Published in final edited form as:

Vaccine. 2013 January 7; 31(3): 559–565. doi:10.1016/j.vaccine.2012.10.118.

Safety and Immunogenicity of Recombinant Rift Valley Fever MP-12 Vaccine Candidates in Sheep

John C. Morrill^{a,*}, Richard C. Laughlin^b, Nandadeva Lokugamage^a, Roberta Pugh^b, Elena Sbrana^a, William J. Weise^b, L. Garry Adams^b, Shinji Makino^a, and C. J. Peters^a

^aDepartment of Microbiology and Immunology, The University of Texas Medical Branch at Galveston, 301 University Blvd., Room 4.142B MRB, Galveston, Texas 77555-1019, USA

^bDepartment of Veterinary Pathobiology, College of Veterinary Medicine, Texas A & M University, College Station, Texas 77843-4467, USA

Abstract

The safety and immunogenicity of two authentic recombinant (ar) Rift Valley Fever (RVF) viruses, one with a deletion in the NSs region of the S RNA segment (arMP-12ΔNSs16/198) and the other with a large deletion of the NSm gene in the pre Gn region of the M RNA segment (arMP-12ΔNSm21/384) of the RVF MP-12 vaccine virus were tested in crossbred ewes at 30 – 50 days of gestation. First, we evaluated the neutralizing antibody response, measured by plaque reduction neutralization (PRNT₈₀), and clinical response of the two viruses in groups of four ewes each. The virus dose was 1×10^5 plaque forming units (PFU). Control groups of four ewes each were also inoculated with a similar dose of RVF MP-12 or the parent recombinant virus (arMP-12). Neutralizing antibody was first detected in 3 of 4 animals inoculated with arMP-12ΔNSm21/384 on day 5 post inoculation and all four animals had PRNT₈₀ titers of 1:20 on day 6. Neutralizing antibody was first detected in 2 of 4 ewes inoculated with arMP-12ΔNSs16/198 on day 7 and all had PRNT₈₀ titers of 1:20 on day 10. We found the mean PRNT₈₀ response to arMP-12ΔNSs16/198 to be 16- to 25-fold lower than that of ewes inoculated with arMP-12ΔNSm21/384, arMP-12 or RVF MP-12. No abortions occurred though a single fetal death in each of the arMP-12 and RVF MP-12 groups was found at necropsy. The poor PRNT₈₀ response to arMP-12ΔNSs16/198 caused us to discontinue further testing of this candidate and focus on arMP-12ΔNSm21/384. A dose escalation study of arMP-12ΔNSm21/384, showed that 1×10^3 plaque forming units (PFU) stimulates a PRNT₈₀ response comparable to doses of up to 1×10^5 PFU of this virus. With further study, the arMP-12ΔNSm21/384 virus may prove to be a safe and efficacious candidate for a livestock vaccine. The large deletion in the NSm gene may also provide a negative marker that will allow serologic differentiation of naturally infected animals from vaccinated animals.

© 2012 Elsevier Ltd. All rights reserved.

*Corresponding author. Telephone 011 1 409 772 4908; Fax 011 1 409 772 5065. jcmorrill@utmb.edu.

Author Contributions

Conceived and designed the experiments: JCM, LGA, CJP, SM. Performed the experiments: JCM, LGA, RCL, RP, SM, WJW, NL, ES. Wrote the paper: JCM, LGA, RCL, SM, NL. All authors have approved this manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Rift Valley fever; RVF MP-12; arMP-12ΔNSm21/384; sheep

Introduction

Rift Valley fever virus (RVFV, family *Bunyaviridae*, genus *Phlebovirus*) is a segmented, single-stranded RNA virus and the etiological agent of the vector-borne zoonotic disease, Rift Valley fever (RVF) [1–5]. The virus poses a public health and economic threat and outbreaks have significant impacts in affected areas [6–8].

The RVFV genome contains three RNA segments of negative-sense or ambi-sense polarity: L, M and S [9–11]. The anti-viral-sense L-segment (6404 nt) encodes the viral RNA-dependent RNA polymerase [12]. The anti-viral-sense M segment (3885 nt) encodes two major envelope glycoproteins, Gn and Gc, and two accessory proteins, 14-kDa NSm and 78-kDa [13–16]. The NSm and 78-kDa proteins are not essential for RVFV replication in cell culture [14], whereas a RVFV mutant lacking the pre-Gn region is less pathogenic than RVFV carrying the pre-Gn region in rats [13], indicating NSm, which has anti-apoptosis function, affects viral pathogenicity [16–19]. The S segment encodes a nonstructural protein, NSs, a major virulence factor, and nucleocapsid (N) protein [20–24].

While susceptibility of naïve individuals to RVFV infection is presumed high, protection can be achieved by humoral immune responses or colostrum [25,26]. The single administration of a vaccine eliciting a rapid humoral response and a long-term protective immunity in an outbreak scenario would save lives and fiscal resources. While several RVFV vaccines have been developed [27], work still remains to improve safety, responsiveness, effectiveness, environmental stability, genomic integrity and long term immunity.

Here we report the testing, in pregnant ewes, of two recombinant viruses possessing deletions in either the NSs region or NSm region of the RVF MP-12 genome to assess their safety, immunogenicity and potential use as a livestock vaccine.

