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PCR Detection and Partial Molecular Characterization of Feline Herpesvirus-1 in Suspected Cats from Chennai, India

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ABSTRACT

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Feline herpesvirus-1 (FHV-1) is an important viral pathogen of cats and is associated mainly with upper respiratory and ocular disease. The present study aimed to detect FHV-1 in samples collected from suspected cats in Chennai and to characterize the virus molecularly using selected virulence-associated genes. A total of 40 throat swab samples were collected at Madras Veterinary College, Vepery, Chennai, and subjected to DNA extraction and polymerase chain reaction (PCR). PCR assays targeted glycoprotein I (gI), thymidine kinase (TK) and glycoprotein B (gB). Amplicons of approximately 490 bp for gI and 670 bp for TK were detected, whereas the expected gB product was not amplified. The screening recorded three PCR-positive samples among the 40 samples examined. Positive products were purified, sequenced and compared with GenBank sequences using BLAST. The sequences reported in the thesis showed 99.87% nucleotide homology with available FHV-1 sequences. Phylogenetic analysis was performed using MEGA11 and showed close clustering of the study sequences with FHV-1 reference sequences. The findings demonstrate the usefulness of PCR and sequence analysis for molecular detection and characterization of FHV-1 in suspected feline samples and provide baseline molecular information for the sampled population in Chennai.

Introduction

Feline herpesvirus-1 (FHV-1), also known as feline rhinotracheitis virus, is a member of the genus *Varicellovirus*, subfamily *Alphaherpesvirinae*, and family *Herpesviridae*. It is an important viral pathogen of domestic cats and is primarily associated with upper respiratory and ocular disease. The virus was first

isolated from cats exhibiting respiratory disease in the late 1950s (Crandell and Maurer, 1958). FHV-1 is an enveloped, double-stranded DNA virus with a genome of approximately 134–136 kb and a genomic organization characteristic of alphaherpesviruses (Rota *et al.*, 1986; Tai *et al.*, 2010). The viral genome consists of unique long and unique short regions flanked by repeat sequences and encodes a variety of structural and

regulatory proteins. Several envelope glycoproteins are involved in viral attachment, entry, cell-to-cell spread, and interactions with the host immune response (Tai *et al.*, 2010; Yang *et al.*, 2020).

FHV-1 infection is commonly associated with clinical manifestations including fever, depression, anorexia, rhinitis, nasal and ocular discharge, conjunctivitis, keratitis, and, in severe cases, pneumonia. Young cats may develop more severe clinical disease, whereas recovered cats may remain latently infected. Viral reactivation can occur following stress or immunosuppression and may result in renewed viral shedding (Gaskell *et al.*, 2007; Thiry *et al.*, 2009). Transmission occurs predominantly through direct contact with infected respiratory and ocular secretions, while contaminated fomites may also contribute to viral spread. The establishment of lifelong latency, particularly within neural tissues, represents an important biological characteristic of FHV-1 and complicates the interpretation and control of infection (Gaskell and Povey, 1979; Reubel *et al.*, 1993).

Several diagnostic methods have been developed for the detection of FHV-1, including virus isolation, serological assays, fluorescent antibody testing, conventional PCR, and real-time PCR. Molecular methods are particularly useful because viral DNA can be detected in clinical specimens as well as tissues harbouring latent virus. PCR assays targeting conserved viral genes, including the thymidine kinase (TK) and glycoprotein genes, have been employed for molecular detection and characterization of FHV-1 (Reubel *et al.*, 1993; Burgesser *et al.*, 1999; Stiles *et al.*, 1997). Real-time PCR has also been used for the detection and quantification of FHV-1 DNA in ocular specimens (Vögtlin *et al.*, 2002). Sequence-based molecular approaches provide additional information by enabling comparison of field-derived sequences with reference sequences available in public databases and by facilitating assessment of their phylogenetic relationships (Yang *et al.*, 2020).

The present study was undertaken to detect FHV-1 DNA in clinical samples collected from suspected cats in Chennai, India. Molecular detection was performed by conventional PCR targeting the glycoprotein I (gI), thymidine kinase (TK), and glycoprotein B (gB) genes. Selected PCR products were subjected to sequencing and comparative sequence analysis to provide partial molecular characterization of the detected viral DNA.

