Prevalence and molecular characteristics of feline coronavirus in southwest China from 2017 to 2020

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Abstract

Feline coronavirus (FCoV) is the causative agent of feline infectious peritonitis and diarrhea in kittens worldwide. In this study, a total of 173 feline diarrheal fecal and ascetic fluid samples were collected from 15 catteries and six veterinary hospitals in southwest China from 2017 to 2020. FCoV was detected in 80.35% (139/173) of the samples using the RT-nPCR method; these included infections with 122 type I FCoV (87.8%) and 57 type II FCoV (41%). Interestingly, 51 cases (36.7%, 51/139) had co-infection with types I and II, the first such report in mainland China. To further analyze the genetic diversity of FCoV in southwest China, we amplified 23 full-length spike genes, including 18 type I and five type II FCoV. The 18 type I FCoV strains shared 85.9%–100% nucleotide sequence identities between one another and the five type II FCoV strains shared 97.4%–98.9% nucleotide sequence identities between one another. This result suggests that the N-terminal domain (NTD) of 23 FCoV strains showed a high degree of variation (73.6%–80.3%). There was five type I FCoV strains with two aa insertions (159HL160) in the NTD region. In addition, 18 strains of type I FCoV belonged to the Ie cluster, and five strains of type II FCoV were in the IIb cluster based on phylogenetic analysis. It is worth noting that five type I FCoV strains also had recombination in the NTD, and the recombination region was 135–625 nucleotides of the S gene. This study constitutes a systematic investigation of the current infection status and molecular characteristics of FCoV in southwest China.

Prevalence and molecular characteristics of feline coronavirus in southwest China from 2017 to 2020

Running title: FCoV in southwest China

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Summary

Feline coronavirus (FCoV) is the causative agent of feline infectious peritonitis and diarrhea in kittens worldwide. In this study, a total of 173 feline diarrheal fecal and ascetic fluid samples were collected from 15 catteries and six veterinary hospitals in southwest China from 2017 to 2020. FCoV was detected in 80.35% (139/173) of the samples using the RT-nPCR method; these included infections with 122 type I FCoV (87.8%) and 57 type II FCoV (41%). Interestingly, 51 cases (36.7%, 51/139) had co-infection with

types I and II, the first such report in mainland China. To further analyze the genetic diversity of FCoV in southwest China, we amplified 23 full-length spike genes, including 18 type I and five type II FCoV. The 18 type I FCoV strains shared 85.9%–100% nucleotide sequence identities between one another and the five type II FCoV strains shared 97.4%–98.9% nucleotide sequence identities between one another. This result suggests that the N-terminal domain (NTD) of 23 FCoV strains showed a high degree of variation (73.6%–80.3%). There was five type I FCoV strains with two aa insertions (159HL160) in the NTD region. In addition, 18 strains of type I FCoV belonged to the Ie cluster, and five strains of type II FCoV were in the IIb cluster based on phylogenetic analysis. It is worth noting that five type I FCoV strains also had recombination in the NTD, and the recombination region was 135–625 nucleotides of the S gene. This study constitutes a systematic investigation of the current infection status and molecular characteristics of FCoV in southwest China.

KEYWORDS

feline coronavirus, detection; S gene; NTD; recombination

INTRODUCTION

Feline coronavirus (FCoV) is a *Coronaviridae* family member (genus*Alphacoronavirus*), first identified in America in 1968. This identification was followed by outbreaks of the disease in many countries, severely endangering kitten health (Chang, Egberink, Halpin, Spiro, & Rottier, 2012; Pedersen, 2009; Ward, Munn, Gribble, & Dungworth, 1968). FCoV includes two pathogenic biotypes: feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV). FECV infection is characterized by mild diarrhea. In contrast, FIPV efficiently replicates in macrophages/monocytes and can lead to feline infectious peritonitis (FIP), a highly lethal systemic granulomatous disease (Ehmann et al., 2018; Oguma, Ohno, Yoshida, & Sentsui, 2018).

