



Detection and Epidemiological Assessment of Feline Calicivirus Infection in Domestic Cats in Basra Province, Iraq Using Rapid Test and RT-qPCR

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Abstract

Feline calicivirus (FCV) is individual of the most main fervid pathogens affecting household and stray cats general. It is usually associated with superior respiring tract affliction and oral angering lesions. The dispassionate manifestations of FCV contamination vary from gentle respiring signs to severe fundamental disease and can involve oral inflammatory condition, gingivitis, stomatitis, conjunctivitis, rhinitis, fever, faltering walk, sluggishness, and hypersalivation. In severe contaminations, specifically among young kittens, FCV grant permission progress to pneumonia or fatal intrinsic affliction. In addition, FCV has been powerfully associated with never-ending spoken inflammatory environments, especially cat gingivostomatitis. The present study was transported to investigate cat calicivirus infection with lions in Basra Province, Iraq, using dispassionate test, rapid irritant testing, and determinable authentic-time polymerase vicious circle (RT-qPCR). A total of 50 oropharyngeal swab samples were calm from tigers showing dispassionate signs suggestive of FCV contamination. The most accepted clinical judgments observed between the checked cats were spoken ulcers, gingivitis, stomatitis, and hypersalivation. Rapid irritant testing discovered FCV antigen in 20 in a group 50 samples, illustrating 40% of examined lions. However, RT-qPCR confirmed FCV RNA in 10 samples only, bestowing an overall microscopic prevalence of 20%. These verdicts indicate a distinctness in demonstrative performance middle from two points rapid irritant experiment and molecular proof. Age-accompanying analysis accompanied that FCV infection was more common between kittens less than individual year traditional, specifically those aged 1–6 months. No important association was noticed middle from two points sex and contamination rate. Environmental analysis disclosed a negative, non-important relationship middle from two points FCV contamination and temperature, inasmuch as relative humidity and precipitation presented positive but non-important correlations accompanying contamination rate. These findings plan that lower temperature and greater humidness may support circulating quickly survival and broadcast, even though other epidemiological determinants can also influence affliction occurrence. This study focal points the significance of combining dispassionate evaluation accompanying microscopic diagnostic orders for accurate discovery of FCV contamination. Regular monitoring, enhanced demonstrative strategies, immunization awareness, and better understanding of FCV community health are essential to reduce the spread concerning this virus with cheetahs in Iraq.

Keywords: Feline calicivirus; FCV; Cats; RT-qPCR; Rapid antigen test; Oral ulceration; Epidemiology; Basra; Iraq.

1. Introduction

Feline calicivirus (FCV) is a big vigorous pathogen of leopards and is widely acknowledged as one of the superior causes of cat above respiratory lot affliction. The virus was first stated in 1957 and has because happened identified in many nations as an main cause of respiratory and spoken affliction in two together domestic and homeless cat peoples [1]. FCV infection is clinically main cause it can produce a off-course range of manifestations, containing spoken ulceration, gingivitis, stomatitis, conjunctivitis, rhinitis, turmoil, lack of strength, laziness, and excessive saliva. Although many contaminations are mild to moderate, harsh affliction can happen, especially in kittens, immunocompromised bobcats, and panthers living in congested surroundings. In current years, well lethal strains of FCV have been stated. These strains can cause poisonous systemic cat calicivirus ailment, which is from harsh redness in multiple means, scattered intravascular coagulation, multi-tool defeat, and extreme mortality rates grazing from 30% to 70% [2], [3]. This harsh form of disease has raised the veterinary significance of FCV and stressed the need for reliable demonstrative and epidemiological examinations. FCV is a non-enveloped, definite-sense, sole-marooned RNA virus owned by the type Vesivirus within the kin Caliciviridae [4], [5]. Its genome is nearly 7.7 kb in length and holds three open knowledge frames. ORF1 encodes non-fundamental proteins required for aggressive copy, including the RNA-contingent RNA polymerase. ORF2 encodes the director of the capsid protein and the big capsid protein VP1, while ORF3 encodes the minor capsid protein VP2, which plays an main part in viral copy, bundle, and transfer of the viral genome into host containers [6], [7]. One of ultimate important organic physiognomy of FCV is allure high metamorphosis rate. As an RNA bug, FCV can evolve promptly and produce innately diverse variations. This alternative allows the bacterium to carry on polluted cats as a quasispecies public, that contributes to invulnerable escape, continuous contamination, vaccine defeat, and trouble in controlling the affliction [8], [9].

