reservoirs could still be implicated in the recent events of LASV transmission to humans.

Our findings strongly point toward *M. baoulei* mice as a potential candidate for LASV spreading in Benin, Togo, and Ghana. Together with the multimammate mice *M. natalensis* and *Mastomys erythroleucus* and the soft-furred mouse *Hylomyscus pamfi* (*10*), the fourth rodent species reservoir of LASV is *M. baoulei* pygmy mice.

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About the Author

Dr. Yadouleton is a medical entomologist in the Centre de Recherche Entomologique de Cotonou, Benin, head of the Laboratoire des Fièvres Hémorragiques in Cotonou, and a teacher at the University of Natitingou, Benin. His research interests include mosquito control and the diagnosis of viral hemorrhagic fevers.

References

1. Whitmer SLM, Strecker T, Cadar D, Dienes HP, Faber K, Patel K, et al. New lineage of Lassa virus, Togo, 2016. Emerg Infect Dis. 2018;24:599–602. https://doi.org/10.3201/eid2403.171905
2. World Health Organization. Lassa fever–Benin [cited 2016 July 1]. http://wwwwhoint/csr/don/13-june-2016-lassa-fever-benin
3. Fichet-Calvet E. Lassa fever: a rodent-human interaction. In: Johnson N, editor. The role of animals in emerging viral diseases. London: Elsevier; 2014. p. 89–123.
4. Kronmann KC, Nimo-Paintsil S, Guirguis F, Kronmann LC, Bonney K, Obiri-Danso K, et al. Two novel arenaviruses detected in pygmy mice, Ghana. Emerg Infect Dis. 2013;19:1832–5. https://doi.org/10.3201/eid1911.121491
5. Olayemi A, Obadare A, Oyeyiola A, Fasogbon A, Igbokwe J, Igbahenah F, et al. Small mammal diversity and dynamics within Nigeria, with emphasis on reservoirs of the Lassa virus. Syst Biodivers. 2017;15:1–10.
6. Olschläger S, Lelke M, Emmerich P, Panning M, Drosten C, Hass M, et al. Improved detection of Lassa virus by reverse transcription-PCR targeting the 5′ region of S RNA. J Clin Microbiol. 2010;48:2009–13. https://doi.org/10.1128/JCM.02351-09
7. Vieth S, Drosten C, Lenz O, Vincent M, Omilabu S, Hass M, et al. RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. Trans R Soc Trop Med Hyg. 2007;101:1253–64. https://doi.org/10.1016/j.trstmh.2005.03.018
8. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 2012;29:1969–73. https://doi.org/10.1093/molbev/mss075
9. Bowen MD, Rollin PE, Ksiazek TG, Hustad HL, Bausch DG, Demby AH, et al. Genetic diversity among Lassa virus strains. J Virol. 2000;74:6992–7004. https://doi.org/10.1128/JVI.74.15.6992-7004.2000
10. Olayemi A, Cadar D, Magassouba N, Obadare A, Kourouma F, Oyeyiola A, et al. New hosts of the Lassa virus. Sci Rep. 2016;6:25280. https://doi.org/10.1038/srep25280

Address for correspondence: Elisabeth Fichet-Calvet, Bernhard-Nocht Institute for Tropical Medicine, Virology, Bernhard-Nocht Str. 74, Hamburg 20359, Germany; email: fichet-calvet@bnitm.de

Genomic Characterization of Rift Valley Fever Virus, South Africa, 2018

Antoinette van Schalkwyk, Marco Romito

Author affiliation: Onderstepoort Veterinary Institute, Onderstepoort, South Africa

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An isolated Rift Valley fever (RVF) outbreak was reported in 2018 in Free State Province, South Africa. Phylogenetic analyses based on complete genome sequences of 3 RVF viruses from blood and tissue samples indicated that they were related to a virus isolated in 2016 from a man returning to China from Angola.

