

Rift Valley Fever in Namibia, 2010

Federica Monaco, Chiara Pinoni,
Gian Mario Cosseddu, Siegfried Khaiseb,
Paolo Calistri, Umberto Molini, Alec Bishi,
Annamaria Conte, Massimo Scacchia,
and Rossella Lelli

During May–July 2010 in Namibia, outbreaks of Rift Valley fever were reported to the National Veterinary Service. Analysis of animal specimens confirmed virus circulation on 7 farms. Molecular characterization showed that all outbreaks were caused by a strain of Rift Valley fever virus closely related to virus strains responsible for outbreaks in South Africa during 2009–2010.

Rift Valley fever virus (RVFV; family *Bunyaviridae*, genus *Phlebovirus*) is an enveloped RNA virus transmitted mainly by mosquitoes. This virus causes severe disease in humans and animals. The virus was identified in 1930 along the shores of Lake Naivasha in the Great Rift Valley in Kenya (1,2). Although direct transmission through contact with infected tissue might occur and could play a major role in human infection (3), mosquitoes still represent the most common way the virus is spread. Mosquito of several species (mainly *Culex* and *Aedes* spp.) have been considered vectors and reservoirs of the virus (4–6).

In 2010, South African veterinary authorities reported to the World Organisation for Animal Health 489 Rift Valley fever (RVF) outbreaks during the epidemic season; >14,000 cases and 8,000 deaths of animals occurred (7,8). The epidemic started on January 2010 in the eastern Free State Province and progressively spread west to Western Cape and Northern Cape Provinces and reached the border with Namibia. In Namibia, although virus circulation has been demonstrated in humans (9–11), little information is available on the distribution and the molecular characterization of RVFV circulating there. We conducted a study to identify and characterize RVFV strains that caused disease outbreaks in Namibia in 2010.

The Study

During May 9–July 30, 2010, ovine and caprine flocks showing clinical signs compatible with RVFV infection

Author affiliations: Istituto Zooprofilattico dell’Abruzzo e del Molise G. Caporale, Teramo, Italy (F. Monaco, C. Pinoni, G.M. Cosseddu, P. Calistri, U. Molini, A. Conte, M. Scacchia, R. Lelli); Central Veterinary Laboratory, Windhoek, Namibia (S. Khaiseb); and Ministry of Agriculture Water and Forestry, Windhoek (A. Bishi)

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were reported to Namibian Veterinary Service. Blood samples were collected from live animals, and liver, spleen, heart, uterus, kidney, and brain samples were obtained from dead animals. Samples were sent to the Central Veterinary Laboratory in Windhoek, Namibia, for laboratory analysis. Tissue samples (100 mg) were homogenized by using a mortar and sterile quartz pestle and diluted 1:10 in phosphate-buffered saline containing antimicrobial drugs (100 U/mL penicillin, 100 µg/mL streptomycin, 5 µg/mL gentamicin, 50 U/mL nystatin). Tissue debris was removed by low-speed centrifugation.

RNA was purified from blood samples and supernatants of homogenized tissues by using the High Pure Viral Nucleic Acid Extraction Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions. RVFV RNA was identified in samples by using the specific one-step reverse transcription PCR (RT-PCR) described by Battles and Dalrymple (12), which is specific for 369-nt region of the medium (M) segment of RVFV RNA. Laboratory tests confirmed circulation of RVFV on 7 farms in the Hardap and Karas regions (Figure).

Aliquots of samples were shipped to the Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise in Teramo, Italy, where virus isolation was conducted on samples positive for virus by RT-PCR by infecting Vero E6 cell (ATCC CRL-1586 VERO C1008) monolayers (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/19/12/13-0593-Techapp1.pdf). RT-PCR amplicons from virus-positive samples were purified by using the QIAquick PCR Purification Kit (QIAGEN, Valencia, CA, USA) and used for direct sequencing. Sequencing was performed by using the Big Dye Terminator Kit (Applied Biosystems, Foster City, CA, USA). Excess dye was removed by using Cleanseq (Beckman Coulter, Inc., Brea, CA, USA). Nucleotide sequences were determined by using DNA sequencer ABI PRISM 3100 (Applied Biosystems). Amplification and sequencing were repeated twice to avoid introduction of artificial substitutions. Raw sequence data were assembled by using Contig Express (Vector NTI suite 9.1; Invitrogen, Carlsbad, CA, USA), and a 328-nt fragment of the Gn glycoprotein coding sequence were obtained after deletion of primer sequences.