Materials and Methods

Sample Collection

A total of 40 throat swab samples were collected from cats suspected of having FHV-1 infection and presented to Madras Veterinary College, Vepery, Chennai, Tamil Nadu, India. Samples were collected by veterinary clinicians as part of routine veterinary practice. The specimens were selected by convenience sampling and subsequently processed for molecular detection of FHV-1 DNA.

DNA Extraction

The collected swab specimens were suspended in sterile phosphate-buffered saline (PBS), and 200 µL of the resulting suspension was transferred to a sterile 1.5-mL microcentrifuge tube. 50 µL of 10% Sodium dodecyl sulfate (SDS) and 10 µL of proteinase K were added, followed by vortexing and incubation at 56°C for 30 min. Phenol:chloroform alcohol (25:24:1) was subsequently added for removal of proteins and other cellular contaminants. The samples were centrifuged at 10,000 rpm for 5 min at 20°C, and the aqueous phase was transferred carefully to a fresh microcentrifuge tube. 20 µL of 10% sodium acetate Sodium acetate and 1 mL of chilled absolute ethanol were then added to precipitate the DNA. The samples were vortexed and incubated overnight at -20°C, followed by centrifugation at 12,000 rpm for 15 min. The resulting DNA pellet was washed with 500 µL of 70% ethanol and centrifuged at 12,000 rpm for 2 min. The pellet was subsequently air-dried and resuspended in 30 µL of nuclease-free water.

DNA quality and quantification

The quality and integrity of the extracted DNA were assessed by electrophoresis on a 0.8% agarose gel. DNA concentration was determined using a NanoDrop One spectrophotometer (Thermo Scientific, USA).

PCR amplification

Conventional PCR was performed in a final reaction volume of 25 µL. Each reaction contained 12.5 µL of red-dye PCR master mix, 1 µL each of forward and reverse primers, 7.5 µL of nuclease-free water, and 3 µL of template DNA. Three FHV-1 genomic targets, namely gI, TK, and gB, were investigated.

For amplification of the gI target, the PCR programme consisted of an initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. A final extension was performed at 72°C for 7 min.

For amplification of the TK and gB targets, the same cycling conditions were employed, except that the annealing temperature was 50°C.

Agarose gel electrophoresis

PCR amplification products were separated by electrophoresis on a 1.5% agarose gel. Ethidium bromide was used for visualization, and a 1-kb DNA molecular weight ladder was included to estimate the size of the amplified products.

Electrophoresis was performed at approximately 80–120 V. Following electrophoresis, the amplified DNA products were visualized using a UV transilluminator and documented using a Bio-Rad Gel Doc Go imaging system.

PCR Product Purification and Sequencing

PCR products corresponding to the expected amplicon sizes were selected for downstream sequence analysis. The amplified products were excised from the agarose gel and purified prior to sequencing.

Chromatogram files obtained from sequencing were processed using FinchTV, and nucleotide sequences were converted into FASTA format for subsequent analysis.

The nucleotide sequences obtained from the selected PCR products were subjected to nucleotide BLAST (BLASTn) analysis against sequences available in the NCBI GenBank nucleotide database. The closest matching sequences were identified based on nucleotide sequence identity and query coverage.

Phylogenetic analysis

Phylogenetic analysis was performed using MEGA11. FHV-1 reference sequences were retrieved from the NCBI GenBank database and aligned with the nucleotide sequences obtained in the present study. The aligned sequences were subsequently used to construct a phylogenetic tree.

Results and Discussion

DNA Isolation & Quantification

Genomic DNA was successfully extracted from the collected swab specimens. The quality and integrity of the extracted DNA were assessed by agarose gel electrophoresis, while DNA concentration was determined spectrophotometrically using a NanoDrop One spectrophotometer.

PCR Screening

A total of 40 throat swab samples were screened for the presence of FHV-1 DNA using conventional PCR assays targeting the gI, TK, and gB genes. Amplification products of approximately 490 bp and 670 bp were obtained for the gI and TK targets, respectively. The expected approximately 1194-bp product was not detected for the gB target.

