Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Prevalence of *Feline Calicivirus* and *Feline Herpesvirus* -1 among Domestic Cats in Three Egyptian Governorates

Abeer M. Hagag[1], Noura F. AlKhalefa[1], Alaa M. Abdelatty[2], Bassiouni A. Heleil[3] and Asmaa F. Magouz[1]*

[1]*Department of Virology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt*.
[2]*Department of Pathology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt*.
[3]*Department of Theriogenology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt* .

Abstract

UPPER respiratory tract infections are major causes of feline morbidity and mortality. This study describes the prevalence of *Feline herpesvirus type 1 (FHV-1)* and *Feline calici virus (FCV)* among domestic cats in three Egyptian governorates. One hundred and twenty conjunctival or nasal swabs were obtained from diseased cats from private pet animal clinics and shelters located in three Egyptian provinces (Cairo, El Gharbia, and Kafrelsheikh). *FHV-1* isolation was done in the Chorioallantoic membranes (CAMs) of 13-days-old SPF eggs. Infected CAMs showed large white pock lesions. Polymerase chain reaction (PCR) of the thymidine kinase gene (TK) of *FHV-1* was positive in 34/120 (28.3%) of the suspected cases. In comparison, PCR amplification of ORF2 and ORF3 of *FCV* was successful in 32/120 (26.6%) of the suspected cases. Mixed infection with the two viruses were reported in 12/120 (10%) of the cases. Two *FHV1* genes were sequenced, and the TK gene sequences were identical to globally strains in the GenBank. For *FCV* one of the amplified genes were sequenced and the ORF2 and ORF3 genes sequences of the *FCV* isolates shared 81-87% similarity with other reference strains in the GenBank.

Keywords: *Feline Herpesvirus 1*, *Feline Calicivirus*, Cats, PCR, Egypt.

Introduction

Numerous infectious agents produce respiratory diseases in cats. Until effective immunizations were developed, viruses causing feline respiratory tract infections were catastrophic. Diseases caused by respiratory viral infections are most common in circumstances of immunologic impairment, poor husbandry, and overcrowding [1]. Upper respiratory tract disease (URTD) is a leading cause of sickness and mortality in kittens . One or more pathogenic agents are typically the cause of URTD, and infected cats frequently exhibit ocular and/or respiratory symptoms. *Feline herpesvirus 1 (FHV)* and *Feline calicivirus (FCV)* are the main viruses involved in the feline URTD. [2].

Feline rhinotracheitis, which is caused by *Feline herpesvirus type-1 (FHV-1)*, is thought to be the primary cause of ocular lesions in cats [3]. In kittens, pneumonia and high death rates were frequently reported [4]. Young cats have more fatalities and are more vulnerable to primary infections than adult cats [5-6]. Domestic cats are the principal hosts of *FHV-1*, but transmission can frequently occur between closely related hosts [7]. *Feline alphaherpesvirus 1* belongs to *Varicellovirus genus*, within the *Alphaherpesvirinae* subfamily of the *Orthoherpesviridae* family [7]. *Alphaherpesvirus's* full virion comprises of a double-stranded DNA core, a capsid, and an envelope. The nucleocapsid is enclosed in a tegument, which is then wrapped by a lipid envelope. The envelope consists of several glycoproteins that serve numerous functions. For example, glycoprotein D (gD) is principally responsible for receptor identification and attachment to host cells [8]. Three viral proteins have been identified as virulent: thymidine kinase (TK), gE, and gC [9]. Antigenically, all *FHV-1* isolates belong to one serotype, yet some strains have shown minor genetic differences [3]. *FHV-1* is transmitted directly by contacting diseased cats or indirectly through contaminated objects such as food, drink, and cages [10]. The reported incidence of *FHV-1* varies greatly

*Corresponding authors: Asmaa F. Magouz, E-mail: asmaamagouz@gmail.com, Tel.: +201005607748
(Received 19 May 2025, accepted 18 July 2025)
DOI: 10.21608/ejvs.2025.386486.2858

(from 2.6% to 63%), probably due to different sample sites [11]. *FHV-1* can infect nerve cells, rendering the animal a lifetime carrier. Stress can trigger reactivation and spread, so vaccination is critical to reducing the incidence and mortality rate [7]. Commercially available attenuated live vaccines and killed vaccines are generally safe and effective, but they do not prevent virus shedding. Magouz et al [3] published the first detection and molecular characterization of *FHV-1* in diseased cats from northern Egypt

Feline *calicivirus* (*FCV*) is a widespread infectious virus in cats. The prevalence is roughly related to the total number of cats in a group. It is low in cats living in small groups and high in larger cat groups [13]. *FCV* infection results in various clinical issues as it is frequently associated with oral ulcers, especially of the tongue, gingivitis–stomatitis, salivation[14], as well as mild upper respiratory symptoms with low mortalities [15]. *Feline calicivirus* belongs to the *Vesivirus* genus in the *Caliciviridae* family. It is a small, naked icosahedral virus with a positive-sense, single-stranded RNA molecule approximately 7.7 kilobases long [16]. *FCV* varies antigenically because of a continuous evolutionary process that involves the presence of punctate mutations and recombination [17]. *FCV's* remarkable genomic flexibility explains the evolution of numerous variations [16]. Because of the viral properties, which include significant genetic flexibility, strains with greater pathogenicity have a high chance of evolving, and several mutant strains have been discovered and reported. [18]. Cats with acute diseases shed *FCV* primarily through nasal secretions, although it can also be found in urine and fecal matter. Many cats shed for at least 30 days post-infection, and some for several years or even their entire lives. Vaccination is highly recommended; however, the potency of vaccines is influenced by the antigenic diversity of the virus [15]. These vaccines are monovalent, consisting of only one *FCV* strain, or bivalent, containing two strains, although they do not appear to provide good cross-protection against all field strains [19]. Molecular techniques have been developed to detect *FCV* and *FHV-1* genomes in nasal, oral, and conjunctival swabs. To our knowledge, *FHV-1* was detected and molecularly characterized in only one reported research in Egypt [3], while *FCV* has still not been detected or molecularly characterized in Egypt. The present study describes the isolation and molecular characterization of *FHV-1* and *FCV* in cats in Egypt, To investigate the prevalence of the viruses that cause URT illnesses on a large scale.

