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Review

Understanding Rift Valley fever: Contributions of animal models to disease characterization and control

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ABSTRACT

Rift Valley fever (RVF) is a mosquito-borne viral zoonosis with devastating health impacts in domestic ruminants and humans. Effective vaccines and accurate disease diagnostic tools are key components in the control of RVF. Animal models reproducing infection with RVF virus are of upmost importance in the development of these disease control tools. Rodent infection models are currently used in the initial steps of vaccine development and for the study of virus induced pathology. Translation of data obtained in these animal models to target species (ruminants and humans) is highly desirable but does not always occur. Small ruminants and non-human primates have been used for pathogenesis and transmission studies, and for testing the efficacy of vaccines and therapeutic antiviral compounds. However, the molecular mechanisms of the immune response elicited by RVF virus infection or vaccination are still poorly understood. The paucity of data in this area offers opportunities for new research activities and programs. This review summarizes our current understanding with respect to immunity and pathogenesis of RVF in animal models with a particular emphasis on small ruminants and non-human primates, including recent experimental infection data in sheep.

1. Introduction

Rift Valley fever (RVF) was first described in 1931 as an "enzootic hepatitis of sheep" after an outbreak in a farm near the shores of Lake Naivasha in Kenya. By that time it was already known that the disease had existed for some years in the Kenyan Rift Valley and had been responsible for extensive losses in the sheep population, particularly coincidental with "wet" years (Daubney et al., 1931). In fact, a similar unrecognized sheep disease in the same geographical area was reported in 1912 by R. E. Montgomery who described an outbreak of a sheep disease associated to liver necrosis (Montgomery, 1913). Over the last three decades RVF virus has spread throughout Africa and since 2000 the geographic extent of the disease expanded to the Arabian Peninsula (Ahmad, 2000) and some Indian Ocean islands such as Madagascar, Comoros and Mayotte (Andriamandimby et al., 2010; Roger et al., 2011; Sissoko et al., 2009) with the most recent epizootic occurring in Mauritania (Sow et al., 2014) (Fig. 1). These epizootics come with devastating impacts for livestock production, causing particularly high rates of neonatal mortality and abortion in ruminants. Effective live attenuated RVF virus vaccines are available for livestock use, although safety issues preclude their distribution to non-endemic RVF areas (reviewed in Ikegami and Makino, 2009; Indran and Ikegami, 2012).

RVF in humans was initially recognized in individuals involved in sheep herding and in those handling infected animal tissues during investigations of the disease in livestock. These individuals suffered from a flu-like syndrome with fever, joint pains and headache. Human morbidity has since been consistently reported following RVF epizootics in livestock, with a proportion of infected individuals developing severe disease manifestations such as retinitis and transient loss of vision, encephalitis, neurological symptoms and fatal haemorrhagic fever with thrombocytopenia (Ikegami and Makino, 2011). In recent outbreaks, case fatality rates >20% have been reported in different geographical settings (Al-Hazmi et al., 2003; Hassan et al., 2011) but there is currently no licensed RVF vaccine for use in humans. Nonetheless, formaline inactivated vaccines were also developed for human use (Randall et al., 1964; Randall et al., 1962). These vaccines were tested in human volunteers with few adverse reactions but they require several booster doses to maintain serum neutralization titres (Pittman et al., 1999). It is expected that new developments for safer and more efficient human vaccine designs will be brought in the near future.

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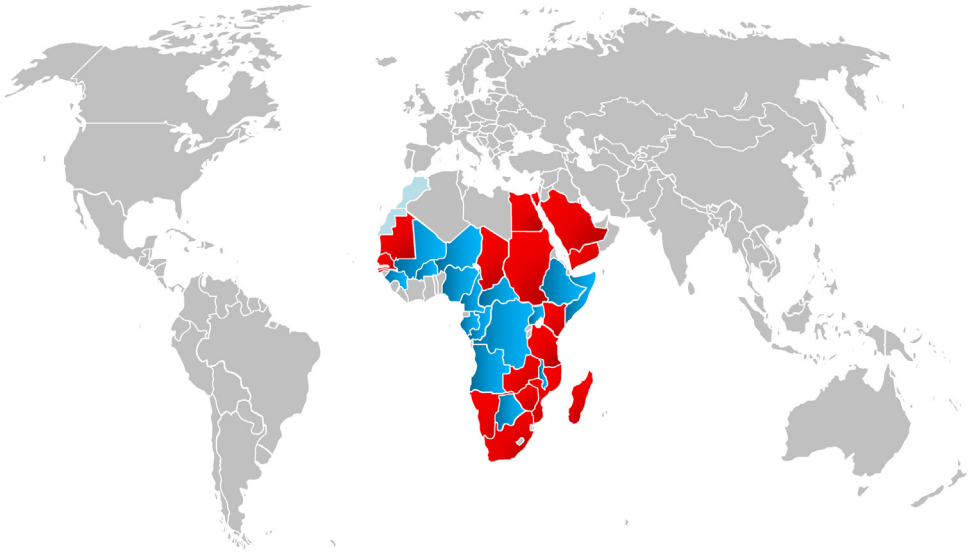

Fig. 1. *Current global distribution of RVF*. After the first description in Kenya the disease has since been reported in many African countries, the Arabian Peninsula, Madagascar and other Indian Ocean islands. Countries where important RVF outbreaks ocurred are shown in red while those countries where both seropositive animals and occasional virus isolation has been reported are shown in blue. In light blue, countries where sero-positive animals (camels) have been recently detected (Di Nardo et al., 2014; El-Harrak et al., 2011) but not reported virus isolation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Rift Valley fever virus

RVF virus (RVFV) is a negative-sense, single-stranded, tripartite RNA phlebovirus that belongs to the family *Bunyaviridae* (Nichol et al., 2005). It can be transmitted by many mosquito species, some with a global distribution, in part explaining its capacity to spread and establish in new geographical settings. In particular, floodwater *Aedes* mosquitoes are believed to transmit RVFV trans-ovarially allowing maintenance of the virus in mosquito eggs for long periods of time (Davies et al., 1985; Logan et al., 1991). This property contributes to the persistence of RVFV in nature during inter-epizootic periods when climatic conditions do not allow egg hatching. Climatic conditions are indeed a major driving force of RVF outbreaks as supported by the strong link between disease occurrence and periods of intense rainfall and flooding (Fig. 2). Certain climate-based risk mapping models have found utility in prediction of future RVF outbreaks (Anyamba et al., 2009, 2010). Upon eclosion, infected Aedine mosquitoes can then bite wild ungulates or free-range livestock. Viremic animals can be bitten also by competent Culicine mosquitoes, therefore amplifying efficiently the virus allowing further spread to animals or humans.

The RVFV particle contains three genomic segments. Studies in mammalian cells concluded that the large (L) segment encodes a viral RNA-dependent RNA-polymerase (RdRp). The medium (M) segment encodes a polyprotein, which is co-translationally processed to give rise to two surface glycoproteins, Gn and Gc, and two non-structural proteins of 78 kDa and 14 kDa (termed NSm). The small (S) segment encodes the viral nucleoprotein (N) and a virulence factor responsible for repressing innate host immune responses (NSs). N and NSs are encoded in an ambisense orientation on the S segment, a characteristic feature of Phleboviruses. While both N and L proteins are essential for viral transcription and replication, both Gn and Gc associate with host cell membranes to constitute the viral envelope (Fig. 3). The NSs protein, expressed very early upon infection, acts as a repressor of host cell transcription in many ways (Table 1) and is involved in the induction of cell-cycle arrest by activation of DNA damage signalling checkpoint protein kinase ATM (Baer et al., 2012).

Recent evidence suggests that the 78 kDa protein may be incorporated in the viral particles when the virus in propagated in mosquito c6/36 cells, perhaps facilitating the ability of the virus to infect mammalian cells upon mosquito bites (Weingartl et al., 2014c). On the other hand the 14 kDa (NSm) protein has been shown to play a role in the suppression of apoptosis in infected cells (Won et al., 2007) and to associate with mitochondrial outer membranes (MOM) (Terasaki et al., 2013). Also, a role in vector competence has also been described for the Nsm protein (Kading et al., 2014) and its interaction with several murine proteins demonstrated, including the cleavage and polyadenylation specificity factor subunit 2 (Cpsf2), the peptidyl-prolyl *cis–trans* isomerase (cyclophilin)-like 2 protein (Ppil2) and the 25 kDa synaptosome-associated protein (SNAP-25) (Engdahl et al., 2012).

Both NSm and NSs proteins are not essential for virus replication and propagation in cell cultures. In fact, natural NSs deletion mutants have being found with an attenuated, avirulent phenotype (Muller et al., 1995). In addition, the availability of reverse genetics techniques for RVFV has allowed obtaining deletion mutants lacking NSm, NSs or both proteins. These mutant viruses have proven stable in propagation in cell cultures while retained their immunogenic properties. They are now considered excellent vaccine candidates since they showed high efficacy in trials using veterinary species (Bird et al., 2011; Dungu et al., 2010; Weingartl et al., 2014b). Besides, it has been possible to manipulate the genome of RVFV in such a way to generate 2 or even 4 RNA segment-containing viruses (Brennan et al., 2011; Wichgers Schreur et al., 2014). This genomic plasticity indicates that RVFV might behave as a viral vector to carry either mutant genes or even foreign antigens, as has been recently described for influenza Ha protein (Oreshkova et al., 2014).

