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Molecular and Clinical Study of Feline Infectious Peritonitis Virus in Iran Shows a Paraphyletic Tree; Emphasizes the "Internal Mutation" Hypothesis

Shahram Jamshidi[1], Farnoosh Momeni[1], Iradj Ashrafi Tamai[2], Omid Madadgar[2] *

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

[2]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

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Abstract

Background: Feline Infectious Peritonitis (FIP) is a severe and often fatal disease affecting feline species globally. Despite the high prevalence of Feline Coronavirus (FCoV) infections, the manifestation of FIP occurs in only a small percentage (1-5%) of cases. The intricate aspects of FIP differential diagnosis persist, and a comprehensive understanding of the molecular mechanisms driving FIP pathogenesis remains elusive.

Objectives: This study aims to conduct a thorough investigation into the characteristics of Iranian FIPV, encompassing sequence analysis, and detailed examination of laboratory and clinical findings. The primary objective is to unravel the hypothesized genesis of the FIP virus, with a specific focus on the M gene level.

Methods: Our methodology involved the examination of abdominal or thoracic fluids from 17 cats suspected of FIP, utilizing biochemical tests such as total serum protein, Albumin to Globulin (A/G) ratio, and the Rivalta test. Additionally, a molecular approach utilizing RT-PCR based on the Membrane (M) gene was employed. Sequence analysis of five crucial residues in the M genes and the subsequent construction of a phylogenetic tree using five sequenced viruses further enriched our investigation.

Results: The study confirmed FIP in 6 out of 17 cats through the Rivalta test, guiding subsequent evaluations. Noteworthy gender disparities in FIP occurrences among young cats (9-30 months old) were observed, with males exhibiting a twofold higher incidence compared to females. Affected cats within the 9-30 months age range consistently exhibited an A/G ratio below 0.66 and total serum protein exceeding 0.43 g/dl. Cavity fluid cytology indicated non-degenerated macrophages and neutrophils against a basophilic background, due to a high protein percentage, confirming FIP diagnosis. Importantly, sequence analysis of five M protein amino acid hotspots revealed negligible differences in nucleotide sequences between FECoV and FIPV, aligning with their biotypic pattern.

Conclusions: the phylogenetic tree generated in this study displayed a paraphilic pattern, emphasizing the "Internal Mutation" hypothesis, which suggests viral mutations occur within the cat's body and there are no significant differences in FECoV and FIPV-generating viruses. These findings contribute valuable insights to the discourse surrounding FIP pathogenesis, potentially guiding future diagnostic and therapeutic approaches.

Keywords: Biochemical Tests, FIPV, Iran, Phylogenetic Analysis, Rivalta test.

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Introduction

Feline Infectious Peritonitis is one of the deadliest infections of the cat population and its causative agent is a *Coronaviridae* family member. Feline Coronavirus (FCoV) include two

separate biotypes; The ubiquitous Feline Enteric Coronavirus (FECV) is more common and causes a self-limiting moderate diarrhea that often cannot be cleared and generates a persistent infection in the cells of the intestinal mucosa and more sporadically type of Feline Infectious Peritonitis Viruses (FIPV) that is highly virulent and deadly (Felten *et al.,* 2017, Li *et al.,* 2019, Aksono *et al.,* 2023).

FIP is a perivascular pyogranulomatous viral infection that may occur in two clinical forms, effusive and non-effusive, which are characterized by the presence of effusions in the body cavities and of pyogranulomatous lesions in organs, respectively (Lorusso *et al.,* 2019). For the development of these lesions, FIPV-infected monocytes and macrophages have been identified as major target cells of FIPVs and are assumed to play a pivotal role in FIP pathogenesis. FIPVs can efficiently infect and replicate in monocytes/macrophages and, the main difference between FIP and moderate diarrhea caused by FECV is the capability of the FIPV to infect monocyte and macrophages (Tekes *et al.,* 2016, Doenges *et al.,* 2016, Decaro *et al.,* 2021).

Clinical signs associated with the FIPV biotype can be quite variable and non-specific, usually including fever, lethargy, anorexia, pica, vomiting, and diarrhea. Hence, differential diagnosis with other infectious diseases is difficult and based on laboratory confirmation. Also, clinical signs can be present in either 'wet', 'dry' or 'mixed' presentations. The wet form of FIP is characterized by an effusion in the abdominal and/or thoracic or pericardial cavities, and the 'dry' form, by the presence of pyogranulomatous lesions (André *et al.,* 2019, Paltrinieri *et al.,* 2021). Antibody responses, Hematology, serum chemistry, and serum protein electrophoresis give only a strongly suggestive diagnosis in the non-pathognomonic pattern of FIP (Felten and Hartmann, 2019) and therefore, identification of related histopathological lesions with immunohistochemical (IHC) detection of FCoV antigen in tissue macrophages is only considered the "gold standard" of FIP diagnosis (Sangl *et al.,* 2019).

