Research Article

Molecular Characterization of VP2 Gene Mutations in Feline Panleukopenia Virus from Surabaya, Indonesia

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Abstract | Feline panleukopenia virus (FPV) remains one of the most contagious and fatal viral infections affecting domestic and wild felids worldwide, including in Indonesia. This study aimed to identify nucleotide and amino acid substitutions within VP2 gene fragment of FPV detected in cats from Surabaya, Indonesia, to understand their genetic variability. Fecal samples were collected from clinically suspected cats and screened using PCR targeting the VP2 gene fragment. Positive amplicons were sequenced and analyzed using bioinformatics tools, including MEGA11 for sequence alignment and homology comparison with global reference strains. Sequence alignment revealed several nucleotide mutations, including A42G, T261A, A565G, and T783A in sample FPV_Surabaya 2_2025, and G324A, G621A, and T928A in sample FPV_Surabaya 5_2025. These changes resulted in amino acid substitutions I189V (both samples) and F310I (FPV_Surabaya 5_2025). Despite these variations, the VP2 gene remained highly conserved, suggesting no major antigenic shift. The detected substitutions occurred within regions associated with host specificity and immune recognition, potentially reflecting local viral adaptation. However, minor point mutations were detected at several nucleotide positions, resulting in limited amino acid substitutions within the capsid region. This study provides baseline molecular data on FPV circulating in Indonesia, particularly in Surabaya, and highlights the importance of continuous molecular surveillance to monitor genetic evolution that may influence viral evolution vaccine effectiveness.

Keywords | Feline panleukopenia, Sequencing, VP2 gene, Mutation, Vaccines in developing countries, Indonesia

Received | November 07, 2025; **Accepted** | December 04, 2025; **Published** | January 20, 2026
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Citation | Muhammad FA, Ramadhanti B, Rantam FA, Sudjarwo SA, Rahmahani J, Puspitasari Y, Hamid IS, Salasia SIO, Wasissa M, Maryono M (2026). Molecular characterization of VP2 gene mutations in feline panleukopenia virus from Surabaya, Indonesia. Adv. Anim. Vet. Sci., 14(1):126-136.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.1.126.136
ISSN (Online) | 2307-8316

INTRODUCTION

Feline panleukopenia virus (FPV) is a highly contagious and frequently fatal parvoviral infection that affects domestic and wild felids worldwide. Belonging to the genus Protoparvovirus within the family Parvoviridae, FPV is a small, non-enveloped, single-stranded DNA virus characterized by remarkable environmental stability

and a high transmission rate. The virus primarily targets rapidly dividing cells in the intestinal crypts, bone marrow, and lymphoid tissues, leading to leukopenia, enteritis, and severe immunosuppression (Truyen *et al*., 2009). Clinical manifestations range from mild gastrointestinal disturbances to acute hemorrhagic enteritis and septicemia, with high morbidity and mortality, particularly among kittens under six months of age (Syarifuddin *et al*., 2025).

Affected cats commonly exhibit vomiting, diarrhea, and loss of appetite, with vomiting unrelated to food intake but resulting from viral destruction of intestinal crypt epithelial cells. The virus targets the Lieberkühn crypts, which contain intestinal stem cells essential for continuous regeneration of the intestinal epithelium. Damage to these cells disrupts mucosal integrity, leading to villous atrophy, intestinal inflammation, and subsequent dehydration and rapid clinical decline (Mahendra *et al*., 2020).

Despite the availability of effective vaccines, FPV outbreaks continue to occur globally, including in well-vaccinated populations. Such cases are often linked to inadequate immunization schedules, waning maternal antibodies, or the emergence of viral variants with altered antigenic profiles (Baroroh *et al*., 2023). For example, a recent investigation in Makassar by Rell *et al*. (2025) identified 2 of the 10 FPV-positive cases among vaccinated cats and could suggest possible vaccine failure because of improper vaccine administration, waning immunity, host-related condition, or exposure to antigenic variants. Transmission occurs predominantly through the fecal–oral route, and indirect spread via contaminated fomites plays a major role in outbreak persistence in shelters and densely populated catteries (Pandey, 2022). The virus's resilience in the environment further complicates eradication efforts, emphasizing the importance of ongoing surveillance and molecular characterization to monitor circulating strains.

Molecular surveillance of FPV in densely populated urban settings is particularly important because the virus's environmental persistence and efficient fecal–oral transmission sustain frequent exposure and outbreak potential in both owned and stray cat populations. Reports indicate that FPV can remain infectious on fomites for extended periods, facilitating indirect transmission in shelters and multi-cat households; combined with variable vaccination coverage and transient maternal immunity in kittens, these factors create conditions conducive to viral maintenance and episodic resurgence. Consequently, region-specific genomic monitoring is essential to detect emerging variants that might affect local disease dynamics and control measures (Pacini *et al*., 2023).

