




Complete Coding Sequences of 23 South African Domestic and Wildlife Rabies Viruses

 Claude Sabeta,^{a,b} Debrah Mohale,^a Baby Phahladira,^a Ernest Ngoepe,^a Antoinette Van Schalkwyk,^a Kgaogelo Mogano,^c George Chirima,^{c,d} Toru Suzuki,^e Kohei Makita^e

^aAgricultural Research Council—Onderstepoort Veterinary Institute (ARC-OVI), Onderstepoort, Pretoria, South Africa

^bUniversity of Pretoria, Veterinary Tropical Diseases Department, Onderstepoort, Pretoria, South Africa

^cAgricultural Research Council, Soil Climate and Water, Pretoria, South Africa

^dCentre for Geoinformation Science, Department of Geography, Geoinformatics and Meteorology, University of Pretoria, Pretoria, South Africa

^eRakuno Gakuen University, Sapporo, Hokkaido, Japan

ABSTRACT South African rabies viruses originating from dogs and jackals (canid viruses) are closely related and highlight cross-species transmission events between the two canine species. Rabies due to the canid lyssavirus variant is a significant public health matter in this country. The complete coding sequences of 23 canid lyssaviruses from South Africa are reported here.

Rabies virus (RABV) is the prototype species of the *Lyssavirus* genus (family *Rhabdoviridae*, order *Mononegavirales*) (1) and causes encephalitis in all warm-blooded vertebrates. In South Africa, RABV infects both domestic and wild carnivore species (2). The domestic dog is the main vector species responsible for the transmission of rabies to humans (3), resulting in at least 59,000 human deaths annually, and the majority of these ($\geq 95\%$) are recorded in Africa and Asia (2).

Specimens were collected by state veterinarians from animals in northern South Africa (Limpopo and Mpumalanga provinces) showing typical signs of central nervous system infection, preserved in glycerol-saline, and transported to the laboratory (see Table 1 for epidemiological information on the samples). The composite brain tissues were then subjected to a direct fluorescent antibody test for lyssavirus antigen (4). Total viral RNAs were extracted from the original lyssavirus-infected brain tissues and prepared for next-generation sequencing (NGS) using the HiSeq (Illumina, San Diego, CA, USA) platform. Briefly, the TRIzol-extracted viral RNA was depleted of host genomic DNA and rRNA using the on-column DNase treatment in the RNeasy Plus kit (Qiagen) and Terminator 5'-phosphate-dependent exonuclease (Epicentre Biotechnologies), as described previously (5–7). Double-stranded (ds) cDNA was synthesized from 50 ng RNA using a random cDNA synthesis system kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. The ds cDNA was purified using AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA), and 1 ng was used for the Nextera XT DNA sample preparation kit (Illumina). A sequencing library was prepared for each sample according to the manufacturer's instructions and sequenced on an Illumina HiSeq with 2×125 -bp paired-end reads following standard Illumina protocols, resulting in between 0.002 and 5.6 Gb data per isolate (Table 1). For each of the isolates, contigs were generated through both *de novo* assembling of all the reads and mapping of the same reads to existing rabies genomes (GenBank accession numbers [KT336433](#) to [KT336436](#)) using default settings in CLC Genomics Workbench v.9 (Qiagen). A single consensus sequence was produced for each sample from an assembly of all the contigs previously generated. The newly obtained consensus sequence was used as a template to map all the original reads in order to estimate

Citation Sabeta C, Mohale D, Phahladira B, Ngoepe E, Van Schalkwyk A, Mogano K, Chirima G, Suzuki T, Makita K. 2020. Complete coding sequences of 23 South African domestic and wildlife rabies viruses. *Microbiol Resour Announc* 9:e00621-20. <https://doi.org/10.1128/MRA.00621-20>.

Editor Kenneth M. Stedman, Portland State University

Copyright © 2020 Sabeta et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](#).

Address correspondence to Claude Sabeta, SabetaC@arc.agric.za.

