



# Detection and Genetic Analysis of Feline Coronavirus in Clinically Suspected Cats

Fia Amalia<sup>1</sup>, Madarina Wasissa<sup>2</sup>, Siti Isrina Oktavia Salasia<sup>2\*</sup>, and Fajar Budi Lestari<sup>3</sup>

<sup>1</sup>Laboratory of Surgery and Radiology, Faculty of Veterinary Medicine, University of Brawijaya, Malang, Indonesia

<sup>2</sup>Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Gadjah Mada, Yogyakarta, Indonesia

<sup>3</sup>Department of Bioresources Technology and Veterinary, University of Gadjah Mada, Yogyakarta, Indonesia

\*Corresponding author's Email: [isrinasalasia@ugm.ac.id](mailto:isrinasalasia@ugm.ac.id)

## ABSTRACT

Feline coronavirus (FCoV) is a common infection in cats, producing outcomes that differ from mild intestinal disease to the fatal form known as feline infectious peritonitis (FIP). Accurate antemortem diagnosis is challenging, and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) targeting the *nucleocapsid* (*N*) gene offers high sensitivity and specificity, while phylogenetic analysis reveals viral variation. The present study aimed to detect FCoV in clinically suspected cats using RT-qPCR and to analyze the phylogenetic relationships of local isolates from Yogyakarta, Indonesia. A total of 45 clinical samples, consisting of 14 ascitic fluid and 31 blood samples, were collected from cats of different breeds (mixed, domestic shorthair, and Persian), aged 0.5-4 years, including 22 males and 23 females, that were presented to seven veterinary clinics in Yogyakarta with clinical signs such as vomiting, diarrhea, abdominal effusion, uveitis, and ataxia. The RT-qPCR revealed 62.2% (28/45) positive cases, with detection rates of 100% (14/14) in ascitic fluid and 45.2% (14/31) in blood. The most frequent clinical findings among FCoV-positive cats were abdominal distension (50%), uveitis (32.1%), ataxia (21.4%), and rhinitis (17.9%). Most infected cats (71.4%) were under two years of age, with a slightly higher prevalence in males (57.1%). Phylogenetic analysis of three isolates from ascitic fluid demonstrated a close genetic relationship with strains from Italy, the United Kingdom, and China, clustering into two distinct clades. The present study highlighted the utility of RT-qPCR targeting the *N* gene as a reliable diagnostic tool for clinical cases, while providing new clinicopathological and molecular data on naturally occurring FCoV infection in Indonesia. These findings contribute to the global understanding of FCoV molecular epidemiology and support future surveillance and control strategies for coronavirus infections in domestic cats.

**Keywords:** Cat, Feline coronavirus, Feline infectious peritonitis, Phylogenetic analysis

## INTRODUCTION

Feline coronavirus (FCoV) belongs to the genus Alphacoronavirus in the family Coronaviridae, order Nidovirales (Theerawatanasirikul et al., 2020), and represents an enveloped, positive-sense, single-stranded RNA virus with a genome size of approximately 27 to 32 kb. The viral genome encodes major structural proteins, including *S*, *M*, *E*, and *nucleocapsid* (*N*), a gene that is highly conserved across strains and abundantly expressed during infection (de Barros et al., 2022; Gao et al., 2023; Shi et al., 2024). This virus is classified into two serotypes, including serotype 1, which predominates in naturally infected cats, and serotype 2, a recombinant form originating from FCoV type 1 and canine coronavirus (Tasker, 2018). Serotyping is primarily based on variations in the *spike* (*S*) gene, which plays a key role in viral entry into host cells. Within each serotype, FCoV occurs in two biotypes, including feline enteric coronavirus (FECV), which usually causes mild self-limiting enteritis, and feline infectious peritonitis virus (FIPV), a virulent variant arising from mutations that allow monocyte and macrophage infection and lead to systemic disease (Pedersen, 2009; Felten and Hartmann, 2019). Approximately 10-12% of FCoV infections can progress to feline infectious peritonitis (FIP), a fatal immune-mediated condition (Tasker et al., 2023).

Feline coronavirus infection is often asymptomatic or presents with mild gastrointestinal signs such as diarrhea and vomiting (Felten et al., 2022). In contrast, FIP occurs in two clinical presentations, including the effusive (wet) form and the non-effusive (dry) form (Tasker et al., 2022). The effusive form is characterized by protein-rich fluid accumulation in the abdominal or thoracic cavities, whereas the non-effusive form is associated with granulomatous inflammatory lesions affecting different organs, particularly the eyes, central nervous system, and abdominal organs (Tasker, 2018; Tasker et al., 2022). Clinical signs of the dry form may include uveitis, ataxia, nystagmus, and chronic rhinitis (Andre et al., 2020; Tasker et al., 2022).

ORIGINAL ARTICLE  
Received: July 08, 2025  
Revised: August 10, 2025  
Accepted: September 07, 2025  
Published: September 30, 2025

Antemortem diagnosis of FCoV-associated conditions, particularly FIP, remains challenging because clinical signs often overlap with those of other feline diseases, and conventional diagnostic tools have limited sensitivity and specificity (Tasker et al., 2022). Although immunohistochemistry for detecting viral antigens in tissues is considered the gold standard, it is invasive and unsuitable for routine clinical use (Felten and Hartmann, 2019). Molecular diagnostics such as reverse transcription quantitative polymerase chain reaction (RT-qPCR) are now widely applied due to the high sensitivity and specificity for FCoV detection (Barker and Tasker, 2020). Several RT-qPCR assays have been developed that target different regions of the viral genome, including the *spike* (*S*) gene, the 3' untranslated region (3'UTR), and the *N* gene (de Barros et al., 2022; Gao et al., 2023; Shi et al., 2024). Among these, the *N* gene is considered a reliable target because it is highly conserved across different FCoV strains and is abundantly transcribed during viral replication, thereby increasing assay sensitivity (Guan et al., 2020; Gao et al., 2023; Shi et al., 2024). Recent studies, including those from Guangxi, China, and northern Vietnam, have successfully employed RT-qPCR assays that include the *N* gene among conserved targets, demonstrating its stability and diagnostic performance across diverse geographical isolates (Dong et al., 2024; Shi et al., 2024).

