Rift Valley Fever

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Key points:

- Rift Valley Fever is a viral disease of domestic ruminants and wild hooved animals of Africa and the Middle East but can also infect humans.
- Rift Valley Fever Virus is spread by several species of mosquitoes and causes intermittent outbreaks.
- Predominantly found in continental Africa, confirmed outbreaks in nearby island nations and the Arabian Peninsula highlight the increasing geographical footprint.
- Animal outbreaks associated with high rates of abortions, neonatal die-offs, and influenza-like illness in people associated with animal care.
- RVF is primarily a hepatic disease but may involve other organ systems.

Rift Valley Fever (RVF) is a zoonotic viral disease that affects domestic and wild ruminants such as cattle, sheep, goats, camels, and buffaloes. Rift Valley fever virus (RVFV), the causative agent of RVF, can also infect humans. RVFV is an arthropod-borne virus (arbovirus) that is primarily spread through the bites of infected mosquitoes. RVFV was first isolated and characterized in the Rift Valley of Kenya in 1931 and is endemic throughout sub-Saharan Africa, including Comoros and Madagascar, the Arabian Peninsula (Saudi Arabia and Yemen), and Mayotte[1]. Highlighting the transboundary nature of RVFV was the first non-African outbreak that occurred in Saudi Arabia and Yemen in 2000 and various serological surveys that revealed its potential expansion to additional African and Middle Eastern countries [2]. Disease prevention in animals is best facilitated by vaccination, vector control programs and restrictions on livestock movement during outbreaks.

Virology

RVFV is a member of the genus *Phlebovirus* in the *Phenuiviridae* family of the Bunyavirales order (reviewed in [3]). Like most bunyaviruses, RVFV is an arbovirus, i.e. a virus transmitted by arthropods, specifically *Aedes* and *Culex spp.* mosquitoes. The virus has a tripartite, single-stranded, negative/ambisense RNA genome consisting of the L, M and S segments (large, medium, and small, respectively) (Figure 1). The viral RNA associates with the RNA-dependent-RNA-polymerase L protein (encoded on the L segment) and the nucleoprotein N (encoded on the S segment) to form the ribonucleoprotein complex. After the completion of replication, RVF viral particles assemble and bud from the Golgi apparatus; the viral particles are enveloped by

host-derived membranes containing the viral glycoproteins Gn and Gc (encoded both on the M segment). The Gn and Gc surface glycoproteins are responsible for virus attachment and host cell entry which allows the release of the viral genetic material into the cytoplasm. RVFV expresses other viral proteins such as the 78 kDa protein (encoded on the M segment), important for mosquito infection, or the NSs and NSm proteins (encoded on the S and M segment respectively), important for modulizations of the host cell environment (NSm) and host immune response (NSs).

Epidemiology

RVFV infections cause disease in domestic and wild ruminants across Africa and parts of the Middle East. Humans living close to livestock are at risk for infection with this zoonotic pathogen [4-6]. Besides sheep, goats, cattle, and camels, RVF is also found in wildlife such as buffalo and springbok; seroepidemiology also identified other susceptible African wildlife species [7] including various rodent species [8]. The protentional role of wildlife in RVFV epidemiology is not well understood, especially with regard to North America, however white-tail deer have been shown to be experimentally susceptible to infection, disease, and transmission of RVFV[9]. Transmission of the virus to mammals is primarily caused via the feeding of infected mosquitoes. Numerous species of mosquitoes from 7 different genera have been shown to be competent for infection and transmission of RVFV, either naturally or in lab settings [10]. Outbreaks of RVF occur unpredictably at 5-35 year intervals and are most often associated with heavy seasonal rainfall and flooding, which are conditions ideal for the hatching of mosquito eggs and mosquito breeding [11]. During interepidemic periods, the virus can be maintained by vertical transovarial transmission of RVFV from infected female *Aedes spp.* mosquitoes to eggs and the resultant adult mosquitoes [12]. This is possible because desiccated eggs of some mosquito species can remain infectious for months to years, depending on the conditions. Transovarial transmission has also been demonstrated experimentally in *Culex tarsalis* [13].Other, yet undetermined, mechanisms for RVFV maintenance in the environment may also play a role. Serological surveys indicate that low levels of livestock and wildlife infections may support the existence of undetected transmission during interepidemic periods [14].

RVF disease was likely first reported in 1912 but the virus was not isolated until 1930 during an outbreak in Tanzania and Kenya [2]; however, molecular epidemiology suggests the virus has been present in Africa since the late 1800s [15-17]. The presence of RVFV has been confirmed by viral isolation or serological surveys in countries in Africa, nearby island nations, and the Arabian Peninsula [1]. Serological surveys of livestock and humans have identified antibodies to RVFV in additional countries around Africa, indicating that the virus could be more widespread; this warrants surveillance for RVFV outside of known endemic areas (Figure 2)[2, 5]. RVFV is not found in North or South America; however, US mosquito species have been shown to be potential vectors [18].

Transmission cycle

Vertebrate hosts can be infected though the bite of infected mosquitoes or by exposure to blood or tissues of infected animals. Mosquito-borne transmission is the most likely infection pathway for livestock. Initial targets of infection are thought to be tissue-resident macrophages

and other antigen-presenting cells [19]. These cells carry the virus to local draining lymph nodes and amplify the infection, causing viremia (infectious virus in the blood) and viral spread to the primary sites of replication: the liver and the spleen [20, 21]. Viremia can occur between 2-4 days post exposure and can last up to 7 days. The fetus and neonate animals are highly susceptible to RVFV infection and can develop viremia in as little as 12-36 hours. Once an animal is viremic it can pass RVFV to a naïve mosquito via blood feeding and the transmission cycle restarts. Another important route of infection is contact exposure to infected tissues and body fluids. This route of infection often occurs in people with close contact to livestock such as butchers, farmers and veterinarians taking care of sick animals, often during abortion storms. While there are no reports of infections from exposure to raw milk, reports indicate milk samples from susceptible animal species have tested positive for RVFV-specific RNA; also, there is a strong association between consuming or collecting raw milk and RVFV seroconversion[22-24].

