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Rift Valley Fever MP-12 Vaccine Phase 2 Clinical Trial: Safety, Immunogenicity, and Genetic Characterization of Virus Isolates

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Abstract

An outbreak or deliberate release of Rift Valley fever (RVF) virus could have serious public health and socioeconomic consequences. A safe RVF vaccine capable of eliciting long-lasting immunity after a single injection is urgently needed. The live attenuated RVF MP-12 vaccine candidate has shown promise in Phase 1 clinical trials; no evidence of reversion to virulence has been identified in numerous animal studies. The objective of this Phase 2 clinical trial was to (a) further examine the safety and immunogenicity of RVF MP-12 in RVF virus–naïve humans and (b) characterize isolates of RVF MP-12 virus recovered from the blood of vaccinated subjects to evaluate the genetic stability of MP-12 attenuation. We found that RVF MP-12 was well tolerated, causing mostly mild reactions that resolved without sequelae. Of 19 subjects, 18 (95%) and 19 (100%) achieved, respectively, 80% and 50% plaque reduction neutralization titers ($PRNT_{80}$ and $PRNT_{50}$) ≥ 1:20 by postvaccination day 28. All 18 $PRNT_{80}$ responders maintained $PRNT_{80}$ and $PRNT_{50}$ ≥ 1:40 until at least postvaccination month 12. Viremia was undetectable in the plasma of any subject by direct plaque assay techniques. However, 5 of 19 vaccinees were positive for MP-12 isolates in plasma by blind passage of plasma on Vero cells. Vaccine virus was also recovered from buffy coat material from one of those vaccinees and from one additional vaccinee.

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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 FY04-33, 06-0-CR-004, Log A-13698, and number FY08-30.

Conflicts of Interest

The authors have no conflicts of interest.

Through RNA sequencing of MP-12 isolates, we found no reversions of amino acids to those of the parent virulent virus (strain ZH548). Five years after a single dose of RVF MP-12 vaccine, 8 of 9 vaccinees (89%) maintained a $PRNT_{80} \geq 1:20$. These findings support the continued development of RVF MP-12 as a countermeasure against RVF virus in humans.

Keywords

MP-12; Rift Valley fever; vaccine; clinical trial

1.0 INTRODUCTION

Rift Valley fever virus (RVFV) can cause serious morbidity and mortality in humans and in animals such as domestic livestock and wild ruminants. Though generally transmitted via mosquitoes, this enveloped RNA virus can also be transmitted by aerosol and other routes and is considered a potential biological weapon [1–3]. Once confined to eastern sub-Saharan Africa, RVF has spread in recent decades to Madagascar, Egypt, and the Arabian Peninsula. An outbreak or deliberate release of RVF in a new region with naïve host populations could have severe public health and socioeconomic consequences [3,4]. And as conflict in the Middle East continues, protection of U.S. troops deployed to this region from the threat of RVF becomes increasingly important [1].

Currently, no licensed drug will alter the course of RVF in humans or animals. The only RVF vaccine currently available for human use is a formalin-inactivated investigational vaccine, TSI-GSD-200, which is used for at-risk laboratory workers at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID). Although this vaccine has relatively good immunogenicity and low reactogenicity, it requires three primary doses, a mandatory boost at 6 months, and periodic boosts to maintain antibody titers at levels thought to be protective [5,6]. A safe and effective RVF vaccine capable of inducing long-lasting protective immunity after a single injection is urgently needed—for the protection of U.S. military personnel, at-risk laboratory workers, and at-risk individuals residing in regions in which RVF is endemic or emerging.

The live attenuated vaccine RVF MP-12 (TSI-GSD-223), produced by 12 serial mutagenesis passages of the ZH548 RVFV strain in the presence of 5-fluorouracil [7], appears to be safe and immunogenic in several species, including sheep [8–11] and nonhuman primates [12,13]. Although teratogenic effects have been reported in one study of ewes vaccinated early in gestation with a high dose of a cell culture passage of the vaccine virus [14], none was seen in studies of MP-12 having the NSm gene deleted [15]. In three Phase 1 studies, the subcutaneous (SQ) or intramuscular (IM) administration of RVF MP-12 (up to $10^{4.7}$ plaque-forming units [pfu] per dose) was followed by infrequent, generally mild local and systemic reactions and only low-level, transient postvaccination viremia. Among subjects receiving the highest doses SQ and those receiving the vaccine IM, the seroconversion rate (approximately 93% [16]) has been comparable to that observed following vaccination with three priming doses of the inactivated vaccine [5,6].

The RVF MP-12 vaccine virus has attenuating mutations in at least two sites [17–20]; thus, reversion of the live attenuated viral vaccine to a virulent disease-causing virus is unlikely [18]. However, the genetic stability of MP-12 attenuation after administration to humans has not been assessed.

In this study, we aimed to (a) further examine the safety and immunogenicity of RVF MP-12 administered as a single IM injection and (b) characterize isolates of RVF MP-12 virus recovered from the blood of vaccinated subjects to evaluate the genetic stability of MP-12 attenuation. We anticipated that (a) RVF MP-12 would display an acceptable safety and immunogenicity profile and (b) the RNA sequences of RVFV isolates would show no evidence of a reversion to virulence.

