ORIGINAL ARTICLE

Veterinary Research Forum. 2023; 14 (11) 583 - 588
doi: 10.30466/vrf.2023.1975795.3701
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Isolation and molecular characteristics of a recombinant feline calicivirus from Qingdao, China

Yongxiang Liu[1]*, Lide Qin[2], Xiaoliang Hu[3], Yanmei Jiang[1]

[1] Department of Veterinary Medicine, College of Life Sciences and Food Engineering, Hebei University of Engineering, Handan, China; [2] Department for Safety Supervision of Animal Products, China Animal Health and Epidemiology Center, Qingdao, China; [3] Department of Veterinary Medicine, Faculty of Agriculture, Forestry and Food Engineering, Yibin University, Yibin, China.

Article Info

Article history:

Received: 06 December 2022
Accepted: 20 May 2023
Available online: 15 November 2023

Keywords:

Feline calicivirus
Isolation
Recombination

Abstract

Feline calicivirus (FCV) is a highly contagious pathogen seriously affecting the upper respiratory tract and producing oral diseases in the feline. Despite widespread vaccination, the prevalence of FCV remains high. In this study, the FCV qingdao (qd)/2019/china was isolated from a domestic feline oropharyngeal swab collected from Qingdao, China. The virus was purified using the plaque assay and identified using the Polymerase chain reaction and indirect immunofluorescence assay methods, the capsid amino acid, *VP1* of qd/2019/china, showed sequence identity with the other isolates ranging between 83.90% (ym3/2001/jp) and 91.10% (CH-JL4). The sequence of the capsid amino acid revealed qd/2019/china to be closely related to CH-JL4 and clustered with CH-JL4 in the phylogenetic tree. The phylogenetic analysis indicated that the complete genomes (GenBank® accession No. MZ322896) of qd/2019/china and CH-JL4 were also classified into the same cluster. The recombination analysis with Simplot indicated that the qd/2019/china originated from the recombination of CH-JL4 and HRB-SS, and the region 3,821 - 5,301 nt originated from HRB-SS. Further, the region 3,821 - 5,301 nt were found to belong to the protease-polymerase (PP) of HRB-SS. Here, we isolated a new recombinant virus, FCV qd/2019/china. Therefore, these results would be beneficial for better understanding of the evolution and epidemiology of FCV.

Introduction

Feline calicivirus (FCV) is one of the most common contagious feline pathogens causing oral ulcers, ocular conjunctivitis and infectious upper respiratory tract disease.[1] The FCV can also infect other members of the Felidae like tigers, cheetahs[2] and lions.[3] It has also been isolated from dog feces.[4-6] Traditionally, FCV infections are usually associated with acute, mild and self-limiting diseases,[7] hence, do not inflict much harm. Therefore, FCV infections do not inflict significant harm if the secondary infection is well-controlled.

The virus belongs to the genus *Vesivirus* of the family *Caliciviridae*[8] comprising many viruses that cannot be readily cultured on cells such as the Norovirus, Sapovirus and Rabbit hemorrhagic disease virus which limit their pathogenic study. The FCV, on the contrary, can be readily cultured on the passaged feline cell lines such as CRFK and F81. There are no strict restrictions on the specific passaged numbers and demonstrating obvious cytopathic effect (CPE), hence, FCV has been established as an *in vitro* model for *Caliciviridae* research.[1]

The FCV is a single-stranded positive-sense RNA virus with a 7.80 kb long genome covered by a capsid protein, however, devoid of an envelope.[9] It has an icosahedral capsid diameter of about 27.00 - 40.00 nm. Due to lower fidelity, the FCV genome bears a high mutation rate and evolves rapidly. The immune system pressure constitutes the most important reason for the mutation power source contributing to its enhanced virulence and vaccination failure.[10]

The low fidelity of the protease and polymerase (PP) constitutes another reason for the mutation power source. The PP is the RNA-dependent RNA polymerase (RdRp) of FCV. The FCV has only one serotype and there is significant diversity in the strains isolated from different countries, regions and periods that needs further investigation.