Material and Methods

Sample collection

One hundred and twenty nasal and conjunctival swabs were obtained from cats exhibiting symptoms at pet animal clinics across three Egyptian provinces: Cairo, El Gharbia, and Kafrelsheikh. The cats displayed respiratory symptoms such as fever, coughing, and serous nasal or eye secretions, conjunctivitis, and corneal and lingual ulcers (Fig. 1). Full details of examined cats and dogs are shown in (Table 1). Swabs were immersed in phosphate-buffered saline (PBS) tubes and transported to the Central Diagnostic Laboratory at the Faculty of Vet Med, Kafrelsheikh University. The samples were centrifuged at 12,000 rpm /15 min, and supernatants were kept at -80.

Virus isolation of Felid herpesvirus 1

Collected supernatants were antibiotic-treated with a stock solution of 100IU/ml penicillin and 100µg/ml streptomycin, then 200 µl were inoculated into the CAMs of 13-day-old SPF eggs [20]. The eggs were maintained at 37°C for a week and monitored twice daily. Embryo deaths within the first 24 hours post inoculation were discarded. After four blind passages, the CAMs were collected, and the cytopathic effect on CAM was evaluated. Infected CAMs were centrifuged, and the supernatants were stored at -20°C till utilized for PCR. Unfortunately, the isolation of *FCV* was not available in our lab.

DNA Extraction and PCR Amplification of (TK) gene of FHV1

Geneaid viral nucleic acid extraction kits (Taiwan) were used to extract viral DNA from 300 µl of nasal or conjunctival swabs, per the manufacturer's protocols. PCR assay for *FHV-1* was conducted by TOPsimple DyeMix-nTaq (enzynomics) (Korea). Primers used are shown in (Table 2) and were manufactured by Synbio Technologies (New Jersey, USA) [21]. The initial phase of PCR starts with denaturation at 94°C for 5 min, then 40X of denaturation at 95°C /1min, annealing at 56 °C /1 min and extension at 70°C/30 sec with terminal extension at 72°C /10 min. PCR bands were detected using 1.5 % gel electrophoresis using a 50 bp DNA marker (Dongsheng Biotech, China).

RNA Extraction and PCR Amplification of ORF2 and ORF3 genes of FCV

Viral RNA was extracted from 300 µl of nasal or conjunctival swab suspensions using Geneaid viral nucleic acid extraction kits (Taiwan) per the producer's protocol. PCR (TOPscript^{TM} RT Dry MIX, Korea) was used to amplify the ORF2 and ORF3 genes of *FCV*. The PCR assay used TOPsimple DyeMix-nTaq (enzynomics) (Korea). Primers used are shown in (Table 2) and were created by Synbio Technologies (New Jersey, USA) [22]. The first PCR step was an initial denaturation at 95°C for 10 min, then 40 X of denaturation at 95°C /30 sec, annealing at 57 °C /30 sec, and extension at 72°C/30 sec with a terminal extension step at 72°C /5

min. PCR bands were detected by 1.5 % gel electrophoresis using the 50 bp DNA marker (Dongsheng Biotech, China).

Histopathology

Feline herpes virus 1 infected Chorioallantoic membranes (CAM) were aseptically harvested for histopathological examination. The harvested CAMs were fixed at once in 10% neutral buffered formalin for 2 days before being routinely processed by dehydration, embedding in paraffin wax, sectioning at 5 µm thickness, and then staining with hematoxylin and eosin (H&E). The sections were examined by light microscope for the detection of viral cytopathic effect.

Electron microscope

Fragments of specimens of the *FHV-1* infected CAMs were immediately immerged in 4F1G in PBS at 4° C for 3 hours for fixation. Then specimens were then post-fixed in 2% OsO_4 at 4°C / 2 hours. The sample was washed in the buffer and dehydrated at 4 °C through an aggraded series of acetone, then soaked in resin to polymerize, then put in the oven at 60 °C for 2 days. Then, the samples were cut into tiny fragments about 90 angstroms thick. Sections were arranged on a grid cobber, stained with uranyl acetate for about 6 minutes followed by lead citrate for 2 minutes [23].

DNA sequencing and Phylogenetic analysis

PCR products of the TK gene of two *FHV-1* isolates and ORF2 and ORF3 genes of *FCV* of one sample were purified and sequenced by Macrogen Clinical Laboratory (Korea). Blast analyses (BLASTn) were performed to create gene homology to GenBank strains. The nucleotide sequences were aligned by the CLUSTAL W tool of MEGA X software. MEGA X software was used to apply a phylogenetic tree using the maximum composite likelihood model with 1000 bootstrap replicates and the neighbor-joining method. [24].

Results

Virus isolation

Infected CAMs showed oedema and thickening with pock lesions at the 6^{th} – 8^{th} days after inoculation Fig 2.

Histopathological findings

The microscopic examination of the H&E-stained infected CAM sections revealed the presence of pathognomonic features of *herpes* infection. As shown in Fig.3A, eosinophilic intranuclear inclusion bodies were observed in the epithelial cells of CAM. These inclusions appeared as homogenous, dense, rounded structures inside enlarged nuclei surrounded by an obvious halo space. Also, multinucleated giant cells (syncytia) were frequently observed in (Fig.3B).The epithelium, further, exhibited varying degrees of cellular degeneration, including cellular vacuolation and focal cell necrosis.

Electron microscope

The presence of *FHV-1* virions was confirmed in infected CAMs using electron microscopy revealed spherical virions with a diameter of about 200 nm (Fig.4)

Molecular identification by PCR

Nasal and/or conjunctival swabs of 34 samples were positive for a 293 bp band of the Thymidine Kinase gene, with a prevalence rate of (28.3%)and 32 samples were positive for *FCV* by amplification of a 324 bp band corresponding to a section of the ORF2 and ORF3 genes with a prevalence rate of (26.6%) **(**Fig. 5).

