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Molecular Detection of Feline Herpesvirus 1 in Cats in Mosul City

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Abstract

The study included 200 cats of various ages, genders, nature of breeding, source, and vaccination programs, as well as health status. They were examined clinically, and the clinical signs appearing on them were recorded, and then swabs from the conjunctiva of the eyes, the oropharyngeal, and the nose were collected and transferred to the laboratory.

The feline herpes virus's DNA molecule was examined using swabs obtained for the investigation. The DNA was extracted, and a specific primer was then used in the polymerase chain reaction technique to identify the thymidine kinase template gene.. The extract DNA for each sample an final reaction volume of 182 bp. After recording positive samples in many samples, 10 positive samples were positive and their DNA was purified. Then the genetic sequence of each sample was performed in the Marcogen laboratory, Korea, and then the similarity to the genetic sequence in the database was determined using the Basic Local Alignment Search program, which is located on the electronic page. For the National Center for Life Technologies (NCBI) (www.ncbi.nlm.nih.gor), the multiple sequence alignment was then created using the Cluster Omega program, and then the phylogenetic tree was created using the MEGA 7 program.

The results of the molecular study showed that there was a higher rate of infection with the feline herpes virus from swabs collected from the pharyngeal area compared to swabs collected from the conjunctiva of the eye. It was found that the highest rate of infection was in the age group less than 6 months, and the rate of infection decreased with getting older. Additionally noted was the greatest infection rate. The molecular study's findings indicated that cats with mouth ulcers and cats who were either raised indoors or imported from them had the highest infection rates, but cats who had received vaccinations had the highest rate of feline herpes virus infection.

The findings from the Thymidin kinase gene genomic sequencing study of the feline herpes virus showed the presence of a genetic sequence for the samples sent in cats in Iraq, as it was found that there were only three isolates out of the total samples sent for genetic sequencing, and they were registered in GenBank with a serial number (OQ935422) (OQ935421) with a degree of similarity between them. It reached 100%.

Feline herpesvirus belongs to the family Herpesviridae within the genus Varicellovirus. The genus Varicellovirus includes the feline herpes virus, which causes upper respiratory diseases in cats. (Alice *et al.*,2020)

The virus is transmitted in several ways, including direct contact with infected cats or indirectly through contaminated tools. The virus has been found to be relatively poorly tolerated in the external environment, and it has been proven that it remains for 18 hours in

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humid environmental conditions and less in dry conditions (Argenta *et al*.,2020).

Feline herpesvirus is considered a common and important pathogen in cats, as it causes severe oral infections and upper respiratory tract infections (URTD) (Asmaa *et al*.,2022), where symptoms include a runny nose, sneezing, inflammation of the conjunctiva, as well as abortion (Becker *et al*.,2020)

The virus is diagnosed by polymerase chain reaction (PCR) and viral isolation (Bergmann *et al*., 2020), as well as serological tests such as ELISA and immunofluorescence testing to detect the virus and its antibodies (Burmeister *et al*.,2015). The disease has been recorded in many countries of the world, including in the United States of America, China, and Australia, and the virus has been recorded in countries neighboring Iraq, such as Iran (Dana *et al*.,2019). Due to the lack of studies on this virus in cats and the extent of its impact and spread in Iraq in general, this study was conducted.

Material and Methods

Swabs

Two swabs were collected from each animal included in the study in a sterile manner. The swabs included the following: swabs from the conjunctiva of the eye and swabs from the oropharyngeal area. The swabs were placed in their own tubes and then 0.5 milliliters of sterile neutral phosphate buffer were added to them and then transferred as quickly as possible to the microbiology laboratory. College of Veterinary Medicine - University of Mosul. After that, the swab was pressed against the walls of the tube and the neutral phosphate buffer was transferred to test tubes and placed in a centrifuge at 1500 rpm for 15 minutes. The sediment was discarded and the supernatant was transferred into Ependorf tubes and stored at -20°C until the study was conducted (Hamdan and Albaroodi, 2022).

Polymerase Chain Reaction

DNA extraction:

Extraction was performed according to the manufacturer's instructions.

