Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

ISSN:3062-357X

2025, Volume 5, Issue 1, Page No: 173-182

Available online at: www.esvpub.com/

Baculovirus-Derived Virus-Like Particles from a Chinese Epidemic FPLV Strain (Ala91Ser, Ile101Thr): Enhanced Immunogenicity and Complete Protective Efficacy in Cats

Ming Liu[1], Wei Wang[1]*, Lei Zhang[1], Na Li [1]

[1]Laboratory of Animal Virology, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei, China.

***E-mail** ✉ wangwei47@163.com

ABSTRACT

Feline panleukopenia (FPL), resulting from infection with the feline panleukopenia virus (FPLV), is an extremely contagious and life-threatening viral disease characterized by high infection and death rates. Immunization continues to be the most reliable measure for limiting the spread and severity of the disease. The viral capsid protein VP2 is the dominant antigen of FPLV and serves as the principal focus for the design of improved vaccines. Recently, virus-like particle (VLP) technology has gained attention as a next-generation vaccine platform due to its excellent immune-stimulating ability and favorable safety characteristics. In the present research, FPLV-VLPs were constructed using a baculovirus expression vector system (BEVS) to express the VP2 protein derived from a Chinese epidemic isolate (Ala91Ser, Ile101Thr) of FPLV. The assembled particles displayed increased antigen recognition and hemagglutination capacity, producing a hemagglutination titer of 1:216. After immunization, cats generated high anti-FPLV hemagglutination inhibition (HI) antibody titers (1:216) and exhibited complete (100%) resistance when challenged with a virulent Chinese field strain (Ala91Ser, Ile101Thr). These results suggest that FPLV-VLPs could serve as effective subunit vaccine candidates for protection against FPLV infection.

Keywords: Feline panleukopenia, FPLV, Baculovirus vector, Virus-like particle, Vaccine development

Received: 15 January 2025
Revised: 16 April 2025
Accepted: 18 April 2025

How to Cite This Article: Liu M, Wang W, Zhang L, Li N. Baculovirus-Derived Virus-Like Particles from a Chinese Epidemic FPLV Strain (Ala91Ser, Ile101Thr): Enhanced Immunogenicity and Complete Protective Efficacy in Cats. Int J Vet Res Allied Sci. 2025;5(1):173-82. https://doi.org/10.51847/CKoRXsNglK

Introduction

Feline panleukopenia (FPL), caused mainly by the feline panleukopenia virus [1], represents one of the earliest documented parvoviral diseases in members of the cat family [2]. The virus is distributed worldwide, infecting nearly all susceptible feline populations, with morbidity surpassing 90% in unvaccinated groups and fatality rates ranging from 50% to 90% among kittens [3]. Immunization is still the most practical and economical method to control and prevent this disease [4, 5].

In China, however, vaccination coverage remains limited, as many cat owners decline inoculation because of high costs or insufficient awareness. The only available commercial option is Fel-O-Vax® PCT, an imported inactivated multivalent vaccine licensed for sale in the country. The shortage of locally produced vaccines restricts stable progress in China's rapidly expanding pet industry.

The introduction of virus-like particles (VLPs) has reshaped vaccine design by linking molecular virology and immunology [6]. These VLPs are formed by the self-assembly of one or more viral structural proteins [7]. Unlike attenuated or inactivated vaccines, they maintain a virus-like morphology but lack genomic material, eliminating

the risk of replication [8]. Their repetitive antigenic surfaces also trigger potent immune responses, setting them apart from other subunit-based formulations [9, 10].

Numerous host systems can be employed for VLP synthesis, allowing considerable flexibility for scalable production [8]. Among them, the baculovirus expression system (BES) has become a preferred approach because it supports correct post-translational processing (such as glycosylation) and is cost-efficient for large-scale application [8, 11].

The present study reports, for the first time, the use of BES to produce VLPs carrying the VP2 protein from a Chinese epidemic strain (Ala91Ser) of FPLV. The structural and immunological characteristics of these particles were evaluated. The obtained results provide essential data for the creation of new-generation FPLV vaccines and potentially contribute to the development of broad-spectrum parvovirus vaccines, including those for canine parvovirus (CPV) and porcine parvovirus (PPV).

Materials and Methods

Virus and cell lines

The feline panleukopenia virus strain designated FPLV-CC19-02 was isolated in Changchun City, Jilin Province, from a cat exhibiting acute diarrhea. This isolate carries an Ala91Ser substitution in the VP2 gene and has been circulating among domestic cats in China since 2017 [12, 13]. Its full-length genome sequence is accessible under GenBank accession number OR921195.1. Spodoptera frugiperda (Sf9) cells were maintained in Sf9-900 II SFM medium (Gibco, Waltham, MA, USA) at 28 °C. Cellfectin™ II (Gibco, Beijing, China) was used for transfection, and the BacPAK Baculovirus Rapid Titer Kit (Takara, Dalian, China) was used for viral quantification. The anti-CPV-2c-VP2 monoclonal antibody (clone 5B18) was produced in our laboratory.