3. RVF pathogenesis

Several mouse models have been used to characterize the pathology associated with RVFV infection, including BALB/c, IFNAR$^{-/-}$, MBT/Pas, 129 and C57BL/6 mice, respectively (reviewed in Ross et al., 2012). Pathology appears to vary with route of exposure to RVFV though the basis of this is not well understood. The pathogenesis of infection caused by exposure to RVFV-infected mosquitoes might be expected to result in circulation of virus or virus-infected cells from the inoculation site to regional lymph

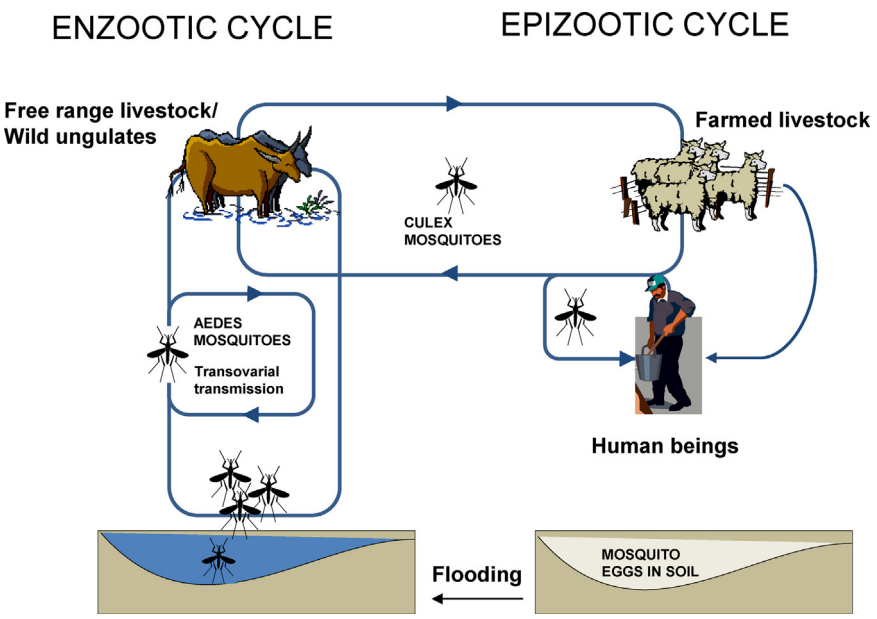

Fig. 2. *Proposed natural history of RVFV infection.* Mosquito species determine the maintenance of the virus in nature (enzootic cycle) or the explosive outbreak (epizootic cycle). Aedine floodwater mosquitoes could bite and transmit the virus transovarially. Infected Aedine eggs would remain in soil until flooding allows hatching. Culicine mosquitoes (as well as other haematophagus diptera) spread the virus from viremic (RVFV-infected) hosts to domestic herds, where the infection is greatly amplified. Humans act as dead ends and can be infected either by mosquito bites or by direct contact to animal tissues or fluids.

nodes by afferent lymphatic vessels. Viral replication could then occur in specific immune cell populations within the lymph nodes, resulting in a primary viraemia, with subsequent spread *via* the bloodstream and widespread dissemination to different organs and tissues (liver, spleen, kidney) as has been shown in mouse models (Smith et al., 2010). On the other hand, the probable succession of events following infection *via* the aerosol route might be an initial expansion in susceptible cells throughout the respiratory tract

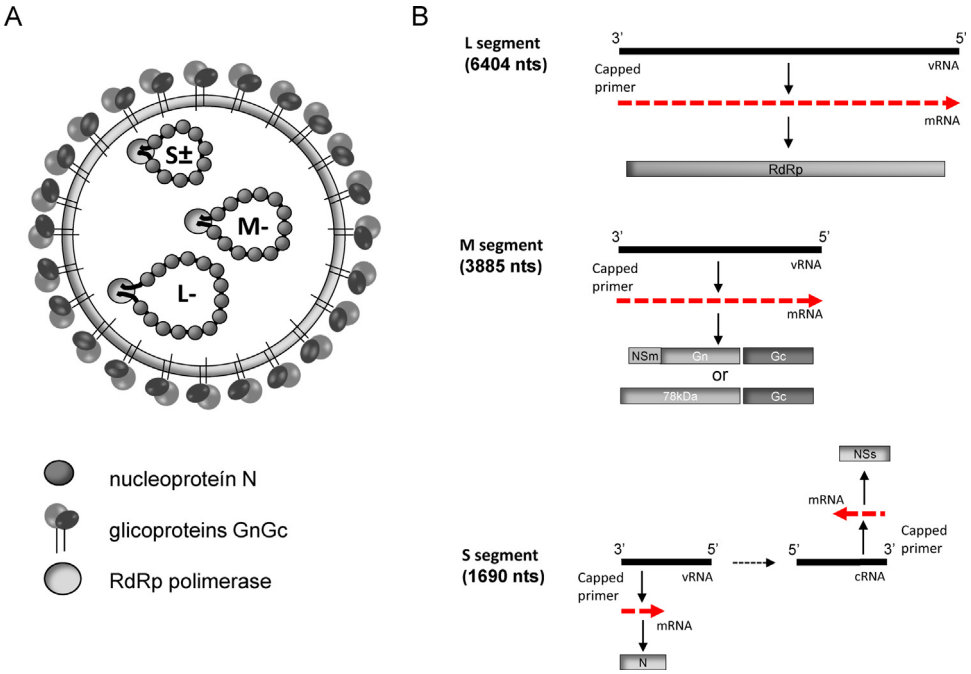

Fig. 3. *Organization of the RVFV viral particle and genome coding strategy.* (A) Schematic representation of the viral particle showing the viral glycoproteins Gn and Gc inserted into the virus envelope. Inside, the three genomic segments of single stranded RNA are shown forming complexes to both the viral RNA dependent RNA polymerase (RdRp) and the viral nucleoprotein N. The ± denotes that both genomic and antigenomic S segments can be encapsidated into the viral particle (Ikegami et al., 2005b). Note the panhandle structures to depict the short stretches of complementary RNA at 5′ and 3′ ends of each RNA molecule. (B) Representation of each transcriptional unit of RVFV and translation strategies. The negative sense, vRNA, is transcribed after sequestration of short host's capped primers by the RdRp (which has endonucleolytic activity). The M segment is transcribed and can be translated into several mature polypeptides (the structural glycoproteins Gn and Gc and two non-structural proteins of 14 kDa (NSm) and 78 kDa) depending on the selective usage of five-in frame start codons. It has been suggested as a strategy for virus adaptation to both insect and mammalian hosts (in fact the 78 kDa protein has been found as a structural component of the viral particles when propagated in insect cell cultures). The S segment has a complex ambisense transcription strategy that includes the replication of vRNA to generate cRNA to act as a template for NSs transcription. The antigenomic S cRNA can be also packaged into viral particles (Ikegami et al., 2005b) thus allowing earlier expression of NSs protein after infection.

Table 1
RVFV NSs-mediated strategies for the inhibition of host's innate immune responses.

Action	Consequence	Reference(s)
Sequestration of p44 and degrading TFIIH p62 subunit and reducing expression of other protein subunits	Blocking type I IFN production. Disruption of host cell transcription	Billecocq et al. (2004), Bouloy et al. (2001), Kalveram et al. (2011), Le May et al. (2004)
Enhanced replication in minigenome system	Regulation of viral RNA synthesis	Ikegami et al. (2005a)
Interaction with Sin3A-associated protein 30 (SAP30)	Repression of IFN-β transcription by maintaining the YY1 repressor complex	Le May et al. (2008)
Downregulation and degradation of PKR	Avoids phosphorylation of eIF2a for sustained translation	Habjan et al. (2009), Ikegami et al. (2009), Kalveram et al. (2013)
Chromatin deregulation	Binding to pericentromeric DNA repeats	Mansuroglu et al. (2010)
Interaction with several promoter regions	Modification of the expression of genes coding for coagulation factors	Benferhat et al. (2012)
Sequestration of nuclear PABP1	Avoids host mRNA polyadenylation favouring viral protein production	Copeland et al. (2013)
Recruiting of FBOX3	Proteasome degradation of the p62 subunit	Kainulainen et al. (2014)

thereby establishing a primary viraemia that eventually reaches the liver, which becomes the principal site for virus replication and virus spread to other tissues including the brain (Dodd et al., 2014; Reed et al., 2013). Earlier studies also raised the possibility that aerosol infection may allow direct invasion of the central nervous system *via* the olfactory nerves (Brown et al., 1981).

Recent studies have indeed found that the development of neuropathology is faster and more severe in mice challenged by aerosol exposure than through parenteral routes (Dodd et al., 2014; Reed et al., 2013). For both parenteral and aerosol routes of exposure the liver is thought to be the main organ capable of sustaining a massive virus replication that would facilitate spread of the virus to secondary target organs. Death appears to be a consequence of liver function failure due to fulminant hepatitis and hepatic necrosis. Whether the liver failure is a direct result of virus replication or due to a strong unregulated immune response is still unclear. Ultrastructural studies in BALB/c mice revealed that viral particles are rarely seen in the liver in spite of the profuse immunostaining of viral antigen detected by immunohistochemistry (Reed et al., 2012) or by chemiluminiscent viruses in IFNAR$^{-/-}$ mice (Gommet et al., 2011). By using GFP or luciferase expressing viruses it was also shown that macrophages, dendritic cells and granulocytes were main target cells for RVFV after intraperitoneal inoculation affecting primarily the thymus, spleen, liver and pancreas. Upon dermal and nasal inoculations, virus was observed in the lymph node draining the injected ear and in the lungs respectively (Gommet et al., 2011).

The immunopathogenesis upon RVFV infection has been also studied in great detail in mice (Gray et al., 2012). Upon infection of C57BL/6 mice a decrease in lymphocytes, monocytes and platelet counts is evident starting from 48 h post-infection (hpi). Coincidental with the increase of granulocyte colony stimulating factor (G-CSF) in serum there is a recovery in the white blood cell (WBC) count. This drop and recovery phase in the immune cell population is consistent with earlier observations made in ruminants and primates infected with RVFV (Easterday, 1965). It was also shown that the level of chemokines in liver (the main target organ), but not that of pro-inflammatory cytokines was significantly elevated (Gray et al., 2012). This would explain the regular infiltrates of monocytes and granulocytes that are seen in infected livers. In agreement with the low level of pro-inflammatory cytokines, the level of IL-10 gene expression in the liver was found to be upregulated in infected mice (Jansen van Vuren et al., 2011). Starting at 48 hpi, serum cytokine levels were found to be elevated for all significant cytokines relative to controls. In particular IL-6, G-CSF and IL-12 (p40) as well as chemokines KC, MCP-1 and MIP-1α showed the highest increase, later decreasing to normal levels at 96 hpi. Significant upregulation of pro-apoptotic and pro-inflammatory genes and downregulation of anti-apoptotic ones was found in livers from infected BALB/c OlaHsd mice (Jansen van Vuren et al., 2011). Later after infection the expression of both IFN-β and IFN-γ genes was heavily upregulated, perhaps contributing to liver damage by means of the exacerbation of cytokine production.

Other rodent models have been used to reproduce the RVFV infection such as laboratory rats (*Rattus norvergicus*), hamsters (*Mesocricetus auratus*) and gerbils (*Meriones ungiculatus*). Each species offer unique features that could make attractive their use by researchers; for example, rats have shown resistant phenotypes and offer an interesting model to explore the genetic basis of host's resistance to RVFV (Anderson et al., 1987; Ritter et al., 2000) and virulence of different RVFV isolates (Anderson and Peters, 1988); Syrian hamsters have been commonly used for experimental transmission using infected mosquitoes (McIntosh et al., 1973) and gerbils may be useful to studies related to neuroinvasiveness and encephalitis and the effect of age in the resistance/susceptibility balance to RVF disease (Anderson et al., 1988). Detailed analysis of the pathology and pros and cons of the use of different animal models, including rodents, have been recently reviewed (Ikegami and Makino, 2011; Ross et al., 2012). Ferrets were among the first non-rodent models ever used for assessing RVFV transmission. Ferrets were inoculated with pharyngeal washes from laboratory assistants that had been accidentally infected while manipulating RVFV infected tissues (Francis and Magill, 1935). "In these ferrets a disease was produced in which pulmonary involvement was the outstanding feature." The ferret model has been used in other infectious diseases such as influenza or SARS experimental vaccines since, unlike some other animal models such as mice, there is no need for prior host adaptation of the human viruses. To our knowledge no further reports using this experimental model for the study of RVFV infection have been published.