Materials and Methods

Animals

Healthy, F1 Suffolk-Rambouillet crossbred ewes, estrus synchronized and time-bred to be 30–50 days of gestation at the time of vaccination were used. The ewes were negative for RVFV neutralizing antibody by plaque reduction neutralization assay at the time of vaccination.

Viruses

The recombinant virus (ar), identified as arMP-12, is genetically identical to the live, attenuated RVF MP-12 vaccine, prepared by the Salk Institute, Swiftwater, PA, for the U. S. Army Medical Research Institute of Infectious Diseases (USAMRIID) for use in humans under an Investigational New Drug (IND) Application [28] and served as the recombinant virus control. The two recombinant viruses tested for safety and immunogenicity, identified as arMP-12ΔNSs16/198 and arMP-12ΔNSm21/384, were generated by reverse genetics techniques [16, 29]. Both viruses contain the MP12 genome but the arMP-12ΔNSs16/198 virus has a deletion in the NSs region of the S RNA segment and the arMP-12ΔNSm21/384 virus has a large deletion of NSm gene in the pre Gn region in the M RNA segment of RVF MP-12.

Experimental Design

The pregnant ewes were housed in a USDA approved ABSL2 Ag biocontainment facility. The ewes were randomized into test groups and acclimated to the biocontainment facility for 14 days. The studies were conducted in two phases. Phase I evaluated immunogenicity and safety of the two recombinant viruses and the arMP-12ΔNSm21/384 was judged to be the most promising vaccine candidate in terms of immunogenicity and safety. Phase II tested escalating doses of arMP-12ΔNSm21/384 for safety and immunogenicity. In Phase I, groups of 4 ewes each were inoculated subcutaneously (s.c.) with approximately 1×10^5 plaque forming units (PFU) of a recombinant virus or RVF MP12. In Phase II, graded doses of arMP-12ΔNSm21/384 were tested in groups of 4 to 10 animals. The ewes were examined daily by a veterinarian or trained veterinary animal specialists. Whole blood was collected prior to inoculation (Day -7), the day of inoculation (Day 0), daily through Day 7 or 14, and periodically thereafter through Day 62 or 69. Rectal temperatures and health status were documented daily. The ewes were euthanized with pentobarbital sodium (120 mg/kg i.v.) prior to term. Necropsies were performed on each ewe and fetus and tissues were collected for virus isolation, histopathologic examination, and immunohistochemistry (IHC). Fetal crown-rump (C-R) measurements were taken to estimate the approximate age of the fetuses [30, 31].

Specimen preparation

Serum was harvested from whole blood and stored at -80°C . Specimens of selected organs were fixed in 10% formalin and processed for histopathological and IHC examination [32]. Formalin-fixed and paraffin-embedded tissue sections were stained with hematoxylin and eosin (H&E). Rabbit anti-N antibody (1:500) was used for IHC staining [14]. Homogenates (10% w/v) of fresh tissues were prepared in Hanks' balanced salt solution (HBSS) pH 7.4, supplemented with 10% fetal bovine serum and antibiotics (200 U penicillin and 50 $\mu\text{g/ml}$ streptomycin sulfate), clarified by low speed centrifugation and stored at -80°C .

Reverse transcription polymerase chain reaction (RT-PCR)

Viral RNA was extracted from 100 μl of 10% tissue homogenate using a high pure viral RNA extraction kit (Roche Applied Science, Indianapolis, IN, USA) according to manufacturer's instructions. The first-strand cDNA was synthesized with Superscript II by using random primers (Invitrogen, Carlsbad, CA). RT-PCR was performed with Platinum Taq Polymerase (Invitrogen) using the following primer sets; S20F (ACA CAA AGC TCC CTA GAG AT) and S1058R (TGC GTT CGG CTT CTG CAA GC) for the S-segment detection, and M19F (ACA CAA AGA CGG TGC ATT A) and M1041R (ACT GCA AAG GGC ACA ACC TC) for the M-segment detection. Using serial dilutions of MP-12, the RT-PCR could detect the S- and M-segments up to 6 PFU (data not shown). The following PCR condition was used; 95°C for 5 min followed by 30 cycles of 95°C for 30 sec, 55°C for 30 sec and 68°C for 1 min, and followed by a final 10 min extension at 68°C . The PCR fragments were subsequently analyzed by agarose gel electrophoresis.

Virus isolation and viral plaque assay

Virus isolation from neat serum and 10% tissue homogenates was accomplished by culturing samples in Vero E-6 cells in 25 cm^2 flasks as previously described [26,33]. The flasks were observed for cytopathic effect daily for 10 days before blind passage in fresh Vero E-6 cells. Virus titers were determined by plaque assay in Vero cell monolayers as previously described [26,33].

Immunology Methods

Serum neutralizing antibody was determined using an 80% plaque-reduction neutralization test (PRNT₈₀) as previously described [33]. Sera were tested for RVFV-specific IgG antibodies using an enzyme immunoassay as previously described [34, 35]. Sera for IgG were tested at an initial dilution of 1:100. The cutoff value for assigning a positive IgG result was determined from a panel of five sera from RVFV IgG negative animals calculated in an adjusted OD₄₁₄ value greater than 3 SD.

Statistical Analysis

All calculations were done using Prism Version 5.0d analysis software (Graphpad Software Inc). Analysis of mean PRNT₈₀ titers and mean serum IgG values were done using a one-way analysis of variance and a post hoc Tukey's Multiple comparison test with a significance level of $\alpha=0.05$.

