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Detection and Genetic Evolution Analysis of Feline Coronavirus in Jinan City, Shandong Province, 2025-2026

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Abstract

Feline coronavirus (FCoV) is the causative agent of fatal feline infectious peritonitis (FIP) and poses a serious threat to the health of cat populations worldwide; however, its epidemiological characteristics and molecular evolution in northern China remain poorly understood. This study aimed to determine the prevalence of FCoV in Jinan, Shandong Province, China, its co-infection patterns with other pathogens, and the genetic and evolutionary characteristics of the spike (S) gene. A total of 65 clinical feline samples were screened in this study for eight common feline viruses. Seven complete FCoV S gene sequences were successfully obtained and used for phylogenetic analysis, recombination analysis, and B-cell epitope analysis. The results showed that the overall prevalence of FCoV was 38.5%, and all positive cases exhibited co-infections. Furthermore, all isolates clustered within feline coronavirus type I, exhibiting high genetic diversity and frequent S-gene recombination, with the N-terminal domain and receptor-binding domain of the S1 subunit showing high genetic variability. Additionally, very few high-confidence B-cell epitopes were shared between local isolates and the reference strain, suggesting the possibility of antigenic drift. These findings provide critical insights for feline coronavirus surveillance and vaccine development in this region.

INTRODUCTION

With the rapid development of the companion animal industry in China, the domestic cat population has expanded continuously in recent years, accompanied by a growing demand for the prevention and control of feline viral diseases. Feline viral infections not only severely threaten the health and welfare of domestic cats, but also cause substantial economic losses to cat owners and the pet industry, and even pose potential public health risks at the human-animal interface. Feline coronavirus (FCoV) is one of the most prevalent viruses in global feline populations, and is the etiological agent of feline infectious peritonitis (FIP), a systemic granulomatous disease with an extremely high fatality rate of nearly 100% in untreated cats. Given the persistent circulation of FCoV in domestic cat populations and its irreversible pathogenic transformation potential, FCoV infection has long been a major challenge in small animal clinical practice worldwide.

FCoV is an enveloped, single-stranded positive-sense RNA virus belonging to the genus Alphacoronavirus, subfamily Orthocoronavirinae, family Coronaviridae, order Nidovirales, as classified by the International Committee on Taxonomy of Viruses (ICTV)[18]. Based on serological characteristics and genetic features, FCoV is divided into two distinct genotypes: type I (FCoV-I) and type II (FCoV-II), with FCoV-I being the dominant epidemic genotype in most regions of the world, accounting for more than 80% of global FCoV isolates. According to pathogenicity, FCoV is further classified into two biotypes: feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV). FECV typically causes self-limiting intestinal infection or asymptomatic persistent shedding in cats, while FIPV arises from in vivo mutation of FECV, acquiring macrophage tropism and thus causing fatal systemic FIP. [11, 19].

Accumulating evidence has confirmed that the spike (S) protein, the major envelope glycoprotein of FCoV, is the core determinant of viral receptor recognition, cell tropism, fusogenicity, pathogenicity, and host

immune response[19].

The S protein is divided into two functional subunits: the S1 subunit, which contains the N-terminal domain (NTD) and receptor-binding domain (RBD) responsible for binding to the host feline aminopeptidase N (fAPN) receptor, and the S2 subunit, which mediates viral-host membrane fusion[3, 24]. Genetic variations, especially non-synonymous mutations and recombination events in the S gene, directly drive the adaptive evolution, antigenic drift, and pathogenic transformation of FCoV[13, 23]. Therefore, systematic characterization of the S gene of circulating FCoV strains is critical for understanding viral evolutionary dynamics and developing targeted prevention and control strategies. It has been demonstrated that the S1/S2 cleavage motif of FECV is generally adapted to intestinal trypsin-like proteases, while frequent amino acid substitutions occur in this region in FIPV, resulting in the evolution of a canonical furin recognition motif (R-X-K/R-R↓)[22]. Key mutational features include the introduction of basic amino acid residues (arginine R or lysine K), such as the highly conserved arginine substitution at the P4 position and the retention of the conserved serine at the P1' position[13]. These mutations enable the FIPV S protein to be pre-cleaved by ubiquitously expressed furin protease in the Golgi apparatus of virus-infected cells, forming a "pre-activated" conformation. The virus is already in a functionally competent state upon budding and release, which significantly enhances the efficiency of subsequent rounds of infection[13].

In recent decades, extensive epidemiological and molecular evolutionary studies of FCoV have been conducted in Europe, North America, and Southeast Asia, revealing the dominant prevalence of FCoV-I, frequent recombination events, and continuous antigenic variation of global epidemic strains. As a major province with a large companion animal population in northern China, Shandong has a well-developed pet breeding and clinical veterinary industry, but the prevalence, co-infection profiles, and molecular evolutionary characteristics of FCoV in the provincial capital Jinan remain largely uncharacterized. In view of the widespread prevalence of FCoV infection and its potential risk of transformation to a highly lethal phenotype, the epidemic characteristics and evolutionary trends of the virus must be systematically and timely characterized. This study can not only provide a scientific basis for early warning and precise prevention and control of the disease, but also offer critical guidance for the iterative research and development of targeted vaccines and antiviral drugs.

RESULTS

Epidemiological detection of FCoV in the Jinan city

To clarify the prevalence and co-infection characteristics of FCoV in domestic and stray cat populations in the Jinan area, nucleic acid detection of common feline viruses—including feline leukemia virus (FeLV), feline calicivirus (FCV), feline herpesvirus (FHV), feline parvovirus (FPV), feline astrovirus (FAstV), feline bocavirus (FBoV), feline immunodeficiency virus (FIV), and FCoV—was simultaneously performed on 65 clinical cat samples obtained from veterinary hospitals in Jinan. The detection results revealed that both single virus infections and mixed infections were common. The infection rate of FeLV was the highest, reaching 76.9% (50/65), followed by FPV (46.2%, 30/65) and FCoV (38.5%, 25/65) (Fig. 1A). Further analysis of the infection patterns among the 25 FCoV-positive samples demonstrated that mixed

infections were present in all FCoV-positive cases; no single FCoV infection was detected, and the mixed infection patterns exhibited a high degree of diversity (Fig. 1B).

Genetic evolution analysis of FCoV isolates

To investigate whether FCoV undergoes changes within the same feline population, the S genes of seven FCoV isolates from two feline populations were successfully obtained by segmented amplification (Feline population 1: F19, F20, F30; Feline population 2: FCoV, F1480, F1481, F1485) (Fig. 2A). A phylogenetic tree constructed based on the nucleotide sequences of the full-length S gene revealed that global FCoV strains could be clearly divided into two independent genotypic clades, FCoV-I and FCoV-II (Fig. 2B). All seven FCoV isolates obtained in this study from Jinan (indicated by red triangles) were clustered within the FCoV-I genotypic clade, exhibiting significant genetic divergence from FCoV-II genotype strains.