With respect to the pathogenesis in species of veterinary interest, fewer data are available and these refer most to the pathology observed upon experimental infection in different breeds of sheep. The main characteristic outcome of the disease in ovines is generalized abortion in pregnant ewes and close to 100% mortality in newborns which usually die in the first 24 h after birth, showing extensive liver necrosis (Daubney et al., 1931). Some young lambs (≥1 week old) may survive although they show clear signs of disease such as diarrhea, nasal discharge, conjunctivitis, fever, prostration and loss of appetite (Easterday et al., 1962). Adult sheep mortality usually ranges around 20%, associated to liver multifocal necrosis. In some cases it has been described the presence of corneal edemas with regular inflammatory infiltration as well as occurrence of horizontal transmission (Busquets et al., 2010; Galindo-Cardiel et al., 2012), perhaps due to the presence of virus in nasal secretions. In other cases rapid hemorrhagic signs in some adult sheep from other breeds, have been described, displaying parameters associated to endothelial damage or disseminated

intravascular coagulation (Olaleye et al., 1996). In a general sense it can be assumed that the susceptibility for adult sheep appears to be more inconsistent than for young animals and between breeds, reflecting of different host's genetic determinants, making direct comparisons more difficult. Other ruminants, including goats, cattle and camels are also susceptible to RVFV disease and some experimental and vaccine trials have been performed on this species (Barnard, 1979; Botros et al., 2006; Coackley et al., 1967a; Morrill et al., 1997; Nfon et al., 2012; Weingartl et al., 2014a; Yedloutschnig et al., 1981; Yedloutschnig et al., 1979).

4. Immune mechanisms of protection: Lessons learned in mice

The ability of RVFV to cause pathology in rodents has prompted the use of these animals for evaluation of candidate vaccines and immune mechanisms that mediate protection. RVFV is highly sensitive to the action of type-I interferon (IFN) as shown in mice and non-human primates following administration of IFN inducers (such as poly I:C) or recombinant human IFN (Morrill et al., 1989; Peters et al., 1986). In addition, mice lacking the alpha/beta interferon receptor (IFNAR$^{-/-}$) are highly sensitive to RVFV, including the naturally avirulent Clone 13 RVFV strain that contains a 549 nucleotide deletion within the gene coding for the NSs protein (Bouloy et al., 2001). NSs protein antagonizes the antiviral response by blocking type I IFN production through a generalized repression of host cell transcriptional activivity (Table 1) though it is non-essential for RVFV replication. As a general repressor of transcription, the NSs protein may also affect the transcriptional activity of hormone regulated genes which could explain the high rates of abortion and teratogenicity observed in infected pregnant animals or the fast and acute disease in newborns (Le May et al., 2004).

As is the case for many bunyaviruses the antibody response elicited against the viral glycoproteins Gn and Gc confers protection against disease thus offering a good marker for evaluation of vaccine potency or naturally acquired immunity to RVF. Neutralizing epitopes have been mapped within the Gn sequence with some anti-Gn monoclonal antibodies showing neutralizing activity *in vitro* against all RVFV isolates tested (Besselaar and Blackburn, 1994) in agreement with the low sequence variation found between isolates (Battles and Dalrymple, 1988). In contrast, whilst the internal nucleoprotein N is a potent immunogen, antibodies raised against N are not able to neutralize RVFV *in vitro*. However, N-based vaccines have been shown to confer partial protection in some vaccination studies, though the underlying mechanisms of immunity are yet to be determined (Jansen van Vuren et al., 2011; Lopez-Gil et al., 2013; Lorenzo et al., 2010; Spik et al., 2006; Wallace et al., 2006).

Relatively little is known about the cellular immune response to RVFV and its role in protection. Both the Gn and Gc glycoproteins have recently been shown to carry immunodominant CD8+ T-cell epitopes identified following immunization of BALB/c mice with recombinant MVA (Lopez-Gil et al., 2013) adenovirus (Warimwe et al., 2013) or DNA vaccines (Bhardwaj et al., 2010). However glycoprotein-specific T-cell responses in the context of class-I molecules in vaccine target species (ruminants and humans) have yet to be identified. Human modelling approaches have allowed identification of potential T-cell epitopes. For example it has been shown that the N protein carries HLA-A2 restricted epitopes that can stimulate a polyfunctional CD8+ T-cell response in HLA-A2 transgenic mice (Xu et al., 2013). A role for CD4+ T-cells in the protection conferred by an attenuated ΔNSs deletion mutant RVFV obtained by reverse genetics has been described (Dodd et al., 2013). In that study mice devoid of CD4+ T-cells showed higher mortality than those devoid of CD8+ T-cells, whereas μMT mice (devoid of B-cells) were not protected at all. These data reinforce the important role of antibodies in immunity to RVF and suggest involvement of a Th2-biased cellular response.

5. Experimental studies of RVF in ruminants

The conclusions drawn from rodent models with respect to the pathogenesis of RVFV infection or vaccine efficacy trials need to be translated to the natural target species. Small ruminants (sheep and goats) have been the main species for experimental infection trials with fewer attempts made in cattle. The pathogenicity, transmission, strain virulence and immune responses upon experimental challenge with RVFV have been analysed in great detail in these species (Busquets et al., 2010; Capstick and Gosden, 1962; Coackley et al., 1967b; Galindo-Cardiel et al., 2012; Olaleye et al., 1996; Swanepoel et al., 1986; Tomori, 1979; Weingartl et al., 2014a; Yedloutschnig et al., 1981). Sheep have by far been the most studied target species due to their versatility, particularly for room economy in Biosafety laboratory (BSL-3) facilities. Infection in sheep consistently results in a marked decrease in the number of leukocytes, with the degree of leukopenia being more severe in young lambs as compared to adult sheep (reviewed in Easterday, 1965). In adult sheep, the lowest counts of leukocytes were usually recorded on the 3rd and 4th days after infection in earlier studies (Easterday, 1965). Leukopenia was usually of short duration; even in animals with a fatal infection, the number of leukocytes began to return to pre-infection levels before death.

Studies in sheep reflect the typical difficulties in standardizing animal studies to allow comparison of data generated in different labs. As with rodent models, the main driving force toward the establishment of infection models in sheep has been the renewed interest in RVF vaccine development (Kortekaas, 2014; Kortekaas et al., 2011). Two sheep infection models have been used for vaccine efficacy studies: (i) a pregnant sheep model, which has inherent challenges related to pregnancy synchronization and the need for spacious high-containment experimental facilities (Antonis et al., 2013; Baskerville et al., 1992; Bird et al., 2011; Dungu et al., 2010; Harrington et al., 1980; Hunter et al., 2002; Morrill et al., 1991a, 2013; Yedloutschnig et al., 1981), and (ii) a non-pregnant sheep model to monitor viraemia levels (Antonis et al., 2013; Busquets et al., 2010; Kortekaas et al., 2012; Soi et al., 2010; Weingartl et al., 2014a; Yedloutschnig et al., 1979). This latter model is probably the easiest to implement though heterogeneity in results can be a problem. For instance, not all experimentally inoculated sheep may develop detectable viraemia and the variations in viraemia titres and duration may be dramatic, mortality is low, particularly in adult sheep, and morbidity usually non-specific. The use of newborn or very young lambs (less than one month old), which are much more sensitive to disease than adult sheep (Coetzer and Ishak, 1982; Easterday et al., 1962; Van der Lugt et al., 1996), also has difficulties related to the maintenance of lambs soon after birth but it remains to be explored further for testing novel vaccine candidates. Besides these challenges, variation in susceptibility of sheep to RVF may also depend on the route of infection (usually subcutaneous, intramuscular or intravenous), breed of animals, the RVFV strain used and its passage history. The difficulties in establishing an adequate large animal infection model have delayed in depth studies on the immunological mechanisms elicited upon infection or after vaccine administration in target species.

Since RVFV is transmitted by mosquito vectors the virus has adapted to grow in both insect and mammalian cells. Transmission by mosquito bites can be considered the natural route of infection. As shown by other arboviruses (Edwards et al., 1998; Reagan et al., 2012; Styer et al., 2011) it has been demonstrated that Aedes

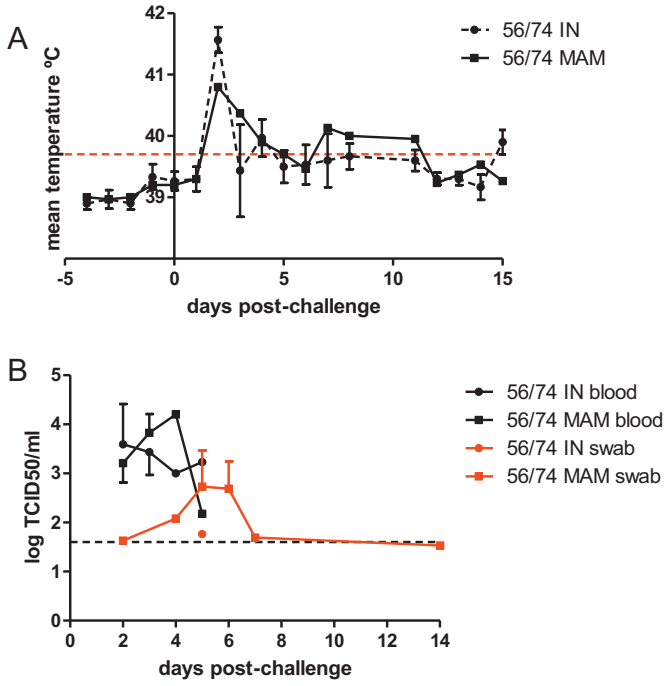

Fig. 4. *Correlates of infection in RVFV challenged sheep.* 6 sheep from an autochthonous breed from the Madrid province area (termed Colmenareña) were housed into the BSL-3 animal facilities and subcutaneously inoculated with 10^5 $TCID_{50}$ of either 56/74 MAM (a Vero cell propagated virus) or 56/74 IN (a C6/36 cell passaged virus). (A) Rectal temperatures of sheep challenged with either 56/74 IN or 56/74 MAM. Animals were considered febrile if rectal temperature was above 39.7 °C (dotted line: mean plus three standard deviations of the rectal temperatures recorded four days before the challenge). (B) Isolation of virus in blood (black lines) or in nasal swabs (red lines) at different times upon challenge with either 56/74 IN or 56/74 MAM. Titres were determined by serial log dilutions in Vero cell cultures by the Reed & Muench method as described in Busquets et al. (2014). A sensitivity threshold was set at 1:40 dilution. Values represent mean ± SD when appropriated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

