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Supplementary information

Feline infectious peritonitis epizootic caused by a recombinant coronavirus

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Feline infectious peritonitis epizootic caused by a recombinant coronavirus

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Guide to supplementary information

Supplementary method relating to extended data figure 6

Bayesian time-resolved phylogenetic trees were estimated using BEAST 1.10.4[1] using the TN93 (Tamura-Nei 93) nucleotide substitution model with a four-category gamma distribution model of site-specific rate variation, a strict clock, the constant population size coalescent tree prior. For this dataset two independent Markov Chain Monte Carol (MCMC) chains were run, each chain consisted of 10,000,000 steps, was sampled every 1,000 steps, and the first 10% of samples were discarded as burn-in. The MCMC settings resulted in a post-burnin effective sample size of at least 200 (which is the accepted standard in BEAST analyses). The two independent chains were further down-sampled by a factor of 18 and combined to make 1,002 posterior trees, which were re-used in BEAST (as empirical trees) along with an asymmetric discrete trait model[2] for Place (5 states) with BSSVS (Bayesian Stochastic Search Variable Selection) and a homogenous Brownian motion diffusion model[3] for latitude and longitude coordinates to enable phylogeographic mapping. The resulting posterior trees from the mapping were summarized to a single Maximum Clade Credibility tree (MCC tree) using TreeAnnotator. The MCC tree was displayed using R-package ape[4] and custom R-scripts, including the addition of the spike deletion information on the tips of the tree.

Supplementary table 1

FIP outbreak Cyprus Jan 2023-Jun 2024; case distribution – sex

Sex	n	Total	Percentage
Female	65	215	33.02
Male	137	215	66.98

Supplementary table 2

FIP outbreak Cyprus Jan 2023-Jun 2024; case distribution – Age stats (years); the age of 51 cases are missing

	Mean_age	Median_age	Min_age	Max_age
Jan 23- Mar-24	**4.6059**	3	1	19
Jan 23- Sep-23	**4.4127**	3	1	16
Oct-23-Mar-24	**5.5423**	4.25	1	19
Apr-24-Jun-24	**5.1250**	3.7	0.6	13.3

Supplementary table 3

FIP outbreak Cyprus Jan 2023 -Jun 2024; case distribution – FIP form

Form	n	Total	Percentage
Non-effusive	22	215	10.23
Neurological	56	215	26.05
Effusive	137	215	63.72

Supplementary table 4

FIP outbreak Cyprus Jan 2023 -Sep 2023; case distribution – FIP form

Form	n	Total	Percentage
Non-effusive	4	167	2.4
Neurological	48	167	28.74
Effusive	115	167	68.86

Supplementary table 5

A) FIP outbreak Cyprus Oct 2023 -Mar 2024; case distribution – FIP form

Form	n	Total	Percentage
Non-effusive	12	34	35.29
Neurological	8	34	23.53
Effusive	14	34	41.18

B) FIP outbreak Cyprus Apr 2024 -Jun 2024; case distribution – FIP form

Form	n	Total	Percentage
Non-effusive	6	14	42.86
Neurological	0	14	0
Effusive	8	14	57.14

Supplementary table 6

FIP outbreak Cyprus Jan 2023-Mar 2024; case distribution – District

District	n	Total	Percentage
Nicosia	58	215	26.98
Famagusta	52	215	24.19
Larnaca	41	215	19.07
Limassol	34	215	15.81
Paphos	30	215	13.95

Supplementary table 7

RNA samples for sequencing; case distribution – Sex

Sex	n	Total	Percentage
Female	54	163	33.13
Male	109	163	66.87

Supplementary table 8

RNA samples for sequencing; case distribution – Age stats (years). The ages of 38 cats are missing.

