



Molecular Detection of Feline Panleukopenia Virus in Domestic Cats in Makassar, Indonesia, Using PCR Targeting the VP-2 Gene

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Abstract | Feline panleukopenia virus (FPV) is a highly contagious and often fatal pathogen affecting domestic cats, especially kittens and unvaccinated individuals. Although clinical cases of feline panleukopenia are frequently observed in Makassar, Indonesia, molecular confirmation using polymerase chain reaction (PCR) has not been documented prior to this study. This study aimed to confirm FPV infection via VP-2 PCR and evaluate associated clinical changes. Ten cats showing clinical signs consistent with feline panleukopenia such as vomiting, diarrhea, anorexia, and fever were selected from four veterinary clinics in Makassar. All cats were tested positive for FPV using a commercial antigen rapid test kit. While rapid antigen kits aided in screening, they were not locally validated against PCR, representing a diagnostic limitation. Without VP-2 sequencing, the possibility of CPV-2 variant infection cannot be fully excluded. PCR amplification of the VP-2 gene from fecal samples showed a consistent band of 974 bp in all ten cats. This study presents the first molecular confirmation of FPV in domestic cats in Makassar and underscores the diagnostic value of integrating clinical observation, rapid antigen kits screening, and PCR-based molecular detection. Limitations include small sample size, urban-only sampling, lack of environmental persistence testing, and absence of vaccine coverage data. However, these findings underscore the need for strengthened FPV surveillance and vaccination programs to prevent outbreaks in the region.

Keywords | Domestic cats, Feline panleukopenia virus, Makassar, Molecular diagnosis, VP-2 gene

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INTRODUCTION

Feline panleukopenia virus (FPV) is a highly contagious and often fatal viral disease affecting domestic and wild felids worldwide (Barrs, 2019). It is caused by a non-enveloped, single-stranded DNA virus in the Parvoviridae family, and primarily targets rapidly dividing cells, including those in the intestinal epithelium, bone

marrow, and lymphoid tissues, leading to severe enteritis, immunosuppression, and panleukopenia (Wilkes, 2022).

This virus can be transferred to other animals directly or indirectly. Indirect transfer of the virus plays an important role in the spread of disease because the virus survives longer in feces or vomiting. When it enters the digestive system, the virus will replicate in enterocytes in the crypts

of the intestinal epithelium, causing hemorrhagic enteritis in infected dogs or cats (Rehme *et al.*, 2022). Clinically affected cats often present with lethargy, vomiting, diarrhea, fever, and anorexia, and mortality rates can exceed 90% in unvaccinated populations (Stuetzer and Hartmann, 2014; Xue *et al.*, 2023).

Genetically, FPV shares a high degree of genetic similarity (>98%) with canine parvovirus type 2 (CPV-2), both are classified under the species carnivore protoparvovirus 1, and without sequence analysis, distinguishing FPV from feline-infecting CPV-2 variants remains challenging (Zhao *et al.*, 2022). This is particularly relevant because CPV-2a, 2b, and 2c strains have been reported in cats (Buonavoglia *et al.*, 2001; Citarová *et al.*, 2024). Despite their similarity, FPV and CPV-2 remain distinct in host tropism and clinical manifestation. The VP-2 capsid protein, which constitutes approximately 90% of the viral particle, is a critical determinant of host range, antigenicity, and diagnostic target (Wang *et al.*, 2022). Because of its genetic stability and high conservation across FPV strains, the VP-2 gene is commonly targeted for molecular diagnostics and epidemiological investigations.

In resource-limited settings, diagnosis is frequently based on clinical signs and commercial rapid antigen tests. However, these methods may lack sufficient sensitivity and specificity, leading to false-positive or false-negative results (Barrs, 2019; Rell *et al.*, 2021). As such, polymerase chain reaction (PCR)-based detection of the VP2 gene has become the gold standard for sensitive and specific confirmation of FPV infection (Awad *et al.*, 2018; Zhao *et al.*, 2022; Xue *et al.*, 2023).

This study aimed to provide the first molecular confirmation of FPV infection in domestic cats in Makassar using VP-2 gene-based PCR and to characterize the associated clinical changes. By integrating clinical observation and molecular diagnostics, this research contributes valuable data to support FPV surveillance, diagnosis, and vaccination strategies in urban cat populations.

MATERIALS AND METHODS

STUDY AREA AND SAMPLE SELECTION

This study was conducted in Makassar City, South Sulawesi, Indonesia. A total of 10 domestic cats showing clinical signs consistent with feline panleukopenia were recruited from four veterinary clinics. Cats were selected based on clinical presentation and positive results from a commercial FPV antigen rapid kit. Fecal and blood samples were collected from each cat for molecular analysis, respectively.

CLINICAL EXAMINATION AND PRELIMINARY DIAGNOSIS

Each cat underwent a complete physical examination performed by a licensed veterinarian. The observed clinical signs including vomiting, diarrhea, fever, and anorexia were documented for each case. Initial diagnosis was supported using a commercial rapid antigen test kit (FPV Ag Test Kit®, VetD-Plus, China), following the manufacturer’s instructions. This kit has published manufacturer performance data but was not independently validated in our laboratory against PCR, which we acknowledge as a limitation. A sample was considered positive if both the control (C) and test (T) lines appeared within the specified time frame.