The origin of FIP has been a controversial issue among scientists for decades and in general, two main hypotheses are the "internal mutation" theory that is based on the mutations in FECV and

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consequently its capability to enter and multiply in macrophage as the main step of FIP pathogenesis and second theory of "circulating virulent–avirulent FCoV" that consider two different strains in virus population (Tekes *et al.,* 2016, Myrrha *et al.,* 2019). This study, which encompasses an analysis of Iranian Feline Infectious Peritonitis Virus (FIPV) characteristics, including sequence analysis, as well as laboratory and clinical findings, aims to systematically investigate these hypotheses at the M gene level in Iranian FIP viruses.

Materials and Methods

Samples

Samples included the abdominal and/or thoracic fluids of 17 cats with symptoms of anorexia, lethargy, weight loss, and increased abdominal volume, which had been confirmed by clinical examination or ultrasound. The rest of the samples after biochemical and Rivalta tests were preserved at -20 °C.

Biochemical examinations and Rivalta test:

Albumin and total protein measurements were conducted utilizing an autoanalyzer (Selectra, ElitechGroup, Netherlands). Subsequently, the albumin value was subtracted from the total protein value to determine the globulin amount in abdominal fluid. Additionally, the globulin amount was calculated by deducting albumin values from total protein, and the ratio of albumin to globulin was computed.

For the Rivalta test, a mixture of 98% acetic acid with 8 ml of distilled water in a 10 ml clear test tube was prepared. Subsequently, a drop of abdominal fluid was added. A negative test result was determined if the drop disappeared and its components separated quickly in the liquid. Conversely, a positive result was recorded if the drop retained its shape, remained attached to the surface, and moved slowly down the solution.

Cellular Examination

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To observe cell contents, 10 µl of abdominal fluid supernatant, centrifuged at 130 g for 10 minutes, was prepared for staining using the Giemsa method. The presence of white blood cells in abdominal fluid smears was assessed through observation under a microscope.

Molecular Investigations

RNA extraction and Reverse Transcription Reaction:

A 1.5 ml microtube containing abdominal fluid from each sample was centrifuged at 130g for 5 minutes. The resulting supernatant underwent RNA purification using the RNXTM-Plus Kit (CinaGen, Tehran, Iran) following the manufacturer's instructions. Briefly, 150 µl of the supernatant was mixed with 1 ml RNX and left for at least 5 minutes at 48°C. After adding 200 ml chloroform and thorough mixing, the liquid was clarified by centrifugation at 12,000g at 48°C for 15 minutes. The supernatant was then transferred into a new tube and mixed with an equal volume of isopropanol, followed by centrifugation at 12,000g at 48°C for 15 minutes. The pellet was washed with 1 ml of 70% ethanol. Finally, RNA was eluted using 50 µl of 1 mM RNase-free DEPC-treated water.

Subsequently, cDNA synthesis was performed using the Maxime RT Premix Kit (iNtRON, Seoul, South Korea). Eight microliters of extracted RNA were added to a ready-to-use tube and filled to 20 µl with DEPC-treated water. The mixture was heated for 60 minutes at 45°C, followed by 5 minutes at 95°C, and the resulting cDNA was immediately transferred to -20°C.

PCR

Polymerase Chain Reaction was conducted following the method outlined by Barker et al., 2013, for the amplification of a 1040 bp segment of the FIPV M gene (Barker et al., 2013). In brief, 12.5 µl of PCR master mix (Cinnaclone, Iran), 2 µl of cDNA, and 1 µl each of forward and reverse primers were combined with 8.5 µl of distilled water to achieve a total volume of 25 µl

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for the PCR reaction. Subsequently, the PCR reaction was carried out using a Thermocycler (Techne, England) with the following protocol: initial denaturation at 98°C for 30 seconds, followed by 40 cycles of denaturation at 98°C for 10 seconds, annealing at 59°C for 20 seconds, extension at 72°C for 30 seconds, and a final cycle of extension at 72°C for 2 minutes. The PCR product (5 µl) was then subjected to analysis by electrophoresis on a 2% agarose gel and stained with ethidium bromide.

Sequencing of M gene, sequence analysis, and generation of the phylogenetic tree:

Moreover, nucleotide sequencing was conducted using an automatic sequencer (ABI-377; Applied Biosystems, Foster City, CA, USA) for the remaining PCR product after purification from the gel. The obtained sequences were analyzed and compared using current algorithms from the National Center for Biotechnology Institute (NCBI, Rockville Pike, Bethesda MD, USA). Multiple alignments were performed using the Clustal W program, and the sequences were scrutinized to compare important hotspot nucleotides with a reference sequence extracted from NCBI GeneBank (Accession number: JN183882) using CLC sequence viewer 6.

