Review article

Rift Valley fever virus (*Bunyaviridae: Phlebovirus*): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention

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Abstract – Rift Valley fever (RVF) virus is an arbovirus in the *Bunyaviridae* family that, from phylogenetic analysis, appears to have first emerged in the mid-19th century and was only identified at the begininning of the 1930s in the Rift Valley region of Kenya. Despite being an arbovirus with a relatively simple but temporally and geographically stable genome, this zoonotic virus has already demonstrated a real capacity for emerging in new territories, as exemplified by the outbreaks in Egypt (1977), Western Africa (1988) and the Arabian Peninsula (2000), or for re-emerging after long periods of silence as observed very recently in Kenya and South Africa. The presence of competent vectors in countries previously free of RVF, the high viral titres in viraemic animals and the global changes in climate, travel and trade all contribute to make this virus a threat that must not be neglected as the consequences of RVF are dramatic, both for human and animal health. In this review, we present the latest advances in RVF virus research. In spite of this renewed interest, aspects of the epidemiology of RVF virus are still not fully understood and safe, effective vaccines are still not freely available for protecting humans and livestock against the dramatic consequences of this virus.

Rift Valley fever / molecular epidemiology / vector / pathogenesis / diagnostic

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1. INTRODUCTION

Rift Valley fever virus (RVFV; *Bunyaviridae: Phlebovirus*) is primarily transmitted by mosquitoes and causes a potentially severe disease among both humans and animals. The virus was first identified in 1930, during an outbreak of sudden deaths and abortions among sheep along the shores of Lake Naivasha in the greater Rift Valley of Kenya [51, 52]. The geographic distribution of the virus has since grown significantly and now includes most countries of the African continent and Madagascar [100]. It emerged for the first time outside Africa in the Arabian Peninsula in 2000–2001 and caused a large outbreak in livestock and humans [11]. More recently, it was detected for the first time in the Archipelago of Comores, located between Mozambique and Madagascar, on the French Island of Mayotte [242]. Due to the increasing range of the virus, the high numbers of competent vector species present in currently RVF-free regions, such as Europe [199] and the USA [95, 266], the intensification of international trade in live animals, and the unknown impact of climate change, several national and international agencies have issued warnings about the heightened risk of introduction of RVFV into RVF-free countries [27, 37, 57, 69, 92, 140, 170, 224, 229, 280, 291]. These reports conclude unanimously that coordinated efforts to better prepare for a possible emergence of RVFV are needed.

This review provides a comprehensive update on RVFV, with particular attention devoted to the molecular epidemiology, virus genetics, vectors, diagnostic techniques and the pathogenesis of this significant veterinary and public health threat [22, 26, 85, 100, 253].

2. GENETIC ORGANIZATION OF THE RVFV

Like all bunyaviruses, RVFV is an enveloped RNA virus characterized by a genome composed of three segments designated L, M and S of negative or ambisense polarity. All the replication steps occur in the cytoplasm of infected cells and virions mature by budding in the Golgi compartment [207].

2.1. Structure of RVF virion and its genome

2.1.1. The virion

Early ultrastructural studies by electron microscopy and negative staining described RVFV particles measuring 90–110 nm in diameter [77]. The envelope is composed of a lipid bilayer containing the G_n and G_c glycoproteins forming surface sub-units, 5–8 nm in length, regularly arranged on its surface, similar to those reported for the related Uukuniemi phlebovirus [273]. The viral ribonucleoproteins (RNP) corresponding to each of the three genomic segments, associated with numerous copies of the nucleoprotein N and the RNA dependent RNA polymerase L, are packaged into the virion. More recent studies by cryo-electron microscopy on Uukuniemi virus and RVFV have modified the former view that phleboviruses are pleiomorphic [89, 118, 212, 238]. Instead these studies indicated that virions are likely to have an icosahedral symmetry: the structure is highly ordered and the surface covered by a shell of 120–122 glycoprotein capsomers arranged in an icosahedral lattice with $T = 12$. Three dimensional reconstructions at 22 or 27 Å resolution revealed that the capsomers resemble hollow cylinders situated at five- and six-coordinated positions. Inside the envelope, a layer of RNP is located proximal to the inner leaflet of the membrane, strongly suggesting an interaction between the cytosolic tail of the glycoproteins and the RNP which would compensate for the absence of matrix protein in the viruses of this family.

2.1.2. The viral genome

The RVFV genome is composed of three segments, L, M and S, which are presumed to be packaged together in the virions in the form of RNP. Sequencing indicated that the 3′ and 5′ terminal sequences are complementary to each other, forming panhandle structures, and explains the finding that RNP are circular when observed by electron microscopy [75]. The L and M segments are of negative polarity, coding respectively for the L protein, which is the viral RNA-dependent RNA polymerase [200], and

for the precursor to the glycoproteins [46, 47]. The S segment utilizes the ambisense strategy to code for two proteins, the nucleoprotein N and a nonstructural protein called NSs [104]. The coding capacity of the genome is depicted in Figure 1. The general view that only the viral genome is incorporated into the mature particle has been revisited since a small but significant fraction of the antigenomes i.e. replicative intermediates have been detected in purified RVFV particles [123]. These data correlate with past studies on Uukuniemi virus showing that the S segment of genomic and antigenomic polarities could be detected in purified virions [241].

2.2. The viral replication cycle: role and function of viral genes and their products

2.2.1. Transcription and replication

The general features of RVFV transcription and replication are similar to those of other negative stranded RNA viruses [74, 75]. During the replication cycle, each segment is transcribed into mRNA and is replicated through a process which involves the synthesis of the exact copy of the genome, called complementary RNA (cRNA) or antigenome. For phleboviruses, and RVFV in particular, the cRNA representing the copy of the S ambisense segment serves as a template for the synthesis of the NSs mRNA. Since the S cRNA is present in the input virus, the protein is expressed early, a good indication that it has an important role during infection. Messenger RNA synthesis is initiated through a cap-snatching mechanism whereas the synthesis of cRNA is initiated with 5′ nucleoside triphosphates. Furthermore, cRNA is the complete copy of the vRNA whereas mRNAs terminate in the non-coding region before the 5′ end of the template for the L and M segments or in the intergenic region for the S segment. The switch between the two activities of the L polymerase remains unknown; although several polymerase consensus motifs were found in the L protein [200], the different domains of the L protein responsible for the activities of cap-snatching or transcription termination have not been determined yet.
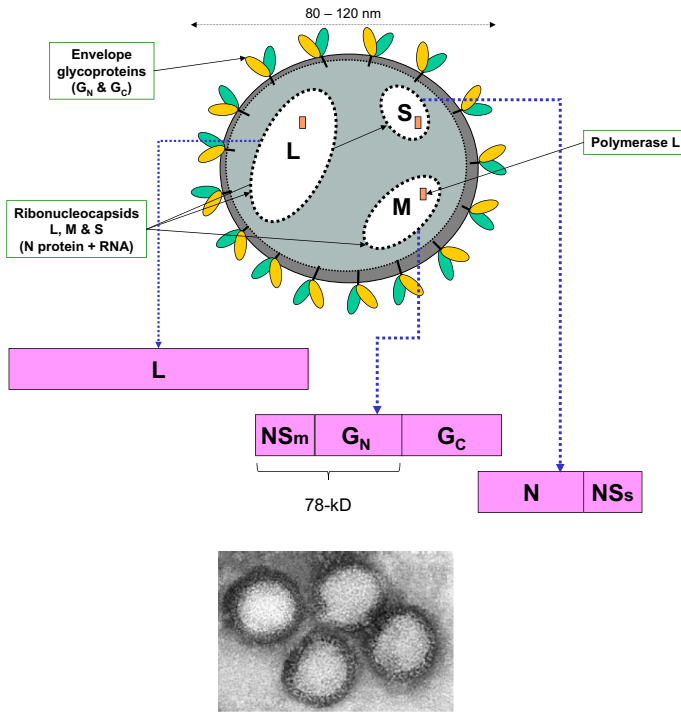
Figure 1. Schematic diagram of Rift Valley Fever virus (electron micrograph from Linda Stannard [258]). (A color version of this figure is available online at www.vetres.org.)

Systems to manipulate the RVFV genome and allow for the rescue of infectious viruses from cDNA have now been established by several different groups [17, 103, 107, 124]. Besides being an essential step in the development of reverse genetics, minigenomes mimicking a genome segment in which the viral ORF is replaced by a reporter gene have been helpful to analyze various steps in RNA synthesis, i.e. transcription, replication, transcription termination and packaging. Minigenomes can be expressed from T7 or PolI promoter-based plasmids. Expression under the control of the T7 promoter requires transfection in cells expressing the T7 RNA polymerase such as the BSR-T7/5 [48] or BHK/T7-9 cells [129] whereas expression from the PolI promoter is species specific so that the cells used for the study must correspond to the species of the promoter sequence [205]. Studies with minigenomes clearly established that transcription

and replication of the viral-like RNA requires the expression of the N and L proteins, which are necessary to reconstitute RNP, thus confirming the concept [161] that naked RNA cannot be transcribed. These data also indicate that L protein by itself or in association with N is able to assume both transcription and replication, excluding the possibility that the L protein had to be modified by a viral factor to function as a replicase. One should note however, that the RVFV NSs was reported to promote viral RNA replication and transcription in a minigenome system [122] but this particular characteristic was not confirmed[1]; instead, RVFV NSs was found to be inhibitory, like the NSs of the related Bunyamwera virus [281]. This difference may be due to the experimental conditions utilized by the two groups: RVFV

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[1] Bouloy M., unpublished data.

minigenome expression as a T7 versus PolI transcript in 293 versus BSR cells.

When compared to each other, the L, M and S segment-based minigenomes do not express identical levels of reporter gene, indicating differential promoter activities associated with the non-coding regions [97]. Although the mutagenesis was not as extensive as the one carried out for Uukuniemi virus [84], it appeared that some of the conserved nucleotides in the non-coding regions, particularly the first 8 terminal nucleotides and the purine at position 13, play an important role in promoter activity and the regulation of gene expression [226].

In contrast with most viral or cellular mRNA, bunyavirus mRNA are not polyadenylated. The M segment derived mRNA of the Sin Nombre hantavirus seems to be the only exception reported to date [120]. The bunyaviral mRNA terminate prematurely on their template, strongly suggesting that the transcriptases recognize a transcription termination signal. Preliminary data suggested that a conserved motif is present in the intergenic region of the S segment of Toscana, sandfly fever Sicilian and RVF viruses [104, 106], contrasting with other ambisense viruses where RNA hairpin structures serve as termination signals [162]. To further understand the termination process, Albarino et al. [3] mapped precisely the 3′ end of the 4 RVFV mRNA: N mRNA, NSs mRNA and the M- and L-derived mRNA, and identified a conserved sequence motif "3′-C_{1-3}GUCG/A" on both M and ambisense S segment sequences of several phleboviruses of the sandfly serogroup. To demonstrate the role of this sequence, they created a recombinant RVFV lacking the termination motif in the S segment by reverse genetics. Analysis of the mRNA synthesized in the cells infected with this mutated virus showed that the transcriptase failed to terminate the S mRNA correctly. Using a method of RNase protection assays to map the 3′ end, Ikegami et al. [125] came to a similar conclusion on the termination of the S and M segments. However, the two reports presented conflicting data on the L segment, which lacks the pentanucleotide motif in the 5′ non-coding region. Albarino et al. [3] reported that L mRNA represents the complete copy of its template whereas Ikegami et al. [125] claimed that the L mRNA lacks the last 16–41 nucleotides when compared to the full-length cRNA copy and that the termination signal corresponds to two 13-nucleotide-long complementary sequences present in the 5′ non-coding region of the L genomic segment.