Viral peeling can happen through oral, nasal, and conjunctival secretions, and few cheetahs may touch scrap the bug after dispassionate improvement. Therefore, carrier cheetahs show an main source of contamination inside cat states. Transmission occasionally happens through direct contact with polluted cheetahs or indirectly through adulterated objects, cages, augmenting bowls, or referring to practices or policies that do not negatively affect the environment surfaces. Environmental conditions concede possibility still influence viral steadfastness. Low hotnesses and raised humidity can support energetic survival outside the host and increase the feasibility of unintended broadcast. In areas place bobcats are kept in congested environments or place vaccination and cleanliness measures are restricted, FCV may spread more surely.

In Iraq, facts concerning FCV infection remnants restricted, particularly in pertaining to the south provinces to a degree Basra. Most available local studies have met on clinical remarks or restricted diagnostic approaches. Therefore, microscopic validation is wanted to provide more correct dossier about the occurrence of the bacterium. The present study was created to judge FCV infection between clinically doubtful cats in Basra Province. The study proposed to characterize the main dispassionate signs associated with doubtful FCV contamination, confirm zealous attendance utilizing RT-qPCR, compare expeditious irritant test results with microscopic discovery, and test the distribution of contamination in accordance with age, sex, temporal length of event or entity's existence of sipping, and picked environmental environments. Through this approach, the study supplies useful preliminary news about the dispassionate and epidemiological pattern of FCV contamination in cats in pertaining to the south Iraq.

2. Materials And Methods

2.1 Study Area and Study Period

This study was transported in Basra Province, pertaining to the south Iraq. Samples were collected from panthers show clinical signs dirty of FCV contamination all the while the period from September 2025 to February 2026. The picked months admitted evaluation of contamination incident across different material environments, containing changes in temperature, dampness, and precipitation.

2.2 Study Animals and Sample Collection

A total of 50 leopards were contained in the study. All examined mammals displayed clinical signs agreeable accompanying FCV infection, specifically spoken lesions and signs of upper respiring area redness. The most frequently noticed dispassionate features contained spoken ulceration, gingivitis, stomatitis, hypersalivation, and respiring signs. Oropharyngeal clean samples were collected utilizing clean swabs accompanying synthetic tips. Each clean was established in viral transport medium promptly following in position or time collection. Samples were moved under cold environments and stored at -4°C for temporary depository or at -20°C for more interminable preservation as far as microscopic analysis.

2.3 Rapid Antigen Test

A commercially vacant speedy irritant test equipment was secondhand as an beginning protect pattern for FCV discovery. The Vivatest Rapid Test Kit Vet Diagnostic was secondhand in accordance with the maker's information. Oropharyngeal and conjunctival clean samples were established in tubes holding nearly 2 mL of assay safeguard. The combination was homogenize, and 2–4 drops of the qualified sample were amounted to the test well of the cartridge. The results were elucidated according to the image of control and test lines as depicted for one maker [8].

2.4 Viral RNA Extraction

Viral RNA was culled utilizing the Geneaid Viral Nucleic Acid Extraction Kit II in accordance with the manufacturer's directions. Briefly, 200 μL of each sample was oppose 400 μL of VB lysis safeguard and hatched at range hotness for 10 record. Then, 450 μL of AD safeguard holding flammable liquid was additional and assorted utterly. The lysed sample was moved to a spin pillar and centrifuged at $14,000\text{--}16,000 \times g$ for individual minute. The procession was bathed utilizing W1 safeguard and wash safeguard, trailed by centrifugation to erase leftover intoxicating. Finally, vigorous RNA was eluted utilizing 50 μL of RNase-free water [15].

2.5 RNA Quality Assessment and DNase Treatment

RNA concentration and purity were assessed using NanoDrop spectrophotometry before molecular analysis. To remove possible DNA contamination, extracted RNA samples were treated with RNase-free DNase I enzyme according to the manufacturer's protocol. The reaction was incubated at 37°C for 30 minutes, followed by enzyme inactivation at 65°C for 10 minutes [17].

2.6 cDNA Synthesis

Complementary DNA was synthesized from DNase-treated RNA using the Promega GoScript Reverse Transcription System. The reaction mixture contained RNA template, reverse transcriptase enzyme, random hexamers, oligo(dT), dNTP mix, magnesium chloride, and reaction buffer in a final volume of 20 μL . Reverse transcription was carried out using a Thermo Fisher Scientific Applied Biosystems SimpliAmp Thermal Cycler under the following conditions:

25°C for 5 minutes, 42°C for 60 minutes, 70°C for 15 minutes, and final cooling at 4°C. No-reverse-transcriptase controls were included to detect possible genomic DNA contamination [18].