***Correspondence:**

Yongxiang Liu. PhD
Department of Veterinary Medicine, College of Life Sciences and Food Engineering, Hebei University of Engineering, Handan, China
E-mail: liuyongxiang@hebeu.edu.cn

Random genetic drift, mutation and recombination are known to be the most common ways of viral evolution and variation. The high plasticity of the RNA genome in the RNA viruses has a high mutation rate during replication due to low fidelity, lack of proofreading and post-replicative repair activities of the viral RNA polymerase.[11] Recombination events like canine enteric coronaviruses,[12,13] porcine reproductive and respiratory syndrome virus,[14,15] and influenza virus[16] have been reported in several viruses, however, there have been very few studies on the recombination of FCV.

However, in recent years, the health of cats has been seriously threatened by the highly lethal strains due to the high degree of FCV variability.[17] Occasional outbreaks of virulent-systemic FCV (VS-FCV) infections have been reported not only in the USA but also in Europe during recent years and also in different regions of China like Heilongjiang and Shanghai.[18] The VS-FCV has been characterized by cutaneous edema, ulcerations of the head and feet, occasionally jaundice, and even death. Owing to the limited knowledge of FCV genetics and variation, the isolation and identification of new isolates and the analysis of the FCV genome sequence are of particular significance.

This study reported the isolation and sequence analysis of a recombinant FCV isolate qd/2019/china from the oropharyngeal swab of a dead domestic cat that exhibited serious mouth and tongue ulceration.

Materials and Methods

Sample information and treatment. In October 2019, a dead domestic cat oropharyngeal swab was submitted for laboratory investigation. The apparent clinical symptom comprised the eyes and nose covered with purulent discharge, severe tongue damage, the whole tongue layer falling off and ulceration of the hard palate. Necropsy of the cat showed hemorrhagic enteritis and lung edema. The oropharyngeal swab was collected with 0.80% sodium chloride (Beijing Solarbo Technology Co. Ltd., Beijing, China) and stored at – 80.00 ˚C until FCV was isolated.

Isolation and purification of the virus. The protocol for virus isolation follows the usual operation. Briefly, the CRFK cells (ATCC : CCL94) were grown in DMEM (Gibco, Carlsbad, USA) containing 10.00% fetal bovine serum (FBS) and 1.00% penicillin-streptomycin (Gibco). The cells were incubated at 37.00 ˚C in a 5.00% CO_2 humidified cabinet. The collected oropharyngeal swab was treated with a 0.22 μm filter and inoculated into the CRFK monolayer after which the CPE was observed at 1st passage (P1) virus generation. When the CPE was obvious, the cultures were freeze-thawed 2 cycles to release the virus then harvested and stored at – 80.00 ˚C. The virus stocks were purified using the plaque assay three cycles to reach out for purified virus. The CRFK cells were grown in DMEM containing 10.00% FBS and 1.00% penicillin-streptomycin on 6-well plate allowing the cells to reach the 80.00% - 90.00% confluency. The growth medium was removed and diluted virus was added to each well using multiple wells per dilution. The cells were coated with overlay medium, containing 1.00% agar No. 1 (Oxoid, Basingstoke, UK) and DMEM. The cell monolayers were observed every day for the presence of foci or plaques, the single foci or plaque were collected with a sterile pipette tip. At last, the virus titer of the purified FCV P2 virus generation was 10^7 median tissue culture infectious doses (TCID50) per 100 μL.