Construction of recombinant baculoviruses

The nucleotide sequence encoding the VP2 protein from FPLV-CC19-02 (OP471917.1) was optimized for Spodoptera frugiperda 9 (Sf9) cell expression and synthesized by Nanjing Zoonbio Biotech Co., Ltd. (Nanjing, Jiangsu, China). The primers employed were: VP2-F, 5′-GATTATTCAACCGTCCCACCATCGGGCGCGGATCCGCCACCATGCTGCTGGTGAACAGAGCCACCAG-3′, and VP2-R, 5′-GCTGATTATGATCCTCTAGTACTTCTCGACAAGCTTTTAGGCGTAGTCAGGCACGTCGTAGGGGTAGTA-3′. The optimization aimed to enhance transcriptional and translational efficiency in insect cells.

The amplified gene was integrated into the donor plasmid pFastBac through homologous recombination. Successful insertion was verified by dual restriction digestion (BamH I and Hind III) and PCR analysis. The resulting recombinant plasmid (pFastBac-FPLV-VP2) was introduced into E. coli DH10Bac competent cells to generate the recombinant bacmid (rBacmid-FPLV-VP2). Positive recombinant colonies were screened using blue–white selection and confirmed via PCR with M13 primers. The bacmid DNA was purified using the Endo Free Plasmid Maxi Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol.

Recovery of recombinant baculoviruses

A suspension of Sf9 cells (5×10^6 cells/mL) was distributed into six-well culture plates at 2 mL per well and allowed to adhere for 12 h at 27 °C. To prepare the transfection mix, 4 µg of bacmid-FPLV-VP2 DNA and 8 µL of Cellfectin™ II (Gibco, Baltimore, MD, USA) were each diluted in 100 µL of antibiotic- and serum-free Grace's medium and pre-incubated for 30 min at 37 °C. Both solutions were then combined carefully and left for another 30 min at room temperature before being overlaid onto the cells. The inoculated cultures were maintained for 4 h at 28 °C, the mixture was removed, and replaced with 2 mL of fresh Sf-900 II SFM. After two rounds of amplification, characteristic cytopathic signs such as cell swelling and detachment confirmed the successful generation of the recombinant virus.

Immunofluorescent verification of recombinant baculoviruses

Sf9 cell suspensions (2×10^6 cells/mL) were seeded into six-well plates and infected with fourth-passage recombinant baculovirus stocks. Parallel uninfected cultures served as negative controls. After 48 h, the medium was discarded and cells were rinsed three times (5 min each) with PBST. Fixation was performed using 4% paraformaldehyde for 45 min at ambient temperature, followed by permeabilization with 0.5% Triton X-100. The

cells were washed again and blocked with 5% skim milk at 37 °C for 1 h. Primary labeling was carried out with an in-house monoclonal antibody against CPV-2c (clone 5B18, 1:200) for 1 h at 37 °C. After three PBST washes, FITC-conjugated goat anti-mouse IgG (H + L) (1:2000) was applied for another 1 h at 37 °C. Slides were washed repeatedly and examined on a Leica AF 6000 fluorescence microscope (Leica Microsystems, Wetzlar, Germany).

Assembly and Isolation of FPLV-VP2 virus-like particles

For large-scale production, 100 mL of an Sf9 suspension containing 5×10^8 cells was inoculated with fourth-generation recombinant baculovirus at a multiplicity of infection (MOI) of 1. Cultures were maintained at 28 °C for 4–5 days and then centrifuged at 8000 × g for 20 min (rotor F-34-6-38, Eppendorf, Germany). The pellet was resuspended in 25 mM $NaHCO_3$ and incubated at 4 °C for 2 h. Following a second centrifugation, the clarified supernatant containing the VLPs was collected. Expression of VP2 was verified by SDS-PAGE and immunoblotting with the laboratory-produced anti-CPV-2c monoclonal antibody (5B18).

Determination of FPLV-VP2-VLP antigenicity

Antigen recognition of the purified VP2 particle preparation (sample P4) was verified using commercial feline panleukopenia virus antigen test strips supplied by SHANGHAI QUICK-ING Biotech Co., Ltd. (Shanghai, China).

Measurement of hemagglutination activity

The hemagglutination property of the VP2 VLPs was quantified via a standard HA test [14] in 96-well V-bottom plates. Each well of rows A–G and columns 1–4 received 25 µL of 0.1 M PBS (pH 6.4). Twenty-five µL of the VLP suspension was added to the first well, then serial two-fold dilutions were prepared ($1:2^1$ to $1:2^{20}$). PBS (25 µL) was added to every test well, followed by 50 µL of 1% porcine red-blood-cell suspension. Plates were agitated at 150 rpm for 2 min and left at 4 °C for 45 min. Agglutinated wells formed diffuse mats (positive), whereas sharply defined pellets were considered negative. The hemagglutination titer corresponded to the reciprocal of the final dilution, showing visible clumping.

Visualization of FPLV-VP2-VLPs by transmission electron microscopy

The structure of the purified VP2 particles (P4) was observed by transmission electron microscopy (TEM) after negative staining as outlined by Gao *et al*. [15]. One milliliter of VLP suspension was mixed with 20 µL of $CaHPO_4$ solution, vortexed, and kept for 10 min at room temperature. The mixture was centrifuged at 15, 000 rpm for 15 min, and the sediment was resuspended in 15 µL of EDTA-saturated solution. A drop of the sample was placed on a copper grid for 20 min, stained with 2% phosphotungstic acid (pH 6.8) for 1 min, blotted, and air-dried. The samples were analyzed using a JEOL 2010 TEM (JEOL Ltd., Tokyo, Japan) operated at 100 kV.