Mean_age	Median_age	Min_age	Max_age
4.21	3	1	16

Supplementary table 9

RNA samples for sequencing; case distribution – FIP form

Form	n	Total	Percentage
Non-effusive	6	163	3.68
Neurological	42	163	25.77
Effusive	113	163	70.55

Supplementary table 10

RNA samples for sequencing; case distribution – District

District	n	Total	Percentage
Nicosia	46	163	28.22
Famagusta	46	163	28.22
Larnaca	31	163	19.02
Limassol	21	163	12.88
Paphos	17	163	10.43
UK	2	163	1.23

Supplementary table 11

RNA samples for sequencing; case distribution – Collection dates

District	n	Total	Percentage
October-21	1	163	0.61
November-21	1	163	0.61
December-21	1	163	0.61
February-22	1	163	0.61
March-22	1	163	0.61
August-22	1	163	0.61
December-22	1	163	0.61
January-23	7	163	4.29
February-23	23	163	14.11
March-23	36	163	22.09
April-23	19	163	11.66
September-23	28	163	17.18
October-23	14	163	8.59
November-23	5	163	3.07
Unknown	1	163	0.61

Supplementary table 12

RNA samples for sequencing; case distribution – Sample type used for extraction

District	n	Total	Percentage
Peritoneal	90	163	55.21
Pleural	24	163	14.72
CSF	43	163	26.38
Nasal Swab	1	163	0.61
Tissue/LN[a] intestinal	5	163	3.07

[a]LN – lymph node

Supplementary table 13

Successfully sequenced spike protein; case distribution – Sex

Sex	n	Total	Percentage
Female	25	63	39.68
Male	38	63	60.32

Supplementary table 14

Successfully sequenced spike protein; case distribution – Age stats (years). The ages of 14 cats are missing.

Mean_age	Median_age	Min_age	Max_age
4.44	3	1	16

Supplementary table 15

Successfully sequenced spike protein; case distribution – FIP form

Form	n	Total	Percentage
Non-effusive	5	63	7.94
Neurological	10	63	15.87
Effusive	48	63	76.19

Supplementary table 16

Successfully sequenced spike protein; case distribution – District

District	n	Total	Percentage
Nicosia	17	63	26.98
Famagusta	24	63	38.10
Larnaca	7	63	11.11
Limassol	9	63	14.29
Paphos	4	63	6.35
UK	2	63	3.17

Supplementary table 17

Successfully sequenced spike protein; case distribution – Collection dates

Date	n	Total	Percentage
January-23	3	63	4.76
February-23	8	63	12.70
March-23	20	63	31.75
April-23	12	63	19.05
May-23	5	63	7.94
June-23	3	63	4.76
July-23	3	63	4.76
August-23	5	63	7.94
September-23	1	63	1.59
October-23	2	63	3.17
November-23	2	63	3.17

Supplementary table 18

Successfully sequenced spike protein; case distribution – Sample type used for extraction

Form	n	Total	Percentage
Peritoneal	41	63	65.08
Pleural	8	63	12.70
CSF	10	63	15.87
Tissue/LN[a] intestinal	4	63	6.35

[a]LN – lymph node

Supplementary table 19

Preliminary primer sequences used during first amplifications of the FCoV-23 genome. Primers were designed either using primal scheme or through manual design following multi-sequence alignment of available FCoV whole genomes on NCBI with Mafft[1] (v7.490). This is not a finalised scheme and Supplementary table 20 primers should be used for sequencing FCoV-23.