Received 21 July 2020

Accepted 24 August 2020

Published 17 September 2020

TABLE 1 Epidemiological information of the canine viruses sequenced in this study

Virus no.	Lab ref no. ^a	Yr of submission	Species from which sample originated	Size of raw data (GB)	No. (%) of reads mapped to new consensus sequence	Sequence length (bp)	% G+C content	BioProject accession no.	GenBank accession no.
1	125/15	2015	<i>Canis familiaris</i>	2.62	159,506 (0.76)	11,923	45.4	SRR12012256	MT454631
2	155/15	2015	<i>Proteles cristatus</i>	2.30	7,365 (0.04)	11,902	45.5	SRR12012255	MT454632
3	361/15	2015	<i>Proteles cristatus</i>	2.73	4,365 (0.02)	11,908	45.6	SRR12012244	MT454633
4	471/15	2015	<i>Canis familiaris</i>	2.65	72,025 (0.34)	11,923	45.4	SRR12012240	MT454634
5	682/15	2015	<i>Canis familiaris</i>	5.63	31,529 (0.07)	11,923	45.3	SRR12012239	MT454635
6	516/16	2016	<i>Canis familiaris</i>	1.73	217,555 (1.57)	11,923	45.3	SRR12012238	MT454636
7	583/16	2016	<i>Canis familiaris</i>	2.53	309,360 (1.53)	11,923	45.4	SRR12012237	MT454637
8	631/16	2016	<i>Otocyon megalotis</i>	4.16	6,656 (0.02)	11,923	45.4	SRR12012236	MT454638
9	635/16	2016	<i>Canis familiaris</i>	1.39	166,479 (1.5)	11,923	45.3	SRR12012235	MT454639
10	676/16	2016	<i>Otocyon megalotis</i>	3.53	5,640 (0.02)	11,923	45.4	SRR12012234	MT454640
11	690/16	2016	<i>Canis familiaris</i>	0.002	37,321 (0.22)	11,923	45.3	SRR12012254	MT454641
12	725/16	2016	<i>Proteles cristatus</i>	2.90	6,971 (0.03)	11,923	45.5	SRR12012253	MT454642
13	269/17	2017	<i>Canis familiaris</i>	4.40	218,019 (0.62)	11,922	45.6	SRR12012252	MT454643
14	400/17	2017	<i>Canis familiaris</i>	1.94	37,211 (0.24)	11,922	45.6	SRR12012251	MT454645
15	454/17	2017	<i>Canis mesomelas</i>	2.80	123,359 (0.55)	11,922	45.6	SRR12012250	MT454646
16	460/17	2017	<i>Canis mesomelas</i>	2.95	21,259 (0.09)	11,922	45.6	SRR12012249	MT454647
17	466/17	2017	<i>Canis mesomelas</i>	4.43	10,620 (0.03)	11,923	45.3	SRR12012248	MT454648
18	474/17	2017	<i>Canis mesomelas</i>	1.58	26,616 (0.21)	11,922	45.6	SRR12012247	MT454649
19	477/17	2017	<i>Otocyon megalotis</i>	2.14	92,643 (0.54)	11,922	45.6	SRR12012246	MT454650
20	480/17	2017	<i>Canis mesomelas</i>	2.90	53,270 (0.23)	11,922	45.6	SRR12012245	MT454651
21	483/17	2017	<i>Canis mesomelas</i>	2.40	7,664 (0.04)	11,923	45.3	SRR12012243	MT454652
22	502/17	2017	<i>Canis mesomelas</i>	2.26	81,394 (0.45)	11,923	45.3	SRR12012242	MT454653
23	503/17	2017	<i>Canis mesomelas</i>	2.21	70,808 (0.4)	11,923	45.3	SRR12012241	MT454654

^a Sample submitted to Agricultural Research Council—Onderstepoort Veterinary Institute (ARC-OVI) for laboratory confirmation of RABV.

the average coverage and possible variants (see Table 1). The genetic organization of the full genomes of the South African RABVs was consistent with that of other previously characterized lyssaviruses (5–7). The sequence length and percent G+C content of each of the samples are listed in Table 1. Each sequence contains the complete coding regions of the nucleoprotein (N), glycoprotein (G), matrix protein (M), phosphoprotein (P), and the RNA-dependent polymerase (L) found in rabies viruses. Genetic analysis of these canid RABVs demonstrated that they are of the Africa 1-b lineage and have a high degree of sequence similarity ($\geq 96.5\%$) (mean distance, 0.023; standard error [SE], 0.001), irrespective of their host species and locality of origin (1, 7–9).

