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RESEARCH ARTICLE

The First Comprehensive Phylogenetic Characterization of Feline Coronavirus Types I and II in Türkiye

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ABSTRACT

Feline coronavirus (FCoV), together with an inadequate host immune response, causes feline infectious peritonitis (FIP), one of the most fatal infectious diseases in cats worldwide. This study investigated, for the first time in Konya, Türkiye, the presence and phylogenetic relationships of FCoV Types I and II in cats. Fecal and effusion samples were collected from 30 cats suspected of having FIP. Viral RNA was extracted, and FCoV-I/II genomes were detected using nested reverse transcriptase-polymerase chain reaction (RT-PCR). Six positive samples were sequenced for phylogenetic and amino acid analyses, and results were statistically evaluated. Nested PCR results showed that 30% (9/30) of effusion samples were positive for FCoV-I, 13.33% (4/30) for FCoV-II, and 6.66% (2/30) were positive for both FCoV types. Among fecal samples, 33.33% (10/30) were positive for FCoV-I, while no FCoV-II was detected. No statistically significant association was found between FCoV positivity and factors such as gender or age. This study demonstrates, for the first time at the molecular level, the coexistence of FCoV-I and FCoV-II strains in Türkiye and their genetic similarity with global variants. Evaluating fecal and effusion samples together enhances diagnostic accuracy and enables the use of alternative specimens in FIP diagnosis. These findings are crucial for understanding FCoV evolution, controlling disease spread, and developing effective therapeutic and preventive strategies. Moreover, this study provides the first comprehensive phylogenetic characterization of FCoV Types I and II in Türkiye, contributing valuable data to global feline coronavirus epidemiology and vaccine research.

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INTRODUCTION

Feline coronavirus (FCoV) is a significant viral pathogen commonly found in cats and can lead to a fatal disease known as Feline Infectious Peritonitis (FIP) (Felten and Hartmann, 2019). First identified in the United States in 1963, FIP remains one of the most prevalent, deadly, and contagious feline diseases worldwide (Hu *et al*., 2024). The disease arises from a virulent form of feline coronavirus combined with an inadequate immune response by the host (Tekes and Thiel, 2016). According to the International Committee on Taxonomy of Viruses (ICTV), FCoV belongs to the order *Nidovirales*, the family *Coronaviridae*, and the species *Alphacoronavirus 1*. It is genetically related to canine coronavirus (CCoV) and the porcine transmissible gastroenteritis virus (TGEV) (Tekes and Thiel, 2016; Millet *et al*., 2021; Hu *et al*., 2024).

Feline coronavirus comprises two distinct biotypes: Feline Enteric Coronavirus (FECV) and Feline Infectious Peritonitis Virus (FIPV) (Pedersen, 2014; Tekes and Thiel, 2016; Felten and Hartmann, 2019). FECV typically causes mild and transient enteritis and is often asymptomatic, whereas FIPV leads to fatal systemic infections (Pedersen, 2014). Clinically, FECV is a major cause of acute diarrhea in cats—especially kittens—although cats of any age may be affected, and mortality is rare. Chronic FCoV infections have also been associated with persistent diarrhea (Addie *et al*., 2023). The FIP biotype, which arises from spontaneous mutations, causes progressive and lethal disease. FIP usually begins with

nonspecific signs such as anorexia, lethargy, weight loss, biphasic fever, and depression, later progressing to ascites, pleural effusion, granulomatous lesions in abdominal organs, and ultimately death (Pedersen, 2014; Felten and Hartmann, 2019).

Feline coronaviruses are further classified into two distinct **serotypes—Type I and Type II—**based on serological and sequence analyses (Pedersen, 2014; Tekes and Thiel, 2016). Type I FCoV is entirely feline-specific and is the most commonly detected in natural infections, whereas Type II results from recombination between canine coronavirus and feline coronavirus Type I (Tekes and Thiel, 2016; Addie *et al*., 2023). Both serotypes can cause FIP, although Type I variants appear more likely to lead to clinical FIP (Pedersen, 2014; Tekes and Thiel, 2016). Recent research on FIP pathogenesis has focused on the Spike (S) gene, which plays a critical role in receptor binding and viral entry. Since the transition from FECV to FIPV involves a shift in cell tropism, mutations in the S gene may contribute to this biotype conversion. In particular, M1058L and S1060A mutations in the fusion peptide region are thought to enhance monocyte/macrophage tropism (Pedersen, 2014; Tekes and Thiel, 2016).

Feline infectious peritonitis is a severe systemic disease and a rare but fatal outcome of FCoV infection. It is commonly observed in young cats and has been more frequently associated with males and certain breeds (Felten and Hartmann, 2019). Clinical signs—such as anorexia, weight loss, fever, lethargy, and neurological or ocular abnormalities—are highly nonspecific and may resemble many other conditions. Therefore, diagnosing FIP presents major clinical and laboratory challenges (Pedersen, 2014; Felten and Hartmann, 2019). Among the samples used for FIP diagnosis, the most common are effusion fluids (abdominal or thoracic), blood, feces, tissue biopsies, and ocular discharge (Pedersen, 2014; Barker *et al*., 2017; Felten and Hartmann, 2019). Effusion fluid is particularly important in diagnosing the wet form of FIP and exhibits characteristic features, including high protein content and low cellularity. While blood samples are useful for hematological and biochemical assessment, their diagnostic specificity is low. Molecular tests—particularly reverse transcriptase polymerase chain reaction (RT-PCR) and nested RT-PCR performed on effusion samples—offer higher sensitivity and specificity for detecting viral RNA. However, a positive PCR result alone is insufficient for definitive diagnosis and must be interpreted alongside clinical and laboratory findings (Pedersen, 2014; Felten and Hartmann, 2019).

A definitive diagnosis typically requires postmortem histopathological and immunohistochemical examination (Felten and Hartmann, 2019). For ante-mortem diagnosis, clinical signs, imaging findings, hematology, biochemical parameters including the albumin/globulin ratio, RT-PCR findings, and—if necessary—more invasive sampling methods should be evaluated together. High diagnostic specificity is crucial to avoid misdiagnosing other diseases as FIP (Pedersen, 2014; Felten and Hartmann, 2019). FCoV can be detected in feces, blood, or tissue via PCR-based tests. The advantage of RT-PCR lies not only in identifying infection in suspected FIP cases but also in detecting asymptomatic FCoV carriers (Pedersen, 2014).

