Japanese Journal of Veterinary Research 73(1): 10–21, 2025

JJVR

REGULAR PAPER Experimental Research

Risk factors and feline coronavirus gene mutations associated with clinical characteristics of feline coronavirus infection

Sisupa Pongsiwat[1,†], Benjaporn Bhusri[2,†], Siriporn Tangsudjai[2], Aeknarin Saechin[2], Chalisa Mongkolphan[2], Boonyarat Detruengsri[1], Witthawat Wiriyarat[3] and Ladawan Sariya[2,*]

[1] Department of Global PetCare Innovation, i-Tail Corporation Public Company Limited, Thasai sub-district, Muangsamutsakhon District, Samut Sakhon 74000, Thailand

[2] Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom 73170, Thailand

[3] Department of Pre-Clinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom 73170, Thailand

† These authors contributed equally to this work.

Received for publication, September 10, 2024; accepted, January 7, 2025

Abstract

In domestic cats, feline coronavirus (FCoV) infection is widespread, and its outcomes range from no signs to severe or potentially fatal disease. This study investigated the prevalence of infection and associated factors in a population of 277 owned cats in Thailand. The overall proportion of FCoV infections was 45.8%, with positive detections observed for all sample types. The positivity rates were 60.6% for rectal swabs, 51.3% for body fluids, and 11.1% for EDTA blood samples. Although the positive rate was slightly higher in males and more common in younger cats, neither difference was statistically significant. Mathematical modeling revealed a significant association between FCoV infection and the sampling period, with the highest positivity observed in April and June. Through S gene sequencing, positive samples from rectal swabs and body fluids [feline infectious peritonitis (FIP)-cats] were identified as FCoV genotype I. All rectal swab samples had a methionine codon at position 1058 of the S gene, and 62.5% of body fluid samples exhibited a notable substitution (M1058L). Additionally, analysis of the S1/S2 cleavage motif revealed that most FCoV-positive samples contained a polybasic R-R-S/A-R-R-S recognition motif. Notably, 37.5% of rectal swabs exhibited a mutation at the P1 residue (arginine to serine) and 14.3% of FIP-suspected cats exhibited a mutation at the P4 residue (arginine to glycine). These findings contribute to our understanding of the epidemiology of FCoV and its behavior in infected cats. Such insights are crucial for developing effective diagnostics, understanding the FCoV transmission dynamics, and informing potential control strategies.

Key Words: Feline coronavirus, Feline enteric coronavirus, Feline infectious peritonitis, Feline infectious peritonitis virus, Risk factors

* Corresponding author: Ladawan Sariya
Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom 73170, Thailand
E-mail: ladawan.sar@mahidol.edu
doi: 10.57494/jjvr.73.1_10

Introduction

Feline coronavirus (FCoV) is an enveloped single-stranded RNA virus belonging to the *Coronaviridae* family. The FCoV genome is approximately 29 kilobases in length and contains 11 open reading frames encoding 4 structural proteins and 7 nonstructural proteins[16,25]. The viral structure comprises a nucleocapsid (N) surrounding membrane (M), envelope (E), and spike (S) proteins. The S protein is involved in viral attachment to host cellular receptors[13] and is a key factor in the virus's entry into host cells. The accessory proteins 3a, 3b, 3c, 7a and 7b interact with the host immune system. Replicase 1a and 1b proteins are involved in viral replication. FCoVs are classified into two genotypes based on the sequence of S protein: FCoV I and FCoV II. FCoV I is a prominent virus and the most common *Felidae* infection worldwide, whereas FCoV II originates from a combination of canine coronavirus (CCoV) and FCoV I and is less common[9,11,25]. In the United States and Europe, the seroprevalence of FCoV infection in healthy cats is approximately 50%. Similarly, FCoV RNA is detected in 53% of cases, with FCoV type I being more commonly identified, while FCoV type II being relatively rare[10,17,22,28]. In contrast, the prevalence of FCoV in Asia is notably higher. In Malaysia, RT-PCR analysis of clinically ill cats reported an FCoV incidence rate of 95%, with 97% of cases classified as FCoV type I and 3% as FCoV type II[2]. In China, the prevalence of FCoV was 77% in healthy cats and 76% in cats suspected of feline infectious peritonitis (FIP). Among the FCoV-positive samples, 96% were determined to be type I, while 4% were type II[18,38]. In contrast, the reported prevalence of FCoV RNA in Thailand was approximately 40% in healthy and FIP-suspected cats, with FCoV type I being the most frequently detected strain (46%). Additionally, the overall seroprevalence of FCoV in Thailand was recorded at 56%[21,33]. Both FCoV genotypes can be further divided into two biotypes based on their pathogenicity: feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV)[6,11,23]. Infection by an avirulent strain (FECV) typically results in no or mild gastrointestinal signs. Many cats infected with FECV may excrete the virus in their feces without developing severe illness. Infected cats can shed the virus for up to three months[1]. In contrast, FIPV is the more severe form. The clinical signs of severe form vary in the location of vasculitis and granulomatous lesions and include multiple pyogranulomatous lesions in various organs, abdominal swelling, and fluid accumulation in the body cavities. FIPV results from the spontaneous mutations of FECV with modifications to the spike glycoprotein and accessory proteins 3c and 7b have been reported[5,19,37].

