Molecular Investigation of Feline Calicivirus Infection in China, 2019-2020

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Research Article

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Abstract

Feline calicivirus (FCV) is a highly contagious viral pathogen of upper respiratory infections and oral disease in cats. To investigate the prevalence and gene characteristic of FCV in China, a total of 1739 clinical swabs of cat eyes and nasal were collected from 19 cities in China from 2019 to 2020. The FCV from clinical samples were isolated in F81 cells, and the gene sequences of the isolated FCV's capsid proteins were phylogenetically analyzed by constructing the phylogenetic tree with the FCV vaccine strain F9 and reference strains of other countries. Results revealed a prevalence of 13.0% (226/1739) for FCV in China in this study, and samples from Langfang showed the highest prevalence in the cities. The 74 FCV strains isolated from clinical samples shared the nucleotide identity of 73.4%-79.1% and the amino acid identity of 83%-90% comparing with the F9 strain. Phylogenetic analysis reveals two branches of these FCV strains from China, which distinct from the vaccine strains of F9 and 255, and other reference strains. Structurally, the highly variable sites of capsid protein were exposed on the protein surface between circulating strains in China and the vaccine strain F9. Overall, this study would promote the understanding of the FCV prevalence and gene characteristics in China.

Main Text

In cats, acute or chronic stomatitis, gingivitis, nasal and eye inflammation, pneumonia, lameness, and neurological symptoms were the most frequent clinical manifestation associated with feline calicivirus (FCV) infection [2, 5, 16, 22, 23, 31]. More seriously, the infection of specific FCV strains could lead to virulent systemic disease with high mortality rates, charactered by dyspnea, transient lameness, and depression [3, 12, 21, 26, 28].

FCV belongs to the family Caliciviruses, harboring a single-stranded positive-sense RNA with a genome of about 7.7 kb in size [8]. The genome contains 3 open reading frames (ORF), ORF1 encodes a large polyprotein precursor that cleaved by viral protease to produce 6 non-structural proteins, which is contributed to the protein translation and RNA transcription[29]. Downstream of ORF1, ORF2 encodes a 73 KDa precursor protein that forms the main virion and stimulates the immune response [4], which divided into 6 regions, the conserved regions of A, B, D and F, and the variable regions of C and E. Although some commercial vaccines were applied for the prevention of FCV, the protective effect against the field isolates infection is insufficient, which may be caused by the genetic diversity of FCV [14, 24]. Meanwhile, the widespread use of the feline calicivirus vaccine may cause genetic variations in FCV. The reactivity between the reference serum and FCV field isolates varied greatly, some reference serum only showed low neutralization titer [15, 17, 18].

To investigate the genetic variation of FCV prevalence in China, 1739 swab samples of cat eyes and nasal collected from 19 cities (on physical examination; from 16 provinces and municipalities) during 2019-2020 were detected (Fig. 1). The clinical samples were collected in sterile phosphate buffer saline (PBS, 0.01M, pH=7.2) and stored at - 20 ℃.

Reverse transcription PCR (RT-PCR) method was applied in FCV nucleotide detection. The primers were designed based on FCV strain s298 (Genbank Accession number: DQ182631) and be synthesized (GENEWIZ, Beijing, China): FCV-f: 5'-CAARGGAGAAAATTCDGACGA-3', FCV-r: GTATTTWAGCACGTTAGCGCAGGT (nt 83-103 and 415-392), which amplify a conserved fragment of about 330 bp in the ORF1 gene. The clinical swabs were homogenized in a vortex and the viral RNA was extracted by viral nucleic acid extraction kit Ⅱ (Geneaid, Taiwan, China). After that, Easyscript One-Step RT-PCR SuperMix (TransGen Biotech, Beijing, China) were used in the reverse transcription (RT) PCR assay, and the cycle was as follows: 42 ℃ for 45 min, 95 ℃ for 3 min, followed by 30 cycles of 95 ℃ for 30 sec, 56 ℃ for 30 sec, 72 ℃ for 45 sec, and with a final extension at 72 ℃ for 10 min. The detail of the samples and positive rate of FCV was shown in Table 1.