Furthermore, sixty-four M gene sequences of Feline Infectious Peritonitis Virus (FIPV) from various regions worldwide, extracted from GenBank, were selected for comparison with the five M genes obtained in this study (FIT1-FIT5). Finally, a phylogenetic tree was generated using the neighbor-joining method with MEGA 7 software with a bootstrap value of 1000.

Results

Clinical and biochemical:

Of the cats included in this study, eight (47%) were female, and nine (53%) were male. The age range of these cats varied from 4 to 14 months. The total protein content in abdominal fluids ranged from 3.3 g/dL to 10.6 g/dL, while the ratio of albumin to globulin (A/G ratio) spanned

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from 0.12 to 0.9. Additionally, apart from abdominal fluids, two out of the seventeen studied cats exhibited fluid accumulation in the thoracic area. Notably, only one of these cats (5%) had exclusive fluid accumulation in the thoracic area (refer to Table 1).

Table: Clinical, biochemical, and PCR result

Number	Sex*	Type of secreted fluid**	A/G***	Total protein (g/dl)	Rivalta test	RT-PCR result	Age(month)
1	M	A, T	0.66	4.31	+	+	15
2	M	A	0.33	6.07	+	+	11
3	M	A	0.24	5.63	+	-	21
4	F	A	0.9	3.42	-	-	9
5	F	A	0.22	9.41	+	-	4
6	F	T	0.28	6.23	+	-	7
7	F	A	0.22	10.17	+	+	9
8	F	A	0.36	9.53	+	+	30
9	M	A	0.32	7.51	+	-	50
10	M	A	0.16	10.2	+	-	10

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11	F	T	0.8	3.3	-	-	5
12	M	A	0.35	5.4	+	+	20
13	M	A	0.72	3.95	-	-	25
14	F	A	0.26	7.28	+	-	8
15	M	A	0.12	10.6	+	+	18
16	M	A	0.36	5.31	+	-	36
17	F	A	0.24	8.59	+	-	40

*F: Female, M: Male. **A: Abdominal, T: Thoracic

***A/G: Albumin to Globulin ration

Cellular examination:

Cytologic examination of abdominal and thoracic fluid revealed the presence of non-degenerate macrophages and neutrophils. Additionally, granules and basophilic protein strands were observed in the background (Figure 1).

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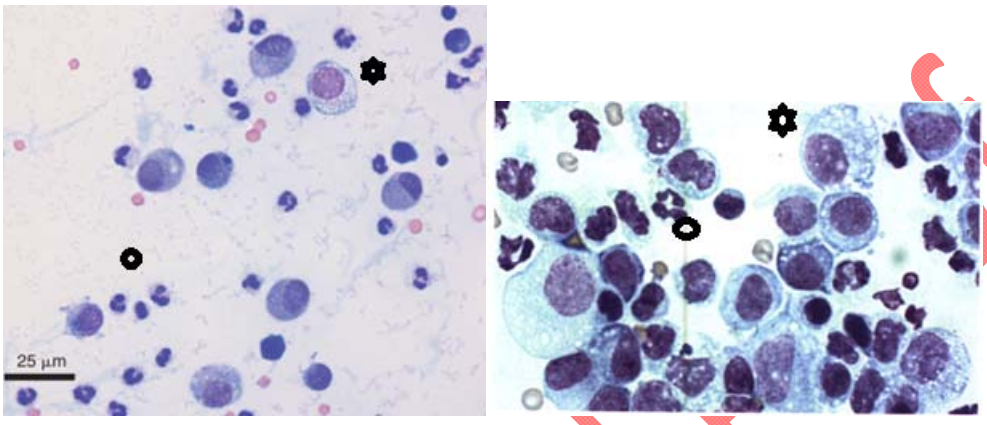

Figure 1: Cytologic examination of fluids shows non-degenerated macrophages and neutrophils. *Black stars indicate Macrophages and black circles show neutrophils.

PCR

The RT-PCR result was positive in six out of the 17 cats (35%), displaying 1040 bp bands (Figure 2).

