



## A study on Feline Upper Respiratory Tract Disease with Clinico-molecular Profiling of the associated Feline Herpesvirus and Calicivirus Infections in Domestic Cats

Nedaa A. El-Zaky<sup>1</sup>, Atef F. Oreiby<sup>1, 2</sup>, Amin A. Tahoun<sup>1</sup>, Hazim O. Khalifa<sup>2, 3</sup>,  
Magdy H. Al-Gaabary<sup>1</sup> and Salama A. Osman <sup>\*1</sup>

<sup>1</sup>Department of Animal Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheik 33516, Egypt

<sup>2</sup>Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain, P.O. Box 1555, United Arab Emirates.

<sup>3</sup>Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt.

### Abstract

**T**HIS cross-sectional study aimed to investigate feline upper respiratory tract disease (FURTD) in domestic cats, with a focus on the associated feline calicivirus (FCV) and feline herpesvirus type 1 (FHV-1). To the best of our knowledge, there are no previous studies on FCV in domestic cats in Egypt, and studies on FHV-1 are very limited. A total of 850 cats were included, signs of FURTD were detected in 101 cats in a percentage of 11.9%. Significant variations were existed among different ages and breeds. PCR targeting FHV-1 and RT-PCR targeting FCV were performed for 30 diseased cats and showed ten and eleven positive cases in a percentage of 33.3% and 36.7%, respectively. Most of the molecular identified cases existed as a singleton infection, except of two cases of mixed infection in a percentage of 6.7%. Obvious respiratory signs were detected for both viruses with a notable ocular involvement of FHV-1 infected cases and Oral lesions and tongue ulcers of FCV infected cases. Five FHV-1 strains and three FCV strains were sequenced for phylogenetic analysis on both the genomic and protein levels which are presented in the results section. In conclusion, FURTD is prevalent among cats in the study area and FHV-1 and/or FCV infection exists in a considerable proportion of these cases which are associated with variable clinical signs and case fatality rates. In addition, phylogenetic analysis is important for traceability and detection of relationship between these pathogens.

**Keywords:** Feline, Herpesvirus, Calicivirus, Cats, Respiratory.

### Introduction

Cats provide companionship and emotional support, maintaining their health is of significant concern [1]. One of the most common health issues affecting them is Feline Upper Respiratory Tract Disease (FURTD), a multi-factorial condition primarily targeting the upper respiratory system. However, in severe cases, it can extend to the lower respiratory tract, posing a life-threatening risk [2].

The disease is a multi-factorial complex condition which usually results from the dynamic interaction between stress-induced immune-suppression, respiratory bacteria and viruses [3]. The primary cause is viral pathogens, of which feline herpesvirus type 1 (FHV-1), responsible for feline viral rhinotracheitis, and feline calicivirus (FCV) being the

most prevalent causative agents accounting for approximately 80-90% of cases [4]. The bacteria involved in such respiratory conditions were reported to carry antimicrobial resistance elements in pets as well as in respiratory diseases of other animal species [5,6]. The close contact between pets and humans facilitates the transmission of antimicrobial resistance [7]. The respiratory symptoms of such cases associated with FHV-1 and FCV vary from sub-clinical, mild to severe, however in advanced cases both viruses can become life-threatening [8]. The diseased cats can shed viruses throughout their lives and become persistent carriers [9]. The disease is more common in cat populations than individual household cats.

Differentiating between FURTD caused by FHV-1 and that caused by FCV can be challenging in the

\*Corresponding authors: Salama A Osman, E-mail: salama2068@yahoo.com, Tel.: 00201144174371

(Received 05 July 2025, accepted 27 August 2025)

DOI: 10.21608/ejvs.2025.400505.2949

©National Information and Documentation Center (NIDOC)

early stages, as both viruses cause similar symptoms, including upper respiratory distress and conjunctivitis [2,10]. However, FHV-1 infections can progress to severe conditions such as pneumonia, gastritis, and necrotizing bronchiolitis, sometimes leading to diffused fibrino-necrotic bronchopneumonia [11]. In addition, FHV-1 can cause Corneal ulcers are typically associated with ocular pain and cause visual impairment [12]. On the other hand, FCV infections are commonly manifest as oral ulcers and mild upper respiratory disease, though severe cases can lead to pneumonia, particularly in kittens [13]. FCV is also associated with conditions such as limping syndrome, paw and mouth disease, and virulent systemic disease [14]. Diagnosis is initially based on clinical signs and rapid test strips, followed by more powerful confirmatory tests such as serology, virus isolation, and PCR [15]. Molecular investigations and sequencing are pivotal for understanding virulence and epidemiology of such pathogens.

In Egypt, there is only very limited research investigations on FHV-1 such as that [16] who reported a prevalence of 11.4% in household cats. On the other hand, there are no studies on FCV in Egypt, and based on our knowledge this may be the first study in this respect. Therefore, the current study aimed to investigate FCV and FHV-1 associated FURTD in domestic cats in Egypt where studies are rare or lacking, focusing on their differential clinical presentations, risk factors, and molecular characteristics.

## **Material and Methods**

### *Animals*

A total of 850 cats of different ages, sex and breeds from three pet clinics to which pets arrive from different geographical regions of Kafr El-Sheikh (n=200) and Tanta (n= 650) districts were included in this study which was conducted from January to December 2024. The collected data included age, sex, breed, vaccination status, and presenting clinical signs.

### *Samples*

Out of the detected clinical cases, 30 (25 from Tanta and 5 cases from Kafr El-Sheikh districts) were randomly selected and either nasal or oropharyngeal was collected from each case. Each swab was immersed in 1% sterile phosphate-buffered saline (PBS), identified and stored at -20°C at the same clinic of collection for a maximum of two days before long-term preservation at -80°C in the laboratory.

### *Epidemiological and clinical Investigation*

Epidemiological parameters including morbidity rate, age and sex susceptibility, as well as the seasonality of cases were determined, and all

examined cats underwent a comprehensive clinical evaluation considering both respiratory signs and other signs which were found to co-exist in some of these cases [17].