FCoV is an enveloped, single-stranded, positive-sense RNA virus of approximately 29 kb in size, containing 11 open reading frames encoding four structural proteins: spike (S), envelope (E), membrane (M), nucleocapsid (N), and seven nonstructural proteins (Dye & Siddell, 2005; Pedersen, 2009). The S protein of FCoV is a membrane glycoprotein thought to be a viral regulator of binding and entry to the cells (Bosch, van der Zee, de Haan, & Rottier, 2003; Jaimes & Whittaker, 2018; Millet & Whittaker, 2015). This protein is also involved in FCoV tropism and virulence and is the switch from enteric disease to FIP (Belouzard, Millet, Licitra, & Whittaker, 2012; Jaimes & Whittaker, 2018; B. N. Licitra et al., 2013). The S protein comprises two amino acid (aa) subunits, S1 and S2, with the S1 being more genetically diverse than S2 (Yang et al., 2020). S1 contains a receptor-binding domain and is responsible for the initial attachment of the virus to the surface of host cells (Belouzard et al., 2012; Jaimes & Whittaker, 2018; Wu, Li, Peng, & Li, 2009). Based on the amino acid sequence of the S protein, FCoV is classified into two genotypes, types I and II (Motokawa, Hohdatsu, Hashimoto, & Koyama, 1996; Takano, Satomi, Oyama, Doki, & Hohdatsu, 2016). Type II FCoV emerged via double recombination between type I FCoV and type II canine coronavirus (CCoV) (Herrewegh, Smeenk, Horzinek, Rottier, & de Groot, 1998). As a result of this recombination, the spike (S) gene and adjacent regions of type I FCoV were replaced by the corresponding part of the CCoV genome (Herrewegh et al., 1998; Lin, Chang, Su, & Chueh, 2013; Terada et al., 2014).

FCoV has a worldwide distribution (Amer et al., 2012; An et al., 2011; Klein-Richers et al., 2020; Li et al., 2019; Luo, Liu, Chen, & Chen, 2020; McKay et al., 2020; Soma, Wada, Taharaguchi, & Tajima, 2013). Infections with FCoV are mostly type I FCoV, while type II FCoV occurs primarily in southeast Asia (An et al., 2011; Li et al., 2019; Luo et al., 2020). With a gradual increase in the number of cats raised in China, coronavirus-related diseases have become more and more complex and diverse in cats. Nevertheless, the prevalence and genetic diversity of FCoV in southwest China remain unclear. Therefore, in this study, we recorded the current infection status of FCoV in southwest China and analyzed the sequences of currently circulating FCov strains.

2. MATERIALS AND METHODS

2.1. Samples collection

One hundred seventy-three samples were collected from cats under one year of age from 15 catteries and six veterinary hospitals in southwest China from 2017 to 2020, of which 93 samples were from catteries, and 80 samples were from veterinary hospitals. Of the 173 samples, 150 fecal samples were obtained from cats with diarrhea, and 23 samples of ascetic fluid were obtained from FIP-suspected cats. The cattery samples were collected from pet breeding centers and stray animal shelters in southwest China, both considered multi-cat environments. The samples collected at the veterinary hospital were individual, home-owned pets considered to come from single-cat environments. The cat breeds included British Shorthair, Nulla luctus felis, Ragdoll, Garfield, and Persian. The samples were shipped on drikold and stored at –80 °C.

2.2. RNA extraction and cDNA synthesis

The fecal and ascetic fluid samples were fully resuspended in phosphate-buffered saline (1:5 w/v) and centrifuged at 10,000 × g for 10 min. According to the manufacturer's instructions, viral RNA was extracted from 300 μL of the fecal suspension using RNAios Plus (TaKaRa Bio Inc). The cDNA was synthesized using the PrimeScript RT Reagent Kit according to the manufacturer's instructions (TaKaRa Bio Inc) and then stored at –20 °C until required.

