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SHORT COMMUNICATION

Molecular Characterization and Phylogenetic Analysis Reveal a Distinct Genotype I FCoV Sub-Lineage in Tangerang, West Java, Indonesia

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ABSTRACT

This study aimed to detect Feline Coronavirus (FCoV) in clinically suspected Feline Infectious Peritonitis (FIP) cases using RT-PCR and to perform phylogenetic analysis of the S gene. A total of 30 samples from 23 symptomatic cats were collected from Tangerang and Bogor, West Java, Indonesia. Sample types included ascites, pleural effusion, rectal swabs, plasma, and feces. FCoV detection was performed using RT-PCR targeting the 3′ UTR and S genes, followed by sequencing. Nineteen samples tested positive for FCoV, and 17 isolates from Tangerang were successfully sequenced. Pairwise homology of the local isolates of the partial S gene showed nucleotide similarities ranging from 88.45% to 100%, with most isolates exhibiting the highest identity (88.72%–91.68%) to an Indian FCoV strain (PP254209). All isolates exhibited adenine (A) at position 23,531, consistent with the wild-type enteric strain that resemble sequences commonly found in FECV. Phylogenetic analysis confirmed that all local FCoV isolates belong to the dominant Genotype I strain, forming a distinct region-specific monophyletic sub-lineage in Tangerang, Indonesia.

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INTRODUCTION

Feline coronavirus (FCoV), a member of the *Coronaviridae* family, infects domestic and wild cats and causes disease ranging from mild enteric illness to fatal systemic infection. The prevalence of FCoV in pet cats is estimated at 25–40% globally, occurring most frequently in young cats aged 0–1 years (52.1%) and male domestic cats (62.4%) (Benetka *et al.*, 2004). FCoV consists of two genotypes, type I and type II, which occur as two biotypes: Feline Enteric Coronavirus (FECV), responsible for mostly mild or asymptomatic enteric infections, and Feline Infectious Peritonitis Virus (FPIV), the highly pathogenic biotype causing FIP. Distinguishing FECV from FIPV is difficult because differences are mainly associated with pathogenicity rather than antigenicity or genome sequence (Gao *et al.,* 2023).

Feline Infectious Peritonitis remains one of the most severe viral diseases in cats (Pedersen, 2009). Its clinical and laboratory abnormalities are nonspecific, and definitive diagnosis traditionally requires histopathology. Serological assays commonly used in Indonesia, including rapid antibody tests which is lack diagnostic accuracy due to cross-reactivity with non-pathogenic FECV strains (Felten & Hartmann, 2019). RT-PCR provides rapid and sensitive detection of FCoV, but results must be interpreted alongside clinical signs, as PCR alone cannot confirm FIP (Sharif *et al.,* 2010). PCR assays frequently target conserved regions such as the 3′UTR, Pol, and 7b genes (Herrewegh *et al.,* 1995; Benetka *et al.,* 2004). The Spike (S) gene is also widely used due to its variability and utility for serotype differentiation (Lin *et al.,* 2022).

In Indonesia, scientific literature on FCoV remains limited, with only nine publications to date (Wasissa, 2021; Jayanti *et al.,* 2021; Sumule *et al.,* 2022; Diana *et al.,* 2022; Hartono *et al.,* 2022; Aksono *et al.,* 2023; Amalia *et al.,* 2023; Permatasari *et al.,* 2024; Fitrawati *et al.,* 2025). Among these, only seven employed PCR, and only two

included phylogenetic analysis (Wasissa, 2021*;* Aksono *et al.,* 2023). Therefore, this study aimed to detect FCoV in symptomatic cats using RT-PCR targeting the 3′UTR and S gene and to perform phylogenetic analysis of selected S-gene segments.

MATERIALS AND METHODS

Ethical clearance: This study was approved by the Veterinary Ethics Commission, School of Veterinary Medicine and Biomedical Sciences, IPB University (Certificate No. 255/KEH/SKE/IX/2024).