2.2.2. The glycoproteins and their role in cell entry and particle formation

During the viral cycle, the glycoproteins play an essential role for the penetration of the virus and their proper processing is crucial for the maturation and budding of the virion. The glycoproteins, being the most exposed components of the virus during infection, are recognized by the immune system and induce the production of neutralizing antibodies, which play a predominant role in protection. The glycoproteins also mediate virus entry into many cell types through specific receptors which, in the case of RVFV and many other bunyaviruses, remain to be identified. Entry is predicted to employ a class II fusion mechanism that is activated by low pH following endocytosis of the virion [82]. Little is yet known regarding the early phases of infection that precede the release of virus RNP into the cytosol.

The RVFV M segment codes for a polyprotein precursor which, after cleavage, generates G_n (encoded by amino-terminal sequences of the precursor) and G_c (encoded by carboxy-terminal sequences) as well as two nonstructural proteins, the 78 kDa (also called NSm1) and the 14 kDa (NSm2) proteins. The question was raised of whether the 78 kDa protein is a structural protein [227], but a more recent work indicated that this protein was not detected in purified particles [89]. The NSm1 and NSm2 nonstructural proteins are produced by alternative use of the first or the second of the 5 in-frame AUG codons present at the 5′ end of the M mRNA and located upstream of the G_n sequence (Fig. 2). The first AUG initiates the synthesis of the 78 kDa NSm1 product which includes the sequence of G_n while the second AUG is utilized for the synthesis of the 14 kDa NSm2 which terminates before G_n. The first and fourth AUG precede
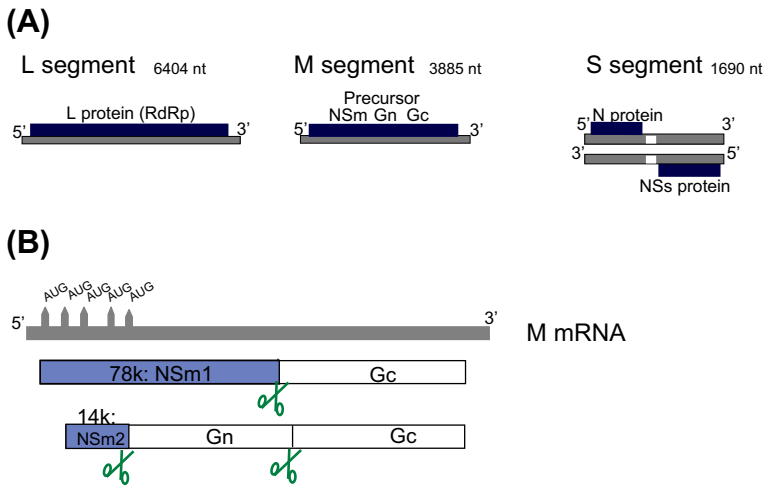
(A)

(B)

Figure 2. (A) Schematic representation of the Rift Valley fever virus genome. The antigenomic sense RNA and the encoded open reading frames (blue box) are represented. For the ambisense S segment, the genome and its open reading frame are represented (below). (B) Schematic diagram of the mRNA transcribed from the segment M of Rift Valley fever virus. (A color version of this figure is available online at www.vetres.org.)

a sequence typical for signal peptides, suggesting that NSm1 and G_n could be directed to the endoplasmic reticulum (ER). Wasmoen et al. [278] showed that, regardless of the presence of NSm1 and NSm2, G_n and G_c are localized in the Golgi complex, suggesting that the signals for Golgi localization reside in G_n and/or G_c. However, when expressed in the absence of G_n, G_c localizes to the ER due to the presence of a lysine-based ER retrieval signal at its C terminus [101]. This suggests that G_c moves to the Golgi apparatus via its physical association with G_n. Localization of the glycoproteins in the Golgi complex is essential for proper maturation of the bunyavirus particles which bud into the lumen of the Golgi. RVFV-infected hepatocytes seem to represent the only known exception as budding of particles was observed to occur at the plasma membrane as well as at the Golgi vesicles [5]. Late in infection, in most vertebrate cells, the Golgi complex undergoes morphological changes, with vacuolization and dispersion of small and large vesicles in the cytoplasm. Virions are then transported within these vacuoles to the cell surface where fusion of the vacuole and plasma membranes allows for the release of virus into the extracellular medium.

Minigenome systems, developed to assess transcription and replication activity, were also used to evaluate packaging activity. The glycoproteins, which are co-expressed together with the transcription machinery, allow formation of the viral like particles (VLP) which are released into the medium [108]. When observed by electron microscopy and negative staining, these particles resemble RVFV particles in size and morphology. They were able to infect naïve cells and to undergo the first step of the replication cycle, i.e., primary transcription. If both L and N are provided *in trans*, replication of minigenomes occur [204]. Interestingly, similar RVFV VLP were produced in insect cells infected with a dual baculovirus vector expressing G_n/Gc glycoproteins and the N protein. Another vector expressing N and only G_c was constructed but was found to produce particles of a more pleiomorphic nature, suggesting that both G_n and G_c contribute to the assembly process and likely interact with N [159].

2.3. The nonstructural proteins and their role in evasion of the innate immune response

2.3.1. The role of NSs in virulence

Remaining undetermined for many years, NSs has been identified as a major factor of virulence, primarily characterized as an interferon antagonist. The molecular mechanisms sustaining this phenomenon involve several cellular proteins interacting with NSs. One of them, SAP30 belongs to the Sin3A/NCoR/HDAC repressor complexes which intervene in gene transcription regulation. Moreover, it was shown that SAP30 interacts directly with YY1, a transcription factor involved in the regulation of expression of numerous genes, including IFN-ß [151]. Through a series of co-immunoprecipitation confocal microscopy and chromatin immunoprecipitations, it was demonstrated that NSs, SAP30, YY1, HDAC3 and Sin3A-associated corepressor factors are recruited on the IFN-ß promoter, excluding CBP (a coactivator known as CREB binding protein) loading and preventing histone acetylation and transcriptional activation. To ascertain the role of NSs interacting with SAP30 in this mechanism, a recombinant ZH548 RVFV, containing the specific domain of NSs required for the interaction with SAP30, was produced by reverse genetics. In contrast with the virulent ZH548 RVFV, this mutant, ZH548-NSsΔ210-230, was able to induce IFN-ß expression and was avirulent in the mouse model.

Efforts made during the last few years to investigate the role of NSs led to the concept that it is a multifunctional protein, enabling RVFV to evade the host antiviral response. A strategy to circumvent the host response relies on the interaction of the p44 subunit with the TFIIH basal transcription factor, which is sequestered by the NSs filamentous structure so characteristic of RVFV infection [50, 150]. As a consequence TFIIH cannot assemble and its concentration drops rapidly, explaining the drastically reduced transcriptional activity of cells infected with RVFV expressing NSs. Interestingly the two functions of NSs complement each other: first, the specific inhibition of IFN-ß gene transcription is implemented as early as 3–4 h post-infection (p.i.) a time at which IFN-ß would normally be synthesized. Later during the replication cycle, at a relatively late time (after 8 h p.i.), a second mechanism mediated through the interaction of NSs and TFIIH takes place to inhibit the general transcription of the infected cells.

The RVFV NSs protein is unique among bunyaviruses as it forms a filamentous structure in the nucleus, which is unexpected for a virus replicating in the cytoplasm [248, 250]. Investigations into the formation of the NSs filament showed that cellular DNA is predominantly excluded from NSs filaments except for some specific DNA regions of the host genome such as clusters of a pericentromeric gamma-satellite sequence where NSs interacts with DNA [167]. Targeting of these sequences by NSs was correlated with the induction of chromosome cohesion and segregation defects in RVFV-infected murine and ovine cells. Such phenomena may be responsible for the foetal deformities and abortions observed in infected animals, in addition to the necrosis of the placentome.

Very recently, a novel function of NSs was described simultaneously by Ikegami et al. [127, 128] and Habjan et al. [109]: NSs promotes post transcriptional downregulation of dsRNA-dependent protein kinase (PKR) and therefore prevents phosphorylation of eIF2α (eukaryotic initiation factor 2 alpha), phosphorylated eIF2α being known to lead to suppression of host and viral translation. Virulent RVFV was largely resistant to the antiviral action of PKR because NSs triggers the specific degradation of PKR via the proteasome. Together with the data showing that NSs suppresses cellular transcription, these studies on the downregulation of PKR highlight different strategies of NSs to prevent the innate antiviral host responses.

It was also observed that this activity is specific to RVFV NSs but not shared with other phleboviruses like sandfly fever Sicilian virus or the orthobunyavirus, LaCrosse virus [109]. This conclusion was based on the production of a recombinant ZH548 RVFV in which the NSs sequence was replaced by the heterologous NSs from the phlebovirus or the orthobunyavirus. One should note that, in contrast with the

PKR degradation activity, the IFN antagonistic activity of NSs is conserved among orthobunyaviruses and phleboviruses [76].

Altogether, it appears that NSs has multiple functions to counteract the host cell interferon response, either at the transcriptional or at the translational levels by degrading PKR, which in turn facilitates translation of the viral products [127].

2.3.2. The role of the NSm protein as a suppressor of virus-induced apoptosis

To determine the biological function of NSm1 and NSm2, an M segment-deletion mutant arMP12-del 21-384 was produced in which the first 3 initiating AUG were deleted. This virus was unable to synthesize the two nonstructural NSm proteins. Although its growth was similar to that of the parental virus arMP12, it induced extensive cell death and produced larger plaques than the parent [287]. Further analyses indicated that the deletion mutant triggered apoptosis through the caspase 3, 8 and 9 pathway, thus revealing that NSm has an anti-apoptotic function and contributes to pathogenesis. Interestingly, a study on Maguari virus showed that the NSm protein of this orthobunyavirus, which has no sequence homology with the NSm protein of phleboviruses or common coding strategy [148, 202], is not essential for growth in cultured cells [225]. It is not yet known if these NSm proteins from two distantly related viruses from distinct genera (Orthobunyavirus and Phlebovirus) share the same anti-apoptotic function. However, in this case, the same evolutionary pathway could have occurred to conserve the functions of both NSm and NSs among genera.

3. MOLECULAR EPIDEMIOLOGY

3.1. Influence of widespread virus movement, reassortment and introduction across natural barriers on RVFV genomics

The evolutionary history of RVFV is complex and has been influenced greatly by dramatic changes to the environment throughout Africa in the past approximately 150 years. Over that time, RVFV gene flow has been influenced on multiple levels ranging from the macroscopic (i.e., geographic dispersal) to the molecular (i.e., reassortment events) [14, 19, 21, 231–234, 239, 288]. Overall, the virus can be subdivided into at least 7 major genetic lineages (Fig. 3). While no exclusive correlation of virus genotype and geographic location can be observed, representatives from one area do tend to cluster together within each lineage [19]. However, virus strains with distant origins can be found within each of the 7 main lineages, which provide strong evidence of widespread dispersal and movement of RVFV genotypes throughout Africa. Striking examples of the magnitude of this long distance translocation can be found by the monophyletic linkage of isolates from regions as distant as Egypt, Madagascar and Zimbabwe or Kenya, Mauritania, Burkina Faso, Zimbabwe and South Africa (Fig. 3).

