RESEARCH ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2025.2024-136
Cyprus J Med Sci 2025;10(Suppl 1):1-3

Development of a qRT - PCR Diagnostic Kit for the Detection of FCoV

Gülten Tuncel Dereboylu[1], Gökçe Akan[1], Melis Kalaycı[1], Cenk Serhan Özverel[1,2], Tamer Şanlıdağ[1]

[1]DESAM Research Institute, Near East University, Nicosia, North Cyprus
[2]Department of Basic Medical Sciences, Near East University Faculty of Dentistry, Nicosia, North Cyprus

Abstract

BACKGROUND/AIMS: In recent years, feline infectious peritonitis (FIP), which led to the death of more than 3,000 cats on the island of Cyprus, has become a significant concern. FIP is a fatal disease in cats caused by feline coronavirus (FCoV). Due to its rapid spread among cats, its potential to pose a pandemic threat, and its presentation of symptoms similar to other diseases, accurate and rapid diagnostic methods are essential.

MATERIALS AND METHODS: This study reports the development of a novel quantitative reverse-transcription polymerase chain reaction (qRT-PCR) diagnostic kit specifically designed to detect the presence of FCoV-specific nucleic acid in anal/fecal swab samples. Sequence-specific primer and probe sequences were designed and optimized to specifically target FCoV membrane and *open reading frame 7* genes. The kit was evaluated using clinical samples from suspected FCoV cases.

RESULTS: Designed kit demonstrated high sensitivity and specificity on tested samples. Target genes in all FCoV-positive samples were successfully amplified with no false-positive or false-negative results.

CONCLUSION: Our findings indicate that the designed qRT-PCR kit provides a reliable tool for the rapid and early diagnosis of FCoV infection in cats, thereby aiding in clinical decision-making as well as disease management.

Keywords: Feline coronavirus, FCoV, FIP, qRT-PCR

INTRODUCTION

Feline infectious peritonitis (FIP) is a severe, often fatal systemic disease caused by a virulent strain of feline coronavirus (FCoV).[1] FCoV is an enveloped, positive-sense single-stranded RNA virus belonging to the Coronaviridae family. The FCoV genome is approximately 29.4 kb in length and consists of several open reading frames (ORFs) that encode structural proteins, including the spike (S), envelope, membrane (M), and nucleocapsid proteins, as well as non-structural proteins, which are involved in viral replication and pathogenesis.[2] The *S protein* gene has been reported as the main factor responsible for the adhesion of the virus to the cells that it will attack. The other important region in the genome of FCoV is the ORF, which encodes non-structural proteins.[3] It is reported that non-structural proteins are responsible for viral replication and transcription. FCoV is reported as highly contagious. It can spread rapidly among cats, particularly in multi-cat environments such as shelters and catteries.[4,5] In view of its fast transmission rate in cat populations, the importance of early and accurate detection is crucial for proper disease management and prevention of possible outbreaks.[5] FCoV is mainly transmitted by fecal-oral transmission, where infected cats excrete the virus in their feces or semen.[6] Close contact with an infected cat, shared litter boxes, or contaminated environments can also be listed as a transmission route for FCoV.

To cite this article: Tuncel Dereboylu G, Akan G, Kalaycı M, Özverel CS, Şanlıdağ T. Development of a qRT - PCR diagnostic kit for the detection of FCoV. Cyprus J Med Sci. 2025;10(Suppl 1):1-3

ORCID IDs of the authors: G.T.D. 0000-0002-3332-471X; G.A. 0000-0002-3878-1135; M.K. 0000-0002-5779-5532; C.S.Ö. 0000-0001-9932-4774; T.Ş. 0000-0001-9932-4774.

Corresponding author: Tamer Şanlıdağ
E-mail: tamer.sanlidag@neu.edu.tr
ORCID ID: orcid.org/0000-0002-6015-8032

Received: 10.12.2024
Accepted: 19.01.2025
Publication Date: 04.06.2025

Current testing methods for FCoV rely on antigen testing. However, this method carries a high risk of cross-reactivity, especially with other viruses from the same family, causing false-positive results. Also, antigen tests have lower sensitivity compared to amplification-based polymerase chain reaction (PCR) technology. In this study, we present a novel quantitative reverse-transcription (qRT)-PCR diagnostic kit specifically designed to detect FCoV nucleic acids from anal/fecal swab samples. The kit targets the M and ORF genes, which are known to be crucial for viral replication and indicate the presence of the virus.[7] By utilizing highly specific primer and probe sets, this PCR kit aims to provide a sensitive and accurate tool for early diagnosis of FCoV.