Beyond molecular detection, phylogenetic analysis plays a pivotal role in understanding the genetic diversity and evolutionary relationships of circulating strains, thereby supporting epidemiological surveillance and informing preventive measures (Decaro and Lorusso, 2020). In Indonesia, reports on the molecular epidemiology of FCoV remain limited. Wasissa et al. (2021) detected FCoV in clinically suspected FIP cases and identified two genetic clusters of local isolates. Aksono et al. (2023) analyzed FCoV isolates in Surabaya, Indonesia, and demonstrated genetic similarities with strains from Europe and North America. More recently, Fitrawati et al. (2025) reported the detection of FCoV in imported cats, highlighting the risk of cross-border introduction.

Despite significant advances, comprehensive molecular characterization of naturally occurring FCoV cases in high-density pet populations, such as those in Yogyakarta, Indonesia, remains rare. Addressing this knowledge gap is essential not only for regional veterinary diagnostics but also for advancing the global comprehension of FCoV diversity and evolution. Based on the most recent available information, this investigation is the first study to combine clinical observations with RT-qPCR detection and phylogenetic analysis of naturally occurring FCoV infections in domestic cat in Indonesia. Therefore, the present study aimed to detect FCoV in clinically suspected cats in Yogyakarta, Indonesia, using RT-qPCR targeting the *N* gene and to perform phylogenetic analysis of local viral isolates.

## MATERIALS AND METHODS

### Ethical approval

All experimental procedures were approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia (00113/EC-FKH/Int./2021). Informed consent was obtained from both veterinarians and cat owners before sample collection.

### Study duration and location

The present study was conducted from June 2021 to September 2022 at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

### Sample collection

A total of 45 clinical samples (14 ascitic fluid, 31 blood) were obtained from cats of different breeds (mixed, domestic, Persian), aged 0.5 to 4 years (22 males, 23 females), with signs of FCoV-associated disease (vomiting, diarrhea, abdominal effusion, uveitis, and ataxia). Samples were collected from 7 veterinary clinics in Yogyakarta, Indonesia. With the consent of owners, cats underwent clinical examinations, anamnesis, and sample collection conducted by veterinarians. Blood samples (2 mL) were drawn into tubes containing EDTA, while effusion fluids were collected in sterile tubes. All samples were subsequently processed and analyzed at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, University of Gadjah Mada, Yogyakarta, Indonesia.

### Reverse transcription-quantitative polymerase chain reaction for feline coronavirus detection

Viral RNA was extracted using the Quick-RNA Viral Kit (Zymo Research, California, USA) according to the manufacturer's protocol. All RNA samples were stored at -40°C before analysis. Feline coronavirus detection was conducted using RT-qPCR targeting the *N* gene, with the following specific primers including forward 5'-TGCTTCGGCTAACTTTGGTG-3' and reverse 5'-CAATCATCTCAACCTGTGTGTCAT-3' (Guan et al., 2020).

The RT-qPCR reaction was performed in a total volume of 20 µL, consisting of 10 µL One Step SYBR Green RT-qPCR buffer 2X, 0.8 µL ABScript II One Step Enzyme Mix, 0.8 µL of forward and reverse primers, 0.4 µL ROX Dye

50X, 6 µL RNase-free ddH<sub>2</sub>O, and 2 µL of template RNA. Amplification was performed using a Bio-Rad CFX96 PCR machine (Bio-Rad, California, USA) as follows. Reverse transcription was carried out at 50°C for 5 minutes, followed by initial denaturation at 95°C for a minute. The reaction then proceeded through 40 cycles of denaturation at 95°C for 15 seconds and annealing at 60°C for 31 seconds, concluding with a melting curve analysis. Samples were considered positive for FCoV when the cycle threshold (Ct) was <35 and the melting curve peaked at 80°C ( $\pm 5^\circ\text{C}$ ; Sun et al., 2021). Each RT-qPCR and RT-PCR run included positive and negative controls to ensure assay reliability. The positive control (K+) consisted of RNA from a 2-year-old female cat with ascites previously confirmed as FCoV-positive, which consistently amplified the *N* gene and 5'UTR. The negative control (K-) consisted of nuclease-free water to monitor for potential contamination. A negative biological control using RNA from Specific Pathogen Free cats was not included in this study.

### Phylogenetic analysis

To investigate the genetic characteristics of local FCoV circulating in Yogyakarta, five RT-qPCR-positive samples were selected for DNA sequencing. Selection criteria included high viral load (lower Ct values) and diversity in clinical presentation and sample type, including blood and ascitic fluid, to ensure representative coverage of positive cases. Conventional RT-PCR was then performed on these samples using the same primer pair. Reactions were carried out with the MyTaq One-Step RT-PCR Kit (Bioline, London, UK) according to the manufacturer's instructions, in a total volume of 50 µL containing 25 µL MyTaq One-Step Mix 2X, 2 µL of each primer, 0.5 µL reverse transcriptase, 1 µL RNase inhibitor, 15.5 µL DEPC-treated water, and 4 µL template RNA.