There have been at least three separate introductions of RVFV across significant natural geographic barriers. The large 1977–1979 Egyptian "virgin-soil" outbreak marked the first time the virus was recognized north of the Sahara desert and was associated temporally with construction of the Aswan High Dam along the Nile river [133, 183]. Later, the virus was isolated for the first time outside of continental Africa in 1979 across the Indian Ocean in Madagascar where the virus is now endemic [194–196]. More recently in 2000, the virus was introduced across the Red Sea into Saudi Arabia and Yemen and precipitated outbreaks among livestock and humans [10]. In each of these examples, firm phylogenetic linkages were established between RVFV collected during each outbreak and virus strains circulating a few years earlier within continental Africa [19, 234, 239]. The Arabian Peninsula introduction in 2000 is particularly worrisome, as it appears from the genomic data that the origins of this "virgin-soil" outbreak were closely linked to the large 1997–1998 east African epidemic/epizootic (Fig. 3) [19, 239]. After introduction, the virus likely circulated below the threshold of detection by livestock and public health authorities until favorable climatic conditions in 2000 provided an
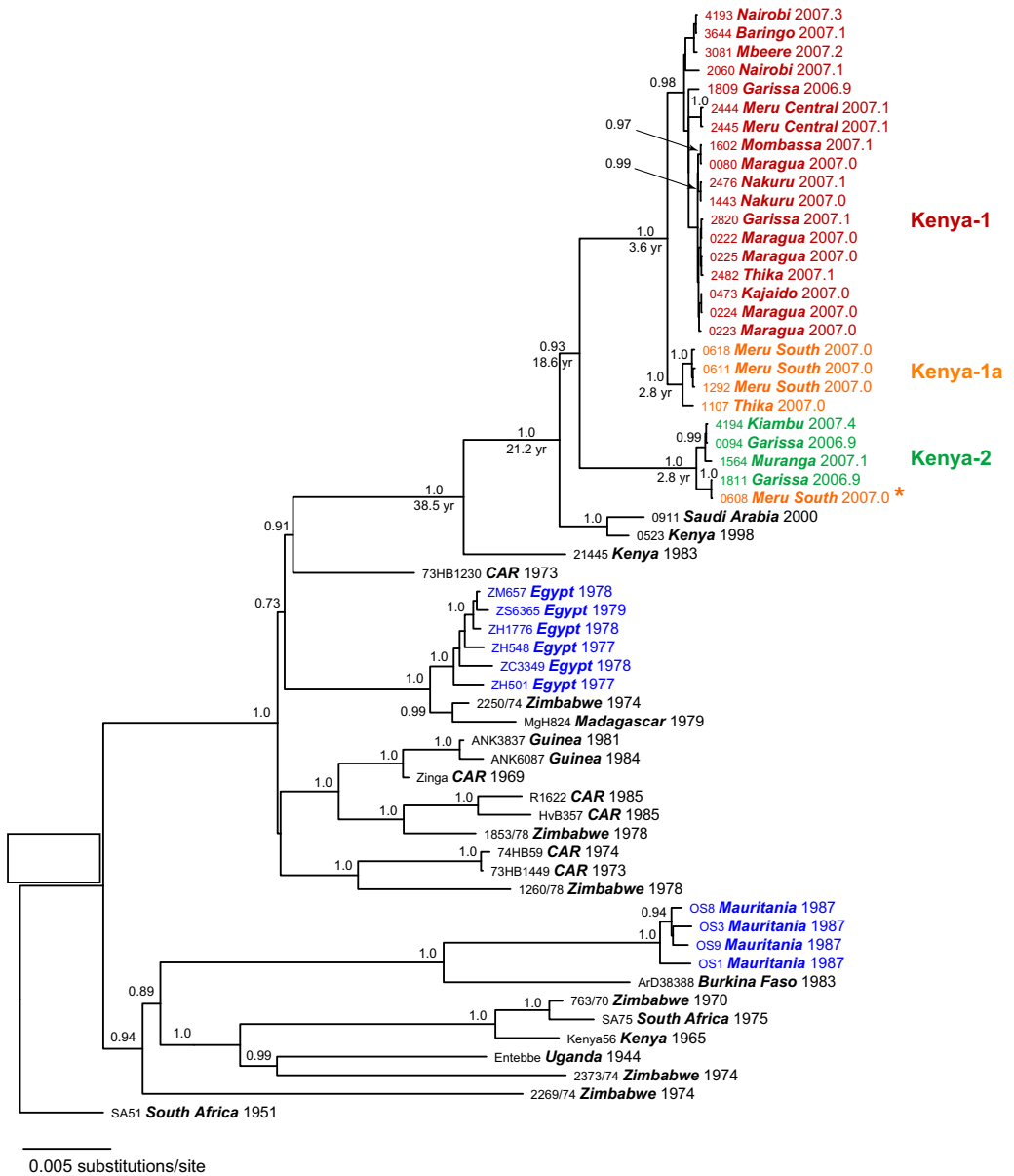
Figure 3. Rift Valley fever virus M segment maximum a posteriori clade credibility tree, MCMC chain length 9.0×10^7 steps, 2.25×10^6 steps (25%) removed as burn-in. Posterior support values (highest posterior density, HPD) are indicated as integers (i.e., 100% support = 1.0) above each node respectively. The calculated mean times to the most recent common ancestor (TMRCA) are indicated below each respective node and are enumerated as years before the collection date of the last outbreak specimen (May, 2007). The 2006–2007 Kenyan outbreak specimen M segment reassortant (strain #0608) is indicated by an asterisk. Adapted from [21]. (A color version of this figure is available online at www.vetres.org.)

opportunity for increased virus activity. It is clear that RVFV has crossed several significant physical barriers with impunity in the past and the potential exists for further introductions, especially given ongoing increases in the global movement of humans, livestock and mosquitoes.

Genomic rearrangement via RNA segment reassortment or homologous recombination are both potent mechanisms to promote genetic diversity and can allow for the emergence of novel RVFV strains. The reassortment of RNA genome segments among viruses of the family *Bunyaviridae* has been reported frequently in both in vitro and in vivo studies [15, 23, 28, 34, 102]. Reassortment among RVFV strains has also been well documented [19, 21, 231, 234]. However in sharp contrast, no evidence of homologous recombination among RVFV has been reported [19]. Potential reassortant events have been identified involving each of the three RVF genome segments (i.e., S segment: Lineage B viruses, M segment: Kenya 2006–2007 strain #0608, L segment: CAR strain 73HB1230) [21, 234]. The impact of these reassortment events on RVFV replication, fitness and, most importantly, host virulence is not fully known and requires further detailed study.

3.2. Genomic diversity and molecular evolutionary rate

A key feature of RVFV genomics is the relatively low genetic diversity: approximately 4% and 1% at the nucleotide and protein coding levels, respectively [19, 21, 231, 239]. This low diversity contrasts sharply with other bunyaviruses, including the tick-borne Crimean Congo haemorrhagic fever virus which was found to have approximately 32% diversity at the nucleotide level [31, 59]. The low genomic diversity of RVFV suggests that the virus either has a very low tolerance for mutation within its genome (i.e., an inherently slow molecular clock, or a "double-filter" selection mechanism) or alternatively that the extant viruses collectively identified today as RVFV have a relatively recent common ancestor. The molecular evolutionary rates (measured as nucleotide substitutions per site per year) were recently calculated for 60 complete genomes using a relaxed clock Bayesian algorithm [21, 67, 68]. The mean evolutionary rates and 95% highest posterior probability distributions (shown in parentheses), were 3.9×10^{-4} (2.4–5.5×10^{-4}), 3.6×10^{-4} (2.6–4.6×10^{-4}) and 2.8×10^{-4} (1.8–3.9×10^{-4}) nucleotide substitutions/site/year for the S, M and L segments, respectively, and were comparable with other arthropod-borne or mammalian host-restricted single-stranded negative-sense RNA viruses [21, 65, 132].

3.3. Recent ancestry and the influence of environmental change

Using the known date of collection for each virus specimen, the molecular evolutionary rate and the overall genomic diversity, it was possible to estimate the number of years prior to the present that the progenitor of the known RVFV was in circulation [67, 68]. Complete genome data from 60 naturally occurring RVFV specimens [19] revealed that the time to the most recent common ancestor (TMRCA) occurred in the recent past, with mean values of the TMRCA coalescing towards 120–130 years before the present, i.e., approximately 1880–1890. Such a contemporary origin was surprising, but is broadly consistent with the earliest case reports from Kenya in the early 1900s of a disease resembling RVF among animals [253]. During that time, major ecological changes were occurring in eastern and southern Africa, with the establishment of colonial agriculture systems and the importation of large numbers of highly susceptible European breed livestock [110, 163].

Taken together, the TMRCA and veterinary case reports support a hypothesis that at some time between 1850 and 1910, a previously unrecognized arbovirus ancestor of what we now know as RVFV exploited a newly formed ecological niche created by the sudden appearance of large concentrations of susceptible livestock. Since that time, the virus has subsequently established itself throughout large portions of eastern, western and southern Africa. The strong phylogenetic linkage of virus strains from distant locations suggests that, during the intervening years, the movement of

infected livestock and the natural dispersal of mosquitoes could have allowed the spread of RVFV throughout continental Africa, Madagascar and the Arabian Peninsula.

3.4. Insights into RVFV molecular epidemiology from the east African 2006–2007 outbreak

The large 2006–2007 epizootic/epidemic that occurred in Somalia, Kenya and Tanzania provided the first opportunity to conduct a detailed examination of RVFV molecular epidemiology during an outbreak [206]. During the epidemic period, a total of 1 062 human cases and 315 fatalities were reported throughout the region, along with dramatic losses to livestock production [285]. As part of the outbreak response, approximately 3 250 animals from Kenya (including cattle, sheep, goats, camels and various wildlife species) were tested for RVFV [21]. Of these, 289 (9.2%, primarily sheep, cattle, goats and African buffalo) were found to be acutely infected with RVFV by RT-PCR, antigen detection and IgM ELISA. The complete sequences of the S, M and/or L genome segments were obtained from a total of 31 virus specimens representing all affected regions of Kenya and spanning the entire known outbreak time period (December 2006 until May 2007).

All 31 virus specimens were monophyletic with a virus specimen (strain 0523) collected during the previous 1997–1998 east African outbreak and clustered with a larger east African lineage of RVFV (Fig. 3). This lineage has been present in Kenya since at least the early 1980s, as evidenced by monophyletic grouping of the S, M and L segments (with 100% support) with a RVFV (strain 21445) collected in 1983. Among the 2006–2007 viruses analyzed, two separate and highly supported sub-lineages (Kenya-1 and Kenya-2) were observed (Fig. 3). In contrast with more limited data from previous outbreaks, there was increased genomic diversity (~1.6% at the nucleotide level) relative to that observed among RVFV collected during the Egyptian 1977–1979 (0.3%) and Mauritanian 1987 (0.3%) outbreaks (Tab. I). This finding was unexpected and may reflect differences in the ecological/epidemiological factors that initiated each outbreak. The 2006–2007 Kenya outbreak occurred in a known endemic area, whereas the Egyptian and Mauritanian outbreaks likely resulted from limited or single introductions of RVFV onto "virgin-soil".

