

# Feline Calicivirus Prevalence among Cats in Turkey's Kayseri Province

Abayli, H.,<sup>1\*</sup> Can-Sahna, K.,<sup>1</sup> Ozbek, R.<sup>1</sup> and Karaca Bekdik, I.<sup>2</sup>

<sup>1</sup> Department of Virology, Faculty of Veterinary Medicine, Firat University, Elazig, Turkey.

<sup>2</sup> Department of Internal Medicine, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey.

\* **Corresponding Author:** Dr. H. Abayli, Department of Virology, Faculty of Veterinary Medicine, Firat University, 23110 Elazig, Turkey.  
Contact number: +904242370000-3993, Email: habayli@firat.edu.tr.

## ABSTRACT

Feline calicivirus (FCV) is a highly contagious pathogen, which is common in the cat population. The present study investigated the prevalence of FCV and potential risk factors for infection in the cat population in Turkey's Kayseri province. The study group comprised clinically healthy cats ( $n=67$ ) and cats displaying signs of FCV infection ( $n=26$ ). The animals varied in term of breed, age, gender, vaccination status, and housing status (shelter vs. outdoor). Swab samples were obtained and tested for FCV using a commercial enzyme-linked immunosorbent assay (ELISA) kit and semi-nested reverse-transcription polymerase chain reaction (RT-PCR) assay with a new primer design. From a total of 93, ten samples were positive for FCV according to the ELISA, and 18 samples were positive according to the semi-nested RT-PCR test. The positivity rate was found to be higher in semi-nested RT-PCR than ELISA. The overall prevalence of FCV infection was 19.35% (18/93). A risk factor analysis revealed that breed, age, gender, housing status and vaccination status were not risk factors for FCV. There was a statistically significant difference only in clinically healthy cats and symptomatic cats ( $p < 0.001$ ). The study revealed the presence of FCV in both asymptomatic cats and vaccinated cats. The semi-nested RT-PCR with newly designed primers may be beneficial for researchers.

**Keywords:** Feline Calicivirus; Semi-Nested RT-PCR; ELISA; Turkey

## INTRODUCTION

Feline calicivirus (FCV) is a single-stranded, positive-sense RNA virus, which belongs to the Vesivirus genus of the Calicivirus family (1). FCV is a highly contagious pathogen, which is common in the cat population worldwide (2). FCV usually infects animals aged 6–84 days, with the prevalence of infection generally highest among kittens aged 56–84 days (3). FCV is spread through oral, ocular, and nasal secretions and is transferred via direct contact with secretions of acutely infected and carrier cats (4-5). In treated cats, virus shedding may continue for a number of days (30 days) or a number of years (2). As the survival of the FCV is relatively short lived outside the host, the environment is rarely a source of long-term infection (3). As the tropism and virulence of FCV varies between strains, infection is accompanied by a large spectrum of clinical symptoms (oral

ulcers, chronic stomatitis, limping syndrome, conjunctival signs, etc.) and respiratory symptoms (6-7). Previous research demonstrated the presence of FCV-like viruses in dogs, which indicates a potential risk for interspecies transmission (8).

Although FCV has a single serotype and genotype, different variants resulting from genetic diversity lead to severe and acute virulent systemic diseases. Mortality of up to 50–60% has been reported following infection with highly virulent FCV strains (9-10). Most attenuated or inactivated vaccines do not prevent re-infection by homologous or heterologous FCV variants. Therefore, the prevalence of FCV remains high among cats in the same household or cats housed in shelters (11).

For FCV detection, conjunctival swabs, oral swabs, blood samples, skin smears, or lung tissue samples are tested using

reverse-transcription polymerase chain reaction (RT-PCR), nested PCR, and quantitative PCR methods (12-13).

The present study aimed to investigate FCV prevalence-related risk factors and to compare the sensitivity of a commercial enzyme-linked immunosorbent assay (ELISA) and semi-nested RT-PCR method with a new primer design in the diagnosis of the disease.