Results

Phase I

This Phase was a test, in groups of 4 ewes each, of the clinical and immunological response of two vaccine candidates compared to the recombinant parent virus, arMP-12 and MP-12. The individual neutralizing antibody responses of the ewes are listed in Table 1 and the mean PRNT₈₀ responses of the four groups shown in Figure 1A. Figure 2A shows the serum IgG responses of the four groups. The time to seroconversion and intensity of the neutralizing antibody response was markedly delayed and of lesser intensity in the arMP-12 Δ NSs16/198 group when compared to the other groups. During the study, mean rectal temperatures of the groups did not exceed 39.5°C (data not shown).

Ewes #17–20 inoculated with arMP-12 Δ NSs16/198—Ewes #17, 18, 19 and 20 experienced no significant adverse clinical events; however, ewes #17 and 18 were not pregnant. At necropsy, ewe #19 had a single viable fetus with a C-R measurement of 30 cm and ewe #20 had viable twin fetuses with C-R measurements of 32 and 34 cm respectively. We estimated those three fetuses to be between 15–18 weeks of age. No significant gross or histopathologic lesions were observed in any of the ewes or their fetuses. No viremia was detected and no virus or viral RNA was recovered from or detected in any tissues from ewes or fetuses.

Ewes #21–24 inoculated with arMP-12 Δ NSm 21/384—No significant adverse clinical events were observed in this group during the study. Ewe #22 was not pregnant at the time of inoculation but the other 3 pregnant ewes each had a single viable fetus at the time of necropsy. The three fetuses had C-R measurements of 31 cm, 32 cm and 33 cm respectively, and we estimated those fetuses to also be between 15 and 18 weeks of age. No significant gross lesions or anomalies were observed in any of the ewes or their fetuses, and no vaccine virus was recovered from or detected in serum or tissues from any of these ewes or their fetuses.

Ewes #25–28 inoculated with RVF MP-12—There were no significant adverse clinical events noted in this group during the study. When the study was terminated and the animals necropsied, Ewe #26 had a dead, autolyzed fetus measuring 39 cm C-R. We estimated this fetus to be approximately 18–20 weeks of gestation. PCR analysis of brain tissue from that fetus was weakly positive for vaccine viral RNA but we were unable to recover viable virus. The other three ewes had live, viable fetuses. Ewe # 25 had twins measuring 31 cm C-R each (15–18 weeks); Ewe #27 and Ewe #28 each had a single fetus measuring 37 cm and 40

cm respectively (18–20 weeks). No virus was recovered from or detected in serum or tissues from any of the ewes or their fetuses.

Ewes #29–32 inoculated with arMP-12—No significant adverse clinical events were observed in this group during the study however, at necropsy, Ewe #31 had a dead, autolyzed fetus with a C-R measurement of 12 cm suggesting the fetus was 6 to 8 weeks of gestation. The other three ewes had viable fetuses (two had single fetuses and one had twins) measuring 31 or 32 cm C-R indicating they were approximately 15 to 18 weeks of gestation. No viremia was detected and no virus or viral RNA was recovered from or detected in any tissues from ewes or fetuses.

Phase II

The comparatively weak neutralizing antibody response to arMP-12ΔNSs16/198 vaccine caused us to abandon this culture as a livestock vaccine candidate. The arMP-12ΔNSm21/384 vaccine showed a rapid and vigorous serologic response together with no untoward clinical events. We elected to perform a dose escalation study of this vaccine candidate to determine the optimum safe and immunogenic dose. The animals in this Phase were terminated at 62 days after inoculation. Tables 2 through 4 show individual PRNT₈₀ titers of the four groups of test animals and Figures 1B and 2B show the mean serum PRNT₈₀ and serum IgG responses of the four groups.

Group #1 - Ewes #37 – 40 inoculated with 1×10^2 PFU of arMP-12ΔNSm 21/384 (N=4)—This group of sheep (Table 2) received the lowest dose of any of the test animals and they experienced no adverse clinical events during the course of the study. Rectal temperature recordings were unremarkable with no evidence of pyrexia in this group of sheep. At the termination of the study, all four ewes had a single, morphologically normal and viable fetus with C-R measurements of 30 or 31 cm of 15–18 weeks of fetal age. No viable virus was recovered by direct plaque techniques from any tissues, including sera, from the ewes or their fetuses.

Group #2 - Ewes #41–44 inoculated with 1×10^3 PFU of arMP-12ΔNSm21/384(N=4)—Although post inoculation clinical observations were unremarkable with no evidence of pyrexias in this group (Table 3), a virus titer of 2×10^1 PFU/ml, determined by direct plaque assay, was detected in the serum of ewe #41 on Day 4 only. PCR analysis of the recovered virus, after amplification in Vero E6 cells, showed a partial deletion in the M segment. RNA sequencing of that virus was not done. In contrast, no virus or viral RNA was recovered from the other three ewes. At termination of the study, the PRNT₈₀ titer of the viremic ewe was 4-fold higher than the cohorts (Table 2). The viremic ewe was not pregnant and a 10 cm retroperitoneal abscess was observed that adhered to the uterus and large and small intestine. Ewes #42, #43 and #44 each had a single, morphologically normal and viable fetus with C-R measurements of 26, 31 and 31 cm respectively. We estimated those fetuses to be approximately 14–18 weeks of age. No virus or viral RNA was recovered from or detected in any tissues from the ewes or their fetuses at necropsy and no neutralizing antibody was found in the fetal sera.

