Rift Valley Fever Virus

Ramon Flick[1,*] and Michèle Bouloy[2,*]

[1]*University of Texas Medical Branch, Department of Pathology, Center for Biodefense and Emerging Infectious Diseases, 301 University Boulevard, Galveston, TX 77555-0609, USA*

[2]*Institut Pasteur, Unité de Génétique Moléculaire des Bunyaviridés, 25 rue du Dr Roux 75724 Paris Cedex 15, France*

Abstract: Rift Valley fever is considered to be one of the most important viral zoonoses in Africa. In 2000, the Rift valley fever virus spread to the Arabian Peninsula and caused two simultaneous outbreaks in Yemen and Saudi Arabia. It is transmitted to ruminants and to humans by mosquitoes. The viral agent is an arbovirus, which belongs to the *Phlebovirus* genus in the *Bunyaviridae* family. This family of viruses comprises more than 300 members grouped into five genera: *Orthobunyavirus, Phlebovirus, Hantavirus, Nairovirus,* and *Tospovirus*. Several members of the *Bunyaviridae* family are responsible for fatal hemorrhagic fevers: Rift Valley fever virus (*Phlebovirus*), Crimean-Congo hemorrhagic fever virus (*Nairovirus*), Hantaan, Sin Nombre and related viruses (*Hantavirus*), and recently Garissa, now identified as Ngari virus (*Orthobunyavirus*). Here are reviewed recent advances in Rift Valley fever virus, its epidemiology, molecular biology and focus on recent data on the interactions between viral and cellular proteins, which help to understand the molecular mechanisms utilized by the virus to circumvent the host cellular response.

INTRODUCTION AND HISTORY

Rift Valley fever (RVF) was first described in 1931 by Daubney *et al.* [1] near Naivasha in the Rift Valley of Kenya. The authors isolated the causative viral agent by inoculating lambs with the serum from a moribund sheep. Rift Valley fever virus (RVFV) was classified later as a member of the *Bunyaviridae* family, genus *Phlebovirus* [2]. It was probably endemic in this region of the world as early as 1911, when an unidentified outbreak was reported associated with the death of lambs and sheep [3, 4]. Major outbreaks have been recorded in Kenya in 1968, 1978/79 and 1997/98 as well as in Southern Africa, South Africa, Zimbabwe, Zambia and Madagascar [5-11]. In West and Central Africa, RVFV was not isolated prior to 1974 [12] but it manifested itself later on, causing major outbreaks in Mauritania in 1987 and 1998 [13]. The virus has also been isolated in Senegal, Mali, Guinea and Central Africa. RVFV was not reported to circulate beyond the Sub-Saharan countries before 1977, when it provoked a sudden and dramatic outbreak in Egypt. It reappeared in the same region in 1993 and 1997 [14-16]. Manifestations of the virus were observed in Madagascar in 1990/91. During the Kenyan outbreak in 1997/98 [17, 18] RVFV spread to Tanzania and Somalia. Direct contact with animals, particularly with sheep body fluids was the most important risk factor for RVFV infection. During this outbreak, another bunyavirus, Garissa, was found to be the cause of human cases of hemorrhagic fevers [19]. This orthobunyavirus is in fact, an isolate of Ngari virus, which, in turn, is a Bunyamwera virus reassortant, which acquired the L and S segments of Bunyamwera virus [20].

In 2000, two simultaneous epizootics occurred in Yemen and Saudi Arabia [21, 22]. This was the first time that RVFV was observed outside of the African continent. Infected mosquitoes or viremic animals might have played a role in the spread of virus.

Phylogenetic analyses indicated that the numerous RVFV isolates collected over some fifty years are grouped into three major lineages; Western Africa, Eastern/Central Africa and Egypt [23]. Interestingly, the virus isolated in Madagascar in 1990 was located in the same lineage as the Kenyan isolate of 1997 [24]. All the strains isolated from the Arabian Peninsula were closely related to each other and mapped together with the Kenyan isolate [25-27]. The phylogenetic studies also revealed the ability of this segmented RNA virus to exchange genetic material between isolates from different geographic areas [28].

TRANSMISSION

The virus is normally transmitted by the bite of infected mosquitoes. Occasionally, humans can be infected by contact with infected tissues, or aerosols. Persons in contact with animals, such as veterinarians and workers in the livestock industry are at risk. There is also circumstantial evidence that infection can occur very rarely through contact with raw milk [9, 10]. In Africa, 23 species of mosquitoes

*Address correspondence to these authors at the [1]University of Texas Medical Branch, Department of Pathology, Center for Biodefense and Emerging Infectious Diseases, 301 University Boulevard, Galveston, TX 77555-0609, USA; Tel: +1-409-747 2962; Fax: +1-409-747 2437; E-mail: raflick@utmb.edu

[2]Institut Pasteur, Unité de Génétique Moléculaire des Bunyaviridés, 25 rue du Dr Roux 75724 Paris Cedex 15, France; Tel: +33 (0) 1 40 61 31 57; Fax: +33 (0) 1 40 61 31 51; E-mail: mbouloy@pasteur.fr

1566-5240/05 $50.00+.00

in the genera *Aedes, Culex, Anopheles, Eretmapodites* and *Mansonia,* have been found to be involved in RVFV transmission. Numerous strains of RVFV were isolated from various species of mosquitoes captured in the wild, as well as from *Culicoides* (biting midges)*, Simuli* (black flies) and occasionally from ticks (*Rhipicephalus*). However, in some cases, the virus may be transmitted mechanically.

The most important epidemics/epizootics occurred after periods of unusually heavy rains or in association with construction of dams. RVF outbreaks were observed in 1997/98 after the El Niño floods in the horn of Africa, after the flooding of the Aswan and Diama dams in Egypt in 1977, and along the Senegal River in 1987, respectively. The presence of water is an important factor for the establishment of insect vector breeding sites. Using the records of rainfall and vegetation index data coupled with satellite observations, it may be possible to predict the outbreaks up to 5 months in advance in East Africa [29, 30]. During inter-epidemic periods, the virus is maintained in nature *via* transovarial transmission in mosquitoes, as was shown in *Aedes lineatopennis* in Kenya [31] and in *Aedes vexans* in Senegal [32]. It is not known if there is a vertebrate reservoir such as wild rodents, which are able to propagate the virus.