Scheme location	Forward (5'-3')	Reverse (5'-3')	Specific target (where applicable)	Start (consensus genome)	End (consensus genome)	Expected length	Note
28 to 32	GACGCAGACTTCAGTGTTA	ACCATTATGCCATTRTARTA	Spike	19728	23341	3613	[a]
23	TGCCCAGCTGARATTGTTAARACAG	CATAGTTGTAAGYTCAAGACCACC	ORF1b region	16376	17426	1050	
35	TGTCTBAGTACTGGHTGYTGTGG	AGCATAGGGTCTACAAAATGCAAC	Orf3c/E/M region part 1	23592	24616	1024	
36	GATGGCATTGTKACAAYAACTGTCTT	ARCCGAACATTACATATCTGGAAACTT	Orf3c/E/M region part 2	24380	25489	1109	
1	GGACACCAACTCGAACTAAACGA	GTCCARTCACCDACACCACTACT		0	992	992	
2	GTAGCACCRCCAGTCAAGARRAAYTC	GTRGCRAARAATGCACTATCAAGRCC		801	1775	974	
3	GTGAARGCHTTYGATGTYTTCACACA	TACRGGCACACTATGCAGTCTTAA		1605	2676	1071	
3 to 4	TTGTCAAGCTTGTCAGTGT	ATTGAGCATCGTCTCCAA		1705	3726	2021	
5 to 7	TGGGCTGYTGCTGTYGAYGAACA	GTGTTTAAGYGCGAGAACTGMCTT		3438	6408	2970	
8 to 9	CAGTACMTYAACCTGTGHTRTC	CCACCRAAGCAAAAACCAGCT		6270	8135	1865	[b]
9	GGTAAGTGCATGACTTTYGATGC	CCACCRAAGCAAAAACCAGCT		7191	8135	944	
9 to 11	GGTAAGTGCATGACTTTYGATGC	ACCYACTGACCACARGTACCAGC		7191	9342	2151	
10 to 14	TGGTTATTAAGAAYGGTRTYGTTCAACC	TGYCTAAYCTTGGAAAGCACTTCA		7567	11363	3796	
14 to 16	GCTTACCATGTTGATAAGTCTTACTACAAA	AGACTTGCTCATGGTCCATAACG		10313	12501	2188	
17	TTAAACGAGTGCGGGGTTCTAG	GGYACACCATCWATRTGRACTTTACG		12282	13264	982	
18 to 19	ATGGGAATKACTTCATGTTTAGAR	CAGAAACAGCTTGAAAGATGT		12977	14352	1375	
20	ACAACYAGYGGTGATGGTACTACA	CCAAACACATTACCATTAGCACAGAG		14285	15316	1031	
21 to 22	GGCATGTGTGTTGTTTGTGGTT	TGTARATDACATAATCRTACTCACTACC		15050	16684	1634	
23	TGCCCAGCTGARATTGTTAARACAG	CATAGTTGTAAGYTCAAGACCACC	ORF1b region	16376	17426	1050	
24 to 25	TTTGCTATGCGTAATGTDAGAGCRTG	TGGCACTRAGYCCYTTCACAGC		17072	18835	1763	

26 to 27	AATRGYAAAGCMCTCCARAGT*	GARTTTCKCCAAAATATATAATTGGCATGC		Not present	20120	1648	[c]
28 to 32	GACGCAGACTTCAGTGTTA	ACCATTATGCCATTRTARTA	Spike	19728	23341	3613	[d]
33	GCCCTTAAYCTTGGYGCRCGTMT	ACRCATATTCCTGACCATGCCG		Not present	Not present		[e]
34 to 35	GCAGCACTTAAYGCBTATGYGTC	AGCATAGGGTCTACAAAATGCAAC		Not present	24616		[f]
35	TGTCTBAGTACTGGHTGYTGTGG	AGCATAGGGTCTACAAAATGCAAC	ORF3c/E/M region part 1	23592	24616	1024	
36	GATGGCATTGTKACAAYAACTGTCTT	ARCCGAACATTACATATCTGGAAACTT	ORF3c/E/M region part 2	24380	25489	1109	
36 to 37	GATGGCATTGTKACAAYAACTGTCTT	CTTATTACCTATTCCYTTGGGAACAA		24380	25407	1027	
38	CYGGTGATTACTCAACAGAAGCA	GTTTTGGCATCATCYTTGGCAGG		25779	26805	1026	
38 to 40	CYGGTGATTACTCAACAGAAGCA	ACATTTTAAACAATCACTAGATCCAGACG		25779	28167	2388	
40	CARCTTTTGARRCCAGACTGYC	ACATTTTAAACAATCACTAGATCCAGACG		27161	28167	1006	

[a]Also amplifies GHR, deletion variant; shorter. [b]Poor performance. [c]Amplification works, however F primer was not incorporated into the draft genome possibly due to variation in the amplified viruses. [d] Also amplifies GHR, deletion variant shorter. [e] Poor performance. [f] Very poor performance

Supplementary table 20

Tiled amplicon scheme for the amplification of the FCoV-23 genome. Primers were designed using primal scheme[2] and manual design using a composite FCoV-23 genome generated using primers in supplementary table 20. Amplicon lengths are designed for 800 bp lengths with 80 bp overlap. We recommend to first assess presence of full-length (FL) spike or domain 0 deletion using primers 36A-F and 37A-R.

