**Safety and Immunogenicity of a Mutagenized, Live Attenuated Rift Valley Fever Vaccine,
MP-12, in a Phase 1 Dose Escalation and Route Comparison Study in Humans**

Phillip R. Pittman*, David McClain, Xiaofei Quinn, Kevin M. Coonan, Joseph Mangiafico,
Richard S. Makuch, John Morrill, Clarence J. Peters

U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), 1425 Porter Street,
Fort Detrick, MD 21702-5011

* Corresponding Author:

Phillip R. Pittman, M.D., M.P.H., email: phillip.r.pittman.civ@mail.mil

Running Head: RVF MP-12 Vaccine Phase 1 Clinical Trial

ABSTRACT

Rift Valley fever (RVF) poses a risk as a potential agent in bioterrorism or agroterrorism. A live attenuated RVF vaccine (RVF MP-12) has been shown to be safe and protective in animals and showed promise in two initial clinical trials. In the present study, healthy adult human volunteers (N=56) received a single injection of (a) RVF MP-12, administered subcutaneously (SQ) at a concentration of $10^{4.7}$ plaque-forming units (pfu) (SQ Group); (b) RVF MP-12, administered intramuscularly (IM) at $10^{3.4}$ pfu (IM Group 1); (c) RVF MP-12, administered IM at $10^{4.4}$ pfu (IM Group 2); or (d) saline (Placebo Group). The vaccine was well tolerated by volunteers in all dose and route groups. Infrequent and minor adverse events were seen among recipients of both placebo and RVF MP-12. One subject had viremia detectable by direct plaque assay, and six subjects from IM Group 2 had transient low-titer viremia detectable only by nucleic acid amplification. Of the 43 vaccine recipients, 40 (93%) achieved neutralizing antibodies (measured as an 80% plaque reduction neutralization titer [PRNT₈₀]) as well as RVF-specific IgM and IgG. The highest peak geometric mean PRNT₈₀ titers were observed in IM Group 2. Of 34 RVF MP-12 recipients available for testing 1 year following inoculation, 28 (82%) remained seropositive (PRNT₈₀ $\geq$ 1:20); this included 20 of 23 vaccinees (87%) from IM Group 2. The live attenuated RVF MP-12 vaccine was safe and immunogenic at the doses and routes studied. Given the need for an effective vaccine against RVF virus, further evaluation in humans is warranted.

Keywords: MP-12, Rift Valley fever, vaccine, clinical trial

1.0 INTRODUCTION

Rift Valley fever (RVF) is an arthropod-borne viral disease responsible for considerable morbidity and mortality among domestic animals and humans in both historically endemic and newly involved parts of the world [1]. The etiologic agent, RVF virus (RVFV), is considered a potential bioterror and agroterror weapon [1].

The current inactivated vaccine (TSI-GSD-200; BB-IND 365), developed by the U.S. Army to protect at-risk employees from occupational exposure [2], requires three inoculations over a 4-week period and a mandatory boost at month 6 to maintain antibody titers at levels thought to be protective; many recipients require periodic boosters thereafter [3–5]. Mass immunization with a multi-dose vaccine, particularly in regions of the world lacking an efficient public health infrastructure, poses a logistical challenge.

The need for an improved RVF vaccine has prompted several lines of research. Among the live attenuated vaccine candidates, Clone 13—a plaque-purified clone of RVFV containing a large deletion within the small (S) segment of the tripartite RVFV genome—has proven highly immunogenic in mice, sheep, and goats [6,7]. A recombinant RVF vaccine with complete deletions of the major virus virulence factors has been found to be safe, immunogenic, and protective in rats and sheep [8,9]. The only live attenuated RVF vaccine candidate to have undergone clinical trials is the MP-12 vaccine, developed by the U.S. Department of Defense [10].

Two Phase 1 trials of RVF MP-12's safety and immunogenicity are summarized briefly here (greater detail is provided in the Investigator's Brochure [11]). In the first Phase 1 trial, undiluted RVF MP-12 ($10^{4.4}$ plaque-forming units [pfu]) was administered subcutaneously (SQ) to four RVFV-naïve subjects. All four subjects developed IgM and persistent IgG responses, as measured by an enzyme-linked immunosorbent assay (ELISA); three of these subjects also developed neutralizing antibodies. Vaccine virus was recovered from one vaccinee by nucleic acid amplification, though we found no evidence of a reversion to virulence. The severity of adverse events (AEs) was categorized as Grade 1 (mild), 2 (moderate), 3 (severe), or 4 (potentially life threatening); most AEs were Grade 1 or 2. All four RVFV-naïve vaccinees experienced transient Grade 1 or 2 elevations in alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST). All clinical and laboratory abnormalities resolved without sequelae.