Although evidence of FCoV infection has been reported among cat population in Thailand, information on the prevalence of FCoV and gene mutations remains limited. This study investigated FCoV in a population of owned cats, including those with suspected FIP and healthy individuals. FCoV mutations were analyzed and compared with respect to the clinical characteristics. Generalized linear models were also used to investigate the influence of age, sex, breed, and the time of sampling on the proportion of FCoV infection to better understand the potential risk factors of FCoV infection and its association with clinical outcomes. The findings can contribute to a better understanding of the management and control strategies for FCoV.

Materials and Methods

Study design and subject selection

A retrospective study of 277 owned cats collected from various regions across Thailand (including central, eastern, western, and southern parts) was conducted using data from the Monitoring and Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals (MoZWE) at the Faculty of Veterinary Science, Mahidol University, Thailand, in 2023. The cats were analyzed for FCoV infection using reverse transcription polymerase chain reaction (RT-PCR) and classified into two groups based on their clinical status: suspected FIP or healthy. Then, 54 EDTA blood samples and 152 body fluid

samples were collected from cats with suspected FIP without rectal swabs. The diagnosis of suspected FIP was based on a combination of clinical symptoms and diagnostic testing. A case was considered to be suspected FIP if it presented two of the following criteria: clinical indicators, including pleural effusion and/or ascites for effusive FIP, or ocular and neurological signs for non-effusive FIP; a positive result for Rivalta's test in effusion samples; and an albumin/globulin (A/G) ratio below 0.6[4,7,15]. However, effusion may also be present in other conditions. Therefore, abdominal neoplasia, cholangitis, peritonitis associated with intestinal rupture, and heart failure were excluded. Seventy-one rectal swab samples were collected from clinically healthy cats. These clinically healthy cats were gathered for disease prevention testing for companion trading by a veterinarian. All cats in the study tested negative for feline immunodeficiency virus and feline leukemia virus infections. For the 277 cats, information on sex (250/277), age (186/277), and breed (201/277) are shown in supplementary table S1.

Sample RNA extraction and FCoV detection

RNA was extracted from EDTA blood, body fluid (including abdominal fluid and plural fluid), and rectal swab samples using a Total RNA Mini Kit (GeneAid, New Taipei City, Taiwan). The oligonucleotide primers used in this study were designed based on the highly conserved membrane protein (M) gene sequence of the FCoV genome using the NCBI Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). To confirm if RNA extraction process was done successfully, primers specific to the housekeeping gene (β-actin gene) were also included in the RT-PCR mixture. The RT-PCR primers are presented in supplementary table S2. The RT-PCR mixture consists of 5 µl of template RNA, 5 µl of OneStep RT-PCR buffer, 1 µl of 10 mM dNTPs mix, 1 µl of QIAGEN OneStep RT-PCR enzyme mix (QIAGEN, Hilden, Germany), 0.5 µM of each FCoV primer, and 0.32 µM of each β-actin gene primer. The thermal cycling parameters were as follows: 50 min at 50°C for cDNA synthesis; 15 min at 95°C for initial denaturing; followed by 35 cycles of 15 sec at 94°C, 30 sec at 60°C, and 45 sec at 72°C; and termination at 72°C for 10 min. The PCR product sizes of the FCoV fragment and housekeeping gene were 346 and 94 bp, respectively. The PCR products were analyzed using 2% agarose gel electrophoresis followed by GelRed nucleic acid gel staining (Biotium, Inc., Fremont, CA).

Amplification and DNA sequencing analyses of the S gene mutation

Further analysis was performed on a randomly selected subset comprising at least 10% FCoV-positive samples from each sample type. Sequencing focused on the spike protein (S) gene, specifically targeting two regions; the fragment covering amino acid positions 1058 and 1060 and the furin cleavage site located between the receptor-binding (S1) and fusion (S2) domains. The S gene was amplified using nested RT-PCR (primers listed in supplementary table S2). Briefly, FCoV-S-726F and FCoV-S-1464R primers were used in the first round of RT-PCR[31]. The RT-PCR mixture contained 5 µl of template RNA, 5 µl of OneStep RT-PCR buffer, 1 µl of 10 mM dNTPs mix, 1 µl of QIAGEN OneStep RT-PCR enzyme mix (QIAGEN), and 0.5 µM of each primer. The cycling parameters were as follows: 50 min at 50°C for cDNA synthesis; 15 min at 95°C for initial denaturation; followed by 30 cycles of 30 sec at 94°C, 30 sec at 50°C, and 90 sec at 72°C; and termination at 72°C for 10 min. The first round of RT-PCR product was used as a template of the second round PCR. Primers FCoV-UCD1-S-3022 and FCoV-UCD1-S-3636 were used in the second-round PCR to sequence the region covering amino acid positions 1058 and 1060[14]. The PCR mixture consisted of 2 µl of template DNA, 2.5 µl of 10× Mg^{2+}-free buffer, 1.5 mM of Mg^{2+} solution, 1 mM of dNTPs, 2.5 units of i-*Taq* DNA polymerase (iNtRON Biotechnology, Inc., Korea), and 0.5 µM of each primer. The cycling parameters were as follows: 2 min at 94°C for initial denaturing; followed by 30 cycles of 20 sec at 94°C, 20 sec at 47°C, and 60 sec at 72°C; and termination at 72°C for 7 min. The PCR product of the second round of PCR (615 bp in size) was gel-purified and submitted to the U2Bio sequencing service (U2Bio