### *RT-PCR based molecular detection for FCV*

Viral nucleic acid was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Germany). RT-PCR was performed using the primers listed in Table 1. A reaction of 25-μL was prepared containing 12.5 μL of Quantitect Probe RT-PCR Buffer (Qiagen, Germany), 1 μL of each primer (20 pmol), 0.25 μL of RT enzyme, 5.25 μL of nuclease-free water, and 5 μL of template RNA. Reactions were carried out using a Biometra T3 thermal cycler. The reverse transcription was conducted at 50°C for 30 minutes, primary denaturation at 95°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 55°C for 40 seconds, and 72°C for 50 seconds. A final extension step was performed at 72°C for 10 minutes.

PCR products were separated by electrophoresis in 1.5% agarose gel (Applichem, Germany) using 1X TBE buffer at room temperature and voltage gradient of 5V/cm. A GeneRuler 100 bp DNA ladder (Fermentas, Thermo Scientific, Germany) was used for fragment size determination. Gels were documented using an Alpha Innotech gel documentation system (Biometra), and results were analyzed.

### *Conventional PCR based molecular detection for FHV-1*

The DNA was extracted from the samples using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations and using the primers listed in Table 1. Briefly, 200 μl of the sample suspension was incubated with 20 μl of proteinase K and 200 μl of lysis buffer at 56°C for 10 min. After incubation, 200 μl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 μl of elution buffer provided in the kit. Reactions were carried out using a BiometraThermoblock. The primary denaturation was conducted at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 57°C for 40 seconds, and 72°C for 1.2 minutes. A final extension step was performed at 72°C for 10 minutes. The gel electrophoresis was like that of FCV.

### *DNA Sequencing Protocol*

A purified PCR product of five FHV-1 and three FCV bands were sequenced in the forward and reverse directions using an Applied Biosystems 3130 automated DNA sequencer (ABI 3130, USA). The sequencing reaction was performed with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Perkin-Elmer/Applied Biosystems, Foster City, CA;

Cat. No. 4336817), following the manufacturer's instructions. Sequence identity and homologous sequences were conducted through the BLAST® (Basic Local Alignment Search Tool) analysis [18] conducted against GenBank accessions.

#### *Phylogenetic Configuration:*

Phylogenetic analysis of the sequenced isolates was conducted using NGPhylogeny.fr (<https://ngphylogeny.fr/workflows/advanced/phyml-oneclick-imported-from-uploaded-file>). The three FCV isolates identified in this study (C1, C2, and C3) were compared with reference sequences from various countries, including GenBank ID DQ910789 (USA), OQ784660, OQ718383, MW658522, MW658526, and OP203937 (China), and ON595842 (Australia). Similarly, the five FHV isolates identified in this study (HP1–HP5) were compared with GenBank ID D30767 (Japan) and D42113 (Netherlands).

#### *Statistical analysis*

The statistical analysis was performed using Chi-square [19].

### **Results**

Out of the 850 examined cats, 101 exhibited respiratory signs of FURTD, representing an overall prevalence rate of 11.9%. Out of 650 and 200 examined cats, 86 and 15 exhibited respiratory signs of FURTD representing 13.2% and 7.5% of the examined cats in Tanta and Kafr El-Sheikh districts, respectively. Descriptive epidemiological parameters and signs of FURTD cases are shown in Table 3.

Out of the clinical cases, 11 cats were vaccinated with commercial vaccine including FCV, FHV-1, FPV and *Chlamydomydia felis* and presented only mild respiratory signs and all were completely recovered. The remaining 90 un-vaccinated cats displayed varying degrees of clinical signs and responses to treatment.

Out of the PCR investigated animals, 30 cats representing 3.5% out of the total FURTD cases, ten and eleven cases were positive for FHV-1 and FCV infections in a percentage of 33.3% and 36.7%, respectively. Singleton infection by FHV-1 and FCV was found in eight and nine diseased cats in a percentage of 26.7% and 30%, respectively. Mixed FHV-1/FCV infection was detected in two cases in a percentage of 6.7%. The epidemiological parameters of FHV and FCV confirmed cases are shown in Table 4.

Variable survival and case fatality rates were observed among cases infected with FHV-1, FCV, and their mixed infections: five, eight, and one animal survived, while three, one and one cases were died, corresponding to survival rates of 62.5%, 88.8%, and 50%, and case fatality rates of 37.5%,

11.2%, and 50% for the three aforementioned infection categories, respectively.

Variable clinical signs were evident among FHV-1 singleton-infection cases, FCV singleton-infection cases and mixed infection cases and are shown in fig. 4. For FHV-1 singleton-infection cases, the most common clinical signs that recorded were conjunctivitis, eye discharge and nasal discharge. Rough coat was recorded in four cases with percentage 50%. Dehydration and fever were recorded in three cases with percentage 37.5%. Loss vision and anorexia were recorded in two cases with percentage 25%. Sneezing, dyspnea, lateral recumbency, pale or congested mucus membranes and external parasites were recorded in one case with percentage 12.5%.

For FCV singleton-infection cases, the most common clinical signs that recorded were gingivostomatitis, oral ulcer, anorexia, fever and nasal discharge. Dyspnea was recorded in three cases with percentage 33.3%. External parasites and loss epithelial layer of tongue were recorded in one case with percentage 11.1%.

For the two mixed infection cases, the most common clinical signs that recorded were fever, conjunctivitis, eye discharge, gingivostomatitis, oral ulceration, and anorexia but external parasites, rough coat, ear pinnae drooping, dyspnea, and nasal discharge were recorded only in one case with percentage 50%.

The phylogenetic analysis of FCV and FHV-1 strains detected in this study is shown in Figure 1 and 2, respectively. Protein mutations of *VPI* gene from FCV strains, and *gD* gene from FHV-1 strains are shown in Table 2. In addition, the sequence types of detected FCV strains are recorded in GenBank under accession numbers PQ826459, PQ826460 and PQ826461 and those of FHV-1 under the accession numbers PQ623035, PQ623036, PQ623037, PQ623038 and PQ623039.