Cat samples: A total of 30 samples were obtained from 23 domestic cats clinically diagnosed with FIP in Bogor (n= 7) and Tangerang (n=23), with informed owner consent, including ascitic fluid (n=3), pleural effusion (n=2), rectal swabs (n=11), plasma (n=8), and feces (n=6). The discrepancy between the number of samples and the number of animals was due to multiple samples being collected from some individuals (Table 1). Samples were stored at –20°C. The FIP vaccine Primucell-FIP™ (Pfizer Animal Health, USA) was used as a positive control.

Table 1: Sample Description and RT-PCR Results

No.	Sample ID[a]	Origin	Sample type	RNA concentration (ng/uL)	RT-nPCR 3'UTR gene	RT-PCR S gene
1	A	Bogor	pleural effusion	29,1	+	-
2	B	Bogor	ascitic fluid	6,6	+	+
3	C	Bogor	pleural effusion	7,9	+	+
4	D	Bogor	Feces	65	+	-
5	E1	Tangerang	Plasma	7	+	+
6	E2	Tangerang	Feces	53,6	+	-
7	F1	Tangerang	Plasma	-25,7	na[b]	na
8	F2	Tangerang	Feces	11	+	+
9	G1	Tangerang	Plasma	-67,1	na	na
10	G2	Tangerang	Feces	72,3	+	+
11	H1	Tangerang	Plasma	-74,9	na	na
12	H2	Tangerang	Feces	40,4	+	+
13	I1	Tangerang	rectal swab	108,8	+	+
14	I2	Tangerang	Plasma	2,7	+	-
15	J1	Tangerang	rectal swab	20,2	+	+
16	J2	Tangerang	Plasma	27,1	+	-
17	K	Tangerang	rectal swab	5,8	+	+
18	L	Tangerang	rectal swab	8,1	+	+
19	M	Tangerang	rectal swab	6,5	+	+
20	N	Tangerang	rectal swab	80,3	+	+
21	O1	Tangerang	rectal swab	5,2	+	+
22	P2	Tangerang	ascitic fluid	nd	+	+
23	Q2	Tangerang	Plasma	-11,6	na	na
24	Q1	Tangerang	rectal swab	6,6	+	+
25	S	Bogor	ascitic fluid	nd	+	+
26	T	Bogor	Plasma	-0,7	na	na
27	U	Tangerang	Feces	32,5	+	+
28	V	Bogor	rectal swab	-13,2	na	na
29	pB	Tangerang	rectal swab	nd[c]	+	+
30	pE	Tangerang	rectal swab	nd	+	+

[a]The same letters indicate that the samples were collected from the same cat. [b]Not applicable due to low RNA concentration. [c]Not determined

Viral RNA extraction: Viral RNA was extracted using the Viral Nucleic Acid Extraction Kit II (Geneaid, New Taipei City, Taiwan) and quantified with a NanoDrop 2000 (Thermo Scientific, Delaware, USA).

PCR amplification: Detection of the 3′UTR region used RT-nested PCR (Herrewegh *et al.,* 1995) with MyTaq One-Step RT-PCR (Meridian Bioscience, Tennessee, US). RT-PCR used primers p205/p211, followed by nested PCR with p204/p276. Thermal conditions followed established protocols from manufactures, and amplicons (177 bp) were visualized on 2% agarose gel.

For S-gene amplification, cDNA was synthesized with primer FCoV-S4-R and ReverTra Ace-α® (Toyobo, Osaka, Japan). PCR was performed using MyTaq HS Red Mix (Meridian Bioscience, Tennessee, US) with primers FCoV-S4-F and FCoV-S4-R (Shi *et al.,* 2024). Cycling consisted of initial denaturation at 95°C, 35 cycles at 94°C, 58°C, and 72°C, and final extension at 72°C. The expected 1032 bp amplicons were confirmed on 2% agarose gel and sequenced by First Base (Malaysia) using Sanger sequencing.