Most of positive *FHV-1* and FCV cases were from baladi breed cats, about 15 baladi cats are positive for FHV-1(15/34) (44.1%) and 13 baladi cats are positive for FCV (13/32) (40.6%), prevalence rate of infection is high in baladi breed cats as most of them are not vaccinated against these viruses.

Positive non vaccinated cats infected with FHV-1 from different breeds of cats were 18 samples (18/34) (52.9%) and for FCV was about 10 samples(10/32) (31.3%), most of these positive cases show the typical respiratory symptoms such as fever, coughing, serous nasal or eye secretions, conjunctivitis, and FCV cases are characterized by presence of lingual ulcers.

Most of positive cases for *FHV-1* and FCV were more than 12 months of age.

Sequencing and phylogenetic analysis

Alignment and Phylogenetic analysis of the TK gene of isolates *(FHV1Egypt/2025/1)* and *(FHV1Egypt/2025/2)* showed 99.95% similarity with other isolates (strain KANS_10, USA,2018 /strain MILW_12, USA,2018 /strain FHV-1Tur/Kays18, Turkey,2021 /strain LOU-15, USA,2023/isolate 3234-05, Australia,2016 /isolate Yasuharu Suga: A-19-5, Japan, 2025) Fig.6.

Alignment and Phylogenetic analysis of the ORF2 and ORF3 genes amplicon of the isolate *(FCV Egypt/2025)* revealed 92% similarity with Chinese strain (isolate *FCV*32-BJ-2023),91% with the Taiwan strain (NTU/C1463/2023), 87% with the Italian strain (*FCV*/81/2014/ITA) and Chinese strain (isolate TFHLJ-8) While it shared only 81% similarity with the Australian strain (E1/V2/NSW5/Bella) Fig.7

Discussion

Feline upper respiratory infection is a common illness among cat populations. It is mostly caused by

FCV and *FHV-1*, which account for about 90% of the cases, with the remainder caused by bacterial infections. *FCV* infections are generally associated with oral lesions, while *FHV* infections were more frequently associated with nasal and ocular discharges [25]. The clinical symptoms of *FHV-1* and *FCV* are nearly similar. However, *FHV-1* infections are typically indicated by ocular lesions, whereas *FCV* infections are marked by palate and tongue ulcers [3]. *Feline herpesvirus* is a highly contagious infection extensively dispersed globally, resembling a significant hazard to feline populations [26]. Our investigation examined nasal and conjunctival swabs for *FHV* and *FCV* using PCR. The PCR detection frequency for *FHV1* was 28.3%, which is more than that of an earlier study of *(FHV-1)* isolation in cats in Egypt in 2022 (11.4%) [3] and reports in China from 2016 to 2019 (16.3%) and in Southern Italy (9.05%). Lower detection rates were reported in China in 2024 (71.4%) [27]. PCR detection rate for *FCV* was 26.6%, which is higher than studies in Thailand (1.85%) [26] and studies in southwestern China (23.46%) [28]. This wide variation may be explained by the fact that detection rates vary according to the population under study, sample type, detection method, and infection status [3], and the reasons may be also that the nasal and conjunctival swabs used in the current study were preliminary from cats that show the typical symptoms of the disease. Considering that the incidence of *FCV* is generally connected with crowding problems and that higher infection rates occur when a lot of cats are housed together, the high rate of FCV may be explained by the fact that many FCV-positive samples were taken from shelters. As a result, the infection incidence for pet cats housed in low-density environments is often low [28-29]. Conversely, cats in large populations typically have a higher risk of infection (between 25 and 40 percent) [28-30]. Four of the FCV-infected cases in this study tested positive for the virus even after receiving vaccination; this could be explained by the fact that many cats continue to shed after recovering, usually for a month after infection, and some for even their entire lives because it can be detected in asymptomatic carriers [32]. Mixed infections with *FHV -1* and *FCV* are commonly detected in feline medicine. Our study reported 12/120 (10%) cases of *FHV1* and *FCV* co-infection via PCR detection. The overall detection rate in our study corresponds to other studies on mixed infection of these viruses. Based on the population tested, the presence of clinical symptoms, and the diagnostic techniques employed, reported co-infection rates might vary greatly between studies (from a few percent to approximately 10–20% of cats) [31-33]. A shelter-based study in Belgium reported approximately 10% of cats actively co-infected with both viruses at the same time [31]. Despite the lower overall prevalence in pet homes, the UK study noted that nearly 50% of the *FHV-1* positive cats (5 of 9 cats) were shedding *FCV*, emphasizing that even in closely monitored home environments, co-infections can still happen [32]. Generally a higher incidence of *FHV-1* and *FCV* has been associated with group living and dense housing. Crowding is repeatedly identified as a risk factor for infection in outbreak investigations and epidemiologic surveys; endemic circulation of both viruses is frequently observed in large multi-cat colonies and shelters [34], making mixed *FHV/FCV* infections an epidemiologically significant issue in cat populations. Sequencing of *FHV1* DNA revealed 99.59% homology with other isolates (strain KANS_10, USA,2018/strain MILW_12, USA,2018/strain *FHV-1*Tur/Kays18, Turkey,2021/strain LOU-15, USA,2023/isolate 3234-05, Australia,2016/isolate Yasuharu Suga: A-19-5, Japan,2025) and most of the reference *FHV-1* isolates in the GenBank database. The sequences of the TK gene obtained from the present samples and those of reference isolates did not vary significantly, which is noteworthy. Overall, our results indicate that *FHV-1* genomes are extremely conserved, as reported by other studies which stated that all *FHV-1* strains belong to the same serotype and are genetically similar and have a great identity level [35]. FHV-1 has been shown to contain 23 virion associated proteins.Four viral proteins have been considered as virulence factors: thymidine kinase (TK), serine/threonine protein kinase (US3), gE, and gC [3]. The target segment is in a greatly conserved area, and the TK gene is considered a highly conserved gene, as an initial step in the development of recombinant FHVs for use in the vaccination of cats was by identifying the thymidine kinase (TK) gene of this feline-specific alphaherpesvirus, a recombinant FHV bearing a deletion in the identified TK gene was constructed and shown to possess the expected TK- phenotype. The FHV TK gene is located at a position of approximately 40% in the long unique component of the FHV genome [21]. However, more investigation has discovered discrepancies across *FHV-1* isolations, such as rearrangements in the (gC) gene [3]. While sequencing the ORF2 and ORF3 genes sequences of one of the *FCV* samples revealed only 81-87% similarity with other isolates in the GenBank, which is explained by the *FCV*'s exceptional genetic flexibility, resulting in the emergence of several variants [16].