The phylogenetic comparison of Type I and Type II FCoV infections is essential for understanding viral spread, evolutionary dynamics, and biotype differences. Such information contributes to elucidating viral pathogenesis and supports the development of prevention and treatment strategies for severe complications like FIP. However, phylogenetic studies on FCoV in Türkiye are limited, and no comprehensive research has examined the prevalence or evolutionary relationships of the two biotypes. Previous studies in Türkiye have largely focused on serological and clinical aspects, while molecular characterization and phylogenetic analyses remain insufficient. These analyses are critical for understanding the epidemiology of FCoV and clarifying its relationship with global variants. In this context, the first phylogenetic analysis conducted in Türkiye reveals the genetic characteristics of FCoV Type I and Type II strains, sheds light on regional molecular epidemiology, and defines Türkiye's position within global coronavirus diversity. By using both fecal and effusion samples, this study aims to detect FCoV Type I and Type II strains more comprehensively and reliably. This approach not only increases diagnostic accuracy but also facilitates the identification of different clinical forms. Overall, the findings of the current study are expected to make significant contributions to veterinary virology and epidemiology in Türkiye and provide guidance for future strategies regarding the diagnosis and control of FIP.

MATERIALS AND METHODS

All sampling procedures were conducted in accordance with animal welfare and ethical guidelines. This study was approved by the Local Ethics Committee for Animal Experiments of Selcuk University (Ethical approval number 2020/81, dated 18.09.2020). The study design scheme/flow diagram is shown in Table S2.

Sample collection: This study was designed as a prospective observational series with no experimental intervention. Rectal swabs and effusion samples were collected from 30 unvaccinated owned cats suspected of FIP that were brought for diagnosis and treatment to the Veterinary Faculty clinics of Selcuk University and to private veterinary clinics in the Konya region. The sample size of 30 cats was determined based on the availability of suspected FIP cases during the study period and logistical constraints.

All cats presented with pleural effusion, along with one or more additional clinical signs such as diarrhea, ascites, uveitis, or seizures. Rectal swab samples were stored in 2 mL of phosphate-buffered saline (PBS) containing 25,000 U/mL penicillin and 20 mg/mL streptomycin, and transported to the Virology Laboratory using commercial swab sticks. In the laboratory, the tubes were vortexed, and the buffer fluid was transferred into 1.5 mL DNase- and RNase-free tubes. Samples were stored at −20 °C until further processing.

Virus isolation from rectal swab and effusion samples: Frozen samples stored at −20 °C were thawed and centrifuged at 3,000 rpm for 10 min at +4 °C. Viral RNA was extracted from the supernatants using a commercial kit (QIAamp Viral RNA Mini Kit) according to the

manufacturer's instructions. Extracted viral RNA was stored at −20 °C until use.

Reverse transcription (RT) of viral RNA: RNA integrity was assessed by agarose gel electrophoresis, and the extracted RNA was used as a template for complementary DNA (cDNA) synthesis. cDNA was synthesized using a commercial kit containing reverse transcriptase (iScript™ cDNA Synthesis Kit, 100 × 20 µL). The reaction mixture included RNA, buffer, and reverse transcriptase. The thermal profile consisted of 5 min at room temperature for priming, 20 min at 46 °C for synthesis, and 1 min at 95 °C for enzyme inactivation.

Polymerase chain reaction (PCR): Following reverse transcription, all samples were tested using nested PCR targeting the FCoV S gene. To prevent contamination, nuclease-free water was included as a negative control in each PCR run. Nested-I and Nested-II reactions were performed using previously published primers (Addie *et al*., 2003) under standard cycling conditions. Nested-I: 25 µL 2× PCR master mix, 1 µL each of FCoV-F1, CCoV-F1, and Universal-R1 primers, 3 µL cDNA, and 19 µL nuclease-free water. Nested-II: 25 µL 2× PCR master mix, 1 µL each of FCoV-F2, CCoV-F2, and Universal-R2 primers, 2 µL Nested-I product, and 20 µL nuclease-free water. PCR products were analyzed by electrophoresis on 2% agarose gels containing 0.01% GelRed in 0.5× TAE buffer, run at 8 V/cm for 35 minutes. Detailed primer sequences are listed in Supplementary Table S1.

Phylogenetic analysis: Six samples testing positive for FCoV Type I or Type II by PCR were submitted to a commercial company for sequencing. All sequences underwent quality control and trimming before analysis. Reference sequences were selected based on geographic origin and the partial gene region analyzed. Sequence identification was performed using the BLAST service on the NCBI GenBank database. Multiple sequence alignment was carried out with ClustalW in BioEdit version 7.0.9 and AliView. Aligned data were converted to FASTA format and analyzed using the Neighbor-Joining method with 1,000 bootstrap replicates (Felsenstein, 1985) in MEGA X (Kumar *et al*., 2018). Phylogenetic trees and homology matrices were constructed using the Tamura 3-parameter model (Tamura, 1992). Based on these analyses, the presence of FCoV Types I and II in cats from the Konya region was confirmed, and molecular differentiation and typing of the two biotypes were performed.

Statistical analysis: Data were analyzed using SPSS 25.0. The distribution of the data was assessed using the Shapiro–Wilk test. As the data were not normally distributed, non-parametric tests were applied. The Mann–Whitney U test was used to compare FCoV positivity between genders, with effect size reported as r ($r = Z / \sqrt{N}$). The Kruskal–Wallis test was performed to assess differences among age groups, with effect size reported as epsilon-squared (ε^2). Prevalence values are presented with 95% confidence intervals (CI) calculated using the Wilson method. The Chi-square test was used to evaluate differences in positivity between effusion and fecal samples; effect size was reported as Cramér's V (φ). For differences in proportions, Cohen's h was also calculated as a measure of effect size.

RESULTS

Nested PCR results: As a result of the nested PCR performed for the detection of FCoV-I and FCoV-II, the first reaction targeted a 376 bp cDNA fragment specific to FCoV-I and a 283 bp fragment specific to FCoV-II; the second reaction targeted a 360 bp fragment specific to FCoV-I and a 218 bp fragment specific to FCoV-II. In the first step of the nested PCR, 1 of 30 effusion samples (3.33%) tested positive for FCoV-I, and similarly, 1 of 30 fecal samples (3.33%) was also positive for FCoV-I. In the second step, among effusion samples, 30% (9/30) were positive for FCoV-I, 13.33% (4/30) were positive for FCoV-II, and 6.66% (2/30) were positive for both FCoV-I and FCoV-II. Among fecal samples, 33.33% (10/30) were positive for FCoV-I, while FCoV-II was not detected in any fecal sample. These results are presented in Fig. 1, Table 1, and Fig. 2.