Sequence and phylogenetic analysis: Forward and reverse chromatograms were trimmed, assembled into contigs, and analyzed for sequence homology using BLASTn. Heatmaps of similarity values were generated using GraphPad Prism 10.5. Multiple alignments of partial S-gene sequences were performed in MEGA 12.0.10 (ClustalW), including assessment of mutations at site 23,531 relative to HLJ/HRB/2016/10 (KY566209.1). The phylogenetic analysis included 32 global reference S-gene sequences retrieved from GenBank, which were selected because they originated from complete whole-genome sequences. A Neighbor-Joining (NJ) phylogenetic tree was constructed using the Tamura–Nei model with 1000 bootstrap replicates. The NJ method was selected as a distance-based approach suitable for reconstructing phylogenetic relationships among partial gene sequences and for efficient visualization of clustering patterns among the isolates. A canine coronavirus sequence was used as an outgroup. Trees were visualized using MEGA and iTOL.

RESULTS AND DISCUSSION

Of the 30 collected samples, only 24 had sufficient RNA concentration to undergo RT-nPCR. Five plasma samples and one rectal swab sample were excluded from the analysis due to insufficient RNA concentration, likely resulting from RNA degradation or low viral load in the specimens. The exclusion was based exclusively on RNA quality criteria and was unrelated to sample origin or clinical characteristics, thereby reducing the risk of sampling bias. All 24 samples tested positive for FCoV using RT-nPCR targeting the highly conserved 3'UTR gene, indicating the presence of FCoV RNA and validating the reliability of the primers (Herrewegh *et al.,* 1995; Shi *et al.*, 2024). However, only 19 samples yielded positive results for the S gene target, and only 17 isolates were successfully sequenced, all originating exclusively from Tangerang, Indonesia. The generally lower detection rate of the S gene compared to the 3'UTR is likely due to the significant genetic variability in the FCoV S protein, which can reduce primer sensitivity (Sangl *et al.,* 2020).

Pairwise homology analysis of local isolates of the partial S gene showed that these isolates shared nucleotide similarities ranging from 88.45% to 100%, as presented in Fig. 1. BLASTn analysis indicated that most Tangerang isolates exhibited the highest similarity (88.72%–91.68%) to an Indian FCoV isolate (accession no. PP254209). Although these values are lower than the typical high

nucleotide identity values commonly observed in viral sequence comparisons (98–100%) (Edgar, 2018), this finding may be explained by differences in sequence length and genomic regions used for comparison. The reference sequences retrieved from GenBank were derived from whole-genome sequences, whereas the sequences generated in this study correspond to a partial region of the S gene (nucleotides 23,100–24,131) (Shi *et al*., 2024). In addition, the spike (S) gene is known to be one of the most variable regions of the FCoV genome. Therefore, comparisons restricted to this partial and genetically variable segment may result in relatively lower percentage identity values while still indicating a distant genetic relationship between the isolates.

Phylogenetic analysis confirmed that all Indonesian FCoV isolates belong to Genotype I, clustering with reference strains from various international locations (China, Japan, Netherlands, etc.) as presented in Fig. 2. Crucially, the Indonesian isolates formed a distinct monophyletic clade within the Genotype I, grouping with reference strains from various countries, including China, Japan, and the Netherlands, as illustrated in Fig. 2. Notably, thirteen of the Indonesian isolates formed a distinct monophyletic subclade within Genotype I, supported by a bootstrap value of 100%, indicating a well-supported phylogenetic relationship. This clustering suggests genetic divergence within the Indonesian isolates and may reflect local viral evolution or the emergence of region-specific sublineages in Tangerang. Consistent with this observation, homology analysis revealed that several isolates (I1, J1, pE, and U) exhibited comparatively lower nucleotide similarity (88.78%–91.74%) compared to the remaining thirteen isolates, further highlighting the genetic variability within the studied population.