While the shared evolutionary history of the 1997–1998 and 2006–2007 outbreak viruses was apparently based on phylogeny, the TMRCA of the Kenya-1 and Kenya-2 lineages detected in 2006–2007 was found to be shortly before or during the previous 1997–1998 outbreak (Fig. 3). Surprisingly, further population genetics-based approaches revealed that the recent evolutionary history of the Kenya-1 and Kenya-2 lineages differed. Both lineages were more closely related to the 1997–1998 RVFV prototype than to each other, indicating ongoing and separate evolutionary patterns since the previous outbreak (Fig. 4). More detailed population genetics analyses revealed that the Kenya-1 lineage viruses had recently undergone demographic or spatial expansion, whereas the Kenya-2 lineage viruses had likely not (Fig. 4) [21]. Interestingly, the timing of the Kenya-1 expansion event was calculated to have occurred a few (2.1–3.7) years prior to the detection of the 2006–2007 outbreak event (Fig. 3). These population genetics-based estimates were found to correlate closely with data from climate models that incorporate normalized difference vegetation imaging that indicated the potential for enhanced RVFV activity risk during that time period[2]. Taken together, these results suggest that an undetected and significant demographic and spatial expansion of the Kenya-1 lineage occurred during the intervening years between the 1997–1998 and 2006–2007 outbreaks. These differing evolutionary patterns are consistent with potentially important biological differences in reproductive fitness existing between local subpopulations of virus within the natural environment.

During this outbreak, human and veterinary epidemiologic data revealed an apparent temporal course of virus activity shifting from the north-east border of Kenya towards the southwest and extending into Tanzania. At that time,

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[2] ftp://rvf:geis@pengimms.gsfc.nasa.gov

Table I. Comparison of M segment nucleotide (NT) diversity among RVFV collected during enzootic/ endemic and epizootic/epidemic time periods.

Country of origin	Date of collection	Virus activity	% nt difference
Zimbabwe	1970–1978	Endemic	4.7
Central African Republic	1973–1974	Endemic	1.8
Egypt	1977–1979	Epidemic	0.3
Mauritania	1987	Epidemic	0.3
Kenya	2006–2007	Epidemic	1.6
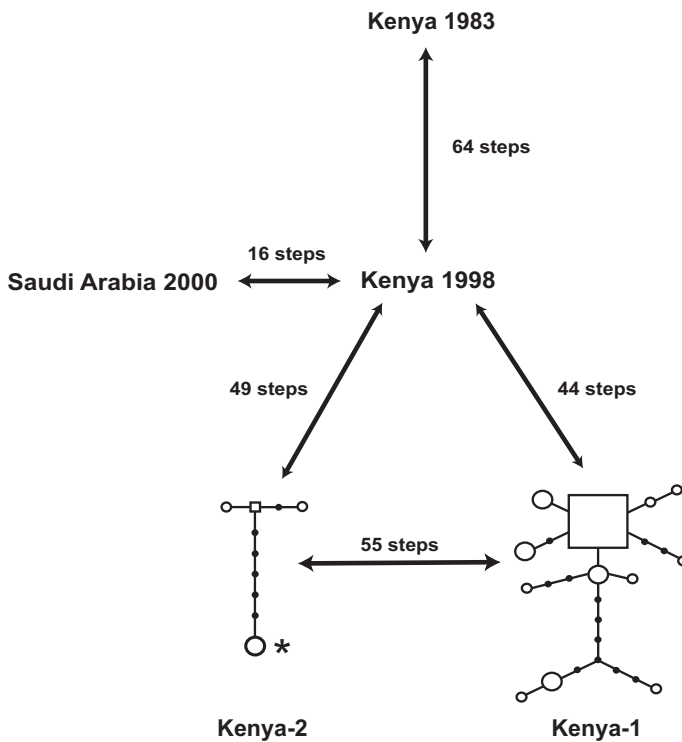
Figure 4. Minimum spanning networks (MSN) visually describing discrete genetic distance between unique haplotypes of the RVFV M genome segment within the greater east African lineage. Each node represents one nucleotide difference between extant (open circle) or inferred (black filled circle) haplotypes. Proportionally larger open circles or squares represent the relative number of extant haplotypes represented in the network. Generally, squares denote the predicted progenitor haplotype for each lineage, whereas circles indicate progeny haplotypes. Note the greater distance as measured in nucleotide changes (steps) between the Kenya-1 and Kenya-2 lineages than with the prototype Kenyan 1997–1998 RVFV strain. Also note the star-like phylogeny of the Kenya-1 lineage indicating the potential for increases in virus population size or geographic range. An asterisk indicates the relative position of the putative M segment reassortant virus (strain #0608). Adapted from [21].

it was unclear whether this shift was due to initial virus emergence in the north-east followed by direct "wave-like" spread of the virus via livestock or mosquito translocation or, rather, due to changes in rainfall patterns throughout the region, allowing for the eruption of local enzootic foci of virus activity. Among the 31 virus specimens analyzed, no significant evidence could be detected of a correlation between genotype, time of collection, or geographic origin [21]. This suggests that changes in rainfall patterns were more likely responsible for the apparent shift in activity rather than "wave-like" spread throughout the region.

The 2006–2007 data provide a unique basis for understanding the complex interplay between the environment, virus and susceptible hosts that allows for the establishment of RVFV endemicity and to precipitate outbreaks. It is clear that within the Kenyan ecosystem particular virus lineages can contribute to multiple periodic outbreak events over relatively long time periods. Although widespread and explosive RVFV outbreaks are hallmarks of the virus life cycle, the importance of a cryptic enzootic transmission cycle cannot be overstated and requires further study.

4. PATHOGENESIS AND IMMUNE RESPONSES

4.1. Natural infection in animals and humans

The natural disease has been well described in susceptible animals and particularly in ruminants since the first identification of RVFV in the Rift Valley of Kenya during an epizootic in 1930 [51, 52]. Numerous and well documented descriptions of the symptoms in naturally-infected animals have been recorded [42, 43, 253] and, consequently, detailed descriptions of the natural disease have not been conducted during more recent epizootics. Instead, the most recent descriptions of RVF epizootics have focused on the analytical and predictive epidemiology of the disease [38–40, 168, 169, 272]. A clear distinction can be made regarding the susceptibility to, and progression of the disease in young animals when compared with adults. The basis for these observed differences has not received clear explanation but might be driven by age-related susceptibility of the primary target cells of the virus that allows for more intense viral replication during early infection. Alternatively, differences in the underlying mechanisms of innate immunity that are stimulated by infection, as has been demonstrated with other infectious pathogens and disease models, may exacerbate susceptibility of young animals to RVFV [294].

The classical hallmark of RVF epizootics is the large number of near simultaneous abortions among pregnant ruminants, regardless of the stage of pregnancy. These massive abortion events have been referred to as "abortion storms" and allow for the differentiation of RVF from many of the other common infectious causes of abortion in ruminants such as: Q fever (*Coxiella burnettii*), chlamydiosis, salmonellosis, listeriosis or toxoplasmosis. In non-endemic countries, an active surveillance strategy employing the use of sentinel herds is cost prohibitive. However, robust passive surveillance-based systems that rely on the detection and rapid reporting of significant abortion events to national authorities (e.g., > 20% of pregnant animals in a herd suddenly aborting with accompanying signs of jaundice among survivors) could provide a cost effective means to detect the emergence of this significant veterinary and human health threat.

RVFV infection of animals can occur by the bite of an infected mosquito or by direct contact with infected animal tissues, bodily fluids and fomites, particularly if associated with abortions. Aborted foetal materials and placental membranes contain large numbers of virus particles which can either contaminate the local environment directly or infect animals in close contact. The RVFV may persist for relatively long periods in the environment as has been demonstrated during in vitro experiments [9, 49, 257].

The relative importance of each mode of transmission varies according to the stage of the epizootic: in the first stage, the bites of infected mosquitoes are the predominant mode of transmission whereas direct contact of animals with infected tissues (foetal or otherwise)

may become predominant during the amplification stage of the epizootic [191]. The relative importance of each mode of transmission still remains a bit controversial as some authors argue that the bites of infected mosquitoes constitute the main means of transmission of the virus, whatever the circumstances and even during a large outbreak [22, 25]. Exposure to infected tissues or bodily fluids constitutes the main route of infection for humans [253]. Transmission via infected mosquitoes remains important for the dissemination of RVFV between herds or flocks over short distances but also allows for the emergence and dissemination of the disease over longer distances, throughout a region or a country, but has to be preceded by the movement of infected animals [36] or by translocation of infected mosquitoes. Mosquito-borne transmission is also the most important mode during an enzootic cycle, i.e., without any clear signs of the disease but with an active circulation of the virus between susceptible animals.

The mechanisms and epidemiological importance of virus shedding from mammalian hosts is also a subject of debate. It is clear that the infectivity of blood during acute infection is high, with extremely high titres of virus up to $10^{8.5}$ mouse LD_{50} per 0.02 mL of blood at the peak of viraemia in sheep [173]). Aborted materials constitute another route of virus transmission, through direct contact with foetal envelopes, placenta and the foetus. Virus within these tissues may stay infectious over a period of a few days as RVFV particles are rather resistant to inactivation when in a protein-rich environment. In contrast, other sources of virus like nasal and lacrymal secretions have been suggested but not confirmed in controlled laboratory studies [274, 275]. The shedding of RVFV into milk has potentially large consequences for public health. The consumption of raw milk, such as during the small epidemic in Mayotte [242], is often reported as a potential risk factor for exposure to the virus. The probability for presence of the virus in milk during the viraemic phase has been confirmed experimentally but in later stages of infection, the presence of virus in milk is questionable

and, if it exists, the viral load would be low [70, 71]. To date, the presence of RVFV in the faeces or urine of infected animals has not been demonstrated, except when contaminated by frank blood.

In humans, detailed studies of RVFV infection were completed during several of the more recent epidemics that occurred in the Arabian Peninsula and in Africa. These recent outbreaks have provided an opportunity to examine confirmed RVF cases and investigate associated disease symptoms more precisely. In the vast majority of cases, infection with RVFV was asymptomatic. For the small proportion with clinical signs, the majority presented with an influenza-like syndrome without any severe sequelae. However, RVFV epidemics can involve hundreds if not thousands of individuals. The manifestation of severe RVF disease in humans is variable. Humans may develop a wide range of clinical signs including hepatitis, retinitis, delayed-onset encephalitis and, in the most severe cases, haemorrhagic disease. The overall case fatality ratio is estimated to be between 0.5% and 2%. Human cases with jaundice, neurological disease, or haemorrhagic complications are at increased risk of fatality [149, 164]. A summary of the frequency and clinical characteristics of a RVF epidemic that occurred in Saudi Arabia in 2000–2001 [11] in 683 patients [164] can be found in Table II. It is worthwhile to note that, in contrast to the massive abortion storms and extremely high fatality observed among young ruminant animals during epizootics, human children, pregnant women and neonatal infants seem to have been spared the disease. During the 2000–2001 RVF outbreak in the Arabian Peninsula, no child under the age of 10 years old was confirmed to have died as a result of RVFV infection. The underlying difference in susceptibility of young and pregnant animals with that observed in humans requires further study and raises an important question: are the dramatic differences in lethality the result of a lack of contact of children with infected mosquitoes or infected animals or are there true differences in susceptibility between young animals and young children?

Table II. Clinical features of 683 patients with laboratory-confirmed RVF in Saudi Arabia in 2000–2001 (adapted from [164]).

Variable	n^a/N^b (%)
Fever	499/539 (92.6)
Nausea	315/530 (59.4)
Vomiting	280/532 (52.6)
Abdominal pain	202/532 (38.0)
Diarrhea	118/530 (22.1)
Jaundice	96/530 (18.1)
CNS manifestations (confusion, lethargy, disorientation, vertigo, coma, tremor, convulsions, …)	81/475 (17.1)
Haemorrhagic manifestations (haematemesis, petechiae, bleeding, purpura, gingival bleeding, epistaxis, …)	35/494 (7.1)
Ocular complications (vision loss, scotomas, …)	10/683 (1.5)
Deaths	95/683 (13.9)

[a] n = Number of patients with symptom.
[b] N = Number of patients under active observation.