2.0 METHODS

2.1 RVF MP-12 Vaccine Candidate

The test article used in this study, labeled “Live-attenuated, Mutagenized (ZH548, MP-12, TSI-GSD-223, Lot 7-2-88),” was manufactured in 1988 by The Salk Institute – Government Services Division (Swiftwater, PA). The dried, lyophilized preparation was grown in MRC-5 cells and maintained in Eagle's minimum essential medium. This vaccine can be administered as an investigational new drug (IND) under BB-IND-4307.

The vaccine was reconstituted with sterile water for injection, U.S. Pharmacopeia. Each 1-mL IM injection of undiluted RVF MP-12 vaccine contained approximately 1×10^5 pfu. Prior to initiating the study, the potency of the vaccine (expressed as viral pfu/mL) was determined to verify the planned dose.

2.2 Subjects and Design

In this open-label, Phase 2, single-center study, conducted from 16 August 2006 (date of enrollment of the first subject) to 1 May 2008 (date of completion of the last subject), we assessed the safety, immunogenicity, and genetic stability of RVF MP-12 vaccine in healthy, RVFV-naïve subjects. The protocol for this study (FY04-33) was approved by USAMRIID's Human Use Committee (HUC), the institutional review board at the University of Texas Medical Branch (UTMB), the Department of the Army's Office of the Surgeon General Human Subjects Research Review Board, and the National Institute of Allergy and Infectious Diseases. An additional protocol for the long-term (years 2–5) follow-up assessments of immune response (FY08-30) was approved by USAMRIID's HUC.

Volunteers were recruited from among the military and civilian populations within and around Fort Detrick and Frederick, Maryland. Among the eligibility requirements, volunteers had to be 18–50 years old and, in the case of women, not pregnant or lactating; in good general health, with no evidence or history of liver disease, thymic disease, immunosuppression, or eye disease (other than refractory changes); RVFV-naïve (no previous infection with RVFV or receipt of an RVF vaccine); no known allergies to any vaccine components; not planning to receive another vaccine within 30 days of RVF MP-12 vaccination; willing to refrain from excessive physical exercise, alcohol consumption, and unprotected sexual activity for at least 2 weeks after vaccination; and, in the case of women,

willing to use a highly effective method of birth control for 3 months after vaccination. A total of 20 eligible volunteers provided written consent and were enrolled in the study. One subject was excluded after receiving another vaccine within 30 days of the intended MP-12 vaccination; 19 subjects received the RVF MP-12 vaccine and completed the study.

Each subject received a single 1-mL IM injection of RVF MP-12 vaccine. For the first 14 days following vaccination, subjects returned for clinical observations and blood draws. On day 21, subjects reported clinical status by telephone. On day 28 (± 5 days), subjects underwent ophthalmic examination and visual acuity testing. At 3 months (± 2 weeks), an electrocardiogram was obtained. At 6 months (± 2 weeks), final reactions to the vaccine were assessed, and at 12 months (± 4 weeks), the final clinical assessment was conducted. To assess the long-term immunogenicity of the vaccine, subjects were asked to return for additional blood draws at postvaccination years 2–5.

2.3 Safety

We evaluated the safety of RVF MP-12 by recording the frequency and severity of local and systemic adverse events (AEs) following vaccination. AEs included any untoward or unfavorable medical occurrence, including clinical abnormalities and clinical laboratory abnormalities—evident via complete blood counts and selected serum biochemistry (enzyme) values—regardless of whether they were causally related to the receipt of MP-12. We quantified the severity of AEs as Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), or Grade 4 (potentially life threatening). We also recorded the rates of hospitalizations and rates of lost duty/work time. Rates of AEs, hospitalizations, and lost duty/work time were analyzed for three periods: (a) onset between day 0 and day 30 postvaccination, (b) onset between day 31 and month 12 postvaccination, and (c) onset between day 0 and month 12 postvaccination.

Safety evaluations occurred daily on 17 days (days 0–14, 21, and 28) of the initial 30 days of the study. From day 31 through month 12, evaluations occurred at months 3, 6, and 12.

2.4 Immunogenicity

We evaluated immunogenicity by measuring 80% and 50% plaque reduction neutralization titer ($PRNT_{80}$ and $PRNT_{50}$) antibodies to RVFV for up to 12 months following vaccination (and up to 5 years after vaccination for those volunteers who participated in the long-term follow-up assessment). We report both the $PRNT_{80}$ and $PRNT_{50}$ titers to allow for comparison with other vaccines. Responders were defined as those having $PRNT_{80}$ and $PRNT_{50} \geq 1{:}20$. We analyzed the immune response prior to vaccination (day 0) and at several points after vaccination: days 1, 2, 3, 7, 10, 14, and 28 (± 5 days); months 3 (± 2 weeks), 6 (± 2 weeks), and 12 (± 4 weeks); and years 2 (± 2 months), 3 (± 2 months), 4 (± 2 months), and 5 (± 2 months). The proportion of subjects exhibiting seroconversion was calculated for each time point after vaccination.