The RT-PCR results showed that the prevalence of FCV in China was 13.0% (226/1739) in this study, and the samples from Langfang showed the highest prevalence of 28.6%, while no positive samples were detected in the samples collected from cities of Shenyang, Harbin and Tangshan. For the samples collected from different seasons, the results showed the highest prevalence in samples collected in winter (17.18%, 95% confidence interval [CI]: 0.137-0.212), and the lowest in autumn (9.63%, 95% CI: 0.071-0.129), the prevalence of the samples collected in spring and summer were 15.49% (95% CI: 0.123-0.192) and 10.57% (95% CI: 0.082-0.135), respectively. In the 1147 samples with age information (12 days to 15 years old), the highest FCV prevalence was found in the 4-6 months-old cat group (16.58%, 95% CI: 0.119-0.226), and lowest in the cat group aged above 12 months (10.92%, 95% CI: 0.084-0.141), , the prevalence of the samples collected from cats aged below 4 months and aged from 7-12 months were 13.94% (95% CI: 0.108-0.178) and 15.83% (95% CI: 0.103-0.234), respectively. In the 498 samples with FCV immunization information (382: immunized; 116: non-immunized), the FCV prevalence in immunized cat group was 11.52% (95% CI: 0.087-0.151), which was lower than that in non-immunized cat group (16.38%, 95% CI: 0.107-0.241).

For FCV isolation, the F81 cells were grown in Dulbecco's modified eagle medium (DMEM) (Gbico, USA) containing 8% fetal bovine serum (FBS), supplied with streptomycin (200 mg/mL) and penicillin (200 U/mL), and then cultured at 37 ℃ in a 5% CO_2 incubator. FCV positive samples identified by RT-PCR were used for virus isolation. After centrifugation at 600 × *g* for 5 min. The supernatant was 5-fold diluted in DMEM, and then filtered using a 0.22 μm filter. The filtrate was subsequently added to confluent monolayers of F81 cells at a ratio (v/v) of 1/10. After incubation for 1 h, the supernatant was discarded and the F81 cells were maintained with DMEM medium supplemented with 2% FBS. The cytopathic effect (CPE) was monitored daily, and cell cultures were harvested when 80%-90% of the cells manifested CPE. The harvested cell cultures were stored at -80 ℃ until use.

The full length of FCV capsid protein gene was amplified using the primers FCV-C-f (5'-CCTACACTGTGATGTGTTCG-3') and FCV-C-r (5'-GCAGCTTTGTCCAATTCAAT-3'), which were designed based on the FCV strain CH-JL4 (Genbank Accession number: KT206207). The total RNA was extracted from cell culture using viral nucleic acid extraction kit Ⅱ (Geneaid) and then the cDNA was synthesized using EasyScript First-Strand cDNA Synthesis SuperMix (TransGen Biotech). The PCR cycle was as

follows: 94 ℃ for 3 min, followed by 30 cycles of 95 ℃ for 30 sec, 55 ℃ for 30 sec, 72 ℃ for 30 sec, and with a final extension at 72 ℃ for 10min. The PCR products were purified using TIANgel Midi Purification Kit (TransGen Biotech) and ligated into pEASY-Blunt cloning vector (TransGen Biotech). The recombinant plasmids were transformed into Trans1-T1 Phage Resistant Chemically Competent Cells (TransGen Biotech). After identification with PCR, the positive clones containing the recombinant plasmids were sent for sequencing (GENEWIZ). The variations of the FCV capsid genes and proteins were compared with the reference strain F9 (GeneBank M86379) and other strains (Supplementary Table 1). Phylogenetic of the FCV capsid protein genomes was performed using MEGA X. Phylogenetic trees based on capsid genes were constructed by distance-matrix and neighbor-joining analyses after bootstrapping to 1000 replicates.

