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Isolation and phylogenetic analysis of feline calicivirus strains from various region of China

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Abstract

Feline calicivirus (FCV) is an important feline pathogen mainly causing upper respiratory tract disease, conjunctivitis, and stomatitis, and it is classified into genotype I and genotype II. To investigate the prevalence and molecular characteristics of FCV, this study collected 337 cat swab samples from animal hospitals in different regions of China from 2019 to 2021. The positive detection rate of FCV was 29.9% (101/337) by RT-PCR. Statistical analysis showed that FCV prevalence was significantly associated with living environment ($p = 0.0004$), age ($p = 0.031$) and clinical symptoms ($p = 0.00$), but not with sex ($p = 0.092$) and breed ($p = 0.171$). The 26 strains of FCV were isolated using F81 cells. Phylogenetic analysis showed that 10 isolates belonged to genotype I, and 16 isolates belonged to genotype II. These 26 isolates were highly genetically diverse, of which HB7 isolate had three same virulence-related amino acid loci with VSD strains. Potential loci distinguishing different genotypes were identified from 26 isolates, suggesting the genetic relationship between different genotypes. In addition, selection pressure analysis based on capsid protein of 26 isolates revealed that the protein is under diversifying selection. This study reveals the genetic diversity of FCV and provides a reference for the screening of vaccine candidate strains and the development of vaccines with better cross-protection effects.

Keywords: Feline Calicivirus, Genetic diversity, Phylogenetic analysis, Selection pressure analysis, ORF2 gene

Introduction

Feline calicivirus (FCV) belongs to family *Caliciviridae* and genus *Vesivirus*. It has a single-stranded positive-sense unsegmented RNA genome without an envelope (Knowles et al. 1990). After it was first reported in 1957, FCV has also been isolated around the world (Sosnovtsev et al. 1998; Wilhelm and Truyen 2006). FCV can cause typical oral respiratory disease (ORD) and mainly infects domestic cats, and it can also infect wild cat animals such as lions, tigers and cheetahs (Dawson et al. 1993; Horimoto et al. 2001). In recent years, FCV infection has caused the outbreaks of malignant systemic diseases in cats (Aboubakr et al. 2018; Guo et al. 2018). For example, virulent systemic disease (VSD) strain can infect adult cats, cause high fever, edema, head and limb ulcers, jaundice symptoms, even resulting in a high mortality rate (Ossiboff et al. 2010).

Currently, FCV exhibits only one serotype, but it has two genotypes, genotype I and genotype II (Pesavento et al. 2008; Sun et al. 2017). It is well known that both genotypes are spread globally, but the specific distribution of these two genotypes of isolates was not clear (Zhao et al. 2017). FCV contains three open reading frames (ORFs). ORF1 encodes the non-structural protein of the virus; ORF2, as the major structural protein, encodes the capsid protein (VP1); ORF3 encodes a smaller structural protein (Geissler et al. 1997; Sosnovtsev et al. 1998). The functional domains of capsid

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protein (VP1) encoded by the ORF2 is divided into six regions: A, B, C, D, E and F (Geissler et al. 2002; Helps et al. 2002). Region A utilizes protease cleavage function to form mature structural protein; regions B, D and F are relatively conserved; regions C and E are highly variable; and E region contains majority of the linear antigenic epitopes and some sites distinguishing VSD strain from ORD strains (Guo et al. 2018; Radford et al. 1999). Specific amino acid sites (438 T, 448 A and 465 S) exist in most VSD strains, but only in a few ORD strains (Brunet et al. 2019).

FCV has high genetic plasticity during replication (Neill 2002). In decades, FCV has mutated into a variety of different strains under immune pressure (Aboubakr et al. 2018). The existing vaccines have poor cross-protection after immunization, resulting in immune evasion, which is one of the main reasons for the current high incidence of FCV (Smith et al. 2020). This poses new challenges to clinical diagnosis and prevention. In China, there are successive reports on VSD and ORD (Kim et al. 2021). To further explore the prevalence of FCV in China and its genetic variation and evolutionary trends, this research performed pathogen isolation, identification, and genetic and phylogenetic analyses of clinical samples from animal hospitals in multiple cities or regions of China. This study will provide a theoretical basis for the development of new FCV vaccines.