Primer number	Forward (5'-3')	Location (FL genome)	LONG Pool	SHORT Pool	x-fold in Pool
1-F	GGACACCAACTCGAACTAAACGA		1	1	0.5
1-R	ARTTCTTCTTGACTGGTGGTGC		1	1	0.5
2-F	ACGGARTAAGTGATCTTAAACCTGTTCT		2	2	0.5
2-R	GAAGTCGTTCAATTCGCCTTCAAA		2	2	0.5
3-F	TCTTTGCTAAYAGTGTGCTCCAA		1	1	1
3-R	AAGCCATATCACCAAYAAYGACA		1	1	1
4-F	GCRCTTGTYAAGCTTGTCAGTG		2	2	1
4A-F	TGACACCACAGAGGACTGAGGC		2	2	1
4-R	AACYTTAAYACCATTACCTAGC		2	2	1
4A-R	TCCARTTCCAGCAGAAGGTCWG		2	2	1
5-F	CCAGTKTGTCTTAAAAACCATGTYGG		1	1	1
5-R	GTACCAATGACYTTTTCAAGCACC		1	1	1
6-F	AGAGATASAACCYGTTACACGTGTC		2	2	1
6-R	TCGTCTCCAAAAGRTATTCTGCATC		2	2	1
7-F	GATGAACAGGAAKCTGAACAACC		1	1	1
7A-F	GAYCCATGGGCTGCTGCTGTT		1	1	1
7-R	TCCKTGGTARAATGARACTTTTCCC		1	1	1
7A-R	CTTTGACGTTCTTCTGCTACRCAC		1	1	1
8-F	TGGATGGTATGGGAATTAAACCTCG		2	2	1
8-R	CACACTWGGYAACTGGTTAGT		2	2	1
9-F	TGTCTTYGTTTACACTGACCARGAG		1	1	1
9-R	CGTARTAGGTGTAATGACCACGYG		1	1	1
10-F	GCTTGKTGAKTTGATGTCRAGTG		2	2	2
10-R	ACTAGTTTTGCATARCGCCA		2	2	2
11-F	GCTGACGTRTTCTTTATGRCTGG		1	1	1
11-R	GCTTTTTRCAGAGTYTACCAGTACC		1	1	1
12-F	TCATGGGATTAYAAGTCAGACCC		2	2	1
12-R	TTGGCATTRAARAGTGCTCCCT		2	2	1
13-F	CARGAAGTGCTTAAGACTATGTTYC		1	1	1
13A-F	TTCACGGCATATGACTATGATG		1	1	1
13B-F	GAAGTATAGTAGTCAGGAAGTGC		1	1	2
13-R	CCATGTAATCATARCCCTCWGCAG		1	1	2
13A-R	CAAATGGTTGAACAACACCGTTC		1	1	2
14-F	CAGATGAAGATYTGCCKTATGAGCG		2	2	2
14-R	CCATCYCCAAACTCTTTATCATAGACA		2	2	2
14A-R	TCAATACACTCTCCGACTCTGC		2	2	2
15-F	TGAGGGTGCTAAGCTTTACAGTG		1	1	0.5
15-R	ATCAGCCTCTCCCATMGAACCA		1	1	0.5
16-F	TCYCTACCATCACTATTCAAACTTAARGT		2	2	1