A total of 74 FCV strains were isolated from the single FCV infection samples identified by RT-PCR (Table 1). The full lengths of 74 FCV capsid protein genes were sequenced. The nucleotide identity of the FCV capsid protein genes ranges from 72.8% to 100%. Identity of FCV capsid protein genes in the same area were Beijing (73.9%~87.2%), Haikou (73.4%~86.1%), Luoyang (74%~88.5%), Qingdao (73.7%~79.3%), Tianjin (72.4%~86.4%), Zhengzhou (73.6%~85%), Chongqing (73.3%~81.1%), Kunming (74.8%~79.6%), Chengdu (73.6%~82.2%). The FCV isolations from China shared the nucleotide identity of 73.4% to 79.1% compared with F9 strain. The identity of the FCV capsid amino acid ranges from 80.3% to 100%. The amino acid identity of the FCV capsid protein in the same area were Beijing (82.2%~93.6%), Haikou (82.1%~92.1%), Luoyang (83.3%~90.6%), Qingdao (82.5%~88.8%), Tianjin (81.2%~93.4%), Zhengzhou (82.1%~93.6%), Chongqing (82.9~92.8%), Kunming (83.4%~90%), Chengdu (82.4%~91.2%). Compared with F9 strain, the FCV isolations from China shared the amino acid identity of 83% to 90%. Phylogenetic analysis based on the full capsid genes showed that a distinct cluster, including most of the FCV reference strains of China, was separated from the strains isolated from other countries, except the strains F2020KM1, F2020KM2, F2019TJ16 and HRB-SS (Fig.2). In the two distinct branches of FCV strains isolated from different areas in China, one branch was closed to the FCV reference strains of other countries, another branch was clustered with the earlier isolated strains in China (Fig.2). No obvious evolutionary difference between isolates from different areas in China.

FCV is a highly contagious pathogen of cats worldwide. Although it is assumed that maybe a high prevalence of FCV in cats in China, there are a few reports about the antigen detection [12, 13, 30, 32]. Here, our study showed that the incidence of FCV was 13.0% in household cats in China. Usually, the co-infection of FCV, Feline panleukopenia virus (FPV) and Feline herpesvirus type 1 (FHV-1) was common in the nucleoid acid detection of samples from household cats. In this study, the positive rates of FPV and FHV-1 were 16.33% and 13.97%, respectively (data not shown), and the FCV infection rate was lower in China than other countries [19].

FCV's activity is highly influenced by temperature, the high temperature in summer could cause the virus inactivity, which might be a reason for the lower FCV prevalence in summer than that in winter. It is common for domestic cats to receive FCV vaccination in recent years. Our results showed that the immunized cats still can be infected with FCV, while non-immunized cats are more susceptible,

suggesting the imported cat triple inactivated vaccine cannot provide completely protection to the domestic FCV epidemic strain, but can reduce the clinical symptoms after infection. Therefore, vaccination is necessary for the prevention and control of FCV in China.

FCV is considered to have only one serotype, but a great diversity was reported based on capsid protein worldwide [9, 11, 27], and the variable antigen of capsid contributed to the immunization failure of commercial vaccine for FCV prevention [25]. FCV could be isolated from cats immunized with the F9, FCV-255 or FC-7, and the serum from the vaccinated cats showed low neutralization titer against the field strains [20]. It is reported that neutralize sites located in the D (ags1, 415-421aa) and E (ags2, 445-451aa; ags3, 452-457aa; ags4, 475-479aa) regions of FCV capsid protein [10]. Domain 1 of feline junctional adhesion molecule 1, a functional receptor for FCV, binds to the outer face of the FCV capsid protein, inducing conformational changes in the viral capsid and the protein surface would be under greater selective pressures [1]. In this study, comparing with the F9 strain, significant diversity in the C-terminal of capsid protein (aa 425-520), including D and E regions, was found (Fig.3). Structurally (based on the three-dimensional structure of capsid protein of FCV F9 strain, PDB: 6GSI [6]; the graphical representation of the amino acid was generated by WebLogo [7]), the highly variable sites of capsid protein were exposed on the protein surface between circulating strains in China and the vaccine strains (Fig.3), indicating an increased risk of FCV infection for cats in China.