DNA EXTRACTION AND POLYMERASE CHAIN REACTION (PCR)

Fecal samples from each cat were subjected to molecular testing using conventional PCR to detect FPV genetic material. PCR set-up followed strict contamination control; DNA extraction, PCR preparation, and post-PCR analysis were performed in separate rooms using dedicated equipment. Positive and no-template negative controls were included in every run. Viral DNA was extracted using the Invitrogen™ PureLink™ Viral DNA/RNA Mini Kit (Thermo Fisher Scientific, USA), following the manufacturer’s protocol optimized for fecal samples. DNA concentration and purity were measured using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific), and samples were stored at -20°C until use. Amplification of the VP-2 gene was performed using a conventional PCR protocol with specific primers previously described by Mochizuki *et al.* (1996) and Buonavoglia *et al.* (2001). The primer sequences and target information are summarized below:

Primer name	Sequence (5’-3’)	Target region	Product size
Hfor	CAGGTGATGAATTTGCTACA	VP-2 gene	974 bp
VPR	TTTCTAGGTGCTAGTTGAG		974 bp

The primers from Mochizuki *et al.* (1996) and Buonavoglia *et al.* (2001) were used without modification of sequence, but annealing temperature and reagent volumes were optimized for our thermocycler and enzyme system to ensure consistent amplification.

The PCR mixture was prepared in a total volume of 17.2 µL, consisting of 10 µL of Invitrogen Rmix solution, 5 µL of Platinum™ Green Hot Start PCR Master Mix (Invitrogen), 0.6 µL of each forward (Hfor) and reverse (VPR) primer, and 1 µL of the extracted DNA template. The thermal cycling was carried out in a programmable thermocycler under the following conditions: An initial denaturation step at 95°C for 7 minutes, followed by 39

amplification cycles consisting of denaturation at 94°C for 45 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step was performed at 72°C for 5 minutes, after which the reaction was held at 4°C until further analysis. Positive and negative controls were included in each run. The positive control was extracted DNA from a previously confirmed FPV-positive sample, and the negative control was nuclease-free water. PCR products were visualized by electrophoresis on a 2% agarose gel stained with ethidium bromide. The gel was run at 100 V for 45 minutes and viewed under UV transillumination. A 100 bp DNA ladder (Invitrogen™) was used as a molecular size marker. A band at 974 bp indicated a positive result for FPV.

RESULTS AND DISCUSSION

This study presents the first molecular confirmation of feline panleukopenia virus (FPV) infection in domestic cats in Makassar, Indonesia, using VP-2 gene-based PCR, and provides supporting clinical evidence of systemic involvement. The combination of clinical observations and molecular detection offers a comprehensive diagnostic profile for feline panleukopenia in the regional context.

CLINICAL PRESENTATION

Ten domestic cats presenting with clinical signs consistent with feline panleukopenia were examined across four veterinary clinics in Makassar, Indonesia. A summary of the clinical characteristics, vaccination status, and rapid test results is provided in Table 1. The most frequently observed signs were vomiting and diarrhea, recorded in all ten cats (100%). Additional symptoms included anorexia (60%) and fever (30%). These signs are consistent with the enteric and systemic pathogenesis of FPV, which targets rapidly dividing cells in the intestinal crypts and lymphoid tissues, leading to mucosal destruction and immunosuppression (Barrs, 2019; Isaya et al., 2021). The ages of the cats ranged from 2 to 10 months, with a predominance of unvaccinated individuals (8/10 cats, 80%). All ten cats tested positive for FPV antigen using a commercial rapid test kit. Although useful for point-of-care screening, rapid test kits have been reported to yield false-positive or false-negative results depending on sample quality and timing of viral shedding (Rell et al., 2021; Pandey, 2022). Given known limitations in rapid antigen test specificity and sensitivity (Allison et al., 2013), we used them only as preliminary screening before PCR confirmation.

MOLECULAR DETECTION

All ten fecal samples were positive for FPV by PCR targeting the VP2 gene. The gel electrophoresis results are shown in Figure 1. A distinct band of approximately 974 base pairs was observed in each sample following

electrophoresis on 2% agarose gel stained with ethidium bromide. No amplification was detected in the negative control. The VP2 gene remains the most widely used and validated target in FPV diagnostics due to its high sequence conservation and functional relevance in viral capsid formation and host specificity (Zhao et al., 2022; Xue et al., 2023). The presence of PCR-confirmed FPV in all symptomatic and rapid test-positive cats strengthens the diagnostic reliability of combining these tools.

Table 1: Clinical and diagnostic characteristics of 10 domestic cats suspected of FPV infection.