Primer number	Forward (5'-3')	Location (FL genome)	LONG Pool	SHORT Pool	x-fold in Pool
16-R	GAGAGCCGTTACCTAATTCTAGATGG		2	2	1
17-F	GTCTGTGAAACCAGGTGAGAGTT		1	1	0.5
17-R	AACAACATTTTATGCTTAATTCCAACGAC		1	1	0.5
18-F	ACGCCTACTGAAGTCATAAGGC		2	2	0.5
18-R	ACTGTGAACTGGTAAACACCACA		2	2	0.5
19-F	CAAAGGACTGGTTTGTTGTTTTTGC		1	1	0.5
19-R	GCGTGCCTCTTTGTACATGCYA		1	1	0.5
20-F	TTGCCTAGCTGGATTGCCTATG		2	2	1
20-R	TATTTAACYTCAGGACCATTARCRCC		2	2	1
21-F	TGCTTATGGTAGYGGTAAAGCGC		1	1	0.5
21-R	AGCTCTACTAACATGGTCTGGATC		1	1	0.5
22-F	GWGGTATGCAGCCAGTTAMTAAYT		2	2	1
22-R	ACTGGRTCAAACCAATCCTTRTT		2	2	1
23-F	TGYAGTTGCTGAACAYGACTT		1	1	1
23-R	GKAGATCTGTCATRGTCAACTTCA		1	1	1
24-F	CATTGTGCYAATTTTAACACRYT		2	2	0.5
24A-F	CTGCATTTGGACCTCTTGTACGTA		2	2	0.5
24-R	CTGTCTCGTYGTCATTGTKGA		2	2	0.5
24A-R	TCCCACAGTGCGAGCTCTAGAC		2	2	0.5
25-F	TTTGGTAAGGCAAGACTTTACTATGAGA		1	1	1
25-R	TCTGCTACATARCCAAGATCWGCA		1	1	1
26-F	GCTTTTAGGAGTRGATTCAAACAC		2	2	1
26A-F	ACAGCGCAAGATATATGACAATTG		2	2	1
26-R	AGCTACACACATAWGGYGTAATAGAC		2	2	1
26A-R	GTAACATCATTAACAGTACAACCATT		2	2	1
27-F	CAAAACACCCTAARCCTGCWTATCAA		1	1	0.5
27-R	GAGTCACTACCATATTCTGACTGCTC		1	1	0.5
28-F	TGTGAAAGCWAAGGAGGARTCTGT		2	2	1
28-R	AARGTTCTAGGWGCTGGRAGTT		2	2	1
29-F	AGGATAATACCTCAAAGAATCAGAGTTGA		1	1	1
29-R	ACCACAAGTTTCAGGTTTTGCYTG		1	1	1
30-F	ACTCGGCKCAAGGTAGTGAGTA		2	2	0.5
30-R	CCATTTTGCCGCAMTCACATTT		2	2	0.5
31-F	TCATGAGGAGRGGTCAAYCYT		1	1	1
31-R	GCATGATTGTTAACATACAACGCAC		1	1	1
32-F	AAYAATGTTAGATGTCTGGAGTAYGA		2	2	1
32-R	AGTTTGAGAAAGGACAGTCCGC		2	2	1
33-F	AGGAACGGACCTACTGACAAGT		1	1	0.5
33-R	TCGTCCAAGAGTATGTCCATATAAGTG		1	1	0.5
34-F	TGGTTTTGAACACGTTGTATTTGGA		2	2	0.5
34-R	ATCACCTGTAACACTGAAGTCTGC		2	2	0.5
35-F	GCTCCTGGTAGTACTGTCYTAAGA		1	1	1
35A-F	GTCAAGTACACTCAGTTGTGTC		1	-	1
35-R	CACCAACAACYACASTTCCTTCT		1	-	1
35A-R	CGTTGCCATCTAATTGTGTTACG		1	-	1
36-F	GGCAARYTACTAAACTTTGGTAACC		2	2	1
36A-F	CTAAGGAAGGGTAAGTTGCTCA		2	2	1
36-R	CCAGTGCAATRTTCATAATCYTCACA		2	1	1