IMPORTANCE FOR ANIMAL AND PUBLIC HEALTH

Initially, when the disease was reported to affect sheep and lambs, it was described as an enzootic hepatitis with extensive necrosis. Before the Egyptian epidemic in 1977, RVF was essentially known as a disease affecting domestic animals, especially sheep, cattle and goats, producing high mortality rates in new-born animals and abortions in pregnant animals. Only a few fatal human cases were reported before 1977. During the epizootic of RVF in South Africa in 1950/51, 100,000 sheep died and 500,000 aborted [5]. In Kenya in 1997/98, a considerable number of livestock died but viruses other than RVFV, such as Bluetongue virus, were also involved [21, 22]. Although camels do not exhibit overt clinical signs, they abort, as do buffaloes. RVF outbreaks caused and still cause great losses in livestock and economic impact resulting from the death of domesticated animals and restriction of trade and export, which may last several months or years after the end of the epizootic.

In humans, the disease leads to a wide variety of clinical manifestations: most often, RVF begins as a dengue-like illness characterized by fever, headache and is often associated with hemorrhages, but in some cases, severe complications like retinitis, encephalitis and hepatitis with fatal hemorrhagic fevers are observed. The Egyptian outbreak in 1977 was the first one involving a considerable number of human cases: some 18,000 to 200,000 cases were estimated with 623 recorded deaths from encephalitis and/or hemorrhagic fevers [15]. In 1987, 224 human patients died from RVF virus in Mauritania [13]. Although it was difficult to estimate the number of human and animal RVF virus cases in 1997/98 in Eastern Africa, due to the circulation of numerous pathogens, more than 89,000 humans were infected, indicating that it was probably the most important epidemic recorded [18]. In the Arabian Peninsula in 2000, the total number of human cases remains unknown, but if the hospitalized patients represent only a small fraction (usually less than 1%) of the total infections, the 884 and 1087 patients hospitalized respectively, in Saudi Arabia and in Yemen, provide a strong indication of the magnitude of this epidemic.

BIOLOGY OF INFECTION

In new-born lambs less than 1 week of age, the mortality rate may be as high as 90%. The incubation period may be as short as 12 hours (in experimental infections) but lasts usually 24 to 36 hours (or even longer in natural infections), after which the animal develops a high fever, exhibits abdominal pain and dies within 24 to 36 hours after the onset of the first clinical signs. Older animals exhibit various clinical signs from inapparent to peracute or acute infection, the latter form being the most frequent one under field conditions. Sick animals exhibit fever, anorexia, nasal discharge, bloody or foetid diarrhoea and in some cases, a severe icterus. Epizootics are often characterized by sudden storms of abortion. For pregnant animals, abortion may occur at any stage of pregnancy. Abortion rates are usually very high, ranging from 40 to 100% in Southern Africa or 80 to 100% in Egypt in 1977. Adult cattle and sheep may suffer mortality rates of 10-30% or higher, depending on the nutritional state of the animal.

In humans, RVF is usually benign resulting in fever, headache and myalgia followed by a complete recovery. However, in some cases, infection progresses to severe and sometimes fatal complications [6, 33-35] such as retinitis, encephalitis, and hemorrhagic fever with acute hepatitis, which was observed in 1% of the cases in Egypt in 1977 [36]. Encephalitis is often associated with confusion and coma. When the patient recovers, there may be sequelae [37]. A high incidence of retinal/ocular involvement was reported during the 2000 epidemic in the Arabian pensinsula [35].

AETIOLOGICAL AGENT AND CLASSIFICATION

RVFV virus is a member of the genus *Phlebovirus* within the family *Bunyaviridae*. Most viruses in this genus are transmitted by phlebotomine sandflies, hence the genus name *Phlebovirus*. The exceptions are RVFV, which is associated with mosquitoes, and

Uukuniemi-related viruses, which are transmitted by ticks.

Like all the *Bunyaviridae*, RVFV has a tripartite single stranded RNA genome consisting of L(arge), M(edium) and S(mall) segments Fig. (**1**). The L and M segments are of negative polarity and express, respectively, the RNA dependent RNA polymerase L and the precursor to the glycoproteins G_N and G_C. Post-translational cleavage of this precursor protein also generates a non-structural protein (NSm) of yet undetermined role. The S segment of phleboviruses uses an ambisense strategy and encodes for the nucleoprotein N in antisense and for the non-structural protein NSs in sense orientation [38] Fig. (**1**). Both open reading frames (ORF) are separated by an intergenic region important for proper transcription termination. The viral genome S segment (vRNA) serves as transcription template for the subgenomic N-encoding mRNA, whereas the replication intermediate (cRNA) is used to generate subgenomic mRNA coding for the NSs protein. Similar to all negative stranded viruses, the RVFV genome is transcribed and replicated only when complexed with N and L, forming ribonucleoproteins (RNPs). In contrast with genomic (vRNA) and antigenomic (cRNA) RNAs, viral mRNAs are capped with 5' extensions acquired by cap snatching and no poly(A) is added at the 3' end. Transcription of the L and M segments terminates before the 5' end of the template at sequences or structures that have not been identified [39]. Synthesis of the mRNAs derived from the S segment terminates in the vicinity of the intergenic region.

Viral genes are flanked by non-coding regions containing important *cis*-acting elements for the regulation of viral genome transcription, replication, encapsidation and packaging into progeny virions Fig. (**1**). Bunyavirus genus-specific highly conserved complementary nucleotide stretches are located at the segment ends. Intra-strand basepair interaction between these terminal nucleotides leads to non-covalently closed, circular RNAs providing the functional promoter region for the interaction of the viral polymerase with the genomic RNA segments.

Although it does not contain any classical nuclear localization signal (NLS), NSs accumulate in the nucleus where it polymerizes and forms filamentous structures [40] Fig. (**2**). However, it should be noted, that the nuclear localization of this protein is unexpected, since all the steps of the viral cycle occur in the cytoplasm. NSs is a phosphoprotein phosphorylated at two serine residues at its C terminus [41]. Furthermore, the protein interacts with itself at the C terminal domain [42], which appears to be responsible for the formation of filaments.