Clinical Disease

Disease susceptibility decreases with age and varies between animal species [25]. Sheep and goats are more susceptible than cattle and camels, and there are differences in susceptibility across breed and genotype within livestock species [26]. Neonatal lambs and kids less than 1 week old are highly susceptible. The relative susceptibility of wild animals has not been established. Experimentally, white-tailed deer have been shown to be highly susceptible to RVFV infection resulting in clinical disease and virus transmission [9].

Most livestock outbreaks are usually associated with high incidence of abortions (abortion storms), newborn die-offs, and spill-over into the human population. RVFV infections can lead to clinical signs ranging from inapparent to peracute. Newborn lambs, kids, and to a lesser extent, calves, develop severe liver disease with high fevers, listlessness, and abdominal pain. Icterus (common in calves) has also been reported. Mortality in very young animals varies from 70-100%. The incubation period can be as short as 12 hours post exposure but is typically 24-36 hours post exposure. Older sheep and goats develop clinical signs 24-72 hours post exposure that include fever, anorexia, listlessness, diarrhea, and icterus with a mortality rate from 10-30% [27]. Adult cattle develop anorexia, diarrhea, dysgalactia, excessive salivation, and nasal discharge. Clinical signs are often not apparent in adult cattle, and mortality is less than 10%. Viremia, an important factor in the transmission cycle of RVFV, lasts for 1-3 days in less susceptible breeds, but can be as long as 10 days in more susceptible ones [27].

Abortion storms are a key indicator of large RVFV outbreaks and cause the main economic impact in livestock. Abortion rates typically reach 90-100% in sheep and goats [27]. Although not well studied, RVF-associated abortions have also been reported in camels and African buffalo [28, 29]. RVFV-induced abortions may also be associated with increased maternal mortality [30]. Horizontal transmission to the fetus *in utero* typically targets the liver and brain of the fetus; *in utero* infection can result in necrotizing viral placentitis resulting in abortion prior to fetal infection [31]. Thus, a failure to identify RVFV or RVFV RNA in fetal tissues does not rule out RVF as an etiology.

There exists a complex symptomatology of RFVF infection in humans, many of which are non-specific [32]. RVFV infections in humans commonly present as self-limiting influenza-like febrile illness that can last 3-4 days but may recrudesce and last for up to 10 days. Viremia can be detected for 3-4 days during the febrile stage of the disease. The overall human mortality rate is estimated at less than 1% [33]. Occasionally, a macular rash, jaundice and bleeding may occur, which is indicative of RVFV-associated hemorrhagic fever, usually with fatal outcome [34, 35]. Additionally, some patients develop hepatic necrosis or ocular disease resulting in partial or total blindness, while some develop encephalitis with neurological signs including neck rigidity, limb weakness, confusion, hyperreflexia, and coma [36, 37]. Vision loss and neurological symptoms can be temporal or permanent and may present in the same patient [38].

Pathogenesis

Severe RVF disease is mainly observed in neonatal lambs and kids while cattle and older sheep and goats exhibit milder disease. The most prominent pathological features of an RVFV infection in ruminants are small, necrotic lesions (1-3 mm size) in the liver (Figure 3)[39]. These lesions can vary in density from multifocal liver foci to coalescing necrotic foci in more severe cases. A peracute infection results in an enlarged, friable, and discolored brown-orange liver with accentuated lobulation [25, 40]. Gall bladder involvement is often seen with notable edema and hemorrhage in the bladder wall and blood in the luminal space [41]. Lymph nodes and kidneys can be enlarged and edematous with petechial hemorrhages; the spleen is also often enlarged and congested [39]. Diffuse pulmonary congestion is often noted. Hemorrhages on the inner and outer surfaces of the heart and on serosal surfaces can be observed.

Microscopically, liver lesions are characterized by multifocal to coalescing, midzonal to periportal necrosis of hepatocytes, accompanied by variable hemorrhages and inflammation (Figure 4). Hepatic foci consist of well-defined foci with loss of architecture and hepatic cords. Hepatocyte drop-out, hepatic swelling and karyorrhexis, cellular lysis and apoptotic cells (councilman-like bodies) are mixed with fibrin and hemorrhage as well cellular and nuclear debris. Often there are infiltrates of neutrophils, macrophages, and lymphocytes within and surrounding necrotic foci [40]. Mixed periportal infiltrates of inflammatory cells and fibrin thrombi in vessels can also occur. Necrotic foci are of varying size, can coalesce and affect entire liver lobes in severe cases. [25]. In some instances, acidophilic intranuclear inclusion bodies are appreciated[41]. When performing ultrastructural analysis, RVFV-infected cells, mainly in fetal and neonate samples, display filamentous intranuclear bodies consisting of the viral NSs protein [42]. Immunohistochemistry (IHC) using RVFV-specific hyperimmune serum/antibodies is used to confirm the presence of RVFV-specific antigen in infected livers. IHC-positive cells are commonly located at the periphery of necrotic lesions **(Figure 4);** however, in fulminant RVF infections, IHC staining can occur throughout the entire hepatic parenchyma [41].