Nucleotide homology analysis revealed that the S gene nucleotide identity among the seven Jinan FCoV isolates ranged from 82.3% to 98.7%, with three isolates sharing over 98% identity, suggesting the presence of a locally circulating dominant FCoV subpopulation in the Jinan area (Fig. 2C). Compared with global FCoV-I reference strains, the nucleotide identity of the Jinan isolates ranged from 76.5% to 97.2%, whereas the identity with FCoV-II reference strains was only 45.2%–51.8%. Amino acid homology analysis yielded a consistent trend with the nucleotide analysis, although the overall identity levels were slightly lower than those observed at the nucleotide level (Fig. 2D).

Recombination analysis of FCoV S gene

To identify recombination events in the S gene of FCoV obtained in this study during its evolutionary history, recombination detection was performed using RDP4 software with a combination of seven algorithms (RDP, GENECONV, Bootscan, Maxchi, Chimaera, SiSscan, and 3Seq), and key recombination events were further validated by Simplot analysis. A total of 18 highly reliable recombination events were detected in the S gene sequences, each supported by at least five algorithms, with some events verified by all seven algorithms, suggesting that recombination may serve as an important molecular mechanism driving the evolution of the FCoV S gene (Table 1). Among the 18 recombination events, 15 were identified with the isolates obtained in this study as the recombinant, indicating that recombination occurred extensively in the S gene region among the circulating strains in the Jinan area (Fig. 3).

Table 1
Recombination analysis of FCoV-S by RDP4

Event Number	Found in	Recombinant	Minor Parent	Major Parent	Detection Methods						
					R	G	B	M	C	S	T
1	1	F20	MW815662.1	Unknown	+	+	+	+	+	+	+
2	1	F1480	PP448849.1	MW815662.1	+	+	+	+	+	+	+
3	1	F30	PV246323.1	Unknown	+	+	+	+	+	+	+
4	1	F19	KY566211.1	Unknown	+	+	+	+	+	+	+
5	1	FCoV	F19	Unknown	+	+	+	+	+	+	+
6	1	MW815662.1	F1485	Unknown	+	+	-	+	+	+	+
7	1	DQ160294.1	F1485	Unknown	+	+	+	+	+	+	+
8	1	F19	PZ043812.1	F1480	+	+	+	+	+	+	+
9	1	PP448854.1	PP464268.1	F1485	+	+	+	+	+	+	+
10	1	F19	F1480	Unknown	+	-	+	+	+	+	+
11	1	PV246297.1	F1485	MW815660.1	+	-	+	+	+	+	+
12	2	PV780394.1	F1485	Unknown	+	-	+	+	-	+	+
13	1	PZ043812.1	PV246323.1	F30	+	+	+	+	+	+	-
14	1	PP448854.1	Unknown	F1485	+	+	+	+	-	+	-
15	1	PZ043812.1	Unknown	F1485	+	-	+	+	-	+	+
16	1	F20	MW815662.1	Unknown	+	+	+	+	+	+	+
17	1	PP448854.1	PZ043811.1	F1480	+	+	-	+	+	+	-
18	1	FCoV	Unknown	PV246323.1	+	-	+	+	+	+	+
Note: RDP(R), GENECONV(G), Bootscan(B), Maxchi(M), Chimaera(C), SiSscan(S), and 3Seq(T).											
+: recombination; -: non- recombination											

Analysis of FCoV S gene

To clarify the functional domain organization and the evolutionary conservation characteristics of different regions of the FCoV S protein, systematic domain annotation and multiple sequence alignment were performed on the full-length S protein sequences of the Jinan isolates and reference strains (Fig. 4A). Multiple sequence alignment revealed marked differences in sequence conservation among different domains of the FCoV S protein (Fig. 4B–E). The heptad repeat1 (HR1) and heptad repeat 2 (HR2) domains in the S2 subunit were highly conserved, with the vast majority of amino acid positions being completely

identical across all strains; in contrast, the NTD and RBD domains in the S1 subunit displayed a higher degree of variation, with numerous amino acid substitution sites, among which the variation in the NTD region was the most pronounced.

The conservation profile of the full-length S protein demonstrated that the S2 subunit was significantly more conserved than the S1 subunit, with the average conservation scores of the FP, HR1, HR2, and transmembrane anchor (TM) regions all exceeding 0.8. Conversely, the NTD and RBD regions of the S1 subunit exhibited the lowest conservation, where multiple hypervariable regions with conservation scores below 0.5 were identified (Fig. 4F).

These results indicate that the S2 subunit of the FCoV S protein, as the core functional region mediating viral membrane fusion, is subjected to stringent functional constraints during evolution and is therefore highly conserved. In contrast, the NTD and RBD of the S1 subunit, serving as the primary targets of the interaction between the virus and the host immune system, are under stronger immune selection pressure and consequently tend to exhibit higher levels of variation.

Analysis of transmembrane region sites of FCOV S gene

The transmembrane region serves as a critical hub linking the extracellular and intracellular domains of the S protein, and its topological structure determines the spatial conformation, membrane localization, and modes of interaction with other molecules. To clarify the transmembrane topological characteristics of the S protein of FCoV isolates from the Jinan area, prediction of transmembrane domains was performed on the S proteins of seven local isolates and classical reference strains using TMHMM. The S proteins of all FCoV strains were found to exhibit a typical type I transmembrane protein topology, with only a single highly conserved transmembrane helical region. The position, length, and topology of the transmembrane domain were completely identical among the seven Jinan isolates, and these features were also highly conserved with those of the FECV and FIPV reference strains; no insertions, deletions, or shift mutations were identified in the transmembrane region (Fig. 5).

Analysis of glycosylation sites of FCOV S gene

Glycosylation is one of the most critical post-translational modifications of the coronavirus S protein, influencing correct protein folding, membrane fusion activity, antigenicity, and immune evasion capacity. To characterize the glycosylation modification features of the S protein of the Jinan isolates, prediction analyses of both N-glycosylation and O-glycosylation sites were performed on the proteins of the local isolates and reference strains.

The N-glycosylation site prediction results revealed that a large number of conserved N-glycosylation sites were present on the S protein of all FCoV strains, and these sites were unevenly distributed across the S1 and S2 subunits (Fig. 6A). The number of N-glycosylation sites in the Jinan isolates was similar to that in the reference strains, with the majority of N-glycosylation sites being highly conserved across all strains and only a few sites exhibiting inter-strain differences.

