Rift Valley Fever Outbreak in Livestock, Mozambique, 2014

José M. Fafetine, Peter Coetzee, Benjamin Mubemba, Ofélia Nhambirre, Luis Neves, J.A.W. Coetzer, Estelle H. Venter

In early 2014, abortions and death of ruminants were reported on farms in Maputo and Gaza Provinces, Mozambique. Serologic analysis and quantitative and conventional reverse transcription PCR confirmed the presence of Rift Valley fever virus. The viruses belonged to lineage C, which is prevalent among Rift Valley fever viruses in southern Africa.

Rift Valley fever (RVF) virus (family Bunyaviridae, genus Phlebovirus) is a mosquito-borne virus that affects ruminants and humans. The virion contains 3 single-stranded RNA genome segments, large, medium, and small. In ruminants, RVF virus infection is characterized by high rates of abortion and of death, particularly in newborn animals. In humans, the infection is usually asymptomatic, but in severe cases, hemorrhage, meningoencephalitis, retinopathy, and death can occur (1).

Some of the most notable RVF epidemics reported in the past 2 decades occurred in eastern Africa and in southern Africa, where Mozambique is located. In 2006 and 2007, outbreaks of the disease occurred in eastern Africa, including Tanzania (2). In 2008 (3) and 2010 (4), epidemics of the disease were reported in South Africa. However, during the same period, no RVF outbreaks were reported in Mozambique. The few confirmed RVF outbreaks in the country occurred in 1969 in Gaza and Maputo Provinces, resulting in the deaths of 220 and 25 cattle in each province, respectively (5). In 1999, cases of abortion in a herd of water buffaloes (Bubalus bubalis) in Zambézia Province were attributed to RVF virus, but no virus was detected or isolated (6).

In 2010, serosurveys were conducted in the Zambézia and Maputo Provinces of Mozambique. Seroprevalences of 9.2% in sheep and 11.6% in goats was recorded in Zambézia Province (7). In Maputo Province, an overall seroprevalence of 36.9% was documented in cattle (8). The results indicated the possible circulation of RVF virus during interepidemic periods without the manifestation of typical clinical signs, as has been described elsewhere (9).

In this article, we report the detection of specific antibodies against RVF virus and the genetic analysis of RVF virus isolates from outbreaks in Mozambique. We also discuss the possible factors associated with the occurrence of this outbreak.

The Study
In late March 2014, after a period of heavy and persistent rainfall in southern Mozambique, particularly in Maputo and Gaza Provinces, abortions and deaths in ruminant offspring were reported on some farms. The owner of a farm located in the Goba District, Maputo Province (26°3′59.73”S, 32°0′23.36”E), informed the veterinary authorities that 16 of 88 goats aborted their fetuses and 5 newborn kids died. The veterinary authorities also received reports from 2 farms in Xai-Xai (25°03′24.1S, 33°41′24.7E) and Chibuto (24°42′01.222S, 33°32′24.822E) in Gaza Province, where 26 goats and 8 sheep aborted, respectively, and a total of 7 newborn animals died on both farms. According to the farmers, no animals had been purchased or brought into the herds for >3 years.

Serum samples were collected from farms in Goba (n = 88), Xai-Xai (n = 26), and Chibuto (n = 13). On the Goba farm, liver and spleen tissue samples were also collected from 1 aborted fetus and from 1 dead newborn goat. All the serum samples were tested for the presence of RVF virus IgM by using the IDvet Screen RVF IgM ELISA (IDvet innovative diagnostics kit; IDVet, Montpellier, France). In addition, the serum samples collected in Goba were further tested for RVF virus IgG by using the RVF recN IgG ELISA kit (Biological Diagnostic Supplies Limited, Edinburgh, Scotland, UK).