Result

FCV positive rate

The FCV positive rate of 337 cat samples was 29.9% (101/337). Rate of cat samples with obvious clinical FCV symptoms was 39.57% (93/235), and that of cat samples without clinical FCV symptoms was 7.84% (8/102). The FCV positive rates of male and female cats were 30.61% (60/196) and 29.08% (41/141), respectively, and the FCV positive rates of cats within 10 months and over 10 months were 34.12% (72/211) and 23.01% (29/126), respectively. The FCV positive rates of group living and solitary living were 39.49% (62/157) and 21.67% (39/180), respectively. FCV positive rate was significantly correlated with living environment ($p=0.0004$), age ($p=0.031$), and clinical signs ($p=0.000$), but not with sex ($p=0.092$) and breed ($p=0.171$). The corresponding test results of the samples were shown in Table 1.

Virus isolation and identification

FCV isolates were identified by cytopathic effects (CPE) such as distinct shrunken, rounded, beaded morphologies (Fig. 1A) and IFA using anti-FCV serum (Fig. 1C) and purified by sucrose gradient centrifugation, followed by electron microscopy observation. The capsid was observed to be centrally-depressed cup-shaped with an icosahedral structure and a diameter of 30–40 nm, which was consistent with morphology of an FCV virion (Fig. 1E).

Table 1 Statistical analysis of FCV positive rate

	Total number of samples	FCV positive number	Positive rate (%)	FCV negative number	Negative rate (%)	χ^2	*p*	OR	95% CI
Clinical status	*n* = 337	101	29.9	236	70.1	34.123	0.000		
FCV suspected cat	235	93	39.57	142	60.43			1.530	1.359–1723
Non- FCV cat	102	8	7.84	94	92.16			0.199	0.100–0.394
Gender	*n* = 337					0.092	0.762		
Male	196	60	30.61	136	69.39			1.031	0.848–1.253
Female	141	41	29.08	100	70.92			0.958	0.725–1.266
Breed	*n* = 337					1.871	0.171		
Mongrel cat	236	76	32.20	160	67.80			1.110	0.963–1.280
Purebred cat	101	25	24.75	76	75.25			0.769	0.522–1.132
Age	*n* = 337					4.637	0.031		
≤ 10 M	211	72	34.12	139	65.88			1.210	1.028–1.425
> 10 M	126	29	23.01	97	76.99			0.699	0.496–0.985
Residential density	*n* = 337					12.693	0.0004		
Single	180	39	21.67	141	78.33			0.646	0.495–0.844
Multiple	157	62	39.49	95	60.51			1.525	1.225–1.899