2.3. Detection and typing of feline coronavirus

The 3'UTR of FCoV was amplified using RT-nested PCR as described by Herrewegh et al. (Herrewegh et al., 1995), and a positive 3'UTR produced a 177 bp amplicon on electrophoresis. Furthermore, for samples in which the 3'UTR gene was detected, genotyping was performed with RT-nPCR as reported by Addie et al. (DD Addie, Schaap, Nicolson, & Jarrett, 2003), providing 360 and 218 bp amplicons of FCoV types I and II, respectively. The PCR detection was performed in a 25 μL volume, including 1 μL of template, 1 μL of each primer (10 pmol), 8 μL of ddH2O, and 12.5 μL of Quick Taq HS DyeMix at 2 × concentration (Toyobo, Japan). The PCR conditions were set at 94 °C for 2 min, 35 repeats of denaturing at 94 °C for 30 s, annealing at 51 °C for 30 s, extension at 68 °C for 1 min, and a final extension at 72 °C for 8 min. The RT-PCR products were analyzed using 1.5% agarose gel electrophoresis and visualized using ultraviolet illumination. Finally, the PCR products consistent with the expected size were sent to Sangon Biotech (Shanghai, China) for sequencing.

2.4. PCR amplification of S gene

The complete S gene was PCR-amplified from samples already known to be FCoV-positive according to the primers in Table S1. All PCR products were purified using the Omega Gel Kit following the manufacturer's instructions. They were ligated to the pMD19-T vector (TaKaRa Bio Inc.) and transformed into DH5α competent *Escherichia coli* cells for sequencing. The S sequences were assembled using SeqMan software (version 7.0; DNASTAR).

2.5. Sequence, phylogenetic, and recombination analyses

The homologies of the nucleotide and deduced amino acid (aa) sequences were determined using the MegAlign program in DNASTAR 7.0 software, and a heat map was constructed based on sequence homology using HemI software (Heatmap Illustrator, version 1.0.3.7); MEGA X 10.2.4 (https://www.megasoftware.net/) was used for multiple sequence alignment and to subsequently build neighbor-joining phylogenetic trees with bootstrap testing (1,000 replicates). Phylogenetic trees were pruned and re-rooted using the Interactive Tree Of Life software, version 5.6.3 (https://itol.embl.de/), an online tool for displaying the circular tree annotation. Recombination events were assessed using the recombination detection program RDP 4.0 (version 4.9.5) with the RDP, GeneConv, Chimaera, MaxChi, BootScan, SiScan, and 3Seq methods. The results were used for Simi larity Plotting analysis using the SimPlot program version 3.5.1 with a window size of 200 bp and a step size of 20 bp.

2.6. Statistical analysis

The collected data were coded and entered into a computer using Epi Info 7 version 7.2.0.1 and were then exported to SPSS version 23.0 for analysis. The chi-square test was used to assess the association of variables with the primary outcome. P -values <0.05 were considered to indicate statistical significance.

3. RESULTS

3.1. Prevalence of FCoV in southwest China

From May 2017 to December 2020, 23 FIP-suspected samples of ascites fluid and 150 diarrheal fecal samples from veterinary hospitals and catteries in southwest China were tested using RT-nPCR amplification. Of the 173 samples, 139 (80.35%, 95 % CI; 73.6%–86%) were positive for FCoV (Table 1), including the positive rates of FCoV in FIP-suspected cat; the rates in diarrheal cats were 95.65% (22/23, 78.1%–99.9%) and 78% (117/150, 95% CI; 70.5%–84.3%), respectively. As shown in Table 2, the positive rates of FCoV in multi-cat environments and single-cat households were 85% (79/93, 95% CI: 76%–91.5%) and 66.67% (38/57, 95% CI: 52.9%–78.6%), respectively. These results suggest that FCoV is highly prevalent in southwest China. The detection rate of FCoV was significantly associated with the cat's living environment ($p = 0.014$) and clinical symptoms ($p = 0.05$).

The 139 positive samples were typed using type I and II FCoV typing primers. Of the 127 successfully typed FCoV-positive samples, type I FCoV was predominant, accounting for 87.77% (122/139, 95% CI: 81.1%–92.7%), and type II FCoV accounting for 41.01% (57/139, 95% CI: 32.7%–49.7%). Eleven other samples failed typing, possibly due to the low viral load in samples or the potential mismatching between primers and templates. Interestingly, co-infection with type I and II FCoV were detected in 51 samples (36.69%, 95% CI: 28.7%–45.3%), the first such report in mainland China, and the detection rate of co-infection with types I and II FCoV were significantly higher in the multi-cat environment than in the single-cat environment ($p<$ 0.01; Table 2).

