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Research Article

Molecular detection of feline calicivirus (FCV) in cats with oral lesions

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ABSTRACT

Feline Calicivirus (FCV) is a major cause of oral lesions in cats with respiratory tract disease (RTD). FCV is a single-stranded, positive-polarity RNA virus that encodes three open reading frames (ORFs). Active virus excretion occurs through the saliva of cats infected with FCV, which belongs to the *Vesivirus* genus of the *Caliciviridae* family. Oral mucosal lesions caused by infectious agents in RTD lead to significant impairment in the quality of life of cats. RTD, which also affects the oral mucosa, is a common problem in cats. Ten cats of different ages, breeds, and genders with ocular lesions were used in this study. At the time of sample collection, the veterinarian performed general and oral examinations on each animal. On oral examination,varying degrees of gingivitis, stomatitis, and ulceration symptoms were noted. Samples were extracted using a commercial viral nucleic acid isolation kit. Three out of ten samples (30%) were found to be positive for FCV using RT-PCR. T
In conclusion, the high sensitivity, specificity, and potential for field sample testing make RT-PCR a very important and inevitable method for research and clinical diagnosis related to FCV infection in cats with oral lesions.

INTRODUCTION

Viral infections in cats represent a significant concern due to their potential impact on various systems. The ability of these infections to target multiple systems, including the digestive, respiratory, immune, genital, and neural systems, underscores the complexity and severity of viral diseases in cats Aydin and Timurkan 2018; Baydar et al. 2014; Karapinar et al. 2024). In recent years, respiratory tract disease (RTD), which also affects the oral mucosa, has been increasingly encountered in cats among these infections (Dağalp et al. 2019; Lee et al. 2019; Schulz et al. 2015). Oral mucosal lesions caused by infectious agents in RTD lead to a significant deterioration in cats' quality of life. In addition, mixed infections with more than one pathogen pose difficulties for veterinarians in terms of diagnosis and treatment methods (Sykes et al. 1997; Cao et al. 2022). The primary pathogens that appear in mixed cases are Feline calicivirus (FCV) feline herpesvirus-1 (FHV-1), Bordetella bronchiseptica, Chlamydia psitacci, and others. (Walter et al. 2020).

FCV is a highly contagious viral pathogen that poses a significant threat to the health of cats worldwide (Abd-Eldaim et al. 2009). Cats infected with FCV often present with a range of clinical signs, including oral lesions that can range from mild gingivitis to severe ulcerative stomatitis, causing discomfort. Detection and diagnosis of FCV in cats with oral lesions is crucial for effective treatment and control of the disease (Cao et al. 2022; Henzel et al. 2012). Studies have shown that FCV is one of the main causes of oral lesions in cats (Fontes et al. 2023). FCV is a single-stranded, positive polarity RNA virus that encodes three open reading frames (ORFs) (Radford et al. 2007). Active virus excretion occurs via the saliva of cats infected with FCV, which belongs to the Vesivirus genus of the *Caliciviridae* family. This shedding can last for several months depending on individual factors (Binns et al. 2000). FCV infection has been reported to be common in housed domestic cats under one year of age and in cats living in shelters. FCV infection occurs in these cats, particularly in chronic stomatitis (Knowles et al. 1989; Fontes et al. 2023). FCV can cause cases of gingivostomatitis in cats and humans because, together with bacterial plaques, they cause lymphocyte infiltration into the oral mucosa (Lommer and Verstraete 2003). FCV, which studies have not yet shown to pose a direct threat to humans, can cause fatal illnesses in cats. The infection caused by this pathogen is particularly common in animal shelters and cat hotels and usually affects young cats. In Türkiye, many cats, including owner cats, have direct or indirect contact with stray animals. This can cause infected cats to transmit infections like FCV more easily. Since FCV, whose nucleic acid was detected in the current project, is a highly variable RNA virus, the data obtained from positive cases will help future molecular characterization studies.