OR odds ratio, *CI* confidence interval

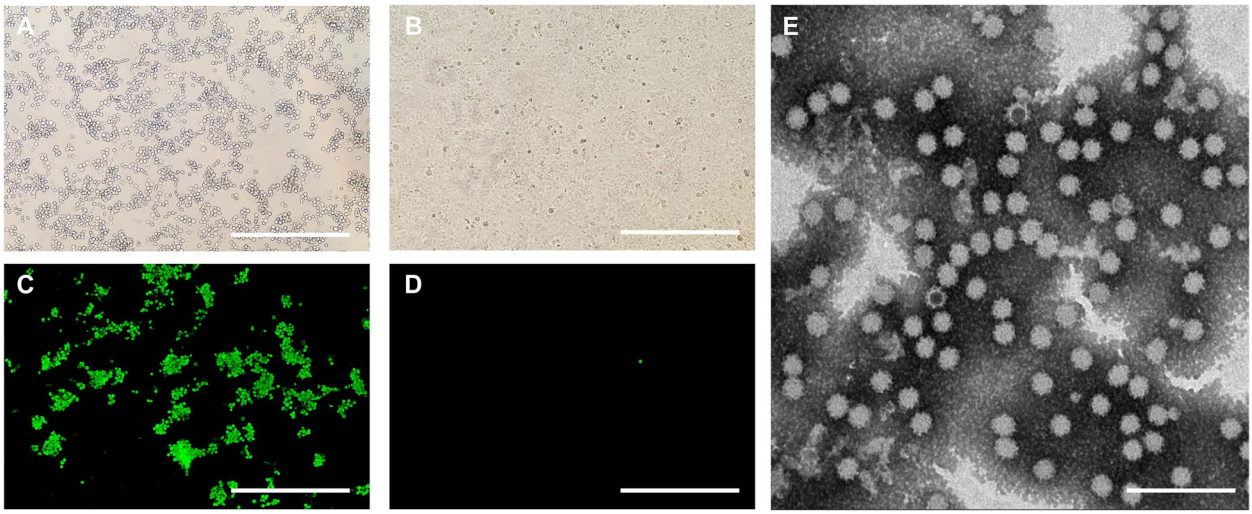

Fig. 1 Isolation and identification of feline calicivirus (FCV). **A** Cytopathic effect (CPE) of F81 cells at 24 h post infection (hpi) with FCV HB7 isolates under microscope (200×). The scale bar is 60 μm. **B** CPE of uninfected cells (negative controls) under microscope (200×). The scale bar is 60 μm. **C** FCV isolate-specific indirect immunofluorescence analysis (IFA, green fluorescence) of F81 cells at 24 hpi. The scale bar is 60 μm. **D** CPE of uninfected cells (negative controls) under fluorescence microscope (200×). The scale bar is 60 μm. **E** Feline calicivirus (FCV) virion under a transmission electron microscope. The scale bar is 200 nm

Comparison of homology of FCV ORF2

Through sequencing and splicing, FCV ORF2 sequences of 26 isolates were obtained from different regions in China (Tables 2 and 3). The ORF2 nucleotide sequence homology was 73.1–100%, and the amino acid sequence homology was 81.4–100% between the new isolates. Overall, all the 26 isolates contained mutations within the ORF2 region compared to that of the reference FCV strains (Fig. S1 in supplementary materials). The nucleotide sequence homology was 70.8–88.0%, and amino acid sequence homology was 80.7–94.4% between these 26 new isolates and representative reference strains. Compared with foreign vaccine strain F9/F4/255, these 26 new isolates exhibited 70.8–77.3%/72.9–78.3%/73.3–77.8% nucleotide sequence homology, respectively, and 82.3–89.8%/80.9–89.7%/81.4–86.7% amino acid sequence homology, respectively. Compared with VSD representative strains, these 26 new isolates displayed 71.8–82.4% nucleotide sequence homology and 80.7–90.4% amino acid sequence homology.

Genetic and phylogenetic analyses of FCV isolates

The ORF2-based phylogenetic tree revealed two main lineages, namely, genotype I and genotype II. According to the evolutionary relationship, genotype I was divided into eight clades, genotype II fell into two clades, and our 26 new isolates were genetically distant from vaccine strains F9, F4, and 255 (Fig. 2). Of these 26 new isolates, 16 belonged to the genotype II lineage, and 10 belonged to the genotype I lineage. Most VSD strains belonged to the genotype I lineage, but they were genetically distant from our 26 isolates.

Comparison of amino acid sites in E region of capsid protein VP1

The comparation of the amino acids of the capsid protein E region found that there was a large genetic diversity among the 26 isolates (Fig. 3A). The comparison between our 10 genotype I isolates and 16 genotype II isolates revealed that 12 specific recognition loci could clearly distinguish more than 80% of 26 isolates (Table 4). Compared with of the vaccine strain F9, 26 isolates exhibited multiple site mutations containing three missing amino acid residues 496-498aa (Fig. 3B). Compared with that of VSD strains and ORD strains, the E region of the capsid protein of 26 isolates showed different amino acids at 7 virulence factor-related loci (Fig. 3B), and HB7 isolate from our 16 genotype II isolates showed three same amino acid sites ($_{G}440_{Q}$, $_{D}455_{T}$ and $_{G}465_{S}$) with the VSD strains (Table 5).

Selective analysis of the capsid protein

To examine selective pressure of the capsid protein, non-synonymous and synonymous single-nucleotide polymorphisms (SNPs) as well as the overall ratio between non-synonymous to synonymous substitutions (dN/dS, also called the ω value) within the ORF2

Table 2 Twenty-six FCV isolates identified in this study

Isolates	Clinical status	Time	Area	Genotypes	Genbank No.
FCV-ZJ2	ORD	2020	Zhejiang	I	OM650775
FCV-HB7	Conjunctivitis, ORD	2020	Hubei	II	OM650776
FCV-HB10	ORD	2020	Hubei	I	OM650777
FCV-FJ1	Conjunctivitis	2021	Fujian	II	OM650778
FCV-AH3	Conjunctivitis, ORD	2021	Anhui	I	OM650779
FCV-LN4	ORD	2021	Liaoning	II	OM650780
FCV-JL18	Conjunctivitis, ORD	2021	Jilin	II	OM650781
FCV-HB28	ORD	2021	Hubei	I	OM650782
FCV-HB29	Conjunctivitis, ORD	2021	Hubei	II	OM650783
FCV-GX43	ORD	2021	Guangxi	II	OM650784
FCV-JS143	ORD	2021	Jiangsu	I	OM650785
FCV-HN183	Conjunctivitis, ORD	2021	Hunan	I	OM650786
FCV-CQ184	ORD	2021	Chongqing	II	OM650787
FCV-SC188	Conjunctivitis	2021	Sichuan	II	OM650788
FCV-YN189	Conjunctivitis	2021	Yunnan	II	OM650789
FCV-GD190	ORD	2021	Guangdong	I	OM650790
FCV-SH191	Conjunctivitis	2021	Shanghai	II	OM650791
FCV-SH192	ORD	2021	Shanghai	II	OM650792
FCV-HN199	Conjunctivitis	2021	Henan	I	OM650793
FCV-HB260	Conjunctivitis, ORD	2021	Hubei	I	OM650794
FCV-GX277	ORD	2021	Guangxi	II	OM650795
FCV-SD348	ORD	2021	Shandong	I	OM650796
FCV-SD369	Conjunctivitis	2021	Shandong	II	OM650797
FCV-HN380	Conjunctivitis	2021	Henan	II	OM650798
FCV-GD383	ORD	2021	Guangdong	II	OM650799
FCV-HB384	Conjunctivitis	2021	Hubei	II	OM650800