Gel Electrophoresis

The PCR products were resolved on 1.5% agarose gels and visualized using a UV-based gel documentation system. A band corresponding to the expected approximately 490-bp gI amplicon was observed in one sample, while two samples exhibited bands corresponding to the expected approximately 670-bp TK amplicon. (Figure 1 & 2)

The expected approximately 1194-bp gB amplicon was not observed in the tested samples.

Sequencing & Phylogenetic analysis

FASTA-formatted nucleotide sequences obtained from the selected PCR-positive products were subjected to BLASTn analysis against the NCBI GenBank nucleotide database. The gI and TK sequences showed high nucleotide sequence identity with FHV-1 reference sequences.

The reported maximum nucleotide sequence identity was 99.87%.

The nucleotide sequences generated in the present study were compared with representative FHV-1 sequences retrieved from GenBank. The study sequences clustered with reference FHV-1 sequences in the phylogenetic

analysis, providing additional molecular support for their identification as FHV-1.

The present study used PCR-based detection and sequence analysis to investigate FHV-1 in suspected cats from Chennai. The detection of FHV-1 DNA in field samples is consistent with previous studies demonstrating that PCR is a sensitive method for detecting FHV-1 in clinical specimens. Reubel *et al.*, (1993) demonstrated that PCR could detect FHV-1 DNA in oral and ocular swabs, while Stiles *et al.*, (1997) compared nested PCR with virus isolation and fluorescent antibody testing in cats with conjunctivitis. Burgesser *et al.*, (1999) similarly evaluated molecular and conventional diagnostic approaches for naturally occurring FHV-1 infection.

The use of gI and TK targets in the present study is biologically relevant because herpesvirus glycoproteins participate in viral entry, cell-to-cell spread and interactions with host immunity, while thymidine kinase is associated with viral nucleotide metabolism and is widely used as a molecular target in herpesvirus diagnostics. Previous molecular studies have used TK and glycoprotein genes for identification and characterization of FHV-1 isolates (Nunberg *et al.*, 1989; Yang *et al.*, 2020). The reported 490-bp gI and 670-bp TK amplicons therefore provided useful targets for downstream sequencing.

The nucleotide sequences obtained from the PCR products showed high sequence similarity to FHV-1 sequences. The maximum reported nucleotide sequence identity was 99.87%. Phylogenetic clustering of the study sequences with reference FHV-1 sequences further supported their molecular identity.

Low genetic diversity among FHV-1 clinical isolates has also been described, including in analyses of historical and contemporary isolates (Vaz *et al.*, 2016), although complete-genome analyses can reveal additional variation and evolutionary relationships (Tai *et al.*, 2010; Marino *et al.*, 2021).

The study shows throat swab testing as a novel aspect of the work. Because FHV-1 is classically associated with respiratory and ocular disease and because detection can be influenced by sampling site. Previous studies have evaluated conjunctival, nasal and other mucosal samples for FHV-1 detection, and sampling method can affect DNA yield and detection rate (Sjödahl-Essén *et al.*, 2008;

Westermeyer *et al.*, 2008;). Accordingly, the present finding provides a basis for further investigation rather than establishing throat swabs as a preferred diagnostic specimen.

FHV-1 establishes lifelong latency and can reactivate during stress or immunosuppression, which complicates interpretation of molecular positivity and control at the population level (Gaskell and Povey, 1979; Thiry *et al.*, 2009).

Vaccination can reduce disease severity and viral shedding, but protection is not absolute. Studies of vaccination and challenge models have demonstrated reductions in clinical signs and/or shedding following vaccination (Lappin *et al.*, 2006; Reagan *et al.*, 2014; Bergmann *et al.*, 2020). The present study did not evaluate vaccination efficacy or clinical outcome, so no direct conclusions about vaccine protection can be drawn from these samples.

Limitations

The present study has several limitations. The sample size was small and based on convenience sampling, limiting the generalizability of the findings. Incomplete clinical and demographic data restricted assessment of the association between FHV-1 DNA detection and clinical disease. Conventional PCR did not allow estimation of viral load or differentiation between active infection and latent infection or low-level shedding. Furthermore, amplification was obtained only for the gI and TK targets, resulting in partial molecular characterization, and sequencing and phylogenetic analysis were limited to a small number of specimens. Future studies should include larger, systematically selected populations, standardized clinical data, quantitative PCR, paired specimens from multiple sites, and multilocus or whole-genome sequencing.