Diagnosis

RVFV infections in humans usually present as influenza-like illness accompanied by hepatic disease or hemorrhaging whereas infections in domestic ruminants are usually accompanied by abortion storms and neonatal deaths. RVF is characterized by hepatic lesions seen during macroscopic and histopathological examinations. Differential diagnoses from other conditions

causing hemorrhagic disease with liver necrosis and death include bacterial septicemia, plant or chemical toxicosis, and other hemorrhagic viral diseases. Laboratory confirmation of the presence of antigen, viral RNA, and/or RVFV-specific antibodies should be made for definitive diagnosis.

Diagnostic specimens should include sera and anti-coagulated whole blood from live animals or samples of liver, spleen, lymph nodes, kidneys, and/or heart from dead animals and aborted fetuses. Also, placenta can be useful in some instances if *post mortem* autolysis is not too advanced. Fetal tissue is often autolyzed, leading to the degradation of viral antigens and genomic RNA material as well as poor tissue morphology. Appropriate personal protective equipment (PPE) should be worn when performing *post mortem* examinations and tissue collections. Samples should be handled with the assumption of the presence of infectious RVFV and executed under enhanced BSL-3 conditions [43]. Samples should be sent to appropriate governmental reference laboratories at 4°C or frozen and in accordance with biological hazard regulations.

RVFV can be isolated from serum, plasma, anti-coagulated blood, or from various organs/tissues using susceptible cell lines such as Vero, BHK, or AP61 cells [44]. Virus-positive cell cultures are identified by the presence of cytopathic effects with confirmatory IHC staining or reverse transcriptase PCR (RT-PCR). RVFV antigen detection is primarily done by enzyme linked immunosorbent assay (ELISA) which can detect RVF viral proteins in blood and tissues or by IHC staining on formalin-fixed, paraffin-embedded (FFPE) tissues. Viral RNA can be detected in FFPE tissues via *in situ* hybridization (ISH) or RT-PCR [45]. Lateral flow devices have proven to be effective and are in development but are not yet currently commercially available. Viral genomic components can be detected by RT-PCR while quantitative real-time RT-PCR is able to quantify viral genome copies, a useful tool in tracking disease stage and progression. There are numerous *in-house* protocols and commercial kits available for either ELISA or RT-PCR, and the World Organization for Animal Health (WOAH, formerly OIE) has a collection of protocols which are utilized by reference labs worldwide [43, 44]. Viral isolation, antigen detection and RT-PCR are useful when virus is present (2-4 days post exposure in blood and up to 8 days in tissues). Detection of RVFV infection after the initial exposure/replication period is accomplished via serological assays for RVFV antibody detection. RVFV antibody detection is primarily done by ELISA using recombinant N or other viral proteins as antigen. ELISAs can differentiate between IgM (early antibodies) and IgG (long term antibodies) antibodies. Indirect immunofluorescence (IFA) using RVFV-infected cells or infected tissue slides can also be used. Neutralization tests (NTs) are also commonly used; they are cell culture-based assays that test a serum sample's ability to block viral infection (virus neutralization assay, VNA) or reduce the number of viral plaques formed (plaque reduction neutralization test, PRNT).

Prevention and control

Due to the sporadic nature of RVFV outbreaks and the unpredictability of heavy rain/flooding, vector control and the movement of livestock to drier areas is not an effective mitigation strategy. The most effective solutions within endemic areas are regular vaccination of highly susceptible livestock and restriction of livestock movement from affected areas to non-affected

areas. Surveillance of susceptible livestock and wildlife near outbreak locations and in areas between endemic and non-endemic regions is important to determine whether control efforts are working and for confirming the onset of outbreaks. The potential movement of RVFV into non-endemic countries including legal and illegal movement of animals, mosquito vectors and humans has been reviewed [18]. Community outreach and educational programs are critical to support the above-mentioned mitigation strategies.

Currently, only a few RVFV vaccines are licensed for veterinary use; there is no human vaccine available. Thus, if RVFV is suspected, since it is zoonotic, proper personal protective equipment such as latex or vinyl gloves, face masks (minimally surgical masks but preferably N95 or Power Assisted Purified Air Respirators (PAPR)), scrubs or coveralls and boots. The most commonly used vaccine in the endemic area in Africa is the Smithburn vaccine, which is a live attenuated vaccine produced from an RVFV isolate derived from a mosquito pool in Uganda; this RVFV isolate was passed more than 100 times in mouse brains [46]. The vaccine is inexpensive, can be produced easily, and provides robust, long-lasting immunity from a single dose application. However, it is not recommended for some animal species and pregnant animals since it causes abortion and teratogenic effects [47, 48]. Like most live attenuated vaccines, there are concerns of reversion to virulence or reassortment with wild-type RVF viruses [49]. Therefore, the Smithburn vaccine is only recommended in endemic areas. The live attenuated Clone 13 RVFV vaccine has been increasingly used across Africa in the past 10 years after licensure in South Africa [49]. Clone 13 is naturally attenuated, which produces a protective and long lasting immune response similar to the Smithburn vaccine; in addition, it is safe to use in pregnant animals when applied as directed on the label [50]. One study showed that an overdose of Clone 13 in pregnant sheep resulted in stillbirths and malformed fetuses; thus, following the recommendation for the Clone 13 vaccine dose is critically important [51]. The MP-12 RVFV vaccine is another live attenuated vaccine that is conditionally licensed in the United States and Canada for emergency use. The MP-12 vaccine induces robust and long-term immunity and has been shown to be safe and efficacious [52]. However, a cell-passaged MP-12 vaccine was shown to induce teratogenic effects when administered to pregnant animals [53].