Immunization and challenge in cats

Twelve British Shorthair cats, confirmed to be seronegative and aged 3–4 months, were randomly allocated into four experimental sets. Group I received the VLP-based vaccine blended with Seppic adjuvant (9:1); Group II was inoculated with the inactivated FPLV formulation using the same adjuvant ratio; Group III was treated with Fel-O-Vax® PCT; and Group IV served as the control, injected with minimum essential medium (MEM). Each cat was given 1.0 mL of its respective preparation through a single dose.

Serum was drawn from all animals on days 0, 7, 14, 21, 24, 28, 35, 42, and 50 after vaccination to evaluate anti-FPLV antibody titers using a hemagglutination inhibition (HI) assay.

At three weeks post-immunization, all cats were orally exposed to 5 mL of the FPLV-CC19-02 strain (HA = 2^{10}, $TCID_{50} = 10^{6.5}$/mL). Each cat was monitored for 10 consecutive days post-challenge (dpc) for changes in activity level, appetite, hydration, fecal quality, and body temperature. These daily records were used to determine the protective immune response generated by each vaccine type and to compare their overall efficacy against infection with FPLV-CC19-02.

For safety assessment, muscle tissue from the injection site of both control and vaccinated animals was excised, fixed in 10% paraformaldehyde, processed into paraffin blocks, sectioned, and stained with hematoxylin and eosin (HE) for microscopic examination. Before tissue sampling, every cat—whether clinically normal or deceased—

was humanely euthanized using propofol (0.6 mg/kg; Jiabo Co., Ltd., Guangdong, China) followed by potassium chloride (100 mg/kg; MACKLIN Co., Ltd., Shanghai, China) via intravenous administration.

Data analysis

Collected values were processed using GraphPad Prism v9.5 (GraphPad Software, San Diego, CA, USA). Results are shown as mean ± standard deviation (SD). Variations among three or more datasets were compared using a one-way ANOVA test.

Results and Discussion

Assembly and confirmation of recombinant bacmid-VP2

A DNA segment of roughly 2000 base pairs (bp) was amplified through overlap-extension PCR, as illustrated in **Figure 1a** (lanes 1–2). The VP2 gene was inserted into the pFastBac-I donor plasmid via homologous recombination to yield pFastBac-VP2, which was confirmed by PCR (~2200 bp; **Figure 1b**, lanes 1–8) and restriction digestion showing fragments of 926 bp and 4671 bp (**Figure 1c**, lane 2). The recombinant plasmid was transformed into E. coli DH10Bac cells to generate the bacmid-VP2. Detection of an amplicon around 4100 bp (**Figure 1d**, lanes 3, 5–10) verified successful recombination.

Characterization of FPLV-VP2 virus-like particles

From SDS-PAGE results, a prominent band corresponding to ~70 kDa appeared in the supernatant of $NaHCO_3$-treated, infected Sf9 cells (**Figure 2a**, lane 2). Immunoblotting further confirmed intracellular expression of FPLV-VP2-VLPs (**Figure 2b**, lane 2, indicated by arrows).

Transmission electron microscopy (TEM) visualized abundant spherical particles, 20–30 nm in diameter, highly similar in shape to native FPLV virions (**Figure 2c**). Even after serial dilution from 100 to 10^{-3}, these VLPs remained reactive in the FPLV antigen rapid detection test, demonstrating strong antigenic stability (**Figure 2e**). The hemagglutination (HA) titer of FPLV-VP2-VLPs (P4) ranged from $1:2^{14}$ to $1:2^{16}$, indicating robust hemagglutinating capacity (**Figure 2d**).