Group #3 – Ewes #45 – 48 and #53 – 58 inoculated with 1×10^4 PFU of arMP-12ΔNSm21/384(N=10)—The only adverse event in this group (Table 3) occurred in ewe #48. At necropsy, this ewe had hematuria and a dead, autolyzed fetus with a C-R measurement of 31 cm (15–18 weeks). The fetus was weakly positive for viral RNA in the liver, spleen, kidney and brain tissues but we were unable to isolate virus from any of those tissues and advanced post mortem autolysis prevented observation of definitive microscopic lesions. Ewes #56 and 57 were not pregnant and the other 7 ewes each had live,

morphologically normal fetuses. Based on their C-R measurements, the viable fetuses were estimated to be approximately 15 to 18 weeks of age. A viremia titer of 3×10^1 PFU/ml in serum was detected in ewe #56 on Day 1 and PCR analysis of that recovered virus showed it to have a deletion in the M segment similar to the vaccine virus. At necropsy, no virus or viral antigen was detected in any tissues from the dams or viable fetuses.

Group #4 - Ewes #59 – 64 inoculated with 1×10^5 PFU of arMP-12ΔNSm21/384 (N=6)—No adverse clinical events occurred in this group but a viremia titer of 3×10^2 PFU/ml of serum was detected in ewe #62 on Day 4. PCR analysis of this recovered virus also showed it to have a deletion in the M segment similar to the input virus. That ewe also had a slightly elevated rectal temperature of 39.6°C and 39.8°C on Day 2 and 3, respectively, but her temperature was within the upper range of normal on Day 4. At necropsy all 6 ewes had live fetuses measuring 29 to 32 cm C-R, with Ewe #63 having twin fetuses. We estimated all of the fetuses to be 15 to 18 weeks of age. No virus was detected in any organs from the dams or fetuses. Ewes #59 and #64 had markedly lower PRNT₈₀ titers than the other ewes by the end of the study (Table 4). Four of six ewes had robust serum IgG responses whereas Ewe #59 had a decidedly lower serum IgG response and ewe #64 did not mount a detectable serum IgG response to vaccination. Ewe #64 had a temperature of 39.8°C at the time of inoculation on Day 0 and was febrile with a temperature of 40°C on Day 4.

Discussion

We tested livestock vaccine candidates produced by altering the genome of MP-12, a BSL-2 agent that has been tested in 62 human volunteers with no significant adverse events and has been shown to be safe and protective against virulent RVFV challenge in livestock, including mid-to late-term pregnant sheep and rhesus macaques[26,28, 33, 36–38]. The Phase I study revealed little difference between arMP-12 and MP-12 in PRNT₈₀ response and adverse events. We were able to detect viral RNA in one of the two dead fetuses but advanced postmortem autolysis precluded visualizing definitive microscopic lesions. The estimated age of the dead fetus in the arMP-12 group suggests it died around the time the ewe was inoculated. We recovered viral RNA from the autolyzed fetus in the MP-12 group but not enough to amplify. Prenatal losses in livestock due to early embryonic or fetal death can be substantial and may be due to a variety of causes and combination of factors [39,40]. Often the cause of fetal death cannot be accurately determined.

The dose escalation study was focused on the two higher doses of arMP-12ΔNSm21/384 vaccine so the numbers of test animals in the 1×10^4 and 1×10^5 PFU doses were increased as much as animal space would permit. There were no marked differences within and between the three higher dosage groups but one animal (ewe #64) in the 1×10^5 PFU dose group developed a low PRNT₈₀ titer of 1:20. MP-12 vaccine is temperature sensitive and perhaps the elevated rectal temperature at the time of inoculation and pyrexia on Day 4 contributed to poor virus replication resulting in a suppressed PRNT₈₀ response and lack of detectable IgG in that ewe [41]. Only one ewe (ewe #38) in the 1×10^2 PFU dose group developed a neutralizing antibody titer >1:20 yet there was no statistically significant difference in the IgG response between the four dosage groups (Figure 2B). The differences in PRNT₈₀ responses between the Phase I and Phase II arMP-12ΔNSm21/384 groups receiving the same dose of vaccine is surprising but may be a reflection of group and individual variability. There was a significant difference in mean PRNT₈₀ response ($P=0.0137$).

Although we were unable to allow the ewes to go to term, we believe that euthanizing the ewes at 62 or 69 days of gestation was sufficient time to allow determination of the age, health and morphology of the fetus after vaccination. The recombinant

arMP-12ΔNSm21/384 virus was highly immunogenic at doses of 1×10^3 through 1×10^5 PFU and was found to be safe and non-teratogenic when inoculated into ewes in early gestation. These studies suggest that the arMP-12ΔNSm21/384 virus may be a promising candidate as a livestock vaccine and the genomic deletion may prove a useful negative marker in the serologic differentiation of vaccinated animals from infected animals [42] but an evaluation of long-term protective immunity should be done.

Acknowledgments

In conducting the research described in this report, the investigators adhered to the guidelines of the Institutional Animal Care and Use Committee of Texas A & M University and the recommendations in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, 1996). The facilities used are fully accredited by the American Association for Accreditation of Laboratory Animal Care. This study was conducted under an approved Texas A & M University animal use protocol number 2007-156, Amendment A.