Additional next generation RVFV vaccines are in development. These include subunit vaccines, vectored vaccines such as a chimp adenovirus vectored vaccine, and a 4-segmented altered genome vaccine [54, 55]. Subunit vaccines are very safe and offer the capability of differentiating infected from vaccinated animals (DIVA) when paired with the appropriate diagnostic testing platform (e.g., DIVA ELISA). Subunit vaccines currently under development require two vaccination doses to be efficacious. While DIVA compatible subunit RVFV vaccines are of value in non-endemic countries; there are concerns for their usefulness in sub-Saharan Africa where nomadic herding practices are used and multi-dose vaccines regimens would be impractical. The hot climate and limited infrastructure and resources of endemic regions in Africa/Arabian Peninsula make vaccine stability and storage conditions important considerations as well. Other vaccine strategies such as DNA or mRNA vaccines are currently being investigated and may result in novel vaccine platforms [56].

Summary
RVFV is an arbovirus endemic to much of the African continent. RVF outbreaks have also occurred in the Comoros Island, Madagascar, Mayotte, and the Arabian Peninsula. The virus is maintained during inter-epidemic periods in *Aedes* mosquitoes via transovarial transmission and through low-level RVFV infections of livestock and wildlife species. Large outbreaks occur sporadically during periods of heavy rainfall and persistent flooding with the emergence of infected mosquitoes breeding in floodwater. A multitude of different mosquito genera and species are competent vectors for RVFV replication and transmission. Sheep, goats, cattle, and camels are susceptible livestock species, and the virus has been also isolated from wild African buffalo and springbok. In domestic livestock, susceptibility is dependent on species, age, and breed. RVFV is a zoonotic pathogen and human cases often accompany livestock outbreaks. The Americas are free of RVF, so vigilant veterinary practitioners are key for first raising concern of a potential introduction.

Outbreaks in livestock are generally recognized by abortion storms and increased neonatal deaths and flu-like symptoms in humans. The virus causes hepatitis and must be differentiated from other hepatic diseases by virus isolation, antigen detection, RT-PCR methods, or by serology. Veterinary practitioners should contact their state and/or federal animal health official should they suspect an unusual disease outbreak of a foreign disease investigation. This is especially important since infected humans may develop hemorrhagic disease, or ocular or neurological signs that can be fatal. Currently, there is no human RVFV vaccine available. In contrast, live attenuated RVFV vaccines are available for livestock in endemic areas and are the best tools for disease control. These live attenuated RVFV vaccines are highly effective but are not safe for use in pregnant animals. Continued improvement of vaccine strategies with the implementation of novel technologies resulting in a safe and DIVA-compatible RVFV vaccine that induces a rapid and long-lasting immunity with a single dose is critical and may allow for improved prevention and control strategies in the future.

Clinics Care Points

- Animals infected with RVFV present clinical symptoms ranging from inapparent to peracute. Indicators include high herd abortion rates and newborn mortality, severe liver disease, fevers, and abdominal pain.
- Zoonotic infections of humans can occur after mosquito bites or handling of infectious animal fluids typically during veterinary care or butchering. People present with febrile illness that can progress in severity to include hepatic disease, hemorrhagic fever, neurologic involvement, or death.
- Diagnostic samples from suspected cases should be handled under enhanced BSL3 conditions. Definitive diagnostic tests include viral isolation, antigen detection, RT-PCR, and or, pathogen specific antibody detection.
- A few vaccines are regionally approved, but current options have drawbacks. Recent advances offer hope for better vaccine options. Outbreak control is achieved by restricting the movement of susceptible animals into or out of affected areas.

Disclosure:

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The conclusions in this report are those of the authors and do not necessarily represent the views of the USDA. USDA is an equal opportunity provider and employer.

References

1. CDC. *RVF distribution map*. 2023; Available from: https://www.cdc.gov/vhf/rvf/outbreaks/distribution-map.html.
2. Bron, G.M., et al., *Over 100 Years of Rift Valley Fever: A Patchwork of Data on Pathogen Spread and Spillover.* Pathogens, 2021. **10**(6).
3. Nair, N., et al., *Rift Valley Fever Virus-Infection, Pathogenesis and Host Immune Responses.* Pathogens, 2023. **12**(9).
4. Johnson, S.A.M., et al., *Evidence of Rift Valley Fever Virus Circulation in Livestock and Herders in Southern Ghana.* Viruses, 2023. **15**(6).
5. Clark, M.H.A., et al., *Systematic literature review of Rift Valley fever virus seroprevalence in livestock, wildlife and humans in Africa from 1968 to 2016.* PLoS Negl Trop Dis, 2018. **12**(7): p. e0006627.
6. Sang, R., et al., *Rift Valley fever virus epidemic in Kenya, 2006/2007: the entomologic investigations.* Am J Trop Med Hyg, 2010. **83**(2 Suppl): p. 28-37.
7. Capobianco Dondona, A., et al., *Rift Valley Fever Virus among Wild Ruminants, Etosha National Park, Namibia, 2011.* Emerg Infect Dis, 2016. **22**(1): p. 128-30.
8. Rostal, M.K., et al., *Rift Valley Fever: Does Wildlife Play a Role?* ILAR J, 2017. **58**(3): p. 359-370.
9. Wilson, W.C., et al., *Susceptibility of White-Tailed Deer to Rift Valley Fever Virus.* Emerg Infect Dis, 2018. **24**(9): p. 1717-1719.
10. Tantely, L.M., S. Boyer, and D. Fontenille, *A review of mosquitoes associated with Rift Valley fever virus in Madagascar.* Am J Trop Med Hyg, 2015. **92**(4): p. 722-9.
11. Davies, F.G., K.J. Linthicum, and A.D. James, *Rainfall and epizootic Rift Valley fever.* Bull World Health Organ, 1985. **63**(5): p. 941-3.
12. Gargan, T.P., 2nd, P.G. Jupp, and R.J. Novak, *Panveld oviposition sites of floodwater Aedes mosquitoes and attempts to detect transovarial transmission of Rift Valley fever virus in South Africa.* Med Vet Entomol, 1988. **2**(3): p. 231-6.
13. Bergren, N.A., et al., *Laboratory demonstration of the vertical transmission of Rift Valley fever virus by Culex tarsalis mosquitoes.* PLoS Negl Trop Dis, 2021. **15**(3): p. e0009273.
14. Rissmann, M., et al., *Mechanisms of inter-epidemic maintenance of Rift Valley fever phlebovirus.* Antiviral Res, 2020. **174**: p. 104692.