For the isolation and identification of *FHV-1*, swab suspensions were cultivated in chick embryos via CAM like other *Herpesviruses* [36]. Infected CAMs showed oedema and thickening with white pocks, which are typical of *FHV-1*, while isolation of *FCV* was not available in our lab because it is best isolated in feline kidney cell line CRFK (Crandell-Rees Feline Kidney) [39] which was not present in our lab ,so it is considered a limitation of the present study, and our recommendation is to have more

further studies on causes of feline viral respiratory diseases specially FCV.

Histopathological examination of chorioallantoic membrane (CAM) inoculated with *feline herpes virus* revealed standard cytopathic figure emulating the nature of *alphaherpesviruses.* The presence of intranuclear eosinophilic inclusion bodies and multinucleated giant cells (syncytia) proves the active replication and cytolytic potential of the virus. Like other *herpesviruses*, *feline herpesvirus* is known for its nuclear replication cycle, leaving its fingerprint inclusion bodies, which indicates the accumulation of DNA of the virus, protein, and cellular debris inside the nucleus [37]. On the other hand, multinucleated syncytial cells are a well-observed outcome of *herpesvirus* infection, which induces cell fusion**.** This acts as a mechanism the virus uses to spread between cells without being exposed to extracellular immunity [38].

Conclusion

Upper respiratory tract infections are major causes of feline morbidity and mortality, for this reason studies and researches about the causative agents specially viruses as *FHV-1* and *FCV* is very important to control these diseases. *FHV-1* and *FCV* are widespread in Egypt in spite of sporadic vaccination. *FCV* needs further studies on isolation and identification as the present study has a limitation in this part. The observed prevalence rates in this study indicates the significance of *FHV1* and *FCV* as a primary etiological agents of upper respiratory tract infections in cats.

Mixed infections with *FHV -1* and *FCV* are commonly detected in feline medicine and this needs a comprehensive diagnostic approaches in clinical cases, vaccination programs and biosecurity measures in clinics, shelters and other breeding environments. There are different methods for detection of these viruses and PCR is considered as a rapid diagnostic method.

The present study describes the isolation and molecular characterization of *FHV-1* and *FCV* in cats in Egypt, which underlines the necessity of further research on feline respiratory diseases, particularly *FCV* in domestic cats. To investigate the prevalence of the viruses that cause URT illnesses on a large scale, more research is needed.

Acknowledgments

The authors thank Dr. Mai Teba for her kind support and efforts in samples collection from her private pet clinic.

Funding statement

Self-funding.

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, Kafr El-Sheikh University, Egypt (approval number KFS – 2024).

TABLE 1. Details of examined samples (bread, age and sex, vaccination and clinical signs)

Number of samples	Breed	Age(months)	Sex	Vaccination status
5	Egyptian mau	Less than 6	Female	Not vaccinated
6	Egyptian mau	6:12	Female	Not vaccinated
2	Egyptian mau	Less than 6	Male	Not vaccinated
2	Egyptian mau	6:12	Male	Not vaccinated
6	Shirazi(Persian)	More than 12	Male	Not vaccinated
4	Shirazi(Persian)	Less than 6	Male	Not vaccinated
9	Shirazi(Persian)	6:12	Male	vaccinated
2	Shirazi(Persian)	Less than 6	Female	Not vaccinated
2	Mixed breed	Less than 6	Female	Not vaccinated
2	Mixed breed	More than 12	Female	Not vaccinated
4	Baladi (Native)	Less than 6	Female	vaccinated
32	Baladi (Native)	Less than 6	Female	Not vaccinated
3	Baladi (Native)	6:12	Female	Not vaccinated
4	Baladi (Native)	More than 12	Female	Not vaccinated
2	Baladi (Native)	Less than 6	Male	vaccinated
27	Baladi (Native)	Less than 6	Male	Not vaccinated
4	Baladi (Native)	6:12	Male	Not vaccinated
4	Baladi (Native)	More than 12	Male	Not vaccinated

TABLE 2. Oligonucleotide primers used in the study

virus	Primer name	Sequence	Target Gene	Amplified product	Reference
Feline herpes	FHV-F	GACGTGGTGAATTATCAGC	TK gene	293bp	[21]
	FHV-R	CAACTAGATTTCCACCAGGA			
Feline Calici	CAL-F-8	CTGCCTCCTACATGGGAAT	ORF2	324 bp	[22]
	CAL-R-8	GTGTATGAGTAAGGGTCRACCC	ORF3		

F= forward, R= reverse primer, TK=Thymidine Kinase

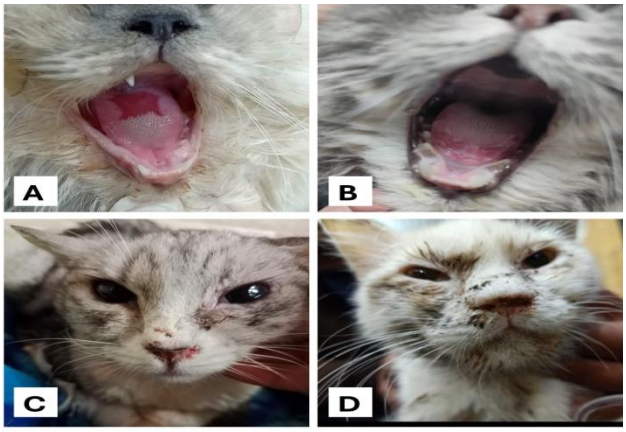

Fig. 1. Cats show signs of FHV-1 and FCV. (A) and (B) lingual erosions and ulcerations in cats infected with FCV. (C) and(D) Nasal and ocular signs of cats infected with FHV-1.