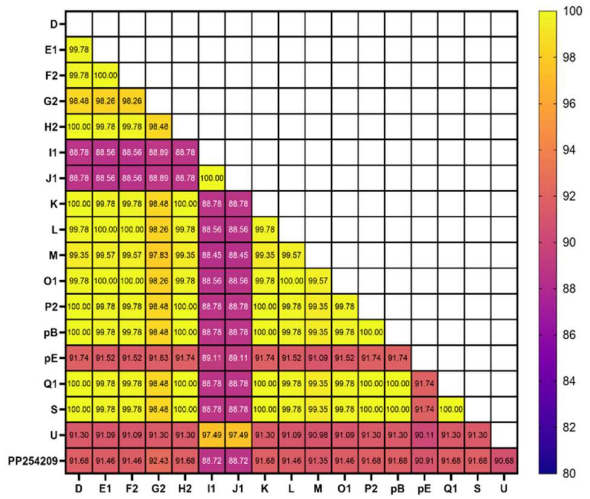

Fig. 1: The sequence identity heatmap illustrates the percentage of nucleotide homology among the FCoV isolates detected in this study, as well as with the reference isolate showing the highest similarity (PP254209 from India) based on BLASTn analysis.

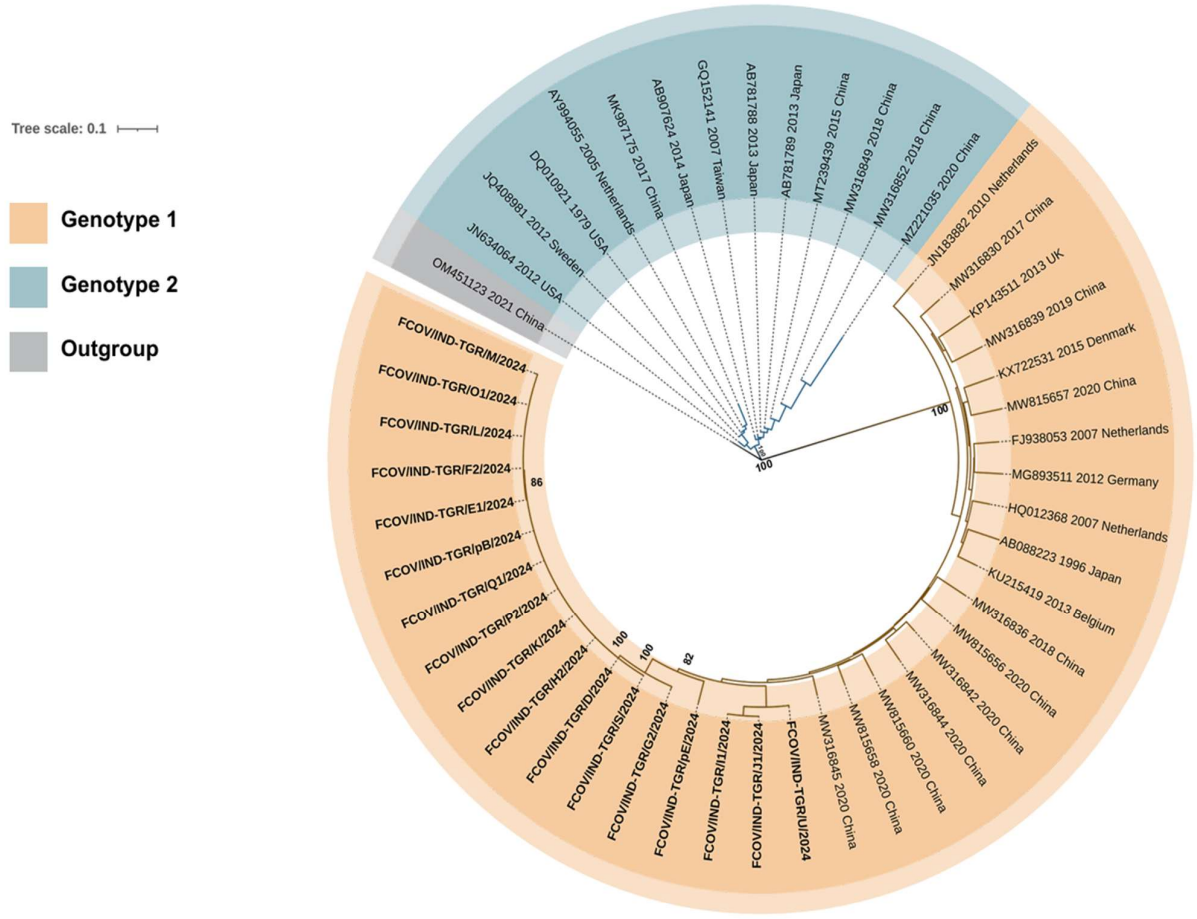

Fig. 2: Phylogenetic analysis based on the partial FCoV S gene nucleotide sequences. The obtained 17 FCoV isolates in bold.

Interestingly, all FCoV isolates exhibited an adenine (A) at nucleotide position 23,531 in the S gene as presented in Fig. 3. This specific site is associated with the wild-type or enteric FCoV strain (FECV), rather than the mutated FIPV forms (Shi *et al.,* 2024). However, the presence of adenine at position 23,531 alone is not sufficient to definitively infer the FECV phenotype, as other mutations have also been associated with the transition from FECV to FIPV. These include nucleotide substitution at position 23,537 (S1060A), alterations in the S1/S2 and S2′ spike cleavage sites, and truncations of the accessory 3c gene (Chang *et al*., 2012; Barker *et al*., 2017; Licitra *et al*., 2013). This finding suggests that the viruses detected in these symptomatic cats resemble sequences commonly found in FECV.

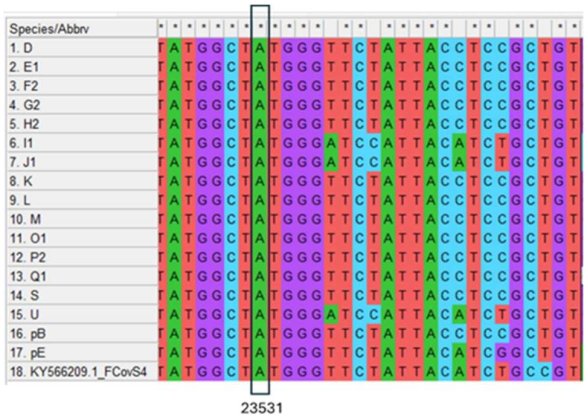

Fig. 3: Amino acid at the 23,531nucleotide site in the S gene of the FCoV isolates from Tangerang, Indonesia.