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The aim of this research article is to prevent the further spread of the virus and improve the welfare of cats by highlighting the value of the RT-PCR method for the detection of FCV in cats with oral lesions.

MATERIALS and METHODS

Animals and Ethics Statement.

This study used ten cats of different ages, breeds and genders with oral lesions between 2022 and 2023. They were transported to the Virology Laboratory the Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University Animal Hospital, and private veterinary clinics. The patients' veterinarians provided information about the cats to which each sample belonged. The animals had a variety of severe symptoms, including gingivitis, stomatitis and ulcers. The same veterinarian examined each animal and recorded its symptoms. This study was approved by the Animal Ethics Committee of Burdur Mehmet Akif Ersoy University under approval number 102/915.

Clinical signs

General and oral examinations were performed on each animal by the veterinarian at the time of sample collection. All findings observed in the cats included in the study were recorded.

Samples

Samples were collected using sterile commercial swabs dipped in PBS with antibiotics. All the liquid in the swabs was transferred to sterile 2 mL microtubes after complete vortex mixing. It was centrifuged for 20 minutes at +4 °C and 3000 rpm. After centrifugation, 500 µL of the supernatant was collected and stored at −80 °C until testing.

Nucleic acid extraction and PCR test

The supernatants were extracted using a commercial viral nucleic acid isolation kit (Roch, Germany). The post-extraction PCR test was performed according to the method of Ohe et al. (2006). Samples found to be positive were aliquoted and stored at −80°C until testing. An extract from a commercial vaccine containing FCV was used as a positive control in the RT-PCR test. Ultra-pure water was used as a negative control.

RESULTS

Clinical signs

A general examination of the cats showed signs of poor oral hygiene (20%), loss of appetite (100%), and reluctance to perform any oral care. After oral examinations, varying degrees of gingivitis (50%), stomatitis (10%), and ulceration (60%) symptoms were found. Overall, three out of ten (30%) cats were detected as positive using RT-PCR. All findings observed during sampling, cat information, and RT-PCR results are listed in Table 1.

Table 1. Cat data, recorded clinical observations and RT-PCR results.

No	Age/ Months	Gender	Symptoms					RT-PCR
			Poor oral hygiene	Loss of appetite	Gingivitis	Stomatitis	Ulceration	
1	6	♂	X	✓	✓	X	✓	+
2	6	♂	X	✓	X	X	✓	-
3	9	♂	X	✓	X	X	X	-
4	5	♀	✓	✓	✓	X	X	-
5	12	♂	X	✓	✓	X	X	+
6	12	♂	X	✓	X	X	✓	-
7	8	♀	X	✓	✓	X	X	-
8	24	♀	X	✓	X	X	✓	-
9	24	♀	✓	✓	X	✓	✓	+
10	12	♂	X	✓	✓	X	✓	-

"✓" marks were used for cats with symptoms and "X" marks for cats without symptoms.

DISCUSSION

Common viral infections in cats include FIP, FIV, FeHV-1, and FCV (Aydin et al. 2018; Baydar et al. 2014; Karapinar et al. 2024; Koc and Oguzoglu, 2020). These viruses can target multiple systems, including the digestive, respiratory, immune, genital, and neural systems, causing a range of symptoms such as fever, pale gums, loss of appetite or weight loss, lethargy and weakness, conjunctivitis, inflammation of the gums and mouth, and respiratory problems (Dağalp et al. 2019; Westman et al. 2022). These viral infections can have significant impacts on a cat's health and well-being, and prevention measures such as vaccination and maintaining good hygiene are crucial in minimizing the risk of infection and transmission. The most common viral pathogens that cause feline respiratory disease complex are FeHV-1 and FCV (Walter et al. 2020). These viruses are responsible for most cases of upper respiratory infections in cats and are often found in combination, with FHV-1 being the primary cause and FCV being more prevalent in some cat populations (Henzel et al. 2012). The clinical symptoms of RTD in cats are similar to those of other feline