4.2. Lessons from animals models of RVF

Animal models of RVFV infection are important for reproducing natural infection in a controlled manner. These models have concerned various target and non-target animal species: ruminants (sheep, goats and cattle), laboratory animals (mice, rats and hamsters) and non human primates [22].

The incubation period following infection, ranges from a few hours to a few days and is dependent on multiple factors, including: the inoculation dose, the virus strain, the route of inoculation, the age of each animal and the animal species tested. When clinical signs do appear, there is a marked febrile response that may be very high (> 42 °C) and may last for 3 days (ranging from 0 to 5 days). During acute infection, the viraemic phase is another almost constant feature of experimental RVFV infection, but may vary in intensity and duration according to the inoculated dose, the virus strain and the degree of natural susceptibility

of the infected animals. Depending on the host's innate susceptibility or resistance, experimental infections often result in three scenarios [41, 56, 189, 198, 259, 289]:

– Scenario 1: Severe acute lethal infection. In this scenario the viraemia is uncontrolled, remains high and the infected animal dies rapidly. A clear relationship has been demonstrated between the viral load in blood (as assessed by real time RT-PCR or LAMP) – for loop-mediated isothermal amplification-PCR; see Section 6: "Old and new diagnostic tools for RVF" – and the final prognosis and outcome of infection [18, 152, 210, 223] in humans and in livestock. In these studies, humans with a high virus load at the time of presentation at hospital were found to be significantly associated with fatal outcome.

– Scenario 2: Mild to asymptomatic infection. The viraemia decreases rapidly, if present at all, and the infected animals recover their initial status without any long term sequelae.

– Scenario 3: Delayed onset complications of infection. After the first phase with fever and viraemia, a second phase can occur with an additional febrile phase and viraemia that may be the result of secondary dissemination of virus into other organs, particularly in the central nervous system, after crossing the blood–brain barrier, and in the retina, leading to a delayed onset of symptoms that are often associated with severe, long term consequences, including blindness, ataxia and potential fatalities.

The patterns observed during experimental infection of animals are similar to those reported for natural infections of animals and humans. In both natural and experimental infections, consistent elevations of liver enzymes and decreases in total leukocyte counts are associated with severe disease.

In both animals and humans, the primary site of RVFV-induced lesions is the liver. This finding is consistent among severe cases and has

been clearly demonstrated by histopathological examination of tissues of experimentally-infected sheep [44, 45]. The rapid onset of severe hepatic damage, particularly in ruminants, may explain many of the early clinical signs associated with severe RVF disease. Although RVFV is primarily hepatotropic, during severe infections the virus can be found in virtually all tissues and cell types [78], indicating that the as yet undiscovered cellular receptor is likely to be ubiquitous. In the absence of an efficient innate response (due to the mechanisms used by the virus for blocking the production of host-cell antiviral proteins induced by type I interferons via the NSs gene product; see previous Subsection 2.3: "The nonstructural proteins and their role in evasion of the innate immune response"), the virus causes marked cytopathic effect very rapidly following infection of the primary target cells, leading to multifocal to coalescing zones of hepatic necrosis with inflammatory infiltrates of immune effector cells. The finding of widespread hepatic necrosis is a classic hallmark of severe RVFV disease both in experimental [43, 44] and natural infections [78].

The primary tropism for the liver, after the presumed initial uptake of virions by immune system sentinel cells, may be altered when an aerosol route of exposure is employed. While not a typical route of exposure, this mode of infection has been described among slaughterhouse and laboratory workers [1, 33, 246]. In mice experimentally infected after inhalation of infectious aerosols, the lungs became the primary site of replication without any clear signs of pneumonia. However, after 48 h following experimental aerosol infection, the virus was isolated from the livers of infected mice that died as a result of a fulminant hepatitis associated, as already described, with a massive hepatic necrosis [29].

While infection via the oral mucosal surfaces has been documented [71, 72], attempts to infect lambs, kittens and puppies with RVFV-contaminated milk were not successful [141]. Infection of lambs via the intestinal tract by ingestion of RVFV-containing gelatin capsules was also unsuccessful but is a possible route of infection. However, the acidity of the stomach lumen is deleterious for RVFV, which is very susceptible to inactivation in solutions with a pH of less than 6.8 [143, 186].

Some animal models, particularly inbred strains of rats, have very striking differences in susceptibility to RVFV infection [4, 221]. These differences are not simply limited to differences between lineages of inbred strains but also depend on the commercial breeding source used in these studies [228]. As an example, Wistar-Furth (WF) rats were found to be very susceptible to experimental RVFV infection and Lewis (LEW) rats were resistant in one laboratory [6, 8, 221]; in another experiment using rats from an European breeding colony (mol), LEW/mol rats developed acute hepatitis and died after infection, whereas WF/mol rats survived the infection [228]. Genetic differences in susceptibility to RVFV infection, such as those observed in experimental infections of inbred rats, may explain in part the conflicting susceptibilities seen among different breeds of sheep under field conditions [80], although these results were not reproduced during controlled experimental infections [211, 259]). Previous experiments with back crosses of inbred rat strains suggest that the number of genes governing these susceptibility differences in the rat may be low [220] and suggests that it may be possible to select for resistant domestic animals through controlled breeding programs.

4.3. Immune responses

A robust innate immune response is critical for control of the initial phase of virus dissemination and eventual animal survival. A vigorous adaptive immune response is developed rapidly following infection, with the production of detectable neutralizing antibodies from the 4th–8th day after infection [117, 188, 193, 216]. These antibodies, which are primarily directed against the viral glycoproteins, G_n and G_c, are also accompanied by the production of IgM and IgG antibodies raised against the nucleoprotein, N, and the nonstructural protein, NSs [171]. The N nucleoprotein is the major immunogen in bunyavirus infection [79, 130] and represents the complement fixing antigen. While the titers of antibodies against the N
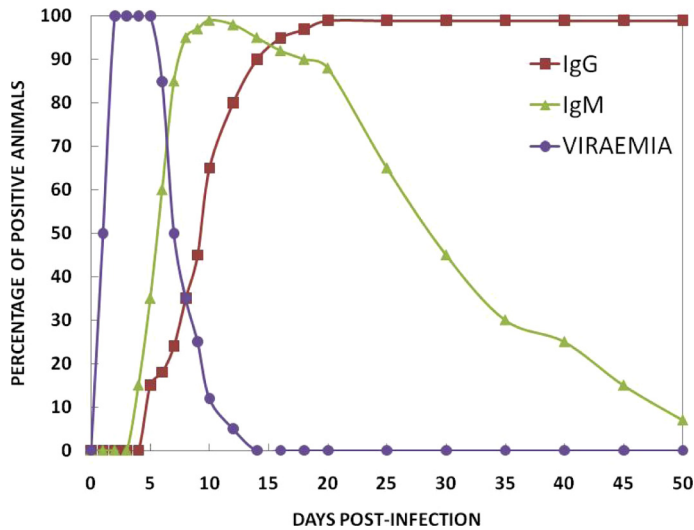
Figure 5. Schematic representation of time course of viraemia and antibody responses against RVFV in experimentally-infected animals. (A color version of this figure is available online at www.vetres.org.)

nucleoprotein are often quite high, the level of antibodies against the NSs protein is typically rather lower. The measurement of anti-N and anti-NSs antibodies can allow a DIVA (differentiation of infected and vaccinated animals) test to be developed as the animals vaccinated with an inactivated or a recombinant live attenuated vaccine such as the candidate clone 13 or one similar to those reported in Bird et al. [22] (which contained partial or complete deletions of the NSs gene [20, 201, 271]) do not produce antibodies against NSs [171] (see also Section 6: "Old and new diagnostic tools for RVF"). The titration of IgM antibodies is critical for detection of acute infections and can be coupled with the results of molecular (RT-PCR) and IgG detection assays to accurately stage the time since infection as IgM antibodies do not persist beyond the 50th day in the majority of cases after infection [209, 213]. However, important individual variation in IgM persistence has been demonstrated in cattle where IgM antibodies may persist for 5 months, so individual animal results must be evaluated within this context [197]. By combining virus isolation in cell culture or molecular detection of viral RNA with IgM and IgG antibody assays, it is possible to determine the stage of infection quite precisely

(see Fig. 5). The neutralizing antibodies are the key factor for the initial and persistent protection of infected animals [222] and are, consequently, a good correlate of the protection induced by vaccines against RVFV: if vaccinated animals produce a high level of neutralizing antibodies, they will very likely be protected against experimental challenge or subsequent natural viral infection under field conditions [73].

Deep knowledge regarding RVFV immunology is lacking and requires further detailed studies of both the innate, humoral and cell-mediated immune pathways.

5. VECTORS OF RVFV

RVFV has the potential to infect a remarkable array of vectors, including ticks and a variety of flies [53, 55, 88, 146, 153, 157, 158, 184, 268], unlike the majority of arboviruses which tend to be adapted to a narrow range of vectors [35, 60, 137, 166, 180, 187, 279, 283]. The propensity of the virus to develop significant viraemia in sheep, goats and cattle and to adapt to anthropogenic alteration of the environment, from development of irrigation schemes for

agriculture (and subsequent concentration of mosquito vectors) to intensive farming with livestock, may be responsible for this adaptability. Vectors of RVFV can be classified into "reservoir/maintenance" vectors, including certain *Aedes* species (spp.) mosquitoes (Diptera: Culicidae) associated with freshly flooded temporary [86, 87] or semi-permanent fresh-water bodies [98], and "epidemic/amplifying" vectors, consisting of *Culex* spp. associated with more permanent fresh–water bodies [178].

Tables III and IV list those species from which RVFV has been isolated in the wild, which have also been shown to be susceptible to and capable of transmitting RVFV in the laboratory. The minimum infection rates (MIR), based on the numbers of isolations per 1 000 adult female mosquitoes, support the epidemiological importance of *Aedes (Neomelaniconion) mcintoshi* (Huang, 1987), *Aedes (Aedimorphus) vexans* subspecies *arabiensis* (Patton, 1905), *Aedes (Aedimorphus) dentatus* (Theobald, 1904), *Culex (Culex) theileri* (Theobald, 1903) and *Culex (Culex) poicilipes* (Theobald, 1903) as RVFV vectors. The vector competence index (VCI) [136] allows for combining infection and transmission rates into a single statistic for comparing the experimental vector competence of vectors. This statistic illustrates the importance of the usual RVFV vectors, except for *Ae. mcintoshi*, the most important maintenance vector of RVFV in east and southern Africa. However, isolation, infection and transmission rates alone are not always sufficient to determine vector capability. Abundance, longevity, distribution and feeding behaviour are all important facets of what constitutes a good vector, as are inherent capabilities such as threshold susceptibility to infective virus. This is illustrated with *Cx. pipiens*, the RVFV vector responsible for the extensive 1977 Egyptian outbreak [115, 182]. Values of the MIR (Tab. III) are consistently low for this species. However, the VCI range from low to extremely high (Tab. IV) for the various experiments. A striking feature of Table IV is the range of IR, TR and VCI for *Cx. pipiens* and *Culex (Culex) antennatus* (Becker, 1903), the significance of which is not readily obvious. The Egyptian *Cx. pipiens* involved in transmission of RVFV is almost

certainly of the variety *molestus*, an unusually anthropophilic, endophilic form of the normally ornithophilic, exophilic type species. To complicate things further, *Culex (Culex) quinquefasciatus* (Say, 1823), which is morphologically similar to *Cx. pipiens* and behaviourally similar to *Cx. pipiens* var. *molestus*, also occurs in this area. The presence of all three taxa in Egypt, the source of the mosquitoes used in the vector competence experiments, may be responsible for these disparate results but this has yet to be demonstrated. Gargan et al. [94] demonstrated that degree of colonization of experimental *Cx. pipiens* had a significant effect on infection and transmission rates, with infection rates increasing (possibly due to artificial selection of more susceptible individuals) and transmission rates decreasing on average. Filial (F) generations of *Cx. pipiens* in the published experiments ranged from F_2 [177] to F_{67} [261]. The one thing the three members of *Cx. pipiens sensu lato* do have in common is widespread and abundant association with the settlements and irrigation canals along the banks of the Nile River and in its delta, in close association with humans and livestock and ideally situated to act as vectors.

