

Table S1. Primer pairs used to amplify and sequence overlapping coding and non-coding genome regions for five historical encephalomyocarditis viruses from South Africa inclusive of two elephants (SPU17/94 and SPU19/94), one *Laelaps* mite (AR3959/61) and two *Mastomys* (AN7402/61 and AN7405/61) strains.

Primer set and orientation (F: Forward/R: Reverse)	Reference	Primer binding site*	Historical virus strains amplified	Expected amplicon size	Targeted genome region	Touch down PCR annealing temperatures and the number of cycles
EMC-AB1: GGCCGAAGCCGCTTGGAATA (F)	van Sandwyk <i>et al.</i> (2013)	219-238	SPU17/94; SPU19/94	285	5'UTR	59°C (2x), 57°C (3x), 55°C (35x)
EMC-AB2: ACGTGGCTTTTGGCCGCAGA (R)	van Sandwyk <i>et al.</i> (2013)	484-503				
EMC-AB1: GGCCGAAGCCGCTTGGAATA (F)	van Sandwyk <i>et al.</i> (2013)	219-238	All	1037	5'UTR-1B	61°C (2x), 59°C (3x), 57°C (35x)
1356R-AB: TGGGTGTTTGACCGTGTT (R)	van Sandwyk <i>et al.</i> (2013)	1232-1251				
687F-AB: GCTCTCCTCAAGCGTATTCA (F)	van Sandwyk <i>et al.</i> (2013)	581-600	SPU17/94; SPU19/94	1886	5'UTR-1B	50°C (2x), 48°C (3x), 46°C (35x)
1356R-AB: TGGGTGTTTGACCGTGTT (R)	van Sandwyk <i>et al.</i> (2013)	1232-1251				
687F-AB: GCTCTCCTCAAGCGTATTCA (F)	van Sandwyk <i>et al.</i> (2013)	581-600	All	1886	5'UTR-1C	57°C (2x), 55°C (3x), 53°C (35x)
2113R-AB: CAGTCCCCACCATGCGGAAGTG (R)	van Sandwyk <i>et al.</i> (2013)	2444-2465				
VP4-F1: CGCCGATCAAGATACGGAGGA (F)	This study	926-946	AN7402/61	3166	L-2B	60°C (2x), 58°C (3x), 56°C (35x)
2B-RM: CGGCAGTAGGGTTTGAGCCATT (R)	van Sandwyk <i>et al.</i> (2013)	4070-4091				
1307F-AB: TCAGACCGAGTGTCTCAAGA (F)	van Sandwyk <i>et al.</i> (2013)	1202-1221	All	1264	1B-1C	59°C (2x), 57°C (3x), 55°C (35x)
2113R-AB: CAGTCCCCACCATGCGGAAGTG (R)	van Sandwyk <i>et al.</i> (2013)	2444-2465				
1307F-AB: TCAGACCGAGTGTCTCAAGA (F)	van Sandwyk <i>et al.</i> (2013)	1317-1336	AR3595/61; AN7402/61; AN7405/61	414	1B	55°C (2x), 53°C (3x), 51°C (35x)
1720R-AB: CTTGGACCATCTGTTGTCCAT (R)	van Sandwyk <i>et al.</i> (2013)	1710-1730				
1904F-AB: GCTTCCTGGACTTTGGTGAT (F)	van Sandwyk <i>et al.</i> (2013)	1914-1933	AR3595/61; AN7402/61; AN7405/61	2178	1B-2B	56°C (2x), 54°C (3x), 52°C (35x)
2B-RM: CGGCAGTAGGGTTTGAGCCATT (R)	van Sandwyk <i>et al.</i> (2013)	4070-4091				
2246MF-JvS: GTACCGTGATCACTAGTCTA (F)	van Sandwyk <i>et al.</i> (2013)	2248-2268	SPU17/94; SPU19/94	985	1C-1D	58°C (2x), 57°C (3x), 56°C (35x)
3204MR-JvS: GACAGCAGGTAGGACAGACAA (R)	van Sandwyk <i>et al.</i> (2013)	3212-3232				
2246MF-JvS: GTACCGTGATCACTAGTCTA (F)	van Sandwyk <i>et al.</i> (2013)	2248-2268	SPU19/94	1729	1C-2B	56°C (2x), 54°C (3x), 52°C (35x)
2B-RM: CGGCAGTAGGGTTTGAGCCATT (R)	van Sandwyk <i>et al.</i> (2013)	3954-3976				
VP3-FM: CACTTCCGCATGGTGGGAACTG (F)	van Sandwyk <i>et al.</i> (2013)	2444-2465	SPU17/94; SPU19/94; AR3595/61; AN7402/61	1533	1C-2B	57°C (2x), 55°C (3x), 53°C (35x)
2B-RM: CGGCAGTAGGGTTTGAGCCATT (R)	van Sandwyk <i>et al.</i> (2013)	3954-3976				