At the genetic level, FPV shares more than 98% nucleotide identity with canine parvovirus type 2 (CPV-2), as both viruses are classified under the species *Carnivore protoparvovirus 1*. Owing to this close genetic relatedness, distinguishing FPV from feline-adapted CPV-2 variants often requires detailed molecular or sequence-based characterization (Zhao *et al*., 2022). This is particularly relevant because CPV-2a, CPV-2b, and CPV-2c strains have occasionally been detected in cats, indicating potential cross-species transmission between FPV and CPV (Citarová *et al*., 2024). However, despite their close genetic relationship, the two viruses can still be distinguished based on host tropism and clinical manifestation, as supported by the clinical characterization of canine parvoviral enteritis described by Akanbi *et al*. (2025).

Canine parvovirus type 2 (CPV-2) itself is a highly contagious viral pathogen that primarily affects puppies, causing severe enteritis with high mortality rates accompanied by various clinical, hematological, and biochemical alterations throughout disease progression (Olaifa *et al*., 2025). In addition, limitations of rapid antigen tests and the clinical signs caused by Feline Panleukopenia Virus often resemble those of other infectious diseases, including viral, bacterial, and parasitic infections, molecular diagnostic approaches such as PCR and sequence analysis are essential for accurate detection and differentiation of FPV from other pathogens (Harelas *et al*., 2022; Azizah, 2023). Whereas point-of-care assays are useful for initial screening, confirmatory PCR and targeted sequencing enable more sensitive detection and allow characterization of nucleotide-level changes that are invisible to immunoassays. Integrating routine molecular characterization with epidemiological surveillance thus provides a more robust framework for assessing vaccine performance and for informing evidence-based adjustments to vaccination and biosecurity protocols in Indonesian settings (Pacini *et al*., 2023).

At the molecular level, the VP2 gene encodes the major capsid protein, accounting for nearly 90% of the viral capsid structure and determining host specificity, tissue tropism, and antigenicity (Chang *et al*., 2020). Even minor nucleotide substitutions in VP2 can influence receptor binding to the feline transferrin receptor (TfR) and alter neutralizing antibody recognition, potentially compromising vaccine efficacy (Li *et al*., 2022). Recent molecular studies in Asia and Europe have identified significant genetic variability within the VP2 gene, including both synonymous and nonsynonymous mutations, suggesting ongoing viral adaptation to local feline populations (Safwat *et al*., 2025).

Molecular data on Feline Panleukopenia Virus (FPV) circulating in Southeast Asia, including Indonesia, remain relatively limited. Previous molecular studies conducted in

Malang, Indonesia have primarily focused on partial VP2 gene characterization and phylogenetic analysis, revealing that local FPV samples are closely related to Asian and global reference strains (Munawaroh *et al*., 2020). VP2-based molecular characterization also conducted in Yogyakarta and Semarang by Raj and Haryanto (2020) analyzed eight clinical FPV samples and demonstrated that all sequences were genetically close to one another and also shared high similarity with parvoviruses detected in raccoons and bobcats. This knowledge gap poses a challenge for effective molecular diagnosis and vaccine optimization. Therefore, this study aimed to identify nucleotide and amino acid substitutions in the VP2 gene fragment of FPV detected in cats from Surabaya, Indonesia. The findings are expected to provide baseline molecular data for local FPV strains, enhance understanding of viral evolution in the region, and contribute to improved vaccine strategies and diagnostic refinement in feline viral diseases.

MATERIALS AND METHODS

Sample collection and clinical examination

A total of 30 domestic cats were sampled from several veterinary clinics distributed across multiple districts in Surabaya, East Java, Indonesia, to ensure representative coverage of urban feline populations. Each cat underwent a clinical examination to identify symptoms characteristic of Feline Panleukopenia Virus (FPV) infection, such as fever, anorexia, vomiting, and hemorrhagic diarrhea. Suspected cases were screened using a commercial rapid antigen test kit specific for FPV. Cats that tested positive on the rapid test were selected for molecular and sequence-based analyses. Rectal swab samples were collected from domestic cats suspected of FPV infection and stored in Viral Transport Medium (VTM) at −80 °C until processing. Viral DNA was extracted using the Geneaid Viral Nucleic Acid Extraction Kit II following the manufacturer's protocol. DNA purity and concentration were assessed using a NanoDrop spectrophotometer.

DNA extraction and PCR amplification of VP2 gene

Amplification of the VP2 gene fragment (999 bp) was performed using specific primers designed based on the reference FPV sequence M38246.1. The primer sequences used were:

Forward primer FPV-VP2-F: 5′- TAC AGG ATC TGG GAA CGG GT -3′

Reverse primer FPV-VP2-R: 5′- AAT GGC CCT TGT GTA GAC GC-3′

These primers target nucleotide positions 2846–3844 bp of the VP2 gene, corresponding to the major capsid protein-coding region. Primer specificity was verified in silico using NCBI BLAST to confirm 100% homology with FPV reference strains and no significant alignment with Canine Parvovirus (CPV) sequences, ensuring accurate differentiation between the two viruses. However, experimental validation using a CPV-positive control was not performed and is acknowledged as a methodological limitation.

Visualization and sequencing

PCR products were visualized on a 2% agarose gel stained with ethidium bromide. Bands with single, distinct, and sharp intensity were selected for sequencing to ensure high template quality. While quantitative viral load measurement was not performed, the use of clear single bands minimized sequencing ambiguities and base-calling errors. Sequencing of the PCR-amplified VP2 fragments was conducted to confirm the identity of each nucleotide position.

