Original Article

Molecular and Clinical Study of Feline Infectious Peritonitis Virus in Iran Showing a Paraphyletic Tree: Emphasizing 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.

Use your device to scan and read the article online

How to Cite This Article Jamshidi, Sh., Momeni, F., Ashrafi Tamai, I., & Madadgar, O. (2025). Molecular and Clinical Study of Feline Infectious Peritonitis Virus in Iran Showing a Paraphyletic Tree: Emphasizing the "Internal Mutation" Hypothesis. *Iranian Journal of Veterinary Medicine, 19*(2), 179-190. http://dx.doi.org/10.32598/ijvm.19.2.1005247

http://dx.doi.org/10.32598/ijvm.19.2.1005247

ABSTRACT

Background: Feline infectious peritonitis (FIP) is a severe and often fatal disease affecting feline species. 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 thoroughly investigate the characteristics of Iranian feline infectious peritonitis viruses (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 membrane (*M*) gene level.

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

Results: The study confirmed FIP in 6 of 17 cats through the Rivalta test, guiding subsequent evaluations. Significant gender disparities in FIP occurrence were observed among young cats (9-30 months old), with males exhibiting a two-fold higher incidence than females. Affected cats within the 9-30 months age range consistently exhibited an albumin to globulin (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 feline enteric coronavirus (FECoV) and FIPV, aligning with their biotypic patterns.

Conclusion: The phylogenetic tree generated in this study displayed a paraphilic pattern, emphasizing the "internal mutation" hypothesis, suggesting that viral mutations occur within

Article info:
Received: 08 Mar 2024
Accepted: 26 Jun 2024
Publish: 01 Apr 2025

*** Corresponding Author:**
Omid Madadgar, PhD.
Address: *Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*
Phone: *+89 (21) 61117053*
E-mail: *omadadgar@gmail.com.*

the cat's body and no significant differences are observed in FECoV and FIPV-generating viruses. These results provide valuable insights into the discourse surrounding FIP pathogenesis, potentially guiding future diagnostic and therapeutic approaches.

Keywords: Biochemical tests, Feline infectious peritonitis viruses (FIPV), Iran, Phylogenetic analysis, Rivalta test

Introduction

Feline infectious peritonitis (FIP) is one of the deadliest infections in the cat population, and its causative agent is the Coronaviridae family member. Feline coronavirus (FCoV) includes two separate biotypes: The ubiquitous feline enteric coronavirus (FECV) is more common and causes self-limiting moderate diarrhea that often cannot be cleared and generates a persistent infection in the cells of the intestinal mucosa, and the sporadic type of feline infectious peritonitis virus (FIPV), which 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. They 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 ability of FIPV to infect monocytes and macrophages (Tekes et al., 2016; Doenges et al., 2016; Decaro et al., 2021).

Clinical signs associated with the FIPV biotype can be variable and non-specific, usually, including fever, lethargy, anorexia, pica, vomiting, and diarrhea. Hence, the differential diagnosis of other infectious diseases is difficult and is based on laboratory confirmation. Also, clinical signs can be present in 'wet,' 'dry' or 'mixed' presentations. The wet form of FIP is characterized by 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 provide only a strongly suggestive diagnosis of the non-pathognomonic pattern of FIP (Felten & Hartmann, 2019); therefore, the identification of related histopathological lesions with immunohistochemical detection of FCoV antigens 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 two main hypotheses are the "internal mutation" theory that is based on the mutations in FECV and, 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 FIPV characteristics, including sequence analysis and laboratory and clinical findings, aims to systematically investigate these hypotheses at the *M* gene level in Iranian FIP viruses.

Materials and Methods

Samples

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

Biochemical examinations and Rivalta test

Albumin and total protein levels were measured using an autoanalyzer (Selectra, Elitech Group, Netherlands). Subsequently, the albumin value was subtracted from the total protein value to determine the globulin concentration 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 and 8 mL distilled water was prepared in a 10 mL clear test tube. 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, or moved slowly down the solution.

Cellular examination

To observe cell contents, 10 µL of abdominal fluid supernatant was centrifuged at 130 g for 10 minutes and stained using the Giemsa method. The presence of white blood cells in abdominal fluid smears was assessed by microscopy.

Molecular investigation

Ribonucleic acid (RNA) extraction and reverse transcription reaction

A 1.5 mL microtube containing abdominal fluid from each sample was centrifuged at 130 g for 5 minutes. The resulting supernatant was subjected to 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 incubated for at least 5 minutes at 48 ˚C. After adding 200 mL of chloroform and thoroughly mixing, the liquid was clarified by centrifugation at 12000 g at 48 ˚C for 15 minutes. The supernatant was then transferred to a new tube, mixed with an equal volume of isopropanol, and centrifuged at 12000 g at 48 ˚C for 15 minutes. The pellet was washed with 1 mL 70% ethanol. Finally, RNA was eluted using 50 µL of 1 mM RNase-free diethylpyrocarbonate (DEPC)-treated water.