Transmission of RVFV was associated primarily with a limited number of mosquito species prior to 1987, even though it had been isolated from a much wider range of arthropods, including *Simulium* spp. blackflies (Diptera: Simuliidae) [268], *Culicoides* spp. midges (Diptera: Ceratopogonidae) [55, 153] and *Amblyomma variegatum* ticks (Acari: Ixodidae) [88]. Experimental susceptibility and transmission studies extended the range of potential vectors to include *Phlebotomus* spp. sandflies (Diptera: Psychodidae) [64, 263], *Rhipicephalus appendiculatus* ticks [52] and a host of mosquito species [91, 96, 135, 176, 260, 261, 264]. However, the recognized vectors responsible for maintenance and amplification of RVFV included members of the subgenera *Neomelaniconion* of *Aedes* and *Culex* of *Culex* [98, 135, 144, 158, 172, 176, 179].

Member species of the mosquito genera, *Anopheles*, *Eretmapodites*, *Coquillettidia* and *Mansonia* have been implicated as vectors after isolations of RVFV had been obtained from

Table III. Minimum infection rates (MIR) for Rift Valley fever virus in adult female mosquitoes sampled in the wild.

Species	Field isolations			
	Locality	Sample size	MIR/1000	References
Ae. (Adm.) dentatus	Zimbabwe 1969	23	43.5	[172]
Ae. (Adm.) vexans arabiensis	Senegal 1991–1996	42055	0.2	[88]
	Saudi Arabia 2000	8091	0.9	[138]
	Saudi Arabia 2000	122	8.2	[185]
Ae. (Neo.) circumluteolus	South Africa 1955	4657	0.4	[144]
	Uganda 1955	1508	0.7	[282]
	South Africa 1981*	695	1.4	[134]
Ae. (Neo.) mcintoshi	Kenya 1981–1984	59644	0.2	[157]
	Kenya 1978–1979	12	83.3	[55]
	Zimbabwe 1969	3842	0.5	[172]
	South Africa 1974–1975	1315	1.5	[176]
Ae. (Neo.) palpalis grp.**	Central African Republic 1969	795	1.3	[63]
Cx. (Cux.) antennatus	Nigeria 1967–1970	unrecorded	(1)***	[153]
	Kenya 1989	250	0	[160]
	Kenya 1981–1984	4988	0.6	[157]
	Egypt 1977	121	0	[182]
Cx. (Cux.) neavei	South Africa 1981	1953	0.5	[179]
Cx. (Cux.) pipiens	Egypt 1977–1978	39150	0.03	[115]
	Egypt 1977	52629	0.04	[182]
	Kenya 1989	4987	0	[157]
	South Africa 1970	4833	0	[172]
	South Africa 1981	63	0	[179]
Cx. (Cux.) poicilipes	Senegal 1998–1999	24327	1.5	[62]
	Mauritania 1998–1999	4691	5.8	[62]
Cx. (Cux.) theileri	South Africa 1953	unrecorded	(2)***	[98]
	South Africa 1956	unrecorded	(2)***	[176]
	Zimbabwe 1969	103	9.7	[172]
	South Africa 1970	1398	2.9	[172]
	South Africa 1970	144	6.9	[172]
	South Africa 1974–1975	12738	0.9	[176]
Cx. (Cux.) tritaeniorhynchus	Saudi Arabia 2000	15428	0.4	[138]
Cx. (Cux.) zombaensis	South Africa 1981	6621	1.1	[179]
	Kenya 1981–1984	2326	0.4	[157]
	Zimbabwe 1978	24	0	[254]
	Kenya 1989	18828	0.3	[157]
Er. chrysogaster s.l./*Er.* spp.****	Uganda 1944	1865	1.6	[243]
Er. quinquevittatus	South Africa 1971	423	2.4	[172]
	Kenya 1981–1984	2660	0	[157]
Oc. (Och.) juppi	South Africa 1984	5425	0	[96]
	South Africa 1974–1975	2945	1.0	[176]
	South Africa 1987	656	0	[135]

* Mostly *Ae. circumluteolus* with only a few *Ae. Luteolateralis*.
** *Ae. palpalis* grp. = *jamoti* 62%: *crassiforceps* 21%: *carteri* 7%: *bolensis/palpalis* 10%.
*** Number of isolates; undisclosed sample size.
**** *Er. chrysogaster* s.l. (*chrysogaster/semisimplicipes/grahami/intermedius/mahaffyi/haddowi/harperi/gilletti*) 75%: *inornatus/penicillatus* 14%: *dracaenae* ssp. *ferox* 11%: *leucopus* ssp. *productus* < 1%.

Table IV. Experimental infection rate (IR), transmission rate (TR) and vector competence index (VCI) for Rift Valley fever virus in mosquito species from which RVFV has been isolated in the wild.

Species	Lab experiments				
	Origins	IR/ % (n*)	TR/ % (n)	References	VCI**
Ae. (Adm.) dentatus	South Africa	87 (39)	35 (34)	[135]	0.31
Ae. (Adm.) vexans arabiensis	Saudi Arabia	positive (15)	1/1 pool	[138]	
Ae. (Neo.) circumluteolus	South Africa	83 (69)	34 (53)	[179]	0.28
	South Africa	29 (63)	0 (4)	[174]	
	Kenya	76 (42)	18 (17)	[267]	0.13
Ae. (Neo.) mcintoshi	South Africa	39 (85)	17 (6)	[176]	0.06
	Zimbabwe	43 (7)	0 (2)	[174]	
	Kenya	50 (355)	12 (97)	[267]	0.06
Ae. (Neo.) palpalis	Central Africa Republic	86 (169)	54 (26)	[267]	0.46
Cx. (Cux.) antennatus	Egypt	84 (25)	38 (16)	[91]	0.32
	Kenya	60 (135)	60 (5)	[267]	0.36
	Egypt	92 (48)	7 (30)	[264]	0.06
Cx. (Cux.) neavei	South Africa	67 (61)	14 (22)	[174]	0.09
Cx. (Cux.) pipiens	Egypt	positive (100)	4/4 pools	[115]	
	Egypt	87 (15)	40 (15)	[182]	0.35
	Egypt	97 (143)	33 (118)	[81]	0.32
	Egypt	91 (64)	100 (8)	[267]	0.91
	Egypt	74 (346)	7 (102)	[264]	0.05
Cx. (Cux.) poicilipes	South Africa	90 (29)	15 (26)	[135]	0.14
	Egypt	56 (9)		[264]	
Cx. (Cux.) theileri	South Africa	96 (93)	55 (20)	[174]	0.53
	South Africa	94 (68)	56 (18)	[178]	0.52
	South Africa	83 (192)	27 (67)	[176]	0.22
Cx. (Cux.) tritaeniorhynchus	Saudi Arabia	73 (40)	17 (36)	[138]	0.12
Cx. (Cux.) zombaensis	South Africa	73 (192)	23 (106)	[179]	0.17
	Zimbabwe	75 (24)	40 (5)	[174]	0.30
	Kenya	71 (72)	16 (61)	[265]	0.12
Er. chrysogaster/intermedius***	Uganda	78 (92)	4 (92)	[245]	0.03
Er. quinquevittatus	South Africa	75 (146)	5 (22)	[174, 176]	0.03
Oc. (Och.) caballus	South Africa	32 (245)	0 (27)	[176]	
Oc. (Och.) caballus/juppi****	South Africa		33 (3)	[98]	
Oc. (Och.) juppi	South Africa	50 (12)	0 (3)	[96]	
	South Africa	49 (143)	5 (16)	[176]	0.03
	South Africa	22 (23)	0 (2)	[176]	

* n = Sample size.
** VCI = IR × TR, with a maximum value of 1.
*** Er. chrysogaster 92%: intermedius 8% based on a limited sample of males.
**** Most likely Oc. juppi as Oc. caballus is rare in the subregion.

field collections [51, 55, 63, 113, 156, 172, 176, 243, 286]. However, supporting evidence for a significant epidemiological role for these species has not emerged; vector competence experiments have shown these mosquitoes to be susceptible to RVFV but incapable of transmis-sion or nearly so [53, 91, 174, 175, 177, 178, 245, 264]. Instead, vectors in the subgenus *Aedimorphus* of *Aedes* were found to be responsible for a large outbreak in West Africa [87, 293], in particular *Ae. vexans* ssp. *arabiensis*. This mosquito was also shown to be the

Table V. Properties of the two existing animal vaccines against Rift Valley fever virus.

	Live-attenuated vaccine	Inactivated vaccine
Origin/Production	– Derived from the Smithburn vaccine strain (origin = Uganda) – Attenuated by successive IC[a] passage in newborn mice and in embryonated eggs (resulting in a neurotropic strain); produced in cell culture since 1971	– Derived from South African field strain – Inactivation with formaldehyde – Contains adjuvant
Advantages	– Needs only one injection – Long duration of protective immunity (entire economic life of animals) – Inexpensive production costs	– No adverse effects – No contraindications
Limitations	– May induce abortions and fœtal malformations → major contraindications for use in pregnant animals – Transient viraemia – Possible residual human pathogenicity – Possible reassortment with field wild–type virus strains	– Need two injections during the first year and booster doses annually – Short duration of protective immunity (necessitating annual booster doses) – Expensive production costs
Recommendations for vaccine use	– Prefer live vaccine in countries/regions where the RVF is enzootic – Vaccinate before the reproductive season	– Prefer inactivated vaccine in countries/regions newly infected or free but with a high risk of RVFV introduction
Common properties	– Do not allow the differentiation of vaccinated and naturally-infected animals (i.e., no « DIVA[b] test » available) – Age of vaccination: > 6 month of age	

[a] IC = Intracranial.
[b] DIVA test = "Differentiation of infected and vaccinated animals".

likely maintenance vector in Saudi Arabia during the emergence of RVFV in 2000 [138, 185]. The epidemic/amplifying vectors in these new foci were once again members of the *Culex (Culex)* subgenus, namely *Cx. poicilipes* [61, 62] and *Culex (Culex) tritaeniorhynchus* (Giles, 1901) [138].