3.2 Amplification and sequence analysis of FCoV S gene

The S protein, encoded by the FCoV S gene, is considered the primary viral regulator in host cell entry; it possesses a receptor-binding domain, while mutations in amino acids of the FCoV S protein are associated with the conversion of FECV to FIPV (Bosch et al., 2003; Jaimes & Whittaker, 2018; Millet & Whittaker, 2015). To further understand the molecular characteristics and genetic variation of the FCoV S gene in southwest China, 18 type I FCoV and five type II FCoV strains were selected to amplify the whole gene of FCoV S according to various years, living environments, and regions. The 18 type I FCoV S genes were 4395–4419 bp in length, each encoding a protein of 1465–1473 aa residues (GenBank accession number MW316830-MW316847). Five type II FCoV S genes were 4353–4362 bp in length, each encoding a protein of 1451–1454 aa residues (GenBank accession number MW316848-MW316852).

A heatmap was constructed using HemI software based on the sequence homology of type I FCoV with classical strain Black and type II FCoV with classical strain 79–1146, respectively (Figure 1). The results showed that the NTD variation in S1 subunit was the highest. The NTD homology between 23 strains and the reference strain was 73.6%–80.3%; the S2 subunit was relatively conserved, the S2 subunit homology between 23 strains and the reference strain was 93.9%–94.2%. The 18 type I FCoV strains shared 85.9%–100% nucleotide sequence identities and 89.4%–100% aa identities. They shared 87.7%–93.8% nucleotide sequence identity and 87.9%–93.1% aa sequence identities with all 64 of the complete type I FCoV S genes available in the GenBank database. Sequence analysis of 105 FCoV strains showed that seven type I FCoV strains (SMU-CD86, SMU-CD77, SMU-CD9, SMU-CD8, SMU-CD60, SMU-CDF12, SMU-CDF19) identified in this study had two aa insertions in the NTD (159HL160), and strains with this insertion pattern also appeared in strains that have been reported in China and the Netherlands (JN183882, KF530123, KY566209, KY566210, KY566211). The five type II FCoV strains shared 97.4%–98.9% nucleotide sequence identities and 95%–98.8% aa identities between one another and shared 86.9%–97.8% nucleotide sequence identity 84.9%–97.7% aa sequence identities with the 18 type II FCoV reference strains. The type II FCoV strain SMU-CQ18 showed three discontinuous unique aa deletions (209N, 214M/L, 217R) in the NTD. These data showed that the nucleotide of the carbohydrate-bound NTD in the 23 S genes had significant mutations, and

there were also insertions and deletions of aa in the NTD.

3.3 Phylogenetic analysis of FCoV S Gene

To track the evolution of FCoV strains identified in southwest China, all FCoV strains in the GenBank database were downloaded, the S genes of 82 FCoV strains, 14 CCoV strains, and one transmissible gastroenteritis virus (TGEV) strain in the GenBank database. The 23 FCoV strains S genes identified in this study were used to construct a phylogenetic tree. The 82 type I FCoV strains were formed five clusters in the S gene-based on the phylogenetic tree (Figure 2), which were tentatively named type Ia, Ib, Ic, Id, and Ie in this study. Type Ia, Ib, and Ic clusters were composed of strains from Europe and North America. The Id cluster consists of strains from Europe and America. All 18 type I FCoV strains belonged to the type Ie cluster, including strains from countries such as Japan, the Netherlands, and Belgium. These results indicate that the type Ie is a mixed cluster consisting of strains from Asia and Europe and is more complex than the other clusters' genetic diversity.

All type II FCoV in the GenBank database form two distinct clusters in the phylogenetic tree (Figure 2), tentatively named type IIa FCoV and type IIb FCoV, respectively. Type IIa FCoV consists of strains from China and the USA. The five type II FCoV in this study clustered with four other type II FCoV strains and five CCoV strains as type IIb FCoV and were most closely related to the isolated CCoV strains in China in 2019. In the present study, 18 type I FCoV belonged to type Ie FCoV, and five type II FCoV belonged to type IIb FCoV.