RVFV are known to bud from Golgi membranes and the budding site seems to be defined by a retention of the glycoproteins G_N and G_C at that particular site [43, 44]. Since bunyaviruses do not have a matrix (M) protein to link the viral surface glycoprotein G_N and G_C with the RNPs, a direct interaction between the RNPs and envelope proteins is expected.

NEW DEVELOPMENTS IN RVFV MOLECULAR BIOLOGY

Recently, it has become possible to genetically manipulate the genomes of negative-stranded viruses and to generate infectious virions entirely

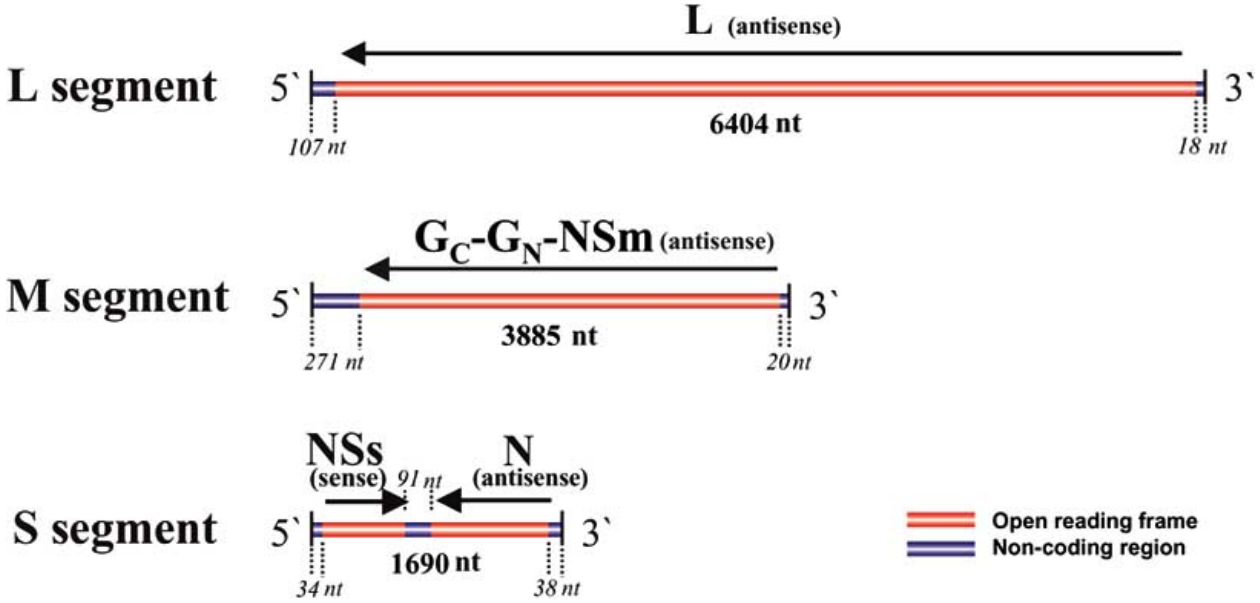

Figure 1. Schematic representation of the genome organization of RVFV MP12 strain. The overall segment length as well as the length of the flanking non-coding regions are indicated. L: viral RNA-dependent RNA polymerase, G_C: glycoprotein located at the C-terminus of the precursor molecule, G_N: glycoprotein located at the N-terminus of the precursor molecule, NSm: M segment-derived non-structural protein, NSs: S segment-derived non-structural protein.

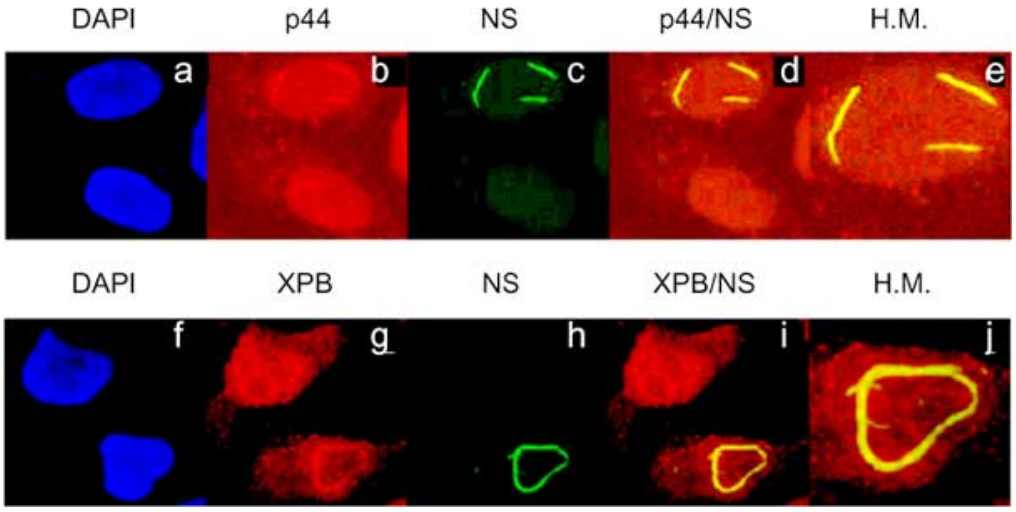

Figure 2. The nonstructural protein NSs interacts with p44 of TFIIH and co-localizes with p44 and XPB in the nuclear filament. Cells expressing the NSs protein of the virulent ZH548 strain of RVFV were analyzed by indirect immunofluorescence using confocal microscopy. The cells were stained with NSs-specific antibodies and with antibodies against p44 and with XPB specific antibodies. Analysis was performed by double immunofluorescence and merging is shown in d-e, i-j. Images are presented at a higher magnification in e and j. (from Le May *et al.* 2004).

from cloned cDNAs. Such "reverse genetics" systems have revolutionized the study of viral gene expression and has enabled the dissection of regulatory elements required for viral transcription and replication steps as well as identified viral components interacting with the host cell. The development of such reverse genetics systems for manipulating the genomes and generating viruses from cloned cDNAs of segmented, negative-strand viruses, e.g. members of the families *Orthomyxoviridae*, *Bunyaviridae*, and *Arenaviridae* have turned out to be very difficult [45-49].