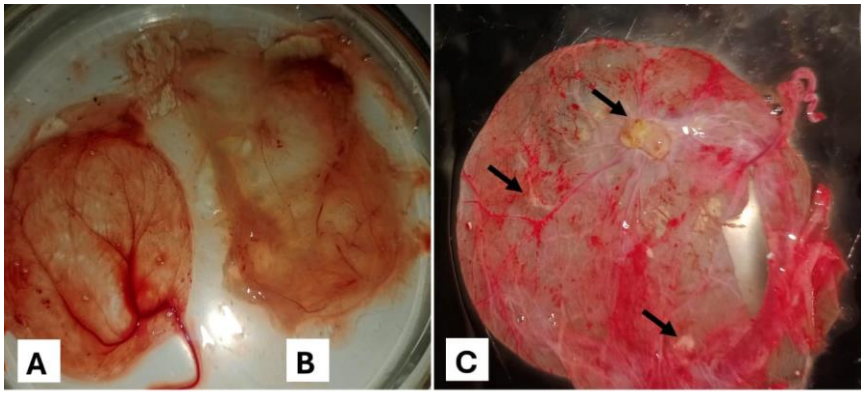

Fig. 2. FHV-1 isolation in chick CAM. (A) Normal non infected CAM. (B) Infected CAM showing cloudiness and thickening, (C) White pock lesions on infected CAM (arrows).

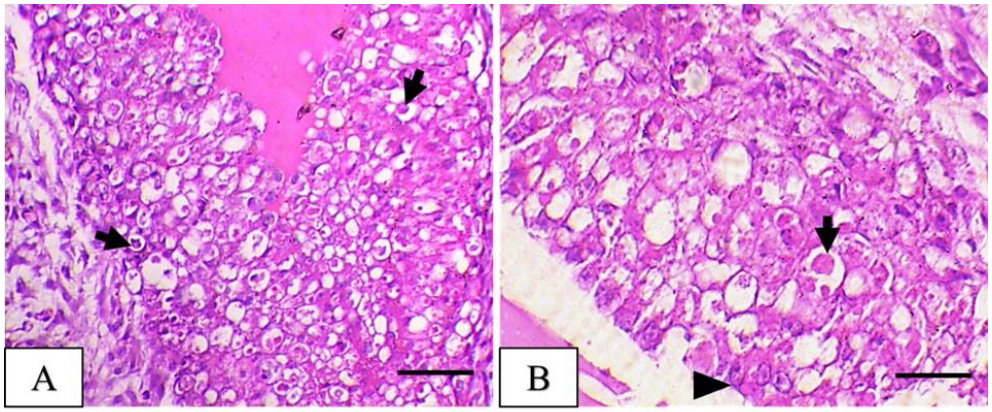

Fig. 3. Histopathological picture of chorioallantoic membrane (CAM) of embryonated chicken eggs inoculated with feline herpesvirus. (A) Eosinophilic intranuclear inclusion bodies (arrows) are evident within the epithelial cells. Stain H&E , Scale bar= 100µm.(B) Formation of multinucleated giant cells (syncytia) (arrowheads) and the presence of intranuclear inclusion bodies (arrow). Stain H&E. Scale bar = 50µm.

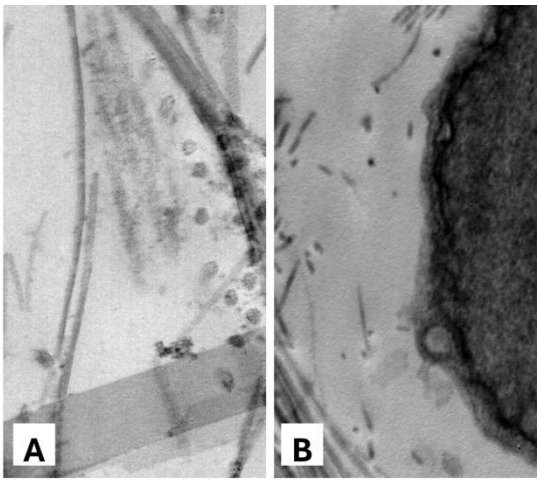

Fig. 4. Electron microscopy of chorioallantoic membrane of embryonated chicken eggs inoculated with FHV-1. (A) FHV-1 virions in the infected CAMs. (B) spherical FHV-1 particles with vesicle membrane with a diameter of about 200 nm.

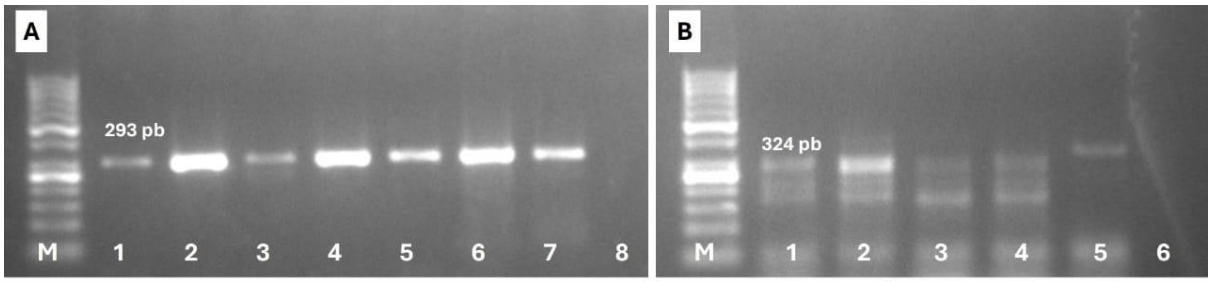

Fig 5. Gel electrophoresis photos of PCR.(A) Detection of TK gene of FHV-1 at 293 bp, (B) Detection of ORF2 and ORF3 genes of FCV at 324 bp, M= 50 bp DNA marker.