Cycling included reverse transcription at 55°C for 30 minutes, initial denaturation at 95°C for a minute, and 35 cycles of 94°C for 15 seconds, 60°C for 15 seconds, and 72°C for 10 seconds, followed by melting curve analysis. Polymerase chain reaction products were visualized on 2% agarose gel stained with Safe Red (ABclonal, Wuhan, China), excised at the expected size (386 bp), purified, and submitted for Sanger sequencing. Conventional RT-PCR was used solely to generate DNA amplicons for Sanger sequencing and phylogenetic analysis, and not to confirm RT-qPCR results.

Nucleotide sequences obtained were aligned using ClustalW in MEGA 11 with default parameters and manually inspected as needed. Nucleotide substitution models were evaluated using the Model Selection tool in MEGA 11 based on the Bayesian Information Criterion (BIC), and the model with the lowest BIC value was selected for constructing the Maximum Likelihood phylogenetic tree. Phylogenetic trees were constructed using the Maximum Likelihood method with 1000 bootstrap replicates, and sequences were compared to global FCoV reference strains from GenBank to assess evolutionary relationships and potential geographic origins.

### Statistically analysis

Associations between sample origin, clinical signs, and viral burden (*N* gene RT-qPCR Ct values) were analyzed using the Mann–Whitney U test, while differences in sex and age groups were assessed with Fisher's exact test; *p*-values <0.05 were considered statistically significant.

## RESULTS

A total of 45 clinical samples were collected from cats suspected of FIP, including 31 blood samples and 14 ascitic fluid samples. RT-qPCR targeting the *N* gene detected FCoV in 28 of 45 samples (62.2%). Notably, all ascitic fluid samples (100%) tested positive, whereas only 14 of 31 blood samples (45.2%) were positive. The detection rate in ascitic fluid was significantly higher than in blood samples ( $p < 0.05$ ). Data regarding sample codes, controls, clinical origin, sex, breed, age, sample type, and *N* gene cycle threshold (Ct) values are presented in Table 1.

Positive ascitic fluid samples exhibited lower Ct values (mean  $22.26 \pm 2.19$ ) compared to blood samples (mean  $24.10 \pm 2.92$ ), indicating higher viral loads, although this difference did not reach conventional statistical significance ( $p = 0.069$ , Mann–Whitney U test), and the results are presented in Table 2. Polymerase chain reaction amplification of the *N* gene is shown in Figure 1. Clinical records indicated that the most common presenting sign in FCoV-positive cats was abdominal distension (50.0%), followed by uveitis (32.1%) and ataxia (21.4%). Cats with abdominal distension had the lowest mean Ct values (22.26), consistent with the highest viral load, whereas uveitis cases exhibited the highest mean Ct values (24.68), suggesting lower viral burden. Of the 28 FCoV-positive cats, 20 (71.4%) were under 2 years of age, showing a significantly higher positivity rate than older cats ( $p = 0.008$ , Fisher's exact test). In terms of sex distribution, 57.1% (16/28) of the infected cats were male, and 42.9% (12/28) were female, with no statistically significant difference in infection rate between sexes ( $p > 0.05$ ).

Partial *N* gene sequences (386 bp) from five selected FCoV-positive samples shown in Figure 2 were successfully amplified and analyzed. Phylogenetic analysis revealed that local Indonesian isolates clustered closely with strains from Italy, suggesting a shared evolutionary origin and possible global transmission. All sequences have been submitted to GenBank (Accession Numbers: PV741167 to PV741171). The phylogenetic tree is shown in Figure 2. The five Indonesian sequences (PV741167 to PV741171) formed a well-supported monophyletic clade (100% bootstrap) with very low pairwise genetic divergence (0.0027-0.0304). This clade grouped with strong support (1000 bootstrap replicates) alongside reference strains from Italy (GU017104.1) and the United Kingdom (KP143512.1), indicating a close evolutionary relationship.

**Table 1.** The RT-qPCR detection of feline coronavirus and confirmation by *N* gene amplification in 45 clinically suspected cats in Yogyakarta during 2021-2022