Seven sequences were obtained, 1 from each of the 7 outbreaks. Sequences showed 100% similarity at nucleotide and amino acid levels. The entire sequence of the M segment of 2 isolates collected (1 in Hardap and 1 in Karas) (online Technical Appendix Table 1) was generated after amplification of 9 overlapping sections. RT-PCR primers used are shown in online Technical Appendix Table 2. Because sequences were 100% identical, the RVFV isolate (Namibia 2010), was considered representative of all isolates. The Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov) was used to identify

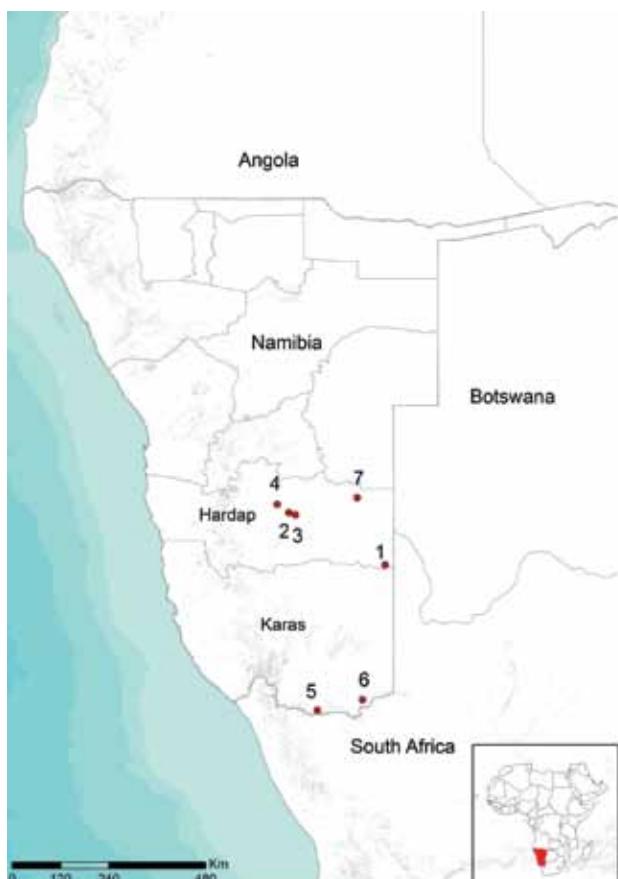


Figure. Location of farms in Namibia with Rift Valley fever virus infection, 2010. Circles and numbers indicate outbreaks from which virus circulation was determined.

homologous regions in sequence databases. Sequences were aligned by using ClustalW (www.clustal.org/) and BioEdit Sequence Analysis Editor version 7.0.5.3 (13). Phylogenetic analysis was conducted by using the entire sequence of the M segment from Namibia 2010 and all homologous sequences available in GenBank (online Technical Appendix, Figure 1). Because of absence of entire sequences from strains that co-circulated in South Africa and Namibia in 2009–2010, we performed phylogenetic analysis of a 490-nt fragment by using a selection of reference strains that had been isolated in different years or countries (online Technical Appendix Table 3) using the maximum-likelihood method in MEGA version 5 (14) with bootstrap support (1,000 replicates) (online Technical Appendix Figure 2). The unique sequence generated was submitted to GenBank under accession no. KC935380.

Overall diversity of partial M segment sequences was low, and bootstrap values for tree nodes were weak in some instances. Phylogenetic analysis showed that isolate Namibia 2010 belongs to the same group of RVFV strains

isolated in South Africa in 2009 (SA404/09) and 2010 (SA85/10, SA1224/10, SA373/10, SA1221/10, SA276/10, SA276/10, SA106/10, SA404/09, SA423/10, SA482/10, SA71/10, and SA54/10). The cluster corresponds to lineage H of RVFV identified by Grobbelaar et al. (11). SPU77/04, which was isolated from a human in Namibia in 2004, is closely related. The number of nucleotide differences between sequences of this group was low (0–3 nt). Isolate Namibia 2010 showed 100% nt identity with SA54/10 and a 1-nt difference with SA85/10, SA482/10, SA71/10, SA106/10, SA404/09, and SA423/10.