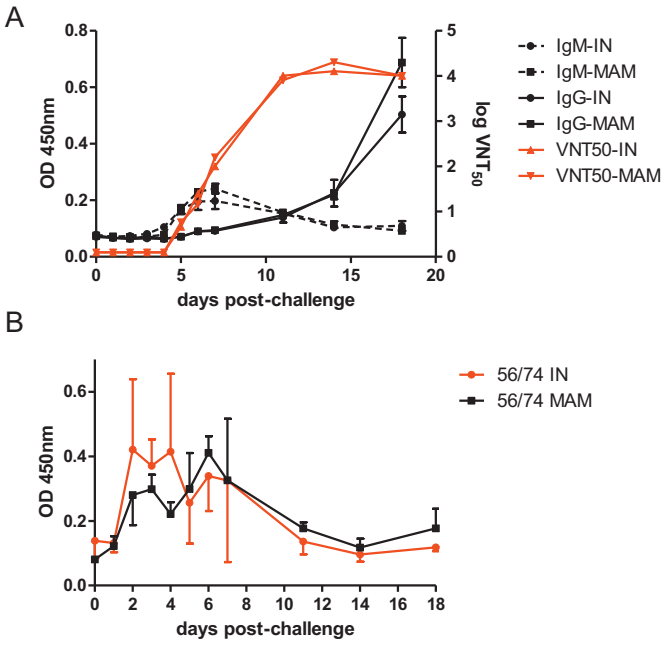

Fig. 5. *Measurement of humoral and cellular responses in RVFV challenged sheep.* (A) Kinetics of anti-RVFV IgM and IgG in serum from sheep challenged with 56/74 IN or 56/74 MAM by virus capture ELISA. The kinetics of induction of VNT 50 neutralization titres in sheep sera are shown by red lines. Values represent mean ± sem. (B) Kinetics of IFN-γ induction in serum from sheep challenged with either 56/74 IN (red lines) or 56/74 MAM (black lines). Values represent mean ± sem. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

saliva significantly increases the pathogenicity of a RVFV challenge in C57BL/6NRJ mice when co-injected intradermally (Le Coupanec et al., 2013). Recent evidence based on the Egyptian ZH501 RVFV isolate suggests increased susceptibility of sheep and goats to infection with virus propagated in insect (*Aedes albopictus*) C6/36 cells when compared to infection with the same virus after propagation in monkey Vero E6 cells (Weingartl et al., 2014a). These authors suggested that the virus propagated in mosquito cells was more efficient initiating the infection upon subcutaneous challenge. We had also conducted a pilot experiment to assess disease susceptibility of adult sheep following infection with a South African RVFV strain (56/74) propagated in mammalian Vero E6 cells (hereafter termed 56/74 MAM) on in the insect C6/36 cells (hereafter termed 56/74 IN). The 56/74 virus was originally obtained from infected cattle. The virus was isolated by serial passage in mouse brain and subsequent bovine cell cultures (Busquets et al., 2010). Whilst no mortality was recorded in either group under the experimental conditions, fever was only evident at day 2 post-infection in the 56/74 IN group whereas the febrile response in the 56/74 MAM group lasted several days (Fig. 4A). The peak of viraemia among those in the 56/74 IN group was earlier (day 2) compared to those in the 56/74 MAM (peak at day 4) (Fig. 4B), though these differences were not statistically significant using non-parametric tests. Interestingly, when viral excretion was tested in nasal swabs virus could be isolated in two sheep inoculated with 56/74 MAM, while only one of six sheep inoculated with 56/74 IN showed viral excretion, although at levels close to the sensitivity threshold of the assay. In both cases detection was produced after the peak of viraemia had occurred as we had described previously (Busquets et al., 2014; Busquets et al., 2010). The presence of virus in nasal swab at late times after infection was unexpected but it was detected only in one sheep and below the threshold level.

The induction of neutralizing antibodies correlated with the presence of IgM as early as 5 days post infection in both groups and these were maintained at high titres starting from 10 days post infection (Fig. 5A). Cellular immune responses were assessed by measuring serum IFN-γ levels by ELISA as described (Lorenzo et al., 2008) on different days post-infection. Starting at day 2 post-infection IFN-γ was readily detectable in serum from sheep in both infection groups and this was maintained at detectable levels at least until day 18 post-infection (Fig. 5B).

These data point to a possible role for IFN-γ in both the early innate antiviral response and in adaptive cellular immunity, and are in agreement with previous studies of the innate immune response to infection with the Egyptian ZH501 RVFV infection in goats (Nfon et al., 2012). Consistent with the antagonistic function of the RVFV NSs protein, the work in goats revealed an earlier induction of systemic IFN-γ and IL-12, but not IFN-α (Nfon et al., 2012). Levels of TNF-α, IL-6 and IL1-β rose later in the course of infection. Changes in the frequencies of certain immune cell types were also observed upon infection as previously described (Easterday, 1965). For instance, baseline counts for $CD172^+$ monocytes and dendritic cells (DCs) dropped only in goats challenged with the insect cell propagated virus but not in goats receiving virus propagated in mammalian cells (Nfon et al., 2012). A similar observation was found for both CD5+ and CD8+ T-cells as well as CD21+ B-cell counts. A plausible explanation for the reduction in the frequencies of monocyte-derived cells could be direct infection of these cells as has been demonstrated in *in vitro* infection experiments of monocyte-derived dendritic cells (Nfon et al., 2012) or monocyte-derived human CD14+ macrophages (McElroy and Nichol, 2012).

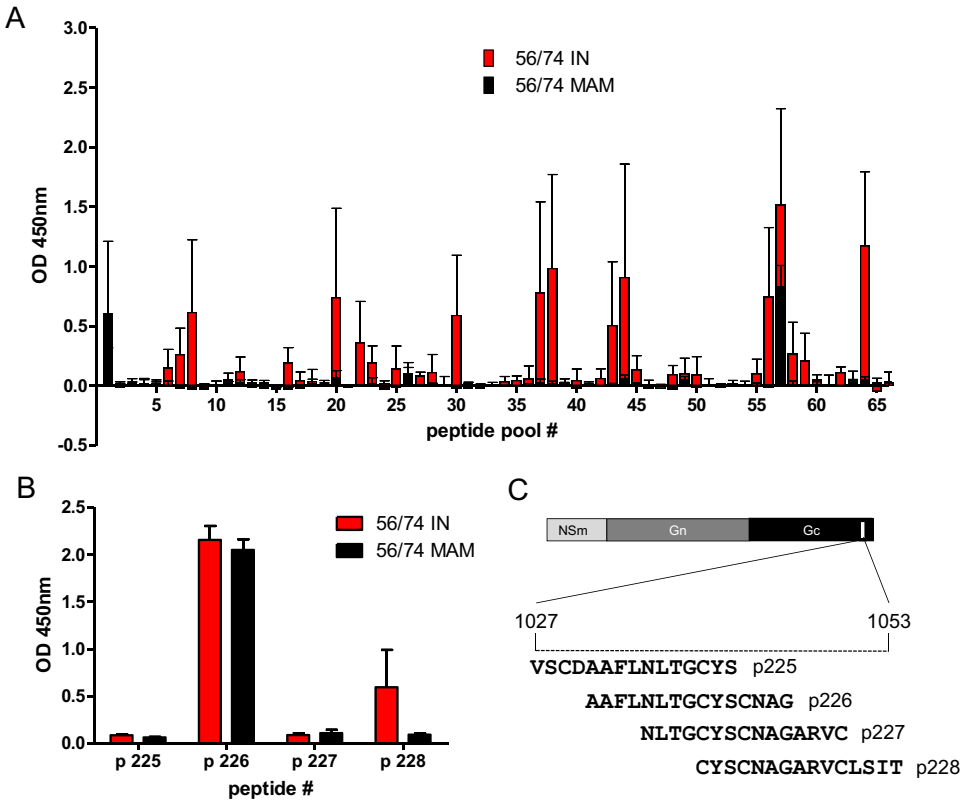

Fig. 6. *Identification of glycoprotein T-cell epitopes by pepscan analysis.* (A) Mean ± sem IFN-γ ELISA values in plasma samples obtained upon blood stimulation with a panel of 66 pools of peptides covering the mature GnGc amino acid sequence. Blood sampling was taken at 7 days after challenge with either 56/74 IN (red bars) or 56/74 MAM (black bars). (B) IFN-γ ELISA values in sheep plasma samples obtained upon blood stimulation with individual peptide components from pool #57. Blood sampling was taken at 14 days after challenge with either 56/74 IN (red bars) or 56/74 MAM (black bars). (C) Location of pool #57 peptide components on the primary amino acid sequence of the M segment (aa numbers corresponding to ORF derived from first AUG codon). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Further, these data are supported by *in vivo* RVFV antigen-specific immunostaining of liver macrophages (Shieh et al., 2012) and dendritic cells in mice (Smith et al., 2010)and are in agreement with the proposed role for DC-SIGN (CD209) as an attachment factor for the entry of RVFV and other phleboviruses (Hofmann et al., 2013; Lozach et al., 2011). DC-SIGN is highly expressed on the surface of dendritic cells from mucosal, lymphoid and dermal tissues and is also present in liver and alveolar macrophages, cells involved in the activation of both innate and adaptive immune responses.

We confirmed that the sustained induction of IFN-γ following infection reflects activation of antigen-specific cellular responses against RVFV antigens using a panel of overlapping 15mer peptide pools spanning the whole mature GnGc sequence (Warimwe et al., 2013). Each pool was used in an *in vitro* stimulation assay of total sheep blood cells taken at 7 days after 56/74 IN and 56/74 MAM RVFV virus challenge and the presence of IFN-γ in the respective plasma measured by sandwich ELISA. Unexpectedly, the frequency of responses to the set of peptide pools was highest in sheep inoculated with 56/74 IN with only two of ten peptide pools stimulating a detectable response in cells from the 56/74 MAM group (Fig. 6A). It is noteworthy that differences in goat immune responses depending on the inoculum used for challenge, *i.e.* whether insect cell or mammalian cell-propagated, were also observed (Nfon et al., 2012). In our studies in sheep only one pool of peptides (#57) was able to stimulate IFN-γ production in cells from all sheep (Fig. 6A). Testing these peptides separately revealed the putative single region/epitope on the Gc polypeptide with characteristics of an immunodominant T-cell epitope (Fig. 6B and C). Further experiments are warranted to confirm whether this peptide is able to re-stimulate IFN-γ production by T-cells from RVFV infected sheep of different breeds or genotypes and to evaluate these responses in relation to protective immunity.