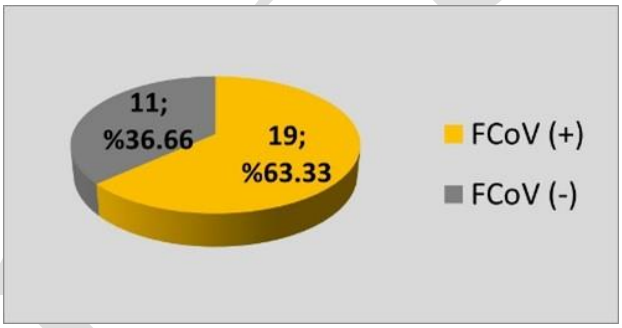

Fig. 1: Detection rate of FCoV using the Nested PCR Method. The pie chart illustrates the distribution of samples tested for feline coronavirus (FCoV). A total of 30 samples were analyzed. FCoV-positive samples (n = 19; 63.33%) are shown in yellow, while FCoV-negative samples (n = 11; 36.66%) are shown in gray. FCoV (+), feline coronavirus–positive; FCoV (−), feline coronavirus–negative.

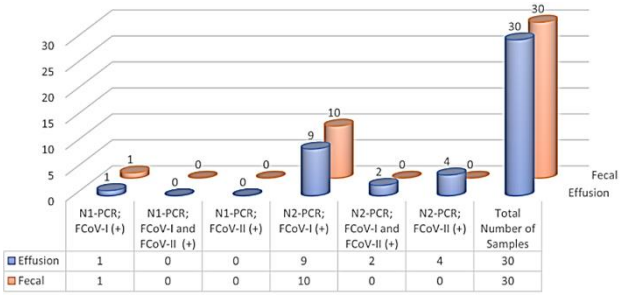

Fig. 2: N1-PCR and N2-PCR represent two RT-PCR assays targeting the S gene region. Samples were further genotyped for FCoV-I and FCoV-II using a type-specific PCR assay. The number of positive effusion (blue) and fecal (orange) samples for each detection and genotyping category is indicated above bars. Total sample numbers for each specimen type are shown on the right. N1; Nested 1, N2: Nested-2.

Table 1: Detection of FCoV type I and type II in effusion and fecal samples from 30 cats using the nested PCR assay.

Sample type	Nested-I tested	Nested-II tested	FCoV-I positive (%)	FCoV-II positive (%)	Both FCoV-I & II (%)
Effusion	30	30	30.0% (9/30)	13.33% (4/30)	6.66% (2/30)
Fecal	30	30	33.33% (10/30)	0% (0/30)	0% (0/30)

The number of samples tested in the first and second rounds of nested PCR and the proportion of samples positive for FCoV type I, FCoV type II, or both types.

Table S1: Primer sets, reaction components, and thermal cycling conditions used for the nested PCR detection of FCoV.

PCR Round	Primer Name	Sequence (5'–3')	Template	Thermal Cycling	Notes
Nested-I	FCoV-F1	GTTTCAACCTAGAAAGCCTCAGAT	cDNA 3 µL	30 cycles: 94 °C 45s, 50 °C	CCoV primers included
	CCoV-F1	GCCTAGTATTATACCTGACTA		45s, 72 °C 45s; initial 94 °C 3	for FCoV-II detection
	Universal-R1	CCACACATACCAAGGCC		min, final 72 °C 10 min	
Nested-II	FCoV-F2	CCTAGAAAGCCTCAGATGAGTG	Nested-I product	Same as Nested-I except	
	CCoV-F2	CAGACCAAACTGGACTGTAC	2 µL	annealing 49 °C	
	Universal-R2	CCAAGGCCATTTTACATA			

Primer pairs targeting feline coronavirus (FCoV) and canine coronavirus (CCoV) were included to enable the detection of both FCoV type I and type II strains. FCoV: Feline coronavirus; CCoV: Canine coronavirus; PCR: Polymerase chain reaction; cDNA: Complementary DNA.

Of the sampled animals, 13 were female and 17 were male. Among these, 8 females (26.7%) and 11 males (36.7%) tested positive for FCoV (Table 3). By age group, 5 of 7 cats aged 0–24 months, 7 of 11 cats aged 25–48 months, 4 of 5 cats aged 49–72 months, and 3 of 7 cats older than 72 months were FCoV-positive (Fig. 3).

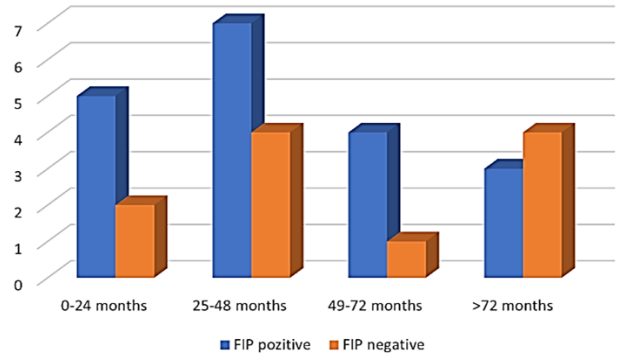

Fig. 3: Distribution of FIP cases across different age groups. The values represent the number of affected animals (counts). FIP, Feline İnfectious Peritonitis.

Results of phylogenetic analysis and homology matrix: Of the 19 positive samples, 6 were included in the phylogenetic tree. Based on S gene sequence analysis, local isolates within each subtype (FCoV-I or FCoV-II) showed 99–100% homology with one another. The TR-E15/KNY FCoV-I strain (accession no. PV266539) exhibited high evolutionary similarity to strains from several countries, including China (OQ196069.1: 96.3%; OQ196094.1: 95.95%), the UK (AY159754.1: 94.93%), Portugal (EU327701.1: 96.55%), Germany (KJ665866.1: 97.57%), and the Netherlands (HQ392469.1: 97.89%). Likewise, TR-R22/KNY FCoV-I (accession no. PV266537) and TR-R5/KNY FCoV-I (accession no. PV266538) clustered on the same branch and were closely related to strains from the Netherlands (HQ392469.1: 95.23%) and China (MW815650.1: 97.26%; MW815662.1: 98.21%).

Local FCoV-II strains—TR-E17/KNY (accession no. PV266534), TR-E10/KNY (accession no. PV266536), and TR-E22/KNY (accession no. PV266535) — showed close genetic similarity, up to 98.48%, with strains from Brazil (OP179857.1: 97.95%), Greece (JQ422598.1), and Italy (GU146061.1, KP981644.1). All reference strains used in the phylogenetic analysis, along with their accession numbers, countries of origin, and subtypes, are listed in Table 2 (Fig. 4; Fig. 5).