The O-glycosylation site prediction results demonstrated that the number of O-glycosylation sites on the FCoV S protein was significantly lower than that of N-glycosylation sites, and these sites were predominantly distributed near the N-terminus of the S1 subunit and the S1/S2 cleavage site (Fig. 6B). A total of 12 potential O-glycosylation sites were identified in the Jinan isolates, among which certain sites (e.g., positions 19, 22, 78, and 804) were present in all strains and were thus considered conserved O-glycosylation sites.

Prediction and conservation analysis of B-cell neutralizing epitopes in FCoV S gene

B-cell epitopes are the core antigenic determinants responsible for inducing host neutralizing antibodies, and their distribution and conservation directly determine the protective efficacy of vaccines and the specificity of diagnostic reagents. To identify dominant B-cell epitopes on the S protein of FCoV circulating strains in the Jinan area and to assess the antigenic similarity between the isolated strains and classical strains, a systematic prediction analysis of linear B-cell epitopes was performed on the S proteins of the local isolates and reference strains.

The distribution of epitope scores across the full-length S protein revealed that the predicted epitope scores of all strains were concentrated in the range of 0.7–0.9 and exhibited a markedly uneven distribution along the S protein (Fig. 7A). The S1 subunit, particularly the NTD and RBD regions, was identified as the epitope-enriched area, where multiple high-scoring antigenic determinants were located. The epitope score heatmap indicated that the overall epitope distribution patterns among different strains were highly similar, but notable inter-strain differences were observed in the hypervariable regions of the S1 subunit, suggesting that these regions are the major sites of antigenic variation in FCoV (Fig. 7B).

Analysis of high-confidence epitope sharing revealed that the number of shared high-confidence epitopes among the local isolates ranged from 35 to 47, indicating high antigenic similarity among the locally circulating strains; in contrast, only 1 to 6 epitopes were shared between the local strains and classical reference strains, suggesting that vaccines developed based on classical strains may confer limited cross-protection against the circulating strains in the Jinan area (Fig. 7C). Epitope distribution analysis indicated that the peak epitope scores of all strains were centered around 0.8, indicating that a large number of B-cell epitopes with moderate confidence exist on the FCoV S protein (Fig. 7D).

Analysis of Furin cleavage site of FCOV S gene

The S protein of coronaviruses can be pre-cleaved into non-covalently linked S1 and S2 subunits by furin protease prior to viral egress from host cells, a post-translational modification that enables the S protein to execute its normal biological functions. Cleavage site score prediction was performed on the S protein of the isolated strains and the reference strain. The results revealed that the scores of all isolates were elevated relative to the reference strain, with the exception of the F19 isolate, which presented a lower score than that of the reference strain. Among these isolates, the F1480 strain displayed the highest score, indicating that its S protein is more susceptible to pre-cleavage and subsequent release of its functional domains (Fig. 8A). Sequence conservation analysis demonstrated that the core canonical motif R-X-R/K-R was highly conserved, with the sequence predominantly composed of basic amino acid residues (Fig. 8B).

DISCUSSION

FCoV is an important pathogen widely prevalent among global feline populations, and its persistent infection together with the pathogenic shift to the lethal FIPV poses a severe threat to feline health worldwide. Previous studies have demonstrated that SARS-CoV-2 can be transmitted from COVID-19 patients to domestic cats, underscoring the critical public health significance of coronavirus surveillance in companion animals at the human–animal interface[7]. The 38.5% FCoV prevalence in Jinan is likely driven by the growing scale of multi-cat housing and persistent fecal shedding from asymptomatic FECV carriers, which facilitate sustained horizontal transmission of FCoV. Notably, all FCoV-positive samples in this study presented mixed infections, with FeLV as the dominant co-infecting pathogen. As an immunosuppressive retrovirus, FeLV impairs host viral clearance, prolongs persistent FCoV replication, and thus increases the risk of pathogenic mutation and viral recombination[9]. Co-infection with enteropathogenic viruses such as feline parvovirus may also synergistically damage the intestinal mucosal barrier, further promoting FCoV invasion and replication. [17, 25]. These results highlight the necessity of routine multi-pathogen detection for suspected FCoV cases to support accurate prognosis assessment and rational clinical treatment.

Phylogenetic analysis showed that all local isolates clustered within FCoV genotype I, with no FCoV-II strains detected, aligning with the global dominant epidemic pattern of FCoV-I[21]. The seven isolates were dispersed across multiple subclades of FCoV-I, rather than forming a single monophyletic cluster, indicating that local circulating strains originated from multiple independent cross-regional introduction events, rather than clonal expansion of a single source. Nucleotide homology analysis further confirmed the high genetic diversity of local strains, with 82.3%–98.7% S gene nucleotide identity among isolates, and three strains sharing over 98% identity suggesting the emergence of a locally dominant subpopulation. In addition to point mutations, recombination serves as a core driving force in coronavirus evolution[16]. Recombination detection was conducted on S gene sequences covering both domestic and international epidemic strains as well as the local isolates, and the results indicateed that 83.3% of the recombination events involved local isolates, indicating that recombination is also a critical factor promoting the generation of genetic diversity and adaptive evolution of FCoV in the Jinan area. Moreover, because the coronavirus RNA-dependent RNA polymerase is inherently deficient in proofreading activity, base insertions, deletions, and mismatches are readily introduced during transcription, providing the intrinsic molecular basis for the high frequency of mutation and recombination events[10].

It has been documented that amino acid variations in the S gene, particularly alterations at key sites within the receptor-binding domain, can directly lead to shifts in viral receptor binding capacity and cell tropism. The high-frequency mutations identified in the S1 subunit in this study therefore provide a core molecular basis for host adaptive evolution and immune evasion of the virus[2]. The majority of N-glycosylation sites were conserved among strains, while O-glycosylation sites showed notable inter-strain differences, which may affect antigen epitope exposure and viral immune evasion[20, 26].

B-cell epitopes, as the core antigenic determinants responsible for inducing host neutralizing antibodies, directly determine the protective efficacy of vaccines and the specificity of diagnostic reagents through

their distribution and conservation[4]. Critically, B-cell epitope analysis showed minimal high-confidence epitope sharing between local isolates and classical prototype strains, indicating significant antigenic drift of local circulating strains.[27]. This antigenic divergence may lead to limited cross-protection of existing vaccines developed based on classical strains, and the sequence data obtained in this study can provide a core basis for the development of region-specific customized FCoV vaccines[5, 8].

In summary, this study confirms a high prevalence of FCoV-I with universal mixed infections, frequent recombination and significant antigenic drift in Jinan. These findings fill the gap of FCoV molecular epidemiological data in northern China, and provide critical guidance for regional FIP prevention and control, as well as targeted vaccine development.