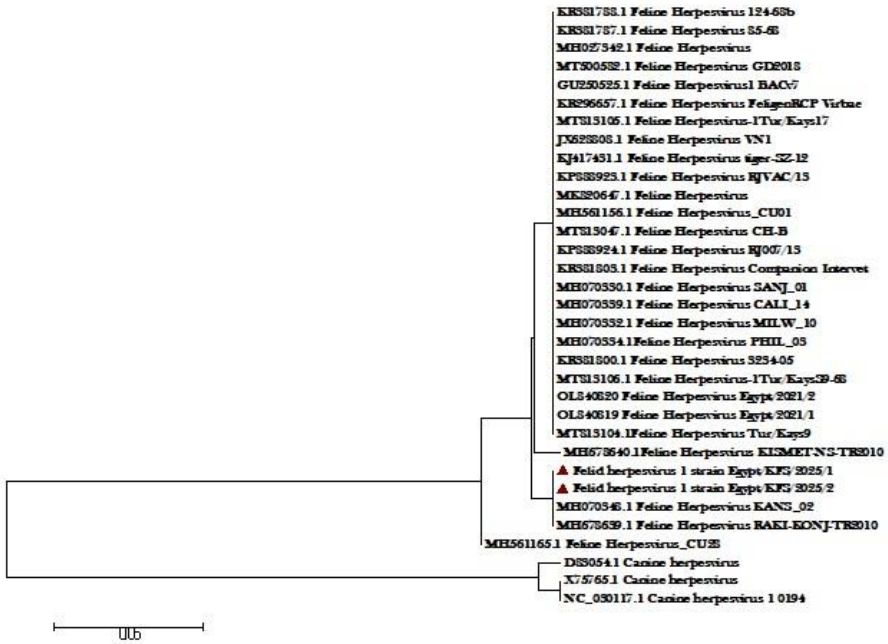

Fig. 6. Phylogenetic tree based on the partial TK gene nucleotide sequences of FHV-1. The isolates of this study is indicated by red triangles. The tree was conducted with the neighbour-joining method and the maximum composite likelihood model with 1000 bootstrap repeats using MEGA X software.

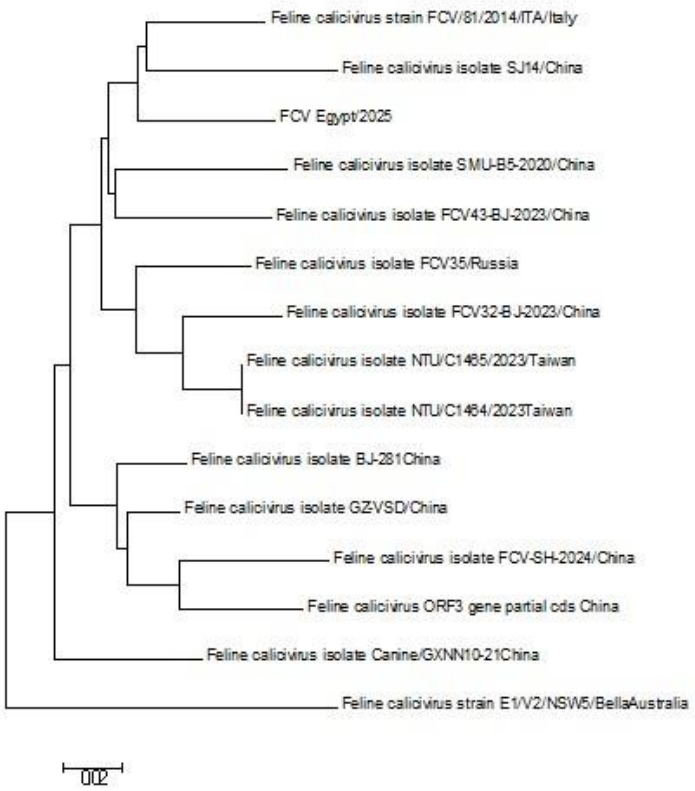

Fig. 7. Phylogenetic tree based on the partial ORF2 and ORF3 genes nucleotide sequences of FCV. The tree was conducted with the neighbour-joining method and the maximum composite likelihood model with 1000 bootstrap repeats using MEGA X software.

References

1. Moise, N.S., Viral Respiratory Diseases, Veterinary Clinics of North America. *Small Animal Practice*, **15**(5), 919-928(1985). https://doi.org/10.1016/S0195-5616(85)50103.
2. Xiao, X., Hao, X., Chen, B., Zhou, P. and Li, S., Two Multiplex PCR Methods for Detecting Several Pathogens Associated with Feline Respiratory and Intestinal Tracts. *Vet. Sci*., **10**(1),14(2022). doi: 10.3390/vetsci10010014. PMID: 36669015; PMCID: PMC9867166.
3. 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. *Vet. Sci*., **9**, 81(2022). https://doi.org/10.3390/ vetsci9020081
4. Holst, B.S., Berndtsson, L.T. and Englund, L., Isolation of feline herpesvirus-1 and feline calicivirus from healthy cats in Swedish breeding catteries. *J. Feline. Med. Surg*., **7**(6),325-31(2005). doi: 10.1016/j.jfms.2005.03.002. PMID: 15914057; PMCID: PMC10822419.
5. Yang, M., Mu, B., Ma, H., Xue, H., Song, Y., Zhu, K., Hao, J., Liu, D., Li, W. and Zhang, Y., The Latest Prevalence, Isolation, and Molecular Characteristics of Feline Herpesvirus Type 1 in Yanji City, China. *Vet. Sci.*, **11**, 417(2024). https://doi.org/10.3390/vetsci11090417
6. Maeda, K., Horimoto, T. and Mikami, T., Properties and functions of feline herpesvirus type 1 glycoproteins. *J. Vet. Med. Sci.*, **60**(8), 881-888(1998), doi: 10.1292/jvms.60.881.
7. Duan, S.H., Li, Z.M., Yu, X.J. and Li, D., Alphaherpesvirus in Pets and Livestock. *Microorganisms*, **13**(1), 82(2025). doi: 10.3390/microorganisms13010082.
8. Ning, Y., Huang, Y., Wang, M., Cheng, A., Yang, Q., Wu, Y., Tian, B., Ou, X., Huang, J., Mao, S., Sun, D., Zhao, X., Zhang, S., Gao, Q., Chen, S., Liu, M., Zhu, D. and Jia, R., Alphaherpesvirus glycoprotein E, A review of its interactions with other proteins of the virus and its application in vaccinology. *Front Microbiol*., **13**, 970545 (2022). doi: 10.3389/fmicb.2022.970545.
9. Yang, D.K., Kim, H.H., Park, Y.R., Yoo, J.Y., Choi, S.S., Park, Y., An, S., Park, J., Kim, J. and Kim H.J., Isolation and molecular characterization of feline herpesvirus 1 from naturally infected Korean cats. *J. Bacteriol. Virol*., **50**, 263-272(2020),.
10. Kim, S., Cheng, Y., Fang, Z., Zhongqi, Q., Weidong, Y., Yilmaz, A., Yilmaz, H. and Umar, S., First report of molecular epidemiology and phylogenetic characteristics of feline herpesvirus (FHV-1) from naturally infected cats in Kunshan, China. *Virol. J.*, **21**(1),115(2024). doi: 10.1186/s12985-024-02391-1.