MATERIALS AND METHODS

Sample Collection and RNA Isolation

By veterinary specialists, a total of 36 blind clinical samples, including anal/fecal swabs, were collected from cats with and without clinical signs of FIP. Near East University Animal Experiments Local Ethics Committee granted the ethical approval for the study (approval number: 2024/192-192, date: 19/12/2024). Control samples were also obtained from healthy cats. RNA isolation from anal swab samples was performed using the commercially available kit, A1 LifeSciences-Diagnovital RTA Viral RNA Isolation Kit (İstanbul, Türkiye), according to the manufacturer's instructions.

Primer and Probe Design

Primers and probes were designed to specifically target the M and *ORF7 protein* genes of the FIP-associated strain of FCoV. The sequences were obtained from the *National Center for Biotechnology Information (NCBI)* gene database. Primer sets were designed using SnapGene and NCBI basic local alignment search tool (BLAST) software. After performing BLAST analysis to ensure that the primers would not result in off-target amplification, had appropriate melting temperatures and no self-annealing risk, the appropriate primer sets were selected.

Polymerase Chain Reaction Assay Development

The PCR assay was developed using a standard protocol with minor modifications to increase sensitivity. The reaction mix included primer and probe sets, PCR mastermix with reverse transcriptase, and extracted RNA. Thermal cycling conditions consisted of an initial reverse transcription step at 55 °C for 15 minimum, followed by an initial denaturation step at 95 °C for 30 30 seconds (sec), then 40 cycles at 95 °C for 10 sec and 58 °C for 30 sec. Fluorescence signals were obtained at Cy5 and FAM channels. The assay was validated using a panel of known FCoV-positive and negative samples. Sensitivity was checked by serial dilution of the target RNA, and specificity was confirmed through in-silico testing against over 30 viral genomes, 20 bacterial genomes, and 10 parasite genomes, along with the feline and Homo sapiens genomes.

RESULTS

The PCR assay successfully amplified the target gene in all FCoV-positive samples, with no amplification observed in negative controls or samples from cats with antigen-negative results (Figure 1). Specificity testing showed no cross-reactivity with non-pathogenic FCoV or other feline pathogens, highlighting the assay's robustness and accuracy.

Among the tested samples, 18 were found to be PCR positive, of which 13 indicated active infection and 5 suggested past infection.

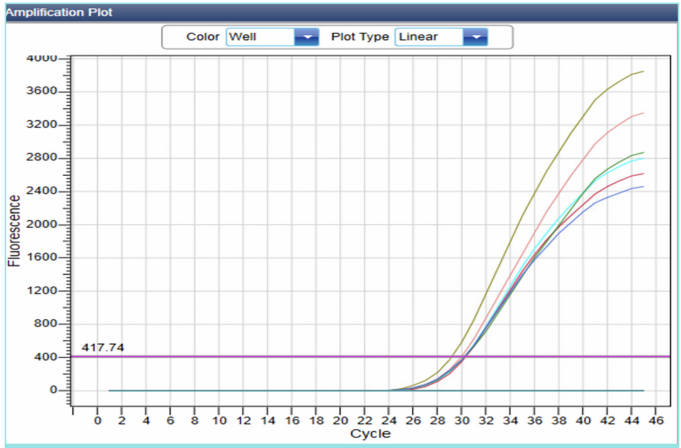

Figure 1. Amplification plot results of FCoV-positive samples.
FCoV: Feline coronavirus.

These findings were consistent with the clinical symptoms observed and antigen results. The kit's performance was further evaluated by comparing its results with serological tests, showing a concordance rate of 100% with serology tests.

DISCUSSION

The ability to diagnose FCoV infections with high specificity and accuracy carries great importance for both clinical and epidemiological purposes since the virus can transmit easily among feline populations worldwide.[4,8] Conventional diagnostic methods, such as serological testing, have notable limitations, including delayed results and a risk of false positives or negatives.[9] A major limitation of this study is the relatively small sample size. Further validation studies of the performance of the kit on a larger feline sample size are necessary. One of the key strengths of this qRT-PCR kit is its use of primers and probes designed specifically to target the genes for FCoV.[8,9] The specificity of this diagnostic method was confirmed not only through laboratory testing but also through in silico analysis against a wide range of viral, bacterial, and parasitic genomes, as well as the feline genome, thus ensuring no cross-reactivity with non-pathogenic strains of FCoV or other common feline pathogens.