ORD oral respiratory disease

Table 3 Sequence homology of FCV ORF2

Strains	ORF2	
	nt	*aa*
New isolates	73.1–100%	81.4–100%
Reference strains (total)	70.8–88.0%	80.7–94.4%
Vaccine F9	70.8–77.3%	82.3–89.8%
Vaccine F4	72.9–78.3%	80.9–89.7%
Vaccine 255	73.3–77.8%	81.4–86.7%
VSD strains	71.8–82.4%	80.7–90.4%

region was calculated using Datamonkey (http://www.datamonkey.org/) with the Fixed Effects Likelihood (FEL) model (Weaver et al. 2018). This approach determined a ω value of 0.496 ($p = 0.0373$) within the region, indicating the protein is under diversifying selection according to the definition of different types of selective pressures based on the ω values (Yang et al. 2000).

Discussion

In recent years, FCV has become the major cause for feline infectious disease (Thomas et al. 2017). Due to the lack of correction function of the viral RNA polymerase, the genome mutation occurs frequently, and the vaccine tends to lose protection in case of VSD strain infection (Guo et al. 2018). Although FCV isolates have been reported in several regions of China (Sun et al. 2017; Tian et al. 2016; Zhao et al. 2017), there are relatively few FCV prevalence data from various regions in China. In this study, we collected 331 clinical cases from different regions in China for risk assessment of FCV epidemic trend, and our identification results showed that the FCV positive rate reached 29.9% (101/337), which was higher than previous report (Guo et al. 2022; Liu et al. 2020; Zhao et al. 2017). This suggests that FCV may be undergoing adaptive mutation, thereby increasing its transmission and making it more prevalent. The FCV positive rate was 39.57% (93/235) for the samples from cats with obvious FCV clinical symptoms and 7.84% (8/102) for the samples from cats without clinical symptoms of FCV.