11. Walter, J., Foley, P., Yason, C., Vanderstichel, R., Muckle, A., Prevalence of feline herpesvirus-1, feline calicivirus, Chlamydia felis, and Bordetella bronchiseptica in a population of shelter cats on Prince Edward Island. *Can. J. Vet . Res*., **84**(3),181-188(2020).

12. Kratzer, G., Lewis, F.I., Willi, B., Meli, M.L., Boretti, F.S., Hofmann-Lehmann, R., Torgerson, P., Furrer, R. and Hartnack, S., Bayesian network modeling applied to feline calicivirus infection among cats in Switzerland. *Front. Vet. Sci*., **7**, 73(2020).

13. Fastier, L.B., A new feline virus isolated in tissue culture. *Am. J. Vet. Res*., **18**(67), 382-389(1957). PMID: 13424932.

14. Hurley, K.F. and Sykes, J.E., Update on feline calicivirus: new trends. *Vet. Clin. North Am. Small Anim. Pract.*, **33**(4), 759-772(2003),. doi: 10.1016/s0195-5616(03)00025-1.

15. Lanave, G., Buonavoglia, A., Pellegrini, F., Di Martino, B., Di Profio, F., Diakoudi, G., Catella, C., Omar, A.H., Vasinioti, V.I. and Cardone, R., An Outbreak of Limping Syndrome Associated with Feline Calicivirus. *Animals*, **13**, 1778(2023),. https://doi.org/10.3390/ ani13111778

16. Palombieri, A., Sarchese, V., Giordano, M.V., Fruci, P., Crisi, P.E., Aste, G., Bongiovanni, L., Rinaldi, V., Sposato, A. and Camero, M., Detection and Characterization of Feline Calicivirus Associated with Paw and Mouth Disease. *Animals*, **13**, 65(2023). https://doi.org/ 10.3390/ani13010065

17. Spiri, A.M., An Update on Feline Calicivirus. *Schweiz .Arch. Tierheilkd*., **164**(3), 225-241(2022). English. doi: 10.17236/sat00346.

18. Duclos, A.A., Guzmán Ramos, P.J. and Mooney, C.T., Virulent systemic feline calicivirus infection: a case report and first description in Ireland. *Ir. Vet. J*., **77**, 1(2024). https://doi.org/10.1186/s13620-024-00262-3

19. Bergmann, M., Speck, S., Rieger, A., Truyen, U. and Hartmann, K., Antibody Response to Feline Calicivirus Vaccination in Healthy Adult Cats. *Viruses*, **11**(8):702(2019). doi: 10.3390/v11080702.

20. Calnek, B., Barnes, H., Beard, C. and Yoder, H. (1997), *Diseases of Poultry*; Iowa State University Press: Ames, IA, USA,.

21. Sun, H., Li, Y., Jiao, W., Liu, C., Liu, X., Wang, H., Hua, F., Dong, J., Fan, S. and Yu, Z., Isolation and identification of feline herpesvirus type 1 from a South China tiger in China. *Viruses*, **6**, 1004–1014(2014).

22. 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. *J .Vet. Sci*., **21**(5), e71(2020),. https://doi.org/10.4142/jvs.2020.21.e71

23. Tahmasebi, P., Javadpour, F. and Sahimi, M., "Three-Dimensional Stochastic Characterization of Shale SEM Images". *Transport in Porous Media*, **110** (3), 521–531(2015).

24. Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K., MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol*., **35**(6),1547-1549(2018),. doi: 10.1093/molbev/msy096.

25. Gao, J., Li, Y., Xie, Q., Al-zaban, M.I., Al-Saeed, F.A., Shati, A.A., Al-Doaiss, A.A., Ahmed, A.E., Nawaz, S., Ebrahem, H., Irshad, I., Kulyar, M.F. 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

26. Khamsingnok, P., Rapichai, W., Rattanasrisomporn, A., Rungsuriyawiboon, O., Choowongkomon, K. and Rattanasrisomporn, J., Comparison of PCR, Nested PCR, and RT-LAMP for Rapid Detection of Feline Calicivirus Infection in Clinical Samples, *Animals*, **14**(16), 2432(2024). https://doi.org/10.3390/ani14162432

27. Deng, M., Liang, H., Xu, Y., Shi, Q., Bao, F., Mei, C., Dai, Z. and Huang, X., Identification, Genetic Characterization, and Pathogenicity of Three Feline Herpesvirus Type 1 Isolates from Domestic Cats in China. *Vet. Sci*., **11**, 285(2024). https:// doi.org/10.3390/vetsci11070285

28. 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**, 1812(2021). https://doi.org/10.3390/ v13091812

29. Wardley, R.C., Gaskell, R.M. and Povey, R.C., Feline respiratory viruses--their prevalence in clinically healthy cats. *J. Small Anim. Pract.*, **15**(9),579-586(1974). doi: 10.1111/j.1748-5827.1974.tb06538.x.