Polymerase Chain Reaction

This test was performed as previously mentioned (Rouhizadeh *et al.,*2016)

By using following primers FehV-F :(5′-GCG AAG TAC CTG GTC AGA GC -3′)

and FehV-R (5′-TAG TGG GCG GTGATA TAG GC -3′)

10 positive DNA samples for the polymerase chain reaction, as well as the positive control represented by the vaccine strain that was diagnosed during the study, were sent to the Marcogen laboratory, Korea, to conduct genetic sequencing for each sample

Determine the similarity of genetic sequences in the database

The Basic Local Alignment Search application, which can be found on the National Center for Life Technologies' internet website, was utilized to search the database using the thymidine kinase gene of the feline herpesvirus genetic sequence in BLAST format. (NCBI) (www.ncbi.nlm.nih.gor)

Create multiple string alignment

Using the Cluster Omega tool, the nucleotide sequence of the thymidine kinase gene of the feline herpesvirus isolates was utilized to generate a sequence alignment in order to track changes and advancements among viral strains globally.

Create a phylogenetic tree

The phylogenetic tree was created according to (Sun *et al*., 2017) using the program MEGA 4

Results

The results of the molecular study, using polymerase chain reaction technology after amplifying the viral DNA template extracted from the swabs collected during the study and using the specialized primer, showed the appearance of clear positive bands depending on the size of the final reaction

The results of the molecular study showed that there was a higher rate of infection with the feline herpes virus from swabs collected from the pharyngeal area compared to swabs

collected from the conjunctiva area, and that there were statistically significant differences between them (Table I).

As for the relationship of age to the rate of infection with feline herpes virus using molecular methods, it was found that the highest rate of infection was in the age group less than 6 months, and the rate of infection decreased with the age of the animals, as the lowest rate of infection was recorded in cats older than a year, and with significant differences between the groups. Less than six months and the age group of six months - one year with the older group

As for cats raised indoors, the highest infection rate was 68.5%, compared to cats raised outside homes, 31.5%. There were no significant differences between them

As for imported cats, they recorded the highest rate of infection with feline herpes virus when compared to local cats, using polymerase chain reaction technology, and without any statistically significant difference between them

Genetic sequencing results of the thymidin kinase gene of feline herpesvirus

According to the findings of the genetic sequencing of the feline herpes virus's Thymidin kinase gene, cats from the city of Mosul had samples that included a genetic sequence. Out of all the samples sent for genetic sequencing, it was discovered that just two isolates existed, and they were assigned a serial number in GenBank. (OQ935421 and OQ935422) (Table V).

Results of the catalytic herpesvirus Thymidin kinase gene's molecular nucleotide sequence in the database. When BLAST was used to compare the nucleotide sequences of the strain to the database, the results for each feline herpesvirus strain that was diagnosed in the study indicated similarity to the nucleotide sequence of the Thymidin kinase gene. The genetic phylogenetic tree was built using the global and diagnostic strains employed in this investigation. The strain registered in the Global Gene Bank, which originated in various countries including America, Australia, and

Table. I The relationship of the rate of infection with feline herpes virus to the type of swab collected from cats using polymerase chain reaction technology

Type of swab	No of swab	No of positive samples	Percentage %
oro pharyngeal	200	89	44.5 a
Eye	200	37	18.5 b

Table II. Relationship of age with the number of positive samples for feline herpesvirus using PCR

Age	No of Animals	No of positive samples	Percentage %
Less than 6 months	87	41	46.1 a
6 months-1 year	59	29	32.6a
More than 1 year	54	19	21.3 b
Total	200	89	44.5

Table III. The relationship of positive samples for feline herpes virus to the nature of cat breeding using PCR.

Animals management	No of animals	No of positive samples	Percentage %
In door	143	61	68.5a
Out door	57	28	31.5a

Table IV. Relationship of feline herpes virus positive samples to feline source using PCR

Source of animals	No of Animals	No of positive samples	Percentage %
Imported	96	47	52.8a
Native	104	42	47.2a

Table V. Nucleotide sequence of the thymidine kinase gene of feline herpesvirus identified local isolates registered in GenBank