Amino acid analysis results: For the FCoV-I strains TR-R22/KNY, TR-R5/KNY, and TR-E15/KNY, the amino acids encoded at positions 8073, 8117, 8143, 8161 and 8189 were identified as serine (S), asparagine (N), glutamine (Q), aspartic acid (D), and leucine (L), respectively. In contrast, the Feline coronavirus UU54 complete genome (GenBank accession no. JN183883.1) encoded asparagine (N), serine (S), arginine (R), asparagine (N), and arginine (R) at the corresponding positions.

Table 2: List of local and reference isolates included in phylogenetic analysis

Isolate / Reference strain	Accession no.	Subtype
Local isolates		
TR-R22/KNY FCoV-I ▼	PV266537	FCoV-I
TR-R5/KNY FCoV-I ▼	PV266538	FCoV-I
TR-E15/KNY FCoV-I ▼	PV266539	FCoV-I
TR-E17/KNY FCoV-II ▼	PV266534	FCoV-II
TR-E10/KNY FCoV-II ▼	PV266536	FCoV-II
TR-E22/KNY FCoV-II ▼	PV266535	FCoV-II
Reference strain		
KP143511.1 (UK)	KP143511	FCoV-I
AY159770.1 (UK)	AY159770	FCoV-I
EU327711.1 (POR)	EU327711	FCoV-I
EU327701.1 (POR)	EU327701	FCoV-I
EU327696.1 (POR)	EU327696	FCoV-I
OQ196069.1 (CHN)	OQ196069	FCoV-I
OQ196094.1 (CHN)	OQ196094	FCoV-I
OQ196101.1 (CHN)	OQ196101	FCoV-I
OQ196071.1 (CHN)	OQ196071	FCoV-I
MW815650.1 (CHN)	MW815650	FCoV-I
MW815657.1 (CHN)	MW815657	FCoV-I
MW815662.1 (CHN)	MW815662	FCoV-I
MW316840.1 (CHN)	MW316840	FCoV-I
FJ917524.1 (USA)	FJ917524	FCoV-I
OR908445.1 (IND)	OR908445	FCoV-I
KJ665866.1 (GER)	KJ665866	FCoV-I
KJ665881.1 (GER)	KJ665881	FCoV-I
HQ392469.1 (NET)	HQ392469	FCoV-I
LC742526.1 (JAP)	LC742526	FCoV-I
AY159754.1 (UK)	AY159754	FCoV-I
KP981644.1 (ITA)	KP981644	FCoV-II
GU146061.1 (ITA)	GU146061	FCoV-II
JQ422598.1 (GRE)	JQ422598	FCoV-II
OP179857.1 (BRA)	OP179857	FCoV-II
MZ320954.1 (CHN)	MZ320954	FCoV-II
KC175341.1 (USA)	KC175341	FCoV-II
KF668590.1 (KOR)	KF668590	FCoV-II
X06170.1 (NET)	X06170	FCoV-II
KY063618.2 (CHN)	KY063618	FCoV-II
X80799.1 (UK)	X80799	FCoV-II
JN634064.1 (UK)	JN634064	FCoV-II
MT114554.1 (CHN)	MT114554	FCoV-II
MT114552.1 (CHN)	MT114552	FCoV-II
MT906865.1 (UK)	MT906865	FCoV-II
MT294701.1 (TR)	MT294701	CCoV
MT294702.1 (TR)	MT294702	CCoV
MT294703.1 (TR)	MT294703	CCoV
MT294704.1 (TR)	MT294704	CCoV

Feline coronavirus (FCoV) isolates and globally sourced reference strains included in the phylogenetic analysis, along with their GenBank accession numbers and subtype designations (FCoV type I, FCoV type II, and CCoV sequences used for comparison). FCoV-I = Feline coronavirus type I; FCoV-II = Feline coronavirus type II; CCoV = Canine coronavirus; TR = Türkiye; UK = United Kingdom; GER = Germany; NET = Netherlands; JAP = Japan; IND = India; GRE = Greece; POR = Portugal; ITA = Italy; USA = United States; CHN = China; BRA = Brazil; KOR = Korea.