CONCLUSIONS

This study systematically investigated the prevalence of 8 common feline viruses in 65 clinical samples from Jinan, China, and performed comprehensive genetic and functional characterization of the *S* gene of 7 local FCoV isolates. The results showed that the FCoV prevalence in Jinan was 38.5%, with universal mixed infections in all positive samples. All local epidemic strains belonged to FCoV genotype I, with abundant genetic diversity, and frequent recombination was identified as the core driver of *S* gene evolution. The local isolates showed significant hypervariability in the S1 subunit and minimal high-confidence B-cell epitopes shared with classical prototype strains, indicating that existing vaccines based on classical strains may provide limited cross-protection against local FCoV strains. Our findings fill the gap of FCoV molecular epidemiological data in northern China, and provide a scientific basis for the development of regional FIP prevention and control strategies and targeted vaccines.

MATERIALS AND METHODS

Total RNA was extracted from the submitted feline serum and pathological tissue samples using a RNA extraction kit (Bioflux,USA). The extracted RNA was reverse-transcribed into cDNA using a reverse transcription kit (Vazyme, China), followed by PCR detection using the primers F (TGCTAGAAGTGCTTCAGCTAAC) and R (CAATCATCTCAACCTGTGTGT). For amplification of the S gene from positive samples, four pairs of specific primers were designed: S1-F (ATGCCAATTATATATTTTGGAGA) and S1-R (AGAARCTYTCTGGTATYTTGCC); S2-F (GGCAARATACCAGARAGYTTYTC) and S2-R (CCATATATGTTRTAGTCAGTRC); S3-F (TGCAGTTTGAAACKYTGTGTTTT) and S3-R (GCAAGTATTTTCTGRTTYTCYTG); and S4-F (GGAGGTATGGCTWTGGGYTCTAT) and S4-R (TTAATGAATGTGAACCTTTTCRAT).

The amplified products were purified by gel extraction (Cwbio, China) and then ligated into the pMD18-T vector (Takara, Japan). The resulting constructs were subsequently sent to Sangon Biotech for sequencing identification.

To elucidate the genetic evolutionary relationships among the amplified strains, a phylogenetic tree was constructed based on the S gene using MEGA12 software. The neighbor-joining method was employed as

the evolutionary model, with 1,000 ultrafast bootstrap replicates. Pairwise nucleotide and amino acid genetic distances among different strains were calculated using the Compute Pairwise Distances function in MEGA12, with 1,000 bootstrap replicates and the p-distance model[12].

To detect potential recombination events, seven algorithms (3Seq, GeneConv, MaxChi, Chimera, RDP, SiScan, and BootScan) implemented in RDP4 software were applied to the sequence alignment under default parameters. A recombination event was considered positive only if it was identified by at least five of the seven algorithms. The identified recombination events were further verified using NCBI BLAST and SimPlot v.3.5.1 software[1, 14, 15].B-cell epitope prediction, glycosylation site analysis, Furin cleaved site analysis, and transmembrane domain analysis were performed using online tools provided by DTU Health Technology[6].

Declarations

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

ETHICS STATEMENT

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Figures

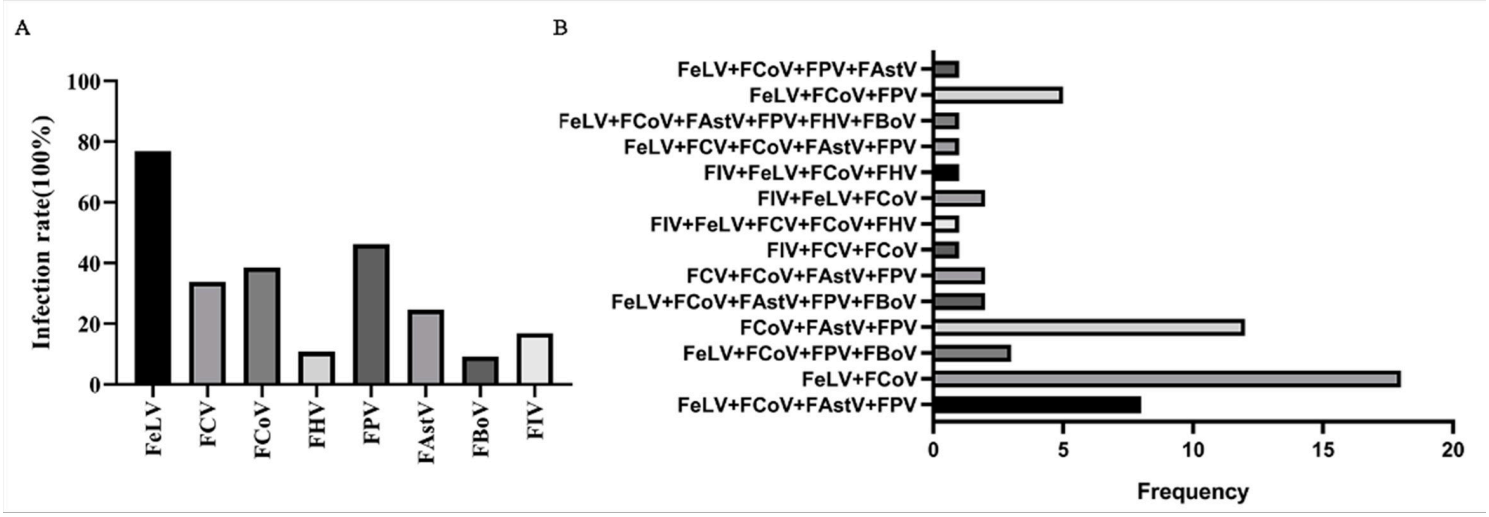


Figure 1

Epidemiological investigation and analysis of cat-borne disease materials submitted for inspection. After RNA was extracted from the samples of cat-borne diseases, reverse transcription was carried out and the pathogens were detected by PCR. **a**: Infection rate of various pathogens; **b**: Detection rate of mixed

infection of pathogen and FCoV. Different color background regions represent different functional domains.