Part of the FCV strains isolated from Tianjin and Hebei shared the aa identity of 100%, indicating the epidemic may be caused by the strains that with the same source. The FCV isolates from China didn't cluster with Japan reference strain ITO but showed a relationship with the South Korea strain 12Q087-1, the finding of high sequence variability of the FCV capsid protein genes in different countries was consistent with the previous reports [27, 30].

In conclusion, the sequence of the FCV isolated from different regions in China showed high diversity compared with the FCV vaccine and other reference strains, indicating a great threaten of FCV infection to cats in China. This study would promote the understanding of the FCV prevalence and gene characteristics in China.

Declarations

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Compliance with ethical standards

Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

All the animal samples were collected according to the protocol approved by the Animal Care and Ethics Committee of National Research Center for Veterinary Medicine (Permit No. 2019011526).

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Tables

Table 1
Prevalence of FCV infection identified by RT-PCR in different provinces in China

Regions	NO. of samples tested	No. of positive samples (%)	Isolations
Tianjin	808	125 (15.47%)	39
Beijing	213	16 (7.51%)	10
Luoyang	96	10 (10.42%)	8
Wuhan	28	3 (10.71%)	0
Haikou	183	23 (12.57%)	11
Shenzhen	59	3 (5.08%)	0
Chengdu	44	4 (9.09%)	1
Chongqing	38	3 (7.89%)	1
Zhengzhou	38	1 (2.63%)	1
Qingdao	29	3 (10.34%)	1
Langfang	78	22 (28.21%)	0
Guangzhou	23	5 (21.74%)	0
Kunming	30	3 (10.00%)	2
Shenyang	15	0 (0.00%)	0
Harbin	9	0 (0.00%)	0
Hefei	9	1 (11.11%)	0
Taizhou	26	3 (11.54%)	0
Jilin	8	1 (12.50%)	0
Tangshan	5	0 (0.00%)	0
Total	1739	226 (13.00%)	74

Table 2
Prevalence of FCV in different seasons.

Seasons	NO. of samples tested	No. of positive samples (%)
Spring	414	64 (15.49%)
Summer	530	56 (10.57%)
Autumn	405	39 (9.63%)
Winter	390	67 (17.18%)

Table 3
Prevalence of FCV infection in different ages.

Age	NO. of samples tested	No. of positive samples (%)
< 4 months	373	52 (13.94%)
4–6 months	187	31 (16.58%)
7–12 months	120	19 (15.83%)
> 12 months	467	51 (10.92%)

Table 4
The prevalence of FCV in vaccinated and unvaccinated cats

Samples	NO. of samples tested	No. of positive samples (%)
Vaccinated	382	44 (11.52%)
Unvaccinated	116	19 (16.38%)

Figures

Figure 1

Locations of sample collection. The area of lightseagreen represents the sampled provinces and municipalities in China, and 19 sampled cities are marked in red.