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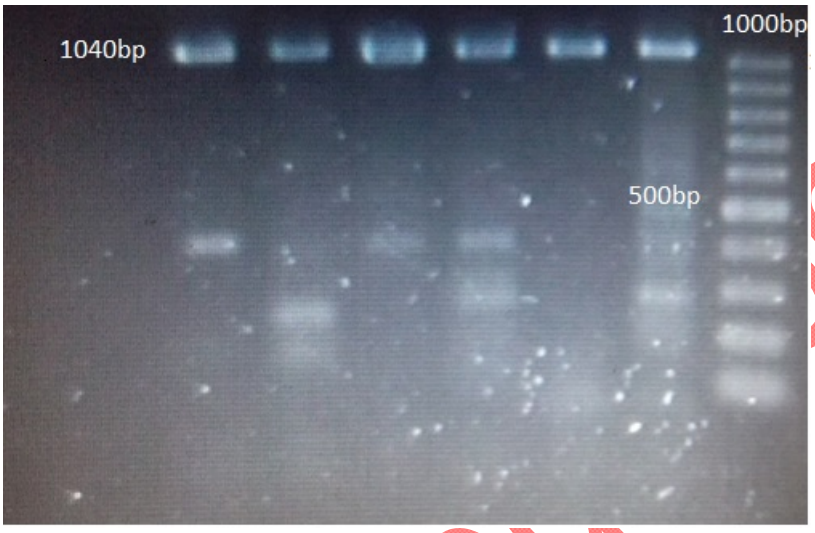

Figure 2: RT-PCR of the fluid cells showed a 1040 bp size band of the M gene.

Protein alignment:

The generated data indicated that the amino acid motif YVIAL (positions 108, 120, 138, 163, and 199, based on the reference sequence for Feline Infectious Peritonitis Virus, GenBank no. JN183882) was predicted for all the viruses analyzed in this study. Therefore, in line with the findings presented by Barker et al., 2013, these crucial amino acids were unable to differentiate between Feline Coronavirus (FCoV) and Feline Infectious Peritonitis Virus (FIPV) in the current study.

Phylogenetic Tree

Nucleotide sequences of cats with FIP and FECoV cats were distributed in paraphilic groups, and different FIPV and FECoV sequences were placed together in different clusters (Figure 3). According to the generated phylogenetic tree, FIT1 is closely related to certain strains of Feline Enteric Coronavirus (FECoV) and Feline Infectious Peritonitis Virus (FIPV) found in the USA,

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Netherlands, and the UK. FIT2 is closely associated with some FECoV strains observed in Japan, and FIT3 shares proximity with certain FECoV and FIPV strains observed in the Netherlands, Brazil, USA, and Germany. FIT4 aligns with some FECoV and FIPV strains from Japan, Taiwan, and some Canine Coronaviruses from Taiwan, the UK, and Italy. Lastly, FIT5 is

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closely related to certain FECoV and FIPV viruses observed in the UK.