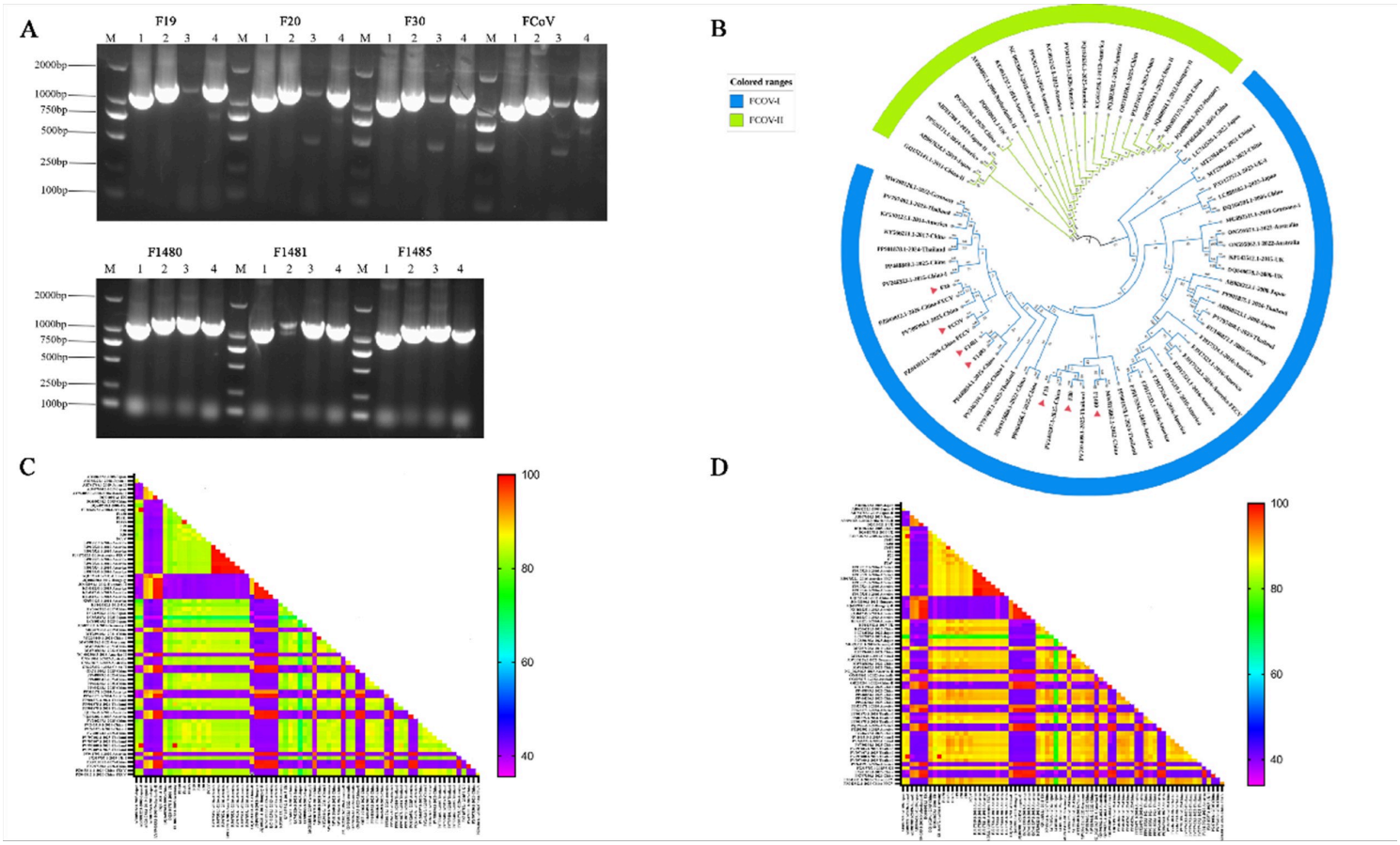


Figure 2

Amplification of FCoV S gene and analysis of nucleic acid and amino acid. a: PCR amplification of FCoV S gene. RNA from diseased materials was extracted and amplified by S gene-specific primers after reverse transcription, and the amplified products were separated by 1% agarose gel electrophoresis. M, DL2000 marker,1-4, S1-S4. **b**: Phylogenetic analysis of FCoV strains using the nucleotide sequences of S genes of FCoV. The tree was constructed using the neighbor-joining method. The green area represents FCoV-I, the blue area represents FCoV-II, and the red triangle marks the isolated strain. The reference sequence from GenBank is represented by strain abbreviation, including GenBank accession number, name and genotype. **c-d**: Homology analysis of nucleic acids and amino acids. Homology analysis was performed by compute pairwise distances.