The $PRNT_{80}$ and $PRNT_{50}$ were determined by preparing serial twofold dilutions of serum in diluent at a starting dilution of 1:5. An equal volume of challenge virus suspension diluted to yield a final plaque dose of 40–100 pfu was added to each serum dilution. After a 1-hour incubation, 6-well plates of Vero cells were inoculated with 0.2 mL of the serum–virus

mixture and overlaid with agarose followed by a second agarose overlay containing 2%–4% neutral red dye on day 4. At 18–24 hours after the second overlay, plaques were enumerated and neutralization titers were calculated. Serial fourfold dilutions of the virus inoculum were plated in a back titration to accurately determine the actual challenge virus dose. The highest serum dilution neutralizing 80% ($PRNT_{80}$) or 50% ($PRNT_{50}$) of the calculated virus plaque dose is the neutralization titer of that serum, as described by Meadors et al. [21]. The upper and lower limits of the PRNT assay are titers of 1:10,240 and 1:10, respectively.

2.5 Genetic Stability

2.5.1 Virus Isolate Recovery—RVF MP-12 virus isolation was conducted at UTMB on plasma and buffy coat specimens collected on postvaccination days 1–14. We used both plasma and buffy coat fractions because we anticipated that virus recovery would be difficult and aimed to determine which tissue would be most useful for this analysis. Standard virus plaque assay and blind, double passage of plasma and buffy coat on Vero cells were applied. Plasma (1 mL) or buffy coat (0.2 mL) suspension was added to a T25 flask of 70%–80% confluent Vero cells from which medium had been decanted. After a 1-hour adsorption (37°C, 5% CO_2), the fluid was decanted and replaced by fresh medium (5 mL). Flasks were incubated (37°C) with daily observation for cytopathic effect (CPE). After 5 days of incubation, the medium in all flasks was replaced with fresh medium. After 10 days of incubation, all flasks negative for CPE underwent a second cell culture passage. Flasks were frozen (−70°C) and rapidly thawed under running cold water. Flask contents were transferred to labeled 15-mL conical tubes and clarified by centrifugation (1,500 rpm, 15 minutes, 4°C). A 1-mL aliquot of each supernatant was then inoculated onto 70%–80% confluent Vero cell monolayers in T25 flasks as in the first passage. After the second 10-day incubation (with medium replacement after 5 days), supernatant from all flasks not exhibiting CPE underwent polymerase chain reaction (PCR) analysis. Flasks exhibiting CPE were frozen (−70°C), thawed, and the supernatant passaged an additional time to amplify the virus, if present. The supernatant from the second passage was tested for the presence of RVFV by plaque assay. Any positive plasma or buffy coats were titrated by inoculating tenfold dilutions of plasma or buffy coat into 24-well culture plates and testing for plaques. Additionally, all isolates underwent indirect fluorescent antibody testing for specific fluorescence using RVF MP-12 immune mouse serum.

2.5.2 RNA Sequencing—We assessed the genetic stability of the vaccine by examining any virus isolates recovered from the blood of vaccinated subjects using non-GLP molecular sequencing techniques and comparing these findings with those from the vaccine virus inoculum. Viral RNA was extracted from the blood of vaccinated subjects using a high pure viral RNA extraction kit (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. The cDNA was synthesized with Superscript II and Random Primers (Invitrogen, Carlsbad, CA). The genome was amplified with Platinum Taq Polymerase (Invitrogen) and genome-specific primer pairs. The PCR conditions followed the general protocol of 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 68°C for 60 seconds, and a final 10-minute extension at 68°C. The amplified products were subjected to gel extraction using a Rapid Gel Extraction Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Nucleotide sequencing

was performed on an AB-3100 Capillary Automated DNA Sequencer (Applied Biosystems, Inc., Foster City, CA). We calculated the mutation rate as (number of mutations/number of nucleotide sequences) × 100.

2.5.3 Virulence Assessment—To test the virulence of RVF MP-12 virus isolates recovered from vaccinated subjects and to compare the virulence of these isolates with that of the RVF MP-12 vaccine, we used a 19-day-old CD-1 mouse model, developed at UTMB (J Morrill, unpublished). Briefly, this model is based on our previous observations that the RVF MP-12 vaccine is fatal or nearly fatal, regardless of dose, in mice aged 14 days and younger but is mostly nonfatal, regardless of dose, in mice aged 21 days or older. In 19-day-old mice, we were routinely able to determine the 50% lethal dose (LD_{50}) to gauge the virulence of the RVF MP-12 vaccine in this model.

2.6 Statistical Analyses

To assess safety, we tabulated time-specific and cumulative rates of local and systemic AEs, rates of hospitalizations, and rates of lost duty/work time overall and by gender. For multiple episodes of the same AE, only the most severe episode was used for further analysis. The proportion of subjects exhibiting AEs was used to assess vaccine tolerability. Fisher's exact tests, with Bonferroni correction for multiple comparisons, were used to compare AE rates between male and female subjects.