Identification of the virus. The viral RNA was extracted from the cell culture supernatant using a TIANamp virus DNA/RNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's protocol, the cDNA was synthesized from the mRNA using the cDNA synthesis kit (TaKaRa, Tokyo, Japan), and the template for Polymerase chain reaction (PCR) identification was used. The PCR primer was synthesized according to the previous report,[19] *FCV-F*: 5′-GTAAAAGAAATTTGAGACAAT-3′, FCV-R:5′-TACTGAAGWTCGCGYCT-3′. The virus isolates were further identified by indirect immunofluorescence assay using a cat polyclonal antibody against FCV.[20]

Genome cloning and sequencing. The genome of the FCV strain qd/2019/china was cloned using a LA Taq PREMIX (LA Taq™ Version 2.0; Takara, San Jose, USA). The primer was obtained based on the conserved regions by multiple sequence alignment analysis of several China FCV isolates (Table 1). The 5′- and the 3′-end sequences were obtained using the 5′ and 3′ rapid amplification of the cDNA ends (5′ and 3′ RACE) kit (Invitrogen, Waltham, USA). The PCR products were cloned into the pMD18-T vector and the positive clones were used for sequencing and BLAST analyses to identify the related reference viruses of the qd/2019/china strain with the differential part of the genome and complete genome. The BLAST analyses (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were performed to identify the related reference viruses and used to study the qd/2019/china strain.

Table 1. Primers used in the genome cloning.

Primer	Sequence（5′ - 3′）	Position *
FCV-TY-20F	GTAAAAGAAATTTGAGACAA	1 - 20
FCV-TY-2440-R	ATGTTGATTGGCGGGTAGTTC	2440 - 2420
FCV-TY-2420-F	GAACTACCCGCCAATCAACAT	2420 - 2440
FCV-TY-5333-R	TTAGCGCAGGTTGAGCACAT	5333 - 5314
FCV-TY-5314-F	ATGTGCTCAACCTGCGCTAA	5314 - 5333
FCV-TY-7709-R	CCCTGGGGTTAGGCGCGA	7692 - 7709

* The position of primers was related to that in FCV strain CH-JL4 (Genbank® accession No. KT206207.1).

Sequence analysis. The capsid gene, *VP1* is the most variable gene in FCV genome. The phylogenetic analysis was usually undertaken based on the alignment of the *VP1* amino acid sequence. The sequence similarity was analyzed using DNAstar software (DNASTAR, Madison, USA). The

phylogenetic analysis of the full-length FCV genome and capsid gene were performed using the MEGA Software (version 6.0; Biodesign Institute, Tempe, USA). The bootstrap values were calculated according to 1,000 replicates of the alignment.

Recombination detection. From the blast analysis, we found that the complete genome of qd/2019/china had the highest similarity with GXNN01-19 (85.70%). While the different parts of the qd/2019/china genome were found to depict results in different search results, suggesting the possibility of recombination of different isolates. Then, the similarity comparisons and phylogenetic analyses of the full-length and part of FCV strain alignments were performed to detect the recombination signals in the FCV genomes. The sequence used was downloaded from NCBI and the Simplot 3.5.1 program with a 500 bp window and a 20 bp step and was used in this study to verify the recombination.

Results

Virus isolation and identification. The PCR assay was carried out to identify the FCV and depicted positive results. The viruses like FCoV, FPLV, and FHV-1 were not detected in the swab. Then the mouth swab was inoculated into the CRFK cells. The CPE could be easily observed 24 hr post-infection. After three rounds of plaque purification, the CPE was found to stably appear on the CRFK cells. The FCV was still positive with the PCR detection. Besides, the qd/2019/china isolate was further identified in the infected CRFK cells using a cat polyclonal antibody against FCV (previously identified by our lab). In this study, one FCV strain qd/2019/china was isolated from the mouth swab and was purified with the plaque assay method (Fig. 1).