### **Discussion**

Respiratory diseases are a substantial healthcare concern worldwide and play a crucial role in morbidity but mortality more common in young kittens [8]. Although FURTD is a multi-factorial condition, FCV and FHV-1 are the major associated viral pathogens [20]. Feline upper respiratory tract disease is of high morbidity and can cause outbreaks especially in high-density cat populations. However, the disease does not usually cause high mortality, kittens and immune-compromised cats can suffer from severe disease which may result in death [21]. FURTD has a high morbidity rate and cats suffer from chronic, long-term form requires high cost for veterinary treatment so these cats exposed to euthanasia in shelters [9].

Compared with FURTD prevalence of 11.9% obtained in the current study, this prevalence is lower than the results which were observed previously in other countries where the prevalence were recorded with percentage of 28%, 25.8% and 39.04% in Canada, USA and China, respectively [4, 22, 23].

The Feline upper respiratory tract disease complex (FURTDC) is multifactorial, and the most frequent related pathogens are FHV, FCV, some bacteria as *Bordetella bronchiseptica*, *Mycoplasma felis* and *Chlamydia felis* may be involved [24]. The FURTDC associated clinical manifestations include ocular discharge, conjunctivitis, sneezing, nasal discharge that is serous, mucoid or mucopurulent, and ulcerations of the lips, tongue, gums, or nasal planum [24]. The cats infected with one of two viruses can become chronic carriers and can continue to shed the viruses for their lifetime [9]. Chronic sinusitis and recurrent upper respiratory tract bacterial infections can result from this chronicity, which can leave nasal passages permanently scarred [25]. Although some authors described the FURTD to be similar regardless of the specific infectious agent [8], etiological agent based clinical variations do exist. The FCV is associated mainly with upper respiratory signs; sneezing, nasal discharge, conjunctivitis and oral ulcers that typically found on the tongue, hard palate, or lips, which can cause significant discomfort and lead to drooling and anorexia. In addition, FCV is associated with limping syndrome in some cases that is characterized by lameness and fever occurring within short period after the oral or respiratory infection [26, 27]. However, the disease is more common and sever in kittens, the highly virulent systemic form of the disease (FCV-VSD) can cause distinct outbreaks particularly in adult cats with mortality rates up to 79% [28] and the associated clinical manifestations are systemic vasculitis and epithelial tissues destruction that leading to head and limbs edema, multiple skin and paw ulcers, jaundice and pneumonia [29]. For FHV-1 associated clinical signs, the typical form is associated with upper respiratory and ocular lesions with nasal discharge, sneezing, ocular discharge and conjunctivitis, but the corneal dendritic ulcers are considered pathognomonic for this disease [21].

In the present study, there were no association between upper respiratory tract affection and sex or season which were previously recorded in other studies, respectively [23, 30]. However, our results should be interpreted carefully because of the low number of cases.

There was an association with cats younger than one year. This finding indicates higher susceptibility of young cats to contract the disease which was reported previously [22]. The higher susceptibility of young cats to respiratory diseases is mainly because of the immature immune system, especially in very

young ages. In addition, young cats are usually facing the active infection of these pathogens for the first time in their life especially if they are non-vaccinated or born from non-vaccinated dams.

In the recent study, we found that FURTD was significantly associated with breed as high percentage were recorded in the Hybrid and Himalayan breeds in percentages of 46.7% and 3.8%, respectively. Breed susceptibility was reported also in other studies [31, 32].

The usual form of FCV is mild and self-limiting signs associated with high morbidity and low mortality. On the other hand, the severe signs of FCV infection are common in kittens [31]. The percentage of FCV positive cases in this study is 36.7%, which based on our knowledge is the first report of FCV in Egypt. Similar result was reported previously in China with percentages of 39.47 [34]. Lower percentage were reported in other studies: 23.46% in Southwestern China [35], 28.9% in Guangdong Province China [36] and 26% in Kunshan China [37]. This noticeable higher percentage of the current study may be because most of the cats involved were non vaccinated. However, higher result than that of this study were also reported in Thailand with percentage of 46.7% [38]. It depends on several factors related to cat immunity, endemicity level of the disease in the study area, the breed or age susceptibility of the involved cat.

The fatality rate among FCV infected cats with single infection was 11.2%. Although it is a disease of low mortality [33], relatively high mortality rates were reported in some studies in Australia, Italy and Ireland with mortality rates 39%, 67% and 86%, respectively [14, 28, 39]. Mortality rate variations between these studies may be related to the severity of the detected cases, where most of cases in the current study belonged to the typical oral self-limiting form, while those of other studies were virulent systemic FCV diseased cases (VS-FCV).

In the current study, infection by FCV was higher in males so the FCV infection was associated with the sex which was previously reported [31]. In contrary, previous study mentioned that the disease is non-sex-linked [40]. All the epidemiological findings of FCV as well as of FHV in the current study should be interpreted carefully because of the low numbers of the involved cases. FCV infection was significantly associated age and breed as the high proportions were recorded in Persian breed, and cats under one year of age. Other reports acknowledged this association [9, 41]. However, future wide scale studies are still required to investigate the breed susceptibility of these cases.

Fever and gingivostomatitis were the most common signs associated with FCV infection, similar result was noted previously [42]. The virus replicates in oropharynx and viremia can occur within three to

four days which is represented clinically by fever [43]. After its first replication, the virus spreads in different tissues of the body, and enter the target cells such as epithelial cells; typically, on the tongue with neutrophile infiltration and developed to vesicle then rupture leaving ulcer; and less common on joints and lung [44]. Feline junctional adhesion molecule A “fJAM-A” is the critical host cell receptor for FCV which present on epithelial cells of oral cavity, respiratory tract, and conjunctiva, epithelial cells in intercellular junctions and immune cells such as high express on platelets and low express on peripheral leukocytes (monocytes and dendritic cells). The receptor presents at the junction between epithelial and endothelial cell causing disruption, leakage and vesicular affection in oral cavity that is represented by oral ulceration [45].

Regarding FHV, nearly similar results to that of the current study (33.3%) were reported in other studies in China and USA with percentage of 32.2% and 28.3%, respectively [23, 31]. Lower values were also reported in China with a prevalence of 21.5% and 15.5%, respectively [37,42]. Higher result was reported previously with a percentage of 51.2% [41]. Several factors may influence the susceptibility of cats to FHV including immunity, vaccinal status, age, breed, degree of endemicity in the study area and the presence or absence of the predisposing factors which is reflected in the prevalence rate of each study.