Subsequently, complementary DNA (cDNA) was synthesized using the Maxime RT Premix Kit (iNtRON, Seoul, South Korea). Eight microliters of extracted RNA were added to a ready-to-use tube, and 20 µL of diethylpyrocarbonate (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.

Polymerase chain reaction (PCR)

The PCR was conducted following the method outlined by Barker et al. (2013) to amplify a 1040 bp segment of the FIPV M gene. Briefly, 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 for the PCR. Subsequently, PCR was performed using a thermocycler (Techne, England) using the following protocol: initial denaturation at 98 ˚C for 30 s, followed by 40 cycles of denaturation at 98 ˚C for 10 s, annealing at 59 ˚C for 20 s, extension at 72 ˚C for 30 s, and a final extension at 72 ˚C for 2 minutes. The PCR product (5 µL) was subjected to electrophoresis on 2% agarose gel and stained with ethidium bromide.

Sequencing of *M* gene, sequence analysis, and phylogenetic tree generation

Nucleotide sequencing was conducted using an automatic sequencer (ABI-377; Applied Biosystems, Foster City, CA, USA) for the remaining PCR products after gel purification. 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 the National Center for Biotechnology Information (NCBI) GenBank (accession number: JN183882) using CLC sequence viewer 6.

Furthermore, sixty-four *M* gene sequences of 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 of the 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 albumin to globulin (A/G ratio) spanned from 0.12 to 0.9. Apart from abdominal fluids, two of the 17 studied cats exhibited fluid accumulation in the thoracic area. Only one percent of these cats (5%) had exclusive fluid accumulation in the thoracic area (Table 1).

Cellular examination

Cytological examination of the abdominal and thoracic fluid revealed the presence of non-degenerate macrophages and neutrophils. Granules and basophilic protein strands were also observed in the background (Figure 1).

Table 1. Clinical, biochemical, and PCR result

No.	Sex	Type of Secreted Fluid	A/G	Total Protein (g/dL)	Rivalta Test	RT-PCR Result	Age (m)
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
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

Abbreviations: PT-PCR: Reverse transcription-polymerase chain reaction; F: Female; M: Male; A: Abdominal; T: Thoracic; A/G: Albumin-to-globulin ratio.

PCR

Reverse transcription-PCR (RT-PCR) results were positive in six out of the 17 cats (35%), displaying 1040 bp bands (Figure 2).

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 FIPV, GenBank No. JN183882) was predicted for all viruses analyzed in this study. Therefore, consistent with the results of Barker et al., (2013) these crucial amino acids could not differentiate between FCoV and FIPV in the current study.

Phylogenetic tree

Nucleotide sequences of cats with FIP and FECoV 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 FECoV and FIPV found in the USA, the 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, the USA, and Germany. FIT4 aligns with some FECoV and FIPV strains from Japan and Taiwan and some Canine Coronaviruses from Taiwan, the UK, and Italy. Finally, FIT5 is closely related to certain FECoV and FIPV viruses observed in the UK.

Discussion

Coronavirus infections are widespread and can often 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, FCOV infection is common in cats worldwide, but most infections are inapparent, and

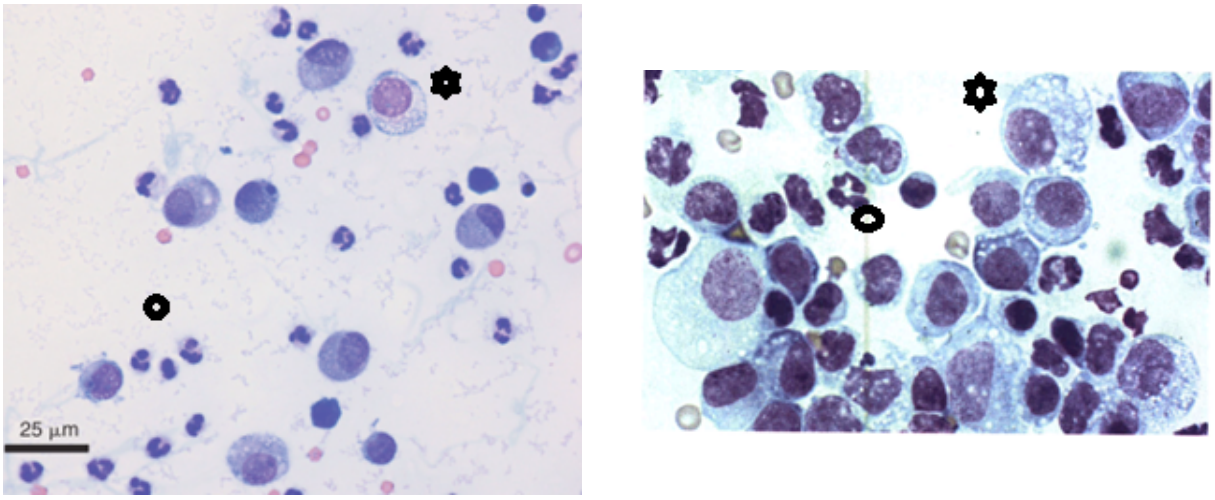

Figure 1. Cytologic examination of fluids showing non-degenerated macrophages and neutrophils