Rift Valley fever (RVF) is endemic to sub-Saharan Africa; major outbreaks were reported in South Africa during the 1950s, the 1970s, and 2008–2011 (*1*). Molecular classification of RVF viruses (RVFVs) isolated from 16 countries showed that these viruses cluster into 15 lineages (A–O) (*2*). Viral sequences from the previous outbreaks in South Africa clustered in lineage C (2008–2009), lineage H (2009–2010), lineage I (1951), and lineage L (1974–1975); 1 isolate in 2009 from Kakamas in the Northern Cape Province was in lineage K (Figure) (*2*). Lineage K contains the hepatotropic Entebbe-44 virus isolated from mosquitoes in Uganda in 1944 and its derivative, the Smithburn neurotropic vaccine strain (SNS) commercially available in South Africa (*2*). RVFV was identified by unbiased deep

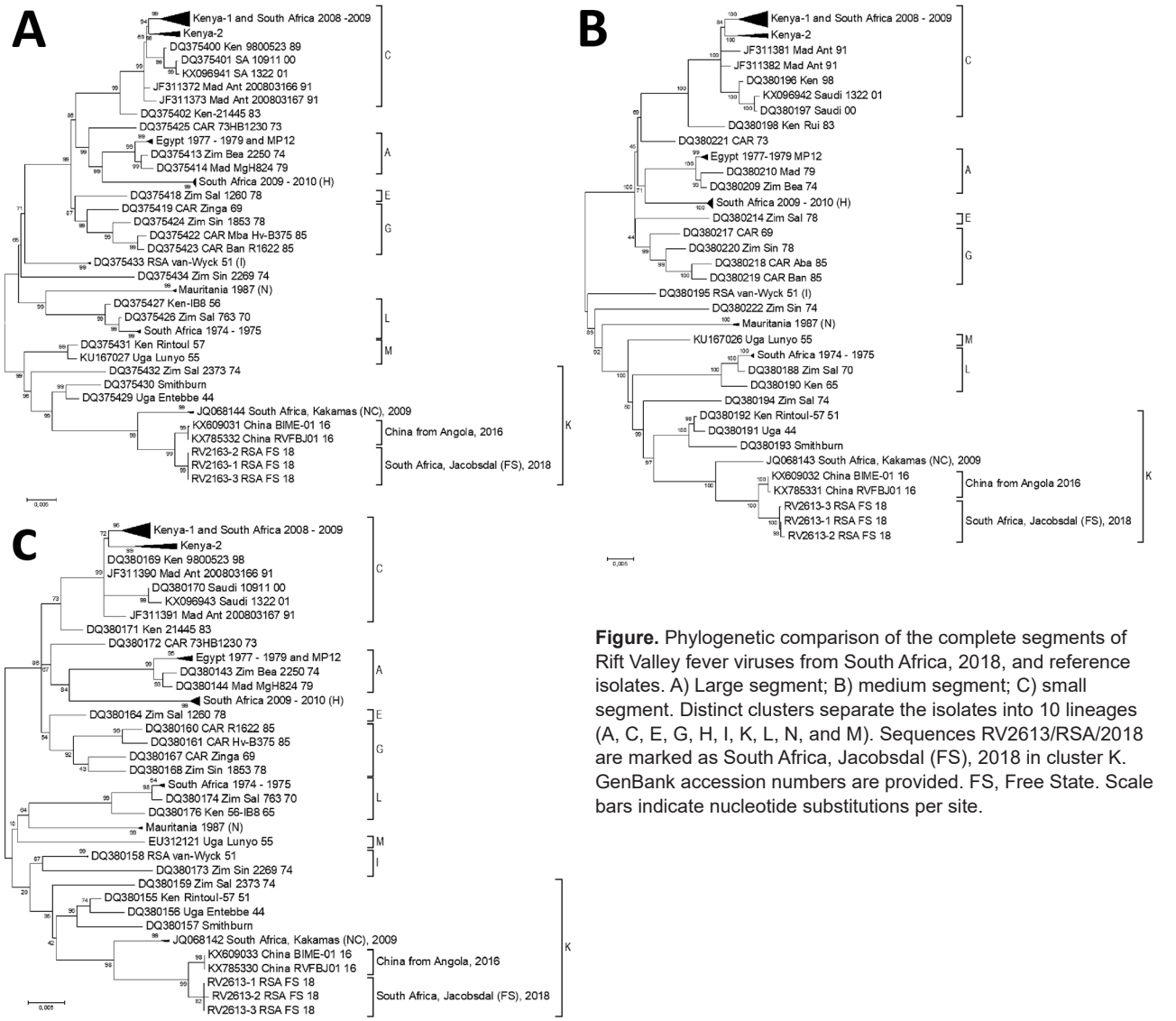

Figure. Phylogenetic comparison of the complete segments of Rift Valley fever viruses from South Africa, 2018, and reference isolates. A) Large segment; B) medium segment; C) small segment. Distinct clusters separate the isolates into 10 lineages (A, C, E, G, H, I, K, L, N, and M). Sequences RV2613/RSA/2018 are marked as South Africa, Jacobsdal (FS), 2018 in cluster K. GenBank accession numbers are provided. FS, Free State. Scale bars indicate nucleotide substitutions per site.

sequencing of the virus genome (isolate BIME-01) from a man returning to China in 2016 with fever and jaundice after a 22-month stay in Angola (*3*). Phylogenetic analysis of the complete RVFV genome of sample BIME-01 and the Vero cell culture isolate of the virus (RVFBJ01) showed that it clustered together with the Kakamas/2009 virus in lineage K (*3*).