6. Studies of RVF in non-human primates

Understanding the pathogenesis and immune response following RVFV infection is key to find novel human vaccines or antiviral treatments. In humans the disease manifests commonly as a self-limiting, febrile illness. However, some patients may develop different outcomes such as neurological disorders, vision loss, hemorrhagic fever, or thrombosis (Al-Hazmi et al., 2003; and reviewed in Ikegami and Makino, 2011). It is interesting to mention that haemorraghic fever usually appears suddenly, soon after the first days of non-specific symptons, while neurologic and ocular disorders are characteristically described after a convalescent phase. In any case, the mechanisms conditioning these outputs are not fully understood and may depend on the route of infection or the heath status of the affected individual. In studies on human patient sera the balance between proinflammatory and immunosuppressive cytokines has been linked with disease severity (McElroy and Nichol, 2012); the proinflammatory chemokine CXCL-1 and the soluble CD40 ligand (sCD40L or CD154) were found elevated in non-fatal versus fatal cases. Conversely, both the IL-1 receptor antagonist (IL-1RA) and IL-10 levels were significantly higher in fatal cases than in non-fatal ones. Since these observations were based on a limited number of sera larger studies are needed to determine the precise role of cytokine signalling upon RVFV infection.

Experimental infections of different non-human primate species could help shed light on the pathogenesis of RVF in humans. Shortly after the first description of the disease in Kenya, George

Marshall Findlay reported the first experimental RVFV infection of monkeys, which was performed by instillation of the virus into the nostrils of Rhesus monkeys (*Macaca mulatta*). Fever, leukopenia and transient viraemia were the characteristic outcomes of the infections "similar to that occurring spontaneously in man". However, no signs of disease or mortality were observed in subsequent experiments using different inoculation routes (Easterday, 1965).

In contrast, intravenous (iv) inoculation of macaques with the Egyptian RVFV ZH501 isolate caused viraemia in most macaques and, for the first time in non-human primates, hemorrhagic fever in 20% of the animals. Hepatic necrosis, disseminated intravascular coagulation, and microangiopathic hemolytic anemia were the pathological features in animals that developed the hemorraghic syndrome (Peters et al., 1988). In a further study, evidence of hemostatic impairment including disseminated intravascular coagulation appeared to be more generalized (Cosgriff et al., 1989). The Rhesus monkey iv RVFV infection model could therefore be useful for the study of human hemorrhagic disease, with applications in the evaluation of therapeutic and prophylactic measures for humans.

Using this model the importance of innate immunity in protection was demonstrated. Intravenous administration to macaques of recombinant or human IFN-α, either 24 h before infection or 6 h after infection, caused complete or marked protection after exposure to infectious RVFV doses (Morrill et al., 1989). Further, use of the Rhesus monkey model also demonstrated a correlation between delayed interferon response and mortality, indicative of the importance of a proper type I IFN response in limiting the severity of disease (Morrill et al., 1990). A protective effect, particularly on reduction of viraemia titres, was also obtained following prophylaxis of monkeys with type II IFN (IFN-γ) 24 h before challenge (Morrill et al., 1991b).

In addition to the antiviral activity of interferons, the Rhesus monkey model has also been used for evaluation of safety and efficacy of candidate human vaccines. In particular, the mutagenized attenuated MP12 vaccine (Caplen et al., 1985) was tested in this model by several routes of inoculation including parenteral, aerosolized, oral and intranasal (Morrill and Peters, 2003, 2011a,b) as a series of preclinical studies towards its experimental use in human volunteers. The immunity provided was studied at the level of induction of neutralizing antibodies and/or serum specific IgM or IgGs by ELISA tests. In these studies the correlation between serum neutralization activity and reduced morbidity was demonstrated. Interestingly, a biphasic neutralizing antibody response of naive (mock-vaccinated) monkeys was observed upon challenge, a characteristic that was not observed previously in other animal models of RVFV infection.

As in sheep, it became clear that the uniformity of RVFV induced morbidity and mortality was difficult to achieve in the Rhesus monkey model, prompting investigation into other alternatives. The common marmoset (*Callithrix jacchus*) has been evaluated more recently as an alternative model for reproducing human RVF (Smith et al., 2012). These animals appear to be more susceptible to RVFV infection than Rhesus monkeys in terms of morbidity, mortality and viraemia, showing typical features of severe human RVF, such as acute hepatitis, delayed-onset encephalitis and hemorrhagic disease, depending on the challenge route used. With respect to the immune response to RVFV infection, few data other than the presence of neutralizing antibody response are available. In this respect, a delayed onset of neutralizing antibody responses to infection could potentially explain the higher mortality rate in marmosets relative to that in Rhesus monkeys. Interestingly, the highest rate of mortality was obtained when inoculation was made intranasally, perhaps reflecting the efficacy of aerosol transmission, which is probably one of the most important routes for human infection. This was confirmed in a recent study (Hartman et al., 2014) in which African green monkeys (*Chlorocebus aethiopicus*) were infected intranasally, with most animals (5/6) succumbing to infection 10 to 11 days after infection. Marmosets were equally sensitive to disease but showed reduced mortality rates (50%). The results obtained in both models indicate they could be useful animal models to reproduce aerosolized RVFV infection, and to evaluate both therapeutic and prophylactic approaches as well.

7. Conclusion and future perspectives

The search for efficient vaccines against RVFV has been the subject of intense research in recent years. This interest has been boosted, at least in part, by the recent examples of novel vector borne diseases appearing in new regions as well as by fears about the potential use of hemorrhagic fever viruses as bioweapons. RVF has been considered a serious zoonotic threat since the unexpected virulence for humans of the RVF Egyptian 1977 outbreak. In addition, many locations of the world could theoretically sustain an endemic establishment of RVFV (according to epidemiologist's predictions considering eco-climatic conditions and presence of mosquito species able to perpetuate the virus in natural settings). In spite of these considerations, no RVF incursions have yet been described out of Africa and the Arabian Peninsula, raising questions about the real spreading potential of RVF in the Mediterranean basin. These uncertainties should question RVF-free countries whether they are prepared to combat the disease in the event of an unwanted disease introduction. These reasons support the case for future research into RVF, particularly directed to the understanding of pathogenic mechanisms and immune response, both of which should aid the design of effective vaccines.

As for many other infectious diseases, mouse models of RVFV infection have greatly contributed to our current understanding of the efficacy of different vaccine approaches as well as the relevance of humoral and cellular immune responses in protection, but extrapolating mouse data to target species is not always possible, particularly those related to cell mediated immunity. Thus, infection and vaccine trials using large animal models are warranted. The most promising vaccine approaches have been tested in ruminants (Bird et al., 2011; Busquets et al., 2014; Dungu et al., 2010; Kortekaas et al., 2012; Oreshkova et al., 2013; Soi et al., 2010; Weingartl et al., 2014b) and some of them have been registered or are under registration trials by pharmaceutical companies. However the availability of reagents for ruminant studies is much more limited, hampering studies aimed at understanding the mechanisms of protection elicited by vaccines in natural target species. On the other hand the studies on the pathogenesis of RVF in ruminants as well as the role of innate immunity in disease outcome are still limited, in part due to the limited access to research facilities and cost of experiments. The use of transcriptomic approaches in infected ruminants would be beneficial to understand pathogenesis and should be performed in a near future.

The benefits of studying RVF immunity and vaccine approaches in ruminants are obvious. Obtaining an effective vaccine for ruminants that could easily be deployed in RVFV-endemic areas would help reduce the impact of the disease, the spread of the virus and the recurrent toll on animal and human lives associated with RVF. On the other hand, a human RVF vaccine would be highly desirable for vaccination of populations at risk in RVFV-endemic settings and, perhaps in the near future, it might be needed in other countries. The lessons learned in large animals about disease pathogenesis and control will inform the selection of better candidate vaccines for human use. Some of these candidates could be tested in preclinical setting using the non-human primate models for RVFV recently developed, particularly those approaches based on safe

technologies already tested in human trials, such as propagation deficient adenoviruses, non-replicating poxvirus, DNA vaccines or subunit and virus-like particle vaccines. In summary, development of animal models to the study of RVF immunopathogenesis is an unconcluded task that should be pursued in the following years.

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References

Ahmad, K., 2000. More deaths from Rift Valley fever in Saudi Arabia and Yemen. Lancet 356, 1422.

Al-Hazmi, M., Ayoola, E.A., Abdurahman, M., Banzal, S., Ashraf, J., El-Bushra, A., Hazmi, A., Abdullah, M., Abbo, H., Elamin, A., Al-Sammani el, T., Gadour, M., Menon, C., Hamza, M., Rahim, I., Hafez, M., Jambavalikar, M., Arishi, H., Aqeel, A., 2003. Epidemic Rift Valley fever in Saudi Arabia: a clinical study of severe illness in humans. Clin. Infect. Dis. 36, 245–252.

Anderson Jr., G.W., Peters, C.J., 1988. Viral determinants of virulence for Rift Valley fever (RVF) in rats. Microb. Pathog. 5, 241–250.

Anderson Jr., G.W., Slone Jr., T.W., Peters, C.J., 1987. Pathogenesis of Rift Valley fever virus (RVFV) in inbred rats. Microb. Pathog. 2, 283–293.

Anderson Jr., G.W., Slone Jr., T.W., Peters, C.J., 1988. The gerbil, *Meriones unguiculatus*, a model for Rift Valley fever viral encephalitis. Arch. Virol. 102, 187–196.

Andriamandimby, S.F., Randrianarivo-Solofoniaina, A.E., Jeanmaire, E.M., Ravololomanana, L., Razafimanantsoa, L.T., Rakotojoelinandrasana, T., Razainirina, J., Hoffmann, J., Ravalohery, J.P., Rafisandratantsoa, J.T., Rollin, P.E., Reynes, J.M., 2010. Rift Valley fever during rainy seasons, Madagascar, 2008 and 2009. Emerg. Infect. Dis. 16, 963–970.

Antonis, A.F., Kortekaas, J., Kant, J., Vloet, R.P., Vogel-Brink, A., Stockhofe, N., Moormann, R.J., 2013. Vertical transmission of Rift Valley fever virus without detectable maternal viremia. Vector Borne Zoonotic Dis. 13, 601–606.

Anyamba, A., Chretien, J.P., Small, J., Tucker, C.J., Formenty, P.B., Richardson, J.H., Britch, S.C., Schnabel, D.C., Erickson, R.L., Linthicum, K.J., 2009. Prediction of a Rift Valley fever outbreak. Proc. Natl. Acad. Sci. U.S.A. 106, 955–959.