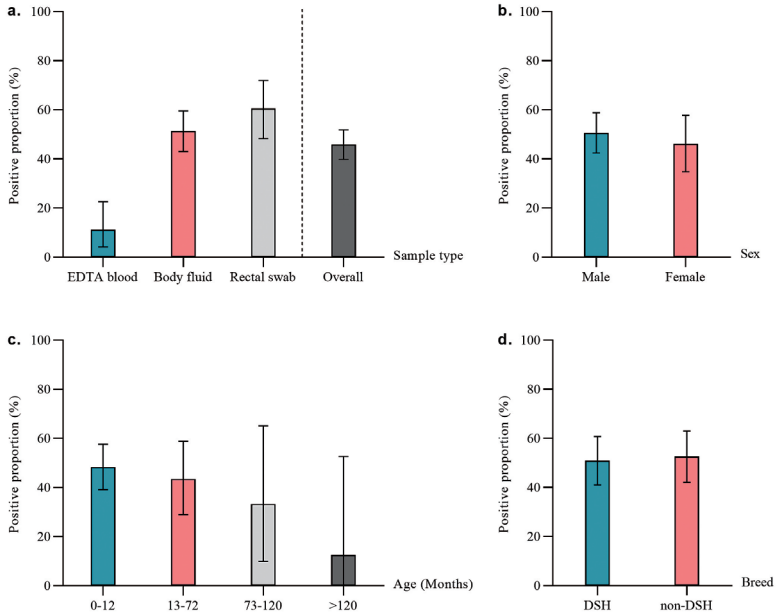


Fig. 1. Feline coronavirus (FCoV) infection according to sample type (a), sex (b), age (c), and breed (d).

Co., Ltd., Korea). To sequence the S1/S2 cleavage site, the nested RT-PCR protocol was followed as described above, with the exception that in the second-round PCR, the primers S1/S2 cleavage site-F and S1/S2 cleavage site-R were used, and the annealing temperature was set to 55°C[19]. The 160 bp PCR product was gel purified and submitted to the U2Bio sequencing service (U2Bio Co., Ltd., Korea).

To identify FCoV genotype, the phylogenetic analysis was conducted using MEGA 11 version 11.0.13 with the neighbor-joining method and a bootstrap value based on 1,000 replicates[32]. The partial S sequences were compared with the corresponding nucleotide sequences of other FCoV isolates retrieved from GenBank. All sequences obtained in this study were deposited in the GenBank database with accession numbers PP601319–PP601335 and PQ641430–PQ641444.

Statistical analyses

The data were analyzed using SPSS 26 (IBM, Armonk, NY, USA). The proportion of FCoV infection was reported with a 95% confidence interval (CI) using Pearson χ^2. Logistic regression analysis was used to assess the correlation between FCoV infection and the demographic of cats, such as age, sex, breed, and month of sampling. Outcomes were expressed as odds ratios (OR) and 95% CI. A *P*-value of < 0.05 was considered statistically significant.

Results

In total, 277 owned cats were investigated for FCoV infection. The overall FCoV infection proportion was 45.8% (127/277; 95% CI: 39.9%–51.9%). Among the positive cases, rectal swab samples showed an FCoV detection rate of 60.6% (43/71; 95% CI: 48.2%–72.0%), body fluid samples had a rate of 51.3% (78/152; 95% CI: 43.1%–59.5%) and EDTA blood samples showed a rate of 11.1% (6/54; 95% CI: 4.2%–22.6%) (Fig. 1a and Supplementary Table S1). FCoV infection by sex showed that the positive rate was higher in males [50.7% (77/152); 95% CI: 42.4%–58.8%] than in females [46.1% (36/78); 95% CI: 34.8%–57.8%], but the difference was not statistically significant (*P* = 0.518) (Fig. 1b and Supplementary Table S1).

Table 1. Univariable analysis of variables associated with the positive proportion of FCoV.

Demographic of cat		Wald	df	*P*-value	OR	95% CI	
						Lower	Upper
Age (month)		3.888	3	0.274			
	Kitten (0–12)	3.002	1	0.083	6.548	0.782	54.865
	Young Adult (13–72)	2.302	1	0.129	5.385	0.612	47.390
	Mature Adult (73–120)	1.034	1	0.309	3.500	0.313	39.153
	Senior (>120)	-	-	-			
Sex	Male	0.418	1	0.518	1.198	0.693	2.070
	Female	-	-	-	-	-	-
Breed	DSH	0.057	1	0.811	0.935	0.537	1.627
	Non-DSH	-	-	-	-	-	-
Sampling period by month		25.567	11	0.008[a]			
	Jan	2.453	1	0.117	2.868	0.767	10.717
	Feb	2.179	1	0.140	2.786	0.715	10.857
	Mar	3.018	1	0.082	3.250	0.860	12.284
	Apr	4.991	1	0.025[a]	6.500	1.258	33.578
	May	2.266	1	0.132	2.955	0.721	12.107
	Jun	9.203	1	0.002[a]	7.583	2.048	28.074
	Jul	3.134	1	0.077	3.575	0.872	14.649
	Aug	2.985	1	0.084	3.656	0.840	15.913
	Sep	1.308	1	0.253	0.342	0.054	2.150
	Oct	.908	1	0.341	2.000	0.481	8.318
	Nov	.037	1	0.847	1.161	0.255	5.286
	Dec	-	-	-	-	-	-
Sampling period by season		2.924	2	0.232			
	Summer	2.776	1	0.096	1.800	0.901	3.594
	Rainy	1.302	1	0.254	1.371	0.797	2.359
	Winter	-	-	-	-	-	-