We thank Ms. Nicolette Ward for her technical assistance and Dr. Clay Ashley and the staff at the TAMU Veterinary Research Park for their assistance in handling and maintaining the animals. We greatly appreciate Dr. Tetsuro Ikegami's input.

Support: This study was supported by funds awarded to LGA from Texas AgriLife Research Project 203367-00000-10000 and funds from the U. S. Department of Homeland Security, National Center of Excellence for Foreign Animal and Zoonotic Disease (FAZD) Defense Project ONR-N00014-04-1-0660. A portion of this work (JCM, NL, ES, SM, and CJP) was supported by a grant through the National Institutes of Health (NIH) grant number NIH-NIAID-DMID-02-24 Collaborative Grant on Emerging Viral Diseases. WJW was supported by a grant from the Meriel Veterinary Scholars Program. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Bird BH, Ksiazek TG, Nichol ST, Maclachlan NJ. Rift Valley fever virus. J Am Vet Med Assoc. 2009; 234:883–893. [PubMed: 19335238]
2. Ahmed J, Bouloy M, Ergonul O, Fooks A, Paweska J, et al. International network for capacity building for the control of emerging viral vector-borne zoonotic diseases: ARBO-ZOONET. Euro Surveill. 2009;14.
3. Al-Afaleq AI, Hussein MF. The status of Rift Valley fever in animals in Saudi Arabia: a mini review. Vector Borne Zoonotic Dis. 2011; 11:1513–1520. [PubMed: 21923257]
4. Marco Di Luca FS, Toma Luciano, Romi Roberto. Twenty Years of the Presence of Aedes albopictus in Italy – From the Annoying Pest Mosquito to the Real Disease Vector. European Infectious Disease. 2011; 2:98–101.
5. Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, et al. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature. 2010; 468:647–652. [PubMed: 21124449]
6. Hightower A, Kinkade C, Nguku PM, Anyangu A, Mutonga D, et al. Relationship of Climate, Geography, and Geology to the Incidence of Rift Valley Fever in Kenya during the 2006–2007 Outbreak. Am J Trop Med Hyg. 2012; 86:373–380. [PubMed: 22302875]
7. Anyamba A, Linthicum KJ, Small J, Britch SC, Pak E, et al. 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. 2010; 83:43–51. [PubMed: 20682905]
8. Christou L. The global burden of bacterial and viral zoonotic infections. Clin Microbiol Infect. 2011; 17:326–330. [PubMed: 21129102]
9. Ikegami T, Makino S. The pathogenesis of Rift Valley fever. Viruses. 2011; 3:493–519. [PubMed: 21666766]
10. Bishop DH, Calisher CH, Casals J, Chumakov MP, Gaidamovich SY, et al. Bunyaviridae. Intervirology. 1980; 14:125–143. [PubMed: 6165702]
11. Walter CT, Barr JN. Recent advances in the molecular and cellular biology of bunyaviruses. J Gen Virol. 2011; 92:2467–2484. [PubMed: 21865443]

12. Ikegami T, Won S, Peters CJ, Makino S. Rift Valley fever virus NSs mRNA is transcribed from an incoming anti-viral-sense S RNA segment. *J Virol.* 2005; 79:12106–12111. [PubMed: 16140788]
13. Bird BH, Albarino CG, Nichol ST. Rift Valley fever virus lacking NSm proteins retains high virulence in vivo and may provide a model of human delayed onset neurologic disease. *Virology.* 2007; 362:10–15. [PubMed: 17412386]
14. Won S, Ikegami T, Peters CJ, Makino S. NSm and 78-kilodalton proteins of Rift Valley fever virus are nonessential for viral replication in cell culture. *J Virol.* 2006; 80:8274–8278. [PubMed: 16873285]
15. Gerrard SR, Bird BH, Albarino CG, Nichol ST. The NSm proteins of Rift Valley fever virus are dispensable for maturation, replication and infection. *Virology.* 2007; 359:459–465. [PubMed: 17070883]
16. Won S, Ikegami T, Peters CJ, Makino S. NSm protein of Rift Valley fever virus suppresses virus-induced apoptosis. *J Virol.* 2007; 81:13335–13345. [PubMed: 17913816]
17. Kakach LT, Wasmoen TL, Collett MS. Rift Valley fever virus M segment: use of recombinant vaccinia viruses to study Phlebovirus gene expression. *J Virol.* 1988; 62:826–833. [PubMed: 3339714]
18. Kakach LT, Suzich JA, Collett MS. Rift Valley fever virus M segment: phlebovirus expression strategy and protein glycosylation. *Virology.* 1989; 170:505–510. [PubMed: 2728348]
19. Gerrard SR, Nichol ST. Synthesis, proteolytic processing and complex formation of N-terminally nested precursor proteins of the Rift Valley fever virus glycoproteins. *Virology.* 2007; 357:124–133. [PubMed: 16963099]
20. Pepin M, Bouloy M, Bird BH, Kemp A, Paweska J. Rift Valley fever virus(Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. *Vet Res.* 2010; 41:61. [PubMed: 21188836]
21. Ikegami T, Narayanan K, Won S, Kamitani W, Peters CJ, et al. Rift Valley fever virus NSs protein promotes post-transcriptional downregulation of protein kinase PKR and inhibits eIF2alpha phosphorylation. *PLoS Pathog.* 2009; 5:e1000287. [PubMed: 19197350]
22. Muller R, Saluzzo JF, Lopez N, Dreier T, Turell M, et al. Characterization of clone 13, a naturally attenuated avirulent isolate of Rift Valley fever virus, which is altered in the small segment. *Am J Trop Med Hyg.* 1995; 53:405–411. [PubMed: 7485695]
23. Bouloy M, Janzen C, Vialat P, Khun H, Pavlovic J, et al. Genetic evidence for an interferon-antagonistic function of rift valley fever virus nonstructural protein NSs. *J Virol.* 2001; 75:1371–1377. [PubMed: 11152510]
24. Habjan M, Pichlmair A, Elliott RM, Overby AK, Glatter T, et al. NSs protein of rift valley fever virus induces the specific degradation of the double-stranded RNA-dependent protein kinase. *J Virol.* 2009; 83:4365–4375. [PubMed: 19211744]
25. Niklasson BS, Meadors GF, Peters CJ. Active and passive immunization against Rift Valley fever virus infection in Syrian hamsters. *Acta Pathol Microbiol Immunol Scand C.* 1984; 92:197–200. [PubMed: 6507105]
26. Morrill JC, Jennings GB, Caplen H, Turell MJ, Johnson AJ, et al. Pathogenicity and immunogenicity of a mutagen-attenuated Rift Valley fever virus immunogen in pregnant ewes. *Am J Vet Res.* 1987; 48:1042–1047. [PubMed: 3631685]
27. Dingu B, Louw I, Lubisi A, Hunter P, von Teichman B, Bouloy M. Evaluation of the efficacy and safety of the Rift Valley Fever Clone 13 vaccine in sheep. *Vaccine.* 2010; 28:4581–4587. [PubMed: 20470792]
28. Bettinger, GE.; Peters, CJ.; Pittman, P.; Morrill, JC.; Ranadive, M.; Kormann, RN.; Lokugamage, N. Rift Valley fever MP-12 vaccine: a university, government, & industry collaborative development. Rift Valley Fever Workshop; 2009.
29. Ikegami T, Won S, Peters CJ, Makino S. Rescue of infectious Rift Valley fever virus entirely from cDNA, analysis of virus lacking NSs gene, and expression of a foreign gene. *J Virol.* 2006; 80:2933–2940. [PubMed: 16501102]
30. Mufti AM, Wani GM, Wani NA, Buchoo BA, Khan MZ. Prenatal development of ovine fetus. *Sml Animal Res.* 2000; 38:87–809.