Feline herpes virus 1 usually causes upper respiratory tract and ocular symptoms, deaths may be evident in young or immunocompromised adult cats. The case fatality rate was calculated in the current study to be 37.5%. In the same context, the mortality rate was recorded with percentage of 50% in kittens [46].

Our results revealed that FHV-1 were not significantly associated with sex and Similar result was reported previously [10], breed and Similar result was reported [9] and age and similar result was reported previously [31]. However, all epidemiological parameters of FHV should be interpreted with caution because of the limited number of cases.

Inflammation of eyes and the upper respiratory tract are the most common signs associated with FHV positive cases reported in this study. Similar result was reported previously [37]. FHV1 replicate in nasopharynx, nasal turbinate and conjunctival mucosa which lead to cell damage and necrosis then clinical signs associated with eye and upper respiratory tract appear such as nasal and ocular discharges, conjunctivitis, ocular ulcers and sneezing and secondary bacterial infection may be also involved in such cases [43].

The percentage of FCV and FHV1 co-infection was found in 6.7% of cases. Nearly similar result was

recorded previously in a percentage of 10% [47]. On the contrary, higher percentage of co-infection was recorded previously with a percentage of 31.5% [38]. In this study, the clinical manifestation associated with co-infected cases were fever, oral ulcer, conjunctivitis and ocular discharge but in late stage there were subnormal temperature and recumbency in dead cases.

Besides the phylogenetic relationship between the identified viral strains in the current study to other GenBank strains, there is a detectable variation in the similarity percentage on the protein level. Feline calicivirus C1 (accession number PQ826459) has the highest protein similarity percentage of 96% with a strain from Italy detected in 2022 (accession number WCB27221). While FCV C2 (accession number PQ826460) have the highest protein similarity +percent of 97% with a strain from China detected in 2023 (accession number WNH25311). For the third FCV strain detected in this study (FCV C3 - accession number PQ826461), it has the highest protein similarity percentage of 96% with a strain from Italy detected in 2022 (accession number UMB28143) which was related to limping syndrome. For FHV-1, Our isolates HP1 and HP2 (accession number PQ623035 and PQ623036) have a protein similarity percentage of 99% with a previously detected strain from a tiger in China in 2014 (accession number AHZ31390). While FHV-1HP3 strain (accession number PQ623037) is of 99% protein similarity with a previously detected strain from a domestic cat in USA in 2010 (accession number YP\_003331589). For FHV-1HP4 strain of the current study (accession number PQ623038), it has a protein similarity percentage of 75% with previously detected strain from a domestic cat in USA in 2010 (accession number YP\_003331589). For the fifth strain FHV-1HP5 (accession number PQ623039) is of a protein similarity percentage of 88% with a previously detected strain from a domestic cat in China in 2020 (accession number QQG63382). Detection of the phylogenetic relationships between different viral strains of the same species is very important to be conducted not only on the genomic sequence basis, but also on the protein basis which gives a deeper insight into the true mutations and deeper analysis.

### **Conclusion**

Feline upper respiratory disease is endemic and prevalent among domestic cat populations of different origins in the study area based on the current clinic-based study. In addition, FHV-1 and/or FCV infection and co-infection exist in a considerable proportion of FURTD cases which are associated with variable clinical signs and case fatality rates. General upper respiratory tract clinical signs were evident with tendency for ocular involvement in FHV-1 infected cases and a tendency for oral involvement in FCV infected cases. Finally,

phylogenetic analysis is important for traceability and detection of relationship between these pathogens. Future wide scale studies are still required for better characterization of this disease condition and its associated pathogens.

#### *Acknowledgement*

Not applicable.

#### *Funding Statements*

This research has no financial support.

#### *Conflict of interest*

The authors declare that there is no conflict of interest.

#### *Ethical of approval*

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt (Number: KFS-IACUC/284/2025).

**TABLE 1. Primer sequences, target gene and amplicon size.**

Target agent	Target gene	Primer sequence (5'-3')	Length of amplified product (bp)	Reference
These primers specific for FHV-1	gD gene	FHV-F: AACTGCCCTCCATTCTACTC	1269 bp	[48]
		FHV-R: TTGGTCCAGACTCCAACCTAT		
Feline calicivirus	VP1	FCV-F: ATGTGCTCAACCTGCGCTAA	848	[49]
		FCV-R: TCAGTGTCAGACATAAGGTGGT		

**TABLE 2. Protein mutation identified in the sequenced VP1 gene from FCV strains, and gD gene from FHV-1 strains**

Viral strains	Protein identities	Colset sequence ID:
C1	252/263(96%)	WCB27221
C2	251/259(97%)	WNH25311.1
C3	248/259(96%)	UMB28143.1
HP1	363/365(99%)	AHZ31390.1
HP2	288/289(99%)	AHZ31390.1
HP3	344/345(99%)	YP_003331589.1
HP4	277/368(75%)	YP_003331589.1
HP5	220/250(88%)	QQG63382.1

TABLE 3. Epidemiological parameter of FURTD among the examined cases.

Parameter	Total Examined cats	FURTD cases	%
<b>Total examined</b>	850	101	11.9%
<b>Gender</b>			
Male	465	57	12.3%
Females	385	44	11.4%
<b>Age</b>			
Under 1 year	484	76a	15.7%
Over 1 year	366	25b	6.8%
<b>Season</b>			
Autumn	262	41	15.6%
Winter	157	17	10.8%
Spring	256	16	6.3%
Summer	175	27	15.4%
<b>Breed</b>			
Balady	300	25a	8.3%
Persian	475	57a	12%
Hybrid	30	14b	46.7%
Himalayan	26	1a	3.8%
British short hair	19	4ab	21.1%

\* Statistically significant at  $p \leq .05$

Different letters of the same column are significantly differs.

TABLE 4. Epidemiological parameters of FHV and FCV confirmed cases.