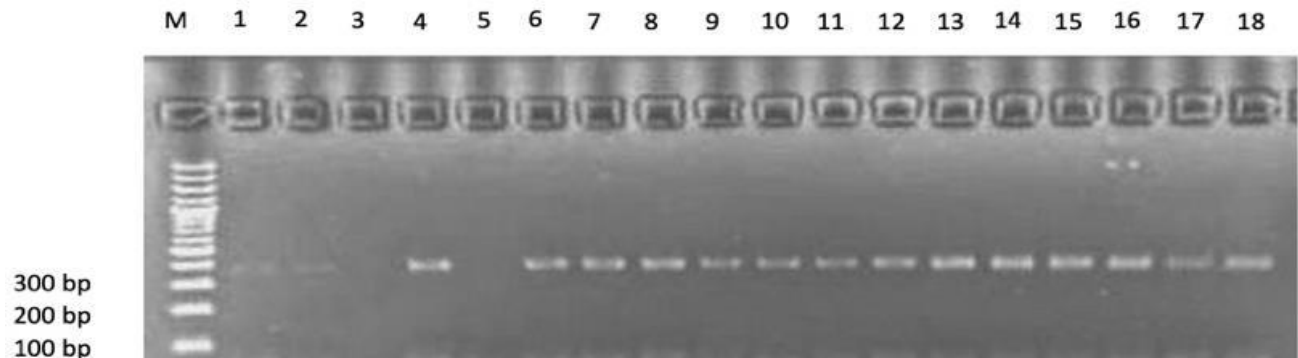
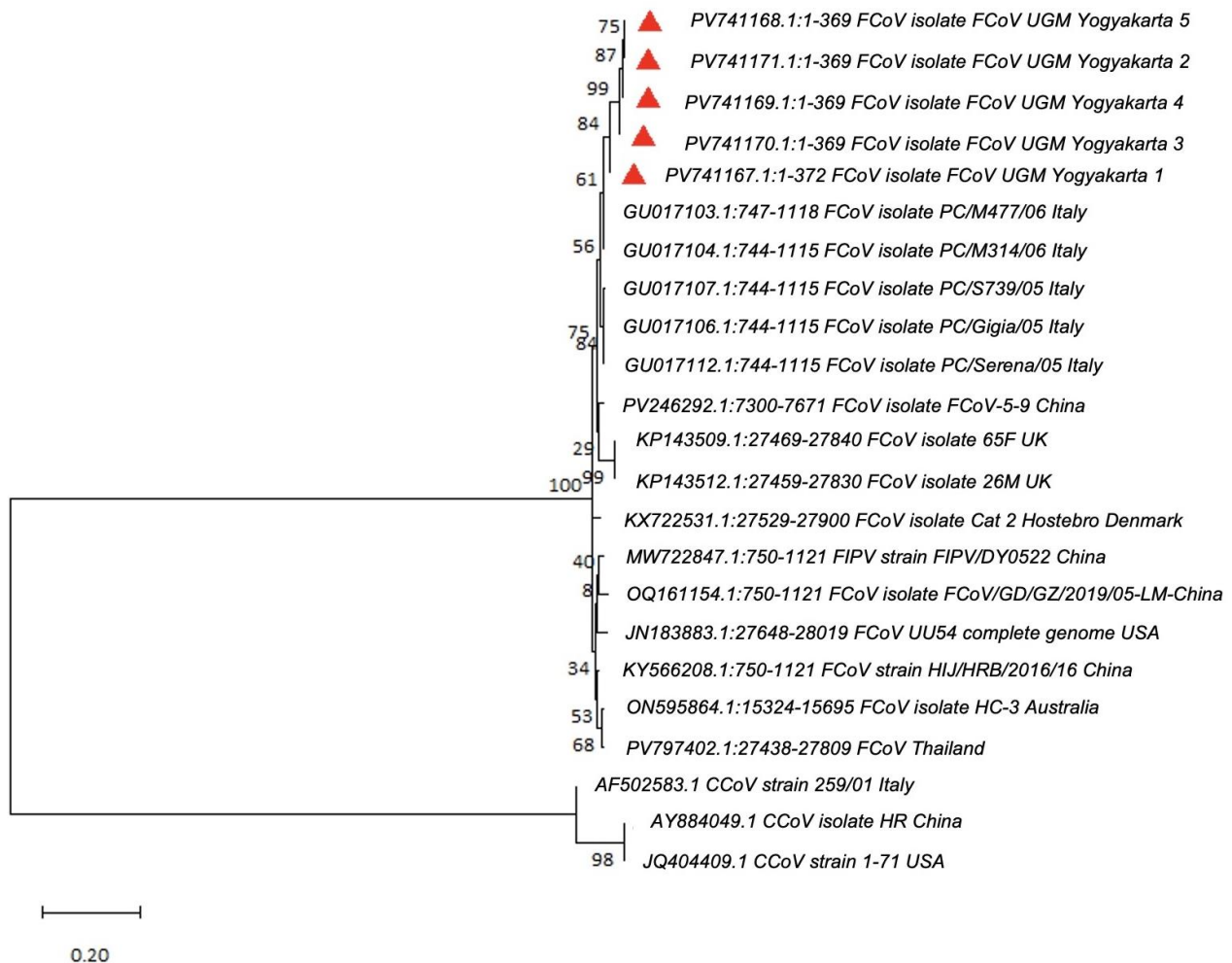
Sample code	Sex	Breed	Age (year)	Sample type	Sample source	Cycle threshold ( <i>N</i> gene)	Cycle threshold (5'UTR)	Result
K+*	Female	D	2	Ascites	KP	21.17	21.41	Positive
K-	-	-	-	PCR Water	-	N/A	N/A	Negative
K-01	Female	M	1	Ascites	SWK	20.84	28.18	Positive
K-02	Female	M	1	Ascites	SWK	22.05	26.7	Positive
K-03	Female	M	1	Ascites	SWK	28.17	27.16	Positive
K-04	Female	M	2	Ascites	SWK	24.54	26.91	Positive
K-05	Female	P	1	Blood	NP	22.34	28.14	Positive
K-06	Female	M	3	Blood	NP	N/A	N/A	Negative
K-07	Male	D	2	Blood	NP	25.4	29.7	Positive
K-08	Male	M	2	Blood	DPC	30.55	28.23	Positive
K-09	Male	D	3	Blood	DPC	N/A	N/A	Negative
K-10	Male	D	1	Blood	DPC	N/A	N/A	Negative
K-11	Male	M	1	Blood	DPC	25.24	29.25	Positive
K-12	Male	M	1	Blood	DPC	26	29.07	Positive
K-13	Male	M	1	Blood	DPC	21.83	28.65	Positive
K-14	Female	D	3	Blood	DPC	N/A	N/A	Negative
K-15	Female	P	1	Blood	DPC	N/A	N/A	Negative
K-16	Female	M	1	Blood	DPC	22.11	29.53	Positive
K-17	Male	M	0.5	Blood	DPC	20.14	29.06	Positive
K-18	Male	D	1	Blood	DPC	21.46	23.4	Positive
K-19	Male	P	3	Blood	DPC	N/A	N/A	Negative
K-20	Female	M	1	Blood	DPC	N/A	N/A	Negative
K-21	Male	D	0.5	Blood	DPC	N/A	N/A	Negative
K-22	Female	P	3	Blood	DPC	N/A	N/A	Negative
K-23	Female	M	1	Blood	DPC	N/A	N/A	Negative
K-24	Female	M	2	Blood	DPC	N/A	N/A	Negative
K-25	Female	D	4	Blood	DPC	N/A	N/A	Negative
K-26	Female	D	3	Ascites	SWK	20.9	20.37	Positive
K-27	Male	D	2	Ascites	SWK	21.87	26.95	Positive
K-28	Male	P	2	Ascites	SWK	22.36	28.66	Positive
K-29	Female	P	0.5	Blood	SWK	28.35	30.79	Positive
K-30	Female	D	2	Ascites	SWK	20.28	23.9	Positive
K-31	Female	M	1	Ascites	SWK	20.28	23.6	Positive
K-32	Male	M	1	Ascites	KNG	21.53	24.16	Positive
K-33	Male	D	1	Blood	NP	21.05	25.2	Positive
K-34	Male	P	1	Ascites	PPC	20.17	25.51	Positive
K-35	Male	P	2	Ascites	PPC	20.67	13.73	Positive
K-36	Male	M	2	Blood	KHJ	N/A	N/A	Negative
K-37	Male	M	1	Blood	KHJ	24.64	21.17	Positive
K-38	Male	D	3	Blood	KHJ	N/A	N/A	Negative
K-39	Female	P	2	Blood	KHJ	N/A	N/A	Negative
K-40	Female	P	1	Blood	KHJ	N/A	N/A	Negative
K-41	Female	M	1	Ascites	KP	24.9	25.58	Positive
K-42	Female	P	4	Blood	KP	23.1	25.71	Positive
K-43	Male	D	1	Blood	NP	24.06	24.35	Positive
K-44	Female	D	1	Blood	NP	24.21	28.39	Positive
K-45	Male	P	1	Ascites	KNG	23.1	29.06	Positive