To study transcription and replication mechanisms of segmented negative strand RNA viruses as well as to identify viral proteins required for genome transcription, replication and packaging, minigenome rescue systems are commonly used [47, 48]. For this, a viral genome segment is reverse transcribed into a cDNA and inserted into a proper transcription plasmid (i.e. bacteriophage T7- or RNA polymerase I-driven). To facilitate detection and quantification of transcription and replication processes, the viral gene is replaced by a reporter gene (e.g. **C**hloramphenicol **A**cetyl **T**ransferase: CAT or **G**reen **F**luorescent **P**rotein: GFP). Transfection of such minigenome constructs together with appropriate viral protein expression plasmids results in detectable reporter gene activity.

Previous studies have demonstrated that minigenome rescue systems are a powerful tool for directed mutagenesis of viral RNA genome segments as well as for analyzing viral proteins required for genome encapsidation, transcription, replication and packaging. A T7-driven minigenome rescue system was developed for RVFV by inserting the CAT reporter gene in the antisense orientation flanked by the 3' and 5' terminal non coding sequence of the S segment of the RVFV MP12 strain. The S-CAT minigenome could be transcribed by the RVFV transcriptase complex when both the N and L proteins were expressed, suggesting that the RNA must be tightly associated with the nucleoprotein and the L RNA polymerase to be transcribed [50]. Introduction of mutations at the 3' end of the RNA template allowed determining the crucial role of the conserved eight nucleotide sequence and the purine at position 14 [51].

However, the system was relatively inefficient and it was not possible to clearly demonstrate whether replication occurred. For this reason, an RNA polymerase I (pol I)-driven system [52, 53] was developed and found to be more efficient and functional for transcription and replication (Gauliard, Billecocq, Flick and Bouloy, in preparation). This system was also recently used for the identification and evaluation of potential RVFV-specific antivirals (Flick, unpublished data). Further optimization of such a reverse genetics technology will lead to the establishment of an infectious clone system allowing the reconstitution of RVFV entirely from cDNA components, thereby circumventing a major problem encountered in a helper virus-mediated system – that of separating the helper from the recombinant virus [47, 48].

PATHOGENESIS

RVFV affects primarily the liver with rapid hepatocellular changes progressing to massive necrosis. Hepatic necrosis is the most striking microscopic lesion of RVFV in domestic animal and humans Fig. (**3**). Primary foci of necrosis comprise dense aggregates of cytoplasmic and nuclear debris. Destruction of hepatocytes may be so marked that

most of the normal architecture of the liver is lost. Rod-shaped or oval eosinophilic intranuclear inclusions are commonly found in RVFV-infected livers. These intranuclear inclusions will be shown later to contain the NSs protein [54]. In lambs and sheep, hemorrhages can be observed in the liver. The young animals are the most susceptible. Furthermore, RVFV can infect a wide range of animals from laboratory mice to frogs and tortoises. Experimental infections of susceptible animals (mice, hamsters, rats and monkeys) species have helped to better understand RVF pathogenesis. Infections of mice, hamsters and some strains of rats by peripheral routes with virulent RVFV strains lead to a transient viremia followed by an acute hepatitis and death. In other strains of rats, RVFV infection provokes encephalitis, while others are completely resistant with asymptomatic infection [55, 56]. Resistance was shown to be governed by a dominant Mendelian gene. Rhesus monkeys represent an excellent model for human infection, exhibiting a variety of clinical symptoms including hemorrhagic forms with disseminated intravascular coagulation [57].

Infections with RVFV lead to characteristic hematological and serum chemistry values with a profound leucopenia, elevated serum enzymes associated with severe liver damage and thrombocytopenia. In the development of the hemorrhagic state, the critical lesions are vasculitis and hepatic necrosis [58]. Severe liver damage reduces the production of coagulation proteins and promotes the occurrence of disseminated intravascular coagulopathy and impaired blood flow. Disseminated intravascular coagulation with features like prolonged bleeding time, prothrombin time, and activated partial thromboplastin time, elevated fibrin degradation products, and decreased fibrinogen has been found in severe cases [59].

Similar to other arboviruses, it is possible that RVFV is transported from the site of inoculation to regional lymph nodes by lymphatic drainage (for a review see [8, 9]). The virus replicates in the lymph nodes and is spread into the circulation to produce the primary viremia, leading to infection of the target organs. The major sites of virus replication are the liver, the spleen and often the brain in animals dying from encephalitis. RVFV replicates in hepatocytes in the liver, in the walls of small vessels (adrenocortical cells and glomeruli of the kidney). In the aborted foetus, virus may be recovered from the visceral organs, the brain, and often from the fresh placenta. The serosanguinous fluid found in the thoracic cavity of arbortuses, especially late gestation abortuses, is loaded with virus (10^9 PFU). However, the low pH associated with advanced post mortem autolysis precludes virus recovery from many organs.

THE IMMUNE RESPONSE

As with most viral infections, RVFV is expected to induce adaptive and innate immune responses.