Anyamba, A., Linthicum, K.J., Small, J., Britch, S.C., Pak, E., de La Rocque, S., Formenty, P., Hightower, A.W., Breiman, R.F., Chretien, J.P., Tucker, C.J., Schnabel, D., Sang, R., Haagsma, K., Latham, M., Lewandowski, H.B., Magdi, S.O., Mohamed, M.A., Nguku, P.M., Reynes, J.M., Swanepoel, R., 2010. Prediction, assessment of the Rift Valley fever activity in East and Southern Africa 2006–2008 and possible vector control strategies. Am. J. Trop. Med. Hyg. 83, 43–51.

Baer, A., Austin, D., Narayanan, A., Popova, T., Kainulainen, M., Bailey, C., Kashanchi, F., Weber, F., Kehn-Hall, K., 2012. Induction of DNA damage signaling upon Rift Valley fever virus infection results in cell cycle arrest and increased viral replication. J. Biol. Chem. 287, 7399–7410.

Barnard, B.J., 1979. Rift Valley fever vaccine—antibody and immune response in cattle to a live and an inactivated vaccine. J. S. Afr. Vet. Assoc. 50, 155–157.

Baskerville, A., Hubbard, K.A., Stephenson, J.R., 1992. Comparison of the pathogenicity for pregnant sheep of Rift Valley fever virus and a live attenuated vaccine. Res. Vet. Sci. 52, 307–311.

Battles, J.K., Dalrymple, J.M., 1988. Genetic variation among geographic isolates of Rift Valley fever virus. Am. J. Trop. Med. Hyg. 39, 617–631.

Benferhat, R., Josse, T., Albaud, B., Gentien, D., Mansuroglu, Z., Marcato, V., Soues, S., Le Bonniec, B., Bouloy, M., Bonnefoy, E., 2012. Large-scale chromatin immunoprecipitation with promoter sequence microarray analysis of the interaction of the NSs protein of Rift Valley fever virus with regulatory DNA regions of the host genome. J. Virol. 86, 11333–11344.

Besselaar, T.G., Blackburn, N.K., 1994. The effect of neutralizing monoclonal antibodies on early events in Rift Valley fever virus infectivity. Res. Virol. 145, 13–19.

Bhardwaj, N., Heise, M.T., Ross, T.M., 2010. Vaccination with DNA plasmids expressing Gn coupled to C3d or alphavirus replicons expressing gn protects mice against Rift Valley fever virus. PLoS Negl. Trop. Dis. 4, e725.

Billecocq, A., Spiegel, M., Vialat, P., Kohl, A., Weber, F., Bouloy, M., Haller, O., 2004. NSs protein of Rift Valley fever virus blocks interferon production by inhibiting host gene transcription. J. Virol. 78, 9798–9806.

Bird, B.H., Maartens, L.H., Campbell, S., Erasmus, B.J., Erickson, B.R., Dodd, K.A., Spiropoulou, C.F., Cannon, D., Drew, C.P., Knust, B., McElroy, A.K., Khristova, M.L., Albarino, C.G., Nichol, S.T., 2011. Rift Valley fever virus vaccine lacking the NSs and NSm genes is safe, nonteratogenic, and confers protection from viremia, pyrexia, and abortion following challenge in adult and pregnant sheep. J. Virol. 85, 12901–12909.

Botros, B., Omar, A., Elian, K., Mohamed, G., Soliman, A., Salib, A., Salman, D., Saad, M., Earhart, K., 2006. Adverse response of non-indigenous cattle of European breeds to live attenuated Smithburn Rift Valley fever vaccine. J. Med. Virol. 78, 787–791.

Bouloy, M., Janzen, C., Vialat, P., Khun, H., Pavlovic, J., Huerre, M., Haller, O., 2001. Genetic evidence for an interferon-antagonistic function of Rift Valley fever virus nonstructural protein NSs. J. Virol. 75, 1371–1377.

Brennan, B., Welch, S.R., McLees, A., Elliott, R.M., 2011. Creation of a recombinant Rift Valley fever virus with a two-segmented genome. J. Virol. 85, 10310–10318.

Brown, J.L., Dominik, J.W., Morrissey, R.L., 1981. Respiratory infectivity of a recently isolated Egyptian strain of Rift Valley fever virus. Infect. Immun. 33, 848–853.

Busquets, N., Lorenzo, G., Lopez-Gil, E., Rivas, R., Solanes, D., Galindo-Cardiel, I., Abad, F.X., Rodriguez, F., Bensaid, A., Warimwe, G., Gilbert, S.C., Domingo, M., Brun, A., 2014. Efficacy assessment of an MVA vectored Rift Valley Fever vaccine in lambs. Antiviral Res. 108, 165–172.

Busquets, N., Xavier, F., Martin-Folgar, R., Lorenzo, G., Galindo-Cardiel, I., del Val, B.P., Rivas, R., Iglesias, J., Rodriguez, F., Solanes, D., Domingo, M., Brun, A., 2010. Experimental infection of young adult European breed sheep with Rift Valley fever virus field isolates. Vector Borne Zoonotic Dis. 10, 689–696.

Caplen, H., Peters, C.J., Bishop, D.H., 1985. Mutagen-directed attenuation of Rift Valley fever virus as a method for vaccine development. J. Gen. Virol. 66 (Pt 10), 2271–2277.

Capstick, P.B., Gosden, D., 1962. Neutralizing antibody response of sheep to pantropic and neutrotropic Rift Valley fever virus. Nature 195, 583–584.

Coackley, W., Pini, A., Gosden, D., 1967a. Experimental infection of cattle with pantropic Rift Valley fever virus. Res. Vet. Sci. 8, 399–405.

Coackley, W., Pini, A., Gosden, D., 1967b. The immunity induced in cattle and sheep by inoculation of neurotropic or pantropic Rift Valley fever viruses. Res. Vet. Sci. 8, 406–414.

Coetzer, J.A., Ishak, K.G., 1982. Sequential development of the liver lesions in newborn lambs infected with Rift Valley fever virus. I. Macroscopic and microscopic pathology. Onderstepoort. J. Vet. Res. 49, 103–108.

Copeland, A.M., Altamura, L.A., Van Deusen, N.M., Schmaljohn, C.S., 2013. Nuclear relocalization of polyadenylate binding protein during Rift Valley fever virus infection involves expression of the NSs gene. J. Virol. 87, 11659–11669.

Cosgriff, T.M., Morrill, J.C., Jennings, G.B., Hodgson, L.A., Slayter, M.V., Gibbs, P.H., Peters, C.J., 1989. Hemostatic derangement produced by Rift Valley fever virus in rhesus monkeys. Rev. Infect. Dis. 11 (Suppl. 4), S807–S814.

Daubney, R., Hudson, J., Garnham, P.C., 1931. Enzootic hepatitis or Rift Valley fever: an undescribed virus disease of sheep, cattle and man from East Africa. J. Pathol. Bacteriol. 34, 545–557.

Davies, F.G., Linthicum, K.J., James, A.D., 1985. Rainfall and epizootic Rift Valley fever. Bull. World Health Organ. 63, 941–943.

Di Nardo, A., Rossi, D., Saleh, S.M., Lejlifa, S.M., Hamdi, S.J., Di Gennaro, A., Savini, G., Thrusfield, M.V., 2014. Evidence of Rift Valley fever seroprevalence in the Saharawi semi-nomadic pastoralist system, Western Sahara. BMC Vet. Res. 10, 92.

Dodd, K.A., McElroy, A.K., Jones, M.E., Nichol, S.T., Spiropoulou, C.F., 2013. Rift Valley fever virus clearance and protection from neurologic disease are dependent on CD4+ T cell and virus-specific antibody responses. J. Virol. 87, 6161–6171.

Dodd, K.A., McElroy, A.K., Jones, T.L., Zaki, S.R., Nichol, S.T., Spiropoulou, C.F., 2014. Rift valley Fever virus encephalitis is associated with an ineffective systemic immune response and activated T cell infiltration into the CNS in an immunocompetent mouse model. PLoS Negl. Trop. Dis. 8, e2874.

Dungu, B., Louw, I., Lubisi, A., Hunter, P., von Teichman, B.F., Bouloy, M., 2010. Evaluation of the efficacy and safety of the Rift Valley fever clone 13 vaccine in sheep. Vaccine 28, 4581–4587.

Easterday, B.C., 1965. Rift valley fever. Adv. Vet. Sci. 10, 65–127.

Easterday, B.C., Mc, G.M., Rooney, J.R., Murphy, L.C., 1962. The pathogenesis of Rift Valley fever in lambs. Am. J. Vet. Res. 23, 470–479.

Edwards, J.F., Higgs, S., Beaty, B.J., 1998. Mosquito feeding-induced enhancement of Cache Valley Virus (Bunyaviridae) infection in mice. J. Med. Entomol. 35, 261–265.

El-Harrak, M., Martin-Folgar, R., Llorente, F., Fernandez-Pacheco, P., Brun, A., Figuerola, J., Jimenez-Clavero, M.A., 2011. Rift Valley and West Nile virus antibodies in camels, North Africa. Emerg. Infect. Dis. 17, 2372–2374.

Engdahl, C., Naslund, J., Lindgren, L., Ahlm, C., Bucht, G., 2012. The Rift Valley Fever virus protein NSm and putative cellular protein interactions. Virol. J. 9, 139.

Francis, T., Magill, T.P., 1935. Rift Valley Fever: a report of three cases of laboratory infection and the experimental transmission of the disease to ferrets. J. Exp. Med. 62, 433–448.

Galindo-Cardiel, I., Busquets, N., Velarde, R., Abad, F.X., Solanes, D., Rivas, R., Valle, R., Brun, A., Domingo, M., 2012. Lymphoplasmacytic endotheliitis and anterior uveitis in sheep infected experimentally with Rift Valley fever virus. J. Comp. Pathol. 146, 40–43.

Gommet, C., Billecocq, A., Jouvion, G., Hasan, M., Zaverucha do Valle, T., Guillemot, L., Blanchet, C., van Rooijen, N., Montagutelli, X., Bouloy, M., Panthier, J.J., 2011. Tissue tropism and target cells of NSs-deleted Rift Valley fever virus in live immunodeficient mice. PLoS Negl. Trop. Dis. 5, e1421.

Gray, K.K., Worthy, M.N., Juelich, T.L., Agar, S.L., Poussard, A., Ragland, D., Freiberg, A.N., Holbrook, M.R., 2012. Chemotactic and inflammatory responses in the liver and brain are associated with pathogenesis of Rift Valley fever virus infection in the mouse. PLoS Negl. Trop. Dis. 6, e1529.

Habjan, M., Pichlmair, A., Elliott, R.M., Overby, A.K., Glatter, T., Gstaiger, M., Superti-Furga, G., Unger, H., Weber, F., 2009. NSs protein of Rift Valley fever virus induces the specific degradation of the double-stranded RNA-dependent protein kinase. J. Virol. 83, 4365–4375.