VP3-FM: CACTTCCGCATGGTGGGAACTG (F)	van Sandwyk <i>et al.</i> (2013)	2444-2465	SPU17/94	1597	1C-2A	64°C (2x), 62°C (3x), 60°C (35x)
EMC2C-IR: CGTCCTCGTTGGCATCGAGCAA (R)	This study	4019-4040				
EMC2B-F4: CCACAAGGGATTGGAGGTTAGA (F)	This study	3526-3547	SPU19/94	515	2A-2B	60°C (2x), 58°C (3x), 56°C (35x)
EMC2C-IR: CGTCCTCGTTGGCATCGAGCAA (R)	This study	4019-4040				
EMC-2BF2: GGTCAGTTGTGGGCTGAAACA (F)	This study	3744-3764	SPU19/94	297	2A-2B	61°C (2x), 59°C (3x), 57°C (35x)
EMC2C-IR: CGTCCTCGTTGGCATCGAGCAA (R)	This study	4019-4040				
EMC-2BF2: GGTCAGTTGTGGGCTGAAACA (F)	This study	3744-3764	SPU17/94	2089	2A-3AB	60°C (2x), 58°C (3x), 56°C (35x)
EMC3C-IR: GTGTGACTAACACCACGCACTAC (R)	This study	5810-5832				
EMC-2BF3: GCAGGATATTTCACGGATCTC (F)	This study	4026-4046	AR3595/61; AN7402/61;	2843	2B-3D	60°C (2x), 58°C (3x), 56°C (35x)
EMC-3DR3: CCAATCTCTCTGCTGCATAT (R)	This study	6848-6868	AN7405/61			
4049-JvSF: GGAGCGGCAGTGTCAAT (F)	This study	4053-4072	SPU17/94;	1780	2B-3AB	59°C (2x), 57°C (3x), 55°C (35x)
EMC3C-IR: GTGTGACTAACACCACGCACTAC (R)	This study	5810-5832	SPU19/94			
4518-FAB: CGGAATGGAATGGCTGCCTATGT (F)	This study	4641-4663	SPU17/94;	1192	2C-3C	62°C (2x), 60°C (3x), 58°C (35x)
EMC3C-IR: GTGTGACTAACACCACGCACTAC (R)	This study	5810-5832	SPU19/94			
EMC2C-IF: GCGCCAGTCAGTATATTCTCTTC (F)	This study	4770-4792	SPU17/94;	1063	2B-3AB	61°C (2x), 59°C (3x), 57°C (35x)
EMC3C-IR: GTGTGACTAACACCACGCACTAC (R)	This study	5810-5832	SPU19/94			
EMC-3CF1-VvM: GCCCTATTGATTTTGTGTAT (F)	This study	5696-5716	SPU17/94;	1286	3C-3D	54°C (2x), 52°C (3x), 50°C (35x)
EMC-3D2R: CCGGTTGGGTCTGGAAC TT (R)	This study	6963-6981	SPU19/94			
EMC-3CF2-VvM: GCCGCGTCAATAATTTACAAA (F)	This study	6202-6222	SPU17/94;	780	3C-3D	58°C (2x), 56°C (3x), 54°C (35x)
EMC-3D2R: CCGGTTGGGTCTGGAAC TT (R)	This study	6963-6981	SPU19/94			
6453F-VVM: CCCTCCAGTGT TAGAATGGTT (F)	This study	6451-6472	SPU19/94	1343	3D	58°C (2x), 56°C (3x), 54°C (35x)
EMC-3DRAB: CTTACCGGTAACGCGTTGT (R)	van Sandwyk <i>et al.</i> (2013)	7774-7793				
6793F-VvM: CCCACCATTTGAGCATTGCAT (F)	This study	6793-6813	SPU17/94;	464	3D	58°C (2x), 56°C (3x), 54°C (35x)
7256R-VvM: CGCCATAAGACAAGATCTTCACAT (R)	This study	7233-7256	SPU19/94			