The results obtained from clinical samples in this study also emphasize the kit's diagnostic effectiveness. Among the 36 tested samples, the qRT-PCR assay identified 13 active infections and 5 past infections in cats. These findings were consistent with the clinical signs and antigen test results, confirming the kit's effectiveness in identifying not only active infections but also previous exposures to FCoV on account of the designed primers and probes. This capability is especially valuable for disease surveillance in multi-cat environments, such as streets or shelters, where rapid identification and isolation of infected cats are essential for controlling possible outbreaks.[4,8] From a research perspective, this diagnostic kit study can be a pioneering study for future research and will help in understanding the molecular mechanism of FcoV.[4]

Study Limitations

The main limitation of this study was the small sample size, which may affect the generalizability of the findings; future research could address this by including a larger and more diverse sample.

CONCLUSION

Development of an in-house qRT-PCR kit for the detection of FCoV is an important step for the field of veterinary diagnostics. The kit is designed to specifically target the viral genes which are M and ORF7. Primer and probe sets are designed to ensure high specificity and sensitivity. During optimization studies, the detection performance of the in-house designed kit has been studied with clinical samples suspected for FCoV. Importantly, the results were in concordance with the serological test results. The results prove that the designed kit can be used as a reliable diagnostic tool. Future studies will focus on optimizing the kit for routine use and exploring its applicability across diverse clinical settings.

MAIN POINTS

- The designed diagnostic kit provides a valuable tool for the early and accurate detection of feline coronavirus (FCoV).
- The kit offers rapid and reliable results.
- It is aimed to be prepared for a possible pandemic by targeting the FCoV which can transmit rapidly.
- Sensitivity tests were carried out in comparison with serological tests and completely compatible results were obtained.

ETHICS

Ethics Committee Approval: Near East University Animal Experiments Local Ethics Committee granted the ethical approval for the study (approval number: 2024/192-192, date: 19/12/2024).

Informed Consent: Not available.

Footnotes

Acknowledgements

The authors would like to extend their sincere gratitude to Near East University Animal Hospital and Hospital Veterinary Clinic for providing clinical samples crucial for the completion of this project.

Authorship Contributions

Concept C.S.Ö., T.Ş., Design: G.T.D., G.A., M.K, Data Collection and/or Processing: G.T.D.,, G.A., M.K, Analysis and/or Interpretation: G.D.T., G.A., M.K, Literature Search: G.T.D., G.A., M.K, Writing: G.T.D., G.A., M.K.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: Near East University.

REFERENCES

1. Sherding RG. Feline Infectious Peritonitis (Feline Coronavirus). Saunders Manual of Small Animal Practice. 2009; 132-43.
2. Lutz M, Steiner AR, Cattori V, Hofmann-Lehmann R, Lutz H, Kipar A, et al. FCoV viral sequences of systemically infected healthy cats lack gene mutations previously linked to the development of FIP. Pathogens. 2020; 9(8): 603.
3. Jaimes JA, Whittaker GR. Feline coronavirus: insights into viral pathogenesis based on the spike protein structure and function. Virology. 2018; 517: 108-21.
4. Gao YY, Wang Q, Liang XY, Zhang S, Bao D, Zhao H, et al. An updated review of feline coronavirus: mind the two biotypes. Virus Res. 2023; 326: 199059.
5. Sykes JE. Feline Coronavirus infection. Canine and Feline Infectious Diseases. 2013; 195-208.
6. Stranieri A, Probo M, Pisu MC, Fioletti A, Meazzi S, Gelain ME, et al. Preliminary investigation on feline coronavirus presence in the reproductive tract of the tom cat as a potential route of viral transmission. J Feline Med Surg. 2020; 22(2): 178-85.
7. Pedersen NC. An update on feline infectious peritonitis: diagnostics and therapeutics. Vet J. 2014; 201(2): 133-41.
8. Roman, N. (2024, January 8). Feline infectious peritonitis. MSD Veterinary Manual. https://www.msdvetmanual.com/generalized-conditions/feline-infectious-peritonitis/feline-infectious-peritonitis
9. Sharif S, Arshad SS, Hair-Bejo M, Omar AR, Zeenathul NA, Alazawy A. Diagnostic methods for feline coronavirus: a review. Vet Med Int. 2010; 2010: 809480.