Accession no	3 5	gene	Isolates
OQ935421	ACTTACTACTTCCCAGAACCAATGCTATACTGGCGTAGTCTCTTT-GAAACTGATGTTGTCGGTGGTATCTATGCCGTCCAGGACCG-GAAACGACGTGGTGAATTATCAGCTGAAGATGCTGCCTATATCAC-CGCCCACTATCAAGCAAGATTTGCCGCACCATACCTTCTTT-TACATTCCAGACTATCCACAATAACAGGATATCAGAAAGTTG-TATGTGAGGAACACCCCGACGTGACCCTAATCATAGATAGA-CACCCTCTCGCCTCTCTGGTCTGTTTCCCACTCGCAAGATAT	Thymidin kinase	WSB-FH3-23
OQ935422	ACTTACTACTTCCCAGAACCAATGCTATACTGGCGTAGTCTCTTT-GAAACTGATGTTGTCGGTGGTATCTATGCCGTCCAGGACCG-GAAACGACGTGGTGAATTATCAGCTGAAGATGCTGCCTATATCAC-CGCCCACTATCAAGCAAGATTTGCCGCACCATACCTTCTTT-TACATTCCAGACTATCCACAATAACAGGATATCAGAAAGTTG-TATGTGAGGAACACCCCGACGTGACCCTAATCATAGATAGA-CACCCTCTCGCCTCTCTGGTCTGTTTCCCACTCGCAAGATAT	Thymidin kinase	WSB-FH4-23

China, had the highest percentage of similarity at 100% with the local isolates that carried the registration number in the GenBank, according to the results (Table VI)

The results of the study showed that the phylogenetic tree that was created showed that the tkgene of the two genotypes that were isolated during the study has phylogenetic and evolutionary characteristics with the global genotypes, knowing that the strains that have characteristics similar to the local strains are declining between China and the United States of America, (Fig 1.).

Discussion

Feline herpes virus is one of the main pathogens of respiratory infection in cats. Disease symptoms vary according to many factors, including the virulence of the strain causing the infection, the immune status of the animal, age, source of the animal, and other environmental and epidemiological factors.

A molecular study using a conventional polymerase chain reaction test revealed differences between the presence of virus nucleic acid in the swabs, which was higher in oral and pharyngeal swabs compared to swabs collected from the conjunctiva of the eye.

When comparing these results with other studies, it turns out that there are many theories that prove this, some of which agree with our results and others contradict them, as the researchers mentioned (Litster *et al*., 2015) that the sites of the mechanism of continued infection with the feline herpes virus are the epithelial cells in the other oral and pharyngeal tissues in cats infected with the infection. In addition, these tissues in the body provide some protection for the virus from the immune system, allowing it to replicate in them, but at low standards. Another reason for the differences is the variation in the shedding patterns of feline herpes virus during the sampling period,However, the virus is shed relatively steadily over long periods of time (consistent shedding), while some virus strains are shed intermittently (intermittent shedding), as they do not shed the virus all the time (Liu *et al*., 2020). Our results are in line with the findings of researchers (Hoferer *et al*., 2017), who indicated high rates in swabs taken from the conjunctiva.

The eye compared to swabs taken from the mouth, pharynx, and nose, While the researchers indicated (Rouhizadeh *et al*. 2016) showed an infection rate of 45% from samples represented by oral, pharyngeal, nasal and conjunctival swabs collected from 200 cats infected with upper respiratory tract diseases and from 19 different regions using polymerase chain reaction technology. While a different pattern of infection with feline herpes virus was demonstrated by researchers (Weigler *et al*.,