Variable	Number
Age	
2–10 months	10
Sex	
Male	6
Female	4
Vaccination status	
Vaccinated	2
Not vaccinated	8
Common clinical signs	
Vomiting	10
Diarrhea	10
Anorexia	6
Fever	3
Rapid test result	
Positive	10
Negative	0

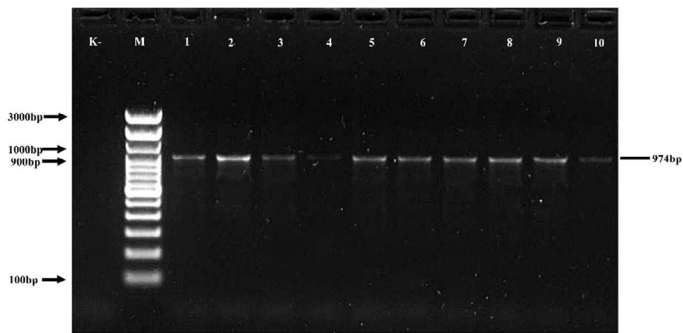


Figure 1: Gel electrophoresis of PCR products targeting the VP2 gene from 10 fecal samples. A 974 bp band was visible in all lanes. Lane M: 100 bp DNA ladder; Lanes 1–10: FPV-positive samples.

Importantly, this study did not perform sequence analysis of the VP2 gene, which would help differentiate between FPV and potential CPV-2 variants (e.g., CPV-2a/2b/2c) that have occasionally been reported in cats (Zhao et al., 2022; Wang et al., 2022; Citarová et al., 2024). While PCR confirmed the presence of VP-2 gene fragments in all cases, sequencing was not performed, so we cannot

exclude the possibility of CPV-2 variants infecting cats, as previously reported in several countries (Citarová *et al.*, 2024). Nevertheless, considering the clinical context and species involved, confirmed as FPV, and the PCR findings confirm active viral shedding and systemic infection.

EPIDEMIOLOGICAL AND PREVENTIVE CONSIDERATIONS

Despite the availability of FPV vaccines, 2 of the 10 infected cats in this study had a history of vaccination. This could suggest possible vaccine failure, improper administration, waning immunity, or exposure to antigenic variants (Jakel *et al.*, 2012; Bergmann *et al.*, 2018). This finding highlights the importance of evaluating immunization protocols and ensuring compliance with recommended vaccination schedules in domestic cats.

All positive cases originated from urban clinics across Makassar, suggesting that FPV is likely circulating widely in the local cat population. All samples were collected from urban clinics; rural prevalence and strain diversity remain unknown. The fecal–oral route of transmission, combined with the virus’s environmental resilience, increases the risk of both direct and indirect transmission in densely populated urban environments (Rehme *et al.*, 2022). Although FPV can persist in the environment for months (Barrs, 2019), environmental samples from clinics or homes were not tested. These findings underscore the urgent need for improved public awareness, stricter vaccination compliance, and biosecurity measures in both clinical and community settings.

However, this study’s sample size is relatively small ($n = 10$), limiting statistical inference. We also lacked data on local vaccine coverage or seroprevalence, which limits direct policy recommendations. Future studies should incorporate larger sample sizes, longitudinal surveillance, and genetic sequencing to monitor viral diversity and potential vaccine escape mutants in Indonesian cat populations.

CONCLUSION

This study provides the first molecular confirmation of feline panleukopenia virus (FPV) infection in domestic cats in Makassar, Indonesia, using polymerase chain reaction (PCR) targeting the VP2 gene. All ten clinically suspected cats tested positive by both rapid antigens testing and PCR. Limitations include absence of local antigen kit validation, lack of VP-2 sequencing, urban-only sampling, no environmental persistence testing, and no vaccine coverage data. These findings underscore the need for enhanced diagnostic capacity, strict vaccination compliance, and effective surveillance systems to prevent and control FPV outbreaks in urban feline populations.

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NOVELTY STATEMENT

This study provides the first molecular confirmation of Feline Panleukopenia Virus (FPV) infection in domestic cats in Makassar, Indonesia, using polymerase chain reaction (PCR) targeting the VP-2 gene. The findings establish a baseline for FPV molecular surveillance in urban Indonesian cat populations and underscore the need for improved vaccination strategies and diagnostic protocols in resource-limited settings.

AUTHOR’S CONTRIBUTION

All the authors contributed to the study design, data collection, data acquisition, analysis and reporting, and manuscript preparation.

ETHICAL CONSIDERATIONS

All animal procedures in this study were conducted with informed consent from pet owners and were approved by the Ethical Committee of the Animal Hospital, Hasanuddin University (No. 005/UN4.1.RSHUH/B/PP36/2025). Owners provided written informed consent, including acknowledgement of FPV’s high mortality risk and the potential need for intensive care.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

Generative Artificial Intelligence (AI) and AI-assisted technologies were not used in the conception, data collection, analysis, or interpretation of this study. AI tools (such as language models) were used solely for English language refinement and formatting assistance under the direct supervision of the corresponding author, ensuring that all scientific content, interpretations, and conclusions are entirely the authors’ original work.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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