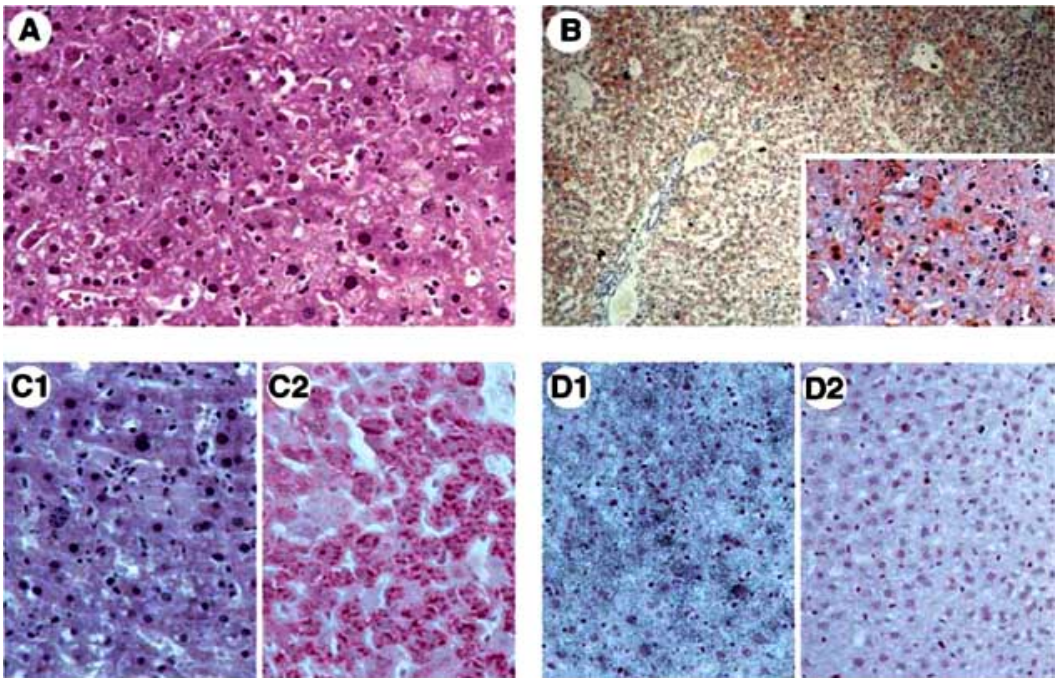

Figure 3. Fulminant hepatitis caused by attenuated Clone 13 in IFN-deficient mice. Histology, immunostaining, and in situ hybridization of post mortem liver sections from IFNAR2/2 mice inoculated with 10^4 PFU of Clone 13. (A) Hematoxylin-eosin staining showing perivascular coagulative necrosis and numerous apoptotic nuclei around the portal area. (B) Immunostaining for viral N protein. (C) Loss of glycogen as revealed by periodic acid Schiff staining. (D) In situ hybridization detecting virus-specific nucleic acids in infected (D1) or uninfected (D2) hepatocytes. Magnifications: (A and C) 3360; (B) 390; (inset) 3225; (D) 3225. (from Bouloy *et al.* 2001).

Innate immunity mediates defense at times preceding activation of adaptive defense mechanisms and also contributes to shaping downstream immune responses. Adaptive response is required to clear infection and mediate long-term memory immunity. Although little is known about the cellular immune response, it is a common feature among bunyavirus infections that the antibody response plays an important role in protection. The internal nucleoprotein appears as the major immunogen but antibodies are also raised against the surface glycoproteins G_N and G_C, which carry neutralizing epitopes [39, 60]. It is well documented that neutralizing antibodies have a protective effect against a virulent RVFV challenge. For this reason, the induction of neutralizing antibodies is a good criterion for the development of a RVFV live attenuated vaccine.

With respect to innate immunity, much attention has been recently drawn on this aspect of the host response (for reviews see [61-63]). Recent work elucidated the mechanisms used by RVFV NSs protein to antagonize the host response [64, 65]. Parallel studies on the orthobunyavirus Bunyamwera, also revealed the major role in virulence played by the non-structural protein NSs [66, 67]. This non-structural protein antagonizes the antiviral response by blocking type I interferon production [46]. NSs appears to be non-essential for the RVFV cycle, since there exists a natural virus Clone 13, in which 70% of the NSs ORF is deleted [68, 69]. This virus was found to be avirulent as well as highly immunogenic in the mouse model. The major role played by type I interferon (IFN) was demonstrated by inoculating mice lacking the alpha/beta interferon receptor with Clone 13: these IFN-deficient animals died very rapidly [65]. Also, avirulence correlates with the production of type I IFN, a property contrasting with the situation encountered with virulent strains of RVFV in which the animals did not produce IFN. In fact, in infected cells, NSs interacts with and sequesters p44 Fig. (**2**), a subunit of the transcription factor TFIIH [70]. Thus, the sequestration of p44 (associated with XPB, its natural partner) by NSs affects the assembly of new molecules of TFIIH complex. As a consequence, cellular transcription is dramatically inhibited. Being a general inhibitor of transcription, NSs must have a wide range of action on cellular transcription: it not only inhibits the antiviral response and prevents the synthesis of IFN ß, but it also may affect the transcriptional activity in response to hormonal stimuli as may be the case for aborted or malformed fetuses.

DIAGNOSIS AND SURVEILLANCE

Since RVF epizootics/epidemics are usually preceded by heavy rains, a high density of mosquitoes, and frequent abortions in sheep and cattle, these environmental criteria should be considered when circulation of the virus is suspected. Liver lesions found by histopathological examinations provide a good indication of RVFV infections Fig. (**3**). When liver sections were observed under electron microscope, many hepatic cells were disorganized with nuclei containing rod or fibre-like structures composed of NSs protein. During an outbreak, the clear demonstration of the presence of the RVFV must be obtained by virological and serological methods. Initially, RVFV was isolated by inoculating lambs with infectious sera but soon after the first isolation, Findlay and Daubney [71] showed that mice were susceptible to RVFV. Thus, mouse inoculation became a routine tool for virus isolation. More recently, mice were replaced by tissue cultures; Vero (green monkey kidney) or mosquito cells being found as sensitive as mice [72]. In addition to virus isolation, which is considered to be the method of choice to demonstrate RVFV activity, diagnosis can also be performed by detection of RVFV-specific IgM or IgG in animal or human sera, the presence of IgM indicating a recent infection. Enzyme-linked immunoassays (ELISA) are widely used in reference laboratories [73-76] and preferred to the previously established methods of complement fixation, hemagglutination inhibition and plaque-reduction neutralization. More recently, detection of the viral genome by RT-PCR amplification has been developed [77-80] and was found to be very useful for rapid presumptive diagnosis, which could be followed by sequencing to further characterize the viral strains.

PROPHYLAXIS AND TREATMENT

Because of the economic importance of the disease in sheep and cattle, efforts were made to produce a veterinary vaccine. To satisfy this need, the Smithburn neurotropic strain was developed by intracerebral passages of the virulent strain Entebbe in suckling mice and embryonated eggs [81]. However, the strain was not completely attenuated, since this live attenuated vaccine is still neurotropic and provokes a range of anomalies of the central nervous system in fetuses such as porencephaly, hydrancephaly, and microencephaly. Vaccination of ewes may also result in abortion and stillbirth. Teratogenic effects associated with vaccination have been reported in up to 15% of pregnant ewes. For this reason, other attenuated strains have been produced or isolated. One of them, the mutagenized strain MP12, which was derived from a virulent strain isolated in Egypt in 1977, appeared to be a good candidate [82] because of the presence of attenuating mutations in each of the three segments of the genome [83]. Experimental inoculation of ruminants indicated that it is an efficient immunogen in adult and young animals [84, 85], but it was also reported that MP12 was abortogenic and teratogenic in pregnant ewes inoculated during the first trimester [86] and the virus was found neurovirulent for monkeys after intracerebral inoculation [87]. A naturally attenuated strain, Clone 13, isolated from a

benign human case was found highly immunogenic for mice. Interestingly, this virus possesses a large deletion in the gene coding for the non-structural NSs, which is the major determinant for attenuation [69]. The presence of the deletion makes any reversion toward the virulent phenotype quite unlikely. It is being tested in South Africa.

