Investigation of *Feline calicivirus* infection in cats with upper respiratory tract disease in Diyarbakir, Turkey*  

*Investigação de infecção por calicivirus felino em gatos com doença do trato respiratório superior na região de Diyarbakir, Turquia*

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ABSTRACT

*Feline calicivirus* is among the most common pathogenic microorganisms in upper respiratory tract disease (URTD) and oral lesions of cats. It leads to stomatitis, oral ulceration, ocular and nasal discharge, conjunctivitis, fever, lameness, anorexia, hypersalivation, pneumonia, respiratory distress, coughing, and depression in infected cats. This study aimed to determine the role of *Feline calicivirus* (FCV) in cats with the upper respiratory tract disease in the Diyarbakir region, Turkey, to provide treatment for infected cats and contribute to the disease prophylaxis. The study material consisted of 10 cats (control group) considered to be healthy according to the clinical examination and 20 cats with URTD that were not vaccinated against *Feline calicivirus* infection of different breeds, ages, and genders brought to Dicle University Veterinary Faculty Prof. Dr. Servet SEKIN Polyclinic with URTD. After routine clinical examinations of the animals, oral and conjunctival swabs and blood samples were taken. Hematological and biochemical analyzes of blood samples were performed. Swab samples were analyzed by the polymerase chain reaction (PCR) method for the diagnosis of the agent. Oral lesions, hypersalivation, ocular and nasal discharge, coughing, and breathing difficulties were seen in clinical examinations of cats with URTD. *Feline calicivirus* was detected in only one cat's conjunctival swab sample in PCR analyses. As a result, we found that *Feline calicivirus* infection was present in cats with URTD in the Diyarbakir region, and 5% positivity was found in cats with clinical symptoms according to PCR analysis.

**Keywords:** *Feline calicivirus*. Cat. PCR. Upper respiratory tract disease.

RESUMO

O calicivírus felino está entre os microrganismos patogênicos mais comuns nas doenças do trato respiratório superior de gatos, determinando estomatites, ulcerações orais, descarga ocular e nasal, conjuntivite, febre, manqueira, anorexia, hipersalivação, pneumonia, distúrbios respiratórios, tosse e depressão. O presente trabalho foi delineado para determinar o papel do calicivírus felino (CVF) em gatos com doenças do trato respiratório superior na região de Diyarbakir, Turquia. Com o objetivo de orientar a prescrição do tratamento para os gatos infectados e contribuir com a profilaxia da doença. O material de estudo consistiu em 10 gatos saudáveis sem qualquer problema de saúde e 20 gatos acometidos por doenças do trato respiratório superior que não haviam sido vacinados contra a infecção pelo calicivírus felino. Os animais de diferentes raças, idades e géneros foram encaminhados para a Universidade de Dicle, na Faculdade de Veterinária, na policlínica Professor Dr. Servet Sekin. Após o exame clínico de rotina dos animais, foram colhidos swabs orais e de conjuntiva e amostras de sangue. Análises hemotológicas e bioquímicas das amostras de sangue foram realizadas e os swabs foram analisados pelo método da reação em cadeia pela polimerase (PCR) para diagnóstico do agente. Nos gatos infectados foram constatadas: lesões orais, hipersalivação, descargas oculares e nasais, tosse e dificuldade respiratória. O calicivírus felino foi detectado pela técnica de PCR no swab conjuntival de apenas um gato. A conclusão obtida foi que a infecção pelo calicivírus felino foi detectada pela técnica de PCR na região de Diyarbakir, Turquia, em gatos com doença do trato respiratório superior com a frequência de 5%.

**Palavras-chave:** Calicivírus felino. Gato. PCR. Doença do trato respiratório superior.


Introduction

The upper respiratory tract diseases (URTDs) are more common and the cause of deaths in animals kept in overcrowding...
environments due to insufficient ventilation and stress. They are quite common in cats, and microorganisms such as bacteria, viruses, and fungi play a role in their etiology (Gourkow et al., 2013; Norsworthy, 2011).