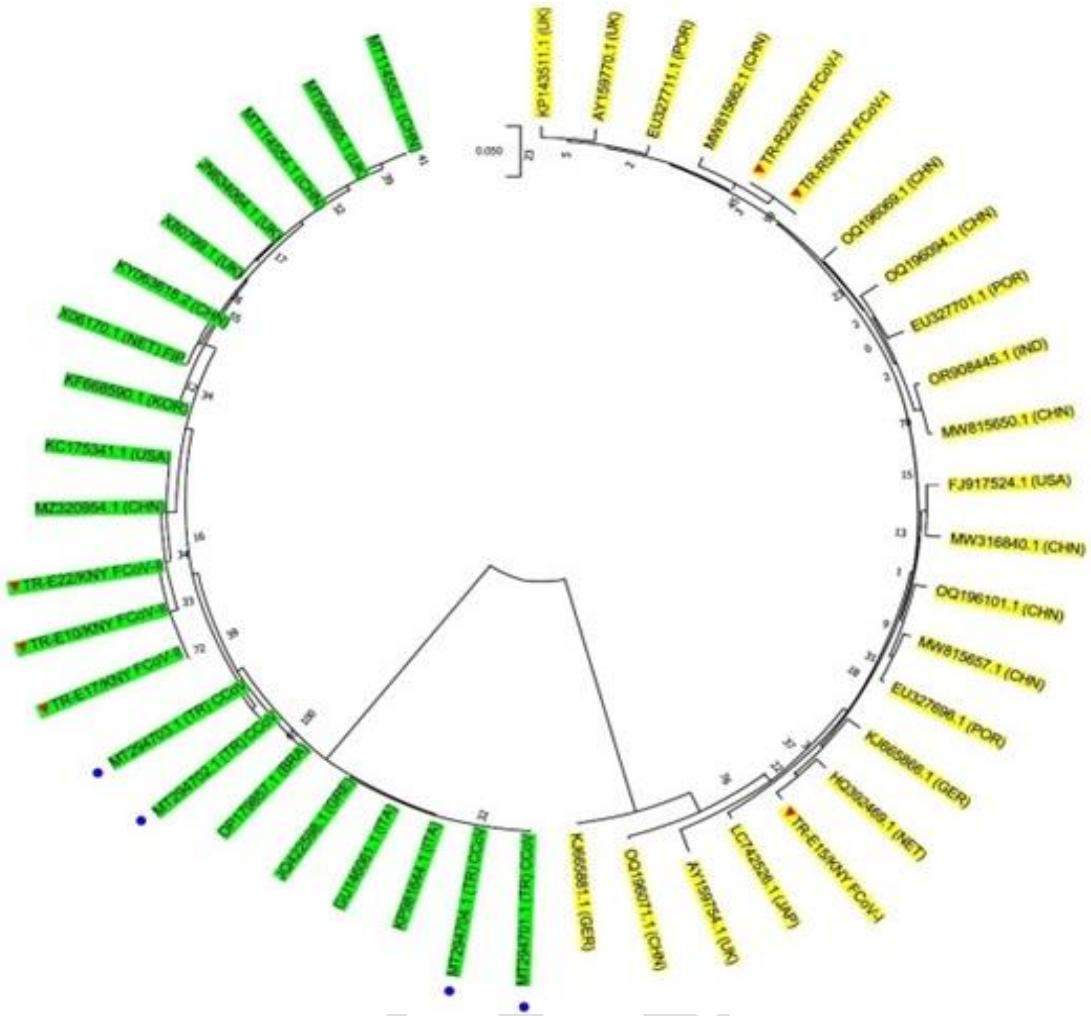

Fig. 4: Phylogenetic analysis of the FCoV Spike (S) gene. Our local isolates are indicated with "▼". TR-E17/KNY FCoV-II: Türkiye – effusion sample number 17, Konya Feline Coronavirus II; TR-R5/KNY FCoV-I: Türkiye – rectal swab sample number 5, Konya Feline Coronavirus I; TR-R22/KNY FCoV-I: Türkiye – rectal swab sample number 22, Konya Feline Coronavirus I; TR-E15/KNY FCoV-I: Türkiye – effusion sample number 15, Konya Feline Coronavirus I; TR-E10/KNY FCoV-II: Türkiye – effusion sample number 10, Konya Feline Coronavirus II; TR-E22/KNY FCoV-II: Türkiye – effusion sample number 22, Konya Feline Coronavirus II. Data highlighted in yellow represent FCoV-I isolates, while those in green represent FCoV-II isolates. Data highlighted in blue represent CCoV isolates from Turkey.

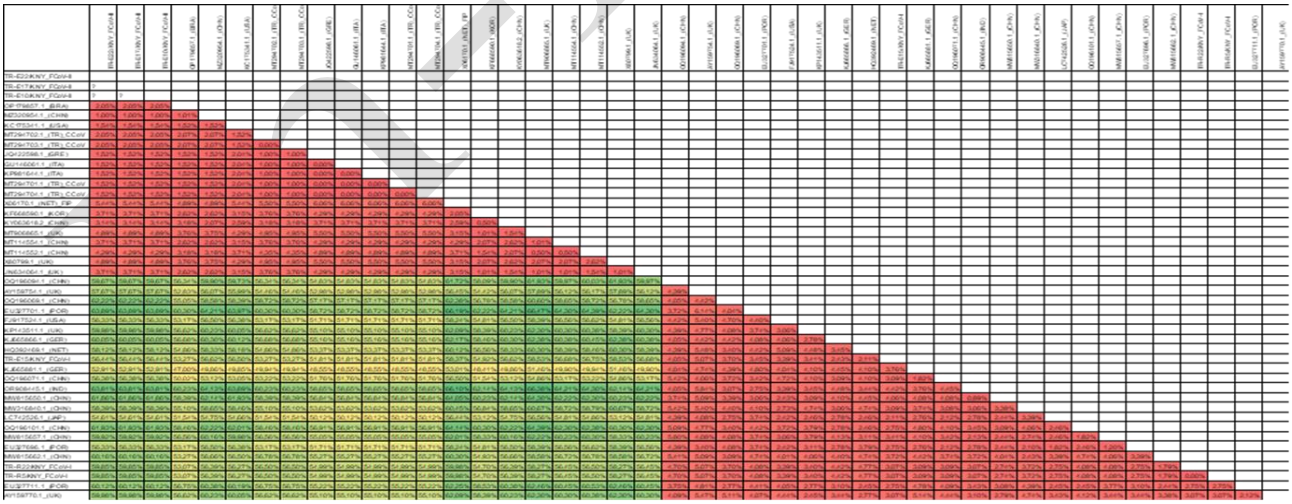

Fig. 5: Genetic distance analysis results based on FCoV-I and FCoV-II sequences.

For the FCoV-II strains TR-E10/KNY, TR-E17/KNY, and TR-E22/KNY, the amino acids encoded at positions 8139, 8167 and 8196 were asparagine (N), histidine (H), and lysine (K), respectively. In comparison, the complete genome of the FCoV/NTU156/P/2007 strain (GenBank accession no. GQ152141.1) (Hsieh *et al.,* 2010) encoded aspartic acid (D), tyrosine (Y), and glutamic acid (E) at these positions. Apart from these substitutions, the remaining amino acid sequences of the local strains were consistent with the corresponding full genomes (Fig. 6).

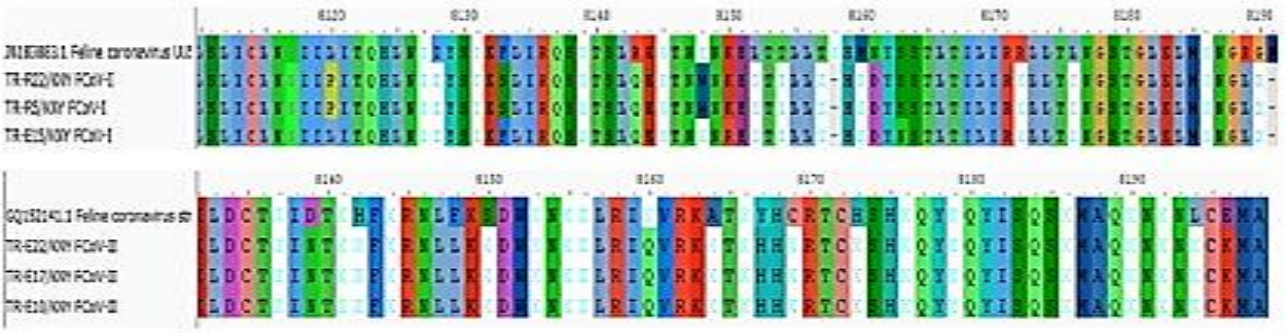

Fig. 6: Amino acid differences observed in local strains based on comparison with complete genomes.