Viral capsid amino acid sequence analysis. The genome of the qd/2019/china strain was successfully sequenced. Since the capsid protein was significantly important for the study of FCV evolution and immune escape, the sequence of the capsid amino acid was analyzed first. About 38 FCV isolates capsid protein sequences were retrieved from NCBI and DNAstar was used to analyze the sequence identity. The comparative pairwise analysis of the complete genome sequence of qd/2019/china *VP1* was conducted with 38 *VP1* sequences. The FCV isolates showed sequence identity ranging from 83.90% (ym3/2001/jp) to 91.10% (CH-JL4) with qd/2019/china that twas consistent with the previously reported genetically different FCV strains. The evolution of the qd/2019/china strain was further investigated by constructing a *VP1* amino acid sequence phylogenetic tree using MEGA. The evolutionary history was inferred using the maximum-likelihood method. The percentage of replicate trees with the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown above the branches.[21] The analysis involved 38 amino acid sequences. The evolutionary tree demonstrated that the qd/2019/china was on the same cluster as CH-JL4. This was consistent with the *VP1* sequence analysis and the complete genome BLAST analysis. The qd/2019/china, CH-JL4, F65, UTCVM-H2, GX01-13, SH2014, UTCVM-H1 were in the same higher cluster (Fig. 2A). Since the FCV CH-JL4 challenge experiment on the kittens indicated typical clinical symptoms which meant that it had a stronger virulence. Since the qd/2019/china was also isolated from the dead cat, it might also be a virulent strain which needed further systematic virus challenge experiments for substantiation.

Complete genome sequence analysis. The complete genome sequence was submitted to GenBank® (accession No. MZ322896) to further understand the genetic origin of the qd/2019/china. The complete genome phylogenetic analysis was conducted using the MEGA6.0 (Fig. 2B). The FCV qd/2019/china strain was closely related to the CH-JL4 sequence and located in the same cluster as CH-JL4. Besides, the other Chinese isolates (SH, XH, WZ-1, HRB-SS, GX01-13), UTCVM-H2 and FCV/DD/2016/GE were in higher-level clusters with the qd/2019/china. The Chinese isolates were segregated into major genetic groups in the phylogenetic trees and designated as genogroups I and II. The strains qd/2019/china, CH-JL4, SH, XH, WZ-1, HRB-SS, GX01-13, UTCVM-H2 and FCV/DD/2016/GE were belonged to the genogroups I.[22]

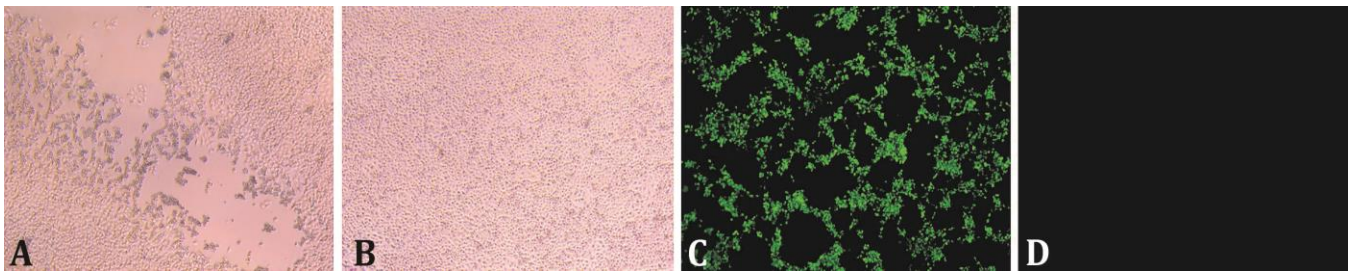

Fig. 1. Virus isolation and identification. **A)** The oropharyngeal swab was inoculated in the CRFK cells. Typical cytopathic effect (CPE), cell aggregation, rounding, falling off and enhanced shading was found (40×), **B)** The physiological saline inoculated CRFK showed no CPE (40×), **C)** Bright green fluorescence was found in the F81 cells infecting FCV (100×), and **D)** Green fluorescence was not found in the normal CRFK cells (100×).