Note: Black stars indicate macrophages and black circles show neutrophils.

only 1%-5% terminate in the highly fatal form of 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 epidemiological 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 Pedersen (2009), reported that the disease is more prevalent in cats aged 6-12 months old cats. Mohammed Ibrahim et al. (2022) studied the seroprevalence of FCOV infection by immunochromatography assay in Ahvaz City in the southeast part of Iran. They found a significantly higher rate of FIP infection in young kittens aged less than 6 months and mean-age cats aged 6 months to 3 years compared to those aged >3 years. Also, it was higher in male cats than in female cats. Mohammed Ibrahim et al. (2022) detected the FCOV genome by real-time PCR in 10 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, the number of males with positive RT-PCR results was twice that of females, and all patients were young 9-30-month-old cats. Therefore, our results are similar and confirm those of other studies (Rohrbach et al., 2001; Pedersen, 2009; Mosallanejad et al., 2012; Mohammed Ibrahim et al., 2022).

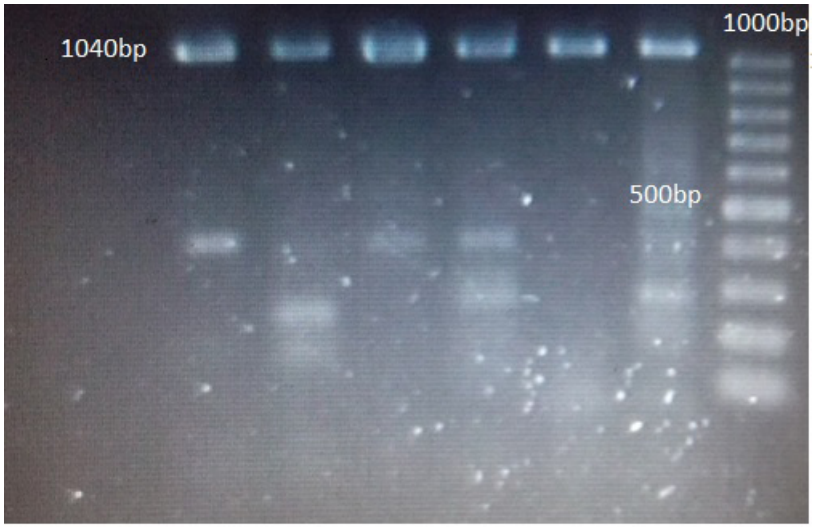

Figure 2. RT-PCR of the fluid cells showing a 1040 bp size band of the *M* gene

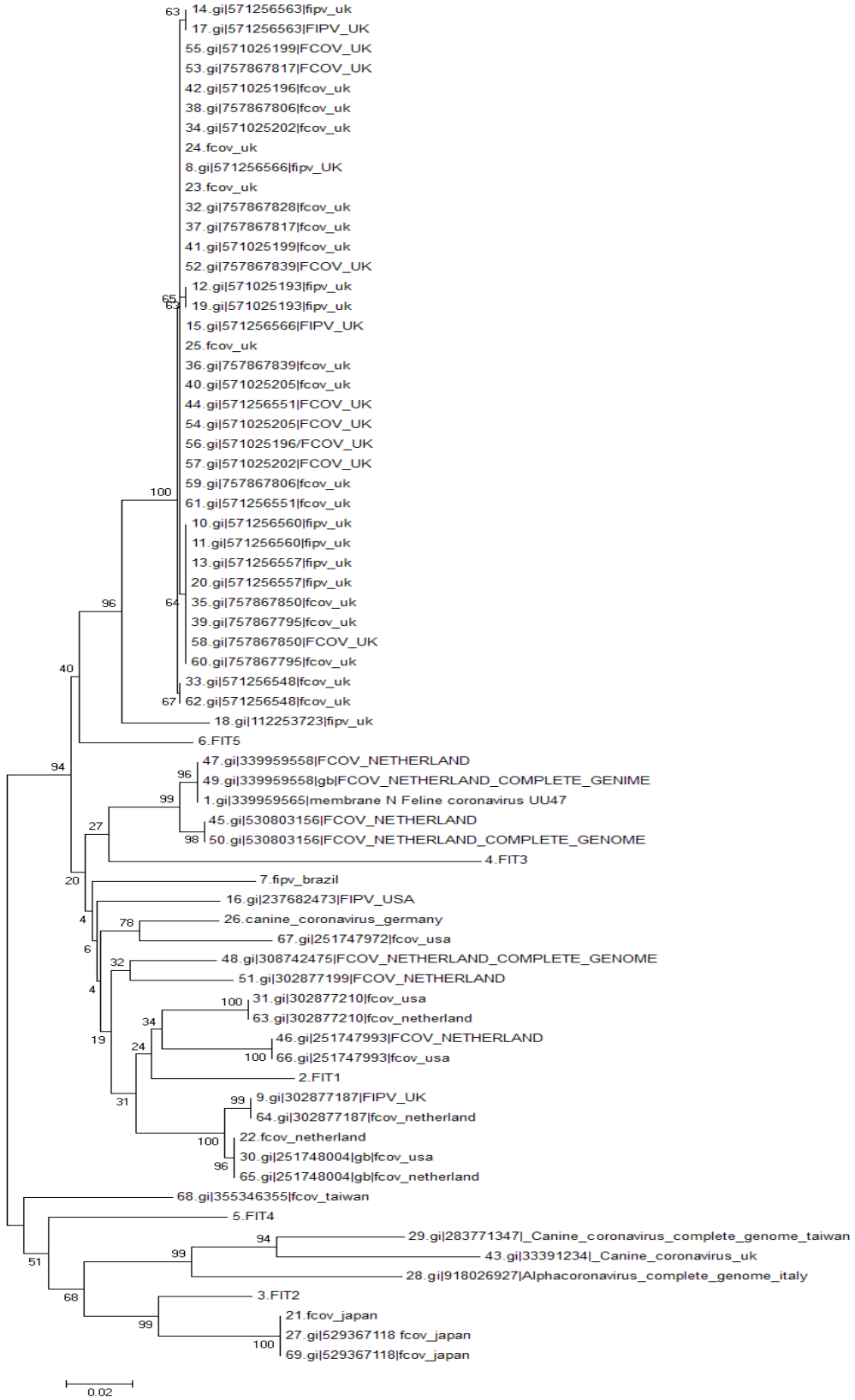

Figure 3. Phylogenetic tree based on *M* gene sequences of FIPVs of this study (FIT1-FIT5) compared to other FIPVs from different regions of the world generated by neighbor-joining algorithm and Mega 7 software with a bootstrap value of 1000

FIP is a cat disease without any specific clinical signs or blood protein profile; therefore, the number of cats executed for an incorrect diagnosis is usually greater than the number of cats dying from the nature of the disease. Therefore, one of the purposes of this study was to reach a reliable protocol and, in other words, to investigate the reliability of each laboratory and clinical finding. According to Tasker (2018), 50% of wet and 70% of dry cats with FIP showed increased serum protein levels. Therefore, serum protein level alone cannot be a definite diagnostic test. Also, Kennedy, (2020) and Addie et al. (1995), indicated that antibody titer against FCOV is not significantly different in healthy vs FIP-affected cats. 