The present study demonstrated the application of conventional PCR and sequence-based analysis for the molecular detection and partial characterization of FHV-1 DNA in clinical samples obtained from suspected cats in Chennai, India. Amplification was obtained for the gI and TK targets, whereas the expected gB amplicon was not detected. Sequence analysis demonstrated high nucleotide sequence identity between the selected study sequences and FHV-1 reference sequences available in GenBank, with a maximum reported identity of 99.87%.

Table.1 Designed Primer sequences used for amplification of FHV-1 targets.

Target	Forward primer (5'–3')	Reverse primer (5'–3')
gI (Glycoprotein I)	TTCTCGACGACCAACCATTAC	GGTTCGGACTCCATCGATTTAT
gB (Glycoprotein B)	GAAATGTTGAGTCGGATAGCCAC	AGTCTTGGTTTTAAGTCAGTATC
TK (Thymidine Kinase)	GCAACACTTCCACGAGAACC	GGGGGGTAATAGGGAACACCA

Figure.1 Agarose gel electrophoresis showing a positive gI (490 bp) PCR product (Lane 3) and molecular marker (Lane 4).



Figure.2 Agarose gel electrophoresis showing a positive TK (670 bp) PCR product (Lane 5 & 6) and molecular marker (Lane 4).

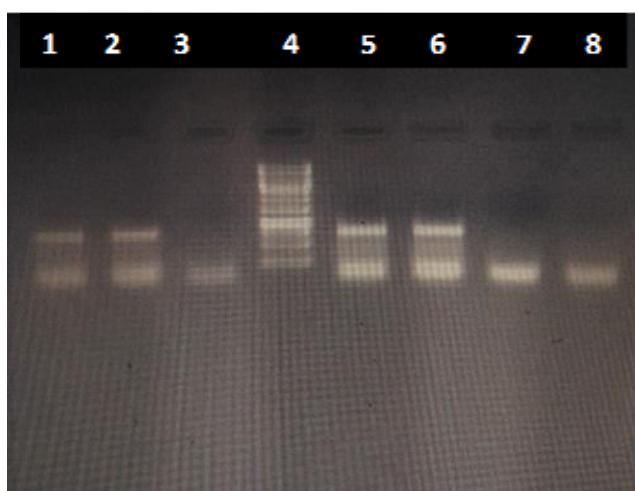
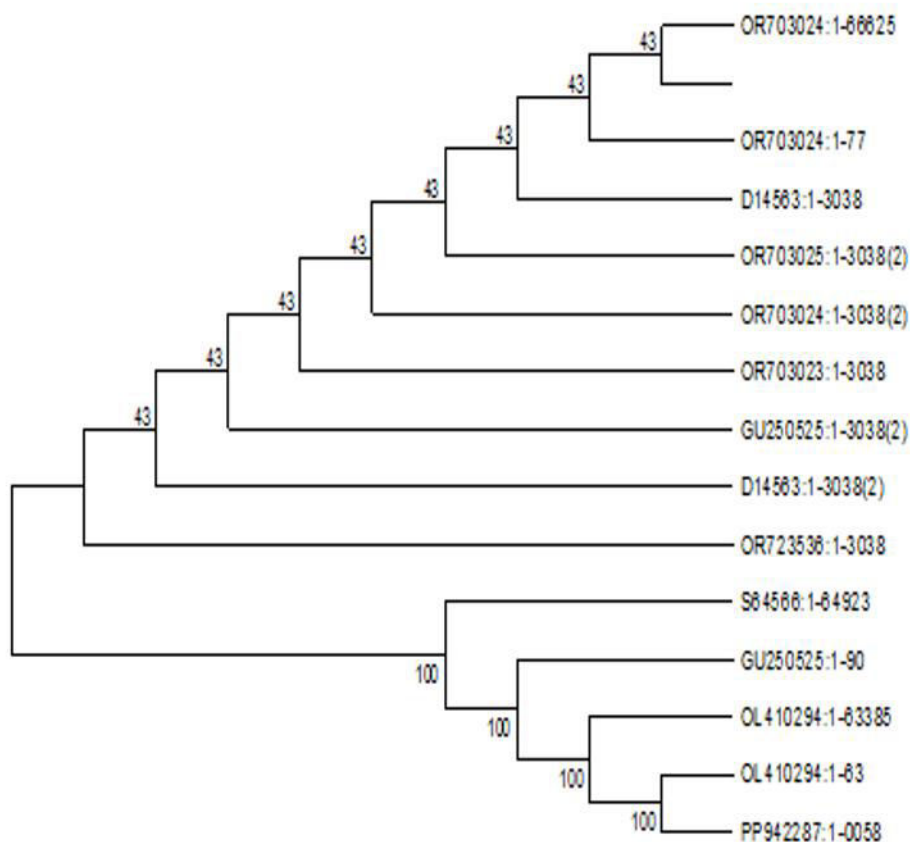