Conclusions

The high degree of sequence identity of related RVFV strains that co-circulated in South Africa and Namibia in 2004–2010 suggests that these strains probably originated from a virus population that circulated between these 2 countries. Molecular data suggest that RVF outbreaks in Namibia in 2010 were caused by possible disseminated infections from South Africa. This hypothesis is further supported by the temporal and geographic location of the outbreaks. Clinical signs were first observed at the beginning of May in southeastern Hardap near the border with South Africa (Figure). The Auob River runs through this area, crosses the border with South Africa, and enters Kalahari National Park. Four outbreaks occurred in central Hardap (Figure) during the second half of May and the beginning of June in an area near the Auroos River and an artificial lake in Hardap that supplies a broad system of water (irrigation) channels. During June 3–14, additional spread of virus was observed in the southern part of Karas near the border with South Africa where 2 outbreaks were confirmed (Figure), again near a water source, the Oranje River, which is the border between Namibia and South Africa.

The large RVF epidemic in South Africa in 2010 was attributed to heavy rainfall during January–February 2010 (15). In Namibia, evidence of intense rainfall was not recorded in the regions where disease outbreaks occurred in 2010 (online Technical Appendix Figure 3). This finding indicates that reactivation of local virus circulation is unlikely. Our findings suggest that control measures along borders of Namibia and other countries should be reinforced and that collaborations between veterinary and public health authorities should be strengthened to reduce the effects of future outbreaks.

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Dr Monaco is a research scientist at Istituto Zooprofilattico dell’Abruzzo e del Molise G. Caporale in Teramo, Italy. Her research interests focus on the molecular epidemiology of arboviruses.

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Address for correspondence: Gian Mario Cosseddu, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale, Campo Boario, 64100 Teramo, Italy; email: g.cosseddu@izs.it

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Technical Appendix

Supplementary Data for Analysis of Rift Valley Fever in Namibia, 2010

Technical Appendix Table 1. Rift Valley fever outbreaks, Namibia, 2010*

Outbreak	Region	Date of report	Animal	Diagnostic results		Medium segment sequence	
				RT-PCR†	Virus isolation	Partial 328 nt	Full-length 3,885 nt
1	Hardap	2010 May 5	Sheep	+	+	+	–
2	Hardap	2010 May 15	Sheep	+	+	+	+
3	Hardap	2010 May 15	Sheep	+	+	+	–
4	Hardap	2010 May 22	Goats	+	–	+	–
5	Karas	2010 Jun 3	Sheep	+	+	+	+
6	Karas	2010 Jun 9	Sheep	+	+	+	–
7	Hardap	2010 Jun 14	Sheep	+	+	+	–

*RT-PCR, reverse transcription PCR.

†Methods for blood and tissues reported by Battles and Dalrymple (1).

Technical Appendix Table 2. Primers used for amplification and sequencing of the entire medium RNA segment of Namibia_10 RVF isolate from Namibia, 2010

RVFV section	Primer	Sequence, 5'→3'
1	RVFM-AFwd	ACACAAAGACGGTGC
	RVF_M_518R	TGCCCTTCCCTGGTCTGT
2	RVF_M_427F	TGACAGTCCTCCAGCCTTAGCAG
	RVF_M_990R	CTTCGCAGACCCCTTTCATTTTTG
3	RVF_M_821F	TTCAGTCAAGTGCCCTCCTAAG
	RVF_M_1356R	GTATCTGCACAATCCCTGACC
4	RVF_M_1262F	TGGGGACGCAGCATTTTTG
	RVF_M_1713R	GCACTAAGCACGGGTCCTG
5	RVF_M_1629F	ATAGGGGTTTCACATGGCACACGA
	RVF_M_2231R	GACCCCTTCAACATCAAACAA
6	RVF_M_2105F	TCAGGCAAGCTCCAGAATC
	RVF_M_2702R	TGCGTCCAGTGAGAGGCTAAC
7	RVF_M_2577F	ATCGACTGGGTGCATAAACTCA
	RVF_M_3107R	ACAAGATACGGCTGCTCCACAAA
8	RVF_M_2866F	GGGCACCAAACCTTATCTCAT
	RVF_M_3601R	TTAGTAGCAGCAAGCCACATTTT
9	RVF_M_2866F	GGGCACCAAACCTTATCTCAT
	RVFM-ARev	ACACAAAGACGGTGC

*RVFV, Rift Valley fever virus.