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 the effusive form and albumin to globulin ratio of the serum of more than 0.8, demonstrating the highest impact in FIP serum diagnostic tests. Shelly et al. determined a cutoff for the FIP diagnostic value of 0.8, but Duthie et al. decreased it to 0.7 (Shelly et al., 1988; Duthie et al., 1997). The results of this study showed that an A/G ratio of less than 0.66 is valuable and reliable for FIP detection and confirms previous results.

Hartmann et al. questioned the utility of serum total protein as a marker of FIP. Their results revealed that a total protein level > 12 g/dL did not reliably indicate the presence of FIP. This ambiguity arises because, 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, A/G ratio, and gamma globulin concentration in abdominal fluids, is much higher than that of serum levels alone. Among them, the total protein level showed the highest diagnostic value, and they demonstrated that a total protein level of more than 0.8 g/dL in serum is crucial for FIP detection (Hartmann et al., 2003). In our study, 50% of FIP-positive cats were matched with this cutoff point, and the other 50% showed a lower total serum protein level. Therefore, our results cannot confirm Hartmann's study results and showed a cutoff of 4.31 g/dL. Paltrini et al. reported a total serum protein level higher than 3.5 g/dL in 87% of FIP-confirmed cases and 67% of non-confirmed cases (with fluid), which is consistent with the results of this study (Paltrinieri et al., 2002). Hartman also evaluated the positive and negative predictive values of the Rivalta test to be 86% and 97%, respectively (Hartmann et al., 2003). The results of this study showed that both were 100%, but Rivalta showed many false positives, which decreased its positive predictive value. Also, Hartmann showed that the positive predictive value is low for reverse transcriptase nested PCR (RT-nPCR) with primers designed for super-conserving three prime untranslated regions (3'UTR) of the FCOV genome in serum but is relatively high in fluids of the cavities. Because we used cavity fluid for RT-PCR, our positive results (based on Hartman's results) would be reliable. However, we may have missed some positive cats due to inhibitors in the cavity's fluids. Finally, Hartman concluded that no reliable test exists for the diagnosis of FIP, that they should be considered together for confirmation of FIP, and that histopathology would be the gold standard of diagnosis (Hartmann et al., 2003).