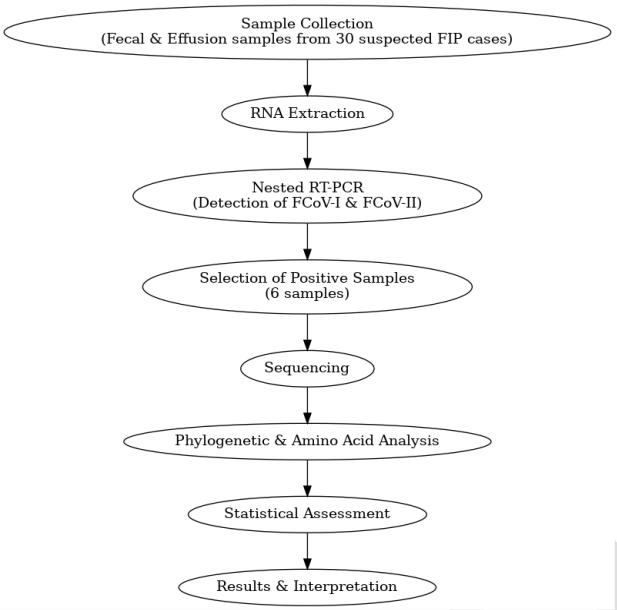

Table S2: Study design schematic/flow diagram

Table 3: The relationship between FCoV prevalence and gender

Sex	Positive (n, %)	Negative (n, %)	Total	95% CI for prevalence	Statistical values
Male	11 (36.7%)	6 (20.0%)	17	41.3–82.7	OR = 0.87 (95% CI: 0.20–3.90)
Female	8 (26.7%)	5 (16.7%)	13	35.5–82.3	ϕ ≈ 0.00 (negligible)
Total	19 (63.3%)	11 (36.7%)	30	45.5–78.1	Mann–Whitney U = 107.0 p = 0.861

FCoV-positive and FCoV-negative cats according to sex, the total number tested, and 95% confidence intervals (CI) for prevalence estimates. Statistical comparisons between male and female cats were performed using the odds ratio (OR), phi coefficient (ϕ), and Mann–Whitney U test to assess differences in infection rates. N: Frequency, %: Percentage, CI: Confidence interval, OR: Odds ratio, ϕ: Phi coefficient.

Table 4: The association between feline coronavirus (FCoV) prevalence and age

Age (months)	Positive (n, %)	Negative (n, %)	Total	95% CI for prevalence	Statistical values
0–24	5 (16.7%)	2 (6.7%)	7	35.9–91.8	H = 1.991; p = 0.574; V = 0.26 (small–moderate)
25–48	7 (23.3%)	4 (13.3%)	11	35.4–84.8	
49–72	4 (13.3%)	1 (3.3%)	5	37.6–96.4	
>72	3 (10.0%)	4 (13.3%)	7	15.8–75.0	
Total	19 (63.3%)	11 (36.7%)	30	45.5–78.1	

The number and proportion of FCoV-positive and FCoV-negative cats in different age categories, along with the total number of animals tested and the 95% confidence intervals (CI) for prevalence estimates. Differences among age groups were evaluated using the Kruskal–Wallis test (H), and the strength of association between age group and infection status was assessed using Cramér's V (V). N: Frequency, %: Percentage, p: Significance level, H: Kruskal-Wallis value, CI: Confidence interval V: Cramér's V.

Table 5: Comparison of FCoV detection rates between effusion and fecal samples using the Chi-square test.

Chi-square test	Fecal (+)	Fecal (–)	Total	Prevalence (%)	95% CI
Effusion (+)	6	9	15	40.0	19.8–64.3
Effusion (-)	4	11	15	26.7	11.5–50.2
Total	10	20	30	33.3	19.2–51.2

The distribution of FCoV-positive and FCoV-negative results in paired effusion and fecal samples from 30 cats, together with prevalence percentages and 95% confidence intervals (CI). A Chi-square test (χ^2) was performed to evaluate whether FCoV detection differed significantly between sample types. Statistical values: $\chi^2 = 0.6$; $p = 0.438$; Cramér's V = 0.19; Cohen's h = 0.34 (small effect). No statistically significant difference was found between effusion and fecal samples based on the Chi-square test (P>0.05).

Statistical analysis results: Data distribution was assessed using the Shapiro–Wilk test, which indicated non-normal distribution ($p < 0.05$); therefore, non-parametric tests were applied. Overall, 19 of 30 cats were FCoV-positive (63.3%, 95% CI: 45.5–78.1). By sex, 8/13 females (61.5%, 95% CI: 35.5–82.3) and 11/17 males (64.7%, 95% CI: 41.3–82.7) tested positive. The Mann–Whitney U test indicated no significant difference between sexes ($p = 0.861$), with an odds ratio of 0.87 (95% CI: 0.20–3.90) and negligible effect size ($\varphi \approx 0.00$).

By age group, positivity rates were 71.4% (5/7; 95% CI: 35.9–91.8) for 0–24 months, 63.6% (7/11; 95% CI: 35.4–84.8) for 25–48 months, 80.0% (4/5; 95% CI: 37.6–96.4) for 49–72 months, and 42.9% (3/7; 95% CI: 15.8–75.0) for >72 months. The Kruskal–Wallis test showed no significant differences among age groups ($p = 0.574$), with a small–moderate effect size (Cramér's V = 0.26).

Regarding sample type, 15/30 effusion samples (50.0%, 95% CI: 33.2–66.8) and 10/30 fecal samples (33.3%, 95% CI: 19.2–51.2) were positive. The Chi-square test showed no significant difference between sample types ($\chi^2 = 1.10$, $p = 0.438$), although a small effect was observed (Cramér's V = 0.19; Cohen's h = 0.34) (Table 3; Table 4; Table 5).

Taken together, while prevalence values varied between subgroups, none of the comparisons were statistically significant, and effect sizes indicated that the observed differences were small in magnitude (P>0.05).

DISCUSSION

FCoV infection is observed worldwide in both domestic and wild cats. In cats infected with FCoV, FIP can develop, a disease characterized by a high mortality rate. Cases of FIP associated with FCoV have also been reported in non-domestic members of the *Felidae* family (Ratti *et al*., 2022).

In the present study, 19 of 30 cats suspected of FIP (63.33%) were found to be FCoV-positive by nested PCR (Fig. 1), and this was further supported by phylogenetic analysis. However, although these methods reliably detect viral infection, a definitive diagnosis of FIP still requires histopathology combined with immunohistochemistry (Felten and Hartmann, 2019). Such confirmatory approaches are recommended in future studies to verify clinical disease and enhance diagnostic accuracy. In clinical practice, when cats present with fluid accumulation in the abdominal cavity, FIP is commonly considered as a preliminary diagnosis. In this study, 15 of 30 effusion samples (50%) and 10 of 30 fecal samples (33.33%) tested positive for FCoV (Fig. 2; Table 1). Although no direct evidence of FCoV cross-species transmission was obtained, from a One Health perspective, its genetic links with CCoV and TGEV suggest that its potential for interspecies spread warrants further investigation. Such work could contribute to an improved understanding of coronavirus ecology and zoonotic risk (Pedersen, 2014; Tekes and Thiel, 2016; Millet *et al*., 2021).