Harrington, D.G., Lupton, H.W., Crabbs, C.L., Peters, C.J., Reynolds, J.A., Slone Jr., T.W., 1980. Evaluation of a formalin-inactivated Rift Valley fever vaccine in sheep. Am. J. Vet. Res. 41, 1559–1564.
Hartman, A.L., Powell, D.S., Bethel, L.M., Caroline, A.L., Schmid, R.J., Oury, T., Reed, D.S., 2014. Aerosolized Rift Valley fever virus causes fatal encephalitis in african green monkeys and common marmosets. J. Virol. 88, 2235–2245.
Hassan, O.A., Ahlm, C., Sang, R., Evander, M., 2011. The 2007 Rift Valley fever outbreak in Sudan. PLoS Negl. Trop. Dis. 5, e1229.
Hofmann, H., Li, X., Zhang, X., Liu, W., Kuhl, A., Kaup, F., Soldan, S.S., Gonzalez-Scarano, F., Weber, F., He, Y., Pohlmann, S., 2013. Severe fever with thrombocytopenia virus glycoproteins are targeted by neutralizing antibodies and can use DC-SIGN as a receptor for pH-dependent entry into human and animal cell lines. J. Virol. 87, 4384–4394.
Hunter, P., Erasmus, B.J., Vorster, J.H., 2002. Teratogenicity of a mutagenised Rift Valley fever virus (MVP 12) in sheep. Onderstepoort. J. Vet. Res. 69, 95–98.
Ikegami, T., Makino, S., 2009. Rift valley fever vaccines. Vaccine 27 (Suppl. 4), D69–D72.
Ikegami, T., Makino, S., 2011. The pathogenesis of Rift Valley fever. Viruses 3, 493–519.
Ikegami, T., Narayanan, K., Won, S., Kamitani, W., Peters, C.J., Makino, S., 2009. Rift Valley fever virus NSs protein promotes post-transcriptional downregulation of protein kinase PKR and inhibits eIF2alpha phosphorylation. PLoS Pathog. 5, e1000287.
Ikegami, T., Peters, C.J., Makino, S., 2005a. Rift valley fever virus nonstructural protein NSs promotes viral RNA replication and transcription in a minigenome system. J. Virol. 79, 5606–5615.
Ikegami, T., Won, S., Peters, C.J., Makino, S., 2005b. Rift Valley fever virus NSs mRNA is transcribed from an incoming anti-viral-sense S RNA segment. J. Virol. 79, 12106–12111.
Indran, S.V., Ikegami, T., 2012. Novel approaches to develop Rift Valley fever vaccines. Front Cell. Infect. Microbiol. 2, 131.
Jansen van Vuren, P., Tiemessen, C.T., Paweska, J.T., 2011. Anti-nucleocapsid protein immune responses counteract pathogenic effects of Rift Valley fever virus infection in mice. PLoS ONE 6, e25027.
Kading, R.C., Crabtree, M.B., Bird, B.H., Nichol, S.T., Erickson, B.R., Horiuchi, K., Biggerstaff, B.J., Miller, B.R., 2014. Deletion of the NSm virulence gene of Rift Valley fever virus inhibits virus replication in and dissemination from the midgut of *Aedes aegypti* mosquitoes. PLoS Negl. Trop. Dis. 8, e2670.
Kainulainen, M., Habjan, M., Hubel, P., Busch, L., Lau, S., Colinge, J., Superti-Furga, G., Pichlmair, A., Weber, F., 2014. Virulence factor NSs of Rift Valley fever virus recruits the F-box protein FBXO3 to degrade subunit p62 of general transcription factor TFIIH. J. Virol. 88, 3464–3473.
Kalveram, B., Lihoradova, O., Ikegami, T., 2011. NSs protein of Rift Valley fever virus promotes posttranslational downregulation of the TFIIH subunit p62. J. Virol. 85, 6234–6243.
Kalveram, B., Lihoradova, O., Indran, S.V., Lokugamage, N., Head, J.A., Ikegami, T., 2013. Rift Valley fever virus NSs inhibits host transcription independently of the degradation of dsRNA-dependent protein kinase PKR. Virology 435, 415–424.
Kortekaas, J., 2014. One Health approach to Rift Valley fever vaccine development. Antiviral Res. 106, 24–32.
Kortekaas, J., Antonis, A.F., Kant, J., Vloet, R.P., Vogel, A., Oreshkova, N., de Boer, S.M., Bosch, B.J., Moormann, R.J., 2012. Efficacy of three candidate Rift Valley fever vaccines in sheep. Vaccine 30, 3423–3429.
Kortekaas, J., Zingeser, J., de Leeuw, P., de La Rocque, S., Unger, H., Moormann, R.J., 2011. Rift Valley fever vaccine development, progress and constraints. Emerg. Infect. Dis. 17, e1.
Le Coupanec, A., Babin, D., Fiette, L., Jouvion, G., Ave, P., Misse, D., Bouloy, M., Choumet, V., 2013. Aedes mosquito saliva modulates Rift Valley fever virus pathogenicity. PLoS Negl. Trop. Dis. 7, e2237.
Le May, N., Dubaele, S., Proietti De Santis, L., Billecocq, A., Bouloy, M., Egly, J.M., 2004. TFIIH transcription factor, a target for the Rift Valley hemorrhagic fever virus. Cell 116, 541–550.
Le May, N., Mansuroglu, Z., Leger, P., Josse, T., Blot, G., Billecocq, A., Flick, R., Jacob, Y., Bonnefoy, E., Bouloy, M., 2008. A SAP30 complex inhibits IFN-beta expression in Rift Valley fever virus infected cells. PLoS Pathog. 4, e13.
Logan, T.M., Linthicum, K.J., Davies, F.G., Binepal, Y.S., Roberts, C.R., 1991. Isolation of Rift Valley fever virus from mosquitoes (Diptera: Culicidae) collected during an outbreak in domestic animals in Kenya. J. Med. Entomol. 28, 293–295.
Lopez-Gil, E., Lorenzo, G., Hevia, E., Borrego, B., Eiden, M., Groschup, M., Gilbert, S.C., Brun, A., 2013. A single immunization with MVA expressing GnGc glycoproteins promotes epitope-specific CD8+-T cell activation and protects immune-competent mice against a lethal RVFV infection. PLoS Negl. Trop. Dis. 7, e2309.
Lorenzo, G., Martin-Folgar, R., Hevia, E., Boshra, H., Brun, A., 2010. Protection against lethal Rift Valley fever virus (RVFV) infection in transgenic IFNAR(−/−) mice induced by different DNA vaccination regimens. Vaccine 28, 2937–2944.
Lorenzo, G., Martin-Folgar, R., Rodriguez, F., Brun, A., 2008. Priming with DNA plasmids encoding the nucleocapsid protein and glycoprotein precursors from Rift Valley fever virus accelerates the immune responses induced by an attenuated vaccine in sheep. Vaccine 26, 5255–5262.
Lozach, P.Y., Kuhbacher, A., Meier, R., Mancini, R., Bitto, D., Bouloy, M., Helenius, A., 2011. DC-SIGN as a receptor for phleboviruses. Cell Host Microbe. 10, 75–88.
Mansuroglu, Z., Josse, T., Gilleron, J., Billecocq, A., Leger, P., Bouloy, M., Bonnefoy, E., 2010. Nonstructural NSs protein of Rift Valley fever virus interacts with pericentromeric DNA sequences of the host cell, inducing chromosome cohesion and segregation defects. J. Virol. 84, 928–939.
McElroy, A.K., Nichol, S.T., 2012. Rift Valley fever virus inhibits a pro-inflammatory response in experimentally infected human monocyte derived macrophages and a pro-inflammatory cytokine response may be associated with patient survival during natural infection. Virology 422, 6–12.
McIntosh, B.M., Dickinson, D.B., dos Santos, I., 1973. Rift Valley fever. 3. Viraemia in cattle and sheep. 4. The susceptibility of mice and hamsters in relation to transmission of virus by mosquitoes. J. S. Afr. Vet. Assoc. 44, 167–169.
Montgomery, R.E., 1913. Report of the Veterinary Department for the Year 1912–1913. Ann. Rep. Dept. Agric., Nairobi, Kenya.
Morrill, J.C., Carpenter, L., Taylor, D., Ramsburg, H.H., Quance, J., Peters, C., 1991a. Further evaluation of a mutagen-attenuated Rift Valley fever vaccine in sheep. Vaccine 9, 35–41.
Morrill, J.C., Czarniecki, C.W., Peters, C., 1991b. Recombinant human interferon-gamma modulates Rift Valley fever virus infection in the rhesus monkey. J. Interferon. Res. 11, 297–304.
Morrill, J.C., Jennings, G.B., Cosgriff, T.M., Gibbs, P.H., Peters, C.J., 1989. Prevention of Rift Valley fever in rhesus monkeys with interferon-alpha. Rev. Infect. Dis. 11 (Suppl. 4), S815–S825.
Morrill, J.C., Jennings, G.B., Johnson, A.J., Cosgriff, T.M., Gibbs, P.H., Peters, C.J., 1990. Pathogenesis of Rift Valley fever in rhesus monkeys: role of interferon response. Arch. Virol. 110, 195–212.
Morrill, J.C., Laughlin, R.C., Lokugamage, N., Pugh, R., Sbrana, E., Weise, W.J., Adams, L.G., Makino, S., Peters, C.J., 2013. Safety and immunogenicity of recombinant Rift Valley fever MP-12 vaccine candidates in sheep. Vaccine 31, 559–565.
Morrill, J.C., Mebus, C.A., Peters, C.J., 1997. Safety and efficacy of a mutagen-attenuated Rift Valley fever virus vaccine in cattle. Am. J. Vet. Res. 58, 1104–1109.
Morrill, J.C., Peters, C.J., 2003. Pathogenicity and neurovirulence of a mutagen-attenuated Rift Valley fever vaccine in rhesus monkeys. Vaccine 21, 2994–3002.
Morrill, J.C., Peters, C., 2011a. Mucosal immunization of rhesus macaques with Rift Valley Fever MP-12 vaccine. J. Infect. Dis. 204, 617–625.
Morrill, J.C., Peters, C., 2011b. Protection of MP-12-vaccinated rhesus macaques against parenteral and aerosol challenge with virulent Rift Valley fever virus. J. Infect. Dis. 204, 229–236.
Muller, R., Saluzzo, J.F., Lopez, N., Dreier, T., Turell, M., Smith, J., Bouloy, M., 1995. Characterization of clone 13, a naturally attenuated avirulent isolate of Rift Valley fever virus, which is altered in the small segment. Am. J. Trop. Med. Hyg. 53, 405–411.
Nfon, C.K., Marszal, P., Zhang, S., Weingartl, H.M., 2012. Innate immune response to Rift Valley fever virus in goats. PLoS. Negl. Trop. Dis. 6, e1623.
Nichol, S.T., Beaty, B.J., Elliott, R.M., Goldbach, R.W., Plyusnin, A., Tesh, R.B., 2005. The Bunyaviridae. In: Fauquet, C., Mayo, M.A., Maniloff, L.S.M., Desselberger, U., Ball, L.A. (Eds.), Virus Taxonomy. Classification and Nomenclature of viruses. 8th Report of the ICTV. Elsevier Academic Press, London, pp. 695–716.
Olaleye, O.D., Tomori, O., Fajimi, J.L., Schmitz, H., 1996. Experimental infection of three Nigerian breeds of sheep with the Zinga strain of the Rift Valley Fever virus. Rev. Elev. Med. Vet. Pays. Trop. 49, 6–16.
Oreshkova, N., Cornelissen, L.A., de Haan, C.A., Moormann, R.J., Kortekaas, J., 2014. Evaluation of nonspreading Rift Valley fever virus as a vaccine vector using influenza virus hemagglutinin as a model antigen. Vaccine 32, 5323–5329.
Oreshkova, N., van Keulen, L., Kant, J., Moormann, R.J., Kortekaas, J., 2013. A single vaccination with an improved nonspreading Rift Valley fever virus vaccine provides sterile immunity in lambs. PLoS ONE 8, e77461.
Peters, C.J., Jones, D., Trotter, R., Donaldson, J., White, J., Stephen, E., Slone Jr., T.W., 1988. Experimental Rift Valley fever in rhesus macaques. Arch. Virol. 99, 31–44.
Peters, C.J., Reynolds, J.A., Slone, T.W., Jones, D.E., Stephen, E.L., 1986. Prophylaxis of Rift Valley fever with antiviral drugs, immune serum, an interferon inducer, and a macrophage activator. Antiviral Res. 6, 285–297.
Pittman, P.R., Liu, C.T., Cannon, T.L., Makuch, R.S., Mangiafico, J.A., Gibbs, P.H., Peters, C.J., 1999. Immunogenicity of an inactivated Rift Valley fever vaccine in humans: a 12-year experience. Vaccine 18, 181–189.
Randall, R., Binn, L.N., Harrison, V.R., 1964. Immunization against Rift Valley fever virus studies on the immunogenicity of lyophilized formalin-inactivated vaccine. J. Immunol. 93, 293–299.
Randall, R., Gibbs Jr., C.J., Aulisio, C.G., Binn, L.N., Harrison, V.R., 1962. The development of a formalin-killed Rift Valley fever virus vaccine for use in man. J. Immunol. 89, 660–671.
Reagan, K.L., Machain-Williams, C., Wang, T., Blair, C.D., 2012. Immunization of mice with recombinant mosquito salivary protein D7 enhances mortality from subsequent West Nile virus infection via mosquito bite. PLoS Negl. Trop. Dis. 6, e1935.
Reed, C., Lin, K., Wilhelmsen, C., Friedrich, B., Nalca, A., Keeney, A., Donnelly, G., Shamblin, J., Hensley, L.E., Olinger, G., Smith, D.R., 2013. Aerosol exposure to Rift Valley fever virus causes earlier and more severe neuropathology in the murine model, which has important implications for therapeutic development. PLoS Negl. Trop. Dis. 7, e2156.
Reed, C., Steele, K.E., Honko, A., Shamblin, J., Hensley, L.E., Smith, D.R., 2012. Ultrastructural study of Rift Valley fever virus in the mouse model. Virology 431, 58–70.
Ritter, M., Bouloy, M., Vialat, P., Janzen, C., Haller, O., Frese, M., 2000. Resistance to Rift Valley fever virus in *Rattus norvegicus*: genetic variability within certain 'inbred' strains. J. Gen. Virol. 81, 2683–2688.
Roger, M., Girard, S., Faharoudine, A., Halifa, M., Bouloy, M., Cetre-Sossah, C., Cardinale, E., 2011. Rift valley fever in ruminants, Republic of Comoros, 2009. Emerg. Infect. Dis. 17, 1319–1320.
Ross, T.M., Bhardwaj, N., Bissel, S.J., Hartman, A.L., Smith, D.R., 2012. Animal models of Rift Valley fever virus infection. Virus Res. 163, 417–423.