To analyze immunogenicity, we measured antibodies against RVFV assessed by $PRNT_{80}$ and $PRNT_{50}$ on serum samples collected from subjects before and after vaccination. Response rates were tabulated by gender and by plaque neutralization metric ($PRNT_{80}$ vs. $PRNT_{50}$). The proportion of subjects exhibiting seroconversion ($PRNT_{80} \geq 1:20$; $PRNT_{50} \geq 1:20$) was evaluated to assess vaccine immunogenicity. Log transformations were applied to $PRNT_{80}$ and $PRNT_{50}$ values, with any titers of < 1:10 replaced by the assay's limit of detection (1:10) divided by the square root of 2. These log-transformed titers were used to calculate geometric mean titers (GMTs) of $PRNT_{80}$ and $PRNT_{50}$. Geometric mean PRNT values were tabulated by gender and for all subjects combined. A mixed-model repeated measures analysis of variance was used to test for differences between male and female subjects in $PRNT_{80}$ and $PRNT_{50}$ values over time. Student's t-tests were used to determine whether men and women differed in peak $PRNT_{80}$, peak $PRNT_{50}$, time to response, and time to peak titer. Fisher's exact tests, with Bonferroni correction for multiple comparisons, were used to compare the response rates of male and female subjects at each time point.

The genetic stability endpoint was the proportion of subjects with a virus isolate that differed genetically (in a manner that would affect virulence) from that found in the vaccine at any time following vaccination. Exact binomial proportions were calculated and tested against the null hypothesis that the proportion of subjects with significant virus mutations was no greater than 0.15.

All statistical analyses were performed using SAS (Cary, NC), version 9.1.3. No corrections for missing data were included in the analyses. The LD_{50} values for the vaccine were calculated from the survivor numbers in the 19-day mouse model [22].

3.0 RESULTS

The 19 subjects who were enrolled and vaccinated included 9 women and 10 men with a mean age of 33 years (range 23–50 years). Most of the subjects were white (89%); none self-identified as Hispanic or Latino. The vaccine's potency (pfu/mL) was identical to the potency calculated when it was prepared in 1988 and maintained as a dry powder at −35°C.

3.1 Safety

Seventeen of the 19 vaccinated subjects reported at least one AE related to the vaccination during the study (Table 1). Within 30 days postvaccination, 67 related AEs were reported: 22 local AEs reported by 13 subjects and 45 systemic AEs reported by 15 subjects. The most common systemic related AE was headache, which occurred in 58% of vaccinees during the first 30 days after vaccination; tenderness was the most common injection site related AE during the first 30 days after vaccination (reported by 47% of subjects).

Most (96%) of the systemic related AEs were classified as Grade 1 in severity, and 4% were classified as Grade 2. The Grade 2 related AEs during this period consisted of headache, experienced by one subject, and flu-like symptoms, experienced by another subject. One subject developed a fever (temperature = 101°F) on postvaccination day 9. All injection site AEs were classified as Grade 1 in severity.

We found no clinically significant changes in complete blood count or platelet count during the course of the study. Elevations in transaminases generally followed creatine phosphokinase (CPK) elevations. Significant increases in CPK always followed extreme exercise. Two subjects experienced Grade 4 elevated CPK levels—in one case on day 0 (prevaccination), 1 day after the subject performed a series of strenuous exercises, and in the other case at postvaccination month 12, 2 days after the subject completed a 50-mile ultramarathon. Both were considered unrelated to vaccination. Ophthalmologic examinations on postvaccination day 28 showed no changes from baseline prevaccination examinations. Electrocardiograms at postvaccination month 3 showed no changes from baseline.

All AEs resolved without sequelae, and no serious AEs were reported. No subjects lost duty/work time or were hospitalized. A Fisher's exact test found no statistically significant differences between men and women in AE rates.

3.2 Immunogenicity

Of the 19 subjects administered a single 1-mL injection of RVF MP-12 (1×10^5 pfu), 18 (95%, including 9 women and 9 men) achieved a $PRNT_{80} \geq 1:20$ by postvaccination day 28 (Figure 1A); all 19 subjects achieved a $PRNT_{50} \geq 1:20$ by postvaccination day 28 (Figure 1B). Mean time to response was 16.6 days (range = 10 to 28 days). The one male nonresponder achieved a maximum $PRNT_{80}$ of 1:10 by day 28. This subject's $PRNT_{50}$ values were 1:30, 1:20, 1:10, and 1:10 on days 28, 90, 180, and 365. Peak response occurred after a mean of 54.2 and 82.2 days for $PRNT_{80}$ and $PRNT_{50}$, respectively. The 18 responders had $PRNT_{80}$ and $PRNT_{50} \geq 1:40$ at postvaccination month 12. Male and female

subjects did not differ significantly in peak $PRNT_{50}$, peak $PRNT_{80}$, time to response, or time to peak titer.

At postvaccination month 12, the $PRNT_{80}$ GMT was 174 overall (203 for men, and 147 for women), the individual peak $PRNT_{80}$ GMT was 496 overall (517 for men, and 474 for women), the $PRNT_{50}$ GMT was 433 overall (570 for men, and 318 for women), and the individual peak $PRNT_{50}$ GMT was 1,403 overall (1,549 for men, and 1,256 for women). Figures 2A and 2B show the GMT and associated standard error of the mean at each time point.