Viral genomic RNA was extracted from ELISA-positive serum samples and tissue samples by using Trizol (Invitrogen, Manchester, UK) according to the manufacturer’s instructions. A quantitative real-time reverse transcription PCR was performed as described (10). Positive samples were subsequently subjected to a conventional RT-PCR to amplify a 490-nucleotide region of the medium segment as described (11). The amplicons were then purified by using the QIAquick Gel extraction kit (QIAGEN, Hilden, Germany) and submitted to Inqaba Biotec (Pretoria, South Africa) for sequencing. The obtained sequences were compared with sequences in GenBank using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence data (480 nt) were

Author affiliations: Eduardo Mondlane University, Maputo, Mozambique (J.M. Fafetine, O. Nhambirre); University of Pretoria, Pretoria, South Africa (P. Coetzee, L. Neves, J.A.W. Coetzer, E.H. Venter); Copperbelt University, Kitwe, Zambia (B. Mubemba)

DOI: http://dx.doi.org/10.3201/eid2212.160310
The farms where the outbreaks occurred are noncommercial, small- to middle-scale farming systems with basic management and with no reports of animal importation. Occasionally, animals are bought from other farms, and the animals known to be imported in the southern part of the country come from the neighboring countries (i.e., South Africa and Swaziland). Animal movement is also reported to occur frequently on the border with adjoining countries. Because no new animals were introduced onto the above mentioned farms and no animal importation was indicated either by the farmers or by the veterinary authorities, we hypothesize that the virus might have been introduced in the past. Then, after a 6-fold increase in rainfall in Maputo (31 mm in November 2013 and 208 mm in December 2013; Umbeluzi Weather Station, pers. comm.), the conditions for an increase in the vector population, and therefore in virus circulation, favored the occurrence of the outbreaks. The high level of seroprevalence reported previously in districts close to the study site (8) may suggest a continuous low level of virus transmission in the region that is exacerbated by above-average rainfall, resulting in RVF virus infection in naive animals.

With this confirmed genetic evidence of RVF virus in Mozambique, all countries on the eastern coastline of Africa, the Indian Ocean islands of Madagascar and Mayotte, and Yemen and Saudi Arabia have all reported the presence of viruses belonging to lineage C (11). The broad geographic distribution pattern of lineage C viruses (in southern and northern Africa) and the related life-cycle dynamics require further investigation to identify the main drivers associated with the circulation and spread of this lineage of viruses.

Conclusions

RVF virus IgM and the molecular detection of RVF virus confirmed the cause of abortions and deaths in sheep and goats in Maputo (Goba) and Gaza (Xai-Xai and Chibuto) Provinces of Mozambique in the first quarter of 2014. Outbreaks of RVF in eastern Africa are usually associated with the circulation of a local virus lineages, triggered by abnormal rainfall that favors the multiplication of the mosquito vectors or by the introduction of virus through animal movement. Sequence comparison and phylogenetic analyses indicated that the Maputo RVF viruses belonged to lineage C, which suggested that the outbreaks had close links to the 2007 and 2010 RVF outbreaks in Sudan.

| Total | Goba District | Xai-Xai District | Chibuto District |
|-------|--------------|-----------------|-----------------|
| No. IgM positive | 26           | 2               | 13              |
| No. IgG positive | 49           | NT              | NT              |
| No. IgM positive only | 2            | NA              | NA              |
| No. IgG positive only | 25           | NA              | NA              |
| No. RT-PCR positive | 12           | 2               | 2               |
| No. RT-PCR and IgM positive | 1            | NA              | NA              |
| No. RT-PCR and IgG positive | 0            | NA              | NA              |
| No. RT-PCR, IgM and IgG positive | 11           | NA              | NA              |

*RVF, Rift Valley fever; RT-PCR, real-time quantitative reverse transcription PCR; NA, not available; NT, not tested.

Acknowledgments

We thank Hermogenes Mucache for help with sample collection.