Usually, infectious agents such as *Feline Herpesvirus type 1* (FHV-1), *Feline calicivirus* (FCV), *Feline reovirus* (F. reovirus), *Chlamydia felis* (C. felis), *Mycoplasma felis* (M. felis), *Bordetella bronchiseptica* (B. bronchiseptica), *Staphylococcus aureus* (S. aureus) and β-hemolytic streptococci play a role in diseases of the upper respiratory tract. All these are associated with high morbidity and mortality, especially in cats that are housed together (Sykes et al., 2001; Dinnage et al., 2009).

Although FCV and FHV-1 are responsible for about 90% of URTD in cats (Di Martino et al., 2007; Edwards et al., 2008; Hawkins, 2009; Wong et al., 2013), FCV is reportedly more common. This high prevalence is due to the antigenic diversity of FCV and the inability of available vaccines to protect against all FCV strains (Gaskell et al., 2012).

FCV, which is in the Vesivirus class of the family Caliciviridae, can be transmitted directly by contact with oral and nasal discharges of patient cats or infected cats, as well as indirectly by contaminated cages, feeding and cleaning tools, and personnel (Gaskell et al., 2004; Radford et al., 2009; Norsworthy, 2011).

The infection by calicivirus leads to stomatitis, oral ulceration, ocular and nasal discharge, conjunctivitis, pyrexia, lameness, anorexia, hypersalivation, pneumonia, respiratory distress, coughing, and depression in cats (Radford et al., 1997; Radford et al., 2009; Pereira et al., 2018). In addition to these clinical findings, a virulent systemic disease (VSD) has also been reported, which occurs in the form of outbreaks in co-living cats, has a high mortality, and causes systemic febrile disease (Coyne et al., 2006; Battilani et al., 2013; Meli et al., 2018).

Since anamnesis and clinical findings are not sufficient for the diagnosis of the disease, virus isolation, serological and PCR methods are used to establish a definitive diagnosis. PCR is the method most commonly preferred for definitive diagnosis because virus isolation takes time, is expensive and virus neutralization does not provide safe results due to vaccine applications (Chander et al., 2007; Radford et al., 2009; Norsworthy, 2011; Litster, 2015).

The prognosis is considered to be good in patients other than cats with no anorexia and dehydration, aggressive treatment, and VSD-FCV-infected cats. The mortality rate in the VSD form of the disease is high (33% -50%) (Litster, 2015).

There are no antivirals approved effective against FCV for the treatment of the infected animals (Radford et al., 2009; Norsworthy, 2011), but there are reports that feline interferon-omega and human interferon-alpha may be useful (Sherding, 2006; Cohn, 2011). Again, to prevent secondary bacterial infections, broad-spectrum antibiotic treatment (combinations of amoxicillin or amoxicillin-clavulanic acid, azithromycin, ampicillin enrofloxacin, and clavulanic acid/amoxicillin plus-fluoroquinolone) is recommended (Norsworthy, 2011; Bilal, 2013).

The treatment may be supported by cleaning the nasal discharge with physiological saline solution several times a day and applying topical ointments (Gaskell et al., 2004; Radford et al., 2009), giving balanced electrolyte solutions by intravenous (iv) or subcutaneous (sc) delivery to prevent the thickening of nasal and ocular secretions as a result of dehydration (Norsworthy, 2011), vitamin C, high-flavored, mixed and heated foods to encourage eating (Norsworthy, 2006; Radford et al., 2007; Radford et al., 2009).

To the authors’ knowledge, no studies report the presence of FCV infection in cats in Diyarbakir, Turkey. This study aimed to determine the occurrence of FCV infection in cats with upper respiratory tract disease in the Diyarbakir region, metropolitan area of Turkey to reveal the status of infection in regional cats and to help prevent the spread of the disease by taking the necessary prophylactic measures.