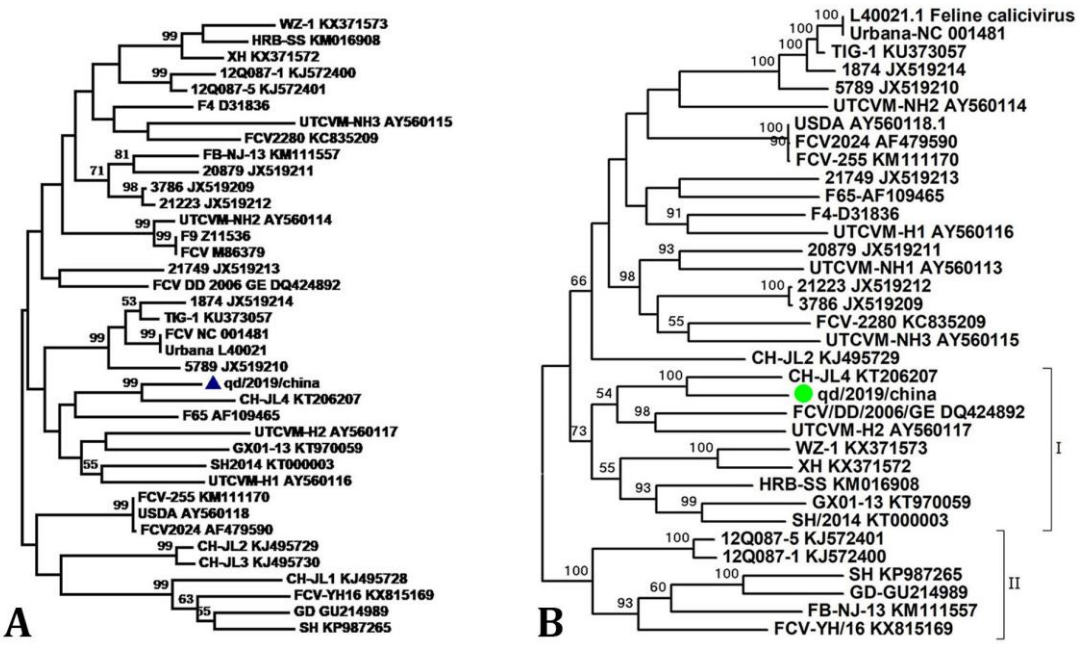

Fig. 2. Sequence analysis of FCV qd/2019/china. **A)** Phylogenetic tree of the feline calicivirus (FCV) *VP1* based on the amino acid sequence. The qd/2019/china was in the same cluster with CH-JL4. The phylogenetic tree was constructed using the maximum-likelihood method based on the JTT matrix-based model. The 1,000 bootstrap repetitions were performed for each analysis. The positions that contained gaps and/or missing data were eliminated. Bootstrap values > 50.00% are indicated, and **B)** The phylogenetic tree of the virus genome was constructed using the neighbor-joining method with 1,000 bootstrap replicates and the Kimura 2-parameter model in the MEGA 6.0 software package. The qd/2019/china is labeled with a green circle. GenBank® accession numbers are indicated on the branches. Bootstrap values > 50.00% are indicated.

Recombination analysis. The result of the BLAST showed that the different part of the FCV genome showed the highest similarity with the different FCV isolate. Given the potential significance of genetic recombination during the evolution of the picorna-viruses,[23] the recombination analysis was conducted with SimPlot. The results provided strong statistical support for an FCV qd/2019/china recombination event. The SimPlot graph revealed the breakpoints that separated the genome of qd/2019/china into three regions of which the two fragments arose from CH-JL4 (regions 1 - 3,821 nt and 5,301 - 7,707 nt) and one fragment originated from HRB-SS (region 3,821 - 5,301 nt). The 3,821 - 5,301 nt was the part of the PP gene based on the FCV CH-JL4 (Fig. 3A). To further validation of the detected recombination signals, a phylogenetic tree of recombination region (the corresponding loci in the genome of qd/2019/china (3821 - 5301 nt) were constructed containing the genogroups I member (CH-JL4, SH, XH, WZ-1, HRB-SS, GX01-13, UTCVM-H2 and FCV/DD/2016/GE) as shown in the tree. The strain qd/2019/china was in the same cluster as HRB-SS (Fig. 3B).