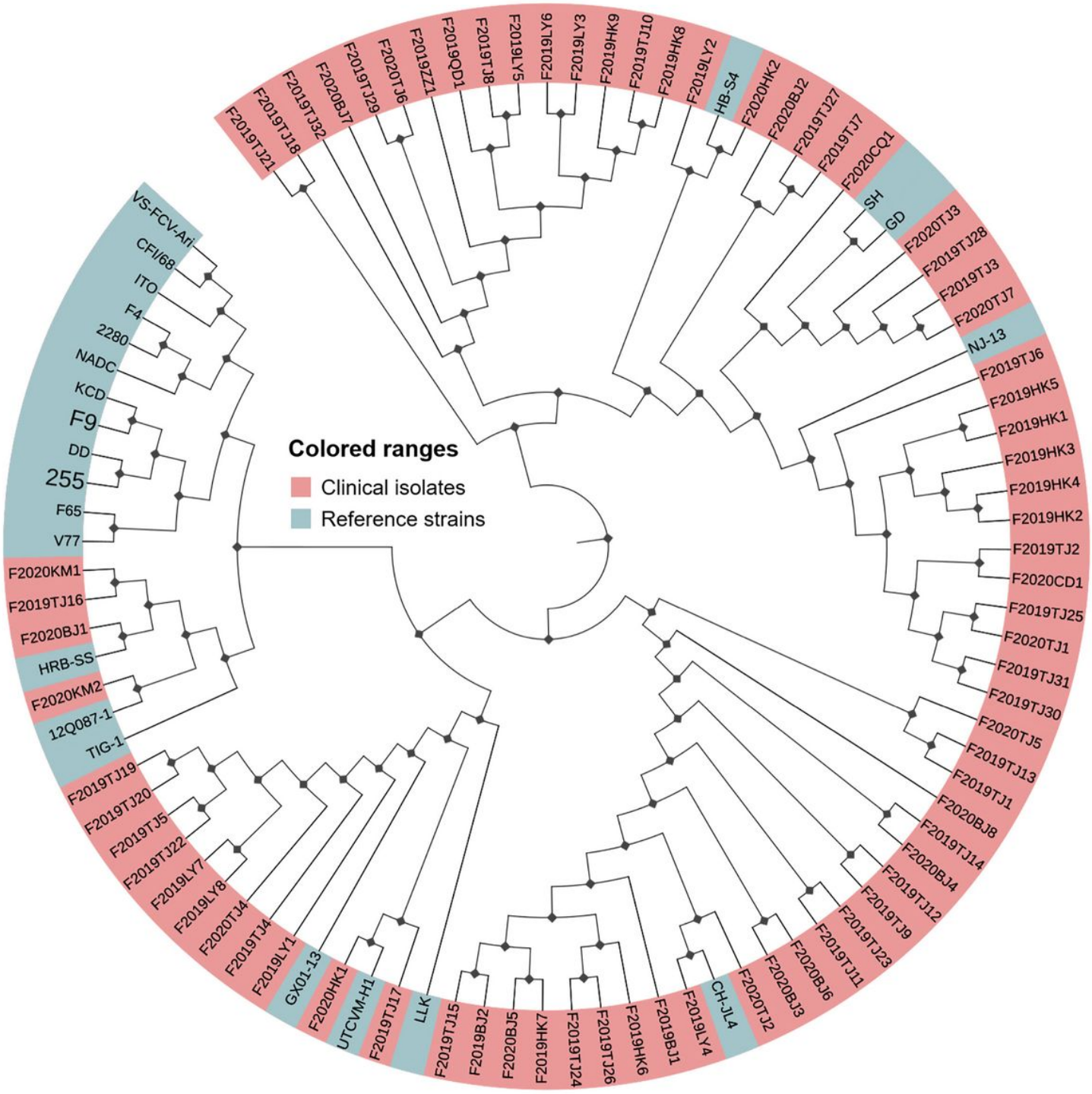

Figure 2

Phylogenetic trees of FCV based on capsid protein gene. The phylogenetic tree was constructed using a distance-based neighbor-joining method with 1000 bootstrap replicates in MEGA X. The red and green background colors represent clinically isolated strains and reference strains, respectively.