Technical Appendix Table 3. Reference strains used in phylogenetic analysis of Rift Valley fever virus, Namibia, 2010*

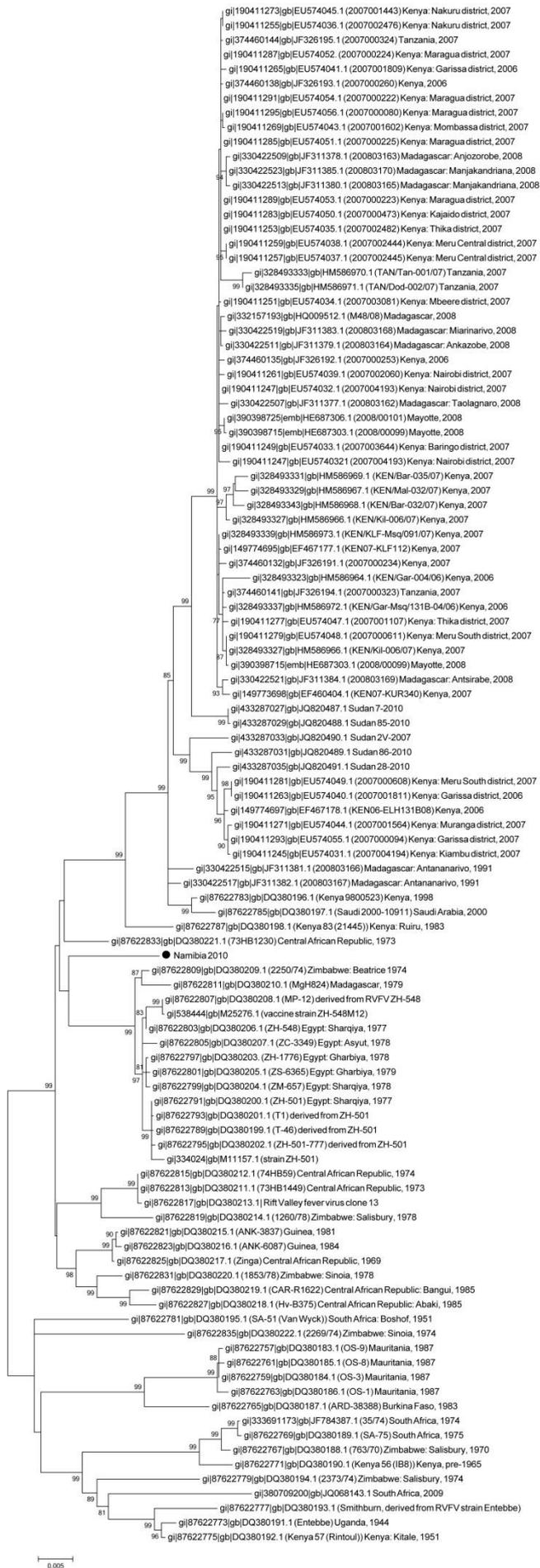
Isolate	Country and year of collection	GenBank accession no.
Namibia 2010	Namibia, 2010	KC935380
SPU204/85	Angola, 1985	HM587076
ARD-38388	Burkina Faso, 1983	DQ380187
ArB1976	CAR, 1969	HM587083
73HB1230	CAR, 1973	DQ380221
73HB1449	CAR, 1973	DQ380211
74HB59	CAR, 1974	HM587082
Hv-B375	CAR, 1985	DQ380218
CAR R1662	CAR, 1985	HM587086
CAR R1752	CAR, 1986	HM587087
AnK3837	Guinea, 1981	HM587084
ZH-548_	Egypt, 1977	AF134508
ZH-1776	Egypt, 1978	DQ380203
ZM-657	Egypt, 1978	DQ380204
ZS-6365	Egypt, 1979	DQ380205
93-Abeer	Egypt, 1993	HM587043

