), “Isolation and characterization of porcine parvovirus in Vietnam”, Veterinary World, 17(7), pp. 1530–1537.

2) Trinh Thi Thu Hang, Vu Khac Minh Duong, Do Van Tan, Pham Trung Hieu and Vu Khac Hung, "Determination of some biological characteristics and immunogenicity evaluation of two parvovirus isolates in Vietnam", Vietnam Journal of Science and Technology, version B (Acceptance letter)