This study was supported by the Southern African Center for Infectious Disease Surveillance–Wellcome Trust (grant no. WT087546MA), Sida Project at the Eduardo Mondlane University, and the National Research Fund (Fundo Nacional de Investigação–Ministry of Science and Technology Mozambique). This study was carried out at the Centro de Biotecnologia/Faculdade de Veterinaria, Eduardo Mondlane.
University, Mozambique, and at the Department of Veterinary Tropical Diseases, Faculty of Veterinary Sciences, University of Pretoria, South Africa.

Dr. Fafetine is assistant professor of microbiology and immunology at the Veterinary Faculty, Eduardo Mondlane University, Maputo, Mozambique. His primary research interest is the diagnosis and epidemiology of zoonotic and emerging diseases.

References

1. Swanepoel R, Coetzer JAW. Rift Valley fever. In: Coetzer JAW, Thomson GR, Tustin RC, editors. Infectious diseases of livestock, 2nd ed. Oxford (UK): Oxford University Press; 2004. p. 1037–70.

2. World Health Organization. Outbreaks of Rift Valley fever in Kenya, Somalia and United Republic of Tanzania, December 2006—April 2007. Wkly Epidemiol Rec. 2007;82:169–78.

3. Archer BN, Weyer J, Paweska J, Nkosi D, Leman P, Tint KS, et al. Outbreak of Rift Valley fever affecting veterinarians and farmers in South Africa, 2008. S Afr Med J. 2011;101:263–6. http://dx.doi.org/10.7196/SAMJ.4544

4. World Health Organization. Rift Valley fever, South Africa—update 1. Wkly Epidemiol Rec. 2010;21:185–6.

5. Valadão FG. Nota prévia sobre a ocorrência de uma nova doença em Moçambique—a doença do vale do Rift. Veterin Moçamb. 1969;2:13–20.

6. Direcção Nacional de Pecuária. Relatório Anual. Ministério da Agricultura e de Desenvolvimento Rural (Maputo, Mozambique); 2002. p. 5–7.

7. Fafetine J, Neves L, Thompson PN, Paweska JT, Rutten VPMG, Coetzer JAW. Serological evidence of Rift Valley fever virus circulation in sheep and goats in Zambézia Province, Mozambique. PLoS Negl Trop Dis. 2013;7:e2065. http://dx.doi.org/10.1371/journal.pntd.0002065

8. Lagerqvist N, Moiane B, Mapaco L, Fafetine J, Vene S, Falk KI. Antibodies against Rift Valley fever virus in cattle, Mozambique. Emerg Infect Dis. 2013;19:1177–9. http://dx.doi.org/10.3201/eid1907.130332

9. Métras R, Cavalerie L, Dommergues L, Mérot P, Edmunds WJ, Keeling MJ, et al. The epidemiology of Rift Valley fever in Mayotte: insights and perspectives from 11 years of data. PLoS Negl Trop Dis. 2016;10:e0004783. http://dx.doi.org/10.1371/journal.pntd.0004783

10. Bird BH, Bawiec DA, Ksiazek TG, Shoemaker TR, Nichol ST. Highly sensitive and broadly reactive quantitative reverse transcription-PCR assay for high-throughput detection of Rift Valley fever virus. J Clin Microbiol. 2007;45:3506–13. http://dx.doi.org/10.1128/JCM.00936-07

11. 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. http://dx.doi.org/10.3201/eid1712.111035

12. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013;30:2725–9. http://dx.doi.org/10.1093/molbev/ms3197

13. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16:111–20. http://dx.doi.org/10.1007/BF01731581

Address for correspondence: José Manuel Fafetine, Eduardo Mondlane University–Universidade Eduardo Mondlane, Faculdade de Veterinária/ Centro de Biotecnologia, Av de Moçambique Km 1.5, C. Postal 257, Maputo, Mozambique; email: jfafetine@yahoo.com
# Rift Valley Fever Outbreak in Livestock, Mozambique, 2014