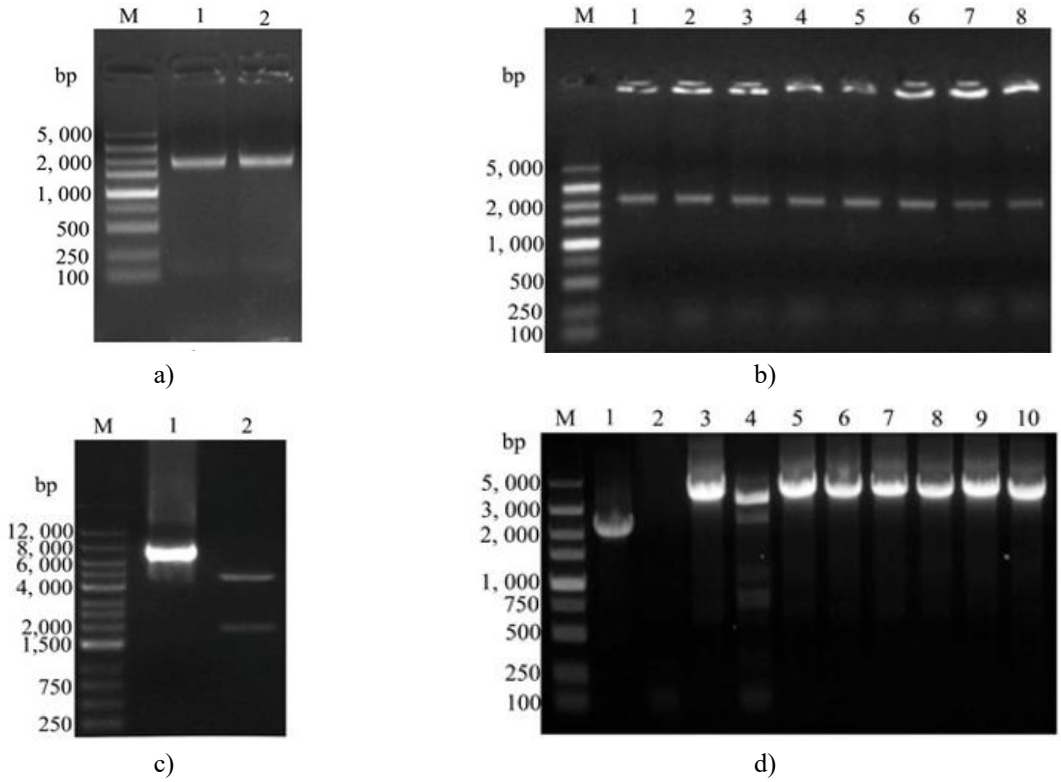

Figure 1. Construction and validation of recombinant bacmid-VP2. (a) Amplified VP2 fragment of FPLV. M: DL 5000 marker; lanes 1–2: PCR product. (b) Screening of positive colonies carrying recombinant plasmids. M: DL 5000 marker; lanes 1–8: transformants from LB medium. (c) Enzymatic digestion of

pFastBac-FPLV-VP2. M: DL 12, 000 marker; lane 1: uncut plasmid; lane 2: product after BamHI and HindIII digestion. (d) PCR verification of bacmid-VP2. M: DL 5000 marker; lane 1: positive control; lane 2: negative control; lanes 3, 5–10: recombinant clones.

Indirect immunofluorescence assay

When Sf9 cells were exposed to the recombinant baculovirus expressing FPLV-VP2-VLP, they became visibly enlarged and emitted strong green fluorescence (**Figures 3b and 3d**). In contrast, cells in the non-infected control group showed neither cytopathic effects (CPE) nor fluorescence (**Figures 3a and 3c**). These results verify that infection with the recombinant baculovirus successfully triggered correct synthesis of the FPLV-VP2 protein in Sf9 cells.

Variation of hemagglutination inhibition (HI) antibody following immunization

The progression of hemagglutination inhibition (HI) antibody titers in all cats after vaccination and challenge is summarized in **Figure 4**. At 7 days post-vaccination (dpv), HI antibodies became measurable in two animals belonging to Group II (FPLV inactivated formulation, red line/scatter). By 14 dpv, both Groups I (green line/scatter) and II (red line/scatter) had developed markedly higher antibody titers. At 21 dpv, increased HI levels were evident in all immunized groups except the control (black line/scatter). After the challenge, antibody titers in surviving cats continued to rise, reaching their maximal levels unless death occurred due to infection.