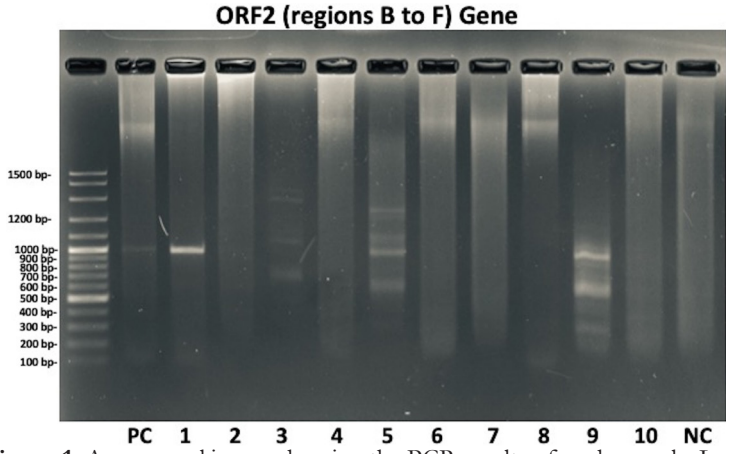

Figure 1. Agarose gel image showing the PCR results of each sample. Lane 1 (Ladder), lane 2 (Positive control), and lane 13 (Negative control) are arranged from left to right. Samples 1, 5 and 9 were positive and the others were negative based on the results of the PCR tests performed.

pathogens, including FCV (Lee et al. 2019; Schulz et al. 2015). FCV infections have become more common in cats worldwide (Mochizuki et al. 2000; Dağalp et al. 2019). A differential diagnosis of these pathogens is required for proper treatment and control (Cao et al. 2022). Diagnostic methods for FCV detection in samples include molecular detection using conventional PCR, virus isolation, and immunofluorescence antibody assay (IFA). Nevertheless, the sensitivity of antigen detection is rather low, although it is quick and inexpensive. Although it can take several days to two weeks, virus isolation is the gold standard. The sensitivity and specificity of conventional RT-PCR assays have also been shown to be high (Sykes et al., 1998). In our study, 30% of cats were found to be FCV positive using RT-PCR. The use of RT-PCR to detect FCV, particularly in cats with oral lesions, provides a valuable approach to identifying the virus and understanding its association with disease severity. These studies add to the growing body of evidence supporting the use of RT-PCR to detect and monitor FCV, particularly in cat populations affected by oral infectious diseases (Henzel et al. 2012; Palombieri et al., 2023; Weeks et al., 2001;). Studies highlight the utility of real-time PCR assays in detecting FCV in cats with stomatitis and highlight the role of molecular diagnostics in guiding targeted therapy as well as the importance of genome sequencing in understanding the genetic diversity of FCV strains (Abd-Eldaim et al., 2009; Palombieri et al., 2023; Radford et al. (2021). Using molecular methods, detection of FCV in cat populations can help identify potential sources of infection and guide targeted interventions. FCV detection in cats with oral lesions using real-time polymerase chain reaction (RT-PCR) is a sensitive and specific method (Schulz et al. 2015; Cao et al. 2022). However, several factors may influencethe accuracy of RT-PCR in detecting FCV in cats with oral lesions. These factors include sampling, sensitivity, cross-contamination, genetic variability, interpretation, and cost and equipment. Obtaining high-quality samples from oral lesions can be challenging due to the presence of debris, blood, or inflammatory exudates that can interfere with the PCR process. Despite its high sensitivity, RT-PCR can still fail to detect FCV in samples with low viral loads, especially in intermittent shedding or carrier states (Henzel et al. 2012; Schulz et al. 2015). The risk of contamination during sample processing and PCR setup can lead to false positive results, highlighting the need for strict laboratory practices to prevent contamination. FCV exhibits genetic diversity, which can create challenges in developing primers that effectively target all circulating strains, potentially leading to false-negative results. Proper interpretation of RT-PCR results requires expertise in distinguishing between active infection, carrier status, or environmental contamination to avoid misdiagnosis. RT-PCR requires specialized equipment and trained personnel, which makes it relatively expensive and less accessible compared to other diagnostic methods (Hofmann-Lehmann et al. 2022; Palombieri et al. 2023). Various interpretations based on the association between cat age and FCV infection have received attention in recent studies. While some studies suggest that younger cats are more likely to be infected with FCV due to factors such as limited access to vaccinations and impairment of maternal antibodies (Zheng et al. 2021), other research suggests that older cats are more susceptible to FCV infections (Tran et al. 2019). One study found that age was significantly associated with FCV transmission and cats older than 3 years were less likely to transmit the virus (Coyne et al., 2006). It has also been reported that kittens less than 8 weeks of age entering shelters were not exposed to FCV, but the transmission rate of the virus increased with age and the highest transmission rate was found in kittens and young cats (Pedersen et al., 2004). On the other hand, a study highlights that older cats are more likely to be infected with FCV than younger cats because the risk of infection is cumulative due to the lifelong nature of infection (Tran et al. 2019). In our study, the age range of cats found positive was 6 to 24 months. Although the exact average age of FCV infection in cats is low, preliminary evidence suggests that young cats may be more susceptible to FCV infection and the shedding rate may increase with age. However, it is clear that studies with larger numbers of cats are needed to show realistic results regarding an association between age and infection.