## Technical Appendix

| No | Isolate     | Country       | Year of isolation | Reference            | GenBank accession |
|----|-------------|---------------|-------------------|----------------------|-------------------|
| 1  | SPU2/99/1   | South Africa  | 1999              | Grobbelaar et al. 2011 | HM587046          |
| 2  | SPU2/99/5   | South Africa  | 1999              | Grobbelaar et al. 2011 | HM587047          |
| 3  | SPU2/99/2   | South Africa  | 1999              | Grobbelaar et al. 2011 | HM587048          |
| 4  | SPU2/99/3   | South Africa  | 1999              | Grobbelaar et al. 2011 | HM587049          |
| 5  | SPU86/09    | South Africa  | 2009              | Grobbelaar et al. 2011 | HM587065          |
| 6  | SA152/08    | South Africa  | 2008              | Grobbelaar et al. 2011 | HM587066          |
| 7  | M37/08      | South Africa  | 2008              | Grobbelaar et al. 2011 | HM587067          |
| 8  | SA52/08     | South Africa  | 2008              | Grobbelaar et al. 2011 | HM587069          |
| 9  | An278       | Saudi Arabia  | 2000              | Grobbelaar et al. 2011 | HM587050          |
| 10 | SA01–1322   | Saudi Arabia  | 2001              | Miller et al. 2001   | AF393745          |
| 11 | H2MAU03     | Mauritania    | 2003              | Faye et al. 2003     | EF160115          |
| 12 | H1MAU03     | Mauritania    | 2003              | Faye et al. 2003     | EF160116          |
| 13 | SPU12/99/21 | Somalia       | 1998              | Grobbelaar et al. 2011 | HM587051          |
| 14 | SPU38/97/1  | Kenya         | 1997              | Grobbelaar et al. 2011 | HM587052          |
| 15 | SPU2/98/1   | Kenya         | 1998              | Grobbelaar et al. 2011 | HM587053          |
| 16 | SPU2/98/9   | Kenya         | 1998              | Grobbelaar et al. 2011 | HM587055          |
| 17 | SPU2/2/7/118| Kenya         | 2007              | Grobbelaar et al. 2011 | HM587062          |
| 18 | SPU2/2/7/125| Kenya         | 2007              | Grobbelaar et al. 2011 | HM587063          |
| 19 | SPU2/2/7/129| Kenya         | 2007              | Grobbelaar et al. 2011 | HM587064          |
| 20 | SPU2/2/7/4  | Kenya         | 2007              | Grobbelaar et al. 2011 | HM587068          |
| 21 | B309        | Kenya         | 1977              | Grobbelaar et al. 2011 | HM587070          |
| 22 | 214445      | Kenya         | 1983              | Grobbelaar et al. 2011 | HM587074          |
| 23 | B1143       | Kenya         | 1977              | Grobbelaar et al. 2011 | HM587076          |
| 24 | VRL2413/98  | Zimbabwe      | 1998              | Grobbelaar et al. 2011 | HM587054          |
| 25 | VRL1187/79  | Zimbabwe      | 1979              | Grobbelaar et al. 2011 | HM587056          |
| 26 | VRL2354/78  | Zimbabwe      | 1978              | Grobbelaar et al. 2011 | HM587058          |
| 27 | VRL1548/78  | Zimbabwe      | 1978              | Grobbelaar et al. 2011 | HM587059          |
| 28 | VRL825/79   | Zimbabwe      | 1979              | Grobbelaar et al. 2011 | HM587071          |
| 29 | VRL2051/76  | Zimbabwe      | 1976              | Grobbelaar et al. 2011 | HM587072          |
| 30 | VRL1217/78  | Zimbabwe      | 1978              | Grobbelaar et al. 2011 | HM587073          |
| 31 | VRL1290/78  | Zimbabwe      | 1978              | Grobbelaar et al. 2011 | HM587077          |
| 32 | MgAn1002    | Madagascar    | 1991              | Grobbelaar et al. 2011 | HM587057          |
| 33 | MgAn991     | Madagascar    | 1991              | Grobbelaar et al. 2011 | HM587060          |
| 34 | MgAn990     | Madagascar    | 1991              | Grobbelaar et al. 2011 | HM587061          |
| 35 | M48         | Madagascar    | 2008              |                       |                   |
| 36 | SPU204/85   | Angola        | 1985              | Grobbelaar et al. 2011 | HM587076          |
| 37 | FI-2        | Madagascar    | 2008              | Ratovonjato et al. 2011  | GU135862          |
| 38 | AL-53       | Madagascar    | 2008              | Ratovonjato et al. 2011  | GU135862          |
| 39 | MgH824      | Madagascar    | 1979              | Grobbelaar et al. 2011 | HM587040          |
| 40 | 93-Abeer    | Egypt         | 1993              | Grobbelaar et al. 2011 | HM587043          |
| 41 | SPU44/85    | Zambia        | 1985              | Grobbelaar et al. 2011 | HM587079          |
| 42 | 74H859      | CAR           | 1974              | Grobbelaar et al. 2011 | HM587082          |
| 43 | SA373       | South Africa  | 2010              | Grobbelaar et al. 2011 | HM587097          |
| 44 | SPU77/04    | Namibia       | 2004              | Grobbelaar et al. 2011 | HM587100          |
| 45 | Ar20364     | South Africa  | 1981              | Grobbelaar et al. 2011 | HM587101          |
| 46 | SA55        | South Africa  | 1955              | Grobbelaar et al. 2011 | HM587120          |
| 47 | SA51        | South Africa  | 1951              | Grobbelaar et al. 2011 | HM587125          |
| 48 | M34_ARB     | Senegal       | 2002              | Soumare et al. 2012    | JN955327          |
| 49 | M38_ARB     | Senegal       | 2003              | Soumare et al. 2012    | JN955343          |
| 50 | Namibia_2010| Namibia       | 2010              | Monaco et al. 2013     | KC935380          |
| 51 | 211977      | Mauritania    | 2010              | Faye et al. 2013       | KF717590          |
References