[a] Significant difference

The proportion of FCoV infection was higher in younger age groups, though the difference was not statistically significant (P = 0.199) (Fig. 1c and Supplementary Table S1). The infection pattern was consistent with a regression model that showed an association with younger age groups and a tendency to decrease in older age groups, as indicated by the declining odds ratios of 6.5, 5.4, and 3.5 compared with the senior group (>120 months) (Table 1). Domestic shorthair (DSH) cats were slightly more susceptible to FCoV infection than non-DSH cats (52.6% vs 50.9%) (Fig. 1d). However, the association between FCoV infection and cat breed was not statistically significant (Table 1). Interestingly, FCoV infection was significantly associated with the sampling period (P = 0.008). The likelihood of FCoV infection was highest in June (OR = 7.6), followed by April (OR =6.5), and significantly lower in September (OR = 0.3) when compared to December. For the sampling period by season, FCoV infection was more common in summer and rainy seasons compared with winter, with an odds ratio of 1.8 and 1.4, respectively, but this difference was not statistically supported (Table 1 and Fig. 2).

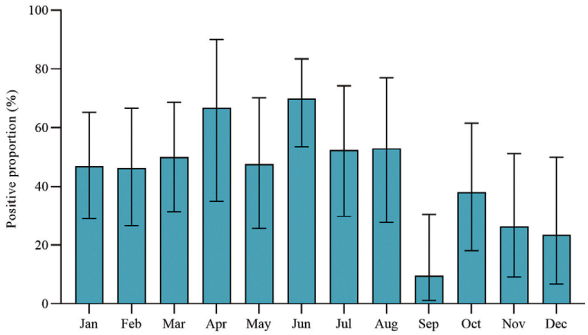
Subsequently, at least 10% FCoV-positive samples from each sample type were further sequenced and analyzed for genotyping and mutation. A phylogram based on the S gene sequence of the FCoV-positive samples identified all samples as the FCoV I genotype (Fig. 3). Sequencing was also performed on FCoV-positive blood samples for genotyping; however, the amplification of long fragments was not successful, likely owing to an insufficient quantity of RNA. To analyze S protein mutations, nucleotide sequences were translated into deduced amino acid sequences. The results revealed a mutation at position 1058 of the S protein, in which methionine (M) was substituted by leucine (L) in five of the eight body fluid samples analyzed (a mutation rate of 62.5%). This substitution was

Table 2. Comparison of the spike protein mutation between FIP-suspected cat and non-FIP cat.

Sample ID		Amino acid at the position 1058	Amino acid at the position 1060	Amino acid in S1/S2 region[a]
FIP-suspected cat				
MW1206/66	Body fluid	M	S	THTRRSRRSTS
MW1344/66	Body fluid	L	S	ND
MW1350/66	Body fluid	L	S	TSPRRARRSTV
MW2397/66	Body fluid	M	S	THTRRSRRSTS
MW2746/66	Body fluid	L	S	ND
MW2801/66	Body fluid	M	S	THTRRSRRSTP
MW3589-99	EDTA blood	ND	ND	TSSRRSRRSLV
MW3798-66	Body fluid	L	S	TSSRRARRSTV
MW4860/66	Body fluid	L	S	ND
MW5299-66	Body fluid	ND	ND	TTSR**G**SRRSVN
Non-FIP cat				
MW12/66	Rectal swab	M	S	TSSRRSRRSTS
MW16/66	Rectal swab	M	S	THTRRSRRSTS
MW50/66	Rectal swab	M	S	ND
MW3404/66	Rectal swab	M	S	TQPRRSR**S**STS
MW3734/66	Rectal swab	M	S	ND
MW3735/66	Rectal swab	M	S	TSSRRARRSTV
MW3829/66	Rectal swab	M	S	TESRRARSSTP
MW3830/66	Rectal swab	M	S	TESRRARSSTP
MW3893/66	Rectal swab	M	S	TQPRRARRSVS
MW5548-66	Rectal swab	ND	ND	TSSRRSRRSTV

[a] Under line indicates the mutation in furin cleavage site. ND = not determined.

not found in all rectal swabs. The serine (S) to alanine (A) substitution at position 1060 of the S protein was not detected in any of the samples (Fig. 4). Furthermore, another region of the S gene, specifically the furin cleavage site within the S1/S2 domain, was analyzed for mutations. Overall, 73.3% (11/15) of FCoV-positive samples analyzed in this study contained a polybasic R-R-S/A-R-R-S recognition motif in the S1/S2 domain. In non-FIP cats (FCoV-positive rectal swab samples), 5 out of 8 (62.5%) cats had the R-R-S/A-R-R-S motif. Mutation of the positively charged basic amino acid arginine (R) to the uncharged amino acid serine (S) at the P1 position of the motif was observed in three cats (37.5%), resulting in the R-R-S/A-R-S-S recognition motif at the cleavage site (Fig. 5a and 5c). For the S1/S2 domain in FIP-suspected cats (FCoV-positive body fluid and EDTA blood samples), 6 out of 7 (85.7%) cats had the R-R-S/A-R-R-S recognition motif. Only one sample (1/7; 14.3%) revealed a mutation at position P4: the positively charged basic amino acid arginine (R) was changed to the nonpolar amino acid glycine (G) (Fig. 5b and 5d).