At years 2, 3, 4, and 5 following vaccination, 13, 9, 8, and 9 subjects, respectively, returned for additional blood draws to enable an assessment of the long-term immunogenicity of a single dose of the RVF MP-12 vaccine. Table 2 shows the $PRNT_{80}$ titers for all subjects at day 28 and month 12 as well as $PRNT_{80}$ titers at years 2–5 for subjects who provided samples at those time points; $PRNT_{50}$ titers at day 28 are shown for comparison. At years 2, 3, and 4, 100% of follow-up subjects had a $PRNT_{80} \geq 1:20$; at year 5, 8 of 9 (89%) subjects had a $PRNT_{80} \geq 1:20$. $PRNT_{50}$ titers were calculated in years 4 and 5 of the long-term follow-up assessment; at both of these time points, 100% of follow-up subjects achieved a $PRNT_{50} \geq 1:20$.

3.3 Genetic Stability

Viremia was undetectable in the plasma of any subject using direct plaque assay techniques. However, from 283 plasma specimens collected on postvaccination days 1–14, nine MP-12 isolates were recovered from five subjects (one to four isolates per subject) on postvaccination days 4–9 via blind, double passage on Vero cells (Table 3). From 283 buffy coat specimens collected on postvaccination days 1–14, two MP-12 isolates were recovered —one from each of two subjects on postvaccination days 7 and 8—by blind, double passage on Vero cells. The proportion of subjects with positive buffy coat isolates (2 of 19 or 0.11) was found not to be statistically significantly greater than 0.15 ($p = 0.4413$). The overall proportion of subjects with positive plasma isolates (5 of 19 or 0.26) was found not to be statistically significantly greater than 0.15 ($p = 0.1444$).

We sequenced a total of nine virus isolates recovered from five volunteers (Figure 3; Table 4). Two of the isolates from the same subject were identical to the RVF MP-12 vaccine virus; in the remaining seven isolates, we identified a total of 10 mutations, none of which was a reversion to the ZH548 sequence at that location. Therefore, the null hypothesis of zero reversion to virulence was accepted. The mutation rate was about 0.00834.

Employing the 19-day-old CD-1 mouse model, we found that the LD_{50} of each of the 11 RVF MP-12 isolates recovered from six subjects (range: $1 \times 10^{2.1}$ to $> 1 \times 10^{5}$ pfu/mL) was greater than the vaccine LD_{50} ($1 \times 10^{1.88}$ pfu/mL) (Table 5). This demonstrates that the virulence of isolates was even less than that of RVF MP-12.

4.0 DISCUSSION

RVF MP-12 was totally stable after 18 years of storage at −35°C. When administered at a dose of 1×10^5 pfu IM to healthy, RVFV-naïve individuals, RVF MP-12 was safe, well tolerated, and immunogenic. AEs were generally mild; elevated transaminase values seen in this study and in prior studies of this vaccine appear to be due to rigorous exercise by study subjects. A single dose of RVF MP-12 produced an immune response in 95% of vaccinees by postvaccination day 28 using the more stringent criterion of $PRNT_{80} \geq 1:20$. $PRNT_{50}$ is presented for ease of comparison to other vaccines. The single subject who did not achieve this minimum titer had a $PRNT_{80}$ of 1:10 and a $PRNT_{50}$ of 1:30 on day 28; by day 90, this subject's $PRNT_{50}$ had decreased to 1:20. Thus, it may be more accurate to consider this subject a low responder rather than a nonresponder. At 1 year after vaccination, all responders achieved a $PRNT_{80}$ and $PRNT_{50}$ of at least 1:20. Most subjects followed for 2–5 years after vaccination retained detectable virus neutralization titers greater than 1:20.

We found no detectable viremia (plasma or buffy coat) by plaque assay in any subject and recovered few virus isolates in vaccinees' plasma or buffy coat specimens after double-blind passage on Vero cells during the first 14 days after vaccination. These results support the low levels of viremia previously found in human RVF MP-12 vaccinees [16] and in animal studies [8,11,13]. Low viremia reduces the likelihood of secondary arthropod spread of the RVF MP-12 vaccine virus [23,24].

RNA sequencing of the nine MP-12 isolates recovered from the plasma of five subjects via double-blind passage on Vero cells showed no SNPs or reversions in the attenuating mutations of the parent virus. The virulence of each of 11 isolates recovered from six subjects, as measured by LD_{50} values in a mouse model, was lower than that of the vaccine. Thus, attenuation appears to be stable in human vaccinees, supporting previous work in other species.

These findings support the continued development of RVF MP-12 vaccine as a potential countermeasure against RVFV and one that is well suited for long-term cold storage as a stockpile medical countermeasure.

