

Emergence of Rabies in Cape Fur Seals, South Africa

Appendix

Viral RNA extraction, RT-PCR and phylogenetic analysis performed during the study

Total RNA was extracted from positive brain samples (n=51) using Tri-Reagent (Sigma Aldrich, USA) as per manufacturer's instructions. A reverse transcription polymerase chain reaction (RT-PCR) was performed by targeting the cytoplasmic domain of the glycoprotein gene and the G-L intergenic region of the viral genome as previously described (1). Since 1995, South African RABVs have been genetically characterized using Sanger sequencing of the G-L intergenic region of the viral genomes. Twenty additional South African rabies viruses (RABVs) nucleotide sequences available in the database were also included for phylogenetic analysis. Phylogenetic analysis was undertaken using a Bayesian Markov Chain Monte Carlo (MCMC) method in the BEAST X version 10.5.0 using a relaxed exponential clock and the sampling dates were included to investigate the linear evolutionary rates of RABVs (2). Runs were performed using the nucleotide substitution model general time reversible (GTR) with gamma plus invariable together with the constant size model as a coalescent tree prior. The phylogenetic analysis relied on three independent Markov chains sampled for 50 million states and a sampling frequency of 5000 was combined after discarding at least the first 10% burn. The posterior distributions were subsequently inspected using the Tracer software (version 1.7.1) to ensure adequate mixing and convergence before the associated statistics were summarized as a maximum clade credibility (MCC) tree using Tree Annotator version 10.5, and the final tree was displayed using FigTree version 1.4.4. The BEAGLE parallel computation library was used to enhance the speed of the likelihood calculations (3).

To investigate the linear evolutionary rates of RABVs in the selected panel of samples, the Bayesian Markov chain Monte Carlo in BEAST X version 10.5.0 was used (2) and incorporated information on the sampling date, year. For these analyses, runs were performed using the nucleotide substitution model general time reversible (GTR) with gamma plus invariable sites under strict molecular clock together with the constant size model as a coalescent tree prior. Fifty million chains were run (discarding the first 5 million steps), and trees were sampled every 5,000 steps. The BEAGLE parallel computation library was used to enhance the speed of the likelihood calculations (3). The resulting trees were summarized in an MCC tree, using Tree Annotator version 10.5 and the final tree was displayed in FigTree version 1.4.4.

References

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Appendix Table. Rabies positive cases obtained from Cape fur seals in the Northern and Western Cape provinces of South Africa

Item number	Laboratory number	Sampling date	Location	Province	Genbank Accession Number
1	186/24	22-May-24	Blouberg beach	Western Cape	PV165423
2	219/24	07-Jan-24	Plettenberg Bay	Western Cape	PV165424
3	220/24	13-Jun-24	Die Dam beach	Western Cape	PV165425
4	223/24	10-Aug-23	Melkbosstrand	Western Cape	PV165426
5	251/24	08-Apr-24	Plettenberg Bay	Western Cape	PV165427
6	256/24	26-Jun-24	Muizenberg	Western Cape	PV165428
7	270/24	27-Jun-24	Glencairn	Western Cape	PV165429
8	287/24	13-Jul-24	Yzerfontein beach	Western Cape	PV165430
9	289/24	13-Jul-24	Yzerfontein beach	Western Cape	PV165431
10	296/24	17-Jul-24	Mossel Bay beach	Western Cape	PV165432
11	332/24	26-Jul-24	Plettenberg Bay	Western Cape	PV165433
12	344/24	01-Aug-24	Pearly Beach	Western Cape	PV165434
13	345/24	01-Aug-24	Pearly Beach	Western Cape	PV165435
14	400/24	27-Aug-24	Groen River Mouth	Northern Cape	PV165436
15	443/24	01-Sep-24	Cliff point	Northern Cape	PV165437
16	445/24	01-Sep-24	Cliff point	Northern Cape	PV165438
17	446/24	01-Sep-24	Cliff point	Northern Cape	PV165439
18	447/24	01-Sep-24	Cliff point	Northern Cape	PV165440
19	448/24	01-Sep-24	Cliff point	Northern Cape	PV165441
20	451/24	01-Sep-24	Cliff point	Northern Cape	PV165442

Item number	Laboratory number	Sampling date	Location	Province	Genbank Accession Number
21	452/24	01-Sep-24	Cliff point	Northern Cape	PV165443
22	458/24	01-Sep-24	Namakwa	Northern Cape	PV165444
23	463/24	01-Sep-24	Kleinzee	Northern Cape	PV165445
24	464/24	01-Sep-24	Kleinzee	Northern Cape	PV165446
25	466/24	01-Sep-24	Kleinzee	Northern Cape	PV165447
26	472/24	01-Sep-24	Kleinzee	Northern Cape	PV165448
27	473/24	01-Sep-24	Kleinzee	Northern Cape	PV165449
28	474/24	01-Sep-24	Kleinzee	Northern Cape	PV165450
29	24/539	19-Sep-24	Velddrif	Western Cape	PV165451
30	24/542	18-Sep-24	Sammy Beach	Northern Cape	PV165452
31	24/545	26-Jun-24	Muizenberg	Western Cape	PV165453
32	24/549	17-Jul-24	Mossel Bay beach	Western Cape	PV165454
33	543/24	23-Oct-24	Clovelly	Western Cape	PV165455
34	549/24	03-Oct-24	Muizenberg	Western Cape	PV165456
35	550/24	24-Oct-24	Sonwabe beach	Western Cape	PV165457
36	511/24	15-Oct-24	Glencairn	Western Cape	PV165458
37	508/24	10-Oct-24	Lambert's Bay	Western Cape	PV165459
38	24/445	16-Aug-24	V&A Waterfront	Western Cape	PV165460
39	24/446	12-Aug-24	Hartenbos	Western Cape	PV165461
40	24/465	25-Aug-24	Groot Brakrivier	Western Cape	PV165462
41	610/24	25-May-24	Klein Brak beach	Western Cape	PV165464
42	589/24	25-Nov-24	Port Nolloth	Northern Cape	PV165465
43	613/24	13-Nov-24	Plettenberg Bay	Western Cape	PV165466
44	576/24	14-Nov-24	Elands Bay	Western Cape	PV165467
45	609/24	10-Nov-24	Buffelsbaai beach	Western Cape	PV165468
46	106/25	13 Mar 2025	Namakwa	Northern Cape	PX718952
47	107/25	13 Mar 2025	Namakwa	Northern Cape	PX718953
48	108/25	13 Mar 2025	Namakwa	Northern Cape	PX718954
49	109/25	12 Mar 2025	Kleinzee	Northern Cape	PX718955
50	111/25	12 Mar 2025	Kleinzee	Northern Cape	PX718956
51	112/25	12 Mar 2025	Kleinzee	Northern Cape	PX718957