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Positive	15	12	15	8	10	28	11	9	2	8	5	4
Total	32	26	30	12	21	40	21	17	21	21	19	17

Fig. 2. Annual positive rate of feline coronavirus (FCoV) based on sampling dates throughout 2023.

A summary of the S gene mutations in each group of cats is presented in Table 2.

Discussion

Continuous circulation of FCoVs within cat populations promotes viral proliferation and mutation. Therefore, the presence of asymptomatic carriers, as well as the continued transmission

Fig. 3. A phylogenetic tree constructed from the S gene of feline coronavirus (FCoV) obtained in the study. Numbers indicate the percentage of bootstraped support for a given branch. Triangles and rhombuses indicate FCoV sequences obtained from fluid and rectal swab samples, respectively.

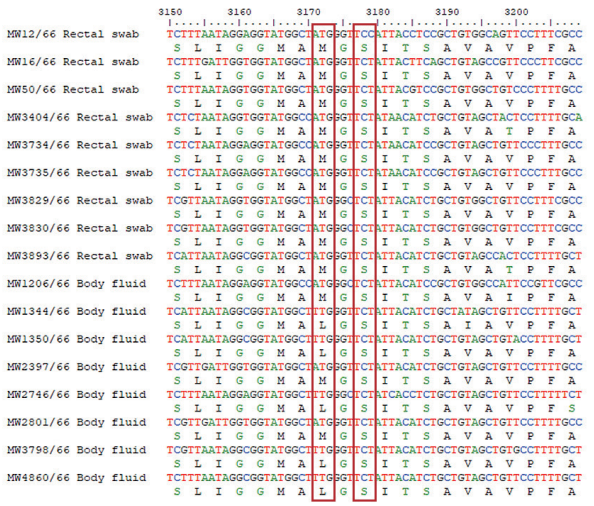


Fig. 4. Schematic representation of mutations in the S protein of feline coronavirus (FCoV) characterized in this study. The scale indicates the nucleotide position in the S gene.

of FCoV within cat populations, can contribute to the increased risk of FIP development. In this study, the overall percentage of FCoV infection in non-FIP and FIP-suspected cats was 46%, which was slightly higher than the previously reported prevalence in Thailand, ranging between 32% and 44%[21,33]. In FIP-suspected cats, the virus was present at higher rates in body fluid samples (51%) than in blood samples (11%). This finding was consistent with study indicated that cats with FIP, which is characterized by effusions in the body cavities, exhibit higher viral loads in the effusions than in the blood[26]. However, we did not collect samples from the same cats over time. Future testing of samples collected from the same cats will be conducted to confirm this finding. Moreover, the distinction between FIPV and FECV infections is crucial, as FIPV-infected cats tend to exhibit higher viral loads than FECV-infected cats. Future studies that directly compare viral loads between rectal swab and body fluid samples from the same FCoV-positive cats will enhance our understanding of the viral dynamics and shedding patterns associated with FCoV infection.

The etiology and pathogenesis of FIP are incompletely understood, and several factors have been implicated in increasing the risk of FIP development in cats. Male sex is a recognized predisposing factor for FIP in cats[27]. Our study identified a higher FCoV infection rate in male cats than in female cats (50.6% vs. 46.1%), in which was consistent with a previous report from central China, where the FCoV infection rate was slightly higher in males than in females (48.4% vs. 44.2%)[24]. This increased prevalence may be attributed to biological or behavioral predispositions that influence the prevalence of FCoV infection in male cats[12,27]. Several reports have demonstrated that FCoV occurs more frequently in younger cats, particularly those less than 2 years of age[17,36]. In this study, the FCoV

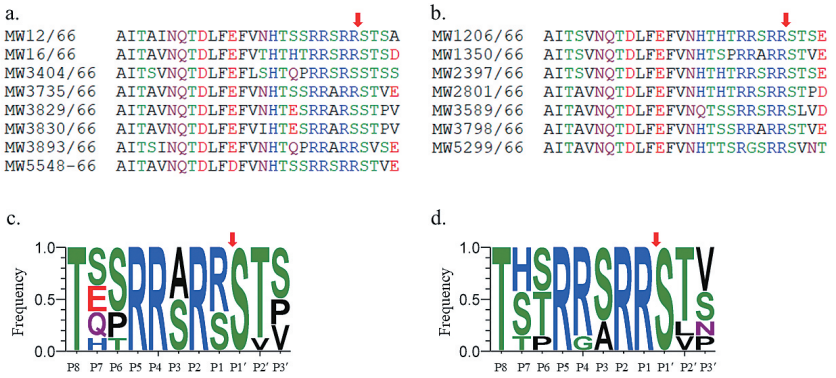


Fig. 5. Sequence analysis of the S1/S2 region detected in FCoV positive samples. Amino acid sequences in the S1/S2 region of the S protein in non-FIP cats (a) and FIP-suspected cats (b). Red arrow indicates the site of furin cleavage. The frequency of each amino acid residue at each position in the S1/S2 recognition motif (P4–P1) of non-FIP cats (c) and FIP-suspected cats (d). The sequences were analyzed and visualized using WebLogo 3.7.12 (https://weblogo.threeplusone.com/create.cgi).