In a follow-on dose escalation study, 22 subjects received a single 0.5-mL SQ injection of either RVF MP-12 or placebo (normal saline); vaccinees received RVF MP-12 either diluted (1:1,000, 1:100, or 1:10) or undiluted. The vaccine proved remarkably safe. The only local AE was injection site tenderness in three of four vaccinees receiving undiluted vaccine, and the most common systemic reaction was headache. Grade 1 or 2 increases in ALT, AST, alkaline phosphatase (ALKP), and lactate dehydrogenase (LDH) were noted, as was a Grade 3 AST elevation in one vaccinee. Elevated creatine phosphokinase (CPK) was observed in two placebo recipients (Grades 1 and 2) and three vaccinees (Grades 1 and 4). Among the four vaccinees receiving undiluted RVF MP-12, two to four individuals seroconverted, depending on the measure used (IgM or IgG via ELISA or 80% or 50% plaque reduction neutralization titer [PRNT₈₀ or PRNT₅₀]).

In the present study, we evaluated the safety and immunogenicity of a single 1.0-mL SQ injection ($10^{4.7}$ pfu) of RVF MP-12. We also evaluated the response to the vaccine delivered intramuscularly (IM) in a dose escalation study.

2.0 METHODS

2.1 Vaccine

The live attenuated RVF vaccine used in this study, MP-12 (lot 7-2-88, TSI-GSD-223, BB-IND 4307), is derived from a strain of RVFV (ZH548) isolated from a human in Egypt in 1977. The viral isolate was subjected to chemical mutagenesis with 5-fluorouracil over 12 serial culture passages in fetal human lung (MRC-5) cells and characterized as previously described [10]. The attenuated virus from the 12th mutagenesis passage (MP-12) was then evaluated in rodents, mosquitoes, sheep, cattle, and nonhuman primates [12–22]. Each 20-mL vial of lyophilized vaccine was reconstituted in 12 mL of sterile water for injection, USP. The placebo in this study was normal saline.

2.2 Subjects and Design

The clinical protocol for this open-label study (U.S. Army Medical Research Institute of Infectious Diseases [USAMRIID] protocol FY96-4) was reviewed and approved by USAMRIID's institutional review board and the Human Subjects Research Review Board of the U.S. Army Surgeon General. Healthy male and nonpregnant female volunteers at least 18 years of age who provided written informed consent were screened using a detailed medical history, complete physical examination, and clinical laboratory evaluation. Exclusion criteria included

any significant screening abnormality, pregnancy, history of prior RVF vaccination or disease, or evidence for such by ELISA or by the presence of neutralizing antibodies to RVFV.

Volunteers were sequentially enrolled into study cohorts and randomly assigned to receive RVF MP-12 or placebo on day 0 of the study. Reconstituted RVF MP-12 or placebo was administered by SQ or IM injection over or into the deltoid muscle as follows: SQ Group, 1-mL SQ injection of $10^{4.7}$ pfu MP-12 (n=10) or placebo (n=5); IM Group 1, 0.5-mL IM injection of $10^{3.4}$ pfu (n=6) or placebo (n=4); and IM Group 2, 0.5-mL IM injection of $10^{4.4}$ pfu (n=10) or placebo (n=4). When preliminary data indicated higher PRNT₈₀ responses in IM Group 2, this regimen was expanded to include an additional 17 volunteers, bringing the IM Group 2 total to 31 volunteers (n=27 vaccinees, n=4 placebo recipients). All placebo recipients were combined into one group for analysis.

Clinical assessments of volunteers were carried out daily for the first week after inoculation and for 3 days during the second week after inoculation. Observations included measurement of vital signs and characterization of any reactions, whether local (erythema, induration, or tenderness at the inoculation site) or systemic (rash, headache, myalgia, arthralgia, joint swelling, odynophagia, or nausea). Serum chemistry and hematology studies were performed at baseline, daily for 7 days after vaccination, and on days 9, 11, 14, 21, and 28 after vaccination. A complete blood count with differential was performed using a Coulter T-540 electronic analyzer (Coulter Electronics, Hialeah, FL). Serum was analyzed using an Ektachem 700XR analyzer (Eastman Kodak, Rochester, NY) for a panel of chemistry determinations, including electrolytes, liver function tests (e.g., LDH, AST, and ALT), and CPK. Specimens for serology were obtained at

baseline; on postvaccination days 1–6, 7, 9, 11, 14, 21, 28, 35, and 42; and at postvaccination months 3, 6, and 12. These samples were frozen at -70°C for subsequent antibody determination by ELISA and PRNT₈₀.

2.3 Viremia Assays

Blood was obtained on days 0–7, 9, 11, and 14 after vaccination for detection of circulating virus. Aliquots frozen at -70°C were assayed for RVFV viremia using the standard viral plaque assay, as described previously [15,23,24].

Virus isolates recovered from volunteers were identified by plaque reduction neutralization using reference RVFV hyperimmune mouse ascitic fluid (HMAF) [25].