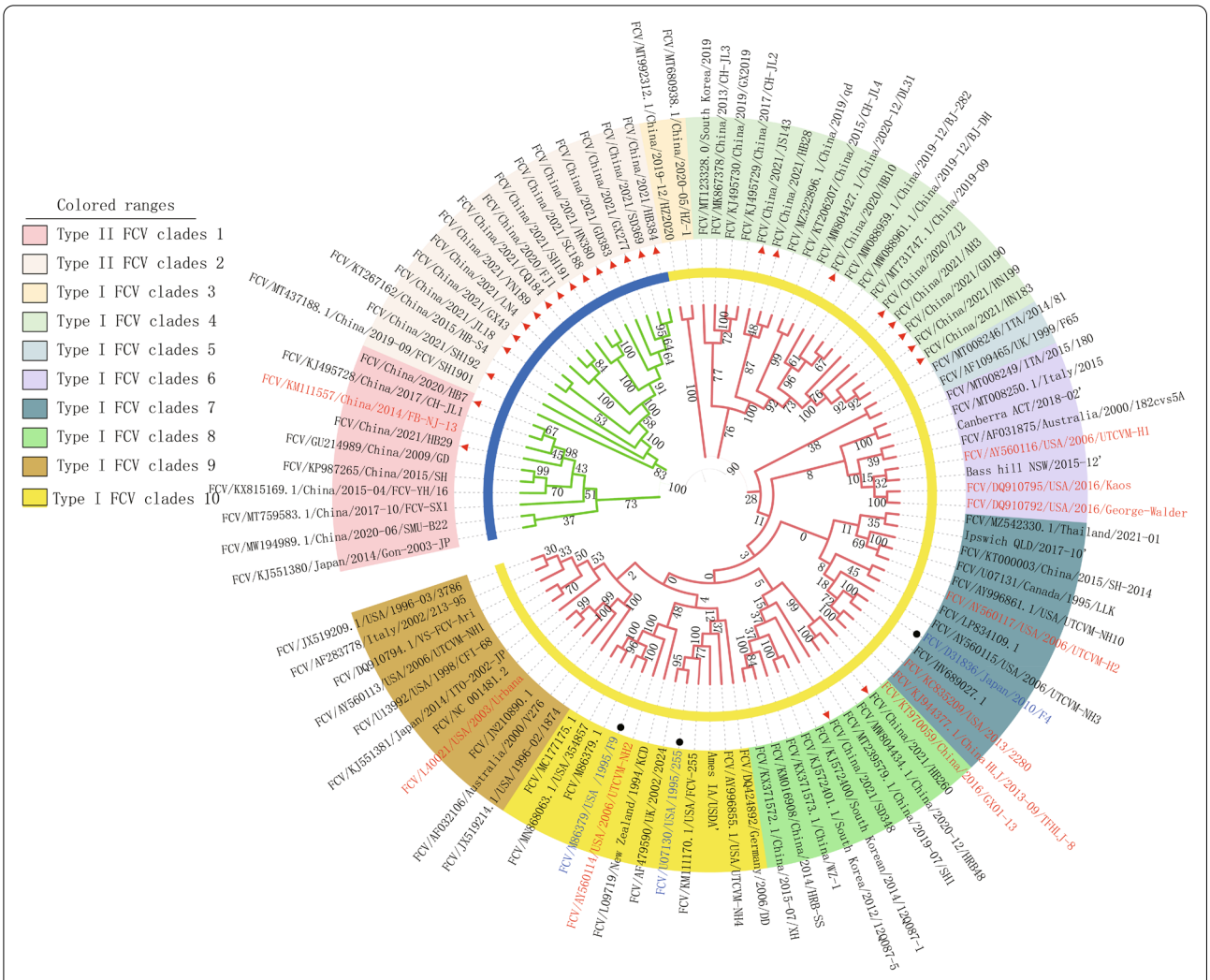

Fig. 2 ORF2 nucleotide sequence-based phylogenetic tree of 26 new isolates and reference strains retrieved from GenBank. Phylogenetic trees were constructed by the neighbor-joining method with 1000 bootstrap replicates using a Kimura 2-parameter model in the MEGA x software package. The 26 new isolates are marked with red triangles (▲), and the vaccine strains are marked with black spot (●). The genotype I branch is marked in red, and the genotype II branch is marked in green. Reference strains and GenBank accession numbers are indicated on the branch, and VSD strain names are in red font

Background information showed although most cases received commercial vaccines, they still developed the disease (details not shown). Our data indicated that FCV prevalence was significantly associated with living environment ($p=0.0004$), age ($p=0.031$), and clinical symptom ($p=0.00$), but not significantly with sex ($p=0.092$) and breed ($p=0.171$). To investigate FCV prevalence and evolutionary trends, positive samples were further isolated and identified. A total of 26 FCV strains were isolated by IFA from different regions of China, and their genetic evolution and pathogenic amino acid loci were analyzed.

The ORF2-based phylogenetic tree revealed that 26 new isolates fell into two lineages, with genotype I lineage containing 10 isolates and genotype II lineage containing 16 new isolates. These 16 genetype II isolates were identical to most previously reported Chinese genetype II isolates. However, there was no obvious association between genotypes and symptoms, and different clinical symptoms were observed among the 26 isolates (Guo et al. 2018; Sun et al. 2017; Zhao et al. 2017). The analyses of ORF2 sequences found that the current prevalent FCV isolates underwent great mutation. Our 26 FCV isolates showed 70.8–88.0% nucleotide homology and 80.7–94.4% amino acid homology