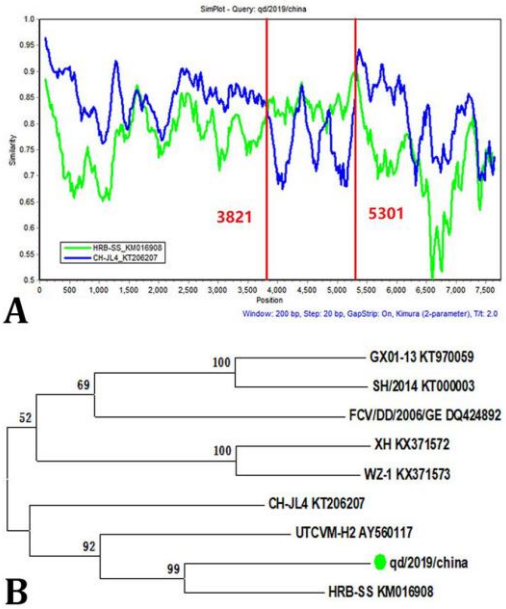

Fig. 3. A) Recombination analysis was based on the whole genome of qd/2019/china. Reference strains, HRB-SS (green), and CH-JL4 (blue) were used as putative parental strains. The X-axis indicates the location of the query sequence and Y-axis indicates the percentage of identity, and **B)** The phylogenetic analysis is based on the genome region 3821 - 5301 nt. The phylogenetic trees were constructed using the MEGA 6.0 software and the maximum likelihood algorithm, with 1,000 bootstrap replications and the Kimura 2-parameter substitution model.

Discussion

The FCV is one of the most common pathogens of domestic cats[1] which accounts for severe respiratory and oral diseases (stomatitis and gingivitis). Despite the cats

being increasingly vaccinated, many strains of the viruses are still isolated in China.[1,24-26] The FCV is a single positive strand RNA virus having high genomic variability for their RdRP low-fidelity. However, the detailed mechanisms of the emergence of the new genetically distinct FCV strains remain unknown.[23] In this study, an FCV qd/2019/china was isolated from a dead cat. This was the first FCV isolate from Qingdao, the eastern coastal areas of China. The sequence analysis of the capsid protein, *VP1*, indicated qd/2019/china to have the highest similarity with CH-JL4 (84.50%).

The northeast part of China has a well-developed pet breeding industry which transports large numbers of cats and dogs to the other parts of the country promoting the spread of the virus. The qd/2019/china might have originated from Jilin indicating that transporting cats across the regions might potentially promote the virus transmission and evolution. Almost at the same time as our first submission, the phenomenon of recombination between different FCV strains had been reported in September 2021. It can be seen that the reorganization of FCV is not a single phenomenon which needs more attention.

The recombination of the virus is a prevalent mechanism of virus evolution reporting the existence of a recombinant FCV circulating within a naturally infected population of cats. The quasispecies represents an effective adaptive strategy for the virus in an extremely heterogeneous viral population evolving with a better replicative capacity.[26] However, the recombination of the FCV virus across the different regions has never been reported. Here, the qd/2019/china might be a recombinant of HRB-SS (from Harbin) and CH-JL4 (from Jilin).

Although there is no licensed live-attenuated FCV vaccine in China, the non-licensed live-attenuated FCV vaccine cannot be prohibited, since it is believed by some clinical veterinarians to be better than the killed vaccine. Here, based on the evidence that the FCV virus can recombine between the different viruses, the virus in the live vaccine was found to be a potential recombinant strain suggesting that the live FCV vaccine registration should be more rigorous.

The FCV shows evolutionary rates higher than those of the other viruses and approximately 1.30×10^{-2} to 2.60×10^{-2} substitutions per nucleotide occur per year in the variable regions of the FCV capsid protein.[27] Thus, the high genetic plasticity of the virus has emerged new variants.[28] The PP protein is the RdRp encoded by FCV and is indispensable for the replication of the viral genome.[29] The PP and its precursor protein also possess the cysteine proteinase activity responsible for the proteolytic processing, cutting the ORF1 encoded large nonstructural proteins into five nonstructural proteins- p5.6, p32, p39, p30, VPg, PP. The PP proteins of FCV 2280 and F9 share 94.00% amino acid identity, however, the strain 2280 grows faster than the strain F9. The challenge experiments in cats showed the strain 2280 to be more virulent than the strain F9. The PP is one of the most important nonstructural protein of FCV. The N-terminal domain of PP can inhibit the host cell protein transcription,[30] therefore, recently PP was found to effectively reduce the mRNA expression by promoting the degradation of the host mRNAs.[31]