3.4 Recombination analysis of type I FCoV S gene

Recombination analysis of the 23 complete S genes showed that recombination events had occurred in five of the type I FCoV strains (SMU-CDF19, SMU-CDF12, SMU-CD8, SMU-CD77, SMU-CD9) based on the RDP 4.0 (six methods) and SimPlot 3.5.1. The recombination breakpoint based on RDP 4.0 identified the beginning of the breakpoint at nucleotide 180 in the fragment (breakpoint 99% confidence intervals: nucleotide positions 1–295 in the fragment) and nucleotide 606 at the end of the breakpoint (breakpoint 99% confidence intervals: nucleotide position 557–1050 in the fragment). For example, the major parental strain of SMU-CD9 was the Belgian strain UG-FH8 (KX722529), and the minor parental strain was the Chinese HLJ/HRB/13 (KY566211), with a recombination score of 0.535 and a predicted recombination breakpoint of nucleotides 135 and 625 (Figure 3). Although the recombination breakpoints predicted by RDP 4.0 and SimPlot differ, both programs showed that the recombination region was located in the NTD of S gene (at nucleotide region 45–804 aa in the full-length FCoV S gene). To further verify this recombinant event, phylogenetic trees of the regions 1–135, 135–625, and 626–4410 nucleotides were constructed. A discrepancy was found between the phylogenetic trees, further confirming the recombination events.

DISCUSSION

FCoV is a lethal infectious agent that causes effusions in the pleural and abdominal cavities in domestic cats. There are no effective vaccines, and management is based on biosafety prevention and control (Haake, Cook, Pusterla, & Murphy, 2020; Pedersen, 2009). FCoV is ubiquitous in cat colonies, with detection rates ranging from 6.6%–95% (Amer et al., 2012; An et al., 2011; Klein-Richers et al., 2020; Li et al., 2019; Luo et al., 2020; McKay et al., 2020). Few epidemiological studies concerning FCoV have been conducted in southwest China. In our study, 173 samples from sick cats were included for FCoV detection, and the positive rate of these samples was 80.35% (139/173), much higher than the prevalence reported in other regions of China and other countries in recent years (Klein-Richers et al., 2020; Li et al., 2019; Luo et al., 2020; McKay et al., 2020). The results suggest that the prevalence of FCoV in cats with diarrhea and FIP is high in southwest China. Several studies reported that living environments are associated with FCoV infection (D Addie et al., 2009; Drechsler, Alcaraz, Bossong, Collisson, & Diniz, 2011; Sharif et al., 2009). In the present study, the infection rate of FCoV in the multi-cat environments was significantly higher than that of the single-cat environment, in line with previous studies (Drechsler et al., 2011; Li et al., 2019).

Type I FCoV is the dominant genotype in most countries, including Austria (86%) (Benetka et al., 2004),

Malaysia (97.5%)(Amer et al., 2012), and China (72.2%) (Li et al., 2019), while type II is less common and is mainly found in southeast Asian countries. Co-infection with types I and II were reported sporadically in Europe a decade ago and in southeast Asia in recent years but has not been reported in mainland China (An et al., 2011; Benetka et al., 2004; Luo et al., 2020; Soma et al., 2013). In this study, in accord with reports from other countries (Amer et al., 2012; Benetka et al., 2004; Li et al., 2019), the dominant genotype of FCoV was also type I FCoV in southwest China (87.77%, 122/139), and the prevalence of type II FCoV was significantly higher than that in other regions (41%, 57/139). We found co-infection of types I and II in southwest China with high prevalence (36.7%, 51/139); this is the first report of co-infection of types I and II FCoV in mainland China.