15. Daubney, R., J.R. Hudson, and P.C. Garnham, *Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep cattle and man from East Africa.* Journal of Pathology and Bacteriology, 1931. **34**(4): p. 545-579.
16. Bird, B.H., et al., *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.* J Virol, 2007. **81**(6): p. 2805-16.
17. Bowring, C.C. and L. Harcourt, *East Africa Protectorate*. Colonial Reports-Annual. 1914, London: Barclay and Fry.
18. Gibson, S., et al., *The increasing threat of Rift Valley fever virus globalization: strategic guidance for protection and preparation.* J Med Entomol, 2023. **60**(6): p. 1197-1213.
19. Lozach, P.Y., et al., *DC-SIGN as a receptor for phleboviruses.* Cell Host Microbe, 2011. **10**(1): p. 75-88.
20. Smith, D.R., et al., *The pathogenesis of Rift Valley fever virus in the mouse model.* Virology, 2010. **407**(2): p. 256-67.
21. Schwarz, M.M., et al., *Lrp1 is essential for lethal Rift Valley fever hepatic disease in mice.* Sci Adv, 2023. **9**(28): p. eadh2264.
22. Gerken, K.N., et al., *Exploring potential risk pathways with high risk groups for urban Rift Valley fever virus introduction, transmission, and persistence in two urban centers of Kenya.* PLoS Negl Trop Dis, 2023. **17**(1): p. e0010460.
23. de Glanville, W.A., et al., *An outbreak of Rift Valley fever among peri-urban dairy cattle in northern Tanzania.* Trans R Soc Trop Med Hyg, 2022. **116**(11): p. 1082-1090.
24. de Glanville, W.A., et al., *Inter-epidemic Rift Valley fever virus infection incidence and risks for zoonotic spillover in northern Tanzania.* PLoS Negl Trop Dis, 2022. **16**(10): p. e0010871.
25. Coetzer, J.A., *The pathology of Rift Valley fever. II. Lesions occurring in field cases in adult cattle, calves and aborted foetuses.* Onderstepoort J Vet Res, 1982. **49**(1): p. 11-7.
26. Olaleye, O.D., et al., *Experimental infection of three Nigerian breeds of sheep with the Zinga strain of the Rift Valley Fever virus.* Rev Elev Med Vet Pays Trop, 1996. **49**(1): p. 6-16.
27. Bird, B.H., et al., *Rift Valley fever virus.* J Am Vet Med Assoc, 2009. **234**(7): p. 883-93.
28. Scott, G.R., et al., *Rift Valley fever in camels.* J Pathol Bacteriol, 1963. **86**: p. 229-31.
29. Fagbo, S., J.A. Coetzer, and E.H. Venter, *Seroprevalence of Rift Valley fever and lumpy skin disease in African buffalo (Syncerus caffer) in the Kruger National Park and Hluhluwe-iMfolozi Park, South Africa.* J S Afr Vet Assoc, 2014. **85**(1): p. e1-e7.
30. Antonis, A.F., et al., *Vertical transmission of Rift Valley fever virus without detectable maternal viremia.* Vector Borne Zoonotic Dis, 2013. **13**(8): p. 601-6.
31. Odendaal, L., et al., *Ovine Fetal and Placental Lesions and Cellular Tropism in Natural Rift Valley Fever Virus Infections.* Vet Pathol, 2020. **57**(6): p. 791-806.
32. Anywaine, Z., et al., *Clinical manifestations of Rift Valley fever in humans: Systematic review and meta-analysis.* PLoS Negl Trop Dis, 2022. **16**(3): p. e0010233.
33. WHO. *Rift Valley Fever Fact Sheet*. 2018 [cited 2023; Available from: https://www.who.int/news-room/fact-sheets/detail/rift-valley-fever.
34. Yassin, W., *Clinico-pathological picture in five human cases died with Rift Valley fever.* J Egypt Public Health Assoc, 1978. **53**(3-4): p. 191-3.