On April 28, 2018, an outbreak of suspected RVF was reported on a sheep farm in the Jacobsdal area of the Free State Province of South Africa. The illness rate was ≈55.8% and the case-fatality-rate 100% (335 sheep died) (*4*). Six persons either working or residing on the farm reported symptoms compatible with RVFV infection, but no human fatalities occurred (*5*). Clinical specimens from affected sheep were submitted to the Onderstepoort Veterinary Institute Agricultural Research Council (Onderstepoort, South Africa) for laboratory confirmation of the outbreak.

We used flocculated nylon swabs (FLOQswabs, COPAN, http://www.copanusa.com) to pierce and swab the tissue pools and then placed the swabs into Eppendorf tubes containing 700 µL phosphate-buffered saline (pH 7.0). After agitation, we removed 200 µL buffer for total nucleic acid extraction. We used either whole blood in EDTA (RV2613-1/RSA/2018) or a combination of tissue swab specimens from liver, spleen, and kidney for extractions (RV2613-2/RSA/2018 and RV2613-3/RSA/2018) using the MagNA Pure 96 (Roche Molecular Systems, https://www.roche.com). We detected the presence of RVFV RNA using real-time reverse transcription PCR (RT-PCR) (*6*). We used the same 3 nucleic acid extracts as templates in 8 individual RT-PCRs (A–H), designed to

overlap the entire genome (Appendix Table 1, http://wwwnc.cdc.gov/EID/article/25/10/18-1748-App1.pdf). We used the SuperScript III One-Step RT-PCR System with Platinum Taq DNA polymerase (Invitrogen, https://www.thermofisher.com) in a 20-µL reaction with 0.25 µmol/L of each primer (Appendix Table 1) at an annealing temperature of 53°C for 45 cycles. The resulting amplicons overlapped regions of all 3 genome segments: large (A, B, C, and H), medium (D, E, and F), and small (H). We submitted the 8 amplicons to Inqaba Biotechnical Industries, Pretoria, South Africa (https://www.inqababiotec.co.za), for Sanger sequencing, using the primers incorporated during the generation of the amplicons and 9 additional primers (Appendix Table 1). We constructed the complete viral genome sequences from the 3 field specimens and submitted them to GenBank (accession nos. MK134834–42).