This study, despite limitations including a small, non-geographically diverse sample size (n=30), the limited number of sequences (n=17) and the use of partial S gene sequencing (limiting mutation analysis to site 23,531), provides vital initial insights into the molecular characteristics of FCoV in Indonesia. The genetic diversity, the dominance of Genotype I, and the presence of the wild-type marker at nt 23,531 highlight the need for continued molecular surveillance using larger, geographically diverse samples and full-length genomic sequences to better understand FCoV epidemiology and evolution in the region.

Conflict of interest: The authors declare that they have no competing interests.

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Authors contribution: ONP contributed to conceptualization, methodology, project administration, funding acquisition, formal analysis, data curation, validation, and writing (original draft and review & editing). EH provided supervision and conceptualization. NLPIM contributed to conceptualization, methodology, and writing (review & editing). RN, RH, LJJ, and DAR carried out the investigation, validation, formal analysis, and data curation, while VAR assisted with investigation and validation.

REFERENCES

Aksono EB, Iradatya KR, Sucipto TH, *et al*., 2023. Phylogenetic analysis of feline infectious peritonitis virus, feline enteric coronavirus, and SARS-CoV-2 in cats from Surabaya, Indonesia. Veterinary World 16(1): 76–81.

Amalia F, Wasissa M, Lestari FB, *et al*., 2023. Clinical and molecular investigation of feline infectious peritonitis treated with GS-441524-containing drug. Asian Journal of Health Sciences 9(2): 386–393.