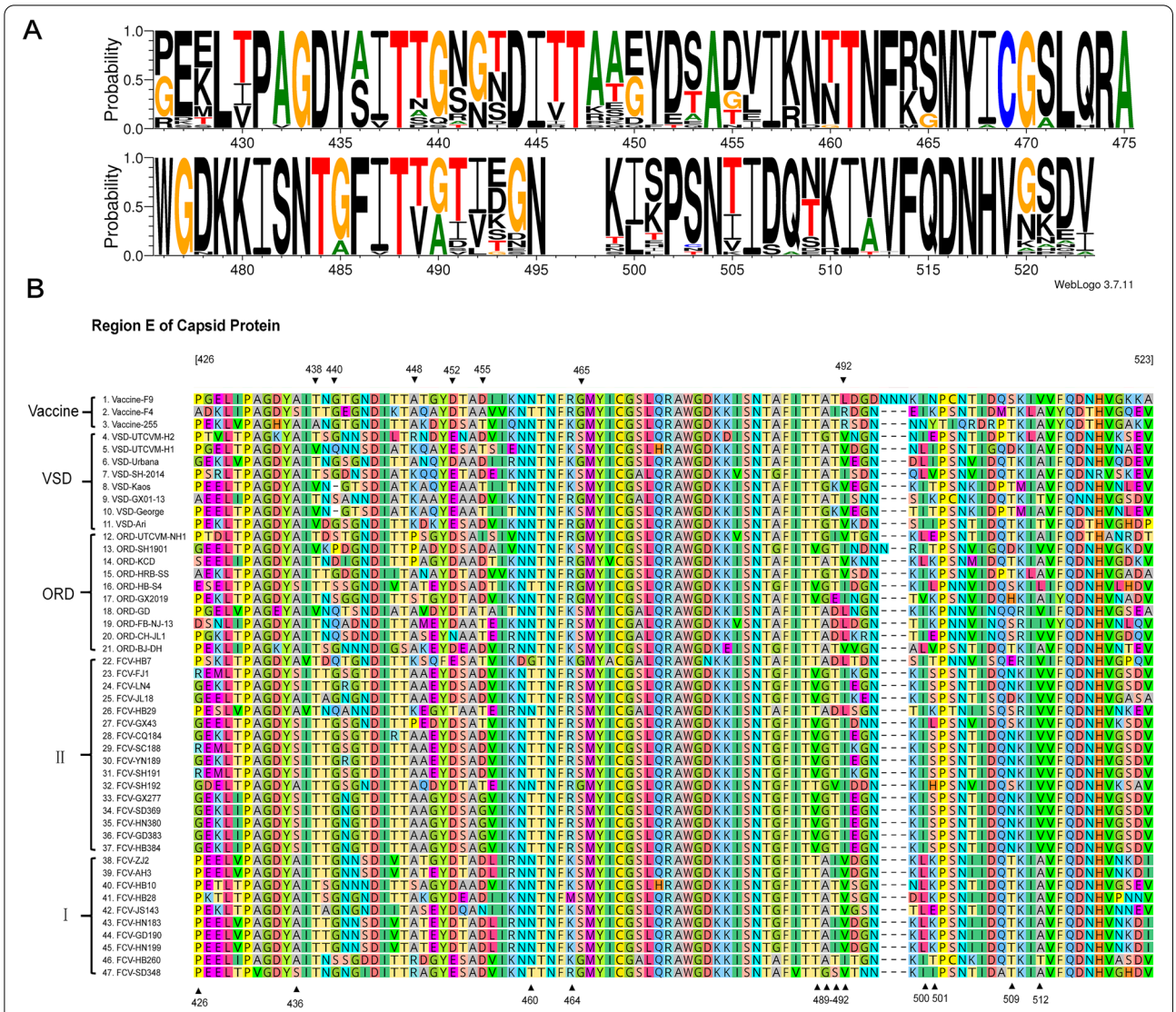

Fig. 3 Comparison of amino acids of capsid protein E region of the 26 FCV isolates. **A** Diversified mutations in the E region of capsid protein, the size of the graphic character at corresponding site is proportional to the occurrence frequency of amino acids at a certain site. **B** Specific identification sites of different genotypes are marked with black triangles at the bottom (▲), seven virulence factor-related sites are indicated by black triangles at the top (▼), "-" indicates missing regions

with the earlier reference strains, indicating that FCV had extensive genetic diversity, which was in line with previous reports (Guo et al. 2018; Sun et al. 2017). Further comparison of the amino acid sites of the capsid protein found that compared with the F9 vaccine strain, our 26 isolates showed multiple amino acid site mutations, including three amino acid site deletions, which was consistent with previous reports (Brunet et al. 2019). This confirmed once again that FCV had a higher degree of genome variation over time, which may be the main reason for the current immune failure against FCV.