Sequence analysis and reference strain collection

Reference strains for nucleotide homology comparison were selected from publicly available FPV sequences in GenBank. A total of 32 strains representing diverse geographical origins (Asia, Europe, North America, Africa, and Australia/Oceania) and spanning collection years 2004–2021 were included to provide broad temporal and regional coverage. Two commercial vaccine strains (Purevax, EU498680.1; Felocell, EU498681.1) were also incorporated to evaluate genetic distance between field samples and vaccine lineages. Accession numbers and origins of all reference strains used in this study are listed in Table 1. Multiple sequence alignment and homology analyses were performed using MEGA X and BioEdit software.

Identification of nucleotide and amino acid substitutions

Aligned sequences were examined to identify nucleotide variations among the local samples and reference sequences. Deduced amino acid sequences were obtained through in silico translation using the Expasy Translate Tool to determine possible amino acid substitutions in the VP2 gene fragment.

Quality control

All molecular and analytical procedures were conducted in physically separated laboratory areas to minimize the risk of cross-contamination. Dedicated pipettes and aerosol-resistant filter tips were used for each workflow step. Each batch of PCR reactions included a previously confirmed FPV-positive control and a nuclease-free water negative control.

Table 1: Global reference FPV strains used for comparative nucleotide homology analysis, representing isolates from Asia, Africa, Oceania, Europe, and the Americas, including two commercial vaccine strains (Felocell and Purevax). The table lists the GenBank accession numbers, geographic origin, year of isolation, and percent identity of nucleotide homology of each reference strain compared with Indonesian samples.

No.	Origin place year	Accession number gen-bank	FPV_ surabaya 2_2025	FPV_ surabaya 5_2025
1.	China 2021	OM885379.1	99.2%	99%
2.	Argentina 2007	EU018144.1	98.6%	98.4%
3.	Hungary 2007	EU360958.1	99.3%	98.9%
4.	Australia 2016	MK570652.1	99.5%	99.1%
5.	Japan 2009	AB000050.1	99.5%	99.1%
6.	USA 2013	KJ813893.1	99.6%	99.2%
7.	Portugal 2014	KT240136.1	99.4%	99%
8.	United Kingdom 2019	MW926314.1	99.6%	99.2%
9.	New Zealand 2017	MK570703.1	99.6%	99.2%
10.	Canada 2018	OM640096.1	99.6%	99.2%
11.	South Korea 2008	HQ184193.1	99.6%	99.2%
12.	Vietnam 2019	MT857273.1	99.5%	99.1%
13.	China 2020	MW017625.1	99.4%	99%
14.	France 2004	AY606131.1	99.5%	99.1%
15.	Italy 2015	KX943318.1	99.3%	99.1%
16.	Turkey 2020	MZ391095.1	99.3%	99.1%
17.	Australia 2017	MK570739.1	99.4%	98.7%
18.	United Arab Emirates 2017	MK570716.1	99.3%	98.9%
19.	Thailand 2018	MW589472.1	99.4%	99%
20.	Russia 2004	AY665655.1	99.6%	99.2%
21.	Egypt 2019	OM937916.1	99%	98.5%
22.	Belgium 2013	KP769859.1	99.3%	99.1%
23.	Thailand 2020	MW589473.1	99.2%	99%
24.	China 2016-2017	MF541135.1	99.3%	98.9%
25	South Korea 2019	OP153927.1	99.3%	98.9%
26.	China 2018	MK266793.1	99.2%	99%
27.	Turkey 2020	MZ391096.1	99.2%	98.7%
28.	China 2020	MZ836375.1	99.3%	98.9%
29.	South Korea 2017	MN400978.1	99.2%	98.7%
30.	India 2018	MH559110.1	98.7%	98.3%
31.	Purevax	EU498680.1	99.5%	99.1%
32.	Felocell	EU498681.1	99.6%	99.2%

RESULT AND DISCUSSION

Nucleotide sequencing of the VP2 gene

A total of 30 fecal or rectal swab samples were collected from cats presented to several veterinary clinics in Surabaya, Indonesia. All samples were initially screened using a commercial rapid antigen test (FPV Ag) to identify potential cases of Feline Panleukopenia Virus infection. Nineteen samples yielded positive results on the antigen test and were subsequently confirmed as FPV-positive by conventional PCR targeting the VP2 gene. These PCR-positive samples were used for further molecular characterization through nucleotide sequencing.

The PCR-amplified products were subsequently subjected to sequencing to determine the nucleotide composition of the targeted VP2 gene fragment. This step was essential to confirm that the amplified fragment corresponded specifically to the intended region of the VP2 gene. Sequencing serves as an important verification step following PCR amplification to ensure specificity and accuracy of the obtained amplicons. Furthermore, sequence verification enables the detection of possible contamination from non-target DNA sequences that may share partial homology with the VP2 gene, thus preventing false-positive or non-specific amplification outcomes (Jansz and Faulkner, 2024).

Among the 19 PCR-positive samples, two amplicons showing clear, single, and intense DNA bands were selected for sequencing analysis. The selection was based on the clarity and intensity of the electrophoretic bands, as well-defined single bands indicate high template quality and specific amplification. This selection criterion follows standard molecular practices to ensure that the resulting sequence data accurately represent the target gene region. In contrast, faint or multiple bands are often associated with non-specific amplification or contamination, which could compromise sequencing quality and data interpretation (Al-Shuhaib and Hashim, 2023).