35. Swanepoel, R., B. Manning, and J.A. Watt, *Fatal Rift Valley fever of man in Rhodesia.* Cent Afr J Med, 1979. **25**(1): p. 1-8.
36. Siam, A.L., J.M. Meegan, and K.F. Gharbawi, *Rift Valley fever ocular manifestations: observations during the 1977 epidemic in Egypt.* Br J Ophthalmol, 1980. **64**(5): p. 366-74.
37. van Velden, D.J., et al., *Rift Valley fever affecting humans in South Africa: a clinicopathological study.* S Afr Med J, 1977. **51**(24): p. 867-71.
38. Ikegami, T. and S. Makino, *The pathogenesis of Rift Valley fever.* Viruses, 2011. **3**(5): p. 493-519.
39. Wilson, W.C., et al., *Experimental Infection of Calves by Two Genetically-Distinct Strains of Rift Valley Fever Virus.* Viruses, 2016. **8**(5).
40. Odendaal, L., A.S. Davis, and E.H. Venter, *Insights into the Pathogenesis of Viral Haemorrhagic Fever Based on Virus Tropism and Tissue Lesions of Natural Rift Valley Fever.* Viruses, 2021. **13**(4).
41. Odendaal, L., et al., *Lesions and Cellular Tropism of Natural Rift Valley Fever Virus Infection in Adult Sheep.* Vet Pathol, 2019. **56**(1): p. 61-77.
42. Barski, M., et al., *Rift Valley fever phlebovirus NSs protein core domain structure suggests molecular basis for nuclear filaments.* Elife, 2017. **6**.
43. Petrova, V., et al., *Rift valley fever: diagnostic challenges and investment needs for vaccine development.* BMJ Glob Health, 2020. **5**(8).
44. WOAH, *Rift Valley Fever*, in *Manual of Diagnotic Tests and Vaccines for Terrestirial Animal*. 2023.
45. Ragan, I.K., et al., *Rift Valley Fever Viral RNA Detection by In Situ Hybridization in Formalin-Fixed, Paraffin-Embedded Tissues.* Vector Borne Zoonotic Dis, 2019. **19**(7): p. 553-556.
46. Smithburn, K.C., *Rift Valley fever; the neurotropic adaptation of the virus and the experimental use of this modified virus as a vaccine.* Br J Exp Pathol, 1949. **30**(1): p. 1-16.
47. Anthony, T., et al., *Vaccination with Rift Valley fever virus live attenuated vaccine strain Smithburn caused meningoencephalitis in alpacas.* J Vet Diagn Invest, 2021. **33**(4): p. 777-781.
48. Botros, B., et al., *Adverse response of non-indigenous cattle of European breeds to live attenuated Smithburn Rift Valley fever vaccine.* J Med Virol, 2006. **78**(6): p. 787-91.
49. Faburay, B., et al., *Current Status of Rift Valley Fever Vaccine Development.* Vaccines (Basel), 2017. **5**(3).
50. Muller, R., et al., *Characterization of clone 13, a naturally attenuated avirulent isolate of Rift Valley fever virus, which is altered in the small segment.* Am J Trop Med Hyg, 1995. **53**(4): p. 405-11.
51. Makoschey, B., et al., *Rift Valley Fever Vaccine Virus Clone 13 Is Able to Cross the Ovine Placental Barrier Associated with Foetal Infections, Malformations, and Stillbirths.* PLoS Negl Trop Dis, 2016. **10**(3): p. e0004550.
52. Ikegami, T., *Rift Valley fever vaccines: an overview of the safety and efficacy of the live-attenuated MP-12 vaccine candidate.* Expert Rev Vaccines, 2017. **16**(6): p. 601-611.
53. Hunter, P., B.J. Erasmus, and J.H. Vorster, *Teratogenicity of a mutagenised Rift Valley fever virus (MVP 12) in sheep.* Onderstepoort J Vet Res, 2002. **69**(1): p. 95-8.

54. Jenkin, D., et al., *Safety and immunogenicity of a ChAdOx1 vaccine against Rift Valley fever in UK adults: an open-label, non-randomised, first-in-human phase 1 clinical trial.* Lancet Infect Dis, 2023. **23**(8): p. 956-964.
55. Wichgers Schreur, P.J., et al., *A single vaccination with four-segmented rift valley fever virus prevents vertical transmission of the wild-type virus in pregnant ewes.* NPJ Vaccines, 2021. **6**(1): p. 8.
56. Kitandwe, P.K., et al., *An Overview of Rift Valley Fever Vaccine Development Strategies.* Vaccines (Basel), 2022. **10**(11).

