

Supplementary table 1: FIP outbreak Cyprus Jan-Aug 2023; case distribution – sex (with NA).

Sex	n	Total	Percentage
Female	54	164	32.93
Male	109	164	66.46
Unknown	1	164	0.61

Supplementary table 2: FIP outbreak Cyprus Jan-Aug 2023; case distribution – sex (without NA)

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

Supplementary table 3: FIP outbreak Cyprus Jan-Aug 2023; case distribution – Age stats (years); the age of 39 cases are missing

Mean_age	Median_age	Min_age	Max_age
4.424	3	1	16

Supplementary table 4: FIP outbreak Cyprus Jan-Aug 2023; case distribution – FIP form

Form	n	Total	Percentage
Non-effusive	2	164	2.44
Neurological	46	164	28.05
Effusive	114	164	69.51

Supplementary table 5: FIP outbreak Cyprus Jan-Aug 2023; case distribution – District

District	n	Total	Percentage
Nicosia	50	164	30.49
Famagousta	48	164	29.27
Larnaca	28	164	17.07
Limassol	19	164	11.59
Paphos	19	164	11.59

Supplementary table 6: RNA samples for sequencing; case distribution – Sex

Sex	n	Total	Percentage
Female	33	93	35.48
Male	60	93	64.52

Supplementary table 7: RNA samples for sequencing; case distribution – Age stats (years). The ages of 21 cats are missing.

Mean_age	Median_age	Min_age	Max_age
4.38	3	1	16

Supplementary table 8: RNA samples for sequencing; case distribution – FIP form

Form	n	Total	Percentage
Non-effusive	2	93	2.15
Neurological	14	93	15.05
Effusive	77	93	82.80

Supplementary table 9: RNA samples for sequencing; case distribution – District

District	n	Total	Percentage
Nicosia	31	93	39.78
Famagousta	27	93	29.03
Larnaca	15	93	16.13
Limassol	8	93	8.6
Paphos	3	93	3.23
Cyprus unk	1	93	1.08
UK	2	93	2.15

Supplementary table 10: RNA samples for sequencing; case distribution – Collection dates

District	n	Total	Percentage
October-21	1	93	1.08
November-21	1	93	1.08
December-21	1	93	1.08
February-22	1	93	1.08
March-22	1	93	1.08
August-22	1	93	1.08
December-22	2	93	2.15
January-23	7	93	7.53
February-23	22	93	23.66
March-23	35	93	37.63
April-23	19	93	20.43
September-23	1	93	1.08
October-23	1	93	1.08
Unknown	1	93	1.08

Supplementary table 11: RNA samples for sequencing; case distribution – Sample type used for extraction

District	n	Total	Percentage
Peritoneal	57	93	61.29
Pleural	18	93	19.35
CSF	14	93	15.05
Nasal Swab	1	93	1.08
Tissue/LN^a intestinal	2	93	2.15
Unknown	1	93	1.08

^aLN – lymph node

Supplementary table 12: Successfully sequenced spike protein; case distribution – Sex

Sex	n	Total	Percentage
Female	19	45	42.22
Male	26	45	57.78

Supplementary table 13: Successfully sequenced spike protein; case distribution – Age stats (years). The ages of 9 cats are missing

Mean_age	Median_age	Min_age	Max_age
4.67	3	1	16

Supplementary table 14: Successfully sequenced spike protein; case distribution – FIP form

Form	n	Total	Percentage
Non-effusive	1	45	2.22
Neurological	5	45	11.11
Effusive	39	45	86.67

Supplementary table 15: Successfully sequenced spike protein; case distribution – District

District	n	Total	Percentage
Nicosia	15	45	33.33
Famagousta	17	45	37.78
Larnaca	5	45	11.11
Limassol	4	45	8.89
Paphos	2	45	4.44
UK	2	45	4.44

Supplementary table 16: Successfully sequenced spike protein; case distribution – Collection dates

District	n	Total	Percentage
January-23	3	45	6.67
February-23	7	45	15.56
March-23	21	45	46.67
April-23	12	45	26.67
September-23	1	45	2.22
October-23	1	45	2.22

Supplementary table 17: Successfully sequenced spike protein; case distribution – Sample type used for extraction

District	n	Total	Percentage
Peritoneal	32	45	71.11
Pleural	7	45	15.56
CSF	5	45	11.11
Tissue/LN ^a intestinal	1	45	2.22

^aLN – lymph node

Supplementary table 18: Primer sequences used during amplification of the FCoV-23 genome. Primers were designed either using primal scheme¹ or through manual design following multi-sequence alignment of available FCoV whole genomes on NCBI² with Mafft³ (v7.490). The greyed-out section highlights the gap in the assembled consensus genome, these primers may work, but not efficiently. The primers that amplify the regions used in Figure 2 amplify very well, and these are listed both at the top of the table and in their place in the draft scheme, the others amplicons have varying levels of efficiency. This should not be considered a finalised scheme.