Herrewegh and Gamble et al., 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 et al. reported that among 79 confirmed FIP cases, 63% had abdominal fluid, 22% had chest fluid, and 15% had both abdominal and chest fluid. In our study, of the six confirmed cases, five (83%) had abdominal fluids, while only one (17%) had chest fluids. This observation highlights a higher prevalence of confirmed positive cases with effusive forms exhibiting abdominal fluids. Also, Paltrini et al. reported that 90% of the confirmed FIP cases exhibited typical cytology profiles. In our study, all six cases demonstrated similar characteristics, indicating that the cytology of fluids, showing 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. evaluated the sensitivity and specificity of the specific modulation frequency (SMF) test in Iran and compared it with electrophoresis and PCR tests to determine its 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).

The unknown origin poses the most significant challenge in the cases of FCoV and FIP. Brown et al. investigated viral sequences obtained from clinically healthy and sick cats infected with 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 analysis. 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 demonstrated distinctive "circulating virulent and avirulent strains" in natural populations. In addition, they reported five membrane protein amino acid residues with functional potential to differentiate 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 "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. studied the ORF *3c* gene of FCOV. They 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 results similar to theirs (Brown et al., 2009). In another study, Barker et al. (2013) examined *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 cluster, indicating an in vivo theory of mutation. The authors also evaluated the amino acid sites identified by Brown et al. as valuable diagnostic sites (Brown et al., 2009). However, they did not confirm Brown's findings in this study (Barker et al., 2013). Recently, Chang et al. showed that *S* gene is vital in changing FECV to FIP, and mutations of M1058L and S1060A are hotspots for this transformation (Chang et al., 2012). Also, Lutz et al. reported another hotspot of I1108T in the heptad repeat one region of the S gene, which is crucial in the FECV to FIPV transition (Lutz et al., 2020). Finally, in this study, no discernible correlation was observed between our confirmed FIP viruses (FIT1-5) and either FECoV or FIPV, the other reason for rejecting the "circulating virulent and avirulent strains" and confirming the" internal mutation" hypothesis.

Conclusion

Numerous uncertainties persist regarding the molecular pathogenesis of FIP. The *S* gene sequence does not explain all the unknowns in FIP pathogenesis, particularly those related to virus entry into cells. Consequently, some recent studies have focused on other genes, such as *3*c, *7b*, and the *M* gene. In this study, five sequences of the *M* gene were subjected to molecular and phylogenetic analyses. Sequence analysis of five hotspot amino acids in the M protein, as reported by Brown et al., revealed that the nucleotide sequences of the *M* genes in FECoV and FIPV did not exhibit separation according to the biotypic pattern. Additionally, the phylogenetic tree displayed a paraphyletic pattern, suggesting that the virus was mutated within the cat's body. Future studies should focus on reverse genetic investigations involving *3c, 7b*, and *M* genes and the crucial residues of these proteins to explore the transition from FECV to FIPV. Additionally, research efforts should be directed toward identifying potential ligands or drugs capable of addressing this transition, ultimately leading to the development of treatments for cats afflicted with FIP.

Ethical Considerations

Compliance with ethical guidelines

This study used non-experimental animals (owned or unowned). Established internationally recognized high standards (best practices) for individual veterinary clinical patient care were followed. Therefore, ethical approval from the committee was not required.

Funding

This study was financially supported by the Ministry of Science, Research, and Technology, and Research Council of the Faculty of Veterinary Medicine, University of Tehran (Project No.: 7508043/6/34).

Authors' contributions

Conceptualization and supervision: Omid Madadgar and Shahram Jamshidi; Methodology: Omid Madadgar, Shahram Jamshidi and Iraj Ashrafi Tamai; Data collection: Farnoosh Momeni, Iraj Ashrafi Tamai; Data analysis: All authors; Investigation, and writing: All authors; Funding acquisition and resources: Omid Madadgar, and Shahram Jamshidi.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors express their gratitude to all the technicians and staff of the Department of Microbiology and Internal Medicine of Small Animals, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

References:

Addie, D. D., Toth, S., Murray, G. D., & Jarrett, O. (1995). Risk of feline infectious peritonitis in cats naturally infected with feline coronavirus. *American Journal of Veterinary Research, 56*(4), 429–434. [DOI:10.2460/ajvr.1995.56.04.429] [PMID]