2.4 Serology

Patient sera were tested for neutralizing antibody to the ZH501 isolate of RVFV using a PRNT₈₀, as described by Meadors et al. [26], but modified for use with RVFV in 12-well plates. Briefly, each serum sample was incubated (56°C – 60°C , 30 min), then diluted 1:10 in Eagle's minimal essential medium containing 10% heat-inactivated fetal bovine serum. A suspension of RVFV, calculated to yield a dose of approximately 40–100 pfu/0.1 mL, was prepared in Hanks balanced salt solution with 40 mM HEPES. Fourfold dilutions of serum samples were then mixed 1:1 with 40–100 pfu of virus suspension and incubated (37°C , 1 h). Following incubation, test samples and controls were inoculated onto cell monolayers for plaque assay. The number of plaques for each test sample was entered into a computer program for determining the PRNT₈₀ using probit analysis. The endpoint titer was expressed as a reciprocal of the highest serum dilution

demonstrating greater than 80% reduction in the number of plaques for the average dose. Undetectable titrations at the initial test dilution of < 1:10 were reported as 1:5 for statistical purposes.

Anti-RVSV IgG antibody was measured by ELISA as previously described [27,28], but modified to use human serum as the test specimen and horseradish peroxidase (HRP)-labeled mouse anti-human IgG (Accurate Chemical Co., Westbury, NY) as the detector antibody. IgM antibody to RVSV was measured using a modification of an antibody capture methodology [27]. Briefly, test sample IgM was captured onto wells of a microtiter plate using goat anti-human IgM, (BioSource, Camarillo, CA). Supernatant from a culture of ZH501-strain RVSV was then added, followed by a 1:2,000 dilution of HMAF. Bound HMAF was then detected using HRP-labeled anti-mouse IgG (Boehringer Mannheim, Indianapolis, IN). Following incubation and washing, 100 µl of ABTS (2,2'-azino-di[3-ethyl-benzthiazoline-6-sulfonate]) substrate (KPL, Inc., Gaithersburg, MD) was added to each well, and plates were read spectrophotometrically at 414 nm. The titer was expressed as the reciprocal of the highest serum dilution exceeding the mean optical density of 4 negative sera plus 3 standard deviations. A titer of less than 1:100 was reported as 1:50.

2.5 Statistical Analyses

Systemic and local reaction rates were compared between all vaccinees and the placebo group using a Fisher's exact test with a permutation adjustment for multiple testing. Geometric mean titers (GMTs), standard errors, and 95% confidence intervals were calculated using log₁₀-transformed titers for PRNT₈₀, PRNT₅₀, IgM, and IgG at each time point. PRNT₈₀ and PRNT₅₀

titers below the lower limit of detection (LLOD) (1:10) were set to 1:5 ($\frac{1}{2}$ LLOD) prior to analysis. IgM and IgG titers below the LLOD (1:100) were set to 1:50 ($\frac{1}{2}$ LLOD) prior to analysis. Exact binomial methods were used to calculate 95% confidence intervals for seroconversion rates over the course of the study and rate of detectable viremia. Seroconversion in PRNT₈₀ or PRNT₅₀ titers was defined as a titer $\geq$ 1:20 after baseline. Seroconversion in IgM or IgG titers was defined as a titer $\geq$ 1:100 after baseline.

All statistical analyses were performed using SAS Version 9.4 (SAS Institute, Inc., Cary, NC).

3.0 RESULTS

Volunteers were predominantly male, Caucasian, and less than 30 years of age (Table 1).

Vaccine and placebo recipients did not differ significantly in demographic characteristics.

3.1 Clinical Observations

Table 2 shows the frequency and percentage of subjects reporting at least one AE during the first 14 days after vaccination. The most frequently reported symptoms (all vaccine groups combined vs. placebo recipients) included headache (25.6% vs. 15.4%, $p = 1.0000$), diarrhea (0% vs. 15.4%, $p = 0.2289$), malaise (14% vs. 0%, $p = 0.8046$), and nausea (4.7% vs. 15.4%, $p = 0.5324$). Systemic side effects were generally minor: one episode each of headache, nausea/vomiting, and chills were classified as Grade 2, and no side effect was deemed Grade 3. No AE required treatment, and none resulted in absence from work. Four vaccine recipients (two in the SQ Group and two in IM Group 2) and one placebo recipient reported trivial, self-limited injection site tenderness.

Self-limited asymptomatic elevations in serum ALT, AST, LDH, and CPK were seen in vaccinees from all study groups (4 vaccinees in the SQ Group, 3 vaccinees in IM Group 1, and 12 vaccinees in IM Group 2) as well as 2 placebo recipients. The vast majority of elevations were Grade 1 or 2. For ALT, AST, ALKP, and LDH, one vaccinee (in IM Group 1) had a Grade 3 elevation in LDH (4,139 U/L [normal range 290–546 U/L]) and a Grade 4 elevation in AST (587 U/L [normal range 10–42 U/L]).

