Complete Genome Sequence of a Natural Mutant of Feline Calicivirus Isolated From a Stray Cat

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Research Article

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Abstract

During the period of the epidemiological surveillance of Feline calicivirus (FCV) isolates, a natural mutant of FCV, designated SH1909, was successfully isolated from a stray cat in Shanghai, China. The complete genome sequence of SH1909 was also determined in this study. Sequence comparison and analysis show that thirteen unique aa residues substitutions and single-aa insertion of N or Y were observed in SH1909 when compared with other FCV isolates. Phylogenetic analysis showed that SH1909 was assigned into a major evolutionary branch and distantly related to vaccine strains. These results indicated that SH1909 was a natural mutant of FCV circulating in China, which has the potential to escape from immunity of FCV vaccine.

Highlights

- The first reported complete genome of FCV strain 1909 in stray cats in China.
- SH1909 has several unique amino acid mutations.
- Most FCVs from China including SH1909 have formed a separate evolutionary branch.
- SH1909 was distantly related to FCV vaccine strains in the phylogenetic tree.

Background

The *Calicivirus* family currently consists of eleven genera: *Bavovirus*, *Lagovirus*, *Minovirus*, *Nacovirus*, *Nebovirus*, *Norovirus*, *Recovirus*, *Salovirus*, *Sapovirus*, *Valovirus*, and *Vesivirus* (<https://www.caliciviridae.com/index.html>). Feline calicivirus (FCV) is a member of the genus *Vesivirus* in the family *Caliciviridae*[6]. Its genome is a single-stranded, positive-sense RNA molecule with a length of about 7.6 kb and a relative molecular mass of $(2.6\text{--}2.8)\times 10^6$, and contains three open reading frames (ORFs) coding for non-structural proteins (NSPs), a capsid precursor that is posttranslationally processed to release the major capsid protein (VP1) and the leader of the capsid (LC) with 124 amino acids (aa) [3, 5], and minor capsid protein (VP2), respectively. The LC protein plays an important role in viral spread and can cause an activation of caspases and a cytopathic effect (CPE) in cell cultures[1]. *VP1* capsid protein have a functional role during the early replication of the calicivirus. The capsid precursor protein (LC-VP1) can further be divided into six regions, termed A to F. Regions B, D and F are more conserved, whereas regions A, C and E are variable. Region E contains two hypervariable regions that are separated by a conserved domain[11]. The E region contains the major B cell epitopes and plays a role in the formation of viral particle antigen structures. And conformational epitopes are probably more important than linear epitopes in viral neutralization[9, 15]. *VP2* is essential for productive replication that results in the synthesis and maturation of infectious virions[4, 13].

Since FCV was firstly isolated and identified in 1957, it has been distributed worldwide in all feline species, such as cats, tigers, lions, and cheetahs, with cats under one year old being the most susceptible[8, 10]. FCV infection is closely related to upper respiratory tract disease, acute mouth

ulceration, and chronic stomatitis. Moreover, most infected or clinically recovered cats can persistently excrete the virus. During the widespread use of commercial vaccines, the cross-protection between the FCV wild strains and the live vaccine strain F9 has been gradually weakened[12], and immunization failure occurs frequently. Although existing vaccines can reduce clinical symptoms and virus excretion, they cannot prevent infection from occurring[2]. Therefore, the persistent surveillance should be performed for the prevalence of FCV isolates and can provide an important insight into the development of novel vaccines. In recent years, there have been some reports of Chinese FCV strains, but few reports of FCV strains from the Chinese stray cats were reported[7, 14]. In view of the larger sphere of activity for stray cats, it is more ready to spread viruses. So, the primary aim of this study was to isolate FCV strains from some stray cats in Shanghai, China and analyze their genomic and evolutionary characterization.

27 fecal samples were collected from the stray cats of Shanghai in September 2019. All samples were respectively homogenized in 1 mL of phosphate-buffered saline and centrifuged at $12,000 \times g$ for 10 min. The clarified supernatant was filtered through 0.22 μm filters (Millipore). Viral RNA was then extracted from the clarified suspension using a TIANamp Virus DNA/RNA Kit (Tiangen, Beijing, China) and reverse transcribed into cDNA using the FastQuant RT Kit (with gDNase) (Tiangen) according to the manufacturer's instructions. Identification of viruses was performed by PCR using the highly conserved primers (Table 1). The results showed three out of 27 samples were positive for FCV. And Crandell feline kidney (CRKF) cells were respectively inoculated with the positive supernatant at 37 °C under 5% CO₂ in 25 cm² flasks. When more than 80% of cells show obvious cytopathic effect, cell suspension was collected and stored at -80°C until use. To amplify the complete genome of FCV, 7 pairs of specific primers (Table 1) were designed based on the conserved regions of the FCV genome sequences retrieved from NCBI. Reverse transcription PCR was performed in a 50 μL reaction mixture comprising 25 μL of Premix Taq (Takara Bio Inc., Shiga, Japan), 2 μL of each primer (10 μM), 2 μL of cDNA template, and 19 μL of ddH₂O. The reaction conditions were as follows: initial denaturation at 95°C for 5 min; , 33 cycles of denaturation at 94°C for 30 s , annealing at 55°C for 30 s, and extension at 72°C for 2 min; and a final elongation step of 72°C for 10min. In addition, the 3' end of the genome was determined by using the method of rapid amplification of cDNA ends (RACE). The PCR products were visualized through electrophoresis on a 1% agarose gel and then purified using the FastPure Gel DNA Extraction Mini Kit (Vazyme) per the manufacturer's instructions. The purified amplicons were cloned into the pMD19-T vector (Takara) for subsequent sequencing (TSINGKE). The obtained partial-genome sequences were sequenced at least three times and assembled using SeqMan Pro v. 7.1.0 (DNASTAR Inc.; Madison, WI, USA). And the assembled full-length genome sequence was analyzed using the Basic Local Alignment Search Tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