Sequencing was performed using a VP2-specific primer, which successfully amplified a 999 base pair fragment of the Feline Panleukopenia Virus VP2 gene. The obtained sequences were aligned and compared with 32 reference strains retrieved from the GenBank database, including two vaccine strains and multiple field isolates originating from Asia, Africa, Australia/Oceania, the Americas, and Europe. Comparative sequence analysis between the FPV samples (designated FPV_Surabaya 2_2025 and FPV_Surabaya 5_2025) and the reference strains revealed several nucleotide and amino acid variations at specific sites, suggesting genetic divergence within the VP2 gene among FPV strains circulating in Indonesia, particularly those detected in cats from Surabaya, East Java.

Nucleotide sequence alignment and variation analysis

Alignment of the nucleotide sequences revealed a small number of point substitution within the VP2 gene fragments of the Indonesian FPV samples (Figure 1).

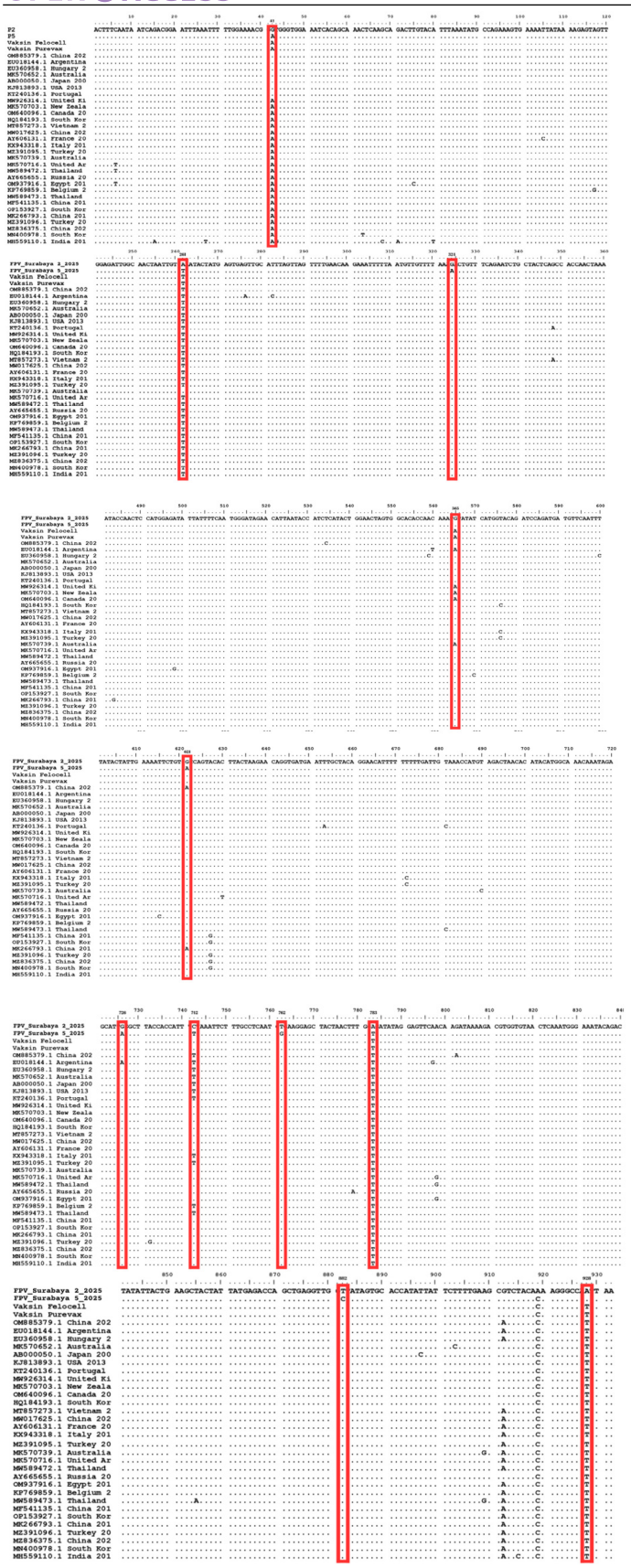

Figure 1: Multiple nucleotide sequence alignment of VP2 gene fragments from local FPV isolates (FPV_Surabaya 2_2025 and FPV_Surabaya 5_2025) and reference strains retrieved from GenBank. Nucleotide mutations unique to the Indonesian isolates are indicated by red boxes at specific positions.

Detailed nucleotide variations detected within the VP2 gene fragments of samples FPV_Surabaya 2_2025 and FPV_Surabaya 5_2025 are presented for clearer visualization of site-specific substitutions (Table 2). Four single-nucleotide changes were detected in sample FPV_Surabaya 2_2025, spanning the A42G–T783A region. These mutations consisted of adenine-to-guanine transitions at positions 42 and 565, and thymine-to-adenine transversions at positions 261 and 783. In contrast, sample FPV_Surabaya 5_2025 exhibited eight nucleotide substitutions distributed across the G324A–T928A region, involving guanine-to-adenine transitions at positions 324, 621, and 726; adenine-to-guanine transition at position 565; cytosine-to-thymine transition at position 742; thymine-to-guanine and thymine-to-cytosine transversions at positions 762 and 882; and a thymine-to-adenine substitution at position 928. These substitutions represent single nucleotide polymorphisms (SNPs), a form of point mutation in which a single base is replaced by another. Such alterations may or may not result in changes to the encoded amino acid, depending on the redundancy of the genetic code.