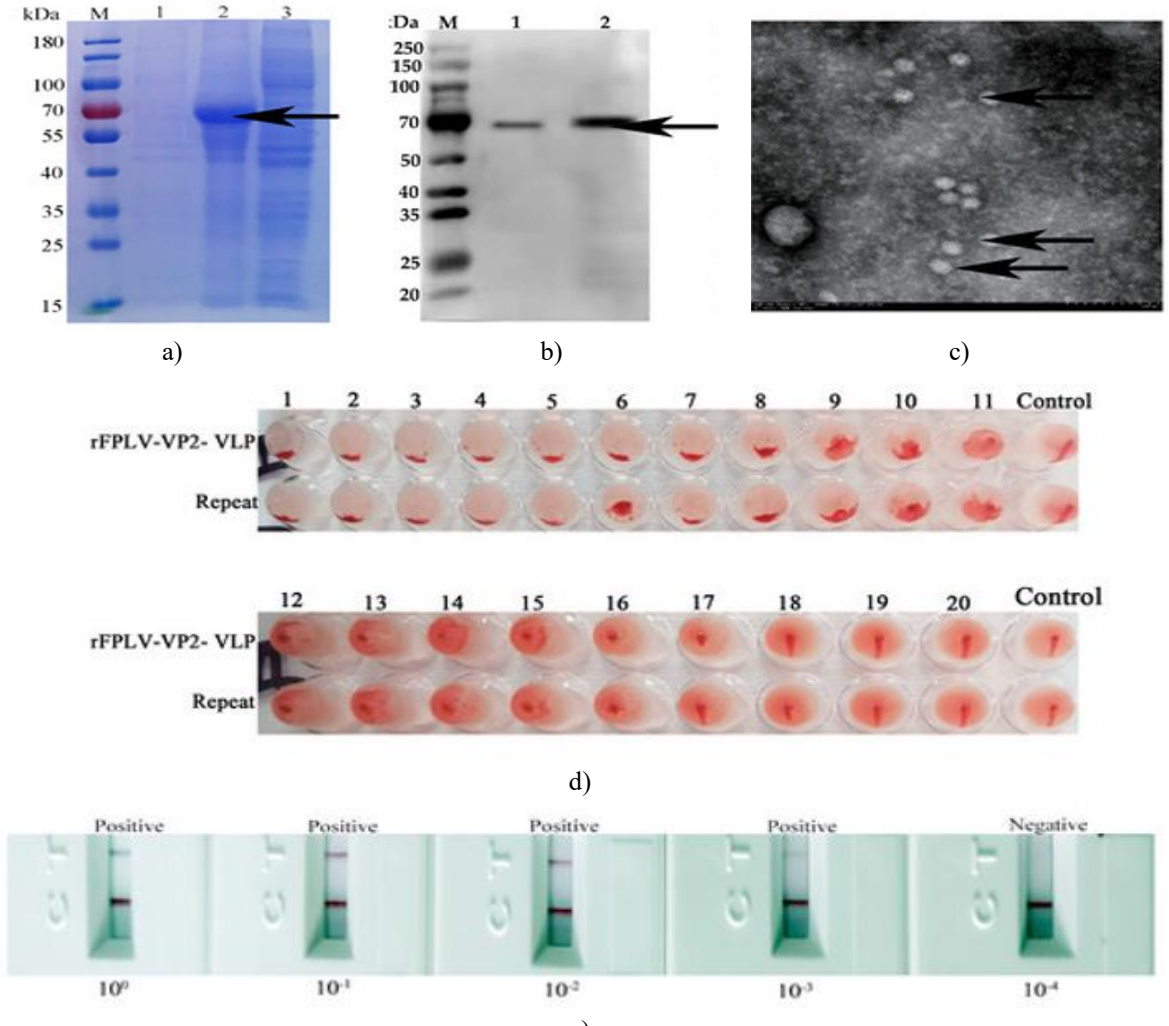

Figure 2. Characterization of FPLV-VP2-VLPs. (a) SDS-PAGE of FPLV-VP2-VLPs. M: PageRuler pre-stained marker; lane 1: supernatant of $NaHCO_3$-treated uninfected Sf9 cells; lane 2: supernatant of infected Sf9 cells; lane 3: pellet of infected Sf9 cells. (b) Immunoblot of FPLV-VP2-VLPs. M: marker; lane 1: pellet of infected Sf9 cells; lane 2: supernatant of infected Sf9 cells. (c) TEM image of FPLV-VP2-VLPs (4000×).

(d) Hemagglutination assay of FPLV-VP2-VLP protein. (e) Antigenicity evaluation of FPLV-VP2-VLP protein.

Clinical observations after viral challenge

Protective outcomes for each group are listed in **Table 1**. Both the VLP-based and the inactivated FPLV vaccines formulated with Seppic adjuvant conferred full (100%) protection to cats. In contrast, the commercial vaccine (Fel-O-Vax® PCT) achieved only 33% protection, while every cat in the unvaccinated control group died within 3–8 days post-challenge (dpc).

Table 1. Protective efficacy in each experimental group.

Groups	Vaccine	Number of Cats	Number of Diseased Cats	Protection Rate
Group I	FPLV-VLP vaccine (Seppic adjuvant)	3	0	3/3 (100%)
Group II	FPLV killed vaccine (Seppic adjuvant)	3	0	3/3 (100%)
Group III	Commercial vaccine (Fel-O-Vax® PCT)	3	2	1/3 (33%)
Group IV	Minimum essential medium	3	3	0/3 (0%)

Post-challenge temperature patterns are illustrated in **Figure 5**. About 67% of cats receiving the commercial vaccine (Group III, pink line/scatter) developed fevers exceeding 40 °C. Body temperatures in Groups I (red line/scatter) and II (green line/scatter) remained within the normal physiological range. In the control group, temperature initially dropped and later spiked above 40 °C, with several animals becoming hypothermic prior to death (pink line/scatter).

Following exposure to the virus, cats in Groups III and IV showed typical feline panleukopenia (FPL) signs—including fever, lethargy, vomiting, diarrhea, loss of consciousness, and death (**Table 2**)—while those in Groups I and II exhibited no symptoms.