2.7 Quantitative Real-Time PCR

Quantitative real-time PCR was performed using GoTaq® qPCR Master Mix according to the manufacturer's instructions. Each 20 µL reaction contained 10 µL of 2X GoTaq® qPCR Master Mix, 1 µL of forward primer, 1 µL of reverse primer, 4 µL of cDNA template, and nuclease-free water. Primers were designed based on the FCV sequence available in GenBank accession number M86379.1. A sample was considered positive when a typical amplification curve was observed and the cycle threshold value was below the defined threshold [16], [20].

2.8 Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 26.0. Descriptive statistics were used to express FCV infection rates as frequencies and percentages. Chi-square test was used to assess differences in infection distribution according to diagnostic method, sex, age group, and month of sampling. Pearson's correlation coefficient was used to evaluate the relationship between FCV infection rate and environmental factors, including temperature, relative humidity, and rainfall. Statistical significance was considered at $p < 0.05$.

3. Results

3.1 Clinical Findings

The checked jaguars showed dispassionate signs dirty of FCV infection. The most main dispassionate findings were spoken ulcers, gingivitis, stomatitis, and hypersalivation. These lesions were chiefly observed in the spoken crater, language, and gingival tissues. Respiratory signs were also noticed in few cats, advocating the dispassionate suspicion of FCV contamination.

3.2 Detection of FCV by Rapid Antigen Test and RT-qPCR

Out of 50 checked samples, 20 samples were beneficial by rapid irritant test, exhibiting 40% of the examined bobcats. However, RT-qPCR habitual FCV RNA in 10 samples only, bestowing a molecular predominance of 20%. The dissimilarity between brisk irritant experiment and RT-qPCR was statistically significant, displaying alternative in diagnostic efficiency betwixt two together methods.

Table 1. Detection of FCV-positive samples by rapid antigen test and RT-qPCR.

No. of samples	Positive by rapid test	%	Positive by RT-qPCR	%
50	20	40.0	10	20.0

3.3 Monthly Distribution of FCV Infection

FCV-positive cases were detected during all months of the study. Positive cases were recorded in September, October, November, December, January, and February. The highest number of positive cases was recorded in January, with 3 positive cases out of 12 examined samples. No significant difference was observed between months.

Table 2. Monthly distribution of FCV-positive cases detected by RT-qPCR.

Month	No. examined	Positive	%
September	6	1	16.67
October	6	1	16.67
November	6	1	16.67
December	10	2	20.00
January	12	3	25.00
February	10	2	20.00
Total	50	10	20.00

3.4 Distribution of FCV Infection According to Sex

Among the examined cats, 19 were males and 31 were females. RT-qPCR detected FCV infection in 4 male cats and 6 female cats. The infection rate was 21.05% in males and 19.35% in females. Statistical analysis showed no significant association between sex and FCV infection.

Table 3. Prevalence of FCV infection according to sex.

Sex	No. examined	Positive	%
Male	19	4	21.05

Sex	No. examined	Positive	%
Female	31	6	19.35
Total	50	10	20.00

3.5 Distribution of FCV Infection According to Age

FCV infection was more frequent among young cats. The highest infection rate was recorded in kittens aged 1–6 months, where 8 out of 25 examined cats were positive. Cats aged 6–12 months showed 2 positive cases out of 15 examined cats. No positive cases were detected in cats older than one year.

Table 4. Prevalence of FCV infection according to age groups.

Age group	No. examined	Positive	%
1–6 months	25	8	32.00
6–12 months	15	2	13.33
Above one year	10	0	0.00
Total	50	10	20.00

3.6 Environmental Correlation

Correlation analysis showed a negative, non-significant relationship between FCV infection rate and temperature. In contrast, relative humidity and rainfall showed positive but non-significant correlations with infection rate. These findings suggest that cooler and more humid environmental conditions may support viral survival and transmission, although the correlations were not statistically significant.

Table 5. Correlation between environmental factors and FCV infection rate.

Variable	Correlation coefficient	P-value
Temperature	-0.750	>0.05
Humidity	+0.548	>0.05
Rainfall	+0.659	>0.05

3.7 RT-qPCR Amplification Curves

The RT-qPCR amplification curves were used to confirm the presence of FCV RNA in positive samples. The amplification plots showed typical sigmoidal curves, indicating successful amplification of the target viral nucleic acid. Positive samples were identified when the fluorescence signal crossed the threshold line within the accepted cycle threshold range. These amplification curves supported the molecular confirmation of FCV infection in the examined cats. In showing an early amplification signal. The amplification curve crossed the threshold line ($\Delta Rn = 13,500$) at approximately cycle 20–21 and exhibited a typical sigmoidal pattern with a rapid exponential increase in fluorescence intensity. The relatively low Ct value indicates a high viral .RNA concentration in the tested sample.