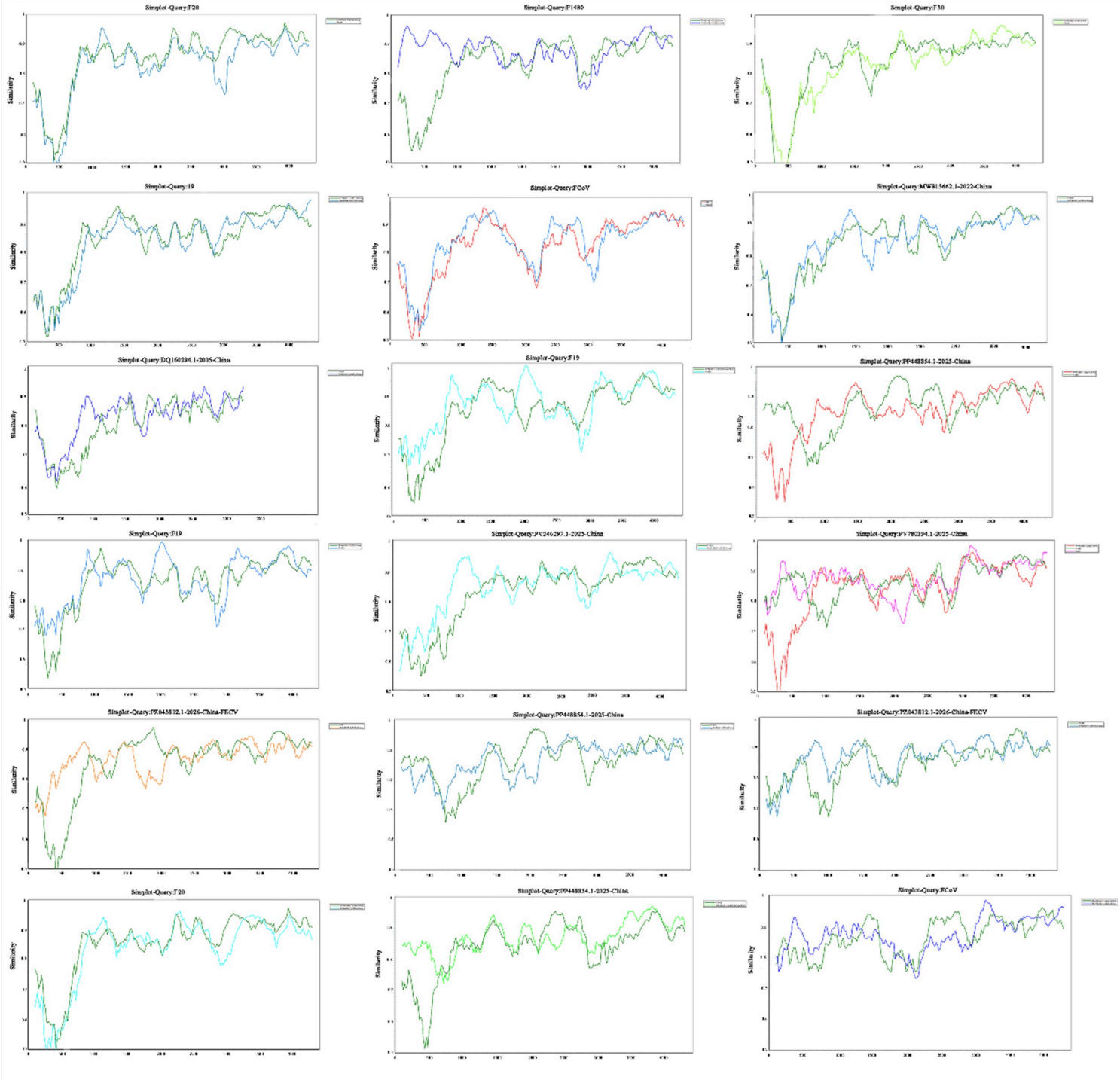


Figure 3

Recombination analysis of isolate strain. SimPlot analysis of the S gene represented by strain isolate strain, window size, 200 bases, step, 20 bases. The vertical axis indicates nt sequence similarity (%) between the query strains and reference strains.

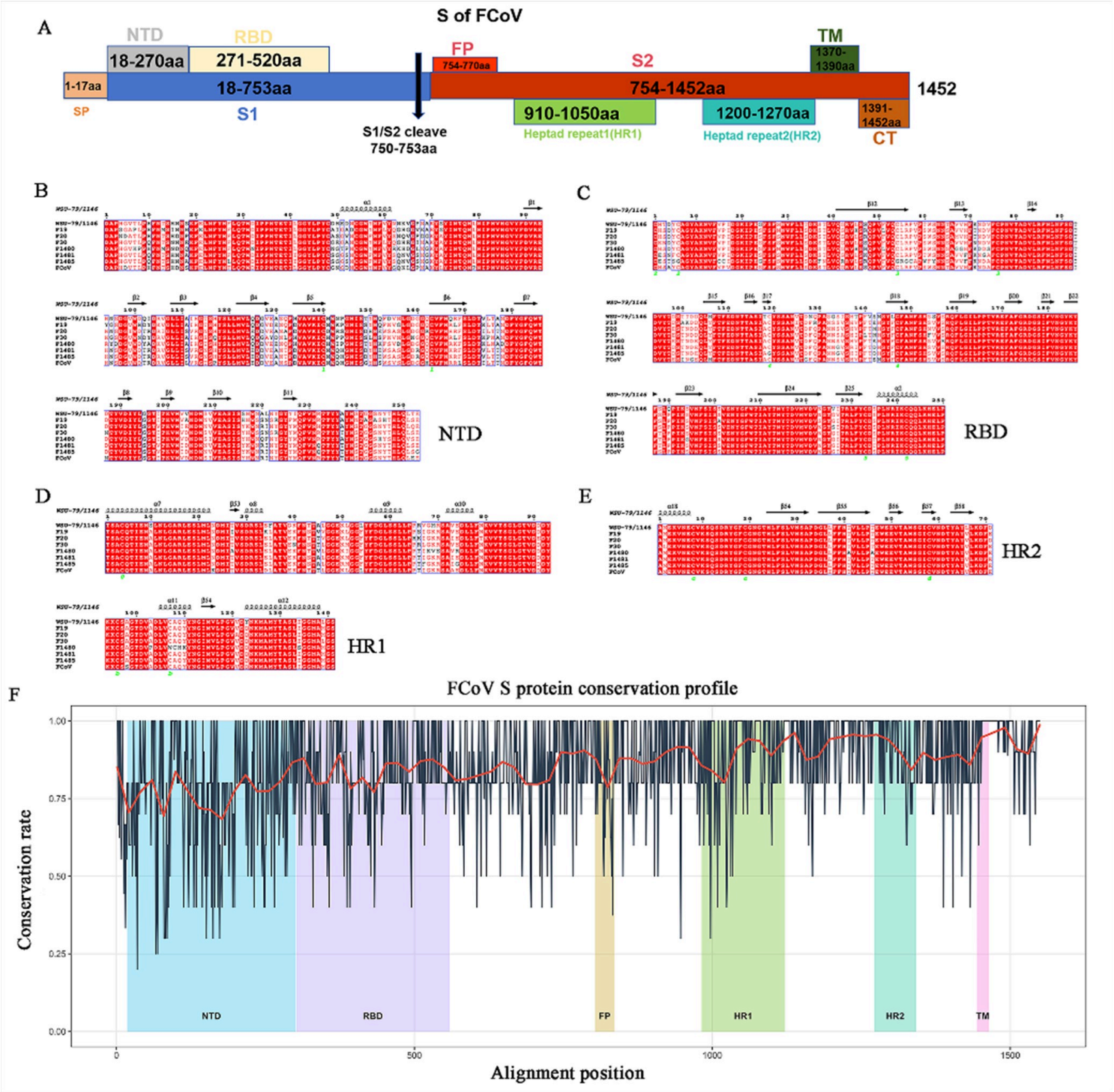


Figure 4

Analysis of amino acid mutation of FCoV S gene. Comparative analysis of amino acids in functional domains of S gene between isolated strain and reference strain. **a**: Annotation diagram of FCoV S protein functional domain. **b-e**: Amino acid sequence alignment of FCoV S protein functional domain. The red area is the position where the amino acids of each group are consistent. **f**: Statistical Analysis of Mutation Probability of Amino Acids in Functional Domains of FCoV S Protein.