K+: Tested positive in PCR and RT-qPCR assays targeting the *N* gene and 5'UTR. RT-qPCR: Reverse transcription-quantitative polymerase chain reaction, PCR: Polymerase chain reaction, FCoV: Feline coronavirus, N/A: Not available, D: Domestic, M: Mixed breed, P: Persian, SWK: Satwakita Vet Clinic, NP: Naroo Pet Vet Clinic, DPC: Dji'o Pet Care Clinic, KNG: Kuningan Vet Clinic, PPC: Panda Pet Care Clinic, KHJ: Jogja Vet Clinic, KP: Prosper Vet Clinic.

**Table 2.** Statistical analysis of feline coronavirus positivity and cycle threshold

Comparison	Test	P-value	Interpretation
Ascitic fluid compared to blood (positivity)	Fisher's exact test	0.00025	Highly significant difference
CT values (ascitic fluid vs blood)	Mann-Whitney U	0.0695	Not significant (trend toward lower Ct in fluid)
Age group and FCoV positivity	Fisher's exact test	0.0081	Significant difference (higher risk in young cats)
Sex and FCoV positivity	Fisher's exact test	0.5420	Not significant

FCoV: Feline coronavirus, Ct: Cycle threshold

**Figure 1.** The feline coronavirus-positive samples from cats with different breeds (mixed breed, domestic, and Persian) in Yogyakarta, Indonesia, during 2021-2022. Lanes 4, 6-18: Showing an expected amplicon size of 386 bp for the *N* gene, Lanes 1-3, 5: Negative samples. M: Marker (100 bp).**Figure 2.** Feline coronavirus isolates (▲) phylogenetic analysis based on the *N* gene compared to reference sequences published in GenBank in Yogyakarta during 2021-2022. The analysis was conducted using MEGA 12 using the Maximum Likelihood model with 1000 bootstrap replicates.



## DISCUSSION

The results of the present study demonstrated the effectiveness of RT-qPCR targeting the *N* gene in detecting FCoV infections. With an overall detection rate of 62.22% (28/45), these findings highlighted the utility of RT-qPCR in identifying FCoV-positive cases with greater sensitivity (He et al., 2024). Notably, all effusion fluid samples (14/14) tested positive, whereas 45.16% (14/31) of blood samples were positive, indicating that the viral load in effusion fluid was significantly higher, supporting its role as a primary diagnostic sample. These findings reinforce the importance of using effusion fluid for diagnosing FCoV, particularly in cases suspected of FIP, the severe manifestation of FCoV infection.

Clinical signs observed in infected cats further validate the RT-qPCR results. Abdominal effusion was noted in 50% (14/28) of positive cases, aligning with the classic presentation of wet FIP, which is characterized by exudate accumulation due to vasculitis (Carossino et al., 2022). This observation is consistent with Jayanti et al. (2020), who reported that 80% of FIP cases in domestic shorthair were the wet form. The underlying mechanism involves virus-laden monocytes triggering an inflammatory response that damages blood vessels, leading to plasma leakage into body cavities (Cerna et al., 2022).

Neurological involvement, particularly ataxia, was detected in 21.42% (6/28) of cases, corroborating Doenges et al. (2016), who reported that approximately 10% of FIP cases develop neurological signs, with ataxia being among the most frequent manifestations. The presence of FCoV in the central nervous system suggested that infected monocytes can cross the blood-brain barrier, leading to neuroinflammation (Elmakaty et al., 2022). Uveitis was observed in 32.14% (9/28) of cases, indicating the occurrence of dry FIP. Carossino et al. (2022) have previously identified FIP as a common cause of feline uveitis, with histopathological evidence of severe vasculitis in ocular tissues.