31. Sivachelvan MN, Ghali Ali M, Chibuzo GA. Foetal age estimation in sheep and goats. *Sml Animal Res.* 1996; 19:69–76.
32. Tseng CT, Huang C, Newman P, Wang N, Narayanan K, et al. Severe acute respiratory syndrome coronavirus infection of mice transgenic for the human Angiotensin-converting enzyme 2 virus receptor. *J Virol.* 2007; 81:1162–1173. [PubMed: 17108019]
33. Morrill JC, Carpenter L, Taylor D, Ramsburg HH, Quance J, Peters CJ. Further evaluation of a mutagen-attenuated Rift Valley fever vaccine in sheep. *Vaccine.* 1991; 9(1):35–41. [PubMed: 2008798]
34. Meegan JM, Yedloutschnig RJ, Peleg BA, et al. Enzyme-linked immunosorbent assay for detection of antibodies to Rift Valley fever virus in ovine and bovine sera. *Am J Vet Res.* 1987; 48(7):1138–41. [PubMed: 3631700]
35. Ksiazek TG, Jouan A, Meegan JM, et al. Rift Valley fever among domestic animals in the recent West African outbreak. *Res Virol.* 1989; 140(1):67–77. [PubMed: 2711047]
36. Morrill JC, Peters CJ. Pathogenicity and neurovirulence of a mutagen- attenuated Rift Valley fever vaccine in Rhesus monkeys. *Vaccine.* 2003; 21:2994–3002. [PubMed: 12798643]
37. Morrill JC, Mebus CA, Peters CJ. Safety of a mutagen-attenuated Rift Valley fever virus vaccine in fetal and neonatal bovids. *Am J Vet Res.* 1997; 58(10):1110–4. [PubMed: 9328663]
38. Morrill JC, Mebus CA, Peters CJ. Safety and efficacy of a mutagen-attenuated Rift Valley fever virus vaccine in cattle. *Am J Vet Res.* 1997; 58(10):1104–9. [PubMed: 9328662]
39. Wilmut I, Sales DI, Ashworth CJ. Maternal and embryonic factors associated prenatal loss in mammals. *J Reprod Fert.* 1986; 76:851–864.
40. Givens MD, Marley MSD. Infectious causes of embryonic and fetal mortality. *Theriogenology.* 2008; 70:270–285. [PubMed: 18502494]
41. Saluzzo JF, Smith JF. Use of reassortant viruses to map attenuating and temperature-sensitive mutations of the Rift Valley fever virus MP-12 vaccine. *Vaccine.* 1990; 8:369–375. [PubMed: 2396475]
42. McElroy AK, Albarino CG, Nichol ST. Development of a RVFV ELISA that can distinguish infected from vaccinated animals. *Virology Journal.* 2009; 6:125. Published online 2009 August 13. 10.1186/1743-422X-6-125 [PubMed: 19678951]

Highlights

We tested 2 recombinant RVF MP-12 vaccine candidates in 30 – 50 day pregnant ewes.