Table 2: Nucleotide substitutions identified in the VP2 gene fragments of Indonesian FPV samples compared to Felocell commercial vaccine virus strain as the reference.

No.	Sample	Position	Reference nucleotide (EU498681.1)	Variant nucleotide
1.	FPV_ Surabaya 2_2025	42	A	G
		261	T	A
		565	A	G
		783	T	A
2.	FPV_ Surabaya 5_2025	324	G	A
		565	A	G
		621	G	A
		726	G	A
		742	C	T
		762	T	G
		882	T	C
		928	T	A

A noteworthy finding in both Indonesian samples (FPV_Surabaya 2_2025 and FPV_Surabaya 5_2025) is the A565G substitution. Multiple global reference strains included in this study also exhibit either adenine or guanine at this position, indicating that site 565 represents a naturally variable nucleotide position. Such recurrent site-level polymorphisms in the VP2 gene have also been observed in recent molecular surveys, which consistentyly demonstrate high overall VP2 conservation despite sporadic local polymorphisms across geographically diverse FPV populations (Pan *et al.*, 2023; Wang *et al.*, 2024). Evolutionary analyses further suggest that identical

substitutions can arise independently in unrelated lineages (homoplasy), particularly at synonymous or neutrally evolving positions, complicating straightforward inference of direct transmission from single shared substitutions (Jantafong *et al.*, 2022). From an epidemiological standpoint, this finding does not strongly support direct transmission between the two cats but rather aligns with the overall pattern of VP2 sequence conservation and natural microevolution.

Although the A565G substitution detected in both FPV_Surabaya 2_2025 and FPV_Surabaya 5_2025 is not unique to Indonesia and represents a naturally variable site in the VP2 gene, its detection remains informative in a regional context. The recurrence of this polymorphism across unrelated global strains reflects the well-documented stability of the FPV genome, in which isolated single-nucleotide changes arise without altering overall antigenic structure or lineage classification. In our dataset, the presence of A565G supports the conclusion that FPV circulating in Surabaya conforms to the global pattern of high VP2 conservation without evidence of emerging antigenic variants. While the mutation itself is not novel, it contributes to defining the local molecular baseline for FPV in Surabaya, where published VP2 sequence data remain extremely limited.

Most nucleotide substitutions identified in this study were synonymous, and only two resulted in amino acid changes (I189V and F310I). The non-conservative substitution (F310I) was observed only in FPV_Surabaya 5_2025. These findings indicate that the partial VP2 gene of the Surabaya samples remains highly conserved, consistent with the well-documented genomic stability of FPV and the functional constraints placed upon the VP2 capsid protein. Rather than reflecting a high degree of genetic variability, the limited number of substitutions observed aligns with previous reports describing the slow evolutionary rate of FPV compared with RNA viruses and the tendency of VP2 to maintain sequence conservation across geographic regions (Wang *et al.*, 2024).

Similar patterns of nucleotide polymorphisms have been described in other FPV and CPV isolates. Safwat *et al.* (2025) identified 32 nucleotide substitutions in Egyptian FPV strains, with 29 being synonymous and only three non-synonymous, whereas Mukhopadhyay *et al.* (2016) reported 27 mutations, which are 16 non-synonymous and 11 synonymous, in CPV/FPV sequences. These comparative findings support the interpretation that VP2 variation is modest and shaped by functional constraints rather than rapid antigenic drift. The VP2 gene encodes the major capsid protein that plays a crucial role in determining the antigenic profile, host specificity, and infectivity of FPV. The tertiary structure of VP2 comprises four major loops (loops 1–4) and a flexible loop, which together form the surface features of the viral capsid. Loops 1, 2, and 4 constitute the top of the threefold spike, while loop 3 forms its lateral "shoulder" region (Li *et al.*, 2022). Spatial interactions between loop 1 and the flexible loop are particularly important, as even a single amino acid substitution within loop 1 can induce conformational changes that affect receptor binding or neutralizing antibody recognition (Zhang *et al.*, 2024).

Therefore, although the exact structural locations of the observed mutations in this study remain undetermined, the detected nucleotide substitutions within the VP2 gene could potentially alter the three-dimensional conformation of the viral capsid, thereby influencing antigenicity, host range, and receptor-binding properties of FPV strains circulating in Indonesia.