30. 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. *Prev. Vet. Med*., **167**, 32-38(2019). doi: 10.1016/j.prevetmed.2019.03.010. Epub 2019 Mar 11.

31. 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. *J. Feline Med. Surg.*, **11**(12), 1023-1027(2009).

32. Chan, I., Dowsey, A. and Lait, P., Prevalence and risk factors for common respiratory pathogens within a cohort of pet cats in the UK. *J .Small Anim. Pract.*, **64**(9), 552–560(2023).

33. Monne-Rodriguez, J., Köhler, K. and Kipar, A., Calicivirus co-infections in herpesvirus pneumonia in kittens. *Vet. J*., **236**,1-3(2018),. doi: 10.1016/j.tvjl.2018.04.004. Epub 2018 Apr 9.

34. Helps, C.R., Lait, P. and Damhuis, A., Factors associated with upper respiratory tract disease caused by feline herpesvirus, feline calicivirus, Chlamydophila felis and Bordetella bronchiseptica in cats: Experience from 218 European catteries. *Vet. Rec*., **156**(21),669-673(2005),

35. Vaz, P.K., Job, N., Horsington, J., Ficorilli, N., Studdert, M.J., Hartley, C.A., Gilkerson, J.R., Browning, G.F. and Devlin, J.M., Low genetic diversity among historical and contemporary clinical isolates of felid herpesvirus. *B.M.C. Genom.*, **17**, 704(2016).

36. Madbouly, H., Tamam, S. and Abd-El-Gaid, A., Isolation and identification of bovine herpes virus-1 (BHV-1) from semen of foreign breeds bulls. *J. Vet. Med. Res*., **18**, 22-27(2008),.

37. Monne Rodriguez, J.M., Leeming, G., Köhler K. and Kipar A., Feline Herpesvirus Pneumonia: Investigations Into the Pathogenesis. *Vet. Pathol*., **54**(6), 922-932(2017). doi: 10.1177/0300985817720982. Epub 2017 Aug 16.

38. Leroy, H., Han, M., Woottum, M., Bracq, L., Bouchet, J., Xie, M. and Benichou, S., Virus-Mediated Cell-Cell Fusion. *International Journal of Molecular Sciences*, **21**(24), 9644(2020). https://doi.org/10.3390/ijms21249644

39. Hofmann-Lehmann, R., Hosie, M.J., Hartmann, K., Egberink, H., Truyen, U., Tasker, S., Belák, S., Boucraut-Baralon, C., Frymus, T. and Lloret, A., Calicivirus Infection in Cats. *Viruses*, **14**, 937(2022). https://doi.org/10.3390/v14050937

مدى انتشار فيروس الكاليسي القطط (FCV) وفيروس الهربس القطط١ (FHV-1) بين القطط في ثلاث محافظات مصرية

عبير محمد يوسف حجاج[١]، نورا فيصل الخليفة[١]، آلاء محمد مصطفى عبدالعاطى[٢]، بسيونى عبدالقادر هليل[٣] وأسماء فوزى معجوز[١]

[١] قسم الفيروسات، كلية الطب البيطري، جامعة كفر الشيخ، مصر.
[٢] قسم الباثولوجيا، كلية الطب البيطري، جامعة كفر الشيخ، مصر.
[٣] قسم التوليد والتناسل والتلقيح الاصطناعي، جامعة كفر الشيخ، مصر.

الملخص

تُعد أمراض الجهاز التنفسي العلوي سبباً مهماً للمرض والوفيات في القطط وتوجد العديد من العوامل الممرضة المسببة لذلك وعادةً ما يكون ناتجاً عن عدوى واحدة أو أكثر من العوامل الممرضة وتُعتبر الفيروسات من أهم هذه المسببات وأشهرهم : فيروس الهربس القططي ١ وفيروس الكاليسى القططي وقد كانت الفيروسات التي تسبب الأمراض في الجهاز التنفسي للقطط مدمرة قبل ظهور اللقاحات الفعالة ولكن في الوقت الحاضر تُلاحظ الأمراض الناتجة عن العدوى الفيروسية التنفسية بشكل شائع في حالات الازدحام (مثل متاجر الحيوانات الأليفة، وملاجئ الكلاب، ومزارع القطط) وسوء الرعاية أو ضعف المناعة. تصف هذه الدراسة العزل والتوصيف الجزيئي لفيروس الهربس القططي من النوع١ وفيروس الكاليسي القططي ومدى انتشارهم في مصر. تم جمع مئة وعشرون مسحات من الملتحمة و/أو الانف من قطط تظهر عليها أعراض مرضية من عيادات وملاجئ الحيوانات الصغيرة الخاصة الواقعة في ثلاث محافظات مصرية (كفر الشيخ، الغربية، القاهرة) وكان تفاعل البلمرة المتسلسل ناجحاً في ٣٤ من أصل ١٢٠ عينة بمعدل (٢٨,٣٪) من الحالات المُشتبه بها لفيروس الهربس القططى من النوع١ بينما كان ناجحا في ٣٢ من أصل ١٢٠ عينة بمعدل (٢٦,٦٪) من الحالات المُشتبه بها لفيروس الكاليسى القططى.

تم اجراء تسلسل للجينات الخاصة بهذه الفيروسات وكانت هذه التسلسلات متشابهة للغاية مع سلالات مرجعية اخرى في قاعدة بيانات الجينات ولكن مازال هناك حاجة الى مزيد من الدراسات حول أسباب التهابات الجهاز التنفسي العلوي في القطط وخاصة المسببات الفيروسية بالاضافة الى دراسات حول فعالية اللقاحات.

الكلمات الدالة: فيروس الهربس القططى١- فيروس الكاليسى القططى- القطط- تفاعل البلمرة المتسلسل- الغشاء المشيمي السُلي- مصر.