## MATERIAL AND METHODS

### Study group

The study was conducted among cats in Turkey's Kayseri province. The study group comprised 26 cats displaying signs of FCV infection and 67 clinically healthy cats. Samples were collected between December 2017 and March 2018 from cats in the animal hospital of the Veterinary Faculty of Erciyes University and in from the municipal animal shelter of Kayseri. The following clinical signs were recorded: nasal and/or ocular discharge, conjunctivitis, gingivitis, oral ulcerations, and edema. Demographic data (age, gender, and breed), housing status (household, n=0, shelter, n=67 or outdoor, n=26), and vaccination history were recorded for each animal. Oral, nasal and conjunctival swabs were obtained from all cats for laboratory diagnosis.

### Preparation of clinical samples

The clinical swab samples were re-suspended in 1 ml of phosphate-buffered saline and stored at -80°C until the ELISA and RNA isolation. Subsequently, the samples were thawed at room temperature. The freezing and thawing procedures were repeated three times. The pellet was then suspended by centrifugation at 400× g for 10 min. The obtained supernatant was used in the ELISA and RNA isolation.

### ELISA

A commercial ELISA kit (Eastbiopharm, Hangzhou, China) was used for the detection of the FCV antigen in swab samples. The test was performed according to the instructions of the manufacturer.

### RNA isolation from clinical swab samples

Total RNA isolation from the supernatants of the clinical samples was performed using GENEzol™ reagent (Geneaid Bio., New Taipei City, Taiwan) according to the manufacturer's instructions.

### Primer design and reverse transcription

First, multiple alignments of the full genome sequences of FCV strains from different geographic regions stored in Genbank were performed with DNA baser software (Heracle Biosoft, Arges, Romania). Subsequently, highly conserved regions were detected within the aligned nucleotide sequences. These regions were then used for primer design using the PrimerQuest® Tool (Integrated DNA Technologies, Iowa, USA). Appropriate internal and external primers were created taking into account melting temperatures and specificity. Primers were tested with BLASTN (<http://www.ncbi.nlm.nih.gov>). The designed primers were synthesized at the Macrogen Company (Seoul, South Korea). The synthesized primers (FCV-P1, FCV-P2, and FCV-P3) are presented in Table 1. These primers and total RNA from oral swabs were used in cDNA synthesis. This step was carried out following the instructions for use of the M-MLV reverse transcriptase kit (Promega, Wisconsin, USA). The reaction mixture containing 1µg of total RNA, 2 pmol of external reverse primer (FCV-P3 primer) and 5µl of ddH2O was first incubated at 70°C for 10min, kept on ice for 3 minutes before adding 5µl of 5 × M-MLV buffer, 2 µl of dNTP mix (10mM of each), 200 U of M-MLV reverse transcriptase. The reaction was conducted at 42°C for 1hour at 70°C for 15 min and terminated the reaction at 4°C.

**Table 1:** Primers used in the semi-nested PCR step and their sequences.

Primers	Nucleotide sequences (5'→3')	Region	Product sizes
FCV-P1	ATGTCTCAAACCTCTGAGCTTC	ORF-1 polyprotein	-
FCV-P2	TGTGAGCTGTTCTTTGCACA	"	396
FCV-P3	ATTGGGTTGTATGATTGCAT	"	1347

### Polymerase Chain Reaction (PCR) and Semi-nested PCR

First-round PCR and semi-nested PCR steps were carried out on the GenAmp 9700 thermal cycler (Thermo Fisher, Massachusetts, USA). In total, 50 µL of the mixture [5 µL of cDNA, 5 µL dNTP mix (of each, 10 mM), 4 µL of FCV-P1 and FCV-P3 primer mixture (20 pmol), 5 µL of KCL added 10× TaqBuffer, 1 unit of Taq DNA polymerase enzyme (Hibrogen, Istanbul, Turkey), and the remaining RNAase-free

water] was prepared for first-round PCR. The amplification parameters were as follows: 3 min of pre-denaturation at 94°C and then 15 sec at 94°C, 30 sec at 52°C, and 90 sec at 72°C for 30 cycles, with a final step of 5 min at 72°C.