Aksono, E. B., Iradatya, K. R., Sucipto, T. H., Fajar, N. S., & Yuniarti, W. M. (2023). Phylogenetic analysis of feline infectious peritonitis virus, feline enteric coronavirus, and severe acute respiratory syndrome coronavirus 2 of cats in Surabaya, Indonesia. *Veterinary World, 16*(1), 76–81. [PMID]

Hajijafari Anaraki, M., Sheikhi, N., Haghbin Nazarpak, H., & Nikbahkt Brujeni, G. (2022). Real time detection of different variant strains of infectious bronchitis virus in trachea, lung and kidney of infected broiler chickens. I*ranian Journal of Veterinary Medicine. 16*(1), 57-63. [DOI:10.22059/IJVM.2021.321594.1005165]

André, N. M., Cossic, B., Davies, E., Miller, A. D., & Whittaker, G. R. (2019). Distinct mutation in the feline coronavirus spike protein cleavage activation site in a cat with feline infectious peritonitis-associated meningoencephalomyelitis. *JFMS Open Reports, 5*(1), 2055116919856103. [DOI:10.1177/2055116919856103] [PMID]

Barker, E. N., Tasker, S., Gruffydd-Jones, T. J., Tuplin, C. K., Burton, K., & Porter, E., et al. (2013). Phylogenetic analysis of feline coronavirus strains in an epizootic outbreak of feline infectious peritonitis. *Journal of Veterinary Internal Medicine, 27*(3), 445-450. [DOI:10.1111/jvim.12058] [PMID]

Brown, M. A., Troyer, J. L., Pecon-Slattery, J., Roelke, M. E., & O'Brien, S. J. (2009). Genetics and pathogenesis of feline infectious peritonitis virus. *Emerging Infectious Diseases, 15*(9), 1445-1452. [DOI:10.3201/eid1509.081573] [PMID]

Chang, H. W., de Groot, R. J., Egberink, H. F., & Rottier, P. J. (2010). Feline infectious peritonitis: Insights into feline coronavirus pathobiogenesis and epidemiology based on genetic analysis of the viral 3c gene. *The Journal of General Virology, 91*(Pt 2), 415–420. [DOI:10.1099/vir.0.016485-0] [PMID]

Chang, H. W., Egberink, H. F., Halpin, R., Spiro, D. J., & Rottier, P. J. (2012). Spike protein fusion peptide and feline coronavirus virulence. *Emerging Infectious Diseases, 18*(7), 1089-1095. [DOI:10.3201/eid1807.120143] [PMID]

Decaro, N., Mari, V., Lanave, G., Lorusso, E., Lucente, M. S., & Desario, C., et al. (2021). Mutation analysis of the spike protein in Italian feline infectious peritonitis virus and feline enteric coronavirus sequences. *Research in Veterinary Science, 135,* 15-19. [DOI:10.1016/j.rvsc.2020.12.023] [PMID]

Doenges, S. J., Weber, K., Dorsch, R., Fux, R., Fischer, A., & Matiasek, L. A., et al. (2016). Detection of feline coronavirus in cerebrospinal fluid for diagnosis of feline infectious peritonitis in cats with and without neurological signs. *Journal of Feline Medicine and Surgery, 18*(2), 104-109. [DOI:10.1177/1098612X15574757] [PMID]

Duthie, S., Eckersall, P. D., Addie, D. D., Lawrence, C. E., & Jarrett, O. (1997). Value of α1-acid glycoprotein in the diagnosis of feline infectious peritonitis. *The Veterinary Record, 141*(12), 299–303. [DOI:10.1136/vr.141.12.299] [PMID]

Farsijani, F., Safi, S., & Shirazi Beheshtiha, S. H. (2023). Comparison of the performance of bioresonance, electrophoresis, and RT-PCR in the diagnosis of feline infectious peritonitis. *Archives of Razi Institute, 78*(3), 1077–1085. [DOI:10.22092/ARI.2023.360790.2606]

Felten, S., & Hartmann, K. (2019). Diagnosis of feline infectious peritonitis: A review of the current literature. *Viruses, 11*(11), 1068. [DOI:10.3390/v11111068] [PMID]

Felten, S., Weider, K., Doenges, S., Gruendl, S., Matiasek, K., & Hermanns, W., et al. (2017). Detection of feline coronavirus spike gene mutations as a tool to diagnose feline infectious peritonitis. *Journal of Feline Medicine and Surgery, 19*(4), 321-335. [DOI:10.1177/1098612X15623824] [PMID]

Gamble, D. A., Lobbiani, A., Gramegna, M., Moore, L. E., & Colucci, G. (1997). Development of a nested PCR assay for detection of feline infectious peritonitis virus in clinical specimens. *Journal of Clinical Microbiology, 35*(3), 673-675. [DOI:10.1128/jcm.35.3.673-675.1997] [PMID]