Figure.3 Phylogenetic analysis of FHV-1 nucleotide sequences.



The PCR findings indicate the presence of FHV-1 DNA in the tested specimens; however, PCR positivity alone should not be interpreted as definitive evidence of active clinical infection because FHV-1 can establish lifelong latency. The molecular findings and phylogenetic analysis provide preliminary evidence supporting the identification of the detected viral DNA as FHV-1 and contribute to the limited molecular information available from the sampled feline population in Chennai. Further studies involving larger sample populations, standardized clinical data, validated molecular assays, quantitative PCR, and multilocus or whole-genome sequencing are warranted to provide more comprehensive information on the molecular epidemiology and genetic diversity of FHV-1 in the region.

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Conflict of interest

The authors declare no competing interests.

Author Contributions

Sowmiya V.: sample processing, molecular assays, sequence analysis and manuscript preparation. Angel Jenefa B: supervision, scientific guidance and manuscript review.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

- Bergmann M, Speck S, Rieger A, Truyen U, Hartmann K. 2020. Antibody response to feline herpesvirus-1 vaccination in healthy adult cats. *Journal of Feline Medicine and Surgery* 22(4): 329–338. <https://doi.org/10.1177/1098612X19845702>.
- Burgesser KM, Hotaling S, Schiebel A, Ashbaugh SE, Roberts SM, Collins JK. 1999. Comparison of PCR, virus isolation, and indirect fluorescent antibody staining in the detection of naturally occurring feline herpesvirus infections. *Journal of Veterinary Diagnostic Investigation* 11(2): 122–126. <https://doi.org/10.1177/104063879901100203>.
- Crandell RA, Maurer FD. 1958. Isolation of a feline virus associated with intranuclear inclusion bodies. *Proceedings of the Society for Experimental Biology and Medicine* 97(3): 487–490. <https://doi.org/10.3181/00379727-97-23783>.
- Gaskell R, Dawson S, Radford A, Thiry E. 2007. Feline herpesvirus. *Veterinary Research* 38(2): 337–354. <https://doi.org/10.1051/vetres:2006063>.
- Gaskell RM, Povey RC. 1979. Feline viral rhinotracheitis: sites of virus replication and persistence in acutely and persistently infected cats. *Research in Veterinary Science* 27(2): 167–174. [https://doi.org/10.1016/S0034-5288\(18\)32823-6](https://doi.org/10.1016/S0034-5288(18)32823-6).
- Lappin MR, Sebring RW, Porter M, Radecki SJ, Veir J. 2006. Effects of a single dose of an intranasal feline herpesvirus 1, calicivirus, and panleukopenia vaccine on clinical signs and virus shedding after challenge with virulent feline herpesvirus 1. *Journal of Feline Medicine and Surgery* 8(3): 158–163. <https://doi.org/10.1016/j.jfms.2005.12.001>.
- Marino ME, Mironovich MA, Ineck NE, Citino SB, Emerson JA, Maggs DJ, Coghill LM, Dubovi EJ, Turner RC, Carter RT, Lewin AC. 2021. Full viral genome sequencing and phylogenomic analysis of feline herpesvirus type 1 (FHV-1) in cheetahs (*Acinonyx jubatus*). *Viruses* 13(11): 2307. <https://doi.org/10.3390/v13112307>.
- Nunberg JH, Wright DK, Cole GE, Petrovskis EA, Post LE, Compton T, Gilbert JH. 1989. Identification of the thymidine kinase gene of feline herpesvirus: use of degenerate oligonucleotides in the polymerase chain reaction to isolate herpesvirus gene homologs. *Journal of Virology* 63(8): 3240–3249. <https://doi.org/10.1128/JVI.63.8.3240-3249.1989>.
- Reagan KL, Hawley JR, Lappin MR. 2014. Concurrent administration of an intranasal vaccine containing feline herpesvirus-1 (FHV-1) with a parenteral vaccine containing FHV-1 is superior to parenteral vaccination alone in an acute FHV-1 challenge model. *Veterinary Journal* 201: 202–206. <https://doi.org/10.1016/j.tvjl.2014.05.003>.
- Reubel GH, Ramos RA, Hickman MA, Rimstad E, Hoffmann DE, Pedersen NC. 1993. Detection of active and latent feline herpesvirus 1 infections using the polymerase chain reaction. *Archives of Virology* 132(3–4): 409–420. <https://doi.org/10.1007/BF01309549>.
- Rota PA, Maes RK, Ruyechan WT. 1986. Physical characterization of the genome of feline herpesvirus-1. *Virology* 154(1): 168–179. [https://doi.org/10.1016/0042-6822\(86\)90439-3](https://doi.org/10.1016/0042-6822(86)90439-3).
- Sjödahl-Essén T, Tidholm A, Thorén P, Persson-Wadman A, Bölske G, Aspán A, Treiberg Berndtsson L. 2008. Evaluation of different sampling methods and results of real-time PCR for detection of feline herpes virus-1, *Chlamydomydia felis* and *Mycoplasma felis* in cats. *Veterinary Ophthalmology* 11(6): 375–380. <https://doi.org/10.1111/j.1463-5224.2008.00661.x>.
- Stiles J, McDermott M, Willis M, Roberts W, Greene C. 1997. Comparison of nested polymerase chain reaction, virus isolation, and fluorescent antibody testing for identifying feline herpesvirus in cats with conjunctivitis. *American Journal of Veterinary Research* 58(8): 804–807.
- Tai SHS, Niikura M, Cheng HH, Kruger JM, Wise AG, Maes RK. 2010. Complete genomic sequence and an infectious BAC clone of feline herpesvirus-1 (FHV-1). *Virology* 401(2): 215–227. <https://doi.org/10.1016/j.virol.2010.02.021>.
- Thiry E, Addie D, Belák S, Boucraut-Baralon C, Egberink H, Frymus T, Gruffydd-Jones T,