For semi-nested PCR, the PCR amplification product obtained at the end of the first-round PCR was used as the template (2 µL). In total, 50 µL of the mixture [5 µL dNTP mix (of each, 10 mM), 4 µL of FCV-P1 and FCV-P2 primer mixture (20 µmol), 5 µL of KCL added 10× TaqBuffer, 1 unit of Taq DNA polymerase enzyme (Hibrogen, Istanbul, Turkey), and the remaining RNAase-free water] was prepared for semi-nested PCR. The amplification parameters were as follows: 15 sec at 94°C, 30 sec at 52°C, 30 sec at 72°C for 35 cycles, followed by 5 min at 72°C. Agarose gel (1.5%) stained with ethidium bromide was visualized after electrophoresis (110 V, 40 min).

### Sequencing

Bidirectional sequence analysis of amplification products of the expected size (396 bp) after semi-nested PCR was performed at the Macrogen Company. In this step, ABI3730XL sanger sequencing device (Applied Biosystems, California, USA), FCV-P1 and FCV-P2 primers and BigDye Terminator v.3.1 cycle sequencing kit were used. The sequence data of all PCR products were compared to the sequence data in Genbank using BLASTN (<http://www.ncbi.nlm.nih.gov>) software and were found to be almost identical to FCV. Two of the sequence data were stored in Genbank. The amino acid sequence of FCV strains in this study was

compared to that of other FCV strains in Genbank using Mega X software (14).

### Statistical analysis

The data were analyzed using the SPSS 21 package program (15). Each parameter was expressed as a percentage. A chi-square test was used for comparison of the data, and a value of  $p \leq 0.05$  was accepted as statistically significant.

## RESULTS

FCV was detected in 10 (10.75%) of the 93 samples according to the ELISA test performed on swab samples and in 18 (19.35%) of the 93 samples according to the semi-nested RT-PCR results. All of the ELISA positive samples were also positive by semi-nested RT-PCR. According to the semi-nested RT-PCR results, FCV-positive samples were confirmed by sequencing. There was no significant difference between the two tests in terms of the number of positive samples ( $p > 0.05$ ).

Figure 1 presents an image of the agarose gel electrophoresis semi-nested RT-PCR products. The band size (396 bp) obtained for the FCV vaccine (Nobivac® Tricat Trio) sample used as a positive control was similar to that of the other samples.

The distributions of breed, sex, and age based on FCV-positive samples detected by the semi-nested RT-PCR are presented in Table 2. Although the distributions differed, the differences were not statistically significant ( $p > 0.05$ ).

**Table 2:** The distribution of breed, sex, and age in FCV-positive cats detected by the semi-nested PCR.

Breed	+/-	%	Age (years)	+/-	%	Gender	+/-	%
Tekir	12/59	16.90	1	15/50	23.07	Female	9/42	17.64
Sarman	1/10	9.09	2	0/6	0	Male	9/33	21.42
Angara	2/2	50.00	3	2/7	22.22			
Siamese	1/1	50.00	4 and older	1/12	7.69			
Mixed	2/3	40.00						
<sup>a</sup> 0.200			<sup>b</sup> 0.192			<sup>c</sup> 0.646		

a, b, c represent  $p$  statistical values

**Table 3:** Clinical, vaccination, and housing status of FCV-positive cats detected by the semi nested-PCR.

Signs of FCV	+/-	%	Vaccination status	+/-	%	Housing status	+/-	%
Yes	11/15	42.30	Vaccinated	3/10	23.07	Shelter	13/54	19.40
No	7/60	10.44	Unvaccinated	15/65	18.75	Outdoor	5/21	20.00
<sup>a</sup> 0.001			<sup>b</sup> 0.714			<sup>c</sup> 0.257		

a, b, c represent  $p$  statistical values



PCR-based studies reported a prevalence of FCV in 2.60% of household cats in Sweden and 28.10% of shelter cats in California, US (22-23). In another study, FCV infection was reported in 29.00% of asymptomatic cats in nine European countries (24). The prevalence of FCV infection in the present study was 23.08% (3/13) in vaccinated animals and 19.23% (15/78) in non-vaccinated animals. As there was no statistically significant difference in the prevalence of FCV infection in vaccinated versus unvaccinated animals. Vaccination status (i.e., non-vaccination) was not considered a risk factor. This finding was consistent with that of previous studies in China (3).