In this study, we found that the region (3,821 - 5,301 nt) of qd/2019/china was recombined from HRB-SS and the region (3,821 - 5,301 nt) belonged to the pp region. The PP is a crucial protein that ensures the fidelity of RNA replication, hence, the replacement or change of PP might greatly change the viral characteristics promoting the evolution of FCV and promoting the emergence of new FCV isolates. Besides, the recombination has been associated with the expansion of the range of the viral host,[32] as many FCV isolates were isolated from non-cat feline.[1]

In this study, an FCV strain qd/2019/china was isolated from Qingdao, China. The phylogenetic analysis of the FCV genome revealed that it had the highest similarity with CH-JL4. The recombination analysis revealed that it might be a recombinant virus of CH-JL4 and HRB-SS and that the recombination area belonged to the PP region. Our results thus, provided new evidence highlighting the contribution of viral recombination to FCV evolution.

Acknowledgments

This work was funded by the Natural Science Foundation of Hebei province （grant No. C2021402005), Science and Technology Research Project of Hebei Colleges and Universities (grant No. ZC2021214). The authors would like to thank all the reviewers who participated in the review and MJEditor (www.mjeditor.com) for their linguistic assistance during the preparation of this paper.

Conflicts of interest

The authors declare no financial or commercial conflicts of interest.

References

1. Tian J, Liu D, Liu Y, et al. Molecular characterization of a feline calicivirus isolated from tiger and its pathogenesis in cats. Vet Microbiol 2016; 192: 110-117.
2. Evermann JF, Heeney JL, Roelke ME, et al. Biological and pathological consequences of feline infectious peritonitis virus infection in the cheetah. Arch Virol 1988; 102(3-4): 155-171.
3. Kadoi K, Kiryu M, Iwabuchi M, et al. A strain of calicivirus isolated from lions with vesicular lesions on tongue and snout. New Microbiol 1997; 20(2): 141-148.
4. Martella V, Pratelli A, Gentile M, et al. Analysis of the capsid protein gene of a feline-like calicivirus isolated