Point mutations, insertions, deletions, and recombination are common in the coronavirus genome, and they are responsible for the emergence of new coronavirus strains. The FCoV S protein is implicated as a regulator of viral binding and entry into cells. Mutations in the S protein of FCoV may cause changes in virus tropism, leading to a switch from enteric disease to FIP (Millet & Whittaker, 2015; Yang et al., 2020). Consistent with previously reported strains, the highly mutated region of the FCoV S gene was the NTD in 23 strains in our study, and the degree of mutation was inconsistent across strains (59.3%–87%). Furthermore, two aa (159HL160) insertions were present in the NTD between ten FCoV strains from China and two strains from the Netherlands (KF530123, JN183882). Previous studies showed that the NTD in the FCoV S1 subunit has sugar-binding functions and aids in host receptor-binding (Belouzard et al., 2012; Jaimes & Whittaker, 2018); however, it remains unclear whether the NTD mutation and insertions of the strains in this study affect the pathogenicity of FCoV and the adaptability of the virus to host receptor.

Genome sequences and subsequent phylogenetic analysis showed that FCoV isolates form geographical clusters (Kipar & Meli, 2014). In Italy and Brazil, investigators reconstructed the origin and spatiotemporal distribution of type I FCoV by phylogeographic analysis and found that from the USA, the virus likely entered Germany and spread to other European countries (Lauzi et al., 2020; Myrrha et al., 2019). In the present study, 81 type I FCoV strains were clustered into five distinct clusters in the phylogenetic tree (types Ia, Ib, Ic, Id, and Ie), with significant geographic differences, and all type I FCoV identified in Asia belonged to Ie FCoV, including some strains identified in Europe. The information from limited samples suggests that the origin of FCoV strains in China may be closely related to strains in Europe.

Type II CCoV is divided into two sub-genotypes, types IIa and IIb, based on differences in the S protein amino acid sequence (Decaro et al., 2010; B. Licitra, Duhamel, & Whittaker, 2014). The amino acid sequence of type IIb CCoV S protein is highly similar to TGEV (Decaro et al., 2010; B. Licitra et al., 2014). The existing type II FCoV strains can be divided into two different subtypes, types IIa and IIb. Using this classification method, five type II FCoV strains belonged to type IIb FCoV. These data suggest that the type I FCoV strains identified in China are more genetically diverse than the type II FCoV strains, in line with previous studies (Li et al., 2019; Luo et al., 2020).

Recombination events are often reported in human and animal coronaviruses, and they play pivotal roles in the evolution of coronaviruses. The S protein is the most common recombination region, and recombination of this protein allows the emergence of new strains with altered virulence and potentially broader host ranges (Boniotti et al., 2016; Decaro et al., 2010; Herrewegh et al., 1998; Na, Moon, & Song, 2021; Qin et al., 2019). Here, we show for the first time recombination in type I FCoV with the recombination region located at the NTD of the S protein. Similar recombination events have been reported in CCoV, porcine epidemic diarrhea virus and infectious bronchitis virus (Ovchinnikova et al., 2011; Qin et al., 2019; Regan et al., 2012). This novel recombination event occurred in five S genes and contributed to the emergence of new variants of FCoV or led to changes in FCoV tropism. The recombination event in S genes of type I FCoV further contributes to our understanding of the evolution and genetic diversity of FCoV from cats.

In conclusion, Type I FCoV strains are the dominant genotype of FCoV in southwest China. The presence of co-infection of types I and type II in feline populations in mainland China were reported for the first time. Genomic analysis indicated that type I FCoV strains showed significant mutations, and there were recombination events. This study helps develop a profile of the current FCoV status and might provide an

outline for future research on the FCoV spike protein gene.

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ETHICAL APPROVAL

In this study, all the experiments were preformed according to the permit guidelines established by Southwest Minzu University, China. The experimental protocols were approved by the Animal Care and Use Committee of Southwest Minzu University. The sampling and data publication were also approved by the cats' owners.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Figure and table legends

Table 1. Detection and typing results of FCoV

Table 2. Correlation of the FCoV prevalence with clinical symptoms and living environments