The FCV vaccine cannot prevent re-infection due to the genetic variability of the virus. These results lead to a discussion of preferred FCV strains in vaccines. According to previous reports, FCV infection is common among both indoor (household cats and cats in shelters) and outdoor (feral) cats in Iranian (25). In the present study, we investigated whether the housing type was a risk factor for FCV infection. The prevalence of FCV infection among cats housed in shelters was 19.40% (13/67), whereas it was 19.23% (5/26) among outdoor cats, with no statistically significant difference between the groups (Table 3).

In common with the findings on housing type, the results showed that breed, age, and gender were not risk factors for infection. According to the chi-square test, there was no statistically significant difference in the distribution of breed, sex, and gender based on FCV-positive samples detected by the semi-nested RT-PCR ( $p > 0.05$ ) (Table 2). Although the prevalence of FCV varied between 0% and 23.07% in cats in different age groups, the difference was not statistically significant ( $p > 0.05$ ). The highest prevalence was observed in the 1-year-old age group (23.07%). Similarly, Wang *et al.* (2017) reported that the prevalence of FCV varied between 28.61% and 38.82% in cats in different age groups in China (3). In the same study, the highest prevalence of FCV infection was found in cats aged  $\leq 1$  y (38.82%), but the finding was not statistically significant. The immune system of older cats is weaker. The lower immune status in this age group as compared with that of younger cats may explain the higher rate of FCV infection. The influence of stressful events, such as vaccination rehousing and castration, may also play a role (3).

In the present study, we also compared the diagnostic sensitivity of ELISA and semi-nested RT-PCR techniques for FCV. The number of positive samples detected with the semi-nested RT-PCR and ELISA techniques differed. As the genetic variability of FCV may lead to failure in PCR amplification (2), the primers used in the semi-nested RT-PCR step were prepared by selecting conserved regions (ORF-1) of the FCV genome. According to the semi-nested RT-PCR results, FCV-positive samples were confirmed by sequencing. Despite the absence of a statistically significant difference, we consider that semi-nested RT-PCR was more sensitive than ELISA due to the higher number of positive samples detected using the semi-nested RT-PCR technique. This technique may also be employed as an alternative to (ELISA) in small-scale laboratory studies or large-scale prevalence studies.

Previous research indicated that genetically different FCV strains were in circulation in various regions and that there was no strong correlation between a particular sequence type and clinical manifestations of the disease (26). The high diversity of the FCV genome poses a challenge in terms of vaccine effectiveness and combating the disease. Future genomic sequencing and molecular characterization studies are needed to reveal the full genome sequence of FCV strains circulating in Turkey. The presence of whole-genome sequences may provide a reference for future epidemiological studies on FCV.

In conclusion, the prevalence of FCV among cats in Turkey's Kayseri province in the present study was 10.75% and 19.35% using the ELISA and semi-nested RT-PCR, respectively. Of note, FCV was detected in both clinically healthy cats and vaccinated cats. To provide protection against FCV, vaccines prepared from circulating strains in the region should be preferred, and diverse strains should be utilized in vaccine preparation. The semi-nested PCR method applied in the present study detected more positivity than the ELISA, and this method has the potential to be used in prevalence studies.

---

#### ACKNOWLEDGMENTS

We are grateful for the contribution of Dr. Ozgur Aslan, a member of the Internal Medicine Department of the Veterinary Faculty of Erciyes University.