We observed three Grade 4 CPK elevations. First, a 21-year-old male vaccinee in IM Group 1 received RVF MP-12 vaccine on 23 January 1996; he had a CPK of 29,740 U/L (normal range 35–374 U/L) on 3 February with corresponding increases in ALT, AST, and LDH. This event followed physical exercise until muscle fatigue. All values normalized by 13 February 1996. The second case was a 21-year-old male placebo recipient who received an injection of normal saline on 23 January 1996; his CPK peaked at 15,506 U/L on 30 January with corresponding increases in ALT, AST, and LDH; all had normalized by 6 February 1996. This event resulted from physical training to the point of muscle failure. The third case was a 36-year-old male placebo recipient who received an injection of normal saline on 27 February 1996. During the initial screening blood draw on 1 February 1996, he had a CPK of 13,165 U/L along with increases in ALT, AST, and LDH; this was attributed to exercising to the point of muscle failure. CPK and other enzymes were normal on 12 February 1996, and he was cleared to participate in the study on 16 February, subsequently receiving an injection of normal saline on 27 February. His CPK peaked at 3,981 U/L on 2 March 1996 along with mildly elevated LDH. The elevations in CPK

and LDH were attributed to the vaccinee starting a new weight lifting program. All values had normalized by 7 March 1996.

Of note, enzyme changes (ALT, AST, ALKP, LDH, and CPK) were observed at similar time points, and to comparable levels, among vaccine and placebo recipients during the postinjection period. No vaccinees from the SQ Group had Grade 3 or 4 laboratory elevations.

Hematologic abnormalities were likewise infrequent and inconsequential. Sporadic elevations as well as depressions of total white blood cell count from laboratory normal ranges were seen in vaccinees from all groups and in placebo recipients. A single occurrence of transient atypical lymphocytosis, possibly attributable to vaccination, was recorded. In no case were changes clinically significant, and all observed abnormalities resolved spontaneously within a few days.

3.2 Viremia

Virus could be recovered only once, at very low titer (1.3 pfu/mL), by direct plating of serum from one vaccinee receiving $10^{4.4}$ pfu IM (IM Group 2) on day 9. Evidence for low-level circulation of RVFV was found on 11 occasions in six vaccinees in IM Group 2 on postvaccination days 6–11 by tissue culture amplification and *in situ* detection via ELISA. No systemic symptoms, enzyme abnormalities, or hematologic changes could be associated with virus detection.

3.3 Serology

Among vaccinees in all groups over the course of the study, 40 of 43 vaccinees (93%) seroconverted by PRNT following receipt of a single dose of RVF MP-12 (Table 3). One vaccinee in IM Group 1 ($10^{3.4}$ pfu) and two in IM Group 2 ($10^{4.4}$ pfu) failed to achieve PRNT₈₀ or PRNT₅₀ titers $\geq 1:20$. The peak GMT in PRNT₈₀ was highest (1:277) in IM Group 2, in which 25 of 27 vaccinees (93%) seroconverted. PRNT antibody kinetics were similar among vaccine groups: titers rose abruptly 14–21 days following inoculation and persisted for the 1-year duration of the study (Figure 1A and 1B). At 6 months after inoculation, 21 of 23 vaccinees (91%) in IM Group 2 from whom serum was available for testing had PRNT₈₀ antibody $\geq 1:20$ detected (96% with titers $\geq 1:10$). At 1 year, 20 of 23 vaccinees (87%) from this group remained seropositive. At the 1-year time point, 28 of 34 vaccinees (82%) from all groups combined who were available for assessment remained seropositive (PRNT₈₀ titer $\geq 1:20$).

Anti-RVFFV IgM antibody was first detected by day 7–9 in vaccinees from all groups, with the exception of one vaccinee in IM Group 2 who had a detectable titer of 1:400 at day 2. The peak GMT was highest (1:10,763) in the SQ Group. Antibody kinetics were similar among vaccinees at all three dose/route combinations (Figure 2A), although titers were noticeably lower among individuals in IM Group 1. Only two vaccinated individuals (both in IM Group 2) failed to develop detectable IgM antibody during the postinoculation follow-up.

Generally, anti-RVFFV-specific IgG followed by several days the appearance of RVFFV-specific IgM in study subjects (Figure 2B). RVFFV-specific IgG began to be detectable starting at day 9 in some vaccinees, reaching peak levels at 5–6 weeks after inoculation. As with IgM, titers were lower among subjects in IM Group 1 than among those in the other two groups. The highest peak

GMT was achieved in SQ Group vaccinees. Two vaccinees (one each in IM Group 1 and IM Group 2) failed to develop RVFV-specific IgG antibody after vaccination.

4.0 DISCUSSION

The rapid spread of RVF from sub-Saharan Africa to Madagascar, Egypt, and the Arabian Peninsula in recent decades [29–33] demonstrated the impact of this disease when introduced to naïve populations and reinforced the need to identify a safe, effective, and efficiently administered vaccine. Like most bunyaviruses, the antibody response—and, in particular, the induction of neutralizing antibodies—plays an important role in protection from RVF [34]. Recent research on the cell-mediated immune response hints at a critical role for CD4⁺ T cells, which stimulate the antibody response and mediate the inflammatory response to RVFV infection [35]. More research is needed to verify these findings.