infection rate was higher in kittens (0–12 months), with a noticeable decline in older age groups. However, these findings were not statistically significant. Although previous reports have suggested that genetic predisposition in purebred cats was more susceptible to FCoV infection[3,24,30], our study did not find evidence of this risk associated with breeding. The absence of statistical significance could be attributed to various factors, such as sample size, population demographics, or environmental influences. Interestingly, our study revealed that the sampling period was associated with FCoV infection, with significantly higher positive rates observed in April and June. These months fall within the summer and rainy seasons, during which FCoV was detected more frequently than in the winter. During this period, Thailand experiences higher temperatures than during other times of the year[35]. Heat stress in cats can weaken their immune systems, increasing their susceptibility to infections. Future studies using longitudinal data and environmental monitoring could further clarify the interplay between environmental factors and FCoV transmission dynamics, thereby enhancing our understanding of seasonal prevalence patterns.

The emergence of the virulent strain of FIPV is attributed to the spontaneous mutation of FECV. Recent research on FIP pathogenesis has increasingly focused on the S gene because of the crucial role of the coronaviral S protein in receptor binding and viral entry. The transition from FECV to FIPV involves a shift in target cell tropism, suggesting that mutations in the S gene, either alone or in conjunction with alterations in other genes, may play a significant role in facilitating the biotype switch[9,34]. M1058L and S1060A mutations have been reported in the S protein in cats with FIP[8]. Several reports have demonstrated that the M1058L mutation is predominantly associated with FIP. Longstaff et al. identified the M1058L mutation in 11 of 17 (65%) effusion samples[20]. Furthermore, among the cats diagnosed with FIP, 23 out of 34 (68%) exhibited amino acid substitutions (M1058L) in the S protein[29]. In our study population, the presence of the M1058L substitution in the S protein was detected in some body fluid samples (62%) (FIP-suspected cats) but not in rectal swab samples (non-FIP cats). These findings suggest that M1058L substitution is associated with specific clinical presentations or disease outcomes. Furthermore, a furin cleavage site in the region between the S1 and S2 domains of the S protein gene in FCoV has been implicated in biotype switching[19]. Among 30 fecal samples collected from FECV-infected domestic cats in the United States by Licitra and colleagues, all samples (30/30; 100%) contained a conserved

furin cleavage motif, specifically R-R-S/A-R-R-S. In contrast, only 1 out of 11 (9%) samples from FIP-positive cats retained this motif; for most FIP-positive cats (10 out of 11; 82%), there was at least one substitution at the cleavage site[19]. Additionally, an analysis of the amino acid sequence at the S1/S2 region of FIPV detected in China revealed that 42% of the sequences had no mutations and 52% had mutations. In comparison, for FECV, 79% of the sequences had no mutations and 21% had mutations. The mutation rate in FIPV was notably higher than that in FECV[29]. However, in this study, the mutation rate of the S1/S2 motif was higher in non-FIP cats than in FIP-suspected cats. Only 14% of FIP-suspected cats exhibited a mutation in the cleavage motif, whereas 86% had no mutation. These findings highlight the limitations of relying solely on S gene mutation analysis in FCoVs to identify the FECV–FIPV biotype switch, as the mutations are neither consistent nor unique. The overlap between FECV and FIPV mutations further complicates diagnosis, suggesting that factors other than spike protein changes must be considered when assessing biotype switching. This underscores the complexity of FCoV infection dynamics and challenges in identifying specific genetic markers of virulence. Therefore, integrating genetic analysis with comprehensive epidemiological investigations is crucial for gaining a deeper understanding of the mechanisms driving the transition from FECV to FIPV and for informing strategies aimed at disease prevention and control.

In conclusion, this study determined the rate of FCoV infection among owned cats, providing valuable insights into the spread of FCoV and the associated factors. The findings revealed a significant association between FCoV prevalence and the period of sample collection. Sequencing analysis suggested that only a subset of cats exhibited specific mutations in the virus, which were associated with the clinical outcomes of FIP. These findings suggest that although gene mutations may play a role in FCoV pathogenesis, other factors likely contribute to the development of FIP. Overall, the present study enhances our understanding of FCoV epidemiology and highlights the importance of considering multiple factors in disease detection, management, and control.

Acknowledgements

This project was financially supported by i-Tail Corporation Public Company Limited. We extend our gratitude to the staff at MoZWE for the data collection. Without your support, this work could not have been undertaken. We would like to thank Assoc. Prof. Dr. Walasinee Sakcamduang and Assoc. Prof. Dr. Ganokon Urkasemsin Andrade Requicha Ferreira for their help in advising on sample collection. We would like to express our sincere gratitude to Asst. Prof. Dr. Chalermpol Lekcharoensuk for his valuable feedback and insightful comments on the manuscript. His efforts greatly improved the quality of this work.

Declarations

Ethics approval

This study was performed after obtaining verbal informed consent from the sample owners and was approved by the Animal Ethics Committee of the Faculty of Veterinary Science, Mahidol University (protocol no. VSMU-2023-10-63).

Competing interests

None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

References

1) Addie DD, Jarrett O. Use of a reverse-transcriptase polymerase chain reaction for monitoring the shedding of feline coronavirus by healthy cats. Vet Rec 148, 649–653, 2001. doi: 10.1136/vr.148.21.649.
2) Amer A, Siti Suri A, Abdul Rahman O, Mohd HB, Faruku B, Saeed S, Tengku Azmi TI.