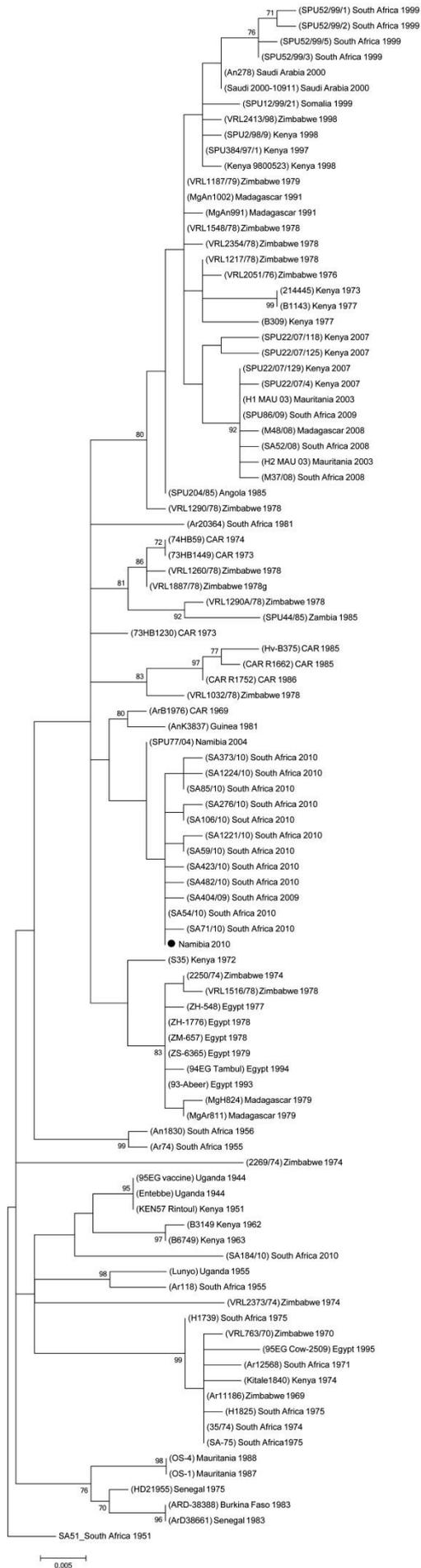
Isolate	Country and year of collection	GenBank accession no.
94EG Tambul	Egypt, 1994	HM587042
95EG Cow-2509	Egypt, 1995	HM587115
KEN57 Rintoul	Kenya, 1951	HM587104
B314	Kenya, 1962	HM587105
B674	Kenya, 1963	HM587106
S35	Kenya, 1972	HM587045
214445	Kenya, 1973	HM587074
Kitale1840	Kenya, 1974	HM587116
B309	Kenya, 1977	HM587070
B1143	Kenya, 1977	HM587075
SPU384/97/1	Kenya, 1997	HM587052
SPU2/98/9	Kenya, 1998	HM587055
Kenya 9800523	Kenya, 1998	DQ380196
SPU22/07/118	Kenya, 2007	HM587062
SPU22/07/125	Kenya, 2007	HM587063
SPU22/07/4	Kenya, 2007	HM587068
SPU22/07/129	Kenya, 2007	HM587064
MgH824	Madagascar, 1979	HM587040
MgAr811	Madagascar, 1979	HM587041
MgAn1002	Madagascar, 1991	HM587057
MgAn991	Madagascar, 1991	HM587060
M48/08	Madagascar, 2008	HQ009512
OS-1	Mauritania, 1987	DQ380186
OS-4	Mauritania, 1988	HM587122
H1 MAU 03	Mauritania, 2003	EF160116
H2 MAU 03	Mauritania, 2003	EF160115
SPU77/04	Namibia, 2004	HM587100
An278	Saudi Arabia, 2000	HM587050
Saudi 2000-10911	Saudi Arabia, 2000	DQ380197
HD21955	Senegal, 1975	HM587123
ArD38661	Senegal, 1983	HM587124
SPU12/99/21	Somalia, 1999	HM587051
SA51	South Africa, 1951	HM587125
Ar74	South Africa, 1955	HM587109
Ar118	South Africa, 1955	HM587120
An1830	South Africa, 1956	HM587108
Ar12568	South Africa, 1971	HM587112
35/74	South Africa, 1974	JF784387
H1739	South Africa, 1975	HM587110
H1825	South Africa, 1975	HM587114
SA-75	South Africa, 1975	DQ380189
Ar20364	South Africa, 1981	HM587101
SPU52/99/1	South Africa, 1999	HM587046
SPU52/99/2	South Africa, 1999	HM587048
SPU52/99/5	South Africa, 1999	HM587047
SPU52/99/3	South Africa, 1999	HM587049
SA52/08	South Africa, 2008	HM587069
M37/08	South Africa, 2008	HM587067
SPU86/09	South Africa, 2009	HM587065
SA404/09	South Africa, 2009	HM587096
SA85/10	South Africa, 2010	HM587098
SA1224/10	South Africa, 2010	HM587099
SA373/10	South Africa, 2010	HM587097
SA1221/10	South Africa, 2010	HM587090
SA276/10	South Africa, 2010	HM587093
SA276/10	South Africa, 2010	HM587093
SA106/10	South Africa, 2010	HM587094
SA482/10	South Africa, 2010	HM587089
SA54/10	South Africa, 2010	HM587092
SA71/10	South Africa, 2010	HM587088
SA184/10	South Africa, 2010	HM587107
SA423/10	South Africa, 2010	HM587095
SA59/10	South Africa, 2010	HM587091
95EG vaccine	Uganda, 1944	HM587103
Entebbe	Uganda, 1944	DQ380191
Lunyo	Uganda, 1955	HM587119
SPU44/85	Zambia, 1985	HM587079
Ar11186	Zimbabwe, 1969	HM587113
VRL763/70	Zimbabwe, 1970	HM587111
2250/74	Zimbabwe, 1974	DQ380209
2269/74	Zimbabwe, 1974	DQ380222
VRL2373/74	Zimbabwe, 1974	HM587121
VRL2051/76	Zimbabwe, 1976	HM587072
VRL1290/78	Zimbabwe, 1978	HM587077
VRL1548/78	Zimbabwe, 1978	HM587059