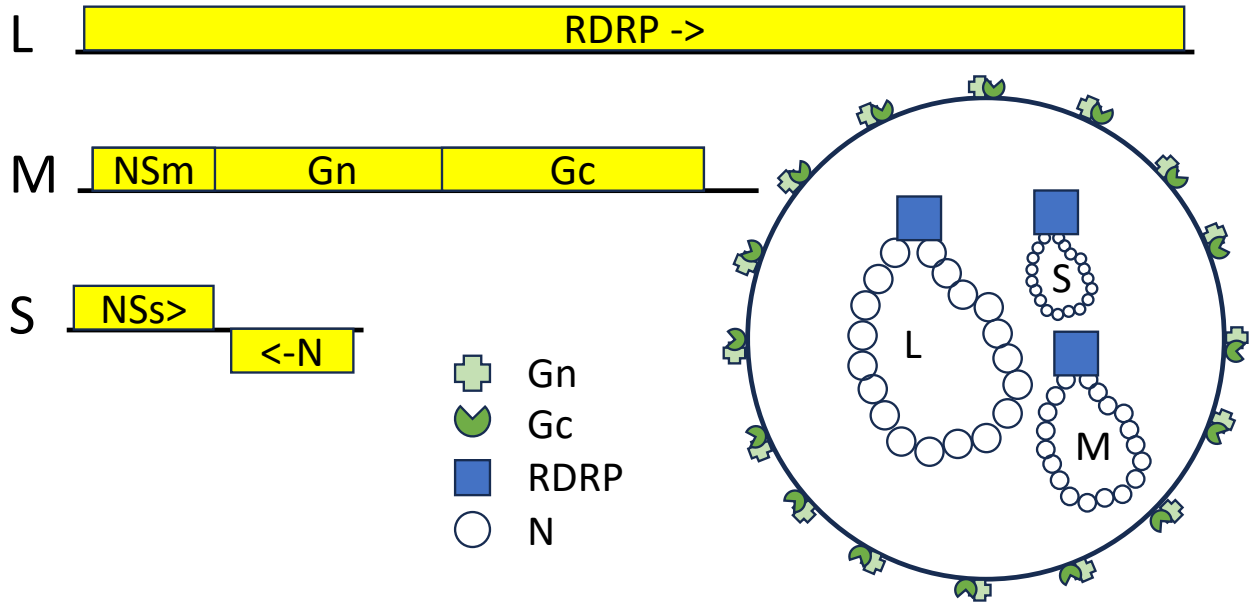

Figure 1. Schematic diagram of RVFV virion and genome structure. Viral RNA is wrapped around the nucleoprotein N (circles) and forms a loop structure with the RNA dependent RNA polymerase L (RDRP, blue square). Gn (green cross) and Gc (green pie) are embedded in the host-derived virus envelope that packages the 3 RNA segments L, M, and S. The L (6.4kb), M (3.9kb) and S (1.7kb) segments encode 8 viral proteins. The RDRP is the only protein produced from the L segment. The M segment encodes the two surface glycoproteins Gn and Gc. Alternate start codons within the M segment are utilized to produce the NSm, NSm', and the 78kDa protein. The S segment is an ambisense RNA that produces the NSs and N proteins.

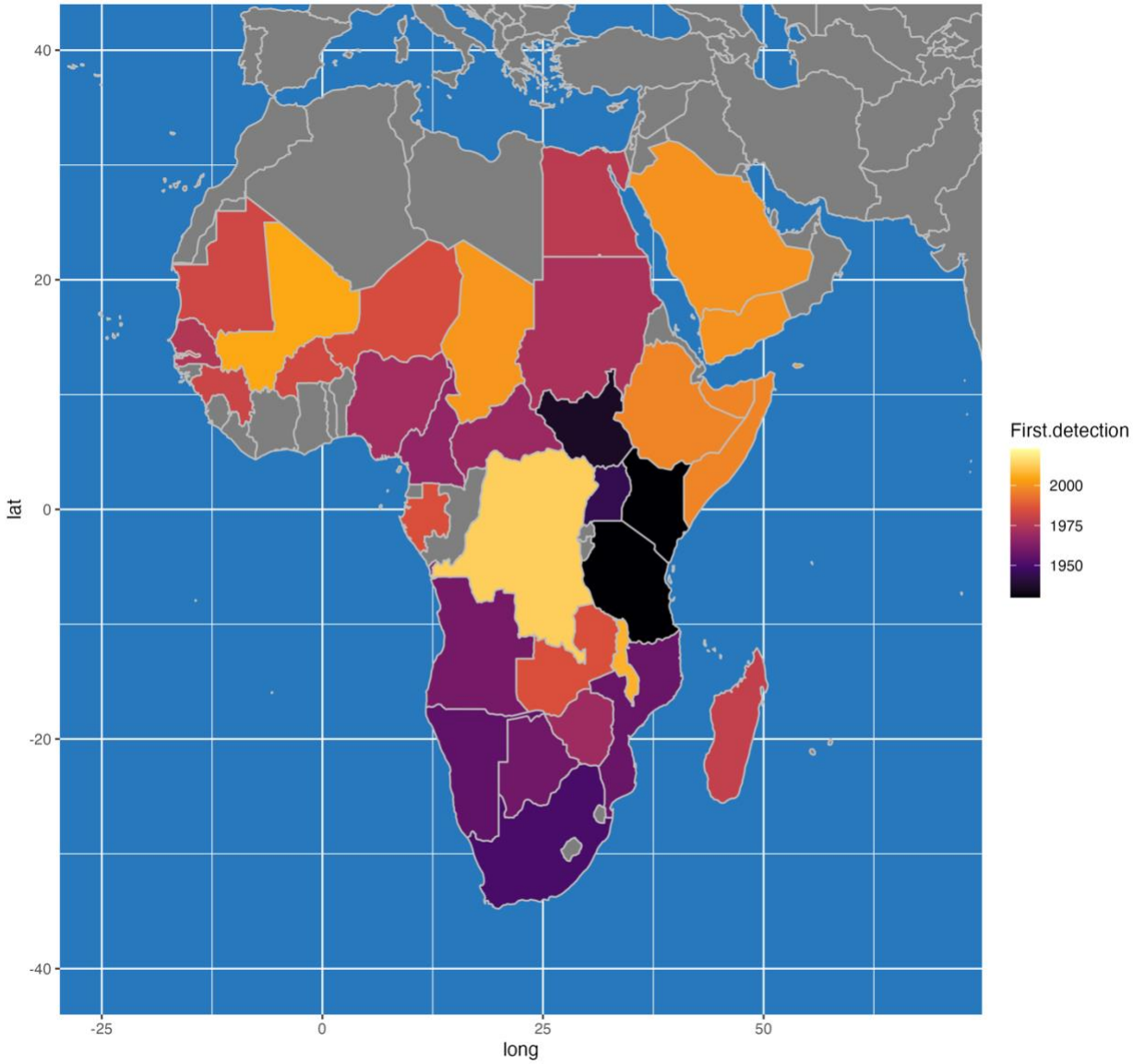

Figure 2. Temporal and spatial spread of Rift Valley Fever from Eastern Africa, starting around 1930. Map of combined serological and virological evidence of RVFV presence across Africa and the Middle East. Colors indicate first year of evidence; color code from earlier (~1930; dark) to later (2000s; light) years. Map based on data from the CDC [1].