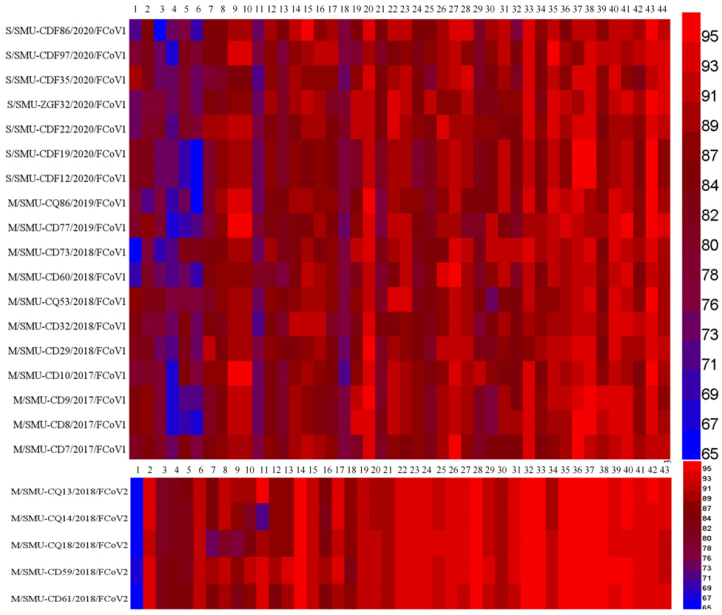
Figure 1. Heatmap based on the nucleotide sequence homology between 23 FCoV strains and reference strains (window size, 100 nucleotides). 1a, based on the nucleotide sequence homology between the 18 type I FCoV strains in this study and the classical strain Black. 1b, based on the nucleotide sequence homology between the five type II FCoV strains in this study and the classical strain 79-1146. The colors represent sequence identity (blue for lower identities and red for higher identities) among different strains. The bar on the right represents the hierarchical similarity among the 43 or 44 meta-clusters.

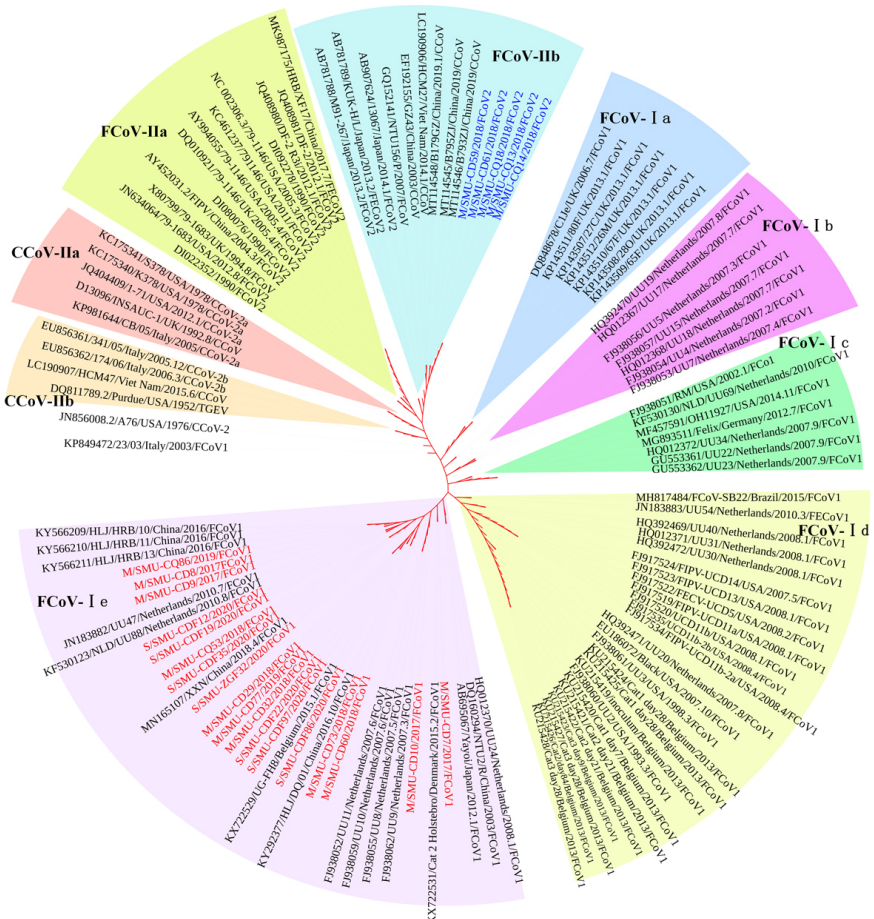
Figure 2. Phylogenetic tree based on the nucleotide sequences of the entire S genes of 23 FCoV strains and other 82 FCoV strains, 14 CCoV strains, and one TGEV strain. The tree was constructed using the neighbor-joining method. The red font indicates the S gene sequences of type I FCoV, and the blue font indicates the S gene sequences of type II FCoV in this study. The reference sequences from GenBank are indicated by strain abbreviations, including GenBank accession number, genotype, country, and collection year.