from a dog. Vet Microbiol 2002; 85(4): 315-322.
5. Di Martino B, Di Rocco C, Ceci C, et al. Characterization of a strain of feline calicivirus isolated from a dog faecal sample. Vet Microbiol 2009; 139(1-2): 52-57.
6. Martella V, Pinto P, Lorusso E, et al. Detection and full-length genome characterization of novel canine vesiviruses. Emerg Infect Dis 2015; 21(8): 1433-1436.
7. Battilani M, Vaccari F, Carelle MS, et al. Virulent feline calicivirus disease in a shelter in Italy: a case description. Res Vet Sci 2013; 95(1): 283-290.
8. Rohayem J, Bergmann M, Gebhardt J, et al. Antiviral strategies to control calicivirus infections. Antiviral Res 2010; 87(2): 162-178.
9. Fino VR, Kniel KE. UV light inactivation of hepatitis A virus, Aichi virus, and feline calicivirus on strawberries, green onions, and lettuce. J Food Prot 2008; 71(5): 908-913.
10. Prikhodko VG, Sandoval-Jaime C, Abente EJ, et al. Genetic characterization of feline calicivirus strains associated with varying disease manifestations during an outbreak season in Missouri (1995-1996). Virus Genes 2014; 48(1): 96-110.
11. Domingo E, Escarmís C, Sevilla N, et al. Basic concepts in RNA virus evolution. FASEB J 1996; 10(8): 859-864.
12. Chen S, Liu D, Tian J, et al. Molecular characterization of HLJ-073, a recombinant canine coronavirus strain from China with an ORF3abc deletion. Arch Virol 2019; 164(8): 2159-2164.
13. Decaro N, Mari V, Campolo M, et al. Recombinant canine coronaviruses related to transmissible gastroenteritis virus of Swine are circulating in dogs. J Virol 2009; 83(3): 1532-1537.
14. Wang HM, Liu YG, Tang YD, et al. A natural recombinant PRRSV between HP-PRRSV JXA1-like and NADC30-like strains. Transbound Emerg Dis 2018; 65(4): 1078-1086.
15. Liu D, Zhou R, Zhang J, et al. Recombination analyses between two strains of porcine reproductive and respiratory syndrome virus in vivo. Virus Res 2011; 155(2): 473-486.
16. He CQ, He M, He HB, et al. The matrix segment of the "Spanish flu" virus originated from intragenic recombination between avian and human influenza A viruses. Transbound Emerg Dis 2019; 66(5): 2188-2195.
17. Hu CJ, Chang WS, Fang ZS, et al. Nanoparticulate vacuolar ATPase blocker exhibits potent host-targeted antiviral activity against feline coronavirus. Sci Rep 2017; 7(1): 13043. doi: 10.1038/s41598-017-13316-0.
18. Guo H, Miao Q, Zhu J, et al. Isolation and molecular characterization of a virulent systemic feline calicivirus isolated in China. Infect Genet Evol 2018; 65: 425-429.
19. Meli ML, Berger A, Willi B, et al. Molecular detection of feline calicivirus in clinical samples: A study comparing its detection by RT-qPCR directly from swabs and after virus isolation. J Virol Methods 2018; 251: 54-60.
20. Wu H, Zhang X, Liu C, et al. Antiviral effect of lithium chloride on feline calicivirus in vitro. Arch Virol 2015; 160(12): 2935-2943.
21. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985; 39(4): 783-791.
22. Zhou L, Fu N, Ding L, et al., Molecular characterization and cross-reactivity of Feline Calicivirus circulating in Southwestern China. Viruses 2021; 13(9): 1812. doi: 10.3390/v13091812.
23. Lukashev AN. Recombination among picornaviruses. Rev Med Virol 2010; 20(5): 327-337.
24. Zhao Y, Chen X, Ying Y, et al. Isolation and phylogenetic analysis of three feline calicivirus strains from domestic cats in Jilin Province, China. Arch Virol 2017; 162(9): 2579-2589.
25. Liu C, Liu Y, Liu D, et al. Complete genome sequence of feline calicivirus strain HRB-SS from a cat in Heilongjiang Province, Northeastern China. Genome Announc 2014; 2(5): e00698-14. doi: 10.1128/genomeA.00698-14
26. Coyne KP, Reed FC, Porter CJ, et al. Recombination of feline calicivirus within an endemically infected cat colony. J Gen Virol 2006; 87(Pt 4): 921-926.
27. Coyne KP, Gaskell RM, Dawson S, et al. Evolutionary mechanisms of persistence and diversification of a calicivirus within endemically infected natural host populations. J Virol 2007; 81(4): 1961-1971.
28. Coyne KP, Christley RM, Pybus OG, et al. Large-scale spatial and temporal genetic diversity of feline calicivirus. J Virol 2012; 86(20): 11356-11367.
29. Wei L, Huhn JS, Mory A, et al. Proteinase-polymerase precursor as the active form of feline calicivirus RNA-dependent RNA polymerase. J Virol 2001; 75(3): 1211-1219.
30. Wu H, Zu S, Sun X, et al. N-terminal domain of feline calicivirus (FCV) proteinase-polymerase contributes to the inhibition of host cell transcription. Viruses 2016; 8(7): 199. doi: 10.3390/v8070199
31. Wu H, Huang J, Liu Y, et al. Feline calicivirus proteinase-polymerase protein degrades mRNAs to inhibit host gene expression. J Virol 2021; 95(13): e00336-21. doi: 10.1128/JVI.00336-21.
32. Simon-Loriere E, Holmes EC. Why do RNA viruses recombine? Nat Rev Microbiol 2011; 9(8): 617-626.