A formalin-inactivated RVFV vaccine, which required three boosters but was safe and efficacious, has been used to protect laboratory workers but at present, the ability to produce this vaccine in the US no longer exists [58].

Concerning treatments, it was shown that administration of antibodies, interferon, interferon inducer or the nucleoside analog ribavirin in experimentally RVFV-infected mice, rats or monkeys was efficient in protecting against the disease [88]. However, these treatments have never been tested to treat RVFV-infected patients.

PERSPECTIVES

Recent epidemics in East and West Africa have emphasized the importance of irrigation and rainfall in the re-emergence of the virus as well as a risk of importation from Africa. Since mosquitoes play an important role in viral transmission and propagation, means for mosquito eradication should be implemented. Owing to its ability to infect many species of mosquitoes, to cause devastating effects and to invade new areas, it is not surprising that RVFV has been classified among pathogens potentially utilized for bioterrorism. Of utmost importance is the control of the circulation of the virus. This can be done through entomological or serological surveys or virus isolation from captured mosquitoes or sentinel herd. Several methods for rapid detection of the virus by immuno-capture ELISA or RT-PCR amplification have or are being developed [73-80]. A very urgent need is the production of an efficient vaccine for the most susceptible animals, i.e. sheep, cattle and goats. It would also be very useful for humans. Therapeutic means would also be valuable to treat patients during epidemics. Research studies during the past years have shown that many viruses encode interferon antagonists [61, 63, 89]. The NSs protein of RVFV plays this role. Recent advances in reverse genetics helped to rationally engineer viruses with modifications in their IFN antagonists. These mutated viruses might be suitable live-attenuated vaccines. This field will hopefully open new perspectives to produce efficacious and safe RVFV vaccines.

ACKNOWLEDGEMENTS

The authors would like to thank all the members of their laboratories for helpful discussions and John Morrill and Norbert Herzog for critical reading the manuscript.

REFERENCES

[1] Daubney, R.J., Hudson, J.R. and Garnham, P.C. (1931) *J. Pathol. Bacterio,* **34**, 545-579.
[2] Elliott, R.M., Bouloy, M., Calisher, C. H., Goldbach, R., Moyer, J. T., Nichol, S. T., Pettersson, R. F., Plyusnin, A. and Schmaljohn, C. S. (2000) San Diego, academic press. 614-616.
[3] Stordy, R.J. (1913) D*ep. Agric. Br. East Afr. Annu. Rep*., 13.
[4] Montgomery, E.R. (1912) *Vet. Pathol. Rep*., 37.
[5] Gerdes, G.H. (2002) *Vet. Clin. North Am. Food Anim. Pract.*, **18**, 549-55.
[6] Morrill, J.C. and McClain, D.J. (1996) Epidemiology and pathogenesis of the Rift Valley fever and other Phleboviruses, in The viruses. Plenum Press: New York and London. 281-293.
[7] Peters, C. and Linthicum, K., *Rift Valley fever*. Handbook series in Zoonoses section B: viral zoonoses, second edition. 1994, Boca Raton, Fl: CRC Press.125-138.
[8] Swanepoel, R. and W, C.J.A., *Rift Valley fever*. Infectious diseases of livestock with special reference to southern Africa, ed. J. Coetzer, G. Thompson, and R. Tustin. 1994, Cape Town: Oxford University Press Southern Africa. 688-717.
[9] Gerdes, G.H. (2004) *Rev. Sci. Tech*., **23**, 613-23.
[10] Shimshony, A. and Barzilai, R. (1983) *Adv. Vet. Sci. Comp. Med.*, **27**, 347-425.
[11] Watts, D.M., MacDonald, C., Bailey, C.L., Meegan, J.M., Peters, C.J. and McKee, K.T. Jr. (1988) *Am. J. Trop. Med. Hyg.*, **39**, 611-6.
[12] Digoutte, J.P., Cordellier, R., Robin, Y., Pajot, F.X. and B., G. (1974) *Annales de l'Institut Pasteur, Microbiol*., **125B**, 451-457.
[13] Digoutte, J.P. and Peters, C.J. (1989) *Res. Virol.*, **140**, 27-30.
[14] Arthur, R.R., el-Sharkawy, M.S., Cope, S.E., Botros, B.A., Oun, S., Morrill, J.C., Shope, R.E., Hibbs, R.G., Darwish, M.A. and Imam, I.Z. (1993) *Lancet*, **342**, 1149-50.
[15] Meegan, J.M. (1979) *Trans. R. Soc. Trop. Med. Hyg.*, **73**, 618-23.
[16] Abd el-Rahim, I.H., Abd el-Hakim, U. and Hussein, M. (1999) *Rev. Sci. Tech.*, **18**, 741-8.
[17] Woods, C.W., Karpati, A.M., Grein, T., McCarthy, N., Gaturuku, P., Muchiri, E., Dunster, L., Henderson, A., Khan, A.S., Swanepoel, R., Bonmarin, I., Martin, L., Mann, P., Smoak, B.L., Ryan, M., Ksiazek, T.G., Arthur, R.R., Ndikuyeze, A., Agata, N.N. and Peters, C.J. (2002) *Emerg. Infect. Dis.*, **8**, 138-44.
[18] (1998) *Can. Commun. Dis. Rep*., **24**, 101-4.
[19] Bowen, M.D., Trappier, S.G., Sanchez, A.J., Meyer, R.F., Goldsmith, C.S., Zaki, S.R., Dunster, L.M., Peters, C.J., Ksiazek, T.G. and Nichol, S.T. (2001) *Virology*, **291**, 185-90.
[20] Gerrard, S.R., Li, L., Barrett, A.D. and Nichol, S.T. (2004) *J Virol*, **78**, 8922-6.
[21] CDC (2000) *MMWR Morb. Mortal Wkly. Rep*., **49**, 1065-6.
[22] CDC (2000) *MMWR Morb. Mortal Wkly. Rep*., **49**, 982-5.
[23] Sall, A.A., de, A.Z.P.M., Zeller, H.G., Digoutte, J.P., Thiongane, Y. and Bouloy, M. (1997) *J. Gen. Virol*., **78 (Pt 11)**, 2853-8.
[24] Sall, A.A., de, A.Z.P.M., Vialat, P., Sene, O.K. and Bouloy, M. (1998) *Lancet*, **352**, 1596-7.
[25] Jup, P.G., Kemp, A., Grobbelaar, A., Lema, P., Burt, F.J., Alahmed, A.M., Al Mujalli, D., Al Khamees, M. and Swanepoel, R. (2002) *Med. Vet. Entomol.*, **16**, 245-52.
[26] Shoemaker, T., Boulianne, C., Vincent, M.J., Pezzanite, L., Al-Qahtani, M.M., Al-Mazrou, Y., Khan, A.S., Rollin, P.E., Swanepoel, R., Ksiazek, T.G. and Nichol, S.T. (2002) *Emerg. Infect. Dis.*, **8**, 1415-20.
[27] Miller, B.R., Godsey, M.S., Crabtree, M.B., Savage, H.M., Al-Mazrao, Y., Al-Jeffri, M.H., Abdoon, A.M., Al-Seghayer, S.M., Al-Shahrani, A.M. and Ksiazek, T.G. (2002) *Emerg. Infect. Dis.*, **8**, 1492-4.
[28] Sall, A.A., Zanotto, P.M., Sene, O.K., Zeller, H.G., Digoutte, J.P., Thiongane, Y. and Bouloy, M. (1999) *J. Virol.*, **73**, 8196-200.
[29] Linthicum, K.J., Anyamba, A., Tucker, C.J., Kelley, P.W., Myers, M.F. and Peters, C.J. (1999) *Science*, **285**, 397-400.
[30] Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S. and Samuel, M.D. (2002) *Science*, **296**, 2158-62.
[31] Linthicum, K.J., Davies, F.G., Kairo, A. and Bailey, C.L. (1985) *J. Hyg., (Lond)*, **95**, 197-209.
[32] Zeller, H.G., Fontenille, D., Traore-Lamizana, M., Thiongane, Y. and Digoutte, J.P. (1997) *Am. J. Trop. Med. Hyg.*, **56**, 265-72.