In the present study, the vaccine candidate RVF MP-12, administered via any of three dose/route combinations, was well tolerated by volunteers. Minimal injection site reactions were recorded in only four vaccinees. Systemic reactions were infrequent, did not generally interfere significantly with everyday activities, and the type and frequency of reported events were essentially indistinguishable from those experienced by placebo recipients. Significant hematologic or biochemical abnormalities were not observed in recipients of RVF MP-12. Although a number of volunteers sustained elevations in serum enzyme values, the vast majority of these changes appeared to be unrelated to receipt of the vaccine; rather, they most likely resulted from tissue breakdown consequent to extreme physical exertion [36]. Those enzyme abnormalities judged to be associated with vaccination were self-limited and of only modest magnitude. Indeed, 2 of the

3 cases of Grade 4 elevation in CPK were in placebo recipients—all were judged to be related to physical exertion to exhaustion. Evidence for viremia was weak and well below the threshold recognized for infection of vector species [12].

A single dose of RVF MP-12 was highly immunogenic: 93% of recipients developed specific anti-RVFP antibodies, as measured by PRNT and ELISA. This seroconversion rate compares favorably with that achieved after three doses of the inactivated TSI-GSD-200 vaccine (90%) [5,37]. In humans receiving the inactivated vaccine, neutralizing antibody titers $\geq 1:40$ are considered acceptable—based on animal data [38–40]—for protection against RVF. USAMRIID requires an RVF PRNT₈₀ titer $\geq 1:40$ for entry into biocontainment suites where RVFP is being studied—a situation in which the potential for exposure is probably substantially greater than that in the natural environment. Niklasson et al. [40] and Harrington et al. [39] found that PRNT₈₀ titers as low as 1:10 were protective in Syrian hamsters and sheep, respectively, after administration of the inactivated RVF vaccine. Compared with an inactivated vaccine, one would expect a live virus vaccine to stimulate a broader, more durable immunologic response—one more similar to that elicited by natural infection [41]. Relatively low neutralizing antibody titers are thought to predict protection after administration of a number of other live attenuated vaccines. For example, the live attenuated TC-83 vaccine against Venezuelan equine encephalitis is thought to be protective with PRNT₈₀ titers as low as 1:20 [42]; the live attenuated yellow fever vaccine has been effective in animal models with a PRNT₅₀ titer as low as 1:10 [43]; and a recent World Health Organization consultation recommended that Japanese encephalitis vaccines should be considered protective with a PRNT₅₀ titer of at least 1:10 [44]. Although the efficacy of RVF vaccines in humans has not been established, we feel that a titer $\geq 1:20$ will prove to be

an acceptable measure of protection. Nevertheless, if one applies the more stringent $\geq 1:40$ criterion, 39 of 43 vaccinees (91%) seroconverted, as measured by PRNT₈₀, following a single RVF MP-12 inoculation.

Although the highest ELISA IgM and IgG antibody titers were seen in volunteers receiving $10^{4.7}$ pfu SQ, neutralizing antibody titers were higher and more sustained among those receiving $10^{4.4}$ pfu IM. Among those vaccinees who received this dose and seroconverted, the vast majority (87%) maintained PRNT titers $\geq 1:20$ 1 year later, suggesting that this route and inoculum are appropriate for further evaluation. Of note, only 35% of individuals receiving the TSI-GSD-200 inactivated RVF vaccine maintained PRNT antibody titers $\geq 1:40$ at 1 year [5].

Further evaluation of RVF MP-12 in human volunteers is clearly warranted.

Acknowledgments

We are most grateful to Elizabeth Brown, Ph.D., for her scholarly review and editing of this manuscript. We thank the skillful assistance of Denise Danner and Julie Hile in the completion of serology and viremia assays. The research described herein was sponsored by the U.S. Army Medical Materiel Development Activity, Medical Research and Materiel Command, U.S. Department of Defense.

Disclaimer

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. Research on human subjects was conducted in

compliance with U.S. Department of Defense, federal, and state statutes and regulations relating to the protection of human subjects and adheres to principles identified in the Belmont Report (1979). All data and human subjects research were gathered and conducted for this publication under IRB-approved protocols, number FY92-1, 94-11, and FY96-4.

Conflicts of Interest

The authors have no conflicts of interest.

REFERENCES

- [1] Mandell RB, Flick R. Rift Valley fever virus: a real bioterror threat. *J Bioterror Biodef* 2011;2:108.

- [2] Eddy GA, Peters CJ, Meadors G, Cole FE Jr. Rift Valley fever vaccine for humans. *Contr Epidem Biostatist* 1981;3:124–41.

- [3] Kark JD, Aynor Y, Peters CJ. A Rift Valley fever vaccine trial: 2. Serologic response to booster doses with a comparison of intradermal versus subcutaneous injection. *Vaccine* 1985;3:117–22.