Isolate	Country and year of collection	GenBank accession no.
VRL2354/78	Zimbabwe, 1978	HM587058
VRL1217/78	Zimbabwe, 1978	HM587073
VRL1032/78	Zimbabwe, 1978	HM587085
VRL1290A/78	Zimbabwe, 1978	HM587078
VRL1887/78	Zimbabwe, 1978	HM587080
VRL1260/78	Zimbabwe, 1978	HM587081
VRL1516/78	Zimbabwe, 1978	HM587044
VRL1187/79	Zimbabwe, 1979	HM587056
VRL2413/98	Zimbabwe, 1998	HM587054

*CAR, Central African Republic.

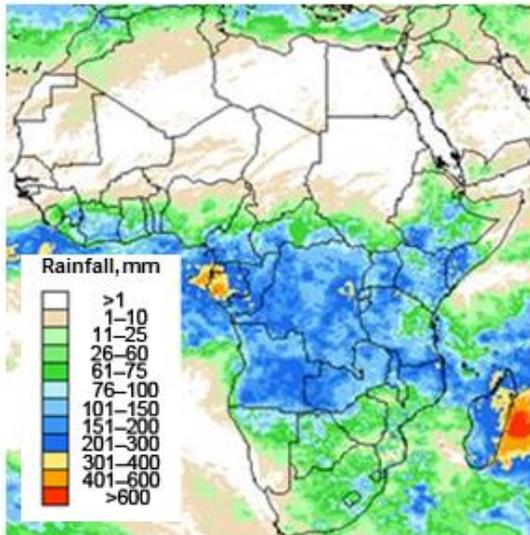
Technical Appendix Figure 1. Phylogenetic tree of complete sequences of Rift Valley fever virus medium RNA segment. Black circle indicates strain isolated in this study. Analysis was performed by using MEGA 5 software (2) and the maximum-likelihood method. Bootstrap support values >70 are shown along the branches (1,000 replicates). Scale bar indicates nucleotide substitutions per site.