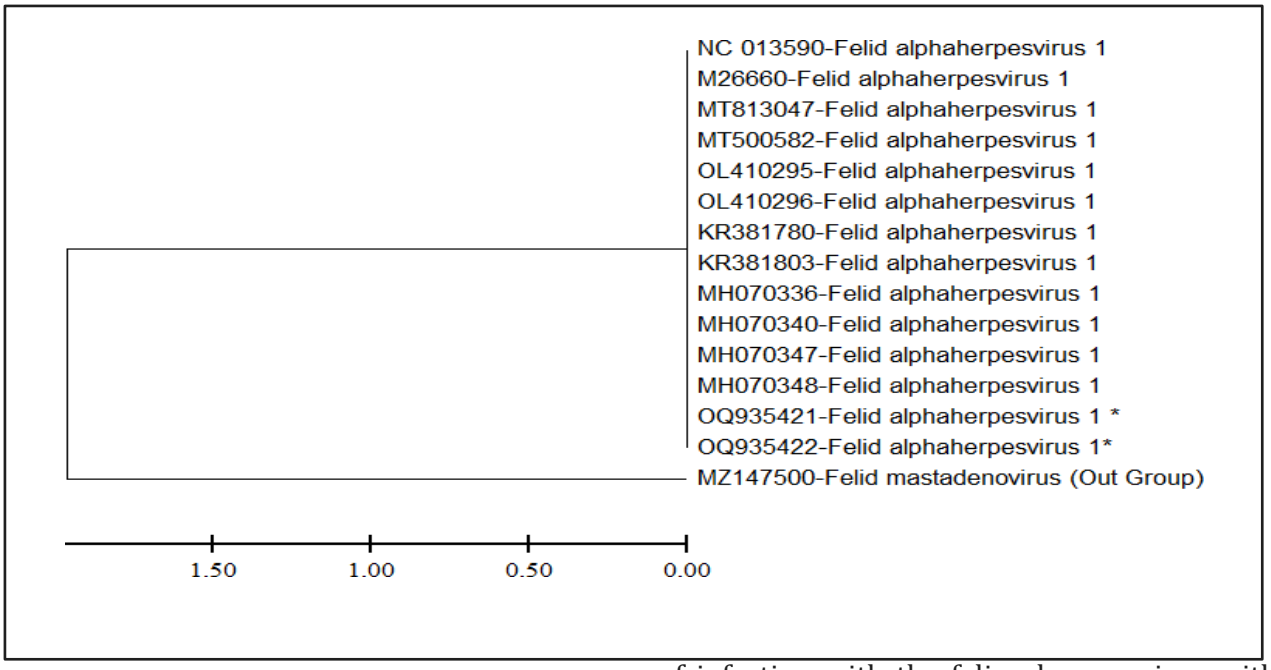

Fig 1. Phylogenetic tree showing that the tkgene of the two genotypes has phylogenetic and evolutionary characteristics with the global genotypes

1997), it was found that there were low rates of infection from swabs collected from the mouth with a low standard of viral particles.

The reason was attributed to the presence of the ribonuclease enzyme in the secretions of the oral mucosa, as well as the genetic difference of the virus, as it affects the detection of the virus in mucous swabs. The aforementioned researcher indicated that the results that gave positive results in the conjunctival swab sample were not detected in the oral swab sample from the same animal, which may result. About the small number of virus particles, the presence of the ribonuclease enzyme in the mucous membrane, the secretions capable of degrading viral RNA, and the genetic difference in the virus strain, while the researchers pointed out (Sun *et al*., 2014). They indicated that the infection rate with the virus was high in swabs taken from nasopharyngeal swabs, reaching 8.5%, while the infection rate in eye swabs reached 2.8%, and this is consistent with the results reached during our studies.

The results of the polymerase chain reaction showed a decrease in the incidence of infection with the feline herpes virus with increasing age. It is consistent with the results reported by researchers (Argenta *et al.,* 2017), who indicated that infection with the virus decreases with increasing age, as the infection rate in ages less than 11 years reached 7%, while the infection rate for ages 1-3 years was 7.4%, and the results of the researchers' study also agreed (Fernandez *et al*., 2020).With our study, the results showed that infection with the virus was high at ages less than 6 months, as these differences were explained by several reasons, including that the mechanism of the immune response to acquire resistance to infection in older cats (more than 3 years) was greater compared to younger cats. Which affects the standard of the virus that is excreted outside the body, which in turn affects the standard of the virus present in the sample. Other reasons are that older cats are exposed to many booster vaccinations against a virus compared to younger cats that were vaccinated mostly once, which leads to the animals acquiring Older animals have a better immune response when compared to young animals (Henzel *et al*., 2014).

While researchers (Hora *et al*., 2013) indicated high rates of infection with the feline herpes virus for cats aged 1-5 years compared to cats less than a year old, while researchers (Nguyen *et al*, 2018) indicated high rates of infection with the virus. In cats aged 3-4 years,

while higher rates of infection were recorded in cats less than 6 months old compared to the rest of the age groups (Porcelatto *et al*., 2018), while researchers (Rodriguez *et al*., 2016) showed that The rate of infection with feline herpes virus varied between 0% and 23.07% in cats in different age groups, without any significant differences between them, with high rates of infection in the 1-year age group. This is consistent with what was mentioned by (Schulz *et al* 2015). He explained these results, except that cats Older cats suffer from the presence of other diseases that lead to a decrease in their immune status compared to young cats. In addition, the aforementioned researcher pointed out the role of stress factors in the extent of the spread of infection with the virus, such as vaccination, castration, and changing shelter locations.