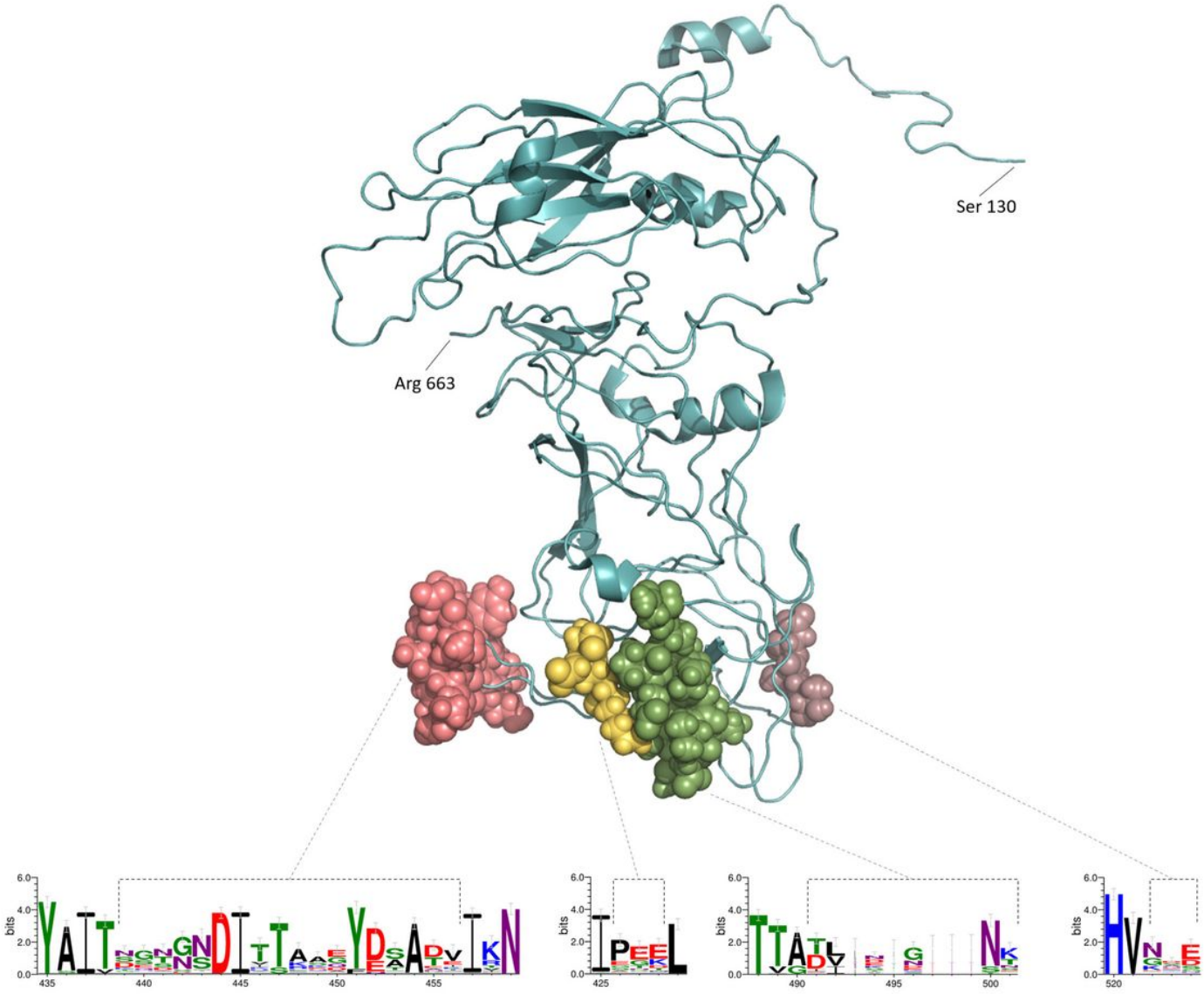

Figure 3

Analysis of amino acid preference of FCV capsid protein between circulating strains in China and the vaccine strain (strain F9), and three-dimensional structure location of hypervariable regions.

Supplementary Files

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- FCVcapsidaaafteralignment.fas
- SupplementaryTableAVFCV.docx