1. Aradaib IE, Erickson BR, Elageb RM, Khristova ML, Carroll SA, Elkhidir IM, et al. Rift Valley fever. Sudan, 2007 and 2010. [Epub 2013 Feb]. Emerg Infect Dis. 2013;19:246–53.

http://dx.doi.org/10.3201/eid1902.120834
2. Bird BH, Khristova ML, Rollin PE, Ksiazek TG, Nichol ST. Complete genome analysis of 33 ecologically and biologically diverse Rift Valley fever virus strains reveals widespread virus movement and low genetic diversity due to recent common ancestry. [Epub 2006 Dec 27]. J Virol. 2007;81:2805–16. PubMed http://dx.doi.org/10.1128/JVI.02095-06

3. Bird BH, Githinji JWK, Macharia JM, Kasiiti JL, Muriithi SG, et al. Multiple virus lineages sharing recent common ancestry were associated with a large Rift Valley fever outbreak among livestock in Kenya during 2006–2007. [Epub 2008 Sep 10]. J Virol. 2008;82:11152–66. PubMed http://dx.doi.org/10.1128/JVI.01519-08

4. Bird BH, Khristova ML, Nichol ST. Direct submission. Submitted 2011 Feb 11. Viral Special Pathogens Branch, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA. http://www.ncbi.nih.gov/nuccore/JF326194

5. Bird BH, Khristova ML, Nichol ST. Direct submission. Submitted 2011 Feb 11. Viral Special Pathogens Branch, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA. http://www.ncbi.nih.gov/nuccore/JF326195

6. Carroll SA, Reynes JM, Khristova ML, Andriamandimby SF, Rollin PE, Nichol ST. Genetic evidence for Rift Valley fever outbreaks in Madagascar resulting from virus introductions from the East African mainland rather than enzootic maintenance. [Epub 2011 Apr 20]. J Virol. 2011;85:6162–7. PubMed