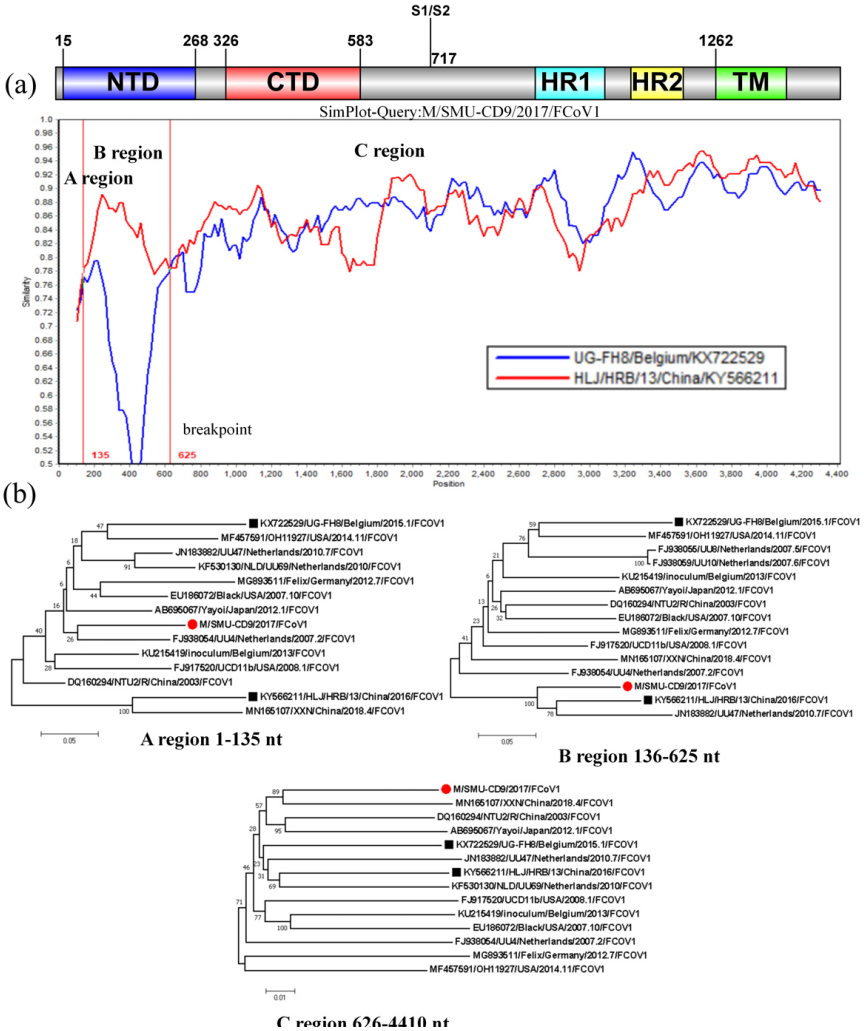
Figure 3. Recombination analysis of the FCoV S gene. (a). SimPlot analysis of the complete genome represented by M/SMU-CD9/2017/FCoV1 strain; window size, 200 bases; step, 20 bases. The vertical axis indicates nucleotide sequence similarity (%) between the query strain and reference strains. (b) Phylogenetic trees for region A (nucleotides 1–135), region B (nucleotides 136–625), and region C (nucleotides 626–4410) of the FCoV strains were constructed using the neighbor-joining method. Red circles indicate the S gene in this study. Black squares indicate major and minor parental strains.

Supporting information legends

Table S1. FCoV S gene amplification primers







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