Primer number	Forward (5'-3')	Location (FL genome)	LONG Pool	SHORT Pool	x-fold in Pool
36A-R	CCATCAGGTATGTAACCTCCC		2	1	1
37-F	GTGGAATGATGAMYTTGTTACAGC		1	-	1
37A-F	ATACCCACGGACAATGGAACGA		1	-	1
37-R	AGGAAATGTGCTAAAGAAATTGTAACCA		1	2	1
37A-R	CCTTTACACTAGGTGGTAATGTTC		1	2	1
38-F	ACAGTGMGTGAGTCTAGTTYTTACA		2	1	1
38-R	CARTTAGCACCAACAGGAYKCA		2	1	1
39-F	ACGTGTATTGCATTCGTTCTAATCAA		1	2	0.5
39-R	GTATCGTGACATTACCRGTGCT		1	2	0.5
40-F	GTRACRCCATGTGATGTAAGCGC		2	1	2
40-R	TGCCATTGTAATATTGAGCACAMAC		2	1	2
41-F	GTGACATCTGGYTTAGGTACAGTCG		1	2	1
41-R	AACRAGTCCRAAAGTGYGATCG		1	2	1
42-F	ACWAGCAGAGGTTAGGGCTAGT		2	1	0.5
42-R	TCTACTGAAAAGAGAATGACAACAGCT		2	1	0.5
43-F	GTCTYAGTACTGGYTGTTGTGG		1	2	1
43-R	GTTRTTGTMACAATGCCATCTATGT		1	2	1
44-F	ACCACATGTTAATACCATAGTACAACAAC		2	1	0.5
44-R	CATGGCGTGCAGGTAGTACTAT		2	1	0.5
45-F	CGTGTCTATGATGTTTCCTAGGGC		1	2	0.5
45-R	TGTAGGTRTGCCATCAAGGGGT		1	2	0.5
46-F	TCGGCTTTAGTGTTGCAGGTG		2	1	0.5
46-R	AGTAGAAGAACCACCTTTCAGGAAG		2	1	0.5
47-F	CCCATTACCCTCGAAACAGGATC		1	2	0.5
47-R	GTRAGYGTGACTTTCACYTGATC		1	2	0.5
48-F	ATGCCAACAAACACASCTGG		2	1	0.5
48-R	CACACWAGGAYTACARCAATCATG		2	1	0.5
49-F	CCTGCTATACATTGTTAGGTGC		1	2	1
49-R	ACATTTTAAACAATCACTAGATCCAGACG		1	2	1

Supplementary table 21

Results of recombination analysis. Several tools run using RDP5 and using a multi sequence alignment between the assembled FCoV-23 genome 2-C11 Re 10276 (PQ133182), a pCCoVII genome (KP981644.1), an FCoVII genome (LC742526.1) and an FCoVI genome (MT239440.1) were used to analyse recombination.

Tool used via RDP5	P- values
RDP5[3]	4e-30 x10^{-300}
GENECONV	4e-30 x10^{-300}
BootScan[4]	8.624 x10^{-269}
MaxChi[5]	1.200 x10^{-199}
Chimaera[6]	2.948 x10^{-64}
SiScan[7]	8.209 x10^{-93}
3Seq[8]	4e-30 x10^{-300}

Supplementary table 22

Results of recombination analysis. Several tools run using RDP5 and using a multi sequence alignment between the assembled domain 0-deletion FCoV-23 genome 2-F12 BW 11350 (PQ133177), a pCCoVII genome (KP981644.1), an FCoVII genome (LC742526.1) and an FCoVI genome (MT239440.1) were used to analyse recombination.

Tool used via RDP5	P- values
RDP5[3]	2.000 x10^{-299}
GENECONV	2.000 x10^{-299}
BootScan[4]	4.414 x10^{-21}
MaxChi[5]	6.000 x10^{-199}
Chimaera[6]	6.000 x10^{-199}
SiScan[7]	9.679 x10^{-87}
3Seq[8]	2.000 x10^{-299}

Supplementary table 23

Comparison of FCoV-23 Spike-2 with determinant mutations. The consensus FCoV-23 sequence was compared with key sequence features that were identified in Zehr *et al.* [9] as positively associated with the FECV biotype. Association with biotype was assessed whether mutations had been previously more likely been associated with one of the biotypes. Amino acid positions below the line are after the recombination breakpoint between pCCoV and FCoV-1.