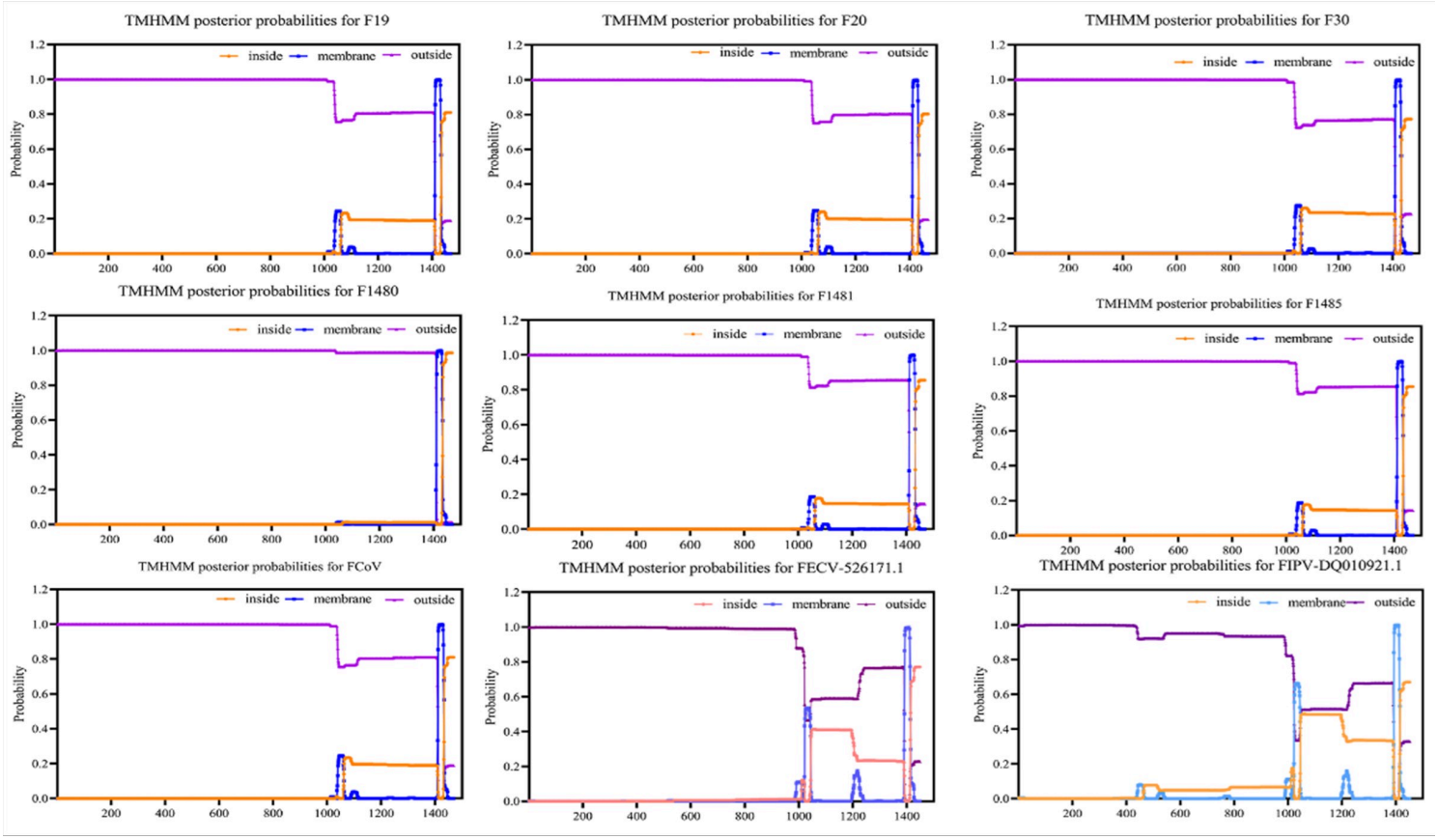


Figure 5

Prediction and analysis of transmembrane region of FCoV S protein. The orange line is the inner membrane area, the blue line is the upper membrane area, and the purple line is the outer membrane area.

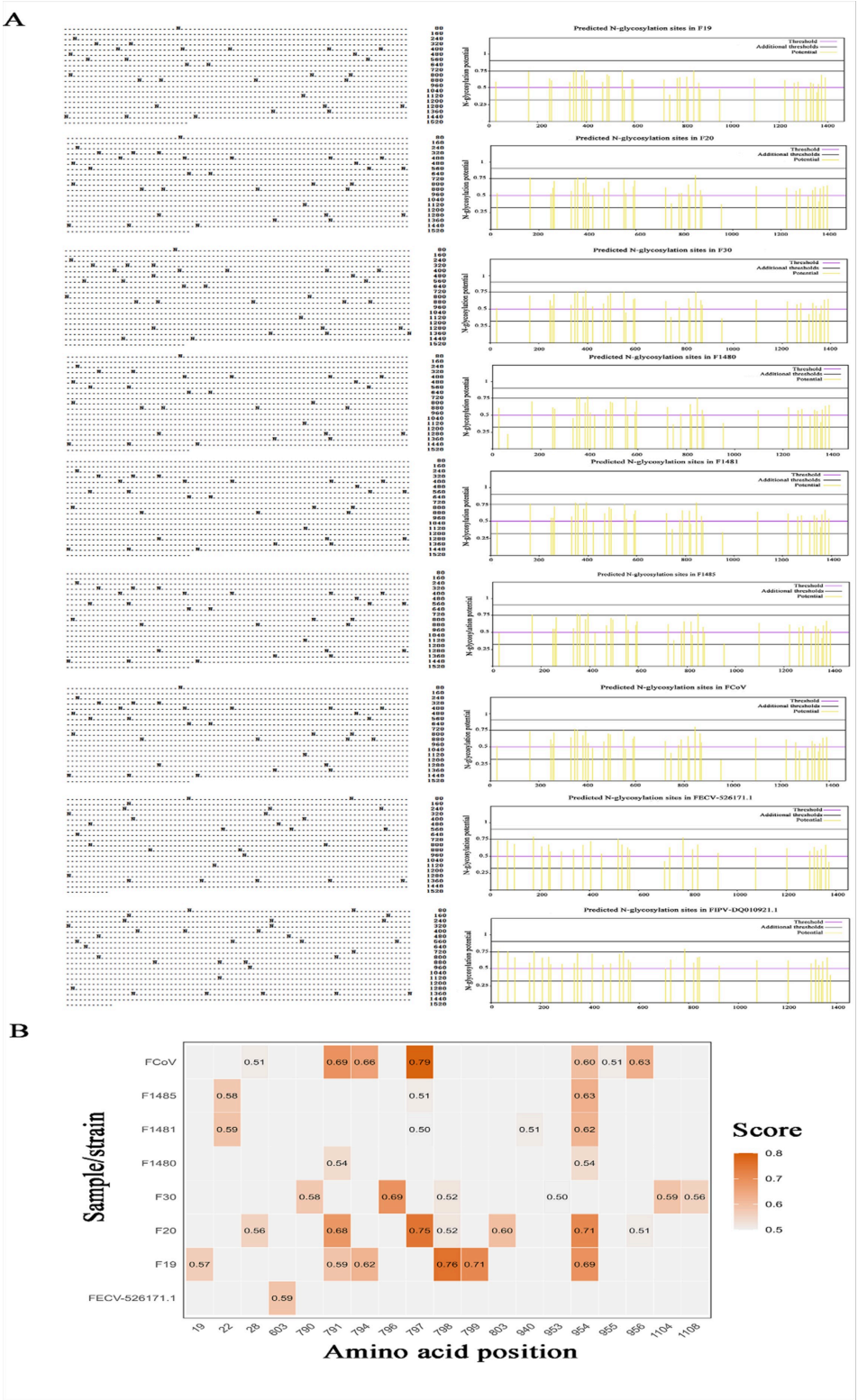


Figure 6

Analysis of glycosylation sites of FCOV S protein. The glycosylation sites of FIPV and FECV reference strains and isolated strains were analyzed. **a**: Analysis of N-glycosylation of S protein. On the left is the analysis of N-glycosylation sites of the S protein, with numbers indicating the position of each row of amino acids; on the right is the analysis of the score for each glycosylation site of amino acids. **b**: Analysis

of O-glycosylation of S protein. The abscissa represents the sites where O-glycosylation occurs, and the ordinate represents the glycosylation score at that site.