[33] 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. and Aqeel, A. (2003) *Clin. Infect. Dis.*, **36**, 245-52.

[34] Borio, L., Inglesby, T., Peters, C.J., Schmaljohn, A.L., Hughes, J.M., Jahrling, P.B., Ksiazek, T., Johnson, K.M., Meyerhoff, A., O'Toole, T., Ascher, M.S., Bartlett, J., Breman, J.G., Eitzen, E.M., Jr., Hamburg, M., Hauer, J., Henderson, D.A., Johnson, R.T., Kwik, G., Layton, M., Lillibridge, S., Nabel, G.J., Osterholm, M.T., Perl, T.M., Russell, P. and Tonat, K. (2002) *JAMA*, **287**, 2391-405.

[35] Madani, T.A., Al-Mazrou, Y.Y., Al-Jeffri, M.H., Mishkhas, A.A., Al-Rabeah, A.M., Turkistani, A.M., Al-Sayed, M.O., Abodahish, A.A., Khan, A.S., Ksiazek, T.G. and Shobokshi, O. (2003) *Clin. Infect. Dis.*, **37**, 1084-92.

[36] Meegan J.M. and C.J., B. (1989) Rift Valley fever. The arboviruses: epidemiology and ecology. M. T.P. Vol. IV, Boca Raton, Fla: CRC Press Inc. 51-76.

[37] (1985) *World Health Organ Tech. Rep. Ser.*, **721**, 5-126.

[38] Giorgi, C., Accardi, L., Nicoletti, L., Gro, M.C., Takehara, K., Hilditch, C., Morikawa, S. and Bishop, D.H. (1991) *Virology*, **180**, 738-53.

[39] Collett, M.S. (1986) *Virology*, **151**, 151-6.

[40] Struthers, J.K., Swanepoel, R. and Shepherd, S.P. (1984) *Virology*, **134**, 118-24.

[41] Kohl, A., di Bartolo, V. and Bouloy, M. (1999) *Virology*, **263**, 517-25.

[42] Yadani, F.Z., Kohl, A., Prehaud, C., Billecocq, A. and Bouloy, M. (1999) *J. Virol.*, **73**, 5018-25.

[43] Gerrard, S.R. and Nichol, S.T. (2002) *J. Virol.*, **76**, 12200-10.

[44] Gerrard, S.R., Rollin, P.E. and Nichol, S.T. (2002) *Virology*, **301**, 226-35.

[45] Fodor, E., Devenish, L., Engelhardt, O.G., Palese, P., Brownlee, G.G. and Garcia-Sastre, A. (1999) *J. Virol.*, **73**, 9679-82.

[46] Bridgen, A. and Elliott, R.M. (1996) *Proc Natl Acad Sci USA*, **93**, 15400-4.

[47] Kawaoka, Y. (2004) *Curr. Top. Microbiol. Immunol.,* **283**.

[48] Walpita, P. and Flick, R. (2005) *FEMS Microbiol. Lett.*, **244**, 9-18.

[49] Neumann, G., Watanabe, T., Ito, H., Watanabe, S., Goto, H., Gao, P., Hughes, M., Perez, D.R., Donis, R., Hoffmann, E., Hobom, G. and Kawaoka, Y. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 9345-50.

[50] Lopez, N., Muller, R., Prehaud, C. and Bouloy, M. (1995) *J. Virol.*, **69**, 3972-9.

[51] Prehaud, C., Lopez, N., Blok, M.J., Obry, V. and Bouloy, M. (1997) *Virology*, **227**, 189-97.

[52] Neumann, G., Zobel, A. and Hobom, G. (1994) *Virology*, **202**, 477-9.