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	POL1b region	16376	17426	1050	
35	TGTCTBAGTACTGGHTGYTGTGG	AGCATAGGGTCTACAAAATGCAAC	Orf3c/E/M region part 1	23592	24616	1024	
36	GATGGCATTGTKACAAYAAGTGTCTT	ARCCGAACATTACATATCTGGAAACTT	Orf3c/E/M region part 2	24380	25489	1109	
1	GGACACCAACTCGAACTAAACGA	GTCCARTCACCDACACCACTACT		0	992	992	
2	GTAGCACCRCAGTCAAGARRAAYTC	GTRGCRAARAATGCACTATCAAGRCC		801	1775	974	
3	GTGAARGCHTTYGATGTYTTCACACA	TACRGGCACACTATGCAGTCTTAA		1605	2676	1071	
3 to 4	TTGTCAAGCTTGTCAGTGT	ATTGAGCATCGTCTCCAA		1705	3726	2021	
5 to 7	TGGGCTGYTGCTGTYGAYGAACA	GTGTTTAAGYGCAGAACTGMCTT		3438	6408	2970	
8 to 9	CAGTACMTYAACCTGTGHRRTC	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	TTAAACGAGTGCAGGGTTCTAG	GGYACACCATCWATRTGRACCTTACG		12282	13264	982	
18 to 19	ATGGGAATKACTTCATGTTTAGAR	CAGAAACAGCTTGAAAGATGT		12977	14352	1375	
20	ACAACYAGYGGTATGGTACTACA	CCAAACACATTACCATTAGCACAGAG		14285	15316	1031	
21 to 22	GGCATGTGTGTTGTTTGTGGTT	TGTARATDACATAATCRCTACTACTACC		15050	16684	1634	
23	TGCCCAGCTGARATTGTTAARACAG	CATAGTTGTAAGYTCAAGACCACC	POL1b region	16376	17426	1050	
24 to 25	TTTGCTATGCGTAATGTDAGAGCRTG	TGGCACTRAGYCCYTTACAGC		17072	18835	1763	
26 to 27	AATRGYAAAGCMCTCCARAGT*	GARTTTCKCAAAAATATATAATTGGCATGC		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	NSP3c/E/M region part 1	23592	24616	1024	
36	GATGGCATTGTKACAAYA AACTGTCTT	ARCCGAACATTACATATCTGGAAACTT	NSP3c/E/M region part 2	24380	25489	1109	
36 to 37	GATGGCATTGTKACAAYA AACTGTCTT	CTTATTACCTATTCCYTTGGGAACAA		24380	25407	1027	
38	CYGGTGATTACTCAACAGAAGCA	GTTTTGGCATCATCYTTGGCAGG		25779	26805	1026	
38 to 40	CYGGTGATTACTCAACAGAAGCA	ACATTTTAAACAATCACTAGATCCAGACG		25779	28167	2388	
40	CARCTTTTGARRCCAGACTGYC	ACATTTTAAACAATCACTAGATCCAGACG		27161	28167	1006	

^aAlso amplifies GHR, deletion variant; shorter. ^bPoor performance. ^cAmplification 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 19: Results of recombination analysis. Several tools run using RDP5 and using a multi sequence alignment between the assembled FCoV-23 genome, a pCCoVII genome (KP981644.1), an FCoVII genome (LC742526.1) and an FCoVVI genome (MT239440.1) were used to analyse recombination.

Tool used via RDP5	P- values
RDP5⁴	1E-30x10 ⁻³⁰⁰
BootScan⁵	3.584x10 ⁻⁸⁰
MaxChi⁶	3.000x10 ⁻²⁰⁰
Chimaera⁷	3.000x10 ⁻²⁰⁰
SiScan⁸	2.390x10 ⁻⁸⁸
3Seq⁹	1E-30x10 ⁻³⁰⁰

Supplementary table 20: 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.*¹⁰ 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 ¹⁰
534	T	New mutation
596	E	New mutation
1404	Not determined	n/a
1405	Not determined	n/a
1416	Not determined	n/a
1434	I	Marginally tentative FIPV

Supplementary table 21: 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.*¹⁰ 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”¹⁰.

Protein	Position in FCoV-23	Amino acid sequence in FCoV-23	Amino acid composition at site associated with biotype ¹⁰
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 22: 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.*¹⁰ 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 ¹⁰
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

References

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