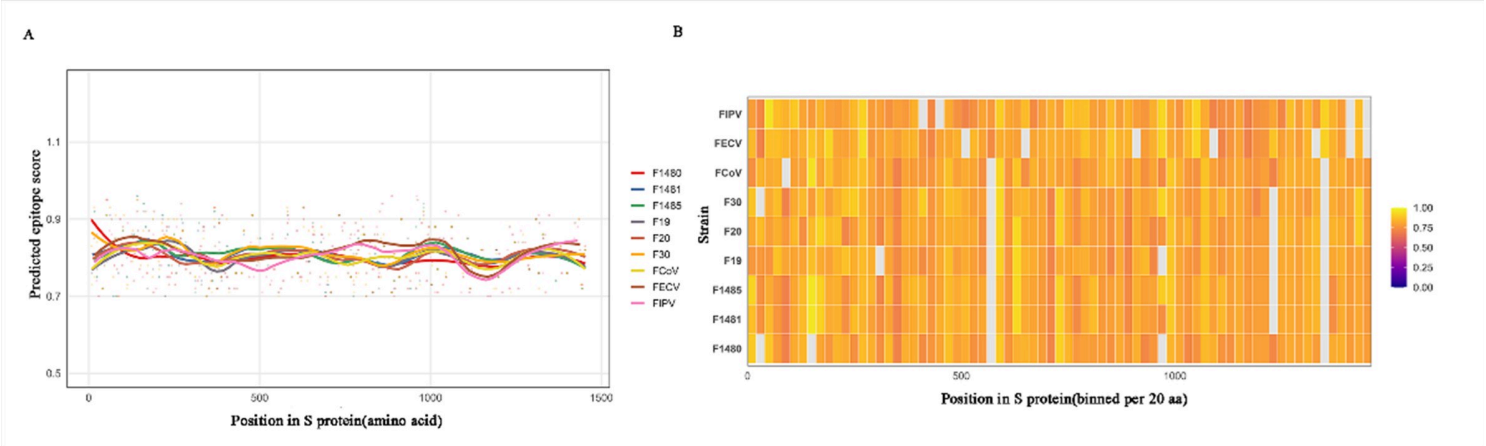


Figure 7

Analysis of B cell sites of FCOV S protein. The amino acids in B cell epitopes of FECV and FIPV reference strains were analyzed, and the reference strains were downloaded by NCBI. **a**: Amino acid analysis of B cell epitope. X-axis is the amino acid site of FCoV S protein, Y-axis is the predicted score, and lines with different colors represent different strains. **b**: Antiepitope amino acid score thermogram. X-axis is amino acid site, 20 amino acids are one step, and the ordinate is the name of each strain, in which the color depth represents the score.X-axis is amino acid site, 20 amino acids are one step, and the ordinate is the name of each strain, in which the color depth represents the score.

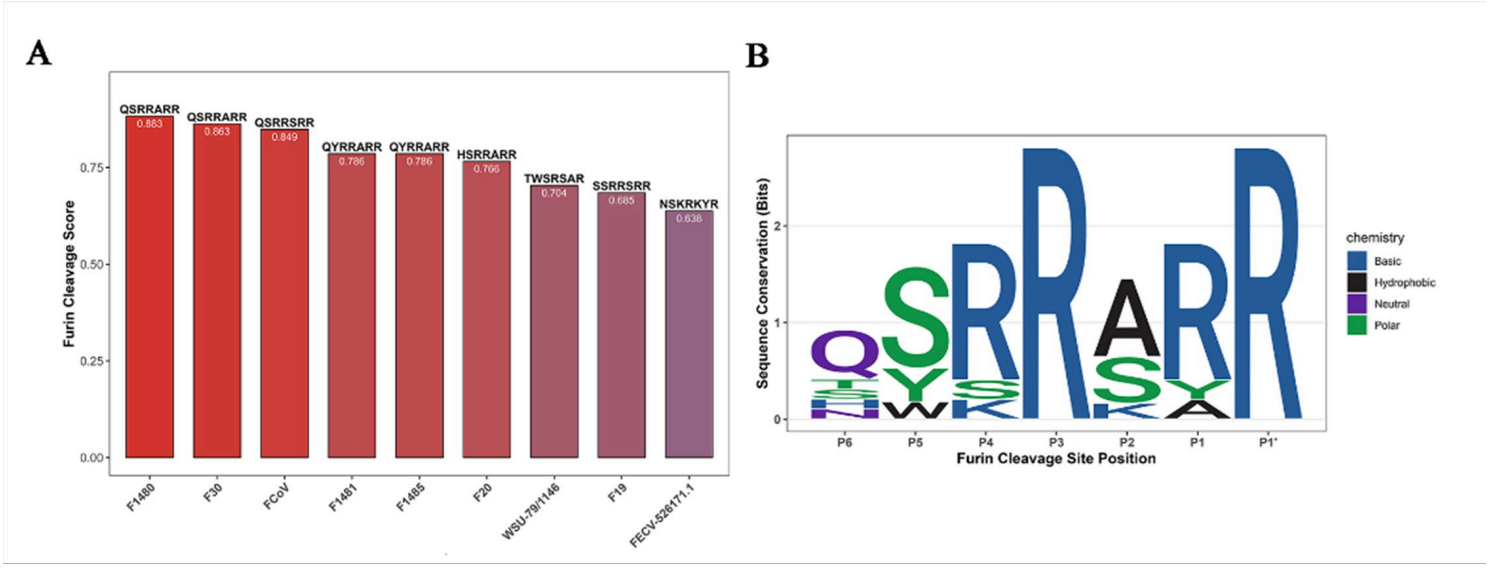


Figure 8

Analysis of furin restriction site of FCoV S protein. a: Enzymatic cleavage sequence and score. The x-axis represents the name of the strain, the ordinate is the Furin cleavage site score, and the top of the histogram is the amino acid sequence. **b**: Furin enzyme cleaves amino acid sites. The x-axis is the

coordinates of P1'-P6 amino acid sites, and the y-axis is the occurrence frequency of amino acids. The larger the letters, the higher the occurrence frequency, and different colors represent different amino acid types.