Hartmann, K., Binder, C., Hirschberger, J., Cole, D., Reinacher, M., & Schroo, S., et al. (2003). Comparison of different tests to diagnose feline infectious peritonitis. *Journal of Veterinary Internal Medicine, 17*(6), 781-790. [DOI:10.1111/j.1939-1676.2003.tb02515.x] [PMID]

Herrewegh, A. A. P. M., Egberink, H. F., Horzinek, M. C., Rottier, P. J. M., & De Groot, R. J. (1995). Polymerase chain reaction (PCR) for the diagnosis of naturally occurring feline coronavirus infections. *Feline Practice. 23*(3), 56-60. [Link]

Kennedy, M. A. (2020). Feline infectious peritonitis: Update on pathogenesis, diagnostics, and treatment. *The Veterinary Clinics of North America. Small animal practice, 50*(5), 1001–1011. [DOI:10.1016/j.cvsm.2020.05.002] [PMID]

Lorusso, E., Mari, V., Losurdo, M., Lanave, G., Trotta, A., & Dowgier, G., et al. (2019). Discrepancies between feline coronavirus antibody and nucleic acid detection in effusions of cats with suspected feline infectious peritonitis. *Research in Veterinary Science, 125,* 421-424. [DOI:10.1016/j.rvsc.2017.10.004] [PMID]

Mohammed Ibrahim, O., Bara Allawe, A., & Ali Kadhim, H. (2022). Isolation and molecular detection of feline infectious peritonitis virus. *Archives of Razi Institute, 77*(5), 1709-1714. [DOI:10.22092/ARI.2022.357997.2135]

Avizeh, R., Mosallanejad, B. and Seyfiabad Shapouri, M. (2012). Antibody detection of feline infectious peritonitis virus (FIPV) in sera of companion cats in Ahvaz, southwest of Iran. *Archives of Razi Institute, 67*(1), 69-74. [DOI:10.22092/ARI.2016.103890]

Paltrinieri, S., Comazzi, S., Spagnolo, V., & Giordano, A. (2002). Laboratory changes consistent with feline infectious peritonitis in cats from multicat environments. *Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine, 49*(10), 503–510. [DOI:10.1046/j.1439-0442.2002.00494.x] [PMID]

Paltrinieri, S., Giordano, A., Stranieri, A., & Lauzi, S. (2021). Feline infectious peritonitis (FIP) and coronavirus disease 19 (COVID-19): Are they similar? *Transboundary and Emerging Diseases, 68*(4), 1786-1799. [DOI:10.1111/tbed.13856] [PMID]

Pedersen, N. C. (2009). A review of feline infectious peritonitis virus infection: 1963-2008. *Journal of Feline Medicine and Surgery, 11*(4), 225-258. [DOI:10.1016/j.jfms.2008.09.008] [PMID]

Li, C., Liu, Q., Kong, F., Guo, D., Zhai, J., Su, M., & Sun, D. (2019). Circulation and genetic diversity of Feline coronavirus type I and II from clinically healthy and FIP-suspected cats in China. *Transboundary and Emerging Diseases, 66*(2), 763-775. [DOI:10.1111/tbed.13081] [PMID]

Lutz, M., Steiner, A. R., Cattori, V., Hofmann-Lehmann, R., Lutz, H., & Kipar, A., et al. (2020). FCoV viral sequences of systemically infected healthy cats lack gene mutations previously linked to the development of FIP. *Pathogens, 9*(8), 603. [DOI:10.3390/pathogens9080603] [PMID]

Mojtahedzadeh, S. M., Jamshidi, S., Ghalyanchi Langroudi, A., Vahedi, S. M., Ashrafi Tamai, I., & Akbarein, H., et al. (2024). Molecular detection of canine distemper virus among dogs showing neurologic and non-neurologic forms of disease. *Iranian Journal of Veterinary Medicine, 18*(2), 203-214. [DOI:10.32598/IJVM.18.2.1005294]

Myrrha, L. W., Silva, F. M. F., Vidigal, P. M. P., Resende, M., Bressan, G. C., & Fietto, J. L. R., et al. (2019). Feline coronavirus isolates from a part of Brazil: insights into molecular epidemiology and phylogeny inferred from the 7b gene. *The Journal of Veterinary Medical Science, 81*(10), 1455–1460. [DOI:10.1292/jvms.19-0090] [PMID]

Ramezanpour Eshkevari, S., Sasani, F., Shokrpoor, S., Mardjanmehr, S. H., Akbarein, H., & Ashrafi, I. (2024). A histopathological study on the changes in the central nervous system of dead cats with neurological symptoms. Iranian *Journal of Veterinary Medicine, 18*(4), 545-554 [DOI: 10.32598/ijvm.18.4.1005436]