**Materials and Methods**

**Animals**

The animal material of the study consists of 10 healthy cats (control group), considered healthy according to the clinical examination, unvaccinated, different breeds, ages, and genders, and 20 cats with URTD that were not vaccinated against *Feline calicivirus* infection of different breeds, ages, and genders brought to Dicle University Veterinary Faculty Prof. Dr. Servet SEKIN Polyclinic with upper respiratory tract disease (such as oral lesions, hypersalivation, ocular and nasal discharge, coughing, difficulty breathing and gingivitis). Samples were collected between March 2018 and January 2019.

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Clinical examination and sampling

After the cat’s anamnesis was taken, routine clinical examinations were carried out and body temperature (T), heart rate (HR), and respiratory rate (R) were recorded. Total leukocyte count (WBC), lymphocyte (Lymph), monocyte (Mon), granulocyte (Gran), lymph (%), Mon (%), Gran (%), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) in erythrocytes, mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) values of the blood samples taken for hematological analysis, were detected by Mindray BC2800 Vet Hematology Analyzer.

After the blood samples taken for biochemical analysis were centrifuged with the Nüve-NF800 brand device, the values of alanine aminotransferase (ALT) activity, blood urea nitrogen (BUN), and creatinine (Cre) were determined from the obtained serum.

Samples of oral and conjunctival swabs were taken from cats with upper respiratory tract disease using sterile swabs. The samples taken were placed in tubes containing 2 ml of Dulbecco’s modified eagle medium (DMEM) solution and delivered to Dicle University Faculty of Veterinary Medicine Genetic Department Laboratory under the cold chain. PCR was performed on the same day. The commercial vaccine (Felocell®-CVR, Zoetis Animal Health, USA) was used as a positive control.

Nucleic acid extraction (RNA isolation)

Two hundred µl was taken from the sample stored in the DMEM solution and transferred to the PCR tube. 200 µl also was added to the PCR tube from the mixture which was previously dissolved with the 'Elution Buffer' and then added to the 'Binding Buffer'. After the mixture was vortexed, it was then incubated for 10 min at +72 °C. After incubation, 100 µl of binding buffer was added to the mixture, and after mixing slowly, the samples were transferred to the filtered collection tubes and centrifuged for 1 min at 8000 rpm. Then, the lower collection tube was removed and a new tube installed, adding 500 µl 'Inhibitor Removal' and again centrifuged at 8000 G for 1 min. 450 µl of 'Wash Buffer' was added by removing the collection tube and installing a new tube and centrifuging for 1 min at 8000 rpm (this process was repeated twice). It was then centrifuged again for 10 sec at maximum speed (at 13,000 rpm). Finally, the filtered tubes were placed in 1.5 ml tubes and a 50 µl 'Elution Buffer' heated to 37 °C was added and centrifuged for 1 min at 8000 rpm. As a result of this process, viral nucleic acid was obtained using 1.5 ml PCR tubes (High Pure Viral Nucleic Acid Kit-Roche).

cDNA synthesis

From the RNAs obtained earlier, 10 µl was taken using a micropipette and transferred to 0.2 ml PCR tubes. After 1 µl Random Hexamer primer and 1 µl ddH2O were added onto it, it was incubated for 5 min at 65° C. After this incubation, 4 µl 5X Reaction Buffer, 1 µl RiboLock RNase inhibitor, 2 µl dNTP Mix, and 1 µl RevertAid m-muLV RT was added and incubated for 60 min at 42 °C, 10 min at 45 °C, and 5 min at 70 °C, respectively.

PCR operations from cDNA

Primers to be used to detect FCV’s cDNAs were first diluted to be 20 pmol/µl. Then, with Tm degrees in mind, gradient PCR was performed at determined degrees, binding degrees were determined for each primer, and the DNAs were amplified. The 677 base pair gene region of the cDNA was replicated for FCV detection. Primers used for the detection of FCV are specified in Table 1 (29). First, in the PCR process, the primers used for the detection of FCV were amplified at different temperatures to determine the binding degrees. Then, PCR protocol was created by determining the degree of binding according to the brightness levels of the DNAs carried in the gel.