Position in FCoV-23	Amino acid sequence in FCoV-23	Amino acid composition at site associated with biotype
534	V	Marginally tentative FIPV
596	Q	Marginally tentative FIPV
1404	L	**New mutation**
1405	V	Marginally tentative FIPV
1416	L	Marginally tentative FECV
1434	L	Marginally tentative FECV

Supplementary table 24

Comparison of FCoV-23 Orf3a,b, and c with determinant mutations. The consensus FCoV-23 sequence was compared with key sequence features that were identified in Zehr *et al.* [9] as positively associated with the FECV biotype. Association with biotype was assessed whether mutations had been previously more likely been associated with one of the biotypes. Sites could previously not be "statistically associated uniquely with one phenotype"[9].

Protein	Position in FCoV-23	Amino acid sequence in FCoV-23	Amino acid composition at site associated with biotype
Orf3a	**30**	L	No indication
Orf3a	**32**	N	No indication
Orf3a	**47**	E	No indication
Orf3a	**58**	Q	No indication
Orf3a	**61-62 gap**	IE	No indication
Orf3a	**64**	S	No indication
Orf3a	**65**	S	**New mutation**
Orf3b	**2**	R	**New mutation**
Orf3b	**64**	K	No indication
Orf3b	**71**	A	No indication
Orf3c	**11**	S	No indication
Orf3c	**71**	G	No indication
Orf3c	**72**	V	No indication
Orf3c	**159**	M	No indication
Orf3c	**165**	T	No indication
Orf3c	**175**	G	No indication

Supplementary table 25

Comparison of FCoV-23 Orf7b with determinant mutations. The consensus FCoV-23 sequence was compared with key sequence features that were identified in Zehr *et al.* [9] as positively associated with the FECV biotype. Association with biotype was assessed whether mutations had been previously more likely been associated with one of the biotypes.

Position in FCoV-23	Amino acid sequence in FCoV-23	Amino acid composition at site associated with biotype
5	V	Marginally tentative FIPV
11	L	No indication
12	A	No indication
19	D	No indication
25	H	No indication
36	Q	No indication
39	V	No indication
41	H	No indication
48	H	No indication
50	I	No indication
63	S	No indication
68	N	No indication
82	I	No indication
89	S	No indication
106	N	No indication
107	Q	No indication
129	T	No indication
131	F	No indication
139	T	No indication
140	Q	No indication
145	R	No indication
147	F	No indication
149	H	No indication
152	S	No indication
159	**I**	**New mutation**
160	H	No indication
167	Y	No indication
168	C	No indication
170	H	No indication
172	L	No indication
187	K	No indication
190	R	No indication
191	S	No indication
194	V	No indication
198	L	No indication
199	N	No indication
200	Q	No indication
202	H	No indication
203	**H**	**New mutation**
204	T	No indication

Supplementary table 26

Reposited full-genome FCoV-23 sequences from this study.

GB Number	Isolate
PQ133176	1-F11 Gi 6590
PQ133177	2-F12 BW 11350
PQ133178	2-A6 Ge 7353
PQ133179	2-A9 Do 9045
PQ133180	2-B6 Lu 7456
PQ133181	2-C8 Wi 8299
PQ133182	2-C11 Re 10276
PQ133183	2-D4 An 7030
PQ133184	2-D8 Ta 8364
PQ133185	2-D9 Ni 9183
PQ133186	2-E6 Bi 7543
PQ133187	2-E8 Ru 8415
PQ133188	2-E9 Mi 9160
PQ133189	2-F3 Je 6971
PQ133190	2-F9 Fe 9265
PQ133191	2-G2 Ka 6882
PQ133192	2-H6 Ka 7790
PQ133193	2-H7 Ja 8138
PQ133194	2-H8 So 8842
PQ133195	2-H9 Mo 9241

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