No abortions or fetal abnormalities were observed.

The arMP-12 Δ NSm21/384 candidate was immunogenic and safe in pregnant ewes.

A dose of 1×10^3 PFU stimulates a PRNT₈₀ response comparable to higher doses.

Figure 1A

Figure 1B

Figure 1.

(A). Mean ($\pm$ SEM) PRNT₈₀ responses of ewes in the Phase I studies that were inoculated s.c. with 1×10^5 PFU of arMP-12ΔNSs16/198 (●), arMP-12ΔNSm21/384 (○), authentic RVF MP-12 (□), or arMP-12 (▲). PRNT₈₀ values of the animals inoculated with arMP-12ΔNSs16/198 were significantly lower ($P < 0.05$) than animals inoculated with the other three viruses. (B). Mean ($\pm$ SEM) PRNT₈₀ responses of ewes in the Phase II studies that were inoculated s.c. with 1×10^2 PFU (●), 1×10^3 PFU (○), 1×10^4 PFU (□) or 1×10^5 PFU (▲) of arMP-12ΔNSm21/384. A significant difference ($P < 0.05$) was detected between the mean PRNT₈₀ values of the animals inoculated with 1×10^2 PFU and the other three doses of arMP-12ΔNSm21/384.

Figure 2A.

Figure 2B.

Figure 2.

(A). Mean ($\pm$ SEM) serum IgG results of Phase I ewes inoculated s.c. with 1×10^5 PFU of arMP-12 Δ NSs16/198 ($\bullet$), arMP-12 Δ NSm21/384 ($\circ$), authentic RVF MP-12 ($\square$), or arMP-12 ($\blacktriangle$). RVFV-specific IgG values were significantly increased ($P < 0.001$) in animals inoculated with arMP-12 Δ NSm21/384 or arMP-12 versus animals inoculated with arMP-12 Δ NSs16/198. Negative cutoff value (dashed line) = 0.317. (B). Mean ($\pm$ SEM) serum IgG results of Phase II ewes inoculated s.c. with 1×10^2 ($n=4$ $\bullet$), 1×10^3 ($n=4$ $\circ$), 1×10^4 ($n=10$ $\blacktriangle$) or 1×10^5 ($n=6$ $\triangle$) PFU of arMP-12 Δ NSm21/384. No statistically significant differences were calculated between the dosage treatments. Negative cutoff value (dashed line) = 0.414.

Table 1

Neutralizing antibody responses of individual ewes in the Phase I study. Each animal was inoculated s.c. with 1×10^5 PFU of the respective vaccine candidate.

Day	#17 [†]	arMP-12 ANSs 16/198					arMP-12 ΔNSm 21/384					MP-12					arMP-12				
		#18	#19	#20	#21	#22	#23	#24	#25	#26	#27	#28	#29	#30	#31	#32					
1	<1:10 [‡]	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
2	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
3	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
4	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
5	<1:10	<1:10	<1:10	<1:10	<1:10	20	10	10	40	<1:10	<1:10	<1:10	<1:10	<1:10	20	<1:10	<1:10	<1:10	20	<1:10	<1:10
6	<1:10	<1:10	10	<1:10	20	40	80	80	80	<1:10	10	<1:10	<1:10	40	20	40	20	40	20	40	20
7	<1:10	10	80	<1:10	80	160	20	160	160	20	20	20	20	80	80	160	80	160	80	160	80
8	20	20	80	<1:10	160	1280	160	320	320	160	80	80	80	320	160	320	160	320	160	320	640
9	20	40	80	<1:10	160	2560	320	320	640	320	160	80	80	640	160	320	640	160	320	640	640
10	20	80	80	20	640	5120	320	320	640	160	1280	160	640	320	640	1280	640	320	640	1280	1280
11	80	80	640	80	160	5120	1280	640	1280	320	640	320	1280	320	1280	640	1280	320	1280	640	640
12	80	80	160	80	640	5120	1280	2560	1280	1280	1280	1280	1280	1280	1280	1280	1280	1280	1280	1280	1280
13	80	80	320	320	640	5120	1280	2560	1280	1280	1280	1280	1280	1280	1280	1280	1280	1280	1280	1280	1280
14	80	80	80	160	1280	10240	2560	5120	1280	1280	1280	2560	2560	640	2560	2560	2560	640	2560	2560	2560
21	80	40	80	320	2560	5120	1280	2560	2560	1280	1280	5120	5120	1280	1280	5120	1280	1280	2560	5120	5120
28	320	20	80	160	2560	1280	1280	5120	2560	1280	1280	10240	10240	1280	1280	2560	1280	1280	2560	5120	5120
35	320	40	80	160	5120	5120	1280	5120	1280	2560	1280	2560	2560	1280	1280	1280	1280	1280	1280	1280	1280
42	320	80	80	80	1280	2560	320	5120	2560	640	1280	1280	1280	640	10240	1280	1280	640	10240	1280	1280
49	320	80	80	80	5120	1280	320	5120	640	1280	1280	5120	1280	1280	1280	1280	1280	1280	1280	1280	1280
56	320	80	80	80	2560	5120	320	5120	1280	2560	2560	5120	1280	1280	10240	1280	1280	1280	10240	1280	1280
63	320	80	80	80	1280	1280	320	2560	1280	2560	1280	2560	1280	1280	10240	1280	1280	1280	10240	1280	1280
69	320	80	80	80	5120	2560	1280	5120	1280	2560	2560	2560	1280	1280	10240	1280	1280	1280	10240	1280	1280

[†] Ewe identification number.