The immunological mechanisms involve immune complex deposition, cytokine-mediated inflammation, and infiltration of virus-infected macrophages, which together disrupt the blood-ocular barrier and drive ocular pathology (Andrew, 2000). Rhinitis was present in 17.85% (5/28) of cases, supporting Andre et al. (2020), who described upper respiratory tract involvement in FIP cases, although FCoV is not typically regarded as a primary respiratory pathogen (Pedersen, 2009).

The present study examined epidemiological factors influencing FCoV infection. Males accounted for 57.14% (16/28) of infected cases, while females constituted 42.85% (12/28). This distribution is consistent with Riemer et al. (2016), who reported that approximately 60-65% of FIP cases occurred in males, and Tasker (2018), who similarly noted that males are more frequently affected than females. Feline infectious peritonitis appears slightly less common in female cats, which may be related to the immunomodulatory effects of estrogen, enhancing antiviral responses (Addie et al., 1995; Pedersen, 2009). However, age, neuter status, breed, and environmental factors also influence susceptibility, indicating that sex differences are likely multifactorial (Worthing et al., 2012). Age also played a critical role, with 71.43% (20/28) of infected cats being under two years old, in line with a study by Guan et al. (2020) and Yin et al. (2021), who reported that younger cats are more susceptible due to an immature immune system and increased exposure to environmental stressors.

Phylogenetic analysis in the present study indicated that the FCoV isolates from Yogyakarta, Indonesia, clustered closely with the Italian isolate (GU017103.1, GU017104.1, GU017107.1, GU017106.1, GU017112.1), supported by phylogenetic clustering and 1000 bootstrap. A similar clustering pattern, as observed with European reference strains, was also reported in isolates from Surabaya (Aksono et al., 2023) and in imported cat cases in Indonesia (Fitrawati et al., 2025). Furthermore, the presence of FCoV in clinically suspected FIP cats in Yogyakarta (Wasissa et al., 2021) indicated that related strains have been circulating locally for several years. These findings suggested that Indonesian FCoV isolates may share a common ancestry with certain European strains, possibly reflecting historical or regional linkage, likely facilitated by international cat travel or trade. A considerable finding of the present study was the close phylogenetic affiliation of Indonesian isolates with European strains from Italy and the UK, rather than with geographically closer Asian strains. This suggested a potential introduction of a European-like strain into Indonesia, likely facilitated by international cat travel or trade, followed by local spread. These findings underscored the global nature of FCoV transmission and highlighted the need for robust genomic surveillance.

The use of RT-qPCR targeting the *N* gene demonstrated reliable sensitivity for FCoV detection in clinical samples, particularly effusion fluid, while partial sequencing of the *N* gene provided valuable insights into the genetic characteristics and phylogenetic relationships of local FCoV strains. Although the present study was conducted in Yogyakarta, Indonesia, the phylogenetic analysis revealed that these isolates shared close genetic proximity to strains originating from Europe and East Asia. This finding underscored the potential of shared transmission pathways, possible cross-border viral introductions, or common evolutionary origins of FCoV strains.

The genetic clustering of Indonesian isolates with international reference strains highlighted the need for continuous, integrated, global surveillance programs to monitor FCoV genetic variation and emergence. The present study represented the first integrated approach in Indonesia that combines clinical observations, RT-qPCR detection, and phylogenetic analysis of naturally occurring FCoV cases in domestic cats, thereby providing novel insights into the molecular epidemiology of FCoV in this region. In a broader context, these results contribute important molecular data from Southeast Asia, a region underrepresented in global FCoV studies, thereby enriching the global coronavirus sequence database and enhancing preparedness for future interspecies transmission events and emerging feline viral diseases. However, the present study was limited by a small sample size for sequencing and only using partial *N* gene fragments, which constrained broader epidemiological analysis conclusions.

## CONCLUSION

The current study confirmed that RT-qPCR targeting the *N* gene is a reliable diagnostic tool for detecting FCoV in clinical samples, especially effusion fluids. Younger cats and effusive cases exhibited higher infection rates, supporting the clinical and epidemiological associations with molecular findings. Phylogenetic analysis indicated close relationships between local isolates and strains from Europe and Asia, suggesting possible transboundary transmission or shared evolutionary origins. These findings not only provided essential data for feline health management in Indonesia but also contributed to the global molecular epidemiology of FCoV, reinforcing the importance of international surveillance and control strategies. Future studies should expand the sample size, include full-genome sequencing, and investigate host immune responses to better understand viral evolution, pathogenesis, and potential risk factors for FIP development.

## DECLARATIONS

### Acknowledgements

The authors express their gratitude to Gadjah Mada University, Yogyakarta, Indonesia, for providing financial support for the current study through RTA No. 5722/UN1.P.III/Dit-Lit/PT.01.05/2022. Additionally, they acknowledge the contributions of all veterinarians, cat owners, and laboratory technicians who supported this study.

### Authors' contributions

Amalia Fia, Wasissa Madarina, Lestari Fajar Budi, and Salasia Siti Isrina Oktavia participated in Conceptualization. Data curation was conducted by Amalia Fia, Wasissa Madarina, Amalia Fia, and Wasissa Madarina. Amalia Fia and Wasissa Madarina conducted the investigation. Amalia Fia and Wasissa Madarina provided the methodology. Project administration was done by Salasia Siti Isrina Oktavia. Amalia Fia and Wasissa Madarina prepared the resources. Amalia Fia and Wasissa Madarina conducted work with software. Salasio Siti Isrina Oktavia supervised the study. Amalia Fia and Wasissa Madarina validated the study. Amalia Fia and Wasissa Madarina participated in the visualization. The original draft was written by Amalia Fia. Salasia Siti Isrina Oktavia conducted the writing, reviewing, and editing. All authors have read and approved the final edition of the manuscript before publication in the present journal.