The 3 sequences (RV2613-1/RSA/2018, RV2613-2/RSA/2018, and RV2613-3/RSA/2018) were similar to one another: no nucleotide differences in the large segment, 2 in the medium segment, and 1 in the small segment. The high sequence identity among these 3 viruses and the lack of segment reassortment, together with the isolated geographic distribution of the outbreak, indicate a single introduction. After phylogenetic analysis, we clustered the 3 viruses into lineage K, with their closest known relatives BIM-01/2016, isolated from a worker from China in Angola, and the virus RVFBJ01/2016 derived from cell culture (Figure). We assessed each genome segment and found <1% sequence difference between any of the 3 South Africa viruses and the virus from Angola and <2.11% sequence difference for Kakamas/2009 (Appendix Table 2). Evolutionary analysis of segment M using Bayesian inference with BEAST version 1.8.1 (https://beast.community) under the Hasegawa-Kishino-Yano substitution model, a strict molecular clock, and a constant population size estimated that RV2613/RSA/2018 and BIM-01/2016 had a common ancestor ≈7 years ago that shared a common ancestor with Kakamas/2009 ≈28 years ago. Virus RV2613/RSA/2018 had a higher sequence identity with the original Entebbe-44 isolate than the SNS vaccine or vaccine-derived Ken Rintoul-57 (Appendix Table 2). This result indicates that Kakamas/2009, BIM-01/2016, and RV2613/RSA/2018 probably evolved from a common ancestor of Entebbe-44 and not from its derivative SNS vaccine.

The sequence data imply that this outbreak was likely the result of a single introduction of virus that probably remained localized to 1 farm because of the onset of colder winter temperatures and a decline in rainfall. The phylogenetic relationship of this virus to known others suggests a persistent, yet largely unnoticed, low-level spread of RVFVs in southern Africa. This finding reemphasizes the importance of active disease surveillance programs with diligent reporting of suspected cases, as well as suitable vaccination regimens.

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About the Author

Dr. van Schalkwyk is a researcher at the Agricultural Research Council, Onderstepoort Veterinary Institute, in South Africa. Her primary research interest is molecular epidemiology and virus evolution.

References

1. Pienaar NJ, Thompson PN. Temporal and spatial history of Rift Valley fever in South Africa: 1950 to 2011. Onderstepoort J Vet Res. 2013;80:384. http://doi.org/10.4102/ojvr.v80i1.384
2. Grobbelaar AA, Weyer J, Leman PA, Kemp A, Paweska JT, Swanepoel R. Molecular epidemiology of Rift Valley fever virus. Emerg Infect Dis. 2011;17:2270–6. https://doi.org/10.3201/eid1712.111035
3. Liu W, Sun F-J, Tong Y-G, Zhang S-Q, Cao W-C. Rift Valley fever virus imported into China from Angola. Lancet Infect Dis. 2016;16:1226. https://doi.org/10.1016/S1473-3099(16)30401-7
4. World Organization for Animal Health (OIE). Rift Valley fever South Africa. 2018 [cited 2018 May 31]. http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=26639
5. Jansen van Vuren P, Kgaladi J, Patharoo V, Ohaebosim P, Msimang V, Nyokong B, et al. Human cases of Rift Valley fever in South Africa, 2018. Vector Borne Zoonotic Dis. 2018;18:713–5. https://doi.org/10.1089/vbz.2018.2357
6. Drosten C, Göttig S, Schilling S, Asper M, Panning M, Schmitz H, et al. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. J Clin Microbiol. 2002;40:2323–30. https://doi.org/10.1128/JCM.40.7.2323-2330.2002

Address for correspondence: Antoinette van Schalkwyk, ARC Onderstepoort Veterinary Institute, MED, Old Soutpan Road, Onderstepoort, Pretoria, Gauteng 0110, South Africa; email: VanSchalkwykA1@arc.agric.za

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Genomic Characterization of Rift Valley Fever Virus, South Africa, 2018

Appendix

Appendix Table 1. Primers used for amplification and sequencing of the Rift Valley Fever virus genomes, South Africa, 2018.