- Hartmann K, Hosie MJ, Lloret A, Lutz H, Marsilio F, Pennisi MG, Radford AD, Truyen U, Horzinek MC. 2009. Feline herpesvirus infection: ABCD guidelines on prevention and management. *Journal of Feline Medicine and Surgery* 11(7): 547–555. <https://doi.org/10.1016/j.jfms.2009.05.003>.
- Vaz PK, Job N, Horsington J, Ficorilli N, Studdert MJ, Hartley CA, Gilkerson JR, Browning GF, Devlin JM. 2016. Low genetic diversity among historical and contemporary clinical isolates of felid herpesvirus 1. *BMC Genomics* 17: 704. <https://doi.org/10.1186/s12864-016-3050-2>.
- Vögtlin A, Fraefel C, Albini S, Leutenegger CM, Schraner E, Spiess B, Lutz H, Ackermann M. 2002. Quantification of feline herpesvirus 1 DNA in ocular fluid samples of clinically diseased cats by real-time TaqMan PCR. *Journal of Clinical Microbiology* 40(2): 519–523. <https://doi.org/10.1128/JCM.40.2.519-523.2002>.
- Westermeyer HD, Kado-Fong H, Maggs DJ. 2008. Effects of sampling instrument and processing technique on DNA yield and detection rate for feline herpesvirus-1 via polymerase chain reaction assay. *American Journal of Veterinary Research* 69(6): 811–817. <https://doi.org/10.2460/ajvr.69.6.811>.
- Yang DK, Kim HH, Park YR, Yoo JY, Choi SS, Park Y, An S, Park J, Kim J, Kim HJ, Lee J, Hyun BH. 2020. Isolation and molecular characterization of feline herpesvirus 1 from naturally infected Korean cats. *Journal of Bacteriology and Virology* 50(4): 263–272. <https://doi.org/10.4167/jbv.2020.50.4.263>.

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