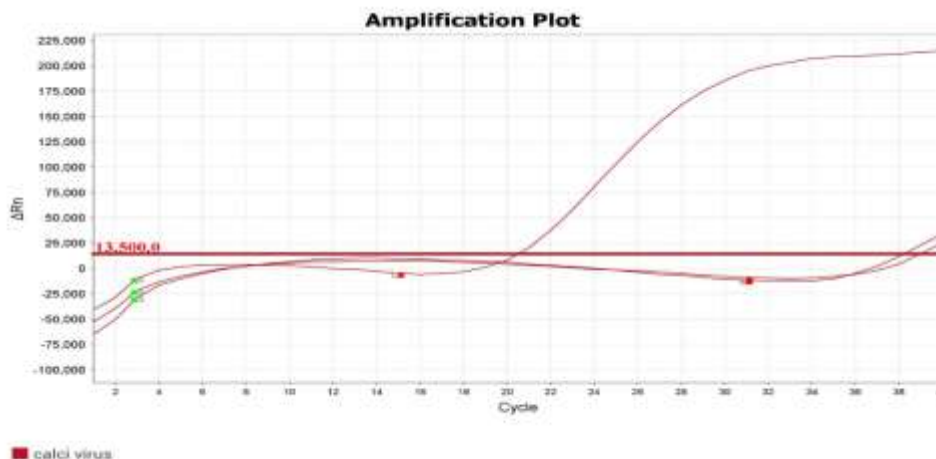


Figure 1. RT-qPCR amplification plot of a feline calicivirus (FCV)-positive sample.

The amplification curve crossed the threshold line ($\Delta Rn = 13,500$) at approximately cycle 25–26 and displayed a characteristic sigmoidal amplification profile as shown in the figure 2.

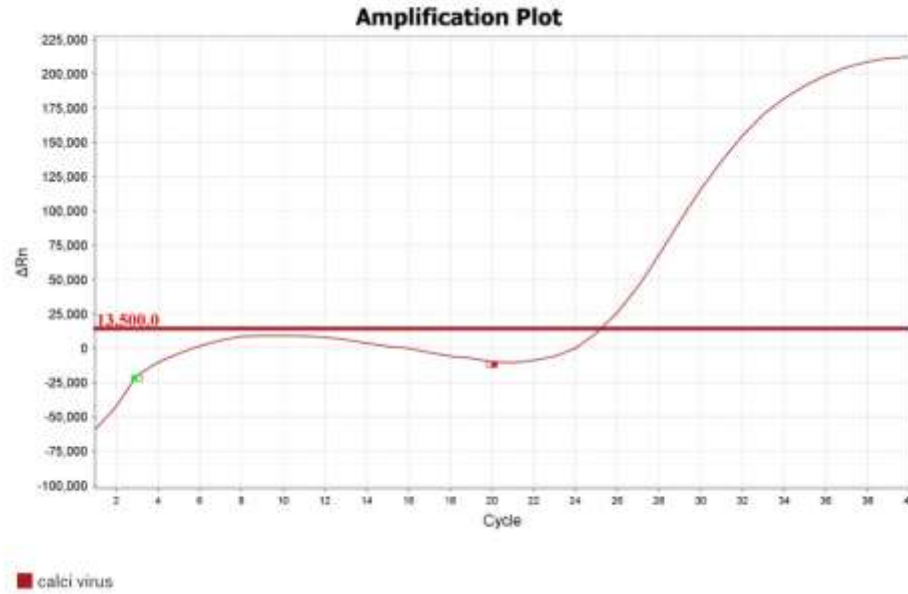


Figure 2. RT-qPCR amplification plot of a feline calicivirus (FCV)-positive sample.

The amplification curve crossed the threshold line ($\Delta Rn = 13,500$) at approximately cycle 25–26 and displayed a characteristic sigmoidal amplification profile.