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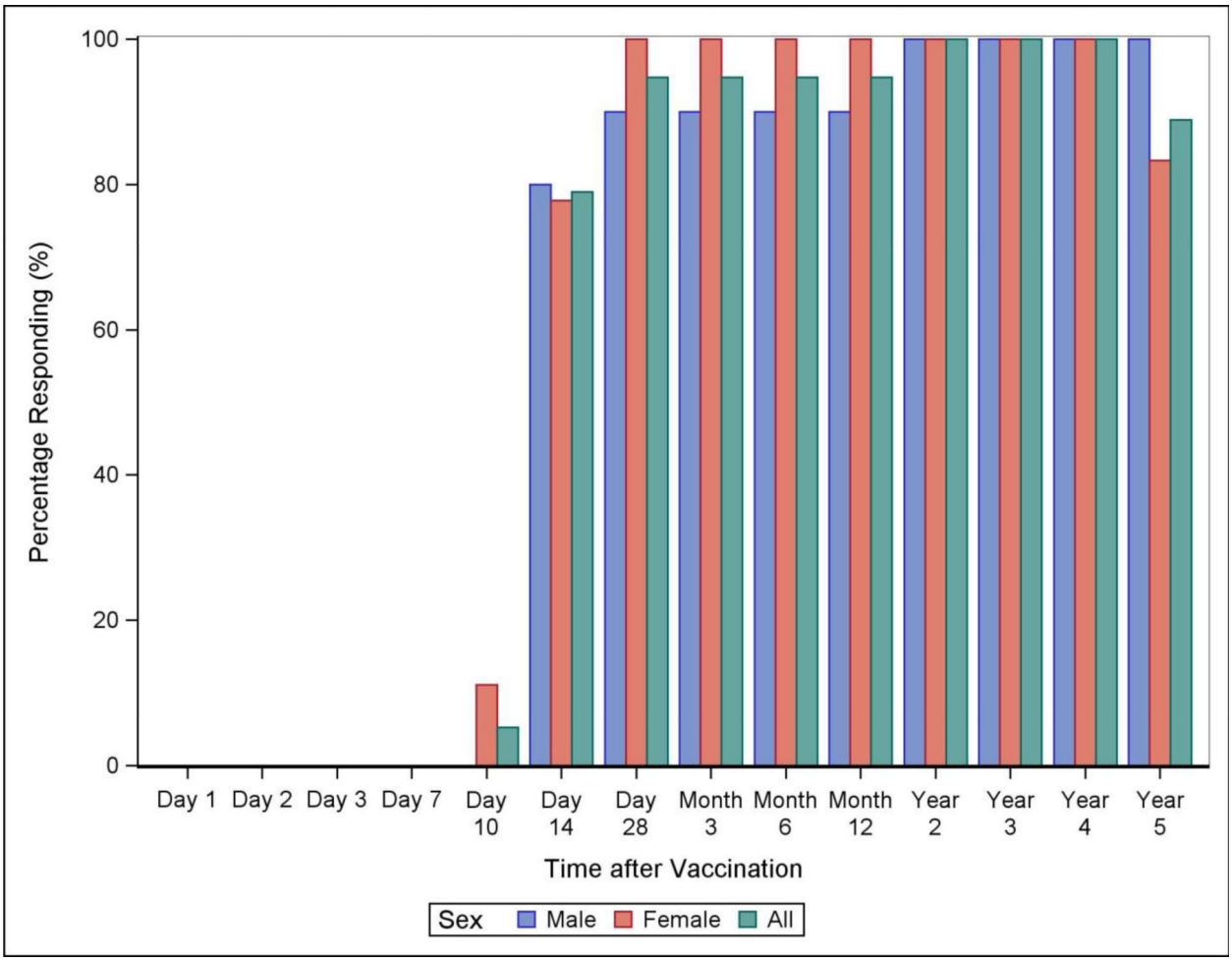