Studies indicate a strong connection between FCV infection and oral health problems in cats (Zheng et al. 2021). FCV-pos-

itive cats show varying degrees of oral symptoms such as gingivitis, stomatitis and ulceration (Druet et al. 2017). Therefore, it is believed that the likelihood of detecting FCV in cats increases when oral symptoms are present. FCV infection can also lead to chronic gingivostomatitis, which is considered an immune-mediated disease, and in extreme cases to virulent systemic FCV (Hofmann-Lehmann 2022). For example, a study of cats with chronic gingivostomatitis (FCGS) found that decreasing FCV burden significantly correlated with clinical improvement and oro-mucousal ulcer outcomes (Druet et al. 2017). However, it is important to note that the exact mechanism by which FCV contributes to oral cavity disease remains unclear. While FCV has been linked to the development of immune-mediated diseases such as FCGS, the specific molecular interactions between FCV and host cells remain poorly understood. In our study, the data provided suggest various signs of poor oral hygiene in cats, such as loss of appetite, reluctance to perform oral care, gingivitis, stomatitis, and ulceration symptoms. This is consistent with existing literature highlighting the common occurrence of dental disease in cats. A study highlights the need for client education and effective communication by veterinary staff to improve cats' oral health. While brushing teeth is recommended as the most effective method for removing plaque, only a small proportion of cat owners reported using this preventive measure regularly (Oskarsson et al. 2021). Such preventive measures and regular dental care can play a crucial role in helping cats avoid FCV infection, which causes gingivitis and stomatitis. Understanding the connection between FCV and oral disease helps inform prevention strategies and early intervention efforts.

CONCLUSION

It is necessary to provide an up-to-date overview of current diagnostic methods for the detection of FCV in cats with oral lesions, incorporating findings from recent literature and studies. Given the successful and rapid detection of FCV in naturally infected cats with oral lesions, our study provided important insights into understanding its association with disease severity. The high sensitivity, specificity, and potential for field sample testing make RT-PCR a crucial and inevitable method for research and clinical diagnosis related to FCV infection in cats with oral lesions. In summary, we believe we can contribute to the advancement of veterinary practice to improve the diagnosis and treatment of FCV-associated oral diseases in cats.

DECLARATIONS

Ethics Approval

All procedures were approved by the Animal Ethics Committee (AEC) Burdur Mehmet Akif University, Türkiye (No:102/915).

Conflict of Interest

Authors do not have any conflict of interests

Consent for Publication

Not applicable.

Competing Interest

The authors declare that they have no competing interests

Author contribution

Idea, concept and design: HSS, ZE

Data collection and analysis: HSS, ZE

Drafting of the manuscript: HSS

Critical review: HSS

Data Availability

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