The accepted transmission paradigm involves survival of RVFV in mosquito eggs through transovarial transmission from parous *Ae. mcintoshi* and *Aedes (Neomelaniconion) circumluteolus* (Theobald, 1908) females to their progeny during periods of drought, when the temporary water bodies dry up completely. These "floodwater" aedines oviposit on the soils surrounding the standing water and the eggs require a period of dehydration before they will hatch, potentially making them the ideal vehicle for survival of RVFV over long periods of time [16, 96, 155, 160]. When such habitats flood after rainfall, biological transmission occurs via infected mosquito saliva to domestic and wild herbivores of the family *Bovidae* (including cattle, buffaloes, sheep and goats) that may be attracted to the water supply. The vertebrate hosts are typically only viraemic for 2–7 days [56, 71, 173, 211], implying that the chronic infection of the invertebrate vector is more important for survival of RVFV from season to season; the vector apparently serves as the reservoir host. Provided the larval habitats remain flooded for more than 2 or 3 weeks, the floodwater *Aedes* are succeeded by *Culex* spp., which oviposit in small egg-rafts on the surface of the water. These eggs are unable to withstand desiccation. However, the egg rafts lead to a population explosion of *Culex* spp. mosquitoes, which become infected upon feeding on viraemic vertebrate hosts. Whereas the

floodwater *Aedes* spp. tend to remain in the immediate vicinity of the larval habitats and only feed at dusk and dawn, the more nocturnal *Culex* spp., e.g., *Culex (Culex) theileri* (Theobald, 1903), are more likely to disperse to find vertebrate hosts to feed on, leading to extensive dissemination of virus and the appearance of epidemics.

Perhaps the single most important habitat requirement in the ecology of RVFV is a shallow depression in the general topography, with water-saturated soil overlaying a poorly porous stratum (e.g., leached sands over granitic bedrock and calcic soils over clay-beds that produces standing water after heavy rainfall) [284]. Such habitats are distributed throughout the bushveld-savanna mosaic and higher-altitude grasslands of sub-Saharan Africa, where they are known colloquially as "dambos" or "pans" [2, 290] and are typically associated with sedges and grasses. However, suitable habitats are also to be found in shallow depressions in the flood-plains of rivers when floodwaters overflow the river-banks, especially in the coastal plains of eastern and southern Africa, as well as in the head-waters to rivers [155].

The West African experience differed from that seen in Kenya, Zambia, Zimbabwe and South Africa. The *Aedes (Aedimorphus)* spp. typically oviposit in the small, temporary ground pools that occur after localized rains. The region on either side of the Mauritanian/Senegalese border had not experienced good rainfall or floods prior to the 1987 outbreak and the typical vectors, *Ae. (Neo.) mcintoshi*, *Ae. (Neo.) circumluteolus* and *Cx. (Cux.)* spp. were not present in high numbers during the entomological investigations [88]. Conditions were more ideally suited to *Ae. (Aedimorphus)* spp., exemplified by *Ae. vexans*, *Ae. ochraceus* (Theobald, 1901), *Ae. dalzieli* (Theobald, 1910) and *Ae. cumminsii* (Theobald, 1903) [36]. At the time, a new dam wall had been constructed on the Senegal River; the subsequent flooding of the river banks was thought to have effected an increase in the vector and livestock population densities in the immediate vicinity of the dam [88]. With the advent of a second outbreak of RVFV in the region, investigators discovered a shift in the dominant mosquito species, namely towards *Mansonia uniformis*

(Theobald, 1901) and *Cx. poicilipes* [62]. *Mansonia* spp. are associated with gently flowing water and oviposit on the underside of the leaves and on the submerged roots and stems of African water lilies and water hyacinth (i.e., floating vegetation), while *Cx. poicilipes* breeds in reed beds in permanent water bodies [116]. It appears that the establishment of the dam on the perennial Senegal River was responsible for the production of *Cx. poicilipes* in sufficient numbers to trigger the second, larger outbreak.

The virus responsible for the 2002 RVF outbreak in the Arabian Peninsula [10, 203] appears to have been caused by introduction of the virus via imported livestock from East Africa [239]. Irrigation for agriculture in the Tihama regions of Yemen and Saudi Arabia and the proximity of the Jizan Dam provided ideal breeding grounds for both *Ae. vexans* and *Cx. tritaeniorhynchus* and grazing of sheep and goats along the wadis and irrigation systems in the foothills of the Sarawat Mountains provided amplifying hosts [138]. The danger of RVFV spreading into the Near and Middle East and, eventually, the European Union has been touted since the 1977 Egyptian outbreak [115] and its emergence in the Red Sea coastal plain of Yemen and Saudi Arabia was seen as a significant step in the northward migration of the virus. However, RVFV has yet to escape the Afrotropical Region, bounded as it is by the Sarawat escarpment and the arid inland plateau of Saudi Arabia. Nevertheless, it is essential that potential vectors are identified in the boundary region of the Mediterranean and Middle Eastern countries, requiring surveillance to monitor species composition, species abundance and vector competence to determine vector candidates. Much still needs to be discovered about the biology of the known as well as the potential vectors, including the hypothesis of transovarial transmission. Laboratory demonstration of transovarial transmission has been hampered by the difficulty of colonizing floodwater *Aedes* mosquitoes. However, field studies have shown infection of both male and female mosquitoes reared from field-collected larvae [157] and this needs to be confirmed and investigated under controlled conditions, especially considering the report [262] of *Cx. pipiens*, *Ae. mcintoshi* and *Ae.*

circumluteolus larvae becoming infected after feeding on RVFV-positive liver from an experimentally inoculated hamster.

6. OLD AND NEW DIAGNOSTIC TOOLS FOR RVF

There are increasing demands for high-quality and procedurally safe diagnostic tests for zoonotic pathogens to ensure the best protection of human and animal populations and to facilitate the free international trade of animals and animal products. The recently documented spread of RVFV and other vector-borne zoonotic pathogens beyond their traditional endemic boundaries has resulted in increased international demand for validated diagnostic tools and specific immunoreagents for the rapid diagnosis of RVF. This demand is greatly challenged by the fact that work with RVFV requires high biocontainment facilities and, preferably, needs to be carried out by vaccinated laboratory staff, but also because the virus is regarded as a potential bioweapon agent. For these reasons, the current capacity for laboratory diagnosis of RVF is restricted to a limited number of reference laboratories worldwide. RVFV belongs to the group of RNA viral haemorrhagic fever (VHF) agents that includes Ebola, Marburg, Lassa (and other arena-), Crimean-Congo haemorrhagic fever, yellow fever, dengue and hanta-viruses. In the absence of haemorrhagic or specific organ manifestations, infections by VHF viruses are clinically difficult to recognise, with the implication that definitive diagnosis depends mainly on reliable laboratory tests. RVF may be suspected when there is a sudden outbreak of febrile illness with headache and myalgia in humans, in association with the occurrence of abortions in domestic ruminants and deaths of young animals. However, cases of RVF in humans are sometimes only recognized late after infection from the occurrence of ocular complications. Late recognition of RVFV infections typically occurs when only sporadic cases are diagnosed during inter-epizootic periods. Haemorrhagic or encephalitic manifestations might be indicative of RVFV infection, especially in the rare instances where residents of other continents develop the illness following a visit to endemic areas in Africa. Experiences from the more recent outbreaks in Africa and the Arabian Peninsula appear to indicate that outbreaks of RVF in livestock are only recognized after diagnosis of the disease in humans.

Diagnosis of RVF is achieved using various techniques, including virus isolation [7, 240], antigen detection [181, 208] and nucleic acid amplification techniques [66, 93, 121] and by detection of specific antibodies [251]. RVFV is readily isolated from serum or whole blood during the febrile stage of the disease as well as from the liver, spleen and brain of fresh carcasses/cadavers or aborted foetuses. Isolation of the virus is achieved in hamsters, infant or adult mice and in various cell cultures [99, 253]. However, virus isolation procedures are lengthy and expensive. Delays in diagnosis based on traditional virus isolation and identification techniques may represent a significant problem for regulatory healthcare authorities faced with a RVF epidemic, especially in countries outside its traditional geographical confines. Hence, considerable efforts have recently been made to develop nucleic acid techniques for the rapid detection and identification of RVFV.

Highly sensitive PCR assays for the detection and quantification of RVFV have been reported, including RT-PCR [93, 121, 235] and real-time detection PCR (RTD-PCR) based on TaqMan probe technology [18, 66]. More recently, real-time reverse-transcription loop-mediated isothermal amplification assays (RT-LAMP) targeting the large RNA segment were developed and evaluated for the detection of a wide spectrum of RVFV isolates and clinical specimens [152, 223]. The RT-LAMP detection limit was reported to be 0.065 $TCID_{50}$ per reaction volume [152] and ~ 10 RNA copies per assay [223], and there was 100% agreement between the RT-LAMP, TaqMan-based RTD-PCR and virus isolation results [152]. Similarly, the assay had a very high diagnostic sensitivity and specificity when testing various clinical specimens from humans and animals that were naturally infected with the virus during recent outbreaks of RVF in Africa. The detection of specific viral genome targets in positive clinical

specimens using the RT-LAMP is achieved in less than 30 min. Apart from high analytical and diagnostic accuracy and speed of detection, another important practical advantage of the LAMP assay is that it utilizes simple and relatively inexpensive equipment which renders it promising for use in resource-poor settings and as a portable device during RVF outbreaks in remote areas [152, 223]. During the 2006 RVF outbreak in Kenya, quantitative real-time RT-PCR (qRT-PCR) was evaluated to identify patients with high viraemia, which is associated with poor prognosis [210]. This was the first report of qRT-PCR being used for case-confirmation and for correlation of RVFV-RNA levels, measured by qRT-PCR, with infectious virus titres. Fatal RVF cases had over 3-fold higher levels of viral RNA (mean = 8.6×10^6 viral RNA copies/mL of serum) and 3-logs higher infectious virus concentrations ($10^{5.2}$ infectious virus particles/mL of serum) when compared to non-fatal cases (means of 2.4×10^6 viral RNA copies/mL and $10^{2.3}$ infectious virus particles/mL of serum). The findings in Kenyan [210] and Saudi Arabian [18] patients sampled during the 2000 outbreak of RVF, indicate that qRT-PCR can be used for the rapid identification of patients with high viraemia and poor prognosis, thereby enabling them to be targeted for special or intensive clinical management.

One has to emphasize, however, that definitive diagnosis or exclusion of RVF, as of any other suspected VHF case, should not rely on a single PCR result. The nucleic acid detection assays should be run in parallel with additional tests, including detection of type-specific antibodies to RVFV. In this context it is important to note that viraemia in RVFV-infected individuals is of very short duration and most infected patients and adult ruminants undergo subclinical or mild infections; however, IgM and IgG antibodies are easily demonstrable shortly after exposure to the virus [213–215]. Furthermore, most nucleic acid techniques require highly specialized laboratory equipment, sophisticated reagents and well trained laboratory personnel, which conditions may not be available when outbreaks occur in remote regions and when rapid diagnosis is necessary.