- [4] Niklasson B, Peters CJ, Bengtsson E, Norrby E. Rift Valley fever virus vaccine trial: study of neutralizing antibody response in humans. *Vaccine* 1985;3:123–7.

[5] Pittman PR, Liu CT, Cannon TL, Makuch RS, Mangiafico JA, Gibbs PH, et al.

Immunogenicity of an inactivated Rift Valley fever vaccine in humans: a 12 year experience.

Vaccine 2000;18:181–9.

[6] Muller R, Saluzzo JF, Lopez N, 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;53:405–11.

[7] Njenga MK, Njagi L, Thumbi SM, et al. Randomized controlled field trial to assess the

immunogenicity and safety of Rift Valley fever clone 13 vaccine in livestock. PLoS Negl Trop

Dis 2015;9(3):e0003550.

[8] Bird BH, Albariño CG, Hartman AL, et al. Rift Valley fever virus lacking the NSs and NSm

genes is highly attenuated, confers protective immunity from virulent virus challenge, and allows

for differential identification of infected and vaccinated animals. J Virol 2008;82(6):2681–91.

[9] Bird BH, Maartens LH, Campbell S, et al. 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 2011;85(24):12901–9.

[10] Caplen H, Peters CJ, Bishop DHL. Mutagen directed attenuation of Rift Valley fever as a

method of vaccine development. J Gen Virol 1985;66:2271–77.

[11] Investigator's brochure: Rift Valley fever vaccine, live, attenuated, mutagenized ZH548, MP-12, TSI-GSD 223. Sponsor: The Surgeon General, Department of the Army. Fort Detrick, MD: U.S. Army Medical Research and Materiel Command (sponsor's representative); 18 January 2012.

[12] Rossi CA, Turell MJ. Characterization of attenuated strains of Rift Valley fever virus. *J Gen Virol* 1988;69:817–23.

[13] Turell MUJ, Rossi CA. Potential for mosquito transmission of attenuated strains of Rift Valley fever virus. *Am J Trop Med Hyg* 1991;44:278–82.

[14] Anderson GW Jr., Lee JO, Anderson AO, Powell N, Mangiafico JA, Meadors G. Efficacy of a Rift Valley fever vaccine against aerosol infection in rats. *Vaccine* 1991;9:710–14.

[15] Morrill JC, Jennings GB, Caplen H, Turrell MJ, Johnson AJ, Peters CJ. Pathogenicity and immunogenicity of a mutagen-attenuated Rift Valley fever virus imunogen in pregnant ewes. *Am J Vet Res* 1987;48:1042–47.

[16] 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;9:35–41.

[17] Hubbard KA, Baskerville A, Stephenson JR. Ability of a mutagenized virus variant to protect young lambs from Rift Valley fever. *Am J Vet Res* 1991;52:50–5.

[18] Baskerville A, Hubbard KA, Stephenson JR. Comparison of the pathogenicity for pregnant sheep of Rift Valley fever virus and a live attenuated vaccine. *Res Vet Sci* 1992;52:307–11.

[19] Hunter P, Erasmus BJ, Verster JH. Teratogenicity of a mutagenized Rift Valley fever virus (MVP 12) in sheep. *Onderstepoort J Vet Res* 2002;69:95–8.

[20] 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;58:1104–09.

[21] Morrill JC, Mebus CA, Peters CJ. Safety of a mutagen-attenuated Rift Valley fever vaccine in fetal and neonatal bovines. *Am J Vet Res* 1997;58:1110–14.

[22] Morrill JC, Peters CJ. Pathogenicity and neurovirulence of a mutagen-attenuated Rift Valley fever vaccine in rhesus monkeys. *Vaccine* 2003;21:2994–3002.

[23] Morrill JC, Peters CJ. Protection of MP-12-vaccinated rhesus macaques against parenteral and aerosol challenge with virulent Rift Valley Fever virus. *J Infect Dis* 2011;204:229–36.

[24] 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;9:35–41.

- [25] Ksiazek TG, Jouan A, Meegan JM, et al. Rift Valley fever among animals in the recent West African outbreak. *Res Virol* 1989;140: 67–77.
- [26] Meadors III GF, Gibbs PH, Peters CJ. Evaluation of a new Rift Valley fever vaccine: safety and immunogenicity trials. *Vaccine* 1986;4:179–84.
- [27] Jouan A, Coulibaly I, Lena P, Sarthou JL, Leguenno B, Meegan J, et al. Filter paper confetti in a serological Rift Valley fever survey. *Res Virol* 1989;140:169–73.
- [28] Morrill JC, Knauert FK, Ksiazek TG, Meegan JM, Peters CJ. Rift Valley fever infection of rhesus monkeys: implications for rapid diagnosis of human disease. *Res Virol* 1989;140:139–46.
- [29] Meegan JM. The Rift Valley fever epizootic in Egypt 1977–1978. I. Description of the epizootic and virological studies. *Trans R Soc Trop Med Hyg* 1979;73:618–23.
- [30] Arthur RR, El-Sharkawy MS, Cope SE, Botros BA, Oun S, Morrill JC, et al. Recurrence of Rift Valley fever in Egypt. *Lancet* 1993;342:1149–50.
- [31] World Health Organization. Rift Valley fever, Yemen (update). *Wkly Epidemiol Rec* 2000;75:329–30.
- [32] Centers for Disease Control. Update: outbreak of Rift Valley fever—Saudi Arabia, August–November 2000. *JAMA* 2000; 284: 2989-2990.