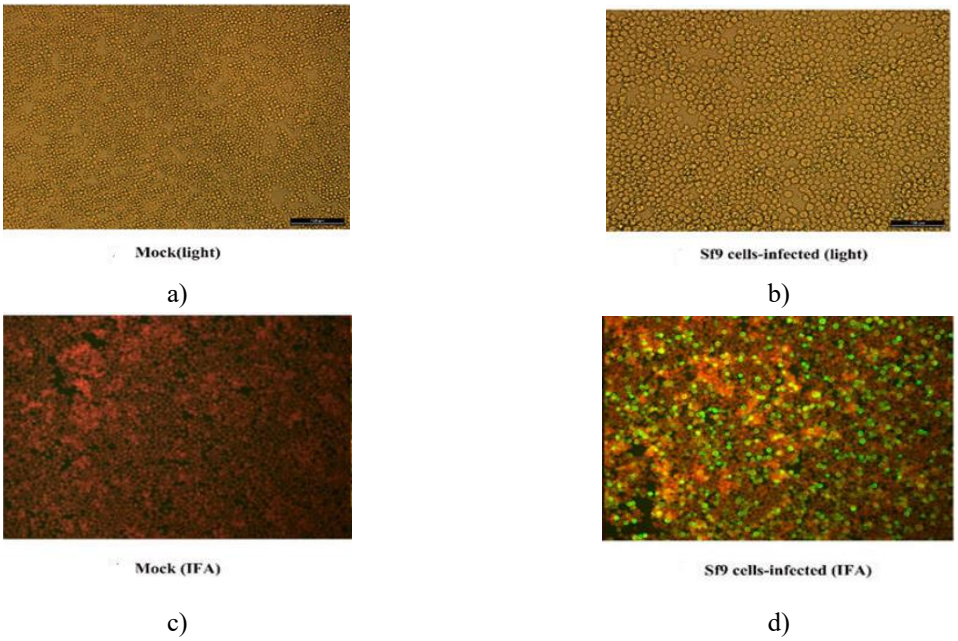

Figure 3. Indirect immunofluorescence assay (IFA) of recombinant FPLV-VP2. (a) Uninfected Sf9 cells (light field); (b) infected Sf9 cells (light field); (c) uninfected Sf9 cells (IFA); (d) infected Sf9 cells (IFA).

Table 2. Clinical scores for cats following viral challenge.

Groups	Vaccine Type	Number of Cats	N Days Post-Challenge (dpc)	−1	0	1	2	3	4	5	6	7	8	9
Group I	FPLV-VLP vaccine (Seppic adjuvant)	1		0	0	0	0	0	0	0	0	0	0	0
		2		0	0	0	0	0	0	0	0	0	0	0

		3	0	0	0	0	0	0	0	0	0	0
Group II	FPLV killed vaccine (Seppic adjuvant)	1	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0	0	0
Group III	Commercial vaccine (Fel-O-Vax® PCT)	1	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	1	1	2	2	3	3
		3	0	0	0	0	0	1	1	1	1	3	
Group IV	Control Group (MEM)	1	0	0	0	0	0	0	1	1	2	3	2
		2	0	0	0	1	3	3					
		3	0	0	0	0	0	1	1	1	2	3	

Legend: “0” = no clinical signs; “1” = slight fever or mild depression; “2” = temperature > 40 °C or unconscious; “3” = death.

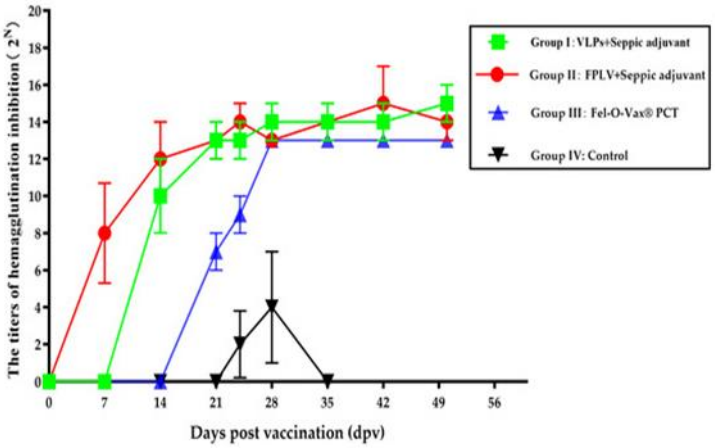

Figure 4. Time-course of HI antibody titers in vaccinated cats before and after FPLV challenge.

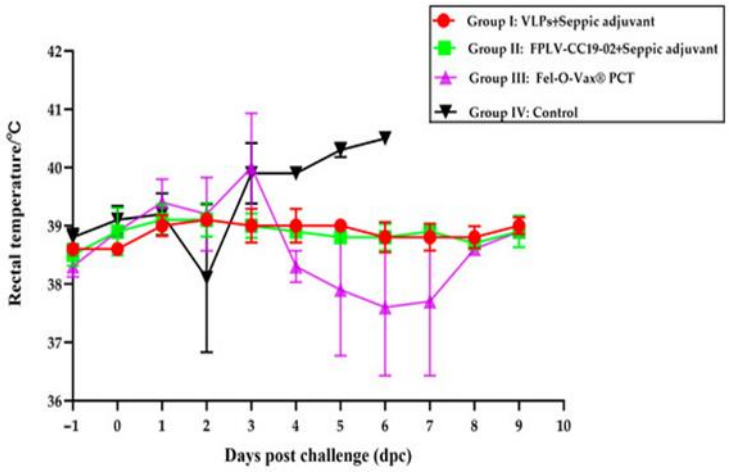

Figure 5. Body temperature changes in cats during days post-challenge (dpc).

Safety assessment of the vaccines

Post-mortem inspection revealed no tumor formation or sarcoma at any injection site (**Figure 6a**). Histological analysis of muscle tissue from both the control (**Figure 6b**) and vaccinated groups (**Figures 6c and 6d**) showed tightly organized fibers with clear cellular outlines and uniform orientation. The alternating light-dark striations were well-defined, connective tissues appeared normal, and no inflammatory cell infiltration was detected in any specimen.