Parameter	FHV cases (n=10)	%	FCV cases (n=11)	%
<b>Gender</b>				
Male	6	60%	8a	72.7%
Females	4	40%	3b	27.3%
<b>Age</b>				
Under 1 years	5	50%	8a	72.7%
Over 1 year	5	50%	3b	27.3%
<b>Breed</b>				
Balady	4	40%	3a	27.3%
Persian	6	60%	8b	72.7%

\*Statistically significant at  $p \leq .05$

(a,b): rows with different letters are Statistically significant

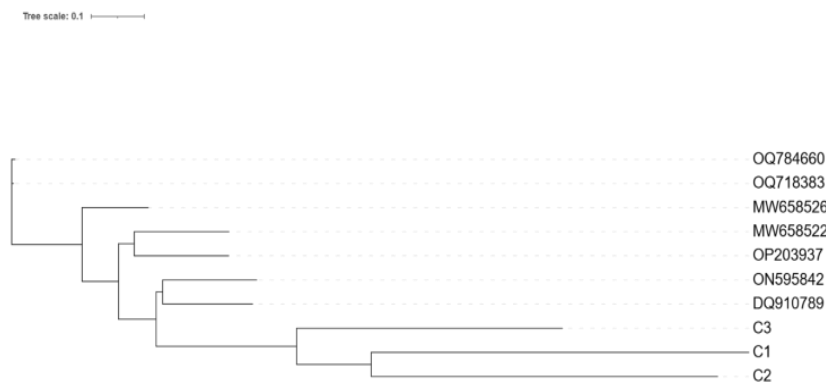
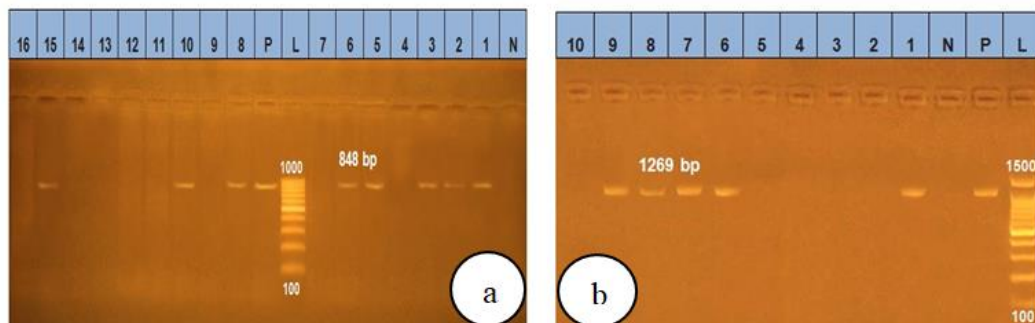


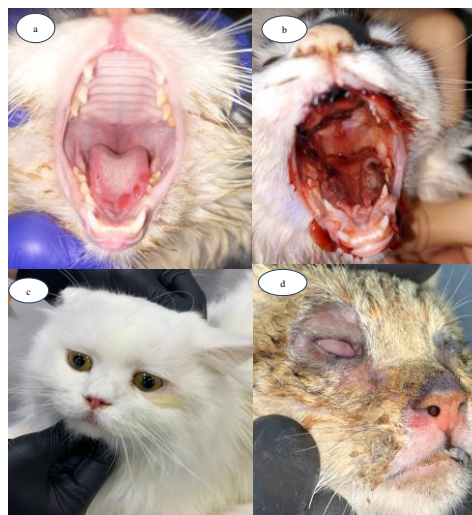
Fig. 1. Phylogenomic tree of the capsid protein VP1 gene from FCV strains C1, C2, and C3 identified in this study, along with seven publicly available FCV VP1 gene sequences retrieved from the NCBI database. Isolates C1 and C2 from this study are genetically closely related to each other but show distinct differences from the third strain.



**Fig. 2.** Phylogenomic tree of the gD gene from FHV-1 strains HP1, HP2, HP3, HP4 and HP5 identified in this study, along with two publicly available close sequence retrieved from the NCBI database. Isolates HP1, HP2 and HP5 from this study are genetically closely related to each other but show distinct differences from the other strains.



**Fig. 3.** conventional and Reverse transcriptase RT-PCR for FHV-1 and FCV detection, respectively. a) Reverse transcription-polymerase chain reaction (RT-PCR) analysis for the detection of Feline calicivirus (FCV) RNA in feline deep nasal or oropharyngeal swab samples. Lane L: 100–1000 bp molecular weight marker; Lane N: negative control (no cDNA template); Lane P: positive control; Lanes 1, 2, 3, 5, 6, 8, 10 and 15: clinical samples showing positive amplification bands (~848 bp). The presence of a 848 bp band indicates a positive result for FCV RNA. b) conventional PCR analysis for detection of feline herpes virus 1 (FHV-1) DNA in feline deep or oropharyngeal swab samples. Lane L: 100–1500 bp molecular weight marker; Lane N: negative control (no cDNA template); Lane P: positive control; Lanes 1, 6, 7, 8 and 9: clinical samples showing positive amplification bands (~1269 bp). The presence of a 1269 bp band indicates a positive result for FHV-1 DNA.



**Fig. 4.** Clinical manifestation of FHV-1 and FCV diseased cases: (a) a cat showing the typical presentation of FCV infection, with an ulcer at the root of tongue. (b) a severe case of FCV infection, characterized by a dropping the most of tongue epithelium. (c) a mild case of FHV-1 infection, with noticeable eye discharge. (d) a severe case of FHV-1 infection, where the eye is severely affected and damaged.