Viral antigen can be detected rapidly in blood and other tissues by a variety of immunological methods, including agar gel immunodiffusion using homogenised tissues and immunostaining on impression smears or on cryostat sections of liver, spleen and brain; these assays allow the specific identification of the RVFV antigen in infected cells. Histopathological examination of the liver of affected animals reveals a characteristic cytopathology [99, 253]. Enzyme-linked immunosorbent assays (ELISA) for the detection of RVFV antigen have also been reported, but most of these assays were based on reagents that are cumbersome and expensive to produce and pose a biohazard risk to laboratory personnel [181, 208, 292]. Recently, Zaki et al. [292] reported on immunofluorescence assays which utilize a pool of mouse IgG monoclonal conjugates reacting with a combination of virus specific antigens (Gs, G_n, N, NSs). Although it was demonstrated to be highly reliable in detecting RVFV in patient sera, its use requires tissue culture amplification and handling of live virus. A number of laboratory infections with RVFV were recorded under circumstances which indicate the virus to be highly infectious for man [83, 142, 246]. To address this problem, a sandwich ELISA for antigen detection (sAg-ELISA) based on an entirely safe procedure, including a set of internal controls based on a recombinant nucleocapsid protein (recNP) for monitoring of routine assay performance, which increases its utility in surveillance and diagnosis in non-endemic areas, was recently reported [131]. The assay was developed for the detection of the nucleocapsid protein (NP) of RVFV in specimens that had been inactivated at 56 °C for 1 h in the presence of 0.5% Tween-20 (v/v) before testing. The sAg-ELISA has been used to detect strains of RVFV, that were isolated in geographically distinct areas of the world over a period of 53 years, with no cross-reactivity with the related African phleboviruses or other members of the family *Bunyaviridae*. The detection limits ranged from $\log_{10}10^{2.2}$ to $10^{3.2}$ TCID$_{50}$/reaction volume. Compared to virus isolation results in sera from RVF patients and experimentally infected sheep, the sAg-ELISA had 67.7% and 70% sensitivity, and 97.97% and 100%

specificity, respectively. The assay was 100% accurate when testing tissues of various organs from experimentally infected mice and naturally infected buffalo foetuses. The assay was able to detect NP antigen in infected culture supernatants 12 to 30 h before cytopathic effects were observed and as early as 8 h after inoculation with $10^{5.8}$ TCID$_{50}$/mL of RVFV. This ability renders the assay suitable for rapid identification of the virus when its primary isolation is attempted in vitro. As a highly specific, safe and simple assay, the sAg-ELISA represents a valuable diagnostic tool for use in less well-equipped laboratories in Africa and for routine differential diagnosis of VHF [131].

Serum specimens are commonly used for RVF diagnosis. Viraemia titers ranging from $10^{5.6}$ to $10^{9.0}$ mouse LD$_{50}$/mL have been recorded in domestic ruminants [12, 51, 111, 188, 252], $10^{8.6}$ mouse LD$_{50}$/mL in humans [220] and $10^{5.4}$ TCID$_{50}$/mL in adult African buffalo [56]. Although viraemia in RVFV-infected individuals reaches high titres, it is of short duration, limiting its use for viral detection systems for RVF outbreak diagnosis. Therefore, attempts to detect recent RVFV infection by ELISA should include a combination of assays which target both viral antigens and IgM antibody. In contrast, RVFV can persist at high titers for 21 days in ovine brain and liver and up to 30 days in spleen [289]. The high diagnostic accuracy of the sAg-ELISA for detecting RVFV in infected tissues, which usually contain virus concentrations at least 10- to 100-fold times above the detection limits determined for the assay [70, 111, 188], thus renders it ideal for testing specimens from aborted foetuses and carcasses/cadavers. Sudden, high abortion and fatality rates in young animals are characteristic features of RVF outbreaks.

Diagnosis of infectious diseases can be made when serological tests are used in combination with clinical observations and, epidemiological history or when seroconversion is demonstrated. Serodiagnostic techniques are also widely used to demonstrate freedom from a disease and in epidemiological investigations. The classical methods for the detection of antibodies to RVFV include haemagglutination-inhibition,

complement fixation, indirect immunofluorescence and virus neutralization tests (VNT) [99, 251]. The disadvantages of these techniques include health risks to laboratory personnel [142, 177, 246, 268] and restrictions for their use outside RVF endemic areas. Diagnosis of recent infection is confirmed by demonstrating seroconversion or a 4-fold or greater rise in titre of antibody in paired serum samples, or by demonstrating IgM antibody activity in an ELISA [215, 217].

Although regarded as the gold standard, the VNT is laborious, expensive and requires 5–7 days for completion. It can be performed only when standardized stocks of live virus and tissue cultures are available. Consequently, it is rarely used and then only in highly specialized reference laboratories. However, from the point of view of using the VNT as a diagnostic discriminator in validation studies, it is important to note that infection with RVFV induces life-long neutralizing immunity and that there is no evidence for the existence of serological subgroups or major antigenic variation between virus isolates of disparate chronologic or geographic origins [253]. The VNT is highly accurate with little or no cross-neutralization with other phleboviruses [54, 249, 251, 255] but since it requires live virus, it can only be done in suitable biocontainment facilities.

Various ELISA formats developed in recent years for the specific detection of IgG, IgM or total antibodies, based on inactivated sucrose-acetone-extracted antigens derived from tissue culture or mouse brain were shown to be highly accurate diagnostic tools in disease surveillance and control programs, import/export veterinary certification and for monitoring of immune response in vaccines [32, 213–216, 219]. As highly accurate, robust and safe tests, they have the potential to replace traditional diagnostic methods which pose health risks and necessitate their use being restricted to high containment facilities outside RVF-endemic areas. However, the production of antigen for these assays also requires bio-containment facilities to limit the risk of exposure of laboratory personnel to infection. To address these problems, an indirect ELISA based on the recNP of RVFV has recently been developed for the detection of

specific antibodies in human and animal sera [79, 130, 217, 218]. The nucleocapsid protein appears to be highly conserved among members of the *Bunyaviridae* family [97, 165, 237, 269] and antigenic cross-reactivity studies in animals [54, 249, 252] and the indirect ELISA based on recNP [217] failed to provide any evidence that other African phleboviruses could obscure the reliable serodiagnosis of RVF. As the most abundant and highly immunogenic viral component in the RVFV virion, NP seems to be the best choice for the development of immunoreagents for antigen detection assays. The fact that a soluble, highly purified recombinant RVF NP can be produced easily in large quantities [130], will allow for less expensive, fully automated mass-screening of sera. Research efforts aimed at the development and validation of a new generation of safe diagnostic immunoreagents and assays, for example ELISA formats based on RVFV recombinant antigens, are strongly recommended since cloning and expression of RVFV antigens would avoid the risk of laboratory infections and of residual virus in the test reagents, making them safe for routine use in RVF-free areas. However, it still remains to be proven through extensive validation studies that recombinant antigen-based ELISA would have at least the same diagnostic accuracy in livestock populations from various geographic regions as ELISA based on the whole inactivated antigen of the virus.

7. PREVENTION AND VACCINES

7.1. Existing vaccines

Because of the socio-economical impact of RVF in Africa, it appeared that vaccines, at least for veterinary use, were urgently needed. The first vaccine, still in use, was developed by Smithburn [244] who adapted the Entebbe isolate by serial intracerebral inoculation of mice to attenuate the virulence, a procedure first used by Theiler to produce the yellow fever 17D vaccine [256]. However, the passaged virus, called the Smithburn strain, had only partially lost its virulence: it induced abortions and tera-

togenesis in ewes, cows and goats [24, 139, 154, 253], so that its use is mainly restricted for use during devastating outbreaks and only in non-pregnant female animals [13, 253]. To circumvent these difficulties, an inactivated vaccine has been produced, allowing the safe vaccination of animals, but this vaccine is not as efficacious as the attenuated vaccines and needs booster inoculations [12] (Tab. V). After the Egyptian outbreak in 1977, USAMRIID initiated research to produce a new RVFV vaccine. The attenuated MP12 was obtained from the virulent Egyptian strain (ZH548) after random mutagenesis with 5-fluorouracil during 12 successive passages [30]. This virus acquired mutations in all three segments and had lost its virulence when tested in mice [236, 270]. Moreover, it was shown to induce a good immunity in ruminants after experimental inoculation [190, 192, 193]. However, trials carried out in South Africa using pregnant ewes revealed some abortions and teratogenesis during the early stages of pregnancy [119]. This might corroborate data reporting that MP12 still had some neurovirulence when inoculated into hamsters, which constitute a very sensitive animal model [230]. At the present time and in spite of these data, MP12 is still being developed as a veterinary and human vaccine. An alternative candidate based on another RVFV isolate, Clone 13, was found to be avirulent due to a large deletion in the NSs protein [201]. This virus grows to high titers in cell cultures and the deletion in the sequence coding for the virulence factor made it attractive due to its inability to revert. Vaccination trials in sheep, including ewes, and in bovines were carried out. Not only did the virus not provoke any deleterious effects but the animals elicited a high antibody response and were protected against a virulent challenge. Pregnant ewes were also protected from abortions, which were observed in the unvaccinated control animals[3].

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7.2. Designing new vaccines

While Clone 13 is a natural virus originating from a mild RVF case, it is now possible to manipulate the viral genome via reverse genetics to produce similar or different viruses, opening new ways to abrogate its pathogenicity [26]. This has been demonstrated by producing a rMP12 in which the S segment carries a mutation identical to the one in Clone 13 [124] or by removing the complete NSs sequence from MP12 or from the otherwise virulent backbone of the strain ZH501 [20]. In addition, because NSm plays a role as an anti-apoptotic factor, a double mutant ΔNSs/NSm abrogating expression of both NSs and NSm proteins was created and shown to be avirulent and to confer complete protection against virulent virus challenge at dosages up to 1.0×10^4 LD$_{50}$ in the rat model [20] and in mice[4].

Sub-unit recombinant vaccine candidates expressing the RVFV glycoproteins have been described: different vectors such as the lumpy skin disease virus [276, 277], an alphavirus [105, 112], a non-replicating adenovirus [114], or the Newcastle disease virus [145] were used. Other groups have developed sub-unit vaccines by expressing the RVFV glycoproteins which can assemble into "empty" virus-like particles or VLP [58, 108, 159, 204]. For efficient production, recombinant baculoviruses were constructed expressing the glycoproteins alone or in association with N [159]. DNA-based vaccine administrated by gene gun has also been described but required several reimmunizations [147, 247, 277].

Interestingly, most of the vaccine candidates, whether live-attenuated virus or sub-unit vaccine, have some genes missing when compared to natural strains circulating in the wild. This was also the case with Clone 13, its truncated NSs being degraded rapidly by the proteasome [271]. This raised the question of vaccine being able to distinguish infected from vaccinated animals (DIVA) [20]. An ELISA based on the detection or non-detection of antibodies against the NSs protein was developed recently [171]. The test was evaluated using sera from experi-

mentally infected rats and from a small number of ruminants which had antibody against the NSs protein, although of low titer. Considering that NSs is poorly immunogenic, a survey of a large number of animals should be done to determine if a high proportion of naturally infected livestock elicits a detectable and long-lasting immune response against this protein.

8. CONCLUSION

This review demonstrates that RVFV has become an important subject of interest over the past three decades and particularly in recent years, as public health agencies have become alerted to the possible emergence of this arbovirus in temperate countries. Climate change [170] and the presence of competent vectors in currently RVF-free temperate countries [199, 266, 280] suggests strongly that RVFV should be included among the most significant emerging viral threats to public and veterinary health. Recent insights into the virus' pathogenesis, molecular epidemiology, diagnostics and the increasing number of vaccine trials using various modern approaches, including reverse genetics, recombinant vectors, VLP, subunit vaccines and DNA vaccines [26, 126], have contributed greatly to our understanding of this significant viral pathogen. Despite these efforts, safe and efficient vaccines, both for medical and veterinary use, are still lacking, even though they are essential for the protection of animal and human populations and reduction of the dramatic health impacts of outbreaks, wherever they may occur. The recent outbreaks in Egypt and in the Arabian Peninsula have illustrated the potential of RVFV to spread to previously unaffected areas, leading to the following warning, cited by Zabransky (2005) [291] by Dr Corrie Brown (University of Georgia, USA): "*If we get Rift Valley fever* [in the USA], *it would make West Nile look like a hiccup.*"

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