[53] Zobel, A., Neumann, G. and Hobom, G. (1993) *Nucleic Acids Res.*, **21**, 3607-14.

[54] Swanepoel, R. and Blackburn, N.K. (1977) *J. Gen. Virol.*, **34**, 557-61.

[55] Anderson, G.W. Jr., Slone, T.W., Jr. and Peters, C.J. (1987) *Microb. Pathog.*, **2**, 283-93.

[56] Ritter, M., Bouloy, M., Vialat, P., Janzen, C., Haller, O. and Frese, M. (2000) *J. Gen. Virol.*, **81**(Pt. 11), 2683-8.

[57] Peters, C.J., Jones, D., Trotter, R., Donaldson, J., White, J., Stephen, E. and Slone, T.W. Jr. (1988) *Arch. Virol.*, **99**, 31-44.

[58] Geisbert, T.W. and Jahrling, P.B. (2004) *Nat. Med.,* **10**(12 Suppl.), S110-21.

[59] Lacy, M.D. and Smego, R.A. (1996) *Adv Pediatr. Infect. Dis.*, **12**, 21-53.

[60] Collett, M.S., Purchio, A.F., Keegan, K., Frazier, S., Hays, W., Anderson, D.K., Parker, M.D., Schmaljohn, C., Schmidt, J. and Dalrymple, J.M. (1985) *Virology*, **144**, 228-45.

[61] Conzelmann, K.K. (2005) *J. Virol.*, **79**, 5241-8.

[62] Garcia-Sastre, A. (2004) *Curr. Top. Microbiol. Immunol.*, **283**, 249-80.

[63] Goodbourn, S., Didcock, L. and Randall, R.E. (2000) *J. Gen. Virol.*, **81**(Pt. 10), 2341-64.

[64] Billecocq, A., Spiegel, M., Vialat, P., Kohl, A., Weber, F., Bouloy, M. and Haller, O. (2004) *J. Virol.*, **78**, 9798-806.

[65] Bouloy, M., Janzen, C., Vialat, P., Khun, H., Pavlovic, J., Huerre, M. and Haller, O. (2001) *J. Virol.*, **75**, 1371-7.

[66] Weber, F., Bridgen, A., Fazakerley, J.K., Streitenfeld, H., Kessler, N., Randall, R.E. and Elliott, R.M. (2002) *J. Virol.*, **76**, 7949-55.

[67] Bridgen, A., Weber, F., Fazakerley, J.K. and Elliott, R.M. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 664-9.

[68] Muller, R., Saluzzo, J.F., Lopez, N., Dreier, T., Turell, M., Smith, J. and Bouloy, M. (1995) *Am. J. Trop. Med. Hyg.*, **53**, 405-11.

[69] Vialat, P., Billecocq, A., Kohl, A. and Bouloy, M. (2000) *J. Virol.*, **74**, 1538-43.

[70] Le May, N., Dubaele, S., Proietti De Santis, L., Billecocq, A., Bouloy, M. and and Egly, J. (2004) *Cell*, **116**, 1-20.

[71] Findlay, G.M. and Daubney, R.J. (1931) *Lancet*, **ii**, 1350-1351.

[72] Digoutte, J.P., Jouan, A., Le Guenno, B., Riou, O., Philippe, B., Meegan, J., Ksiazek, T.G. and Peters, C.J. (1989) *Res. Virol.*, **140**, 31-41.

[73] Niklasson, B., Peters, C.J., Grandien, M. and Wood, O. (1984) *J. Clin. Microbiol.*, **19**, 225-9.

[74] Paweska, J.T., Burt, F.J., Anthony, F., Smith, S.J., Grobbelaar, A.A., Croft, J.E., Ksiazek, T.G. and Swanepoel, R. (2003) *J. Virol. Methods*, **113**, 103-12.

[75] Paweska, J.T., Burt, F.J. and Swanepoel, R. (2005) *J. Virol. Methods*, **124**, 173-81.

[76] Paweska, J.T., Smith, S.J., Wright, I.M., Williams, R., Cohen, A.S., Van Dijk, A.A., Grobbelaar, A.A., Croft, J.E., Swanepoel, R. and Gerdes, G.H. (2003) *Onderstepoort J. Vet. Res.*, **70**, 49-64.

[77] Garcia, S., Crance, J.M., Billecocq, A., Peinnequin, A., Jouan, A., Bouloy, M. and Garin, D. (2001) *J. Clin. Microbiol.*, **39**, 4456-61.

[78] Ibrahim, M.S., Turell, M.J., Knauert, F.K. and Lofts, R.S. (1997) *Mol. Cell Probes*, **11**, 49-53.

[79] Sall, A.A., Thonnon, J., Sene, O.K., Fall, A., Ndiaye, M., Baudez, B., Mathiot, C. and Bouloy, M. (2001) *J. Virol. Methods*, **91**, 85-92.

[80] Drosten, C., Gottig, S., Schilling, S., Asper, M., Panning, M., Schmitz, H. and Gunther, S. (2002) *J. Clin. Microbiol.*, **40**, 2323-30.

[81] Smithburn, K. (1949) *Br. J. Exp . Pathol.*, **30**, 1-16.

[82] Caplen, H., Peters, C.J. and Bishop, D.H. (1985) *J. Gen. Virol.*, **66 (Pt 10)**, 2271-7.

[83] Saluzzo, J.F. and Smith, J.F. (1990) *Vaccine*, **8**, 369-75.

[84] Morrill, J.C., Mebus, C.A. and Peters, C.J. (1997) *Am. J. Vet. Res.*, **58**, 1110-4.

[85] Morrill, J.C., Mebus, C.A. and Peters, C.J. (1997) *Am. J. Vet. Res.*, **58**, 1104-9.

[86] Hunter, P., Erasmus, B.J. and Vorster, J.H. (2002) *Onderstepoort J. Vet. Res.*, **69**, 95-8.

[87] Morrill, J.C. and Peters, C.J. (2003) *Vaccine*, **21**, 2994-3002.

[88] Peters, C.J., Reynolds, J.A., Slone, T.W., Jones, D.E. and Stephen, E.L. (1986) *Antiviral Res.*, **6**, 285-97.

[89] Garcia-Sastre, A. (2001) *Virology*, **279**, 375-84.