Amino acid sequence analysis and substitution mapping

Translation of the VP2 nucleotide sequences yielded a deduced polypeptide consisting of 310 amino acid residues (Figure 2). Analysis of the deduced amino acid sequences revealed two missense substitutions within the VP2 gene fragments of the Indonesian FPV samples (Table 3). These substitutions were further evaluated to assess their potential structural and functional impacts on the VP2 capsid protein. Comparative alignment of the deduced amino acid sequences identified two non-synonymous substitutions within the VP2 fragment, which are an isoleucine-to-valine substitution at position 189 (I189V), observed in both samples (FPV_Surabaya 2_2025 and FPV_Surabaya 5_2025), and a phenylalanine-to-isoleucine substitution at position 310 (F310I), detected exclusively in sample FPV_Surabaya 5_2025. Both substitutions represent missense mutations arising from single-nucleotide polymorphisms (SNPs), indicating that nucleotide variations in the VP2 gene lead to changes in the encoded protein sequence.

Table 3: Amino acid substitutions identified in the VP2 protein fragments of Indonesian FPV samples compared to Felocell commercial vaccine virus strain as the reference.

Sample	Position	Reference amino acid (EU498681.1)	Substituted amino acid	Codon change
FPV_Surabaya 2_2025	189	I	V	ATA -> GTA
FPV_Surabaya 5_2025	189	I	V	ATA -> GTA
	310	F	I	TTT -> ATT

Substitution I189V has been reported in various FPV and CPV-2a lineages and is considered a conservative change, as both residues are hydrophobic (Kathuria et al., 2016).

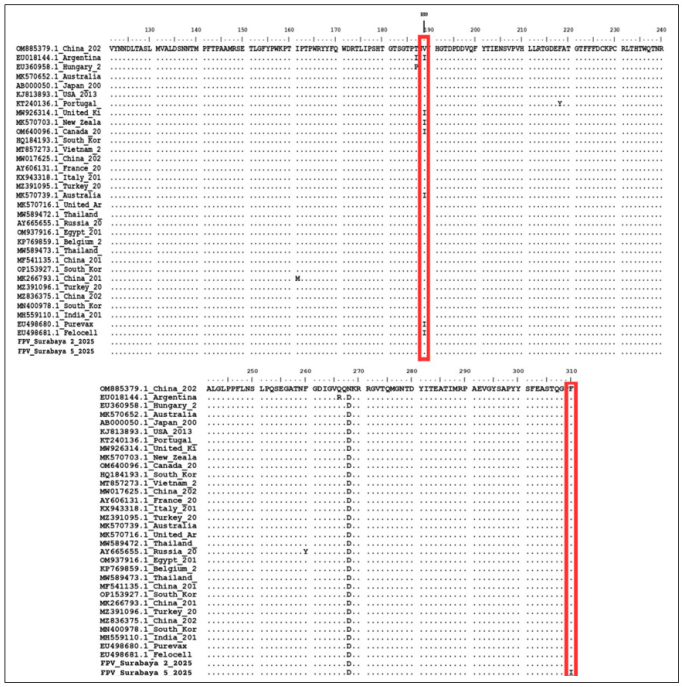

Figure 2: Multiple amino acid sequence alignment of VP2 gene fragments from local FPV isolates (FPV_Surabaya 2_2025 and FPV_Surabaya 5_2025) and reference strains obtained from GenBank. Amino acid substitutions identified in the Indonesian isolates are highlighted by red boxes at specific positions.

In contrast, the F310I substitution represents a non-conservative change, replacing a bulky aromatic residue (phenylalanine) with a smaller aliphatic branched residue (isoleucine). The replacement of phenylalanine by isoleucine could modify antigenic epitopes or influence the affinity of VP2 for the transferrin receptor (TfR), a key determinant of host range and viral infectivity (Halder *et al*., 2012). However, we emphasize that these are mechanistic hypotheses only since we did not perform structural modeling (e.g., homology modeling, solvent-accessibility calculations, or molecular dynamics) nor serological assays to measure antibody binding or neutralization. Therefore, any suggestion that F310I alters capsid topology, receptor affinity, or antigenicity would be premature based solely on sequence data. To clarify the implications of the observed substitution, we recommend follow-up studies including in silico structural analyses to assess whether residue 310 is surface-exposed or situated within a known antigenic loop, and functional assays such as cross-neutralization with vaccine sera or transferrin-receptor binding experiments to determine phenotypic effects. These findings imply that, although both mutations are missense in nature, F310I is more likely to exert measurable effects on capsid structure and biological function than I189V.

Overall, these substitutions highlight that even limited nucleotide variability within the VP2 gene may produce amino acid replacements capable of modulating the chemical and structural properties of the VP2 protein, thereby influencing viral adaptability, antigenicity, and potential immune escape mechanisms. Comparable amino acid changes have been documented in other FPV and CPV isolates. Wang *et al*. (2025) reported mutations at positions A91S and I101T, both located near the receptor-binding interface and overlapping with known antibody recognition sites. Structural modeling demonstrated that I101T induces a local conformational shift through the formation of a new polar interaction with Asp99, possibly reducing the binding efficiency of neutralizing antibodies. Similarly, Zhang *et al*. (2024) emphasized that residues 91 and 101 are positioned adjacent to loop 1 and contribute to the flexibility of this domain, which plays a vital role in receptor attachment. In addition, Wang *et al*. (2024) identified a V232I substitution in multiple strains, suggesting the emergence of novel genetic patterns within the FPV VP2 gene.