Isolation and molecular characterization of type I and type II feline coronavirus in Malaysia. Virol J 9, 278, 2012. doi: 10.1186/1743-422x-9-278.

3) Barua S, Sarkar S, Chenoweth K, Johnson C, Delmain D, Wang C. Insights on feline infectious peritonitis risk factors and sampling strategies from polymerase chain reaction analysis of feline coronavirus in large-scale nationwide submissions. J Am Vet Med Assoc 263, 82–89, 2025. doi: 10.2460/javma.24.03.0208.
4) Berti-Bock G, Vial F, Premuda L, Rullière R. Essudati, trasudati e reazione di Rivalta (1895). Attualità e premesse storiche [Exudates, transudates and the Rivalta reaction (1895). Current status and historical premises]. Minerva Med 70, 3573–3580, 1979. (*in Italian*)
5) Borschensky CM, Reinacher M. Mutations in the 3c and 7b genes of feline coronavirus in spontaneously affected FIP cats. Res Vet Sci 97, 333–340, 2014. doi: 10.1016/j.rvsc.2014.07.016.
6) Decaro N, Mari V, Lanave G, Lorusso E, Lucente MS, Desario C, Colaianni ML, Elia G, Ferringo F, Alfano F, Buonavoglia C. Mutation analysis of the spike protein in Italian feline infectious peritonitis virus and feline enteric coronavirus sequences. Res Vet Sci 135, 15–19, 2021. doi: 10.1016/j.rvsc.2020.12.023.
7) Felten S, Hartmann K. Diagnosis of feline infectious peritonitis: a review of the current literature. Viruses 11, 1068, 2019. doi: 10.3390/v11111068.
8) Felten S, Leutenegger CM, Balzer H-J, Pantchev N, Matiasek K, Wess G, Egberink H, Hartmann K. Sensitivity and specificity of a real-time reverse transcriptase polymerase chain reaction detecting feline coronavirus mutations in effusion and serum/plasma of cats to diagnose feline infectious peritonitis. BMC Vet Res 13, 228, 2017. doi: 10.1186/s12917-017-1147-8.
9) Gao Y-Y, Wang Q, Liang X-Y, Zhang S, Bao D, Zhao H, Li S-B, Wang K, Hu G-X, Gao F-S. An updated review of feline coronavirus: mind the two biotypes. Virus Res 326, 199059, 2023. doi: 10.1016/j.virusres.2023.199059.
10) Hartmann K. Feline infectious peritonitis. Vet Clin North Am Small Anim Pract 35, 39–79, 2005. doi: 10.1016/j.cvsm.2004.10.011.
11) Herrewegh AAPM, Smeenk I, Horzinek MC, Rottier PJM, de Groot RJ. Feline coronavirus type II strains 79-1683 and 79-1146 originate from a double recombination between feline coronavirus type I and canine coronavirus. J Virol 72, 4508–4514, 1998. doi: 10.1128/jvi.72.5.4508-4514.1998.
12) Hu T, Zhang H, Zhang X, Hong X, Zhang T. Prevalence and risk factors associated with feline infectious peritonitis (FIP) in mainland China between 2008 and 2023: a systematic review and meta-analysis. Animals (Basel) 14, 1220, 2024. doi:10.3390/ani14081220.
13) Jaimes JA, Whittaker GR. Feline coronavirus: insights into viral pathogenesis based on the spike protein structure and function. Virology 517, 108–121, 2018. doi: 10.1016/j.virol.2017.12.027.
14) Jähne S, Felten S, Bergmann M, Erber K, Matiasek K, Meli ML, Hofmann-Lehmann R, Hartmann K. Detection of feline coronavirus variants in cats without feline infectious peritonitis. Viruses 14, 1671, 2022. doi: 10.3390/v14081671.
15) Jeffery U, Deitz K, Hostetter S. Positive predictive value of albumin: globulin ratio for feline infectious peritonitis in a mid-western referral hospital population. J Feline Med Surg 14, 903–905, 2012. doi: 10.1177/1098612x12454862.
16) Kipar A, Meli ML. Feline infectious peritonitis: still an enigma? Vet Pathol 51, 505–526, 2014. doi: 10.1177/0300985814522077.
17) Klein-Richers U, Hartmann K, Hofmann-Lehmann R, Unterer S, Bergmann M, Rieger A, Leutenegger C, Pantchev N, Balzer J, Felten S. Prevalence of feline coronavirus shedding in German catteries and associated risk factors. Viruses 12, 1000, 2020. doi: 10.3390/v12091000.
18) Li C, Liu Q, Kong F, Guo D, Zhai J, Su M, Sun D. Circulation and genetic diversity of

feline coronavirus type I and II from clinically healthy and FIP-suspected cats in China. Transbound Emerg Dis 66, 763–775, 2019. doi: 10.1111/tbed.13081.