7. Cêtre-Sossah C, Zeller H, Grandadam M, Caro V, Pettinelli F, Bouloy M, et al. Genome analysis of Rift Valley fever virus, Mayotte. Emerg Infect Dis. 2012;18:969–71. PubMed

8. Faye O, Diallo M, Diop D, Elmamy BO, Ba H, Niang M, et al. Outbreak of Rift Valley Fever in Mauritania, 2003: First emergence of the East-Central Africa virus lineage in West Africa. http://www.ncbi.nlm.nih.gov/nuccore/EF160115

9. Faye O, Ba H, Ba Y, Freire C, Faye O, Ndiaye O, et al. Direct submission. Submitted 2013 Oct 9. Arbovirus Unit, Institut Pasteur-Dakar, Senegal. http://www.ncbi.nlm.nih.gov/nuccore/KF717590

10. Grobbelaar AA, Weyer J, Leman PA, Kemp A, Pawska JT, Swanepoel R. Molecular epidemiology of Rift Valley fever virus. Emerg Infect Dis. 2011;17:2270–6 PubMed http://dx.doi.org/10.3201/eid1712.111035.
11. Miller BR, Godsey MS, Crabtree MB, Al-Mazrao Y, Al-Jeffri MH, Abdoon AM, et al. Direct submission. Submitted 2001 Jun 11. Division of Vector-Borne Infectious Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA. http://www.ncbi.nih.gov/nuccore/AF393745

12. Monaco F, Pinoni C, Cosseddu GM, Khaiseb S, Calistri P, Molini U, et al. Rift Valley fever in Namibia, 2010. Emerg Infect Dis. 2013;19:2025–7. PubMed http://dx.doi.org/10.3201/eid1912.130593

13. Nderitu L, Lee JS, Omolo J, Omulo S, O’Guinn ML, Hightower A, et al. Sequential Rift Valley fever outbreaks in eastern Africa caused by multiple lineages of the virus. [Epub 2010 Dec 14]. J Infect Dis. 2011;203:655–65. PubMed http://dx.doi.org/10.1093/infdis/jiq004

14. Potgieter CA, Smith SJ, Kortekaas J. [Virology, Central Veterinary Institute of Wageningen, University and Research Centre, Lelystad, the Netherlands.]. Direct submission. 2011. http://www.ncbi.nih.gov/nuccore/JQ068143

15. Ratovonjato J, Olive MM, Tantely LM, Andrianaiivolambo L, Tata E, Razainirina J, et al. Detection, isolation, and genetic characterization of Rift Valley fever virus from Anopheles (Anopheles) coustani, Anopheles (Anopheles) squamosus, and Culex (Culex) antennatus of the Haute Matsiatra region, Madagascar. [Epub 2010 Oct 28]. Vector Borne Zoonotic Dis. 2011;11:753–9. PubMed http://dx.doi.org/10.1089/vbz.2010.0031

16. Soumaré POL, Freire CCM, Faye O, Diallo M, de Oliveira JV, Zanotto PM, et al. Phylogeography of Rift Valley Fever virus in Africa reveals multiple introductions in Senegal and Mauritania. PLoS One. 2012;7:e35216. PubMed http://dx.doi.org/10.1371/journal.pone.0035216
Technical Appendix Figure. Maximum likelihood tree generated from sequence data obtained from Rift Valley fever (RVF) viruses from Goba District, Mozambique, and RVF reference sequences available on Genbank (n = 129,480 nt region; medium segment). The evolutionary history was inferred by using the
maximum-likelihood method in MEGA6 (12), based on the Kimura 2-parameter model. The tree that had the highest log likelihood ($-2055.6526$) is shown in the figure. A discrete $\gamma$ distribution was used to model the evolutionary rate differences among sites (5 categories [(+G, parameter = 0.3911)]. Confidence values for the tree topologies were evaluated with a bootstrap analysis of 1,000 replicate datasets (consensus tree; cutoff <70% shown). Lineages were identified as described by Grobbelaar et al. (10).