The ORF2 gene encodes the capsid protein of FCV (Caringella et al. 2019). It is known that the capsid protein is the main structural protein of many viruses including FCV, and this protein plays a very important role in fighting against the host immune systems during the viral pathogenesis (Ohe et al. 2006; Radford et al. 2007; Spiri 2022). Our selective analysis revealed that the dN/dS ratio within the protein was smaller than 1, suggesting that the protein is under diversifying selection as dN/dS ratio (ω value) is commonly used as a measure of purifying versus diversifying selection, and $0 < \omega < 1$ indicate purifying selection (Yang et al. 2000). A purifying

Table 4 Comparison of amino acids of capsid protein E region of 26 FCV isolates

Genotypes	Amino acids of different genotypes of 26 isolates											
	426	**436**	**460**	**464**	**489**	**490**	**491**	**492**	**500**	**501**	**509**	**512**
I	P	A	N	K	T	A	I	V	L	K	T	A
II	G	S	T	R	V	G	T	I	I	S	N	V

Table 5 Comparison of amino acids at seven virulence factor-related sites between 26 isolates and reference strains

Isolates	Amino acids at seven virulence factor-related sites						
	438	**440**	**448**	**452**	**455**	**465**	**492**
VSD-FCV	V/T	Q	K	E	T/D	S	V
ORD-FCV	T	G	A	D	D	G	V
New isolates	T_{26}	$G_{25}Q_1$	$A_{23}K_1P_1R_1$	$D_{22}E_3T_1$	$D_{21}T_4N_1$	$S_{22}G_4$	$I_{16}V_8L_2$

Subscripts indicate the frequency of occurrences among the 26 isolates

selection determined for this protein may be due to its frequently interactions with the host immune systems (Spiri 2022). The most variable E of the capsid protein VP1 contains most of the antigenic linear epitopes of the virus. E region is the main region for phylogenetic analysis and a key region for identifying VSD strains (Di Martino et al. 2020; Glotova et al. 2018). Our comparison of the amino acid sites in the E region of 26 isolates found 12 specific identification sites which could clearly distinguish more than 80% of isolates (including two genotypes). This finding enriched the reported potential specific loci, which contributed to identifying different genotypes (Brunet et al. 2019; Guo et al. 2018). In this study, we also found that 26 new isolates had different amino acids at seven virulence factor-related sites, compared with VSD strains and ORD strains.

The prevalence of VSD strains causing malignant systemic diseases suggests that FCV constantly mutates under immune selection pressure (Lee et al. 2021; Spiri et al. 2021). Our selection pressure analysis based on capsid protein of 26 isolates revealed that the protein is under diversifying selection. This might explain why there are still many cases in China after immunization with a variety of commercial vaccines. It has been reported that the current vaccine strains may have lost their protective power (Barrera-Vazquez et al. 2019; Wang et al. 2021).

Conclusion

Our data indicated that FCV prevalence was significantly associated with living environment ($p = 0.0004$), age ($p = 0.031$), and clinical symptom ($p = 0.000$), but not significantly with sex ($p = 0.092$) and breed ($p = 0.171$). We isolated 26 FCV strains from collected cat swab samples. Phylogenetic analysis showed that 10 strains belonged to genotype I and 16 strains belonged to genotype II. These 26 isolates exhibited high genetic diversity, of which HB7 isolate displayed 3 same virulence-related amino acid sites with VSD strains. Potential loci distinguishing different genotypes were identified from 26 isolates. This provides an insight into the genetic relationship between different genotypes. Selection pressure analysis based on capsid protein of 26 isolates revealed that the protein is under diversifying selection. Our epidemiological investigation of FCV provides valuable reference for the screening of vaccine candidate strains and the development of vaccines with better cross-protection effects.

Methods

Sample collection

A total of 337 clinical cat swab samples were collected from animal hospitals in various regions of China from 2019 to 2021, of which 235 samples were from the cats with typical FCV symptoms (ORD and conjunctivitis) and 102 from the cats without clinical symptoms. The nasal, ocular, and oral samples were collected using sterile cotton swabs and stored in −80°C refrigerators for subsequent RT-PCR.

RNA extraction and identification

RNA extraction of the swab samples was performed with commercially available viral RNA/DNA extraction kits using Takara Biomedical Technology (Beijing, China).

The primers were designed according to NCBI sequences with good specificity. Preliminary detection of FCV in the samples was performed using F1 (GTTGAC

CCTTACTCATACAC) and R1 (CCCTGGGGTTAG GCGC) at an annealing temperature of 52°C. RT-PCR was performed using a commercially available one-step kit (Takara Biomedical Technology, Beijing, China) with the expected product size of 136 bp.