Implications of genetic variation in VP2 for viral evolution and vaccination

The VP2 gene of Feline Panleukopenia Virus (FPV) encodes the major capsid protein that plays a critical role in viral host range determination, cell tropism, and antigenic specificity. Even minor nucleotide or amino acid substitutions within this gene can alter the viral surface topology, influencing its interaction with the host transferrin receptor (TfR) and neutralizing antibodies (Wen *et al*., 2024). The detection of nucleotide and amino acid substitutions in the Indonesian FPV samples suggests that ongoing microevolutionary processes are shaping the genetic diversity of the virus within local feline populations. Such genetic drift events are likely driven by host immune pressure, viral replication fidelity, and regional differences in vaccination coverage and practices (Battilani *et al*., 2011).

Non-synonymous substitutions identified in this study, including I189V in both isolates and F310I in sample FPV_Surabaya 5_2025, represent naturally occurring variations previously reported in global FPV populations. Importantly, neither of these substitutions is located within the well-characterized antigenic regions of the VP2 gene, particularly the 300-series loop structures that govern neutralizing antibody binding and host range specificity in FPV and CPV. This aligns with previous studies showing that peripheral or non-surface-exposed residues tend to maintain the overall structural and antigenic stability of the VP2 protein (Zhang *et al*., 2024). On the contrary, amino acid changes at key VP2 positions modulated receptor binding and cross-reactivity with neutralizing antibodies (Li *et al*., 2022).

From an epidemiological perspective, continuous monitoring

of VP2 genetic variation is essential to detect potential shifts in viral antigenicity that could compromise the protective efficacy of existing vaccines. While most commercial FPV vaccines are based on long-established attenuated strains, regional variants with accumulated mutations may display partial immune escape under certain conditions. Several studies have reported cases of FPV infection occurring in vaccinated cats, indicating that vaccination failure may not be entirely attributable to host-related factors such as poor health or improper vaccine handling. Furthermore, genetic divergence or antigenic incompatibility between vaccine strains and circulating field isolates, may also reduce protective immunity and allow viral persistence in vaccinated populations (Munawaroh *et al*., 2020). The present findings, though limited to partial VP2 fragments, highlight the necessity of integrating molecular surveillance with immunogenicity studies to evaluate vaccine performance against circulating field strains.

In summary, the amino acid substitutions detected in the present samples interpreted as part of the natural microevolutionary landscape of FPV. Nonetheless, sustained molecular surveillance remains important to monitor potential future emergence of mutations at known antigenic sites, especially within the 300-loop region, where even subtle structural shifts may influence antibody recognition. The findings of this study therefore broaden the baseline genomic understanding of FPV circulating in the region while reinforcing the overall antigenic stability of the VP2 gene.

Evolutionary relationship and phylogenetic lineages

The evolutionary relationships among the VP2 sequences were further examined through maximum-likelihood phylogenetic reconstruction (Figure 3). Both of the samples, FPV_Surabaya 2_2025 and FPV_Surabaya 5_2025 were positioned within the broad global FPV cluster, without forming distinct or well-supported lineage groupings. FPV_Surabaya 5_2025 clustered within a moderately supported subclade (bootstrap 87%) containing field isolates from Thailand (2020), Belgium (2013), South Korea (2008), Turkey (2020), and Italy (2015), reflecting its close affinity to commonly circulating FPV strains reported over the past two decades. FPV_Surabaya 2_2025, by contrast, was placed in a separate subcluster with isolates from Australia (2016 and 2017) and Japan (2009), supported by bootstrap values of 60–71%. However, interpretation of lineage divergence must be made cautiously, as this study analyzed only two high-quality sequences. The limited sample size introduces potential sampling bias, and it remains possible that additional, more divergent variants may be circulating but were not detected in this dataset.