[‡] Data are expressed as the reciprocal of plaque-reduction neutralization 80% antibody titer. Minimum detection limit of the assay is 1:10.

Table 2

Neutralizing antibody responses of individual ewes in the Phase II Group #1 and Group #2 studies. These animals received 1×10^2 PFU or 1×10^3 PFU s.c. of the arMP-12 ΔNSm 21/384 vaccine candidate. Ewe #41 was not pregnant but all the other ewes had morphologically normal and viable fetuses at the time of necropsy.

Phase II Group #1				Phase II Group #2					
arMP-12ΔNSm 21/384 (1 × 10 ² PFU)				arMP-12 ΔNSm 21/384 (1 × 10 ³ PFU)					
Day	#37 [†]	#38	#39	#40	Day	#41 [†]	#42	#43	#44
1	<1:10 [‡]	<1:10	<1:10	<1:10	1	<1:10 [‡]	<1:10	<1:10	<1:10
2	<1:10	<1:10	<1:10	<1:10	2	<1:10	<1:10	<1:10	<1:10
3	<1:10	<1:10	<1:10	<1:10	3	<1:10	<1:10	<1:10	<1:10
4	<1:10	<1:10	<1:10	<1:10	4	<1:10	<1:10	<1:10	<1:10
5	<1:10	<1:10	<1:10	<1:10	5	<1:10	<1:10	<1:10	<1:10
6	<1:10	<1:10	<1:10	<1:10	6	<1:10	<1:10	<1:10	<1:10
7	<1:10	<1:10	<1:10	<1:10	7	20	<1:10	<1:10	80
9	<1:10	20	<1:10	<1:10	9	640	80	<1:10	320
13	40	320	20	80	13	1280	80	160	320
20	40	640	20	20	20	5120	80	1280	320
27	20	320	20	20	27	1280	80	640	320
34	20	320	20	20	34	640	320	320	320
41	20	320	<1:10	20	41	1280	320	320	320
48	20	640	<1:10	20	48	1280	320	320	320
55	20	1280	20	20	55	1280	320	320	160
62	20	1280	<1:10	<1:10	62	1280	320	320	320

[†]Ewe identification number

[‡]Data are expressed as the reciprocal of plaque-reduction neutralization 80% antibody titer. Minimum detection limit of the assay is 1:10.

Table 3

Neutralizing antibody responses of individual ewes in the Phase II Group #3 study. These animals received 1×10^4 PFU s.c. of the arMP-12ΔNSm 21/384 vaccine candidate. At necropsy, ewes #56 and #57 were not pregnant and ewe #48 had a dead, partially autolyzed fetus from which PCR products consistent with the input virus were recovered. All other ewes had live, morphologically normal fetuses.

Phase II Group #3												
arMP-12ΔNSm 21/384 (1×10^4 PFU)												
Day	#45 [†]	#46	#47	#48	#53	#54	#55	#56	#57	#58		
1	<1:10 [‡]	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10		
2	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10		
3	<1:10	<1:10	<1:10	<1:10	<1:10	20	<1:10	<1:10	<1:10	<1:10		
4	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10		
5	<1:10	<1:10	20	20	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10		
6	<1:10	<1:10	<1:10	20	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10		
7	20	20	20	80	<1:10	20	20	20	<1:10	20		
9	320	320	1280	1280	<1:10	160	80	320	20	80		
13	640	2560	5120	5120	20	640	320	320	40	160		
20	320	1280	1280	5120	80	320	1280	1280	80	320		
27	320	640	1280	1280	80	320	1280	320	80	320		
34	320	2560	1280	1280	20	320	1280	320	80	320		
41	320	1280	320	320	80	320	1280	320	320	320		
48	80	1280	320	320	80	160	1280	320	80	320		
55	320	320	320	320	80	320	1280	320	80	320		
62	320	1280	320	1280	80	320	1280	320	80	320		

[†]Ewe identification number

[‡]Data are expressed as the reciprocal of plaque-reduction neutralization 80% antibody titer. Minimum detection limit of the assay is 1:10.

Table 4

Neutralizing antibody responses of individual ewes in the Phase II Group #4 study. These animals received 1×10^5 PFU s.c. of the arMP-12ΔNSm 21/384 vaccine candidate. All 6 ewes had live fetuses measuring 29 to 32 cm C-R and ewe #63 had twin fetuses.

Phase II Group #4							
arMP-12ΔNSm 21/384 (1×10^5 PFU)							
Day	#59 [†]	#60	#61	#62	#63	#64	
1	<1:10 [‡]	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
2	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
3	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
4	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
5	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
6	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10	<1:10
7	<1:10	20	20	20	<1:10	<1:10	<1:10
9	20	160	160	320	320	320	20
13	80	640	160	1280	320	320	20
20	80	320	160	5120	1280	1280	20
27	80	1280	160	1280	1280	1280	20
34	80	640	160	1280	1280	1280	20
41	80	1280	320	1280	320	320	20
48	80	1280	320	1280	1280	1280	20
55	80	320	320	1280	320	320	20
62	80	320	320	640	320	320	20

[†]Ewe identification number

[‡]Data are expressed as the reciprocal of plaque-reduction neutralization 80% antibody titer. Minimum detection limit of the assay is 1:10.