Gel electrophoresis technique

To prepare the gel running buffer, 20 ml of EDTA and weighed chemicals were put into a 1000 ml bottle and the top was completed with up to 1000 ml of distilled water. 1X TBE was prepared by taking 200 ml from the 5X TBE prepared for gel preparation and running the gel in the tank, adding 800 ml distilled water to it. For the gel, 1.5 g of agarose was weighed into the 100 ml 1X TBE and heated in the microwave until the agarose in the mixture dissolved. Then, 5 µl of ethidium bromide was added into the prepared gel and poured into the tank with combs in it, and waiting for 45 min for the gel to crystallize. The combs were removed from the frozen gel and placed in the gel runner tank. The inside of the tank was filled with 1X TBE buffer to cover the gel surface. 10 µl from the amplified DNA and 2 µl from the loading buffer were pipetted in a separate

Table 1 - General FCV* primers applied for investigation in cats of the Diyarbakir region, Turkey, between March 2018 and January 2019

<table>
<thead>
<tr>
<th>Amplicon</th>
<th>Primers</th>
<th>Size(bp)</th>
<th>forward/reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer 677 bp</td>
<td>F: 5' - TTGCTGCTTTCTCC-3 R: 5' - TATCAGCATCAATAGACCT-3</td>
<td>677 bp</td>
<td>forward/reverse</td>
</tr>
</tbody>
</table>

*FCV = Feline Calicivirus.
place and loaded into wells in the gel to be 12 µl in total. A 5 µl marker was loaded into the first well to determine the sequencing of DNA based on their weight. The loaded DNAs were run in the tank at 60 amps and 110 volts for 30-35 min. The agarose gel was removed from the tank and transferred to the transilluminator. The bands formed by DNA based on their weight were examined under UV light in the transilluminator.

**Statistical analysis**

The Student's t-test was used to compare groups. The results were expressed as the mean ± standard error of the mean (SEM). P-values of <0.05 were considered statistically significant.

**Results**

**Clinical Examination Findings**

Clinical examination findings of cats with URTD and control group cats are shown in Table 2.

**Laboratory Findings**

The hematological examination findings of cats with URTD and control group cats are shown in Table 3. Serum biochemistry findings are shown in Table 4.

**PCR Results**

Only one conjunctival swab sample taken from cats with URTD was found positive for FCV after PCR analysis, out of a total of 40 oral and conjunctival swab samples. As a result of PCR, samples giving bands in the range of 700 bp and 500 bp were considered positive (Figure 1).

**Discussion**

FCV, which affects cats worldwide and is highly contagious, is among the most common pathogens of upper respiratory tract disease, especially in cats housed together (Radford et al., 2001; Marsilio et al., 2005; Henzel et al., 2012).

FCV reportedly causes stomatitis, oral ulceration, ocular and nasal discharge, conjunctivitis, pyrexia, lameness,
anorexia, hypersalivation, pneumonia, breathing difficulties, coughing, and depression in cats (Radford et al., 2001; Radford et al., 2009; Tian et al., 2016; Pereira et al., 2018). Oral lesions, hypersalivation, ocular and nasal discharge, coughing, respiratory distress, and gingivitis were seen in cats with URTD that constitute the material of this study.

When compared with the control group, the body temperature of the cats with URTD was higher and the respiratory and heart rate were lower. It is thought that a higher breathing and respiration rate in control group cats compared to clinically diseased cats with symptoms compatible with FCV infection is caused by stress and fear because healthy animals are more sensitive to clinical examination.

Mon (%), MCH, MCHC, PLT, and Cre values of the cats with URTD were recorded as statistically significantly lower than the control group, but we determined that the results obtained were in the reference intervals reported for healthy cats (Turgut, 2000).

Sykes et al. (2001) reported detecting FCV infection in 9.6% of swab samples of cats with upper respiratory tract disease in Melbourne and Sydney, Australia. They also reported not finding FCV in any of the samples kept at -70 °C and that they did encounter FCV in 13.7% of the samples kept at +4 °C.

Helps et al. (2005) reported that in the samples taken and kept for analysis at -20 °C from a total of 1,748 cats with and without upper respiratory tract disease in 218 different shelters in 9 different European countries, such as Austria, France, Germany, Italy, Netherlands, Spain, Sweden, Switzerland, and England, they found positivity of 47% and 29%, respectively.