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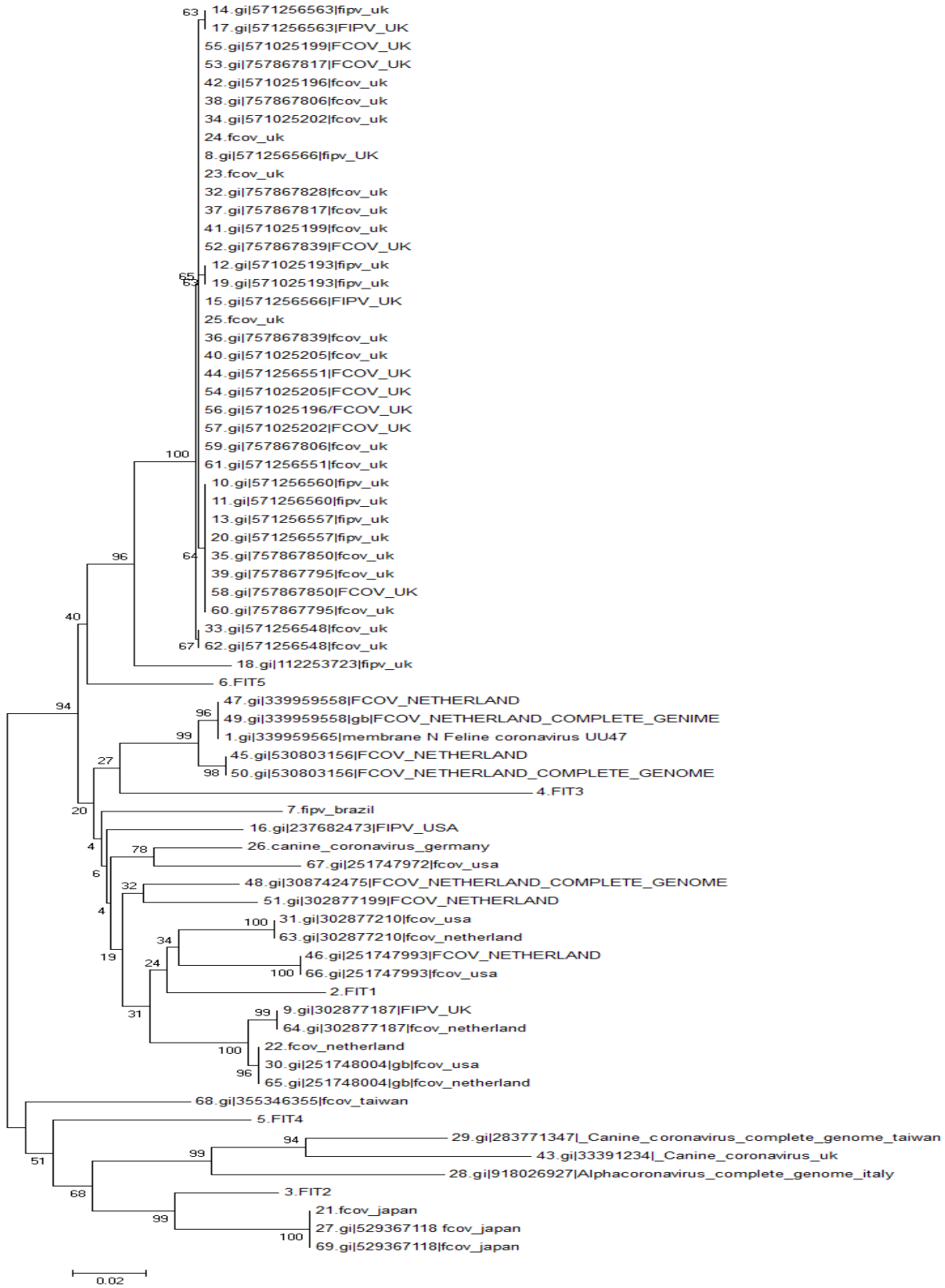
14.gi|571256563|fipv_uk
17.gi|571256563|FIPV_UK
55.gi|571025199|FCOV_UK
53.gi|757867817|FCOV_UK
42.gi|571025196|fcov_uk
38.gi|757867806|fcov_uk
34.gi|571025202|fcov_uk
24.fcov_uk
8.gi|571256566|fipv_UK
23.fcov_uk
32.gi|757867828|fcov_uk
37.gi|757867817|fcov_uk
41.gi|571025199|fcov_uk
52.gi|757867839|FCOV_UK
12.gi|571025193|fipv_uk
19.gi|571025193|fipv_uk
15.gi|571256566|FIPV_UK
25.fcov_uk
36.gi|757867839|fcov_uk
40.gi|571025205|fcov_uk
44.gi|571256551|FCOV_UK
54.gi|571025205|FCOV_UK
56.gi|571025196/FCOV_UK
57.gi|571025202|FCOV_UK
59.gi|757867806|fcov_uk
61.gi|571256551|fcov_uk
10.gi|571256560|fipv_uk
11.gi|571256560|fipv_uk
13.gi|571256557|fipv_uk
20.gi|571256557|fipv_uk
35.gi|757867850|fcov_uk
39.gi|757867795|fcov_uk
58.gi|757867850|FCOV_UK
60.gi|757867795|fcov_uk
33.gi|571256548|fcov_uk
62.gi|571256548|fcov_uk
18.gi|112253723|fipv_uk
6.FIT5
47.gi|339959558|FCOV_NETHERLAND
49.gi|339959558|gb|FCOV_NETHERLAND_COMPLETE_GENIME
1.gi|339959565|membrane N Feline coronavirus UU47
45.gi|530803156|FCOV_NETHERLAND
50.gi|530803156|FCOV_NETHERLAND_COMPLETE_GENOME
4.FIT3
7.fipv_brazil
16.gi|237682473|FIPV_USA
26.canine_coronavirus_germany
67.gi|251747972|fcov_usa
48.gi|308742475|FCOV_NETHERLAND_COMPLETE_GENOME
51.gi|302877199|FCOV_NETHERLAND
31.gi|302877210|fcov_usa
63.gi|302877210|fcov_netherland
46.gi|251747993|FCOV_NETHERLAND
66.gi|251747993|fcov_usa
2.FIT1
9.gi|302877187|FIPV_UK
64.gi|302877187|fcov_netherland
22.fcov_netherland
30.gi|251748004|gb|fcov_usa
65.gi|251748004|gb|fcov_netherland
68.gi|355346355|fcov_taiwan
5.FIT4
29.gi|283771347|_Canine_coronavirus_complete_genome_taiwan
43.gi|33391234|_Canine_coronavirus_uk
28.gi|918026927|Alphacoronavirus_complete_genome_italy
3.FIT2
21.fcov_japan
27.gi|529367118 fcov_japan
69.gi|529367118|fcov_japan
0.02

Figure 3: Phylogenetic tree based on M gene sequences of FIPVs of this study (FIT1-FIT5) compared with other FIPVs from different regions of the world generated by neighbor-joining algorithm and Mega 7 software with a bootstrap value of 1000.