*Primer binding sites correspond to reference sequence L22089 (Mengo isolate M *Macaca mulatta* Uganda 1946).

Table S2: Internal primers, designed during the course of this study, to generate overlapping genome region sequences for both strands of each amplicon for three historical rodent-associated EMC viruses in South Africa.

Primer name and Orientation (F: Forward/R: Reverse)	Primer binding site*	Amplified virus strains
1D-R2: 5' - GGCAAATCCTGACTCCAAACTC - 3' (R)	2590-2611	AN7402/61
EMC-AB2: 5' - ACGTGGCTTTTGGCCGCAGA - 3' (R)	598-617	AR3595/61; AN7402/61; AN7405/61
EMC2C-IR: 5' - CGTCCTCGTTGGCATCGAGCAA - 3' (R)	4019-4040	AR3595/61; AN7402/61; AN7405/61
EMC3C-IR: 5' - GTGTGACTAACACCACGCACTAC - 3' (R)	5810-5832	AR3595/61; AN7402/61; AN7405/61
EMC2C-IF: 5' - GCGCCAGTCAGTATATTCTCTTC - 3' (F)	4770-4792	AR3595/61; AN7402/61; AN7405/61

*Primer binding site determined using the reference sequence L22089 (Mengo isolate M *Macaca mulatta* Uganda 1946).

Table S3. Summary statistics for the three EMCV-1 datasets (i-iii) used for phylogenetic inference in this study.

Dataset	No. taxa	Length (bases)	Va sites	Pi sites	Average nucleotide composition (%): empirical/uncorrected				R	Best-fit model of sequence evolution (under BIC)
					T	C	A	G		
(i) Near-complete genome	41	7418	3511	3189	25.7	24.3	26.4	23.6	1.57	GTR+G+I (G=0.67, I=0.39)
(ii) VP3/1 gene	52	1539	778	729	27.1	27.1	24.2	21.6	1.42	TN93+G+I (G=0.91, I=0.44)
(iii) 3D gene	63	242	105	94	27.2	24.7	27.7	20.4	2.3	K2P+G (G=0.24)

BIC: Bayesian information criterion; No.: Number; V: variable; Pi: parsimony-informative; R: transition: transversion ratio (R). GTR: General Time Reversible; TN93: Tamura-Nei; K2P: Kimura 2-parameter; G: Gamma distribution; I: proportion of invariant sites.

Table S4. Matrix showing the pairwise nucleotide sequence identity (%) between five historical encephalomyocarditis viruses from South Africa inclusive of two elephant (SPU17/94 and SPU19/94), one *Laelaps* mite (AR3959/61) and two *Mastomys* (AN7402/61 and AN7405/61) strains.

	SPU19/94	SPU17/94	AR3595/61	AN7402/61	AN7405/61
SPU19/94					
SPU17/94	99.8513				
AR3595/61	93.6334	93.6469			
AN7402/61	93.5929	93.6064	99.9459		
AN7405/61	93.6064	93.6199	99.9730	99.9459	