The study showed higher rates of infection in animals raised indoors compared to cats raised outside homes. One study indicated the role of the nature of breeding in the rate of infection with the feline herpes virus, as it indicated that increasing the population density, regardless of the type of breeding system, increases the chances of infection with the virus. While another study showed a link between introducing new cats into the home and increasing the rate of infection with the virus (Lee *et al*., 2023), as cats raised outside homes are likely to be a potential source of infection for other cats in the home. Unless uninfected cats can come into contact with infected cats outside, the number of cats inside the home will not make a difference if good sanitary conditions are available inside the homes. It is obvious that the chance of infection will be much greater when cats are able to come into contact with other cats,While the researchers (Spertus *et al*., 2019) indicated high rates of infection with the feline herpes virus in cats raised in groups compared to cats raised individually, while the results of the researchers (Thiry *et al*., 2009) indicated that the infection rate was high in Cats raised indoors compared to cats raised outside, with no significant differences, and the shelter factor does not constitute a risk factor for infection with the feline herpes virus. While researchers (Liu *et al*., 2024) reported that the infection rate of feline herpes virus in cats raised indoors is higher than in cats raised outside the home, as the infection rate in domestic cats was 13.3%, while in cats raised outside the home it was 0%.

This study showed higher rates of infection in imported cats compared to local cats. Where (Davison *et al*., 2009) indicated that there are differences between the breed of cats and the susceptibility to infection with the feline herpes virus, other explanations may include stress factors to which imported cats are exposed during transportation and differences between the virus strains from one country to another. In addition, it has recently been shown that the virus's junctional adhesion molecule 1 (JAM-1), which is considered the receptor for the virus and its various shapes and sizes, has a major role in determining whether cats are resistant or sensitive to infection, which in general is determined by the animal source (Asmaa *et al*., 2022). In addition, the imported cats arriving in the city of Mosul are often young or may not exceed the age of weaning, and this is what makes them sensitive to infection (field observations by the researcher).

The results of the genetic sequencing of the feline herpes virus showed the presence of a genetic sequence for the samples sent in cats in the city of Mosul, where it was found that there were only two isolates out of the total samples sent for genetic sequencing between them. This difference in similarity indicates the genetic diversity of the virus, which is the result of genetic changes and mutations in it, noting that it No strain of feline herpes virus has been previously registered in Iraq.

The results of conducting the genetic sequence of the feline herpes virus, comparing it to the genetic sequence of some international strains registered in GenBank, showed 100% with many isolates in countries of the world.

The various sequence alignment results for the feline herpes virus also revealed the existence of missing, common, and variable areas. These findings are in line with the findings of (Asmaa *et al*., 2022), who suggested that the variation between the global strains and the isolated strains could be caused by geographic differences. The high susceptibility of the virus to causing genetic mutations in the

nucleotide sequence, which benefits the adaptation of the virus during infection to avoid the immune response as well as the emergence of new strains of varying virulence to cause new disease outbreaks, while isolation and differences in environmental conditions are also factors (Lewin *et al*., 2018).

The phylogenetic tree results for the genetic strains included in the study demonstrated that the two strains found belong to the same subgroup and are part of some species that are globally registered in China, America, and Australia. This relationship is clear proof that these species have a high level of genetic relatedness. Simultaneously, the study's isolated strains show that the global and local strains' evolutionary origins are shared from a genetic perspective, as evidenced by their divergence and convergence in the phylogenetic tree. The geographic location might play a part in this.

Therefore, through this study, it becomes clear to us proof of the existence of the feline herpes virus and its danger to the breeding of cats, as one of the most important reasons for its presence is unprogrammed immunization with commercial vaccines without referring to the harmful genetic strains present in the environment of cats and without the presence of a thoughtful vaccination program that suits the spread of the virus and the severity of the infections it causes. In cats in Iraq, random importation is carried out, regardless of whether the animal is vaccinated or not, and without strict quarantine procedures. Also, our study has proven the presence of recombinant genetic strain patterns of the virus resulting from the presence of more than one strain of the virus, and the vaccine strain may have a role in the development of the recombination phenomenon. We referred to it previously in the text of the letter, using documented scientific sources. In addition, the lack of use of anti-viral drugs by veterinary clinics may be one of the reasons for the repeated exacerbation of the presence of the virus in cats, as through field observations it was found that many infections in cats occurred within one year despite Fortifying it more than once (field observations by the researcher)

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