Barker EN, Stranieri A, Helps CR, *et al.,* 2017. Limitations of using feline coronavirus spike protein gene mutations to diagnose feline infectious peritonitis. Veterinary Research 48(60): 1–14.

Benetka V, Kübber-Heiss A, Kolodziejek J, *et al*., 2004. Prevalence of type I and II feline coronavirus in cats with histopathologically confirmed FIP. Veterinary Microbiology 99(1): 31–42.

Chang HW, Egberink HF, Halpin R, *et al*., 2012. Spike protein fusion peptide and feline coronavirus virulence. Emerging Infectious Diseases 18:1089–1095.

Diana RU, Hermawati F and Pramesti MH, 2022. Confirmation of feline infectious peritonitis diagnosis in cats. ARSHI Veterinary Letters 6(2):29–30.

Edgar RC, 2018. Updating the 97% identity threshold for 16S rRNA OTUs. Bioinformatics 34(14):2371–2375.

Felten S, Hartmann K, 2019. Diagnosis of Feline *Infectious* Peritonitis: A Review of the Current Literature. Viruses 11(11):1068.

Fitrawati F, Suwarno, Yudaniayanti IS, *et al*., 2025. Molecular detection of FIPV in imported cats through Soekarno Hatta Airport, Indonesia. Media Kedokteran Hewan 36(1):40–49.

Gao YY, Wang Q, Liang XY, *et al*., 2023. Updated review on feline coronavirus with focus on its two biotypes. Virus Research 326:1-12

Hartono MI, Endrakasih E and Harry H, 2022. Feline infectious peritonitis in a British Shorthair cat at Petvet Animal Clinic, Jakarta. Journal of Applied Veterinary Science and Technology 3(1):1–5.

Herrewegh AAPM, de Groot RJ, Cepica A, *et al*., 1995. Detection of feline coronavirus RNA in feces, tissues, and fluids of naturally infected cats by RT-PCR. Journal of Clinical Microbiology 33(3): 684–689.

Jayanti PD, Gunawan IWNF and Sulabda NLAKMP, 2021. Feline infectious peritonitis virus in male local cats with ascites: a case report. Buletin Veteriner Udayana 13:196–205.

Licitra BN, Millet JK, Regan AD, *et al*., 2013. Mutation in spike protein cleavage site and pathogenesis of feline coronavirus. Emerging Infectious Diseases 19: 1066–1073.

Lin L, Yao D, Wu L, *et al*., 2022. Molecular epidemiology of type I and II feline coronavirus from suspected FIP cases in China (2019–2021). Archives of Virology 167(1):189–194.

Pedersen NC, 2009. Review of feline infectious peritonitis virus infection, 1963–2008. Journal of Feline Medicine and Surgery 11(4):225–258.

Permatasari M, Ardhiansyah RD, Irianti IN, *et al*., 2024. FIP in a domestic male cat with pleural effusion: a case study [Indonesian]. Journal of Mahanakorn Veterinary Medicine 5:77–84.

Sangl L, Felten S, Matiasek K, *et al*., 2020. Detection of FCoV RNA, spike mutations, and antigen in macrophages of aqueous humor for FIP diagnosis. Journal of Veterinary Diagnostic Investigation 32(4):527–534.

Sharif S, Arshad SS, Hair-Bejo M, *et al*., 2010. Diagnostic methods for feline coronavirus: A review. Veterinary Medicine International 28:1-7.

Shi K, He M, Shi Y, *et al*., 2024. Genetic and phylogenetic analysis of feline coronavirus in Guangxi, China (2021–2024). Veterinary Sciences 11(10):1-14.

Sumule C, Jamaluddin AW, Musdalifah M, *et al*., 2022. Feline infectious peritonitis (FIP) in Himalayan cats at Hasanuddin University Veterinary Clinic. Journal of the Indonesian Veterinary Research 6(2):102–106.

Wasissa M, 2021. Clinicopathological study of natural FIP infection [PhD Dissertation, Gadjah Mada University]. UGM Repository.