### Funding

This study is funded by the University of Gadjah Mada, Yogyakarta, Indonesia, through the RTA research contract No. 5722/UN1.P.III/Dit-Lit/PT.01.05/2022.

### Competing interests

The authors declared no conflict of interest.

### Ethical considerations

This manuscript is original, conducted solely by the authors, and it has not been published elsewhere.

### Availability of data and materials

All data supporting the findings of the present study are presented within the published manuscript. Any additional details can be obtained from the corresponding author upon reasonable request.

## REFERENCES

- Addie DD, Toth S, Murray GD, and Jarrett O (1995). The risk of feline infectious peritonitis in cats naturally infected with Feline coronavirus. *American Journal of Veterinary Research*, 56(4): 429-434. DOI: <https://www.doi.org/10.2460/ajvr.1995.56.04.429>
- Aksono EB, Yunus M, Rahman FH, and Suwarno S (2023). Molecular identification and phylogenetic analysis of Feline coronavirus from domestic cats in Surabaya, Indonesia. *Veterinary World*, 16(5): 1212-1219. DOI: <https://www.doi.org/10.14202/vetworld.2023.76-81>
- Andre NM, Miller AD, and Whittaker GR (2020). Feline infectious peritonitis virus-associated rhinitis in a cat. *Journal of Feline Medicine and Surgery Open Reports*, 6(1): 1-6. DOI: <https://www.doi.org/10.1177/2055116920930582>
- Andrew SE (2000). Feline infectious peritonitis. *Veterinary Clinics of North America: Small Animal Practice*, 30(5): 987-1000. Available at: <https://pubmed.ncbi.nlm.nih.gov/11033870/>
- Barker EN and Tasker S (2020). Update on feline infectious peritonitis. *Practice University of Bristol*, 42(8): 372-383. DOI: <https://www.doi.org/10.1136/inp.m3187>
- Carossino M, Del Piero F, Lee J, Needle DB, Levine JM, Riis RR, Maes R, Wise AG, Mullaney K, Ferracone J et al. (2022). Relationship between uveal inflammation and viral detection in 30 cats with feline infectious peritonitis. *Pathogens*, 11(8): 883. DOI: <https://www.doi.org/10.3390/pathogens11080883>
- Cerna P, Lobo D, Bubenikova J, Vrabeloka J, Molinkova D, and Horin P (2022). Shedding persistency and intensity patterns of Feline coronavirus (FCoV) in feces of cats living in breeding catteries in the Czech Republic. *Research in Veterinary Science*, 152: 524-529. DOI: <https://www.doi.org/10.1016/j.rvsc.2022.09.010>
- de Barros A, de Souza ADR, Falcon A, and Melo FL (2022). Feline coronavirus: Insights into viral pathogenesis based on the spike protein structure and function. *Frontiers in Veterinary Science*, 9: 872122. DOI: <https://www.doi.org/10.3389/fvets.2022.872122>
- Decaro N and Lorusso A (2020). Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses. *Veterinary Microbiology*, 244: 108693. DOI: <https://www.doi.org/10.1016/j.vetmic.2020.108693>
- Doenges SJ, Weber K, Dorsch R, Fux R, Fischer A, Matiassek LA, Matiassek K, and Hartmann K (2016). Detection of Feline coronavirus in cerebrospinal fluid for diagnosis of Feline infectious peritonitis in cats with and without neurological signs. *Journal of Feline Medicine and Surgery*, 18(2): 104-109. DOI: <https://www.doi.org/10.1177/1098612X15574757>
- Dong B, Zhang X, Zhong X, Hu W, Lin Z, Zhang S, Deng H, and Lin W (2024). Prevalence of natural Feline coronavirus infection in domestic cats in Fujian, China. *Virology Journal*, 21: 2. Available at: <https://link.springer.com/article/10.1186/s12985-023-02273-y>
- Elmakaty I, Ferih K, Kare O, Ouda A, Elsabagh A, Amarah A, and Malki MI (2022). Clinical implications of COVID-19 presence in CSF: Systematic review of case reports. *Cells*, 11(20): 3212. DOI: <https://www.doi.org/10.3390/cells11203212>
- Felten S and Hartmann K (2019). Diagnosis of feline infectious peritonitis: A review of the current literature. *Journal Viruses*, 11(11): 1068. DOI: <https://www.doi.org/10.3390/v11111068>
- Felten S, Klein-Richers U, Unterer S, Bergmann M, Leutenegger CM, Pantchev N, Balzer J, Zablotzki Y, and Hofmann-Lehmann R (2022). Role of Feline coronavirus as contributor to diarrhea in cats from breeding catteries. *Viruses*, 14(5): 858. DOI: <https://www.doi.org/10.3390/v14050858>
- Fitrawati F, Suwarno, Yudiantiyananti IS, Rahmahani J, Yunus M, Soeharsono, Karo-karo D, and Heriah (2025). Molecular detection of FIPV among imported felines through Soekarno Hatta Airport, Indonesia. *Media Kedokteran Hewan*, 36(1): 40-49. Available at: <https://ejournal.unair.ac.id/MKH/article/view/60486>
- Gao YY, Wang Q, Liang XY, Zhang S, Zhao H, Wang K, Hu GX, Liu WJ, and Gao FS (2023). An updated review of Feline coronavirus: Mind the two biotypes. *Virus Research*, 326: 199059. DOI: <https://www.doi.org/10.1016/j.virusres.2023.199059>
- Guan X, Li H, Han M, Jia S, Feng B, Gao X, Wang Z, Jiang Y, Cui W, Wang L et al. (2020). Epidemiological investigation of feline infectious peritonitis in cats living in Harbin, Northwest China from 2017 to 2019 using a combination of EvaGreen based real-time RT-PCR and serum chemistry assays. *Molecular and Cellular Probes*, 49: 101495. DOI: <https://www.doi.org/10.1016/j.mcp.2019.101495>
- He M, Feng S, Shi K, Shi Y, Long F, Yin Y, and Li Z (2024). One-step triplex TaqMan quantitative reverse transcription polymerase chain reaction for the detection of Feline coronavirus, feline panleukopenia virus, and feline leukemia virus. *Veterinary World*, 17(5): 946-955. DOI: <https://www.doi.org/10.14202/vetworld.2024.946-955>
- Jayanti P, Gunawan I, and Sulabda N (2021). Laporan kasus: *Feline* infectious peritonitis virus pada kucing lokal jantan yang mengalami Asites [Case report: Feline infectious peritonitis virus in male local cat with Ascites]. *Buletin Veteriner Udayana*, 13(2): 96-205. Available at: <http://download.garuda.kemdikbud.go.id/article.php?article=2232169&val=941&title=Laporan%20Kasus%20Feline%20Infectious%20Peritonitis%20Virus%20pada%20Kucing%20Lokal%20Jantan%20Yang%20Mengalami%20Asites>
- Pedersen NC (2009). A review of feline infectious peritonitis virus infection: 1963 to 2008. *Journal of Feline Medicine and Surgery*, 11(4): 225-258. DOI: <https://www.doi.org/10.1016/j.jfms.2008.09.008>
- Riemer F, Kuehner K., Ritz S, Louis CS, and Hartmann K (2016). Clinical and laboratory features of cats with feline infectious peritonitis- A retrospective study of 231 confirmed cases (2000-2010). *Journal of Feline Medicine and Surgery*, 18(4): 348-356. DOI: <https://www.doi.org/10.1177/1098612X15586209>
- Shi K, Li Y, Peng G, Huang J, Fu N, Song X, Sha X, and Zhang B (2024). Genetic and phylogenetic analysis of Feline coronavirus in Guangxi Province of China from 2021 to 2024. *Animals*, 11(10): 455. DOI: <https://www.doi.org/10.3390/vetsci11100455>
- Sun L, Xu Z, Wu J, Cui Y, Guo X, Xu F, Li Y, and Wang Y (2021). A duplex SYBR green I- based real-time polymerase chain reaction assay for concurrent detection of Feline parvovirus and Feline coronavirus. *Journal of Virological Methods*, 298: 114294. DOI: <https://www.doi.org/10.1016/j.jviromet.2021.114294>
- Tasker S (2018). Diagnosis of feline infectious peritonitis: Update on evidence supporting on available test. *Journal of Feline Medicine and Surgery*, 20(3): 228-243. DOI: <https://www.doi.org/10.1177/1098612X18758592>
- Tasker S, Addie DD, Egberink H, Hofmann-Lehmann R, Hosi MJ, Truyen U, Belak S, Boucraut-Baralon C, Frymus T, Lloret A et al. (2022). Feline coronavirus and feline infectious peritonitis ABCD guideline with literature review. *European advisory board on cat disease. ABCD FCoV & FIP Guidelines*, pp. 1-58. Available at: <https://www.abcdcatsvets.org/wp-content/uploads/2022/12/ABCD-FCoV-FIP-Guidelines-April-2022.pdf>
- Tasker S, Addie DD, Egberink H, Hofmann-Lehmann R, Hosie MJ, Truyen U, Belak S, Boucraut-Baralon C, Frymus T, Lloret A et al. (2023). Feline Infectious Peritonitis: European Advisory Board on Cat Diseases Guidelines. *Viruses*, 15(9), 1847. Available at: <https://www.doi.org/10.3390/v15091847>