19) Licitra BN, Millet JK, Regan AD, Hamilton BS, Rinaldi VD, Duhamel GE, Whittaker GR. Mutation in spike protein cleavage site and pathogenesis of feline coronavirus. Emerg Infect Dis 19, 1066–1073, 2013. doi: 10.3201/eid1907.121094.
20) Longstaff L, Porter E, Crossley VJ, Hayhow SE, Helps CR, Tasker S. Feline coronavirus quantitative reverse transcriptase polymerase chain reaction on effusion samples in cats with and without feline infectious peritonitis. J Feline Med Surg 19, 240–245, 2017. doi: 10.1177/1098612X15606957.
21) Manasateinkij W, Nilkumhang P, Jaroensong T, Noosud J, Lekcharoensuk C, Lekcharoensuk P. Occurence of feline coronavirus and feline infectious peritonitis virus in Thailand. Kasetsart journal (Natural sciences) 43, 720–726, 2009.
22) McKay LA, Meachem M, Snead E, Brannen T, Mutlow N, Ruelle L, Davies JL, van der Meer F. Prevalence and mutation analysis of the spike protein in feline enteric coronavirus and feline infectious peritonitis detected in household and shelter cats in western Canada. Can J Vet Res 84, 18–23, 2020.
23) Motokawa K, Hohdatsu T, Hashimoto H, Koyama H. Comparison of the amino acid sequence and phylogenetic analysis of the peplomer, integral membrane and nucleocapsid proteins of feline, canine and porcine coronaviruses. Microbiol Immunol 40, 425–433, 1996. doi: 10.1111/j.1348-0421.1996.tb01089.x.
24) Ouyang H, Liu J, Yin Y, Cao S, Yan R, Ren Y, Zhou D, Li Q, Li J, Liao X, Ji W, Du B, Si Y, Hu C. Epidemiology and comparative analyses of the S gene on feline coronavirus in central China. Pathogens 11, 460, 2022. doi: 10.3390/pathogens11040460.
25) Pedersen NC. A review of feline infectious peritonitis virus infection: 1963-2008. J Feline Med Surg 11, 225–258, 2009. doi: 10.1016/j.jfms.2008.09.008.
26) Pedersen NC, Eckstrand C, Liu H, Leutenegger C, Murphy B. Levels of feline infectious peritonitis virus in blood, effusions, and various tissues and the role of lymphopenia in disease outcome following experimental infection. Vet Microbiol 175, 157–166, 2015. doi: 10.1016/j.vetmic.2014.10.025.
27) Riemer F, Kuehner KA, Ritz S, Sauter-Louis C, Hartmann K. Clinical and laboratory features of cats with feline infectious peritonitis--a retrospective study of 231 confirmed cases (2000-2010). J Feline Med Surg 18, 348–356, 2016. doi: 10.1177/1098612x15586209.
28) Sabshin SJ, Levy JK, Tupler T, Tucker SJ, Greiner EC, Leutenegger CM. Enteropathogens identified in cats entering a Florida animal shelter with normal feces or diarrhea. J Am Vet Med Assoc 241, 331–337, 2012. doi: 10.2460/javma.241.3.331.
29) Sangl L, Matiasek K, Felten S, Gründl S, Bergmann M, Balzer H-J, Pantchev N, Leutenegger CM, Hartmann K. Detection of feline coronavirus mutations in paraffin-embedded tissues in cats with feline infectious peritonitis and controls. J Feline Med Surg 21, 133–142, 2019. doi: 10.1177/1098612X18762883.
30) Sharif S, Arshad SS, Hair-Bejo M, Omar AR, Zeenathul NA, Hafidz MA. Prevalence of feline coronavirus in two cat populations in Malaysia. J Feline Med Surg 11, 1031–1034, 2009. doi: 10.1016/j.jfms.2009.08.005.
31) Song X-L, Li W-F, Shan H, Yang H-Y, Zhang C-M. Prevalence and genetic variation of the M, N, and S2 genes of feline coronavirus in Shandong Province, China. Arch Virol 168, 227, 2023. doi: 10.1007/s00705-023-05816-4.
32) Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Mol Bio Evol 38, 3022–3027, 2021. doi: 10.1093/molbev/msab120.
33) Techangamsuwan S, Radtanakatikanon A, Purnaveja S. Molecular detection and genotype differentiation of feline coronavirus isolates from clinical specimens in Thailand.

Thai journal of veterinary medicine 42, 413–422, 2012. doi: 10.56808/2985-1130.2419.
34) Tekes G, Thiel H-J. Feline coronaviruses: pathogenesis of feline infectious peritonitis. Adv Virus Res 96, 193–218, 2016. doi: 10.1016/bs.aivir.2016.08.002.
35) The Meteorological Department. 2023. Annual Weather Summary. https://www.tmd.go.th/en/climate/summaryyearly [accessed on November 24, 2024].
36) Worthing KA, Wigney DI, Dhand NK, Fawcett A, McDonagh P, Malik R, Norris JM. Risk factors for feline infectious peritonitis in Australian cats. J Feline Med Surg 14, 405–412, 2012. doi: 10.1177/1098612x12441875.
37) Zehr JD, Kosakovsky Pond SL, Millet JK, Olarte-Castillo XA, Lucaci AG, Shank SD, Ceres KM, Choi A, Whittaker GR, Goodman LB, Stanhope MJ. Natural selection differences detected in key protein domains between non-pathogenic and pathogenic feline coronavirus phenotypes. Virus Evol 9, vead019, 2023. doi: 10.1093/ve/vead019.
38) Zhou Q, Li Y, Huang J, Fu N, Song X, Sha X, Zhang B. Prevalence and molecular characteristics of feline coronavirus in southwest China from 2017 to 2020. J Gen Virol 102, 001654, 2021. doi: 10.1099/jgv.0.001654.