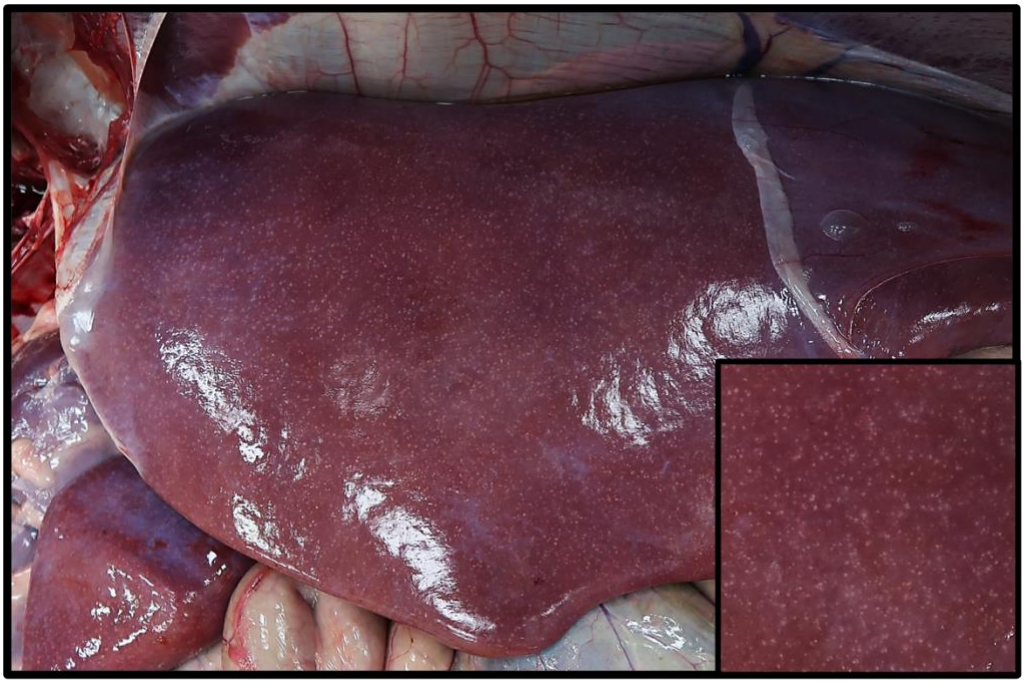

Figure 3. Liver from a calf experimentally infected with wild-type RVFV displaying typical pathological lesions. Liver *in situ* with 1-3 mm pale, necrotic foci (highlighted in insert), accompanied by mild generalized liver enlargement with rounded edges, and slight pallor.

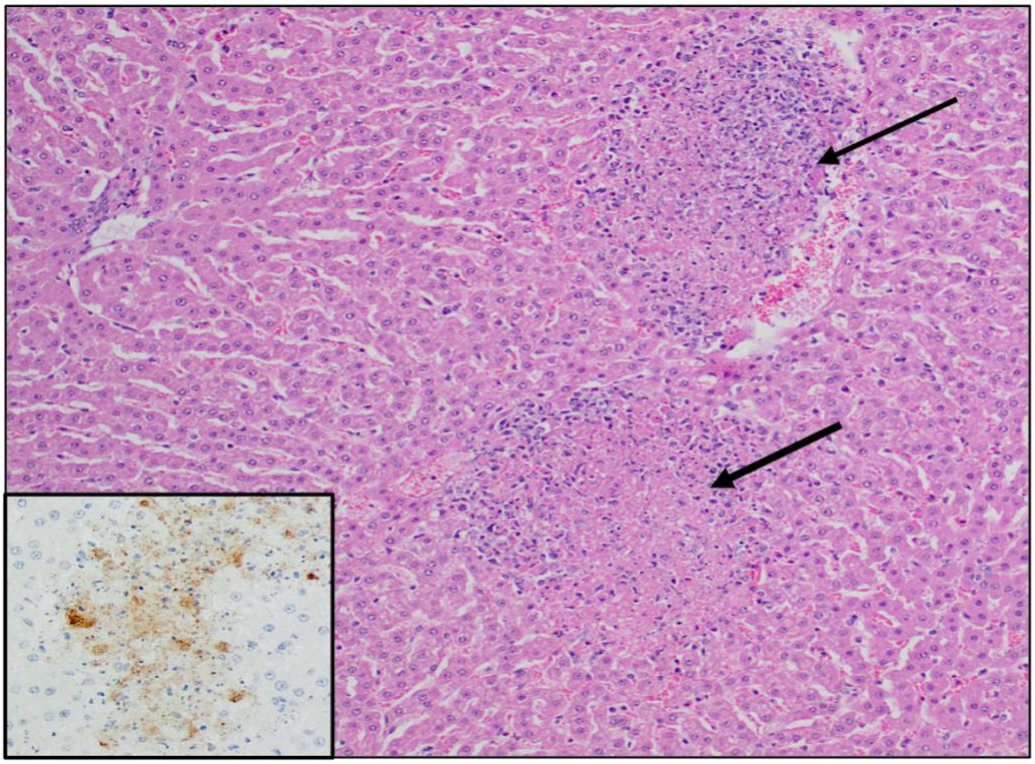

Figure 4. Microscopic hepatic lesions due to RVFV infection. Multiple areas of parenchymal necrosis occur in centrilobular zone or adjacent to central veins. Black arrows denote necrotic foci; the upper lesion contains infiltrates of lymphocytes (40x). Inset: Immunohistochemical staining for RVFV antigen present at the edge of the necrotic lesions (100X).