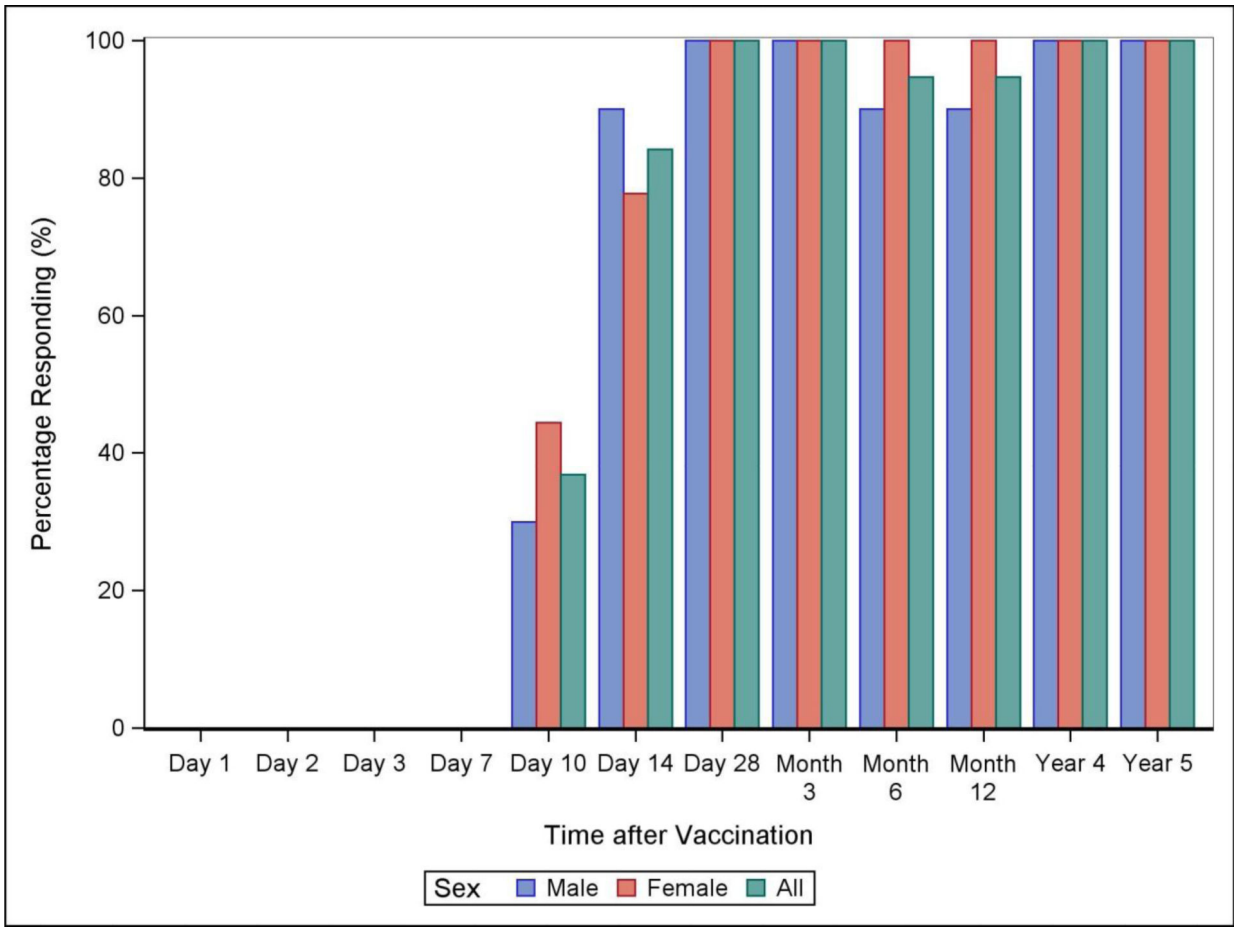
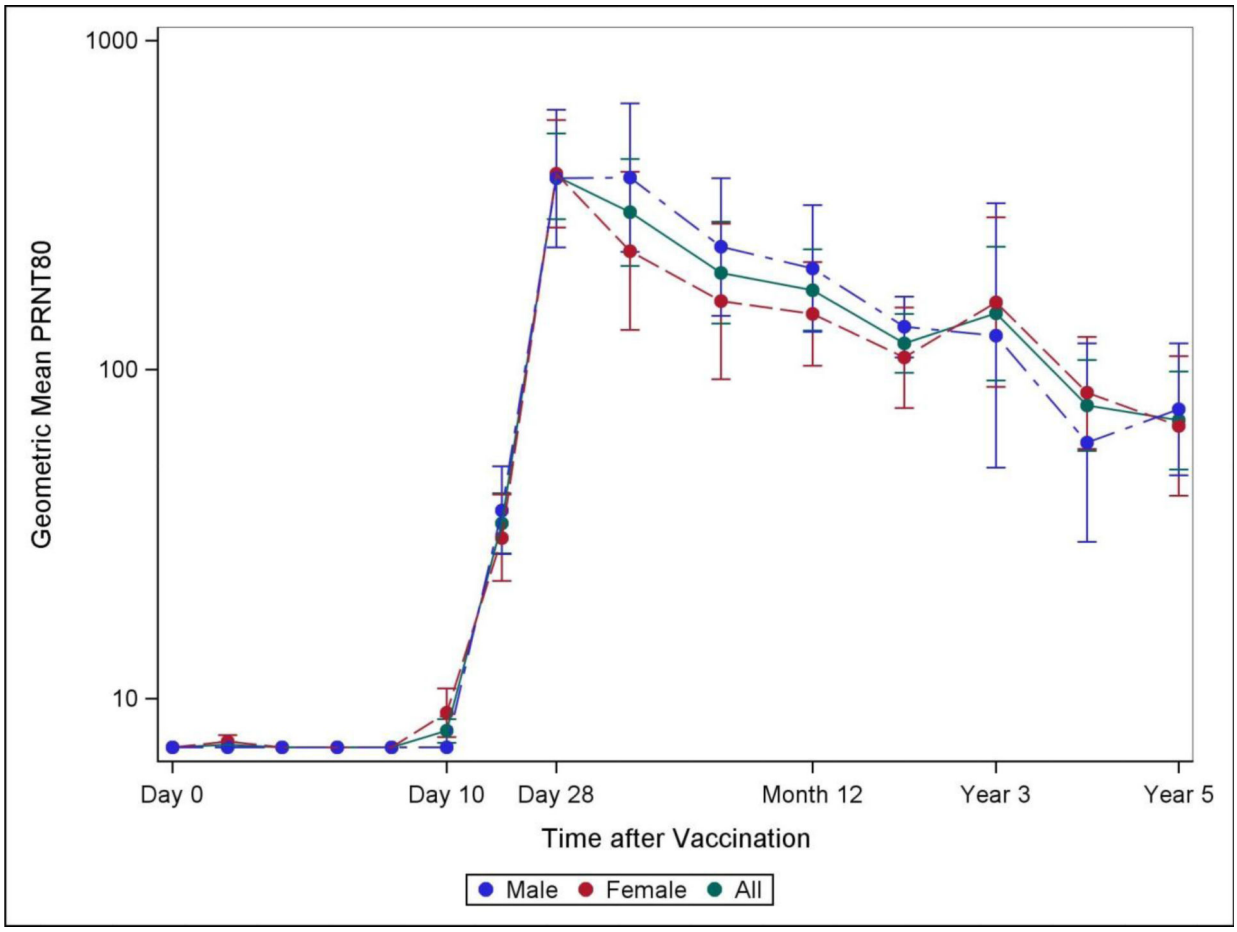


Figure 1.