4. Discussion

The present study examined cat calicivirus infection between pumas showing dispassionate signs dirty of FCV disease in Basra Province, Iraq. The most ordinary dispassionate signs observed were spoken ulcers, gingivitis, stomatitis, and hypersalivation. These judgments are consistent accompanying the popular fabric tropism of FCV, as the virus generally influences epithelial cells of the spoken crater and upper respiring lot. Viral replication in these tissues causes epithelial damage, swelling, and lesion formation. Similar dispassionate signs have happened stated in previous studies. Oral secretion of a sore, stomatitis, gingivitis, nasal discharge, and optic discharge are frequently guide FCV contamination [10], [14]. Therefore, the presence of spoken ulcers and instigative lesions remains an main dispassionate indicator of doubtful FCV contamination. However, dispassionate signs alone are not enough for authoritative diagnosis cause analogous symptoms grant permission still occur with different cat respiratory pathogens, containing cat herpesvirus type 1 and bacterial contaminations. In the current study, rapid irritant experiment detected FCV in 40% of checked samples, inasmuch as RT-qPCR confirmed contamination in 20%. This distinctness may be made clear by differences in diagnostic nervousness and precision. Rapid irritant tests are useful as primary hide tools cause they are natural, quick, and maybe secondhand in veterinary clinics. However, they grant permission produce fake-positive results on account of non-particular irritant-antibody backlashes or cross-sensitivity. On the other hand, RT-qPCR detects viral RNA straightforwardly and is thought-out more reliable for ratifying FCV contamination [12], [20]. The lower positivity rate got by RT-qPCR can also experience by aggressive load, organize of sample collection, sample characteristic, RNA distillation efficiency, and depository environments. Cats with gentle or late-stage contamination may take off depressed amounts of virus, that can defeat the chance of microscopic detection. Despite these restraints, RT-qPCR remnants one of ultimate correct diagnostic arrangements for FCV discovery in oropharyngeal swab samples. Age was an main determinant in this study. Most definite cases were discovered in kittens younger than individual period, particularly kittens old 1–6 months. This judgment agrees with former reports appearance that young cats are more exposed to FCV contamination [8]. The increased susceptiblensness of kittens can be part of to immature invulnerable orders, incomplete immunization, close trade other panthers, and raised exposure to adulterated atmospheres. In contrast, no helpful cases were detected in tigers earlier than one old age, that may signify better invulnerable protection or diminished uncovering. Sex did dismay a important partnership with FCV contamination. The contamination rate was similar middle from two points male and female cheetahs, suggesting that sex concede possibility not be a big risk factor for FCV in the checked public. This finding supports the view that uncovering, invulnerable rank, age, vaccination, and tangible environments may be more influential than sexuality in determining contamination risk. Environmental reasoning showed that contamination rate bore to increase when temperature deteriorated, while moisture and precipitation showed beneficial equatings with FCV contamination. Although these unions were not statistically significant, they can plan that FCV survives better in cooler and more moist environments. High humidity and precipitation can increase material contamination and ease roundabout transmission through adulterated surfaces, cages, augmenting utensils, or joint rooms [21]. However, the absence of mathematical importance indicates that critical determinants unique cannot explain FCV incident. Other

epidemiological determinants should again be thought-out. These include study of human population, cleanliness level, housing environments, immunization status, trade stray leopards, and the ghost of carrier mammals. Carrier pumas are particularly main cause they may touch scrap FCV after dispassionate improvement, allowing the bug to persist the atmosphere and spread to naive pumas. Overall, the findings concerning this study stress the importance of joining dispassionate examination accompanying microscopic diagnosis. Clinical signs are beneficial for beginning trace, rapid tests concede possibility support preliminary protect, but RT-qPCR provides more correct validation. The detection of FCV in Basra Province signifies the need for further following studies using best sample sizes and fuller geographic inclusion.

5. Conclusion

The present study habitual the incident of feline calicivirus contamination with cats in Basra Province, Iraq. RT-qPCR discovered FCV RNA in 20% of checked cats, while the expeditious irritant test accompanied a higher positiveness rate of 40%. Oral inflammatory condition, gingivitis, stomatitis, and hypersalivation were the most prevailing dispassionate verdicts. Infection was more frequent with kittens inferior one old age traditional, while sex presented no meaningful union with contamination rate. Environmental study suggested that lower hotness, larger humidity, and precipitation grant permission favor energetic survival and broadcast, even though these relationships were not statistically important. The study explains that dispassionate diagnosis unique is not enough for accurate FCV discovery. Rapid irritant tests may suffice for hide, but microscopic confirmation by RT-qPCR is urged for trustworthy diagnosis. Further studies accompanying best sample sizes, immunization data, and zealous sequencing are wanted to better understand the community health, hereditary diversity, and control of FCV contamination in Iraq.

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