Statistical analysis of FCV positive rate

Association analyses of FCV infection with clinical symptoms, living environment, sex, breed, and age were performed in 2 × 2 contingency tables using the Chi-square test with Fisher's exact test in SPSS V. 19.0. A multi-cat environment was defined as the number of cats of ≥2. $p < 0.05$ and $p < 0.01$ were considered as statistically significant or highly significant, respectively.

Virus isolation and identification

The RNA-positive clinical samples were homogenized in Dulbecco's modified Eagle's medium (Gibco) containing 100 IU /mL penicillin and 100 μg /mL streptomycin and centrifuged at 5000 rpm for 3 min at 4°C. Supernatants were filtered through 0.22 μm filters (EMD Millipore, Billerica, MA, USA) and inoculated into F81 cells. Cytopathic effects (CPEs) were observed daily. When CPEs reached 80%, viruses were harvested by three freeze–thaw cycles, stored at − 80°C, and were then used for extraction of total RNA. FCV ORF2 gene was then amplified. To identify FCV isolates, indirect immunofluorescence assay (IFA) was performed as follows. F81 cells infected with FCV were incubated first with a mouse monoclonal primary antibody against the FCV VP1 for 3 h at 37°C and then with goat polyclonal secondary antibody to mouse IgG - H&L (Alexa Fluor® 488) (Abcam, USA) for 45 min at room temperature. The incubated cell monolayers were washed three times with PBST. Subsequently, cells were observed under a fluorescence microscope. The virus concentration and purification were conducted by sucrose gradient centrifugation, and samples were negatively stained with 2% sodium phosphotungstic acid.

Mutation detection of ORF2

The primers were designed based on the NCBI sequences with good specificity. FCV isolates were amplified by primers F2 (GTGATGTGTTCGAAGTTTG) and R2 (GAATTCCCATGTAGGAGGC) of the ORF2 gene at an annealing temperature of 58°C with expected product size of 1972 bp. PCR amplification products were subjected to Sanger sequencing by Sangon Biotech Co. Ltd. (Shanghai, China).

Phylogenetic analyses

The sequences of ORF2 gene of typical FCV strains were obtained from the National Center for Biotechnology Information (NCBI) nucleotide database. All details concerning the FCV isolate sequences in this study were submitted to GenBank. ORF2 sequences were aligned at the nucleotide level by Mega X (Pennsylvania State University, USA), whereas capsid protein-coding sequences were aligned at the amino acid level by Mega X and DNAStar (Madison, USA) and manually adjusted. The nucleotide sequence, amino acid sequence, and antigenic epitope domain of the isolated and reference strains were analyzed. A phylogenic tree was constructed with the neighbor-joining method and 1000 bootstrap replicates.

Selective analysis of the capsid protein

Non-synonymous SNPs, synonymous SNPs, and the overall ratio between non-synonymous to synonymous substitutions (dN/dS) within the ORF2 region was calculated using Datamonkey (http://www.datamonkey.org/) with the Fixed Effects Likelihood (FEL) model (Weaver et al. 2018). Different types of selective pressures are determined based on the dN/dS values (also called ω values) as follows: purifying selection ($0 < \omega < 1$), positive selection ($\omega > 1$), and neutral selection ($\omega = 1$) (Yang et al. 2000).

Abbreviations

FCV: Feline calicivirus; ORD: Oral respiratory disease; VSD: Virulent systemic disease; ORFs: Open reading frames; VP1: Capsid protein; NCBI: National Center for Biotechnology Information; RT-PCR: Reverse transcription-polymerase chain reaction; FEL: Fixed effects likelihood; SNPs: Single-nucleotide polymorphisms; CPE: Cytopathic effects; hpi: Hours post infection; dN/dS: Non-synonymous to synonymous substitutions..

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-022-00047-7.

Additional file 1: Fig. S1. Comparison of amino acids of capsid protein E region between 26 isolates and the vaccine strains. "." indicates same amino acids sites. (A) Comparison of amino acids of capsid protein E region between 26 isolates and the F9 vaccine strains. (B) Comparison of amino acids of capsid protein E region between 26 isolates and the F4 vaccine strains. (C) Comparison of amino acids of capsid protein E region between 26 isolates and the 255 vaccine strains.

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Authors' contributions

LC, DZ, and SC contributed to the conception and design of this work; LC, QL, KS, LW, HO, ZY, WD, JY, XH and JL participated in sample collection, laboratory experiments and data analysis; LC drafted the manuscript; and QL, DZ and SC revised the manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials
The data used to support the findings of this study are included in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Author Shenbo Cao has not involved in the journal's review or decisions related to this manuscript. The authors declare that they have no competing interests.

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