## References

1. Cao, N., Tang, Z., Zhang, X., Li, W., Li, B., Tian, Y. and Xu, D. Development and application of a triplex TaqMan quantitative real-time PCR assay for simultaneous detection of feline Calicivirus, feline parvovirus, and feline herpesvirus 1. *Frontiers in Veterinary Science*, **8**, 792322 (2022). <https://doi.org/10.3389/fvets.2021.>
2. Sykes, J.E. Pediatric feline upper respiratory disease. *Veterinary Clinics: Small Animal Practice*, **44** (2), 331–342 (2014).
3. Nguyen, D., Barrs, V.R., Kelman, M. and Ward, M.P. Feline upper respiratory tract infection and disease in Australia. *Journal of Feline Medicine and Surgery*, **21**(10), 973–978 (2019). <https://doi.org/10.1177/1098612X18813248>
4. Gourkow, N., Lawson, J.H., Hamon, S.C. and Phillips, C.J. Descriptive epidemiology of upper respiratory disease and associated risk factors in cats in an animal shelter in coastal western Canada. *The Canadian Veterinary Journal*, **54**(2), 132–138 (2013).
5. Khalifa, H.O., Oreiby, A., Abd El-Hafeez, A.A., Abd El Latif, A., Okanda, T., Kato, Y. and Matsumoto, T. High  $\beta$ -lactam and quinolone resistance of Enterobacteriaceae from the respiratory tract of sheep and goat with respiratory disease. *Animals*, **11**(8), 2258 (2021a). <https://doi.org/10.3390/ani11082258>
6. Khalifa, H.O., Oreiby, A.F., Okanda, T., Kato, Y. and Matsumoto, T. High  $\beta$ -lactam resistance in Gram-negative bacteria associated with kennel cough and cat flu in Egypt. *Scientific Reports*, **11**, 3347 (2021b). <https://doi.org/10.1038/s41598-021-8061-2>
7. Khalifa, H.O., Oreiby, A.F., Abd El-Hafeez, A.A., Okanda, T., Haque, A., Anwar, K.S. and Matsumoto, T. First report of multidrug-resistant carbapenemase-producing bacteria cohabiting mcr-9 associated with respiratory disease complex in pets: Potential of animal-human transmission. *Antimicrobial Agents and Chemotherapy*, **65**, e01890-20(2020). <https://doi.org/10.1128/aac.01890-20>
8. Cohn, L.A. Feline respiratory disease complex. *Veterinary Clinics: Small Animal Practice*, **41** (6), 1273–1289 (2011).
9. Binns, S.H., Dawson, S., Speakman, A.J., Cuevas, L.E., Hart, C.A., Gaskell, C.J. and Gaskell, R.M. A study of feline upper respiratory tract disease with reference to prevalence and risk factors for infection with feline calicivirus and feline herpesvirus. *Journal of Feline Medicine and Surgery*, **2**(3), 123–133 (2000). <https://doi.org/10.1053/jfms.2000.0084>
10. Dall'Ara, P., Labriola, C., Sala, E., Spada, E., Magistrelli, S. and Lauzi, S. Prevalence of serum antibody titres against feline panleukopenia, herpesvirus and calicivirus infections in stray cats of Milan, Italy. *Preventive Veterinary Medicine*, **167**, 32–38 (2019). <https://doi.org/10.1016/j.prevetmed.2019.03.010>
11. Breuer, W. and Hermanns, W. Acute herpesvirus-gastritis in a cat. *DTW. Deutsche Tierärztliche Wochenschrift*, **110** (4), 158–160 (2003).
12. Suga, Y. and Kirisawa, R. Isolation of the feline herpesvirus-1 modified live vaccine strain F2 from one of four cats with dendritic ulcers. *Journal of Feline Medicine and Surgery*, **27** (1), 1098612X241306954 (2025). <https://doi.org/10.1177/1098612X241306954>
13. Slaviero, M., Ehlers, L.P., Argenta, F.F., Savi, C., Lopes, B.C., Pavarini, S.P. and Sonne, L. Causes and lesions of fatal pneumonia in domestic cats. *Journal of Comparative Pathology*, **189**, 59–71 (2021). <https://doi.org/10.1016/j.jcpa.2021.09.005>
14. Bordicchia, M., Fumian, T.M., Van Brussel, K., Russo, A.G., Carrai, M., Le, S.J. and Barrs, V.R. Feline calicivirus virulent systemic disease: clinical epidemiology, analysis of viral isolates and in vitro efficacy of novel antivirals in Australian outbreaks. *Viruses*, **13**(10), 2040 (2021). <https://doi.org/10.3390/v13102040>
15. Kim, S.J., Park, Y.H. and Park, K.T. Development of a novel reverse transcription PCR and its application to field sample testing for feline calicivirus prevalence in healthy stray cats in Korea. *Journal of Veterinary Science*, **21** (5), e71, (2020). doi: 10.4142/jvs.2020.21.e71
16. Magouz, A., Lokman, M.S., Albrakati, A. and Elmahallawy, E.K. First report of isolation and molecular characterization of felid herpesvirus-1 from symptomatic domestic cats in Egypt. *Veterinary Sciences*, **9**(2), 81 (2022). <https://doi.org/10.3390/vetsci9020081>
17. Martin, S.W., Meek, A.H. and Willeberg, P. *Veterinary Epidemiology: Principles and Methods*. Iowa State University Press, Ames (IA), (1987).
18. Altschul, S.F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. Basic local alignment search tool. *Journal of Molecular Biology*, **215**(3), 403–410 (1990). [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
19. Snedecor, G.W. and Cochran, W.G. *Statistical Methods*, 8th ed., Iowa State University Press, Ames (IA), (1980).
20. Yan, M., Shang, J., Zhang, X., Wu, S., Wang, C., Wang, Z. and Feng, E. The establishment and application of a dual Nano-PCR detection method for feline calicivirus and feline herpesvirus type I. *Frontiers in Microbiology*, **14**, 1285268 (2023). <https://doi.org/10.3389/fmicb.2023.1285268>
21. Thiry, E., Addie, D., Belák, S., Boucraut-Baralon, C., Egberink, H., Frymus, T. and Horzinek, M. C. Feline herpesvirus infection. ABCD guidelines on prevention and management. *Journal of Feline Medicine and Surgery*, **11**(7), 547–555 (2009). <https://doi.org/10.1016/j.jfms.2009.05.003>
22. Aziz, M., Janeczko, S. and Gupta, M. Infectious disease prevalence and factors associated with upper respiratory infection in cats following relocation. *Animals*, **8**(6), 91 (2018). <https://doi.org/10.3390/ani8060091>
23. Yang, M., Mu, B., Ma, H., Xue, H., Song, Y., Zhu, K. and Gao, X. The latest prevalence, isolation, and molecular characteristics of feline herpesvirus type 1 in Yanji City, China. *Veterinary Sciences*, **11** (9), 417 (2024). <https://doi.org/10.3390/vetsci11090417>