Data availability. The complete coding sequences were submitted to GenBank and are available under accession numbers [MT454631](#) through [MT454654](#) (Table 1) and BioProject accession number [PRJNA638742](#).

ACKNOWLEDGMENTS

This study was partly supported with funding from the National Research Foundation (NRF) of South Africa (NRF unique grant number 114679, ARC grant number P10000130) and the EU Framework Horizon 2020 Innovation Grant, European Virus Archive (EVAg, grant number 653316).

REFERENCES

- Afonso CL, Amarasinghe GK, Bányai K, Bào Y, Basler CF, Bavari S, Bejerman N, Blasdel KR, Briand F-X, Briese T, Bukreyev A, Calisher CH, Chandran K, Chéng J, Clawson AN, Collins PL, Dietzgen RG, Dolnik O, Domier LL, Dürrwald R, Dye JM, Easton AJ, Ebihara H, Farkas SL, Freitas-Astúa J, Formenty P, Fouchier RAM, Fù Y, Ghedini E, Goodin MM, Hewson R, Horie M, Hyndman TH, Jiāng D, Kitajima EW, Kobinger GP, Kondo H, Kurath G, Lamb RA, Lenardon S, Leroy EM, Li C-X, Lin X-D, Liú L, Longdon B, Marton S, Maisner A, Mühlberger E, Netesov SV, Nowotny N, et al. 2016. Taxonomy of the order Mononegavirales: update 2016. *Arch Virol* 161:2351–2360. <https://doi.org/10.1007/s00705-016-2880-1>.
- Sabeta CT, Bingham J, Nel LH. 2003. Molecular epidemiology of canid rabies in Zimbabwe and South Africa. *Virus Res* 91:203–211. [https://doi.org/10.1016/s0168-1702\(02\)00272-1](https://doi.org/10.1016/s0168-1702(02)00272-1).
- Knobel DL, Cleaveland S, Coleman PG, Fevre EM, Meltzer MI, Miranda MEG, Shaw A, Zinsstag J, Meslin F-X. 2005. Re-evaluating the burden of rabies in Africa and Asia. *Bull World Health Organ* 83:360–368.
- Dean DJ, Ableseth MK, Atanasiu P. 1996. The fluorescent antibody test, p 88–95. *In* Meslin F-X, Kaplan MM, Koprowski H (ed), *Laboratory techniques in rabies*, 4th ed. World Health Organization, Geneva, Switzerland.
- Sabeta C, Phahladira B, Marston DA, Wise EL, Ellis RJ, Fooks AR. 2015. Complete genome sequences of six southern African rabies viruses. *Genome Announc* 3:e01085-15. <https://doi.org/10.1128/genomeA.01085-15>.
- Troupin C, Dacheux L, Tanguy M, Sabeta C, Blanc H, Bouchier C, Vignuzzi

- M, Duchene S, Holmes EC, Bourhy H. 2016. Large-scale phylogenomic analysis reveals the complex evolutionary history of rabies virus in multiple carnivore hosts. *PLoS Pathog* 12:e1006041. <https://doi.org/10.1371/journal.ppat.1006041>.
7. Bruner K, Marston DA, Horton DL, Cleaveland S, Fooks AR, Kazwala R, Ngeleja C, Lembo T, Sambo M, Mtema ZJ, Sikana L, Wilkie G, Biek R, Hampson K. 2015. Elucidating the phylodynamics of endemic rabies virus in eastern Africa using whole-genome sequencing. *Virus Evol* 1:vev011. <https://doi.org/10.1093/ve/vev011>.
8. Ngoepe EC, Fehlner-Gardiner C, Wandeler AI, Sabeta CT. 2014. Antigenic characterisation of lyssaviruses in South Africa. *Onderstepoort J Vet Res* 81:a711. <https://doi.org/10.4102/ojvr.v81i1.711>.
9. Nel LH, Thomson GR, Von Teichman BF. 1993. Molecular epidemiology of rabies virus in South Africa. *Onderstepoort J Vet Res* 60:301–306.