[33] Madani TA, Al-Mazrou YY, Al-Jeffri MH, Mishkhas AA, Al-Rabeah AM, Turkistani AM, et al. Rift Valley fever epidemic in Saudi Arabia: Epidemiological, clinical, and laboratory characteristics. *Clin Inf Dis* 2003;37:1084–92.

[34] Flick R, Bouloy M. Rift Valley fever virus. *Curr Mol Med* 2005;5:827–34.

[35] Dodd K, Bird BH, Metcalf MG, Nichol ST, Albarino CG. Single-dose immunization with virus replicon particles confers rapid robust protection against Rift Valley fever virus challenge. *J Virol* 2012;86(8):4204–12.

[36] Malinoski FJ. Strenuous exercise simulating hepatic injury during vaccine trials. *Vaccine* 1992;10:39–42.

[37] Rusnak JM, Gibbs P, Boudreau E, Clizbe DP, Pittman P. Immunogenicity and safety of an inactivated Rift Valley fever vaccine in a 19-year study. *Vaccine* 2011;29:3222–9.

[38] Anderson Jr. GW, Lee JO, Anderson AO, Powell N, Mangiafico JA, Meadors G. Efficacy of a Rift Valley fever virus vaccine against an aerosol infection in rats. *Vaccine* 1991;9:710–14.

[39] Harrington DG, Lupton HW, Crabbs CL, Peters CJ, Reynolds JA, Slone Jr. TW. Evaluation of a formalin-inactivated Rift Valley fever vaccine in sheep. *Am J Vet Res* 1980;41(10):1559–64.

[40] Niklasson BS, Meadors GF, Peters CJ. Active and passive immunization with Rift Valley fever virus infection in Syrian hamsters. *Acta Pathol Microbiol Immunol Scand C* 1984;92:197–200.

[41] Jahrling PB, Stephenson EH. Protective efficacies of live attenuated and formaldehyde-inactivated Venezuelan equine encephalitis virus vaccines against aerosol challenge in hamsters. *J Clin Microbiol* 1984;19(3):429–31.

[42] Rusnak JM, Kortepeter MG, Aldis J, Boudreau E. Experience in the medical management of potential laboratory exposures to agents of bioterrorism on the basis of risk assessment at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). *J Occup Environ Med* 2004;46(8):801–11.

[43] Gotuzzo E, Yactayo S, Córdova E. Review article: Efficacy and duration of immunity after yellow fever vaccination: Systemic review on the need for a booster every 10 years. *Am J Trop Med Hyg* 2013;89(3):434–44.

[44] Hombach J, Solomon T, Kurane I, et al. Report on a WHO consultation on immunological endpoints for evaluation of new Japanese encephalitis vaccines, WHO, Geneva, 2–3 September, 2004. *Vaccine* 2005;23:5205–11.

Figure Captions

Figure 1. Geometric mean titers (with one standard error) over time, measured by PRNT₈₀ (A) and PRNT₅₀ (B). In each panel, the dotted line indicates PRNT₈₀ or PRNT₅₀ titers below the LLOD (1:10); these values were set to 1:5 (½ LLOD) for analysis. In all groups, PRNT₈₀ and PRNT₅₀ titers were undetectable during postvaccination days 1–11.

Figure 2. Geometric mean titers (with one standard error) over time: IgM (A) and IgG (B). In both panels, the dotted line indicates IgM or IgG titers below the LLOD (1:100); these values were set to 1:50 (½ LLOD) for analysis. IgM titers were undetectable during postvaccination days 1–6 with the exception of one subject in IM Group 2 who had a titer of 1:400 at day 2. In all groups, IgG titers were undetectable during postvaccination days 1–7.