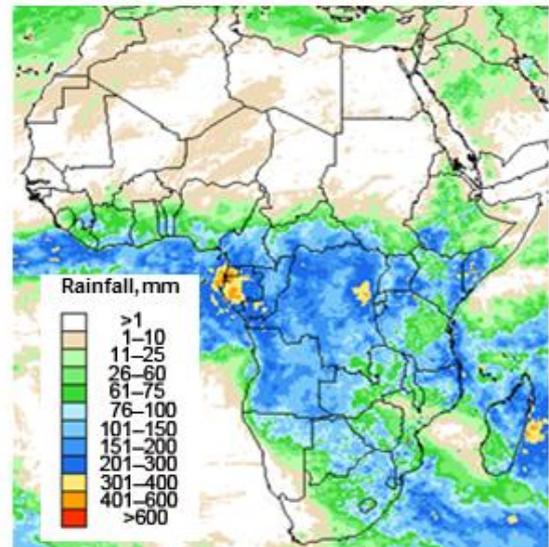


Technical Appendix Figure 2. Phylogenetic tree showing genetic relationships among Rift Valley fever virus (RVFV) isolates. The tree was constructed on the basis of 490-nt sequences of RVFV medium segment. The unique sequence obtained in this study is indicated with a black spot. Viruses are identified by country, year of collection, and nomenclature of RVFV isolates. Black circle indicates strain isolated in this study. GenBank accession numbers are shown in the Technical Appendix. Analysis was performed by using MEGA 5 software (2) and the maximum-likelihood method. Bootstrap support values >70 are shown (1,000 replicates) along the branches. Scale bar indicates nucleotide substitutions per site.

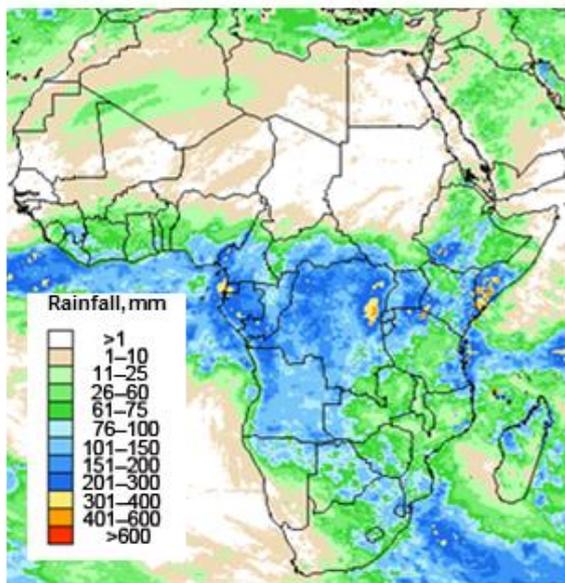
Rainfall in past 30 days as of March 31



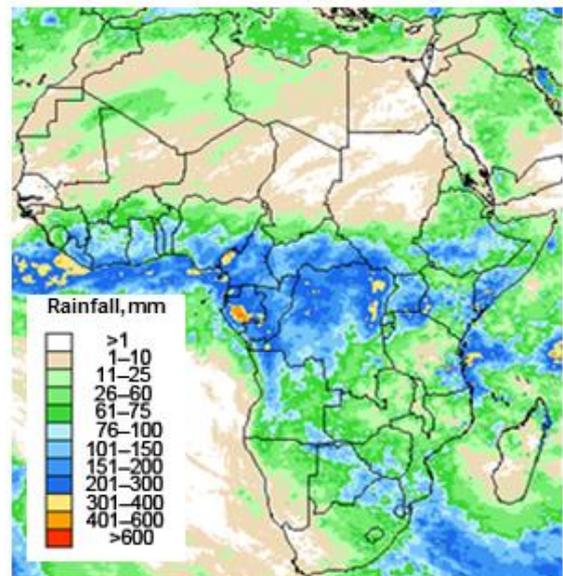
Rainfall in past 30 days as of April 10



Rainfall in past 30 days as of April 20



Rainfall in past 30 days as of April 30



Technical Appendix Figure 3. Rainfall in Africa during the period before outbreaks of Rift Valley fever in Namibia started in May 2010.

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