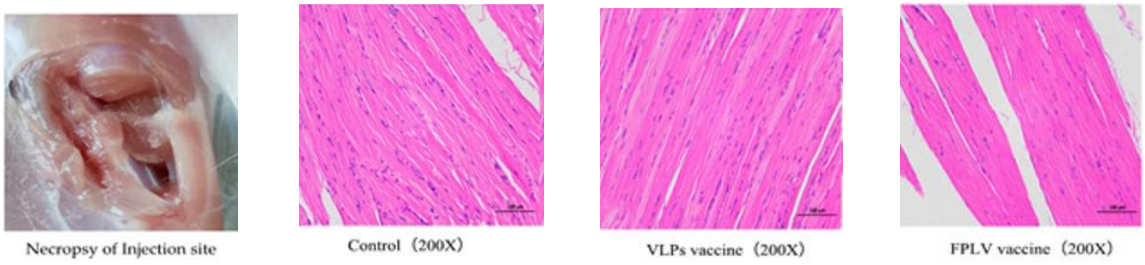

Figure 6. Necropsy and microscopic examination of inoculation sites. (a) Gross observation of the vaccine injection site. (b) Muscle histology in control cats. (c) Muscle histology after VLP-based vaccine. (d) Muscle histology after the inactivated FPLV vaccine.

Feline panleukopenia virus (FPLV) accounts for the vast majority (90–95%) of feline panleukopenia (FPL) cases, whereas infections caused by canine parvovirus variants in cats occur less frequently, representing under 10% of cases. Vaccination remains the most practical and economical method for preventing this disease in feline populations. In China, however, vaccination practices rely solely on a foreign, inactivated multivalent product (Fel-O-Vax® PCT), which is currently the only legally registered option, despite being based on viral strains isolated more than three decades ago.

Recent molecular studies have reported multiple FPLV variants in China with amino acid substitutions in the capsid protein, including Gly299Glu in giant pandas [16], Ala300Pro in canines [17, 18], and Ala91Ser in both cats and dogs [12, 13, 19]. Among these, the Ala91Ser substitution is particularly concerning due to its enhanced infectivity and virulence [19]. This variant has become predominant in Chinese field isolates since 2019 [12, 19], representing over half of clinical detections and, in some regions, reaching up to 86%. Consequently, there is rising concern regarding whether current commercial vaccines can offer adequate protection against such emerging strains, highlighting the urgent demand for safer and more effective vaccines to support the sustainable growth of China's pet industry.

Virus-like particle (VLP) vaccines present distinct advantages—chiefly their structural mimicry of authentic virions [9] combined with the absence of viral genetic material, ensuring non-infectivity. In the present study, we successfully expressed the FPLV VP2 protein using an insect cell baculovirus expression system (**Figure 2a**). The recombinant VP2 protein self-assembled into correctly folded VLPs (**Figure 2c**). Expression and antigenicity were verified through monoclonal antibody detection of the corresponding canine parvovirus 2c VP2 (**Figures 2b and 3d**) and confirmed using FPLV antigen immunochromatographic test strips (**Figure 2e**). These assays confirmed the strong antigenic properties of the recombinant particles. Moreover, the purified VLPs displayed enhanced hemagglutination (HA) activity, achieving titers between 1:214 and 1:216 (**Figure 2d**), exceeding the HA values of circulating FPLV strains (1:28–1:210).

The immunogenic potential of the FPLV-VP2-VLPs was clearly demonstrated, as vaccinated cats developed robust humoral responses. Fourteen days after administration of the Seppic-adjuvanted VLP vaccine, HI antibody titers reached 1:212, in agreement with previous investigations [20]. By 21 days post-vaccination (dpv), all vaccinated cats exhibited HI titers above 210, indicating the induction of a durable immune response. Similarly, cats receiving the inactivated whole-virus formulation showed antibody production, with detectable HI titers emerging as early as 7 dpv. These data suggest that the VLP formulation provides immune protection equivalent to traditional vaccines. Conversely, cats immunized with the Fel-O-Vax® PCT commercial product developed antibodies later, with titers remaining below 28 until 21 dpv (**Figure 4**, blue line/scatter).

Antibody concentration remains a critical indicator of vaccine-mediated protection against viral infection. In this experiment, both the VLP-based and inactivated FPLV vaccines containing Seppic adjuvant provided complete (100%) protection following challenge with virulent FPLV, while the commercial product achieved only 33% protection. This reduced efficacy may reflect inadequate immune stimulation associated with single-dose protocols. These findings are consistent with broader evidence suggesting that HI titers of 1:64 or greater are generally required for effective prevention of feline panleukopenia and that insufficient immunization regimens can impair protective immunity.

The potential link between feline vaccination and sarcoma development has raised considerable concern in recent years [21, 22]. To assess the biosafety of the FPLV-VP2-VLP vaccine, histopathological evaluation was

conducted on muscle samples obtained from inoculation sites. Importantly, no sarcomas, necrotic lesions, or cellular abnormalities were detected in any vaccinated animals (**Figure 6**). Additionally, all immunized cats maintained normal body temperature profiles throughout the observation period. These results collectively indicate that the VLP vaccine possesses a favorable safety margin and does not induce adverse tissue reactions.