Table 1. Demographics of study subjects

	SQ Group	IM Group 1	IM Group 2	Placebo	
	(10 ^{4.7} pfu SQ)	(10 ^{3.4} pfu IM)	(10 ^{4.4} pfu IM)	Group	Total
Characteristic	N=10	N=6	N=27	N=13	N=56
Age, years					
Mean (SD)	29 (6.4)	25 (4.1)	28 (6.2)	27 (6.4)	28 (6.0)
Median	28	26	26	25	26
Min–Max	20–40	20–30	19–43	21–42	19–43
Age groups, n (%)					
19–29	5 (50.0%)	5 (83.3%)	20 (74.1%)	10 (76.9%)	40 (71.4%)
30–39	4 (40.0%)	1 (16.7%)	5 (18.5%)	2 (15.4%)	12 (21.4%)
40–43	1 (10.0%)	0 (0.0%)	2 (7.4%)	1 (7.7%)	4 (7.1%)
Gender, n (%)					
Female	2 (20.0%)	2 (33.3%)	9 (33.3%)	4 (30.8%)	17 (30.4%)
Male	8 (80.0%)	4 (66.7%)	18 (66.7%)	9 (69.2%)	39 (69.6%)
Race, n (%)					
White/Caucasian	9 (90.0%)	3 (50.0%)	20 (74.1%)	10 (76.9%)	42 (75.0%)
Black/African American	0 (0.0%)	2 (33.3%)	4 (14.8%)	3 (23.1%)	9 (16.1%)
Asian	0 (0.0%)	0 (0.0%)	2 (7.4%)	0 (0.0%)	2 (3.6%)
Mixed Race	0 (0.0%)	0 (0.0%)	1 (3.7%)	0 (0.0%)	1 (1.8%)
Other/Unknown	1 (10.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	2 (3.6%)
Ethnicity, n (%)					
Hispanic	1 (10.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	2 (3.6%)

Not Hispanic	9 (90.0%)	5 (83.3%)	27 (100.0%)	13 (100.0%)	54 (96.4%)
--------------	-----------	-----------	-------------	-------------	------------

Table 2. Systemic and injection site reactions

AE Category	SQ Group	IM Group 1	IM Group 2	All Vaccinees	Placebo Group	P-value
	(10 ^{4.7} pfu SQ)	(10 ^{3.4} pfu IM)	(10 ^{4.4} pfu IM)			
	N = 10	N = 6	N = 27	N = 43	N = 13	
	n (%)	n (%)	n (%)	n (%)	n (%)	
Local						
Abscess	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.0000
Arm Motion Limitation	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Cyst	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Edema/Induration	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Erythema	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Necrosis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
SQ Nodule(s)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Tender Axillary Nodes	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Tenderness	2 (20.0%)	0 (0.0%)	2 (7.4%)	4 (9.3%)	1 (7.7%)	
Warmth	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Systemic						
Blurred Vision	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.7%)	0.6497
Chills	0 (0.0%)	0 (0.0%)	2 (7.4%)	2 (4.7%)	0 (0.0%)	1.0000
Decreased Appetite	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.7%)	0.6497
Diarrhea (Stools)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (15.4%)	0.2289
Dizziness	0 (0.0%)	0 (0.0%)	3 (11.1%)	3 (7.0%)	1 (7.7%)	1.0000
Fever	0 (0.0%)	0 (0.0%)	1 (3.7%)	1 (2.3%)	0 (0.0%)	1.0000
Generalized Itching	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.0000
Headache	2 (20.0%)	1 (16.7%)	8 (29.6%)	11 (25.6%)	2 (15.4%)	
Hives (Urticaria)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Joint Aches	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.8046
Malaise	0 (0.0%)	0 (0.0%)	6 (22.2%)	6 (14.0%)	0 (0.0%)	
Muscle Aches	0 (0.0%)	0 (0.0%)	1 (3.7%)	1 (2.3%)	1 (7.7%)	
Nausea	0 (0.0%)	0 (0.0%)	2 (7.4%)	2 (4.7%)	2 (15.4%)	0.5324

Vomiting (Emesis)	0 (0.0%)	0 (0.0%)	2 (7.4%)	2 (4.7%)	0 (0.0%)	1.0000
-------------------	----------	----------	----------	----------	----------	--------

Note: P-values were calculated by Fisher's exact test with permutation adjustment for multiple testing.

Table 3. Seroconversion rates over the course of the study

	IgG ($\geq 1:100$)			IgM ($\geq 1:100$)			PRNT₅₀ ($\geq 1:20$)			PRNT₈₀ ($\geq 1:20$)		
Group	N	n (%)	(95% CI)	N	n (%)	(95% CI)	N	n (%)	(95% CI)	N	n (%)	(95% CI)
SQ Group ($10^{4.7}$ pfu SQ)	10	10 (100%)	(69%–100%)	10	10 (100%)	(69%–100%)	10	10 (100%)	(69%–100%)	10	10 (100%)	(69%–100%)
IM Group 1 ($10^{3.4}$ pfu IM)	6	5 (83%)	(36%–100%)	6	6 (100%)	(54%–100%)	6	5 (83%)	(36%–100%)	6	5 (83%)	(36%–100%)
IM Group 2 ($10^{4.4}$ pfu IM)	27	26 (96%)	(81%–100%)	27	25 (93%)	(76%–99%)	27	25 (93%)	(76%–99%)	27	25 (93%)	(76%–99%)
Total	43	41 (95%)	(84%–99%)	43	41 (95%)	(84%–99%)	43	40 (93%)	(81%–99%)	43	40 (93%)	(81%–99%)

Figure 1A. PRNT₈₀

Figure 1B. PRNT₅₀

Figure 2A. IgM

Figure 2B. IgG