---

## REFERENCES

- Seal, B.S., Ridpath, J.F. and Mengeling, W.L.: Analysis of feline calicivirus capsid protein genes: identification of variable antigenic determinant regions of the protein. *J. Gen. Virol.* 74:2519-2524, 1993. DOI: 10.1099/0022-1317-74-11-2519
- Radford, D.A., Addie, D., Belak, S., Boucraut-Baralon, C., Egbering, H., Frymus, Gruffydd-Jones T., Hartmann, K., Hosie, M.J., Lloret, A., Lutz, H., Marsilio, F., Pennisi, M.G., Thiry, E., Truyen, U. and Horzinek, M.C.: Feline calicivirus infection: ABCD guidelines on prevention and management. *J. Feline Med. Surg.* 11:556-564, 2009. DOI: 10.1016/j.jfms.2009.05.004
- Wang, K., Pei, Z. and Hu, G.: First report of Feline Calicivirus (FCV) infection in stray cats in northeast China. *Pol. J. Vet. Sci.* 20:595-598, 2017. DOI: 10.1515/pjvs-2017-0072
- Coyne, K.P., Gaskell, R.M., Dawson, S., Porter, C.J. and Radford, A.D.: Evolutionary mechanisms of persistence and diversification of a calicivirus within endemically infected natural host populations. *J. Virol.* 81:1961-1971, 2007. doi: 10.1128/JVI.01981-06
- Clay, S., Maherchandani, S., Malik, Y.S. and Goyal, S.M.: Survival on uncommon fomites of feline calicivirus, a surrogate of noroviruses. *Am. J. Infec. Control* 34:41-43, 2006. DOI: 10.1016/j.ajic.2005.05.013
- Di Martino, B., Di Rocco, C., Ceci, C. and Marsilio, F.: Characterization of a strain of feline calicivirus isolated from a dog faecal sample. *Vet. Microbiol.* 139:52-57, 2009. DOI: 10.1016/j.vetmic.2009.04.033
- Poulet, H., Brunet, S., Soulier, M., Leroy, V., Goutebroze, S. and Chappuis, G.: Comparison between acute oral/respiratory and chronic stomatitis/gingivitis isolates of feline calicivirus: pathogenicity, antigenic profile and cross-neutralization studies. *Arch. Virol.* 145: 243-261, 2000. DOI: 10.1007/s007050050021
- Martella, V., Pratelli, A., Gentile, M., Buonavoglia, D., Decaro, N., Fiorente, P. and Buonavoglia, C.: Analysis of the capsid protein gene of a feline-like calicivirus isolated from a dog. *Vet. Microbiol.* 85:315-322, 2002. DOI: 10.1016/s0378-1135(01)00521-1
- Hurley, K.F. and Sykes, J.E.: Update on feline calicivirus: new trends. *Vet. Clin. North. Am. Small Anim. Pract.* 33:759-772, 2003. DOI: 10.1016/s0195-5616(03)00025-1
- Pesavento, P.A., MacLachlan, N.J., Dillard-Telm, L., Grant, C.K. and Hurley, K.F.: Pathologic, immunohistochemical, and electron microscopic findings in naturally occurring virulent systemic feline calicivirus infection in cats. *Vet. Pathol.* 41:257-263, 2004. DOI: 10.1354/vp.41-3-257
- Pereira, J.J., Baumworcel, N., Fioretti, J.M., Domingues, C.F., Moraes, L.F., Marinho, R.D.S.S., Vieira, M.C.R., Pinto, A.M.V. and de Castro, T.X.: Molecular characterization of feline calicivirus variants from multicat household and public animal shelter in Rio de Janeiro, Brazil. *Braz. J. Microbiol.* 49:777-784, 2018. doi: 10.1016/j.bjm.2018.01.003
- Helps, C., Lait, P., Tasker, S. and Harbour, D.: Melting curve analysis of feline calicivirus isolates detected by real-time reverse transcription PCR. *J. Virol. Methods* 106:241-244, 2002. DOI: 10.1016/s0166-0934(02)00167-2
- Wilhelm, S. and Truyen, U.: Real-time reverse transcription polymerase chain reaction assay to detect a broad range of feline calicivirus isolates. *J. Virol. Methods* 133:105-108, 2006. DOI: 10.1016/j.jviromet.2005.10.011
- Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K.: MEGA X: Molecular Evolutionary Genetics Analysis, across Computing Platforms. *Mol. Biol. Evol.* 35:1547-1549, 2018.
- Statistical Packages for the Social Sciences (SPSS 21) (2012): licensed materials property of IBM corporation © copyright IBM corporation and other(s). 1989-2012; International.
- Radford, D.A., Coyne, K.P. and Dawson, S.: Feline calicivirus. *Vet. Res.* 38:319-335, 2007. DOI: 10.1051/vetres:2006056
- Pedersen, N.C., Elliott, J.B., Glasgow, A., Poland, A. and Keel, K.: An isolated epizootic of hemorrhagic-like fever in cats caused by a novel and highly virulent strain of feline calicivirus. *Vet. Microbiol.* 73:281-300, 2000. DOI: 10.1016/s0378-1135(00)00183-8
- Schorr-Evans, E.M., Poland, A., Johnson, W.E. and Pedersen, N.C.: An epizootic of highly virulent feline calicivirus disease in a hospital setting in New England. *J. Feline Med. Surg.* 5:217-226, 2003. DOI: 10.1016/S1098-612X(03)00008-1
- Coyne, K.P., Jones, B.R., Kipar, A., Chantrey, J., Porter, C.J., Barber, P.J., Dawson, S., Gaskell, R.M. and Radford, A.D.: Lethal outbreak of disease associated with feline calicivirus infection in cats. *Vet. Rec.* 158:544-550, 2006. DOI: 10.1136/vr.158.16.544.
- Schulz, B.S., Hartmann, K., Unterer, S., Eichhorn, W., Majzoub, M., Homeier-Bachmann, T., Truyen, U., Ellenberger, C. and Huebner, J.: Two outbreaks of virulent systemic feline calicivirus infection in cats in Germany. *Berl. Munch. Tierarztl. Wochenschr* 124:186-193, 2011.
- Dokuzeylül, B., Kayar, A. and Or, M.N.: Prevalence of systemic disorders in cats with oral lesions. *Vet. Med.* 61:219-223, 2016. doi: 10.17221/8823-VETMED
- Bannasch, M.J. and Foley, J.E.: Epidemiologic evaluation of multiple respiratory pathogens in cats in animal shelters. *J. Feline Med. Surg.* 7:109-119, 2005. DOI: 10.1016/j.jfms.2004.07.004
- Holst, B., Berndtsson, L. and Englund, L.: Isolation of feline herpesvirus-1 and feline calicivirus from healthy cats in Swedish breeding catteries. *J. Feline Med. Surg.* 7:325-331, 2005. DOI: 10.1016/j.jfms.2005.03.002
- Helps, C.R., Lait, P., Damhuis, A., Björnehammar, U., Bolta, D., Brovida, C., Chabanne, L., Egberink, H., Ferrand, G., Fontbonne, A., Pennisi, M.G., Gruffydd-Jones, T., Gunn-Moore, D., Hartmann, K., Lutz, H., Malandain, E., Möstl, K., Stengel, C., Harbour, D.A. and Graat, E.A.: Factors associated with upper respiratory tract disease caused by feline herpesvirus, feline calicivirus, *Chlamydophila felis* and *Bordetella bronchiseptica* in cats: experience from 218 European catteries. *Vet. Rec.* 156:669-673, 2005. DOI: 10.1136/vr.156.21.669
- Najafi, H., Madadgar, O., Jamshidi, S., Langeroudi, A.G. and Lemraski, M.D.: Molecular and clinical study on prevalence of feline herpesvirus type 1 and calicivirus in correlation with feline leukemia and immunodeficiency viruses. *Vet. Res. Forum* 5:255-261, 2014.
- Prikhodko, V.G., Sandoval-Jaime, C., Abente, E.J., Bok, K., Parra, G.I., Rogozin, I.B., Ostlund, E.N., Green, K.Y. and Sosnovtsev, S.V.: Genetic characterization of feline calicivirus strains associated with varying disease manifestations during an outbreak season in Missouri (1995-1996). *Virus Genes* 48:96-110, 2014. DOI:10.1007/s11262-013-1005-0