Discussion

Coronaviral infections are widespread and often can cause life-threatening illnesses in livestock and pets (Anaraki et al., 2022, Ramezanpour Eshkevari., et al., 2023, Mojtahedzadeh, et al., 2023, Rasooli et al., 2023). Among the animal coronaviruses, Feline Coronavirus (FCOV) infection is common in cats worldwide but most infections are inapparent and only 1-5% terminate to highly fatal form of the FIP. It is one of the most serious viral diseases due to its lethality, complexity of pre-death diagnosis, and control of its spread. This investigation is the first study of epidemiology parameters, evaluation of common diagnostic methods, and molecular and phylogenetic analysis of FIP in Iran.

Rohrbach et al., 2001 showed cats with FIP were significantly more likely to be young between 6 months to two years old, purebred, sexually intact males, and significantly less likely to be spayed females, but Pederson., 2009 reported the disease is more prevalent between 6-12 months old cats. Mosallanejad et al., 2012 studied on seroprevalence of feline coronavirus infection by immunochromatography assay in Ahvaz City in the southeast part of Iran and the results showed a significantly higher rate of FIP infection in young kittens less than 6 months and mean-age cats 6 months – 3 years compared with above 3 years. Also, it was higher in male than female cats. Mohammed Ibrahim et al., 2022 detected the feline coronavirus genome by Real-time PCR in 10 out of 50 FIP-suspected cats (20%) in Baghdad, Iraq, and showed the very suitable age of infection was in younger cats with ages lower than 2 years old. In this study number of males with positive RT-PCR results was two times more than females and all patients were young 9-30 months old cats. Therefore, our result is similar and confirms the results of the other studies (Rohrbach et al., 2001, Pedersen, 2009, Mosallanejad et al., 2012, Mohammed Ibrahim et al., 2022).

FIP is one of the cat diseases without any specific clinical signs or blood protein profile and therefore, the number of cats executed for incorrect diagnosis is usually more than the number of

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cats dying from the nature of the disease. So, one of the purposes of this study is to reach a reliable protocol and in other words, to investigate how much each laboratory and clinical finding is reliable. According to Tasker 2018 study, 50% of wet and 70% of dry FIP cats show increased serum protein. So, serum protein level alone cannot be a definite diagnostic test. Also, Kennedy, 2020 and Addie et al., 1995 indicated antibody titer against FCOV is not significantly different in healthy vs FIP affected cats, and therefore the presence of antibodies is not diagnostic for FIP detection as well (Addie *et al.,* 1995, Tasker 2018, Kennedy, 2020).

During 1979-2000, a comprehensive study performed on 488 histopathology-confirmed FIP cases by Hartmann et al., 2003 showed that 81% of cats were affected by effusive form and albumin to globulin ratio of the serum of more than 0.8 demonstrated the highest impact in FIP serum diagnostic tests. Shelly et al., 1988 determined a cutoff for FIP diagnostic value of 0.8 but Duthie et al., 1997 decreased it to 0.7 (Shelly *et al.,* 1988, Duthie *et al.,* 1997, Hartmann *et al.,* 2003). Results of this study show A/G ratio under 0.66 is valuable and reliable for FIP detection and confirms previous results.

In the investigation conducted by Hartmann et al., the utility of serum total protein as a dependable marker for Feline Infectious Peritonitis (FIP) was questioned. Their findings reveal that a total protein level exceeding 12 g/dL does not reliably indicate the presence of FIP. This ambiguity arises due to the observation that within this subgroup, 50% of cats exhibited other infections, such as calicivirus, underscoring the limitations of total protein concentration as a definitive diagnostic marker for FIP. They believe the diagnostic value of measuring all factors, including total protein levels, albumin to globulin ratio, and gamma globulin concentration in abdominal fluids, is much higher than serum levels alone. Among them, the total protein level showed the highest diagnostic value, and they demonstrated total protein level of more than 0.8 g/dl in serum is important for FIP detection. Although in our study 50% of FIP-positive cats were matched with this cutoff point, the other 50% showed a lower total protein level of serum. So, our results cannot confirm Hartmann's study results and showed a cutoff of 4.31 g/dl. Paltrini et al., 2002 reported a total serum protein level of higher than 3.5 g/dl in 87% of FIP-confirmed cases and 67% of non-confirmed cases (with fluid) which their results match with this study

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(Hartmann *et al.,* 2003, Paltrinieri *et al.,* 2002). Hartman also evaluated the positive and negative predictive value of the Rivalta test to 86% and 97% respectively. Results of this study show both are 100% but Rivalta shows many false positives that decrease its positive predictive value. Also, Hartmann showed the positive predictive value is low for RT-nPCR with primers designed for super-conserving 3'UTR part of the FCOV genome in serum, but in fluids of the cavities is relatively high. As we used cavities fluid for RT-PCR, our positive results (based on Hartman's results) would be reliable. However, there is a possibility that we may miss some positive cats due to the presence of inhibitors in the cavity's fluids. Finally, Hartman concluded there is not one test that is reliable for the diagnosis of FIP, and they should be considered together for confirmation of FIP, and histopathology would be the gold standard of diagnosis (Hartmann *et al.,* 2003).