24. Miller, L. and Zawistowski, S. *Shelter Medicine for Veterinarians and Staff*. John Wiley & Sons, 2nd ed., pp. 752 (2012).
25. Pedersen, N.C., Sato, R., Foley, J.E. and Poland, A.M. Common virus infections in cats, before and after being placed in shelters, with emphasis on feline enteric coronavirus. *Journal of Feline Medicine and Surgery*, **6**(2), 83–88 (2004). <https://doi.org/10.1016/j.jfms.2003.08.008>
26. Hurley, K.F. (2005). Virulent systemic feline calicivirus: recognition and control. In *Proceeding of the North American Veterinary Conference*, University of California, Davis, 2005.
27. Radford, A.D., Addie, D., Belák, S., Boucraut-Baralon, C., Egberink, H., Frymus, T., 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. *Journal of Feline Medicine and Surgery*, **11**(7), 556–564 (2009). <https://doi.org/10.1016/j.jfms.2009.05.004>
28. Caringella, F., Elia, G., Decaro, N., Martella, V., Lanave, G., Varello, K. and Buonavoglia, C. Feline calicivirus infection in cats with virulent systemic disease, Italy. *Research in Veterinary Science*, **124**, 46–51 (2019). <https://doi.org/10.1016/j.rvsc.2019.02.008>
29. Reynolds, B.S., Poulet, H., Pingret, J.L., Jas, D., Brunet, S., Lemeter, C. and Boucraut-Baralon, C. A nosocomial outbreak of feline calicivirus-associated virulent systemic disease in France. *Journal of Feline Medicine and Surgery*, **11**(8), 633–644 (2009). <https://doi.org/10.1016/j.jfms.2008.12.005>
30. Maboni, G., Che, S., Tallmadge, R., De Luca, E., Goodman, L.B., Weese, J.S. and Sanchez, S. Feline respiratory disease complex: insights into the role of viral and bacterial co-infections. *Frontiers in Microbiology*, **15**, 1455453 (2024). <https://doi.org/10.3389/fmicb.2024.1455453>
31. Fernandez, M., Manzanilla, E.G., Lloret, A., León, M. and Thibault, J.C. Prevalence of feline herpesvirus-1, feline calicivirus, *Chlamydomydia felis* and *Mycoplasma felis* DNA and associated risk factors in cats in Spain with upper respiratory tract disease, conjunctivitis and/or gingivostomatitis. *Journal of Feline Medicine and Surgery*, **19**(4), 461–469 (2017). <https://doi.org/10.1177/1098612X16634387>
32. Fujiwara-Igarashi, A., Ohshima, T., Kojima, R., Fujita, M., and Nakazawa, Y. Retrospective study of 540 cats with respiratory diseases in Japan (2003–2020). *Veterinary Medicine and Science*, **10**(3), 1456 (2024). <https://doi.org/10.1002/vms3.1456>
33. Berger, A., Willi, B., Meli, M.L., Boretti, F.S., Hartnack, S., Dreyfus, A. and Hofmann-Lehmann, R. Feline calicivirus and other respiratory pathogens in cats with feline calicivirus-related symptoms and in clinically healthy cats in Switzerland. *BMC Veterinary Research*, **11**, 282 (2015). <https://doi.org/10.1186/s12917-015-0595-2>
34. Ye, J., Li, Z., Sun, F.Y., Guo, L., Feng, E., Bai, X. and Cheng, Y. Development of a triple NanoPCR method for feline calicivirus, feline panleukopenia syndrome virus, and feline herpesvirus type I virus. *BMC Veterinary Research*, **18**, 379 (2022). <https://doi.org/10.1186/s12917-022-03460-9>
35. Zhou, L., Fu, N., Ding, L., Li, Y., Huang, J., Sha, X., Zhou, Q., Song, X. and Zhang, B. Molecular characterization and cross-reactivity of feline calicivirus circulating in Southwestern China. *Viruses*, **13**(9), 1812 (2021). <https://doi.org/10.3390/v13091812>
36. Mao, J., Ye, S., Li, Q., Bai, Y., Wu, J., Xu, L. and Li, S. Molecular characterization and phylogenetic analysis of feline calicivirus isolated in Guangdong Province, China from 2018 to 2022. *Viruses*, **14**(11), 2421 (2022). <https://doi.org/10.3390/v14112421>
37. Kim, S., Cheng, Y., Fang, Z., Liu, X., Zhongqi, Q., Weidong, Y. and Umar, S. Molecular epidemiology and phylogenetic analysis of feline calicivirus in Kunshan, China. *Virology Journal*, **21**, 50 (2024). <https://doi.org/10.1186/s12985-024-02319-9>
38. Phongroop, K., Rattanasrisomporn, J., Piewbang, C., Tangtrongsup, S., Rungsipipat, A. and Techangamsuwan, S. Molecular epidemiology and strain diversity of circulating feline calicivirus in Thai cats. *Frontiers in Veterinary Science*, **11**, 1377327 (2024). <https://doi.org/10.3389/fvets.2024.1377327>
39. Duclos, A.A., Guzmán Ramos, P.J. and Mooney, C.T. Virulent systemic feline calicivirus infection: a case report and first description in Ireland. *Irish Veterinary Journal*, **77**(1), 1 (2024). <https://doi.org/10.1186/s13620-024-00262-3>
40. Zicola, A., Saegerman, C., Quatpers, D., Viandier, J. and Thiry, E. Feline herpesvirus 1 and feline calicivirus infections in a heterogeneous cat population of a rescue shelter. *Journal of Feline Medicine and Surgery*, **11**(12), 1023–1027 (2009). <https://doi.org/10.1016/j.jfms.2009.05.023>
41. Nguyen, T.T. and Nguyen, Q.N. (2025). Prevalence of feline panleukopenia virus, feline herpesvirus and feline calicivirus infection in cats at the clinic, Ho Chi Minh City, Vietnam. *Advanced Animal and Veterinary Sciences*, **13**(2), 372–382. <https://dx.doi.org/10.17582/journal.aavs/2025/13.2.372.382>
42. Gao, J., Li, Y., Xie, Q., Al-Zaban, M.I., Al-Saeed, F.A., Shati, A.A. and Li, J. Epidemiological investigation of feline upper respiratory tract infection encourages a geographically specific FCV vaccine. *Veterinary Sciences*, **10**(1), 46 (2023). <https://doi.org/10.3390/vetsci10010046>
43. Gaskell, R. Feline respiratory disease. *Infectious Diseases of the Dog and Cat*, 97–106 (1998).
44. Hofmann-Lehmann, R., Hosie, M.J., Hartmann, K., Egberink, H., Truyen, U., Tasker, S., Belák, S., Baralon, C.B., Frymus, T., Lloret, A., Marsilio, F., Pennisi, M.G., Addie, D.D., Lutz, H., Thiry, E., Radford, A.D. and Möstl, K. (2022). Calicivirus infection in cats. *Viruses*, **14**(5), 937. <https://doi.org/10.3390/v14050937>