Conclusion

This study presents the development of an innovative vaccine candidate targeting feline panleukopenia, demonstrating superior immunogenicity and enhanced safety compared with existing products. The FPLV-VLP formulation represents a promising next-generation vaccine that could be deployed either as an independent immunogen or in combination with other feline vaccines to achieve comprehensive protection against FPL.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Barrs VR. Feline panleukopenia: A re-emergent disease. Vet Clin North Am Small Anim Pract. 2019;49(4):651–70.
2. Fairweather J. Epidemic among cats in Delhi, resembling cholera. Lancet. 1876;108(2765):115–7.
3. Kruse BD, Unterer S, Horlacher K, Sauter-Louis C, Hartmann K. Prognostic factors in cats with feline panleukopenia. J Vet Intern Med. 2010;24(6):1271–6.
4. Jacobson LS, Janke KJ, Ha K, Giacinti JA, Weese JS. Feline panleukopenia virus DNA shedding following modified live virus vaccination in a shelter setting. Vet J. 2022;279:105783.
5. Stone AE, Brummet GO, Carozza EM, Kass PH, Petersen EP, Sykes J, et al. 2020 AAHA/AAFP feline vaccination guidelines. J Feline Med Surg. 2020;22(9):813–30.
6. Mohsen MO, Bachmann MF. Virus-like particle vaccinology: From bench to bedside. Cell Mol Immunol. 2022;19(7):993–1011.
7. Mukhopadhyay E, Hill AVS, Salman AM. Virus-like particle vaccines. In: Vaccinology and Methods in Vaccine Research. Cambridge (MA): Academic Press; 2022. p. 163–76.
8. Gupta R, Arora K, Roy SS, Joseph A, Rastogi R, Arora NM, et al. Platforms, advances, and technical challenges in virus-like particles-based vaccines. Front Immunol. 2023;14:1123805.
9. Zhang L, Xu W, Ma X, Sun X, Fan J, Wang Y. Virus-like particles as antiviral vaccine: Mechanism, design, and application. Biotechnol Bioprocess Eng. 2023;28(1):1–16.
10. Chackerian B. Virus-like particles: Flexible platforms for vaccine development. Expert Rev Vaccines. 2007;6(3):381–90.
11. Chambers AC, Aksular M, Graves LP, Irons SL, Possee RD, King LA. Overview of the baculovirus expression system. Curr Protoc Protein Sci. 2018;91(1):54.
12. Wang J, Yan Z, Liu H, Wang W, Liu Y, Zhu X, et al. Prevalence and molecular evolution of parvovirus in cats in eastern Shandong, China, between 2021 and 2022. Transbound Emerg Dis. 2024;2024(1):5514806.
13. Chen X, Wang J, Zhou Y, Yue H, Zhou N, Tang C. Circulation of heterogeneous Carnivore protoparvovirus 1 in diarrheal cats and prevalence of an A91S feline panleukopenia virus variant in China. Transbound Emerg Dis. 2022;69(6):e2913–25.
14. Wang Z, Jiang Y, Xin T, Yuan W, Guo X, Zhou H, et al. Genetic characterization and evolutionary analysis of canine parvovirus in Tangshan, China. Arch Virol. 2022;167(8):2263–9.
15. Gao T, Gao C, Wu S, Wang Y, Yin J, Li Y, et al. Recombinant baculovirus-produced grass carp reovirus virus-like particles as vaccine candidate that provides protective immunity against GCRV genotype II. Vaccines (Basel). 2021;9(1):53.

16. Yi S, Liu S, Meng X, Huang P, Cao Z, Jin H, et al. Feline panleukopenia virus with G299E substitution in the VP2 protein first identified from a captive giant panda in China. Front Cell Infect Microbiol. 2021;11:820144.
17. Allison AB, Organtini LJ, Zhang S, Hafenstein SL, Holmes EC, Parrish CR. Single mutations in the VP2 300 loop region of the three-fold spike of the carnivore parvovirus capsid can determine host range. J Virol. 2016;90(2):753–67.
18. Li J, Peng J, Zeng Y, Wang Y, Li L, Cao Y, et al. Isolation of a feline-derived feline panleukopenia virus with an A300P substitution in VP2 protein and confirmation of pathogenicity in dogs. Anim Dis. 2024;4(1):5.
19. Xie Q, Sun Z, Xue X, Pan Y, Zhen S, Liu Y, et al. China-origin G1 group isolate FPV072 exhibits higher infectivity and pathogenicity than G2 group isolate FPV027. Front Vet Sci. 2024;11:1328244.
20. Jiao C, Zhang H, Liu W, Jin H, Liu D, Zhao J, et al. Construction and immunogenicity of virus-like particles of feline parvovirus from the tiger. Viruses. 2020;12(3):315.
21. Pereira ST, Gamba CO, Horta RS, de Castro Cunha RM, Lavalle GE, Cassali GD, et al. Histomorphological and immunophenotypic characterization of feline injection site-associated sarcoma. Acta Sci Vet. 2021;49:1789.
22. Brearley MJ. Vaccine-associated feline sarcoma—An emerging problem. J Feline Med Surg. 1999;1(1):5–6.