Name	Segment, amplicon (binding site)	Sequence (5′–3′)
Primers used during amplification and sequencing		
RVF-LA-F	Large, amplicon A (1–17)	acacaaggcgcccaatc
RVF-LA-R	Large, amplicon A (2182–2163)	GGCTCACCTGCTATTCTCTG
RVF-LB-F	Large, amplicon B (2040–2059)	ATCATGGAGGGCTTTGTCTC
RVF-LB-R	Large, amplicon B (3404–3384)	CTACTATCATCTGATCCTTGC
RVF-LC-F*	Large, amplicon C (4216–4233)	ggtgttgtgtcatcattg
RVF-LC-R	Large, amplicon C (6372–6352)	acacaaagaccgcccaatattg
RVF-LH-F	Large, amplicon H (3018–3037)	GCTATGTGAGTTCACCTCTC
RVF-LH-R*	Large, amplicon H (4590–4570)	gtgtgagctagagttgcttc
RVF-MD-F	Medium, amplicon D (1–15)	acacaaagacggtgc
RVF-MD-R*	Medium, amplicon D (1342–1326)	cctgacccattagcatg
RVF-ME-F*	Medium, amplicon E (771–790)	ccaaatgactaccagtcagc
RVF-ME-R	Medium, amplicon E (2284–2264)	GATAGCTCACTTGAGACAGTT
RVF-MF-F	Medium, amplicon F (1714–1732)	ATGGCTGCATAGTGTGTGC
RVF-MF-R	Medium, amplicon F (3885–3870)	cacaaagaccggtgc
RVF-SG-F*	Small, amplicon G (1–22)	acacaaagctccctagagatac
RVF-SG-R*	Small, amplicon G (1693–1677)	acacaaagacccctagtg
Primers used only for sequencing		
RVF-LA-SF	Large, amplicon A (664–682)	AGACTGAGAGAGAGTTGCT
RVF-LA-SR	Large, amplicon A (874–855)	TCATTCCCTTCAGCAAAGAA
RVF-LC-SF	Large, amplicon C (5587–5606)	GCATCAGACAATGATCTCAG
RVF-LC-SR	Large, amplicon C (5744–5725)	tcacatatctcaaggatggc
RVF-MD-S	Medium, amplicon D (690–672)	ctttgcaatgatggtgtc
RVF-MF-SF1	Medium, amplicon F (2412–2431)	GCAGAGTTTTCATTTGTTGG
RVF-MF-SF2	Medium, amplicon F (3021–3040)	GCTTTCTCTAAGGGCTCTGT
RVF-SG-SF	Small, amplicon G (762–781)	CTCCAATCCCAGATGTTGAG
RVF-SG-SR	Small, amplicon G (1025–1007)	agccatgagaagaggagag
*Published primers (*1*).		

Appendix Table 2. Sequence differences between viruses RV2613–1/RSA/2018, RV2613–2/RSA/2018 and RV2613–3/RSA/2018, and other Rift Valley fever viruses with complete genome sequences available in GenBank.*

Closest related isolates					
Isolate	Year isolated	Species; location	No. differences, segment L (%)	No. differences, segment M (%)	No. differences, segment S (%)
RV2613/RSA/2018	2018	Ovine; Jacobsdal, FS, RSA	0	2–5 nt (0.05–0.13)	1 nt(0.06)
BIM-01/2016	2016	Human; Beijing, China	30 nt (0.47)	22–26 nt (0.57–0.67)	7–8 nt(0.41–0.47)
RVFBJ01/2016	2016	Cell culture isolate of BIM-01_2016	29 nt (0.45)	24–28 nt (0.62–0.73)	7–8 nt(0.41–0.47)
Kakamas-09	2009	Ovine; Kakamas, NC, RSA	103 nt (1.61)	78–82 nt (2.01–2.11)	29–30 nt (1.72–1.78)
Entebbe-44	1944	Mosquito; Entebbe, Uganda	167 nt (2.62)	116–120 nt (2.05–2.12)	44–45 nt (2.60– 2.66)
Ken Rintoul-57	1951	Ovine; Kenya	210 nt (3.29)	129–130 nt (3.33–3.43)	40–41 nt (2.36–2.42)
*FS, Free State Province; NC, Northern Cape Province; nt: nucleotide; RSA, Republic of South Africa.					

Reference

1. Grobbelaar AA, Weyer J, Leman PA, Kemp A, Paweska JT, Swanepoel R. Molecular epidemiology of Rift Valley fever virus. Emerg Infect Dis. 2011;17:2270–6. https://doi.org/10.3201/eid1712.111035