Shieh, W.J., Paddock, C.D., Lederman, E., Rao, C.Y., Gould, L.H., Mohamed, M., Mosha, F., Mghamba, J., Bloland, P., Njenga, M.K., Mutonga, D., Samuel, A.A., Guarner, J., Breiman, R.F., Zaki, S.R., 2012. Pathologic studies on suspect animal and human cases of Rift Valley fever from an outbreak in Eastern Africa, 2006–2007. Am. J. Trop. Med. Hyg. 83, 38–42.

Sissoko, D., Giry, C., Gabrie, P., Tarantola, A., Pettinelli, F., Collet, L., D'Ortenzio, E., Renault, P., Pierre, V., 2009. Rift Valley fever, Mayotte, 2007–2008. Emerg. Infect. Dis. 15, 568–570.

Smith, D.R., Bird, B.H., Lewis, B., Johnston, S.C., McCarthy, S., Keeney, A., Botto, M., Donnelly, G., Shamblin, J., Albarino, C.G., Nichol, S.T., Hensley, L.E., 2012. Development of a novel nonhuman primate model for Rift Valley fever. J. Virol. 86, 2109–2120.

Smith, D.R., Steele, K.E., Shamblin, J., Honko, A., Johnson, J., Reed, C., Kennedy, M., Chapman, J.L., Hensley, L.E., 2010. The pathogenesis of Rift Valley fever virus in the mouse model. Virology 407, 256–267.

Soi, R.K., Rurangirwa, F.R., McGuire, T.C., Rwambo, P.M., DeMartini, J.C., Crawford, T.B., 2010. Protection of sheep against Rift Valley fever virus and sheep poxvirus with a recombinant capripoxvirus vaccine. Clin. Vaccine Immunol. 17, 1842–1849.

Sow, A., Faye, O., Ba, Y., Ba, H., Diallo, D., Loucoubar, C., Boushab, M., Barry, Y., Diallo, M., Sall, A.A., 2014. Rift Valley fever outbreak, southern Mauritania, 2012. Emerg. Infect. Dis. 20, 296–299.

Spik, K., Shurtleff, A., McElroy, A.K., Guttieri, M.C., Hooper, J.W., SchmalJohn, C., 2006. Immunogenicity of combination DNA vaccines for Rift Valley fever virus, tick-borne encephalitis virus, Hantaan virus, and Crimean Congo hemorrhagic fever virus. Vaccine 24, 4657–4666.

Styer, L.M., Lim, P.Y., Louie, K.L., Albright, R.G., Kramer, L.D., Bernard, K.A., 2011. Mosquito saliva causes enhancement of West Nile virus infection in mice. J. Virol. 85, 1517–1527.

Swanepoel, R., Struthers, J.K., Erasmus, M.J., Shepherd, S.P., McGillivray, G.M., Shepherd, A.J., Hummitzsch, D.E., Erasmus, B.J., Barnard, B.J., 1986. Comparative pathogenicity and antigenic cross-reactivity of Rift Valley fever and other African phleboviruses in sheep. J. Hyg. (Lond.) 97, 331–346.

Terasaki, K., Won, S., Makino, S., 2013. The C-terminal region of Rift Valley fever virus NSm protein targets the protein to the mitochondrial outer membrane and exerts antiapoptotic function. J. Virol. 87, 676–682.

Tomori, O., 1979. Clinical, virological and serological response of the West African dwarf sheep to experimental infection with different strains of Rift Valley fever virus. Res. Vet. Sci. 26, 152–159.

Van der Lugt, J.J., Coetzer, J.A., Smit, M.M., 1996. Distribution of viral antigen in tissues of new-born lambs infected with Rift Valley fever virus. Onderstepoort. J. Vet. Res. 63, 341–347.

Wallace, D.B., Ellis, C.E., Espach, A., Smith, S.J., Greyling, R.R., Viljoen, G.J., 2006. Protective immune responses induced by different recombinant vaccine regimes to Rift Valley fever. Vaccine 24, 7181–7189.

Warimwe, G.M., Lorenzo, G., Lopez-Gil, E., Reyes-Sandoval, A., Cottingham, M.G., Spencer, A.J., Collins, K.A., Dicks, M.D., Milicic, A., Lall, A., Furze, J., Turner, A.V., Hill, A.V., Brun, A., Gilbert, S.C., 2013. Immunogenicity and efficacy of a chimpanzee adenovirus-vectored Rift Valley fever vaccine in mice. Virol. J. 10, 349.

Weingartl, H.M., Miller, M., Nfon, C., Wilson, W., 2014a. Development of a Rift Valley fever virus viremia challenge model in sheep and goats. Vaccine 32, 2337–2344.

Weingartl, H.M., Nfon, C.K., Zhang, S., Marszal, P., Wilson, W.C., Morrill, J.C., Bettinger, G.E., Peters, C., 2014b. Efficacy of a recombinant Rift Valley fever virus MP-12 with NSm deletion as a vaccine candidate in sheep. Vaccine 32, 2345–2349.

Weingartl, H.M., Zhang, S., Marszal, P., McGreevy, A., Burton, L., Wilson, W., 2014c. Rift Valley fever virus incorporates the 78 kDa glycoprotein into virions matured in mosquito C6/36 cells. PLoS ONE 9, e87385.

Wichgers Schreur, P.J., Oreshkova, N., Moormann, R.J., Kortekaas, J., 2014. Creation of Rift Valley fever viruses with four-segmented genomes reveals flexibility in bunyavirus genome packaging. J. Virol. 88, 10883–10893.

Won, S., Ikegami, T., Peters, C.J., Makino, S., 2007. NSm protein of Rift Valley fever virus suppresses virus-induced apoptosis. J. Virol. 81, 13335–13345.

Xu, W., Watts, D.M., Costanzo, M.C., Tang, X., Venegas, L.A., Jiao, F., Sette, A., Sidney, J., Sewell, A.K., Wooldridge, L., Makino, S., Morrill, J.C., Peters, C.J., Kan-Mitchell, J., 2013. The nucleocapsid protein of Rift Valley fever virus is a potent human CD8+ T cell antigen and elicits memory responses. PLoS ONE 8, e59210.

Yedloutschnig, R.J., Dardiri, A.H., Mebus, C.A., Walker, J.S., 1981. Abortion in vaccinated sheep and cattle after challenge with Rift Valley fever virus. Vet. Rec. 109, 383–384.

Yedloutschnig, R.J., Dardiri, A.H., Walker, J.S., Peters, C.J., Eddy, G.A., 1979. Immune response of steers, goats and sheep to inactivated Rift Valley Fever vaccine. Proc. Annu. Meet. U.S. Anim. Health Assoc., 253–260.