RRasooli, A., Nouri, M., Seyfi Abad Shapouri, M. R., Mohseni-Parsa, S., Baghbanian, H. R., & Lotfi, M., et al. (2023). Serological detection of SRMV, BVDV, BHV-1and BEFV in camels (Camelus dromedarius) in southwest Iran. *Iranian Journal of Veterinary Medicine, 17*(2) 139-147. [DOI:10.32598/ijvm.17.2.1005239]

Rohrbach, B. W., Legendre, A. M., Baldwin, C. A., Lein, D. H., Reed, W. M., & Wilson, R. B. (2001). Epidemiology of feline infectious peritonitis among cats examined at veterinary medical teaching hospitals. *Journal of the American Veterinary Medical Association, 218*(7), 1111-1115. [DOI:10.2460/javma.2001.218.1111] [PMID]

Sangl, L., Matiasek, K., Felten, S., Gründl, S., Bergmann, M., & Balzer, H. J., et al. (2019). Detection of feline coronavirus mutations in paraffin-embedded tissues in cats with feline infectious peritonitis and controls. *Journal of Feline Medicine and Surgery, 21*(2), 133-142. [DOI:10.1177/1098612X18762883] [PMID]

Shelly, S. M., Scarlett-Kranz, J., & Blue, J. T. (1988). Protein electrophoresis on effusions from cats as a diagnostic test for feline infectious peritonitis. *Journal of the American Animal Hospital Association, 24*(5), 495-500. [Link]

Tasker, S. (2018). Diagnosis of feline infectious peritonitis: Update on evidence supporting available tests. *Journal of Feline Medicine and Surgery, 20*(3), 228-243. [DOI:10.1177/1098612X18758592] [PMID]

Tekes, G., & Thiel, H. J. (2016). Feline coronaviruses: Pathogenesis of feline infectious peritonitis. *Advances in Virus Research, 96*, 193-218. [DOI:10.1016/bs.aivir.2016.08.002] [PMID]

مقاله پژوهشی

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

شهرام جمشیدی[1]، فرنوش مومنی[1]، ایرج اشرافی تمای[2]، *امید مددگار[2]

۱. گروه علوم بالینی دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران.
۲. گروه میکروبیولوژی و ایمنولوژی، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران.

Use your device to scan and read the article online

How to Cite This Article Jamshidi, Sh., Momeni, F., Ashrafi Tamai, I., & Madadgar, O. (2025). Molecular and Clinical Study of Feline Infectious Peritonitis Virus in Iran Showing a Paraphyletic Tree: Emphasizing the "Internal Mutation" Hypothesis. *Iranian Journal of Veterinary Medicine, 19*(2), 179-190. http://dx.doi.org/10.32598/ijvm.19.2.1005247

http://dx.doi.org/10.32598/ijvm.19.2.1005247

چکیده

زمینه مطالعه: پریتونیت عفونی گربه‌ها (FIP) یک بیماری شدید و اغلب کشنده است که گونه‌های گربه ها را در سطح جهان مبتلا می کند. علیرغم شیوع بالای عفونت‌های ویروس کرونا (FCoV)، تظاهرات FIP تنها در درصد کمی (۵-۱ ٪) 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 ویروس FIP، با تمرکز خاص بر سطح ژن M است.

روش کار: روش ما بررسی مایعات شکمی یا سینه‌ای از ۱۷ گربه مشکوک به FIP، با استفاده از آزمایش‌های بیوشیمیایی مانند پروتئین کل سرم، نسبت آلبومین به گلوبولین (A/G) و تست ریوالتا بود. علاوه بر این، RT-PCR بر 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.

نتایج: تست ریوالتا FIP را در ۶ گربه از ۱۷ گربه تایید کرد. وقوع FIP در میان گربه های جوان (۹-۳۰ ماهه) در نرها دو برابر بیشتر از ماده ها بود. گربه های مبتلا در محدوده سنی ۳۰-۹ ماهه نسبت A/G کمتر از ۰/۶۶ و کل پروتئین سرم بیش از ۰/۴۳ 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 پروتئین M، تفاوت های ناچیزی در توالی های نوکلئوتیدی FECoV و FIPV، 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 FECoV و FIPV وجود ندارد. این یافته‌ها به شناخت پاتوژنز FIP کمک می‌کنند و رویکردهای تشخیصی و درمانی آینده را هدایت می‌کنند.

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

تاریخ دریافت: ۲۰ دی ۱۴۰۳
تاریخ پذیرش: ۶ تیر ۱۴۰۳
تاریخ انتشار: ۱۲ فروردین ۱۴۰۴

* **نویسنده مسئول:**
دکتر امید مددگار
نشانی: تهران، دانشگاه تهران، دانشکده دامپزشکی،گروه میکروبیولوژی و ایمونولوژی.
تلفن: ۵۸۴۵-۵۰۳ (۰۵۶) ۱+
رایانامه: *omadadgar@gmail.com*

This Page Intentionally Left Blank