Herrewegh and Gamble and their colleagues in different studies reported 95% and 90% positive predictive values of RT-PCR respectively, which matches with our results, and it demonstrates the RT-PCR result of fluids is confirmative (Herrewegh *et al.,* 1995, Gamble *et al.,* 1997). Paltrini and colleagues reported that among 79 confirmed FIP cases, 63% had abdominal fluids, 22% had chest fluids, and 15% had both abdominal and chest fluids. In our study, out of 6 confirmed cases, 5 (83%) had abdominal fluids, while only 1 (17%) had chest fluids. This observation highlights a higher prevalence of positive confirmed cases with effusive form exhibiting abdominal fluids. Also, Paltrini and colleagues reported that 90% of confirmed FIP cases exhibited typical cytology profiles. In our study, all six cases demonstrated similar characteristics, indicating that the cytology of fluids, showcasing non-degenerated macrophages and neutrophils with a basophilic background, serves as a robust and promising marker for confirmation. (Paltrinieri *et al.,* 2002). Recently Farsijani et al., 2023 evaluated the sensitivity and specificity of the specific modulation frequency (SMF) test in Iran and compared it with electrophoresis and polymerase chain reaction (PCR) tests to determine their diagnostic value for FIP infection. They demonstrated 100% sensitivity and 81.1% specificity for SMF and introduced it as an effective and safe test in FIP diagnosis (Farsijani et al., 2023)

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The unknown origin poses the most significant challenge in the case of Feline Coronavirus (FCoV) and Feline Infectious Peritonitis (FIP). In 2009, Brown et al. conducted an investigation on viral sequences obtained from clinically healthy and sick cats infected with Feline Coronavirus (FCoV). The study involved 8 cases of FIP and 48 asymptomatic FECV-infected animals. A total of 735 sequences from four gene segments (S, Pol, M, and 7b) were generated and subsequently subjected to phylogenetic analyses. They showed a monophyletic tree and indicated viral sequences from healthy cats were distinct from sick cats based on genetic distances observed in the membrane and nonstructural protein 7b genes. These data demonstrate distinctive "circulating virulent and avirulent strains" in natural populations. In addition, they reported 5 membrane protein amino acid residues with functional potential for differentiating healthy cats from cats with FIP (Brown *et al.,* 2009). Before and after the publication of this study, many scientists challenged the hypothesis and indicated some kind of "internal mutation" in FCOV strains gives them the capability to replicate in macrophages and monocytes and therefore they can generate FIP infection. Chang et al., 2010 studied the ORF 3c gene of FCOV and reported the nucleotide sequences of FIPV and FECV M genes distributed into paraphyletic patterns rather than in monophyletic clusters indicating the "internal mutation" hypothesis (Chang *et al.,* 2010). They additionally examined the M sequence at five hotspots in Brown's study but did not attain similar results to theirs. In another study, Barker et al., 2013 examined the nsp2, nsp12, S, and M genes, in viruses derived from cats with FIP, and FECV-infected cats. Phylogenetic trees of all three genes showed a paraphilic pattern, and FIPVs and FECVs were placed in the same clusters, indicating the in vivo theory of mutation. They also evaluated the amino acid sites which Brown et al. identified as valued diagnostic sites. However, they did not confirm Brown's findings in this study (Barker *et al.,* 2013). Lately, Chang et al., 2012 showed S gene is the most important gene in changing FECV to FIP and mutations of M1058L and S1060A are hotspots for this transformation (Chang *et al.,* 2012). Also, Lutz et al., 2020 reported another hotspot of I1108T in the heptad repeat 1 (HR1) region of the S gene, which is important in FECV to FIPV transition (Lutz *et al.,* 2020). Finally, and in this study there appears to be no discernible correlation between our confirmed FIP viruses (FIT1-5) and either FECoV or FIPV,

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the other reason for rejecting the “circulating virulent and avirulent strains” and confirming the” internal mutation” hypothesis.

Conclusions

Numerous uncertainties persist regarding the molecular pathogenesis of Feline Infectious Peritonitis (FIP). The S gene sequence is insufficient to explain all the unknowns in FIP pathogenesis, particularly those related to virus entry into cells. Consequently, some recent studies have redirected their focus to other genes such as 3c, 7b, and the M gene. In this study, the five sequences of the M gene were subjected to molecular and phylogenetic analysis. The sequence analysis of five hotspot amino acids in the M protein, as reported by Brown and colleagues, revealed that the nucleotide sequences of the M genes in Feline Enteric Coronavirus (FECoV) and Feline Infectious Peritonitis Virus (FIPV) do not exhibit separation according to the biotypic pattern. Additionally, the phylogenetic tree displayed a paraphyletic pattern, suggesting that the virus mutated within the cat's body. It is recommended that future studies concentrate on reverse genetic investigations involving the 3c, 7b, and M genes, as well as the crucial residues of these proteins, to explore the transition from FECV to FIPV form. Additionally, research efforts should be directed toward identifying potential ligands or drugs capable of addressing this transition, ultimately leading to the development of a treatment for cats afflicted with FIP.