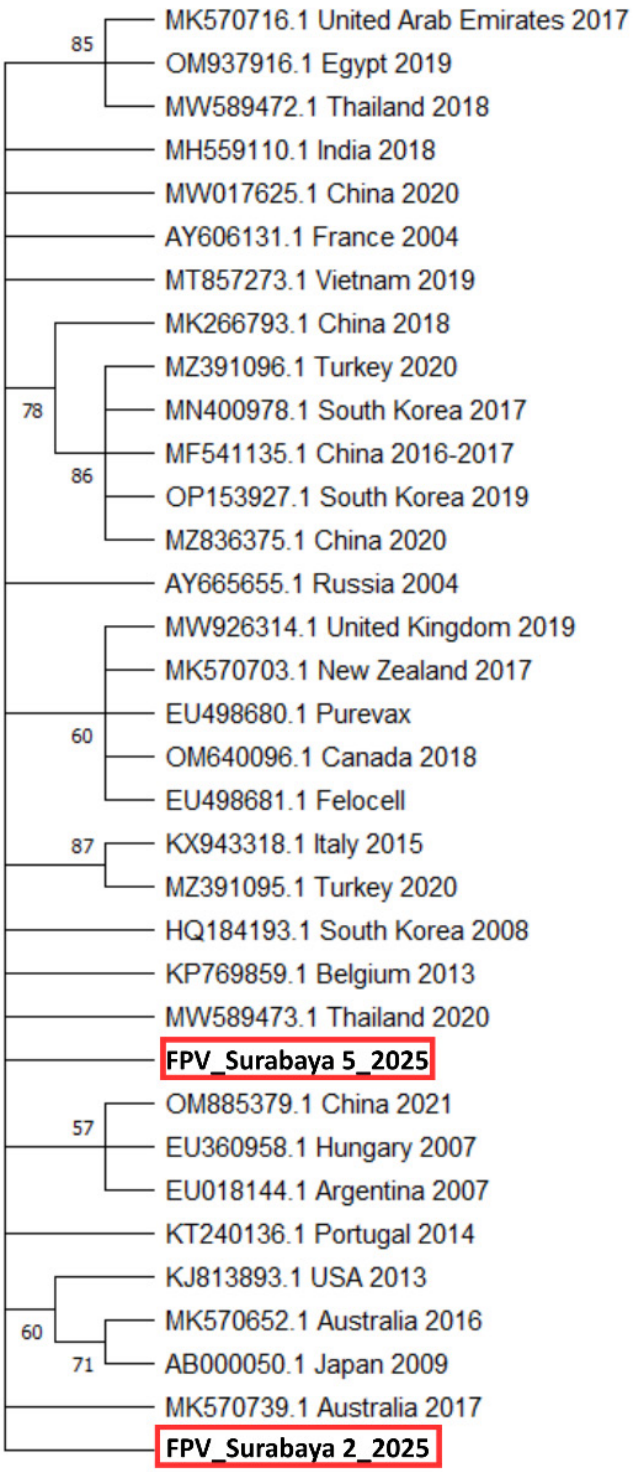

Figure 3: Maximum-likelihood phylogenetic tree based on partial VP2 gene sequences of Feline Panleukopenia Virus (FPV). Local samples (FPV_Surabaya 2_2025 and FPV_Surabaya 5_2025) are indicated with red rectangle. The analysis was conducted using MEGA11 using the Maximum Likelihood model with 1000 bootstrap replicates.

To put these findings into a broader context, similar patterns of phylogenetic divergence have been documented in recent studies. For example, Tang *et al*. (2022) observed geographic clustering of VP2 sequences among FPV

isolates in Beijing, which supports the presence of region-specific lineages. Moreover, detailed molecular–structural work on an emerging FPV strain (ZZ202303) from China showed that this strain forms a distinct clade in VP2 phylogeny, divergent from classical vaccine-lineage strains, and carries an I101T substitution potentially affecting antigenic regions and receptor binding (Wang *et al*., 2025). These observations bolster the interpretation that local FPV populations may consist of multiple co-circulating clades, some of which are more closely related to strains from Southeast Asia and vaccines, while others diverge markedly, underlining the genetic complexity and evolutionary plasticity of FPV even within a restricted geographic area.

CONCLUSIONS

The molecular characterization of the VP2 gene from local Feline Panleukopenia Virus (FPV) samples provides the first VP2 gene characterization of FPV isolates from Surabaya and contributes baseline molecular data for the region. The two sequences analyzed showed limited nucleotide and amino acid variation, consistent with the well-documented genomic stability of FPV worldwide. While these findings demonstrate the presence of natural genetic variation within local field strains, the dataset is too small to infer broader evolutionary trends or confirm continuous microevolution. Instead, the observed substitutions likely represent normal background variation within a highly conserved virus. Broader sampling and longitudinal molecular surveillance will be necessary to determine whether more substantial evolutionary patterns or emerging variants exist in the Indonesian FPV population.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Research Center for Vaccine Technology and Development (RCVTD), Institute of Tropical Disease, Universitas Airlangga, for their permission, technical assistance, and valuable guidance throughout this research. The authors also wish to thank the Viral Diarrhea and Hepatitis Study Group, Institute of Tropical Disease, Universitas Airlangga, for their support and constructive input during the experimental procedures. The authors are also grateful to Ministry of Higher Education, Science, and Technology, Republic of Indonesia for funding this research through BIMA program under the PMDSU Scheme.

NOVELTY STATEMENT

This study provides the first VP2 gene-based molecular characterization of Feline Panleukopenia Virus (FPV) samples from Surabaya, Indonesia. By comparing local field isolates with global reference and vaccine strains, this work establishes an updated regional molecular baseline and confirms the high conservation of the VP2 gene in circulating FPV. The findings contribute novel molecular epidemiological data from an underrepresented geographic region and support future surveillance efforts for FPV in Indonesia.

AUTHORS CONTRIBUTION

FAM and BR: conceptualization, data collection, laboratory analysis, and preparation of the first draft. FAR: supervision, project administration, validation, manuscript review, and correspondence. SAS and ISH: conceptual and methodological guidance, data interpretation, and critical revision of the manuscript. JR and YP: technical assistance, laboratory supervision, and contribution to data validation. SIOS and MW: methodology. MM: supervised the manuscript. All authors have read and approved the final version of the manuscript.

Ethical approval

The animal procedures performed in this study were reviewed and approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga (Ethics Certificate No 1.KEH.175.011.2025).

Generative AI and AI-assisted technology statement

No generative AI or AI-assisted tools were used in the conception, conduct, analysis, or writing of this study.

Conflict of interest

The authors have declared no conflict of interest regarding the publication of this article.

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