- Theerawatanasirikul S, Kuo C, Phetcharat N, and Lekcharoensuk P (2020). *In silico* and *in vitro* analysis of small molecules and natural compounds targeting the 3CL protease of feline infectious peritonitis virus. *Antiviral Research*, 175: 104697. DOI: <https://www.doi.org/10.1016/j.antiviral.2019.104697>
- Wasissa AN, Lestari FB, and Salasia SIO (2021). Molecular detection of Feline coronavirus in clinically suspected feline infectious peritonitis cases in Yogyakarta, Indonesia. *SSRN Preprints*, pp. 1-8. DOI: <http://www.doi.org/10.2139/ssrn.3991766>
- Worthing KA, Wigney DI, Dhand NK, Fawcett A, McDonagh P, Malik R, and Norris JM (2012). Risk factor for feline infectious peritonitis in Australian cats. *Journal of Feline Medicine and Surgery*, 14(6): 405-412. DOI: <https://www.doi.org/10.1177/1098612x12441875>
- Yin Y, Li T, Wang C, Liu X, Ouyang X, Ji W, Liu X, Li J, and Hu C (2021). A retrospective study of clinical and laboratory features and treatment on cats highly suspected of feline infectious peritonitis in Wuhan, China. *Scientific Reports*, 11(1): 52008. Available at: <https://www.nature.com/articles/s41598-021-84754-0>

**Publisher's note:** Scienceline Publication Ltd. remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access:** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <https://creativecommons.org/licenses/by/4.0/>.