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Conflict of interest

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The authors declared no potential conflicts of interest for the research, authorship, and/or publication of this article.

Ethical approval

This work involved the use of nonexperimental animals only (owned or unowned). Established internationally recognized high standards ('best practice') of individual veterinary clinical patient care were followed. Ethical approval from a committee was therefore not necessarily required.

Informed consent

Informed consent (verbal or written) was obtained from the owner or legal custodian of all animals described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

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مطالعه مولکولی و بالینی ویروس پریتونیت عفونی گربه سانان در ایران نشانگر درخت فیلوژنتیک چند شاخه است: تاییدی بر نظریه موتاسیون داخلی

شهرام جمشیدی[۱]، فرنوش مومنی[۱]، ایرج اشرافی تمای[۲]، امید مددگار[۲*]

۱- گروه علوم بالینی دانشکده دامپزشکی دانشگاه تهران.

۲- گروه میکروبیولوژی و ایمنولوژی دانشکده دامپزشکی دانشگاه تهران.

زمینه مطالعه: پریتونیت عفونی گربه ها (FIP) یک بیماری شدید و اغلب کشنده است که گونه های گربه ها را در سطح جهان مبتلا می کند. علیرغم شیوع بالای عفونت‌های ویروس کرونا(FCoV) ، تظاهرات FIP تنها در درصد کمی (5-1%) از موارد رخ می‌دهد. جنبه های پیچیده تشخیص افتراقی FIP همچنان ادامه دارد، و درک جامع از مکانیسم های مولکولی مولد FIP مبهم باقی مانده است.

هدف: بررسی ویژگی‌های FIPV ایرانی، شامل تجزیه و تحلیل توالی و بررسی یافته‌های آزمایشگاهی و بالینی بوده است. هدف اصلی، بررسی فرضیه پیدایش ویروسFIP ، با تمرکز خاص بر سطح ژن M است.

روش کار: روش ما بررسی مایعات شکمی یا سینه‌ای از 17 گربه مشکوک بهFIP ، با استفاده از آزمایش‌های بیوشیمیایی مانند پروتئین کل سرم، نسبت آلبومین به گلوبولین (A/G) و تست ریوالتا بود. علاوه بر این، RT-PCRبر اساس ژن غشایی (M) مورد استفاده قرار گرفت. تجزیه و تحلیل توالی پنج اسید آمینه حیاتی در ژن M و تولید درخت فیلوژنتیک با استفاده از پنج ویروس توالی‌یابی شده، تحقیقات ما را غنی‌تر کرد.

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نتایج: تست ریوالتا FIP را در 6 گربه از 17 گربه تایید کرد. وقوع FIP در میان گربه های جوان (9-30 ماهه) در نرها دو برابر بیشتر از ماده ها بود. گربه های مبتلا در محدوده سنی 9-30 ماهه نسبت A/G کمتر از 0.66 و کل پروتئین سرم بیش از 0.43 گرم در دسی لیتر را نشان دادند. سیتولوژی مایع حفره های بدن ماکروفاژها و نوتروفیل های غیر دژنره شده را در برابر پس زمینه بازوفیل نشان داد که تایید کننده تشخیص FIP است. مهمتر از همه، تجزیه و تحلیل توالی پنج نقطه کانونی اسید آمینه پروتئینM ، تفاوت های ناچیزی در توالی های نوکلئوتیدی FECoV وFIPV ، هم سو با الگوی بیوتیپی آنها را نشان داد.

نتیجه‌گیری نهایی: درخت فیلوژنتیک تولید شده در این مطالعه یک الگوی پارافیلیک که بر فرضیه «جهش داخلی» تأکید دارد را نشان می‌دهد و بنابراین جهش‌های ویروسی در بدن گربه رخ می‌دهد و تفاوت قابل‌توجهی در ویروس‌های مولد FECoVو FIPV وجود ندارد. این یافته‌ها به شناخت پاتوژنز FIP کمک می‌کنند و رویکردهای تشخیصی و درمانی آینده را هدایت می‌کنند.

کلمات کلیدی: آنالیز فیلوژنتیک، پریتونیت عفونی گربه سانان، تست های بیوشیمیایی، تست ریوالتا، ایران.

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