In their study, Barker *et al*. (2017) examined effusion and fecal samples from 102 cats by RT-qPCR and reported positivity rates of 78.4% and 64.6%, respectively. Similar to Barker *et al*. (2017), the present study also found a higher positivity rate in effusion samples (50%), but a lower rate in fecal samples (33.3%) (Fig. 2). In a prospective study conducted in Germany involving 179 cats, RT-qPCR analysis of four consecutive fecal samples revealed that 76.5% of cats shed FCoV at least once, indicating that fecal FCoV positivity is considerably high and that multiple sampling is critical for accurately determining the true prevalence (Klein-Richers *et al*., 2020). In the current study, no statistically significant difference was found between FCoV positivity rates in effusion and fecal samples (Table 5), and this has important clinical implications. Particularly in cases where the patient is severely dehydrated, where fluid collection may induce shock, or in cats with the dry form of FIP or without apparent clinical signs, fecal samples may be preferred as diagnostic material. Establishing a reliable diagnosis using non-invasive fecal samples, instead of relying solely on effusion samples, will greatly facilitate field surveillance of FCoV infection. Moreover, the presence of FCoV infection alone is not sufficient for FIP diagnosis; therefore, molecular approaches aimed at distinguishing between FECV and FIPV have gained importance. Mutations in the S gene may play a critical role in the transformation of FECV to FIPV; however, their exact role in FIP development is still not fully understood. For this reason, S gene mutations can be used as diagnostic markers, but they are not sufficient on their own to confirm FIP (Addie *et al*., 2003; Addie *et al*., 2023).

In the present study, the higher positivity rate observed in the second step of the nested PCR reaction indicates that this method is more sensitive than conventional PCR (Table 1, Fig. 2).

Kopduang *et al*. (2025), in their study on 80 clinical samples, reported that RT-qPCR targeting the FCoV M gene detected 93.75% of positive samples, nested RT-PCR 87.5%, and conventional RT-PCR 61.25%, demonstrating that nested RT-PCR and RT-qPCR provide markedly higher sensitivity and diagnostic reliability compared to conventional RT-PCR, and also emerge as strong and reliable methods for FCoV detection in both fecal and effusion samples.

Panei *et al*. (2024) applied nested PCR to 140 animal samples and 40 positive human samples, specifically targeting the viral N gene. The assay detected SARS-CoV-2 at viral loads as low as 50 copies/µL (Ct 31.5), demonstrating approximately 95% sensitivity and 100% specificity, and showed excellent agreement with real-time RT-PCR (k = 0.829). These findings indicate that nested PCR is a reliable and cost-effective method for detecting low viral loads in animals and can be applied in large-scale surveillance studies. Additionally, nested PCR has been reported to have >90% sensitivity and specificity in detecting FIP from effusion fluid (Pedersen *et al*., 2014). Felten and Hartmann (2019) reported that RT-PCR tests for FCoV in effusion fluid may reach 100% specificity. RT-PCR is frequently used to detect FCoV RNA; however, FCoV RNA can also be detected in the blood of cats that have never had FIP, meaning that RT-PCR cannot distinguish between FECV and FIPV (Sharif *et al*., 2011; Pedersen, 2014). Because information on FCoV genotype, its presentation in wet or dry forms, and whether the infection is transient or persistent is still limited, the progression of FIP is not fully understood. FCoV Type II is thought to arise from recombination between FCoV Type I and CCoV Type II (Tekes and Thiel, 2016; Shi *et al*., 2024).

FCoV positivity rates can vary substantially depending on geographical region, target population, diagnostic methods, and sampling strategies. The positivity rate obtained in the present study (63.33%) is higher than those reported in Italy (7.9%) (Ratti *et al*., 2022) and Taiwan (47.4%) (Yen and Chen, 2021), but lower than those reported in China (80.35%) (Zhou *et al*., 2021), Brazil (64.2%) (Almeida *et al*., 2019), and the United Arab Emirates (65%) (El-Tholoth *et al*., 2023). These differences may be due to variations in diagnostic techniques, sample size, and regional prevalence of FCoV. Therefore, when interpreting FCoV positivity rates, it is crucial to consider study design, sample type, methodology, and local epidemiological conditions together. A limitation of the present study is the lack of information on prior exposure of the cats to other coronaviruses or infectious agents such as FeLV and FIV. Previous infections could influence FCoV susceptibility and viral shedding; thus, future studies incorporating serological screening for these pathogens would provide a more comprehensive understanding of FCoV epidemiology. In the present study, FCoV-I was detected at a higher rate than FCoV-II by nested PCR (Fig. 2). To date, many studies from Korea, China, Japan, the United Kingdom, Austria, and Taiwan have reported FCoV-I as the dominant genotype in cats (An *et al*., 2011; Lin *et al*., 2022; Shi *et al*., 2024; Kim *et al*., 2025). This may be related to the ability of genotype I to persist for years, as noted in previous work (Addie *et al*., 2003). In contrast, genotype II is more likely to cause acute infection and has been reported not to persist after recovery (Lin *et al*., 2022). A study from Switzerland also showed that FCoV Type I is more commonly associated with FIP. The authors suggested that better adaptation of Type I to cats may allow higher viral loads. In that study, FCoV antibodies were

detected in 100% of histopathologically confirmed FIP cases, and most cats had higher antibody titers against Type I than Type II. These findings support the view that Type I may be more frequently associated with FIP development (Kummrow *et al*., 2005).

In this study, cats co-infected with both Type I and Type II were detected at a rate of 6.66% (Table 1). Similar co-infection patterns have been documented in several recent studies. For example, Luo *et al*. (2020) reported nine concurrent Type I/II infections among FIP-suspected cats in Taiwan. Likewise, Yen and Chen (2021) identified simultaneous infections in feline effusion samples using molecular genotyping, emphasizing that although less frequent than single-type infections, mixed infections do occur in natural populations. Additionally, Lin *et al*. (2022) demonstrated the co-circulation of both genotypes across multiple regions in China, indicating the possibility of occasional dual infections in areas where both viral types are endemic. However, it remains unclear which genotype plays a more significant role in the pathogenesis of FIP in such co-infected cases, or whether both genotypes contribute equally.