45. Pesavento, P.A., Stokol, T., Liu, H., van der List, D.A., Gaffney, P.M., and Parker, J.S. Distribution of the feline calicivirus receptor junctional adhesion molecule A in feline tissues. *Veterinary Pathology*, **48**(2), 361–368 (2011). <https://doi.org/10.1177/0300985810375245>
46. Maggs, D.J. Update on pathogenesis, diagnosis, and treatment of feline herpesvirus type 1. *Clinical Techniques in Small Animal Practice*, **20**(2), 94–101 (2005). <https://doi.org/10.1053/j.ctsap.2004.12.013>
47. Wang, D., Zhu, J., Yang, H. and Lyu, Y. Epidemiology and molecular characterization of feline calicivirus in Beijing, China. *Animals*, **15**(4), 494 (2025). <https://doi.org/10.3390/ani15040494>
48. Deng, M., Liang, H., Xu, Y., Shi, Q., Bao, F., Mei, C. and Huang, X. Identification, genetic characterization, and pathogenicity of three feline herpesvirus type 1 isolates from domestic cats in China. *Veterinary Sciences*, **11**(7), 285 (2024).
49. Navarro, C. Feline calicivirus. Molecular Detection with Primers Design. *EC Veterinary Science*, **5**, 54-72 (2020).

## دراسة حول عدوى الجهاز التنفسي العلوي للقطط مع الإشارة إلى الأعراض والتوصيف الجزيئي لفيروس هربس القطط وفيروس الكاليسي المرتبط بالمرض في القطط المنزلية

نداء عبدالعال الزكي<sup>١</sup>، عاطف فتحى عريبي<sup>٢</sup>، امين عبد الهادي طاحون<sup>١</sup>، حازم عمر خليفة<sup>٣</sup>، مجدي حسانين الجعبري<sup>١</sup> و سلامة احمد عثمان<sup>١</sup>

<sup>١</sup> قسم طب الحيوان، كلية الطب البيطري، جامعة كفر الشيخ، كفر الشيخ ٣٣٥١٦، مصر.  
<sup>٢</sup> قسم الطب البيطري، كلية الزراعة والطب البيطري، جامعة الإمارات العربية المتحدة، العين، ص.ب. ١٥٥٥، الإمارات العربية المتحدة.  
<sup>٣</sup> قسم علم الأدوية، كلية الطب البيطري، جامعة كفر الشيخ، كفر الشيخ ٣٣٥١٦، مصر.

### المخلص

هدفت هذه الدراسة إلى الكشف عن إصابة الجهاز التنفسي العلوي للقطط في حالات تم قبولها في ثلاث عيادات للحيوانات الأليفة في محافظتي الغربية وكفر الشيخ، مصر، مع التركيز على فيروس الكاليسيالقططي وفيروس الهربس القططي. ونتيجة لندرة الدراسات عن عدوى الهربس والكاليسي في القطط في مصر تم عمل هذه الدراسة. بفحص عدد ٨٥٠ قطة تم الكشف عن الإصابة بعدوى الجهاز التنفسي العلوي للقطط في ١٠١ قطة بنسبة ١١,٩%. لم تكن هناك أي اختلافات كبيرة في الجنس أو الموسم. من ناحية أخرى. كان هناك تباين كبير في حدوث المرض بين الحيوانات التي نقل أعمارها عن عام بنسبة ١٥,٧% مقارنة بالحيوانات الأكبر في العمر بنسبة ٦,٨%. كانت هناك اختلافات كبيرة لمعدل حدوث المرض بين السلالات مع وجود أعلى وأدنى نسب للحالات في السلالات الهجينة والهيماالايا بنسب ٤٦,٧% و ٣,٨% على التوالي. تم إجراء اختبار تفاعل البوليميراز المتسلسل لفيروس الهربس القططي النوع الأول وأيضاً لفيروس الكاليسي على عدد ٣٠ قطة مريضة وأظهر الاختبار وجود عشر وإحدى عشر حالة إيجابية بنسبة ٣٣,٣% و ٣٦,٧%، على التوالي. معظم الحالات التي تم تحديدها جزيئياً كانت موجودة كعدوى منفردة، باستثناء حالتين من العدوى المختلطة بنسبة ٦,٧%. تم الكشف عن علامات تنفسية واضحة لكلا الفيروسين مع إصابة عينية ملحوظة لحالات الهربس النوع الأول المصابة وأفات الفم وقرح اللسان لحالات فيروس الكاليسي المصابة. كانت معدلات الوفيات ٣٧,٥%، ١١% و ٥٠% من حالات الهربس النوع الأول المنفردة، وفيروس السنوري الكاليسي المنفردة وحالات العدوى المختلطة على التوالي. تم عمل تسلسل لخمس سلالات حالات الهربس النوع الأول وثلاث سلالات لفيروس الكاليسي للتحليل التطوري النسبي على كل من المستويات الجينومية والبروتينية. من خلال النتائج أتضح أن مرض إصابة الجهاز التنفسي العلوي ينتشر بين القطط في منطقة الدراسة وتوجد عدوى حالات الهربس النوع الأول و/أو فيروس الكاليسي في نسبة كبيرة من هذه الحالات التي ترتبط بعلامات سريرية متغيرة ومعدلات وفيات. بالإضافة إلى إن عمل التحليل التطوري هام جداً لإمكانية تتبع واكتشاف العلاقة بين هذه العوامل الممرضة.

**الكلمات الدالة:** القطط، فيروس الهربس، فيروس الكاليسي، القطط، الجهاز التنفسي.