After three serial passages, a strain of FCV, referred to as SH1909, was isolated from a fecal specimen of the stray cat. The full-length genome sequence of SH1909 consisted of 7770 nucleotides (nt) with a 5'-terminal untranslated region (UTR) of 19 nt and a 3'-terminal UTR of 123 nt. Three potential ORFs, including ORF1 (nt 20 to 5320), ORF2 (nt 5321 to 7342), and ORF3 (nt 7339 to 7646), were predicted within the genome of SH1909. The complete genome sequence of FCV SH1909 has been submitted to

the GenBank database under accession number MW356260. Multiple sequence alignments were conducted between SH1909 and other referenced FCV isolates retrieved from NCBI databases using Phylosuite v1.2.1. Pairwise sequence comparison performed using the MegAlign program (DNASTAR Inc). The results showed that NSPs of SH1909 shared 88.6%-95.8% and 88.0%-89.1% aa identities with those of other FCV field isolates from China and vaccine strains, respectively, and the highest aa identity (95.8%) with those of FB-NJ-13. As for the VP1 capsid precursor of FCV, SH1909 shared 83.1%-89.2% and 82.2%-85.4% aa identities with the Chinese FCV isolates and vaccine strains, respectively, and the highest aa identity (89.2%) with HB-S4. The above results indicated that SH1909 has evolved significantly when compared with other FCV isolates and less similarity to the FCV vaccine strains than the field isolates. Compared with other Chinese FCV isolates and vaccine strains, seven unique aa mutations at sites 31 (E/D31K), 214 (K214R), 668 (C/H/N 668F), 751 (V/I751T), 1139 (T/S 1139N), 1416 (E1416D) and 1728 (Y1728F) were observed in the NSPs region, while six aa residues substitutions were displayed in LC-VP1 at positions 10 (K10T), 55 (N/D55K), 402 (K402R), 405 (I/T405Y), 439 (D/N/S/T439K), 497(N/K/T/S/T/E/Q497R). It is worth mentioning that aa substitutions at residues 439 and 497, which located in the N-terminal half and C-terminal half parts of region E respectively, may affect the formation of the antigenic structure of the virus particles, leading to a decrease in the protection of the vaccine strain against it. In addition, SH1909, similar to CH-JL4 and TIG-1, possesses an insertion of an Asparagine at site 495 of LC-VP1, whereas the deletion of a Tyrosine positions at site 1034 of the CH-JL4 and TIG-1 NSPs regions. Phylogenetic trees were constructed using the neighbor-joining method with 1,000 bootstraps replicates by MEGA6.06 based on the complete aa sequences of NSPs and LC-VP1(Fig.1). As shown in Fig 1, all Chinese FCV isolates were distributed in several distinct evolutionary branches based on the phylogenetic analysis of NSPs and LC-VP1. Most FCV isolates including SH1909 were clustered in a monophyletic clade, which was distantly related to the FCV vaccine strains, indicating the commercially available vaccines may be invalid for some Chinese endemic isolates including SH1909.

In conclusion, the complete genome sequence of SH1909, was isolated from a stray cat in China, was determined in this study. Sequence comparison and analysis showed that thirteen unique aa residues substitutions and single-aa insertion of N or Y were observed in ORF1 and ORF2 of SH1909 when compared with other FCV isolates, which revealed that SH1909 was a natural mutant of feline calicivirus. Phylogenetic analysis showed that SH1909 was assigned into a major evolutionary branch and distantly related to vaccine strains, indicating it represents an epidemic isolate circulated in China. These results will further enrich our understanding of the genetic variations involved in evolution.

Declarations

Acknowledgement

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Compliance with ethical standards

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

No experimental work with animals or humans was done in this study.

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Table

Table 1

Primers used for identification and PCR amplification of the complete genome of FCV.

Primer name	Nucleotide sequence(5'-3') ^a	Nt positions ^b	Amplicon size(bp)
FCV-363-F	ATTCGGARTGGGAGGCTTT	5808-6171	363
FCV-363-R	GTRTCAGTRTCRGACATAAR		
FCV-1F	GTAAAAGAAATTTGAGACAATGTCTC	1-2451	2451
FCV-1R	TTACCACATGTTGATTGGCGGGTA		
FCV-2F	CTCCCCCTCCTACCTTTCTTAAC	1987-5601	3614
FCV-2R	ATCTTTCCTGCCTGATCCCAATGCATGGGT		
FCV-3F	ATGTGCTCAACCTGCGCTAAC	5321-7700	2380
FCV-3R	CCCTGGGGTTAGGCGCA		
FCV-3'UTR	AATTGAATTTAGCGGCCGCGAATTGG CCCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT		

^aNucleotide sequences are shown using the single-letter IUB codes for degeneracy: R=A/G purine; Y=T/C pyrimidine; K=T/G.

^b The positions of primers were determined according to the complete genome sequence of FCV strain FCV-SH (GenBank accession no. KP987265)

Figures

Figure 1

Phylogenetic analysis of 58 NSPs and 60LC-VP1 of Feline calicivirus based on amino acid sequences using the neighbor-joining method with the Jones-Taylor-Thornton (JTT) model with 1,000 bootstrap replicates. The reference sequences were retrieved from GenBank. SH1909 isolated in this study is labeled with a filled circle (•). The FCV field isolates from China were indicated by a filled diamond (◆), the

FCV vaccine strains are indicated by a filled triangle (▲). Genbank accession numbers, names of viruses and nations were indicated in the branches. Scale bars indicate amino acid substitutions per site.