Epidemiological studies on cats with FIP have identified several risk factors related to disease development, including age, breed, and sex. In the present study, 56.66% of the sampled animals were male and 43.33% were female. The absence of a statistically significant difference in positivity rates between sex groups is consistent with the findings of Almeida *et al*. (2019), and Lin *et al*. (2022) (Table 3). This suggests that FCoV infection does not show a clear predilection for specific age groups or sexes, and that the risk of infection is likely similar across the general cat population. Consequently, control strategies for FCoV in both domestic and stray cats should adopt a broad, population-level approach rather than focusing on age- or sex-specific interventions.

However, some studies have indicated that male cats are more susceptible to FIP (Hu *et al*., 2024). This has been attributed to greater exposure to stress due to fighting, hormonal influences, and more frequent infections with FeLV and FIV in males compared with females. These factors may weaken the immune system, making males more vulnerable to FIP and increasing their exposure to a wider range of FCoV strains (Tekes and Thiel, 2016).

Many studies (Almeida *et al*., 2019; Lin *et al*., 2022) have reported that the majority of FCoV cases in high-density cat environments occur in young cats (3 months to 3 years old), attributing this to less effective control of FCoV replication in young and immunosuppressed animals. However, other studies (Sharif *et al*., 2011; Almeida *et al*., 2019) have reported no relationship between age and FCoV infection, which is consistent with the results of the present study (Fig. 3; Table 4).

As in many other countries, FIP is a serious problem in domestic cats in Türkiye. The number of studies diagnosing FCoV by PCR methods in Türkiye is still limited. Can-Şahna *et al*. (2007) detected FCoV in 14 of 26 cat blood samples (54%) by PCR, whereas Muz and Muz (2023) found 35 of 150 cats (23.3%) to be PCR positive. Using ELISA, Oguzoglu *et al*. (2010) identified 37 of 53 cats (69.8%) as FCoV-positive, Akar and Yıldırım (2023) reported 22 of 40 cats (55%), and Topçu and Yıldırım (2024) found 41 of 60 cats (68.3%) to be seropositive. To date, there has been no comprehensive molecular characterization of FCoV in Türkiye. In the present study, detection and phylogenetic analysis of FCoV from effusion and fecal samples of 30 cats suspected of FIP were conducted for the first time in Türkiye (Fig. 4). This work is of critical importance for veterinary practice and public health, as it provides guidance for the diagnosis and management of FCoV infections and contributes to controlling disease spread and protecting feline health.

In this study, a region of the S gene (3′ UTR), considered a highly informative part of the viral genome, was amplified and analyzed using nested PCR. It should be noted that only partial S gene sequences were examined, which may limit the detection of all mutations and the full resolution of phylogenetic relationships. As a membrane glycoprotein, the FCoV S protein is responsible for recognizing host cell receptors, mediating viral entry, and inducing neutralizing antibody production (Tekes and Thiel, 2016). Therefore, investigation of the S gene contributes to a better understanding of FCoV genetic diversity. Phylogenetic analysis of the S gene (Fig. 4, Table 2) revealed that local strains from Türkiye (TR-E15/KNY FCoV-I, TR-R22/KNY FCoV-I, TR-R5/KNY FCoV-I, TR-E17/KNY FCoV-II, TR-E10/KNY FCoV-II, TR-E22/KNY FCoV-II) show close genetic relationships with strains from several countries, including those in Europe (United Kingdom, Portugal, Germany, Netherlands) and China (Fig. 5). This suggests direct or indirect viral transmission among these regions. Viruses such as FCoV can spread through international animal trade, pet movement, and the migration or translocation of stray cats. In addition, viral genomes continually accumulate mutations over time, leading to geographic diversification (Phyu *et al*., 2025). The similarity rates observed between the isolates obtained in this study and reference FCoV strains from GenBank (95–98%) imply that these isolates likely share a recent common ancestor, indicating possible global dissemination of specific FCoV-I and FCoV-II lineages.

Turkish FCoV isolates, while largely similar to global strains, exhibit distinct amino acid substitutions that suggest local evolutionary adaptations. The observed changes in FCoV-I (Fig. 6)—at positions 8073 (N → S), 8117 (S → N), 8143 (R → Q), 8161 (N → D), 8189 (R → L) — and in FCoV-II at positions 8139 (D → N), 8167 (Y → H), and 8196 (E → K) can be attributed to ongoing mutation processes in the FCoV RNA genome. Such substitutions at specific residues may reflect natural evolutionary dynamics. Importantly, amino acid changes at critical sites can modify receptor binding, affect infectivity and pathogenicity, and thereby influence viral adaptation (Millet *et al*., 2021). These subtle but consistent mutations may influence viral behavior, including receptor-binding affinity and pathogenic potential, allowing the virus to optimize its fitness within the Turkish feline population while maintaining overall genetic coherence with internationally circulating lineages.

The current study has practical implications for veterinary medicine, as it supports refinements to diagnostic protocols in clinical settings. By highlighting the utility of both fecal and effusion samples for reliable FCoV detection, clinicians can optimize sampling strategies, improve diagnostic accuracy, and implement more

effective surveillance and management plans for feline coronavirus infections. Moreover, in regions with limited resources, access to expensive kits or advanced equipment is often restricted; therefore, nested PCR represents a more accessible, cost-effective, and sensitive method. Consequently, this study provides a model diagnostic workflow for veterinary laboratories. Furthermore, it lays important groundwork for future research using larger sample sizes and broader geographic coverage. Such studies are expected to provide a robust scientific basis for investigating how nucleotide and amino acid–level variations affect FCoV pathogenicity and its interactions with host receptors, thereby guiding more advanced work in this field.

This study has several limitations. First, the sample size was relatively small (n = 30), which may limit the generalizability of the findings to the broader cat population in Türkiye. Second, only partial S gene sequences were analyzed, restricting the ability to detect all possible mutations and to fully resolve phylogenetic relationships; whole-genome sequencing would provide more comprehensive information. Additionally, histopathological or immunohistochemical confirmation of FIP was not performed, which limits the conclusions that can be drawn regarding clinical disease. Future studies with larger sample sizes, additional molecular targets, and more extensive clinical and serological data are needed to validate and expand upon these findings.

Conclusion: In conclusion, this study provides a foundational framework for understanding the molecular epidemiology of FCoV in Türkiye and offers a basis for broader regional surveillance. The data generated here can support more effective monitoring of viral circulation and timely interventions to control outbreaks. Advanced molecular approaches, including whole-genome sequencing, will be critical for identifying specific genetic markers linked to pathogenicity and for guiding vaccine development strategies targeting both serotypes. Such efforts will not only enhance our understanding of FCoV genetic diversity and evolution but also inform effective control and prevention measures within the Turkish feline population.

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