Kang & Park (2008) found that none of the swab samples that were taken from the conjunctiva and oropharynx of 78 cats and maintained at -70 °C until analysis, detected FCV.

Berger et al. (2015) reported detecting positivity in 45% of the oropharyngeal, nasal, and conjunctival swab samples they took from 200 cats with upper respiratory tract disease from 19 different regions of Switzerland.

Najafi et al. (2014) detected FCV infection in all 16 cats (100%), none of whom had been vaccinated, showing clinical symptoms such as sneezing, coughing, oral ulcers, ocular and nasal discharge at the Small Animal Hospital of the University of Tehran.

Gerriets et al. (2012) reported the positivity rate of FCV as 30/99 by RT-PCR method in 99 cats showing clinical signs of upper respiratory tract infection in different parts of Germany.

Marsilio et al. (2005), in their study of cats with upper respiratory tract disease, found positivity rates in 47 oculars and 40 pharyngeal swab samples of 47 cats as 18 and 23, respectively.

Sykes et al. (2001) stated that the swab samples taken to determine the FCV infection are affected by the storage temperatures, and the positivity rate in the samples kept at +4 °C is higher than those kept at -20 °C and -70 °C. The same researchers reported that the seasons affected FCV infection and that the rate of infection was low in samples collected in late winter and early spring.

Marsilio et al. (2005) reported that the small number of virus particles, ribonuclease in mucosal secretions, and the genetic difference of FCV affect the detection of the virus in mucosal swabs.

In the current study, oral and conjunctival swab samples of 20 cats with URTD showing clinical symptoms such as oral lesions, hypersalivation, ocular and nasal discharge, coughing, breathing difficulties, and gingivitis were examined by PCR method. Only one sample showed positivity for FCV. The infection was determined in the conjunctival swab sample of the cat with URTD, an outdoor animal, 6-8 months old. The current study revealed that the FCV infection rate was 5% in cats with URTD in our region.

We noted that this rate lower is than the rate reported by other researchers (Sykes et al., 2001; Marsilio et al., 2005; Gerriets et al., 2012; Henzel et al., 2012; Najafi et al., 2014; Berger et al., 2015; Hou et al., 2016). These differences in the prevalence of infection may be due to geographic, climate, and storage conditions as reported (Sykes et al., 2001, Wang et al., 2017). Radford et al. (2007) reported that FCV can persist longer in colder weather conditions. Sykes et al. (2001) reported that FCV infections are affected by storage temperatures, and the positivity rate in the samples kept at +4 °C is higher than those kept at -20 °C and -70 °C. The low FCV rate in this study may be because the swab samples were analyzed without waiting and the climate of the Diyarbakir region was not too cold.

The fact that the positivity detected in the conjunctival swab sample was not detected in the oral swab sample from the same animal may result from the small number of virus particles, presence of ribonuclease in mucosal, secretions able to degrade viral RNA and the genetic difference of FCV affect the detection of the virus in mucosal swabs, as researchers reported (Marsilio et al., 2005, Kang & Park, 2008).

There has been no URTD of cats, including FCV, in our region. Control of FCV relies on a combination of vaccination and management (Gaskell & Gaskell, 1997). Vaccines prepared from circulating strains in the region should be preferred to protect against FCV with vaccination (Abayli et al., 2020).
Cats should be housed individually unless they come from the same household (Radford et al., 2009).

In conclusion, 5% FCV infection was detected in cats with URTD in the Diyarbakir region, Turkey with the PCR method. We determined that 95% of cats with URTD were caused by other causes (fungi, bacteria, other viruses, and non-infectious causes). Therefore, it would be beneficial to research other infections to provide appropriate treatment and preventive measures.

**Conflict of Interest**

The authors declare no conflict of interest.

**Ethics Statement**

Dicle University Animal Experiments Ethics Committee decided that the permission of the Local Ethics Committee was not required with the letter dated February 19, 2018, Number 17768

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**References**


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