Percentage of subjects responding to RVF MP-12 vaccine over time, as measured by (A) $PRNT_{80} \geq 1{:}20$ and (B) $PRNT_{50} \geq 1{:}20$. $PRNT_{50}$ values were not calculated in year 2 or year 3. In both panels, N = 19 at each time point except at days 1 and 3 (N = 18) and at years 2 (N = 13), 3 (N = 9), 4 (N = 8), and 5 (N = 9).



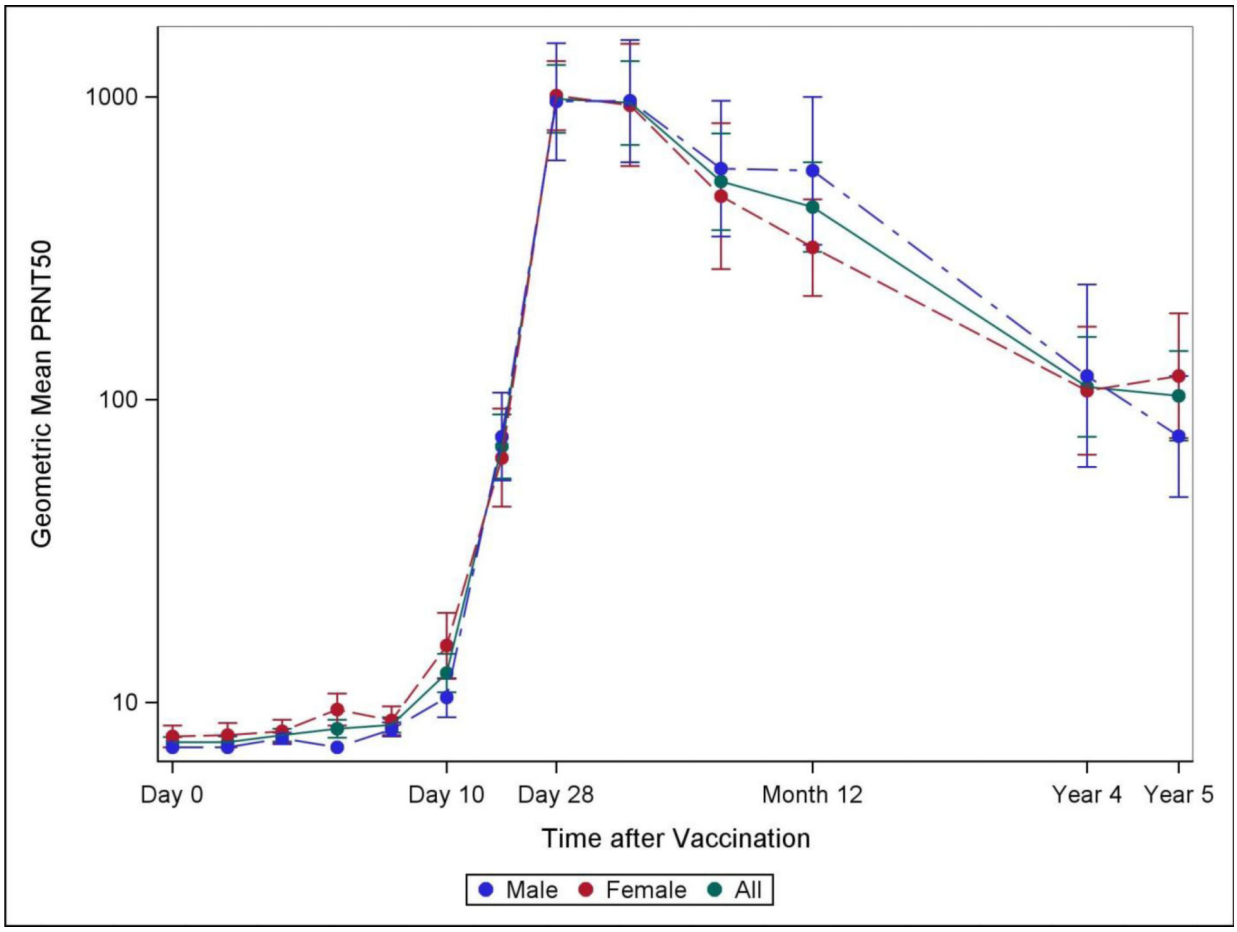


Figure 2.

Geometric mean titers over time, as measured by (A) $PRNT_{80}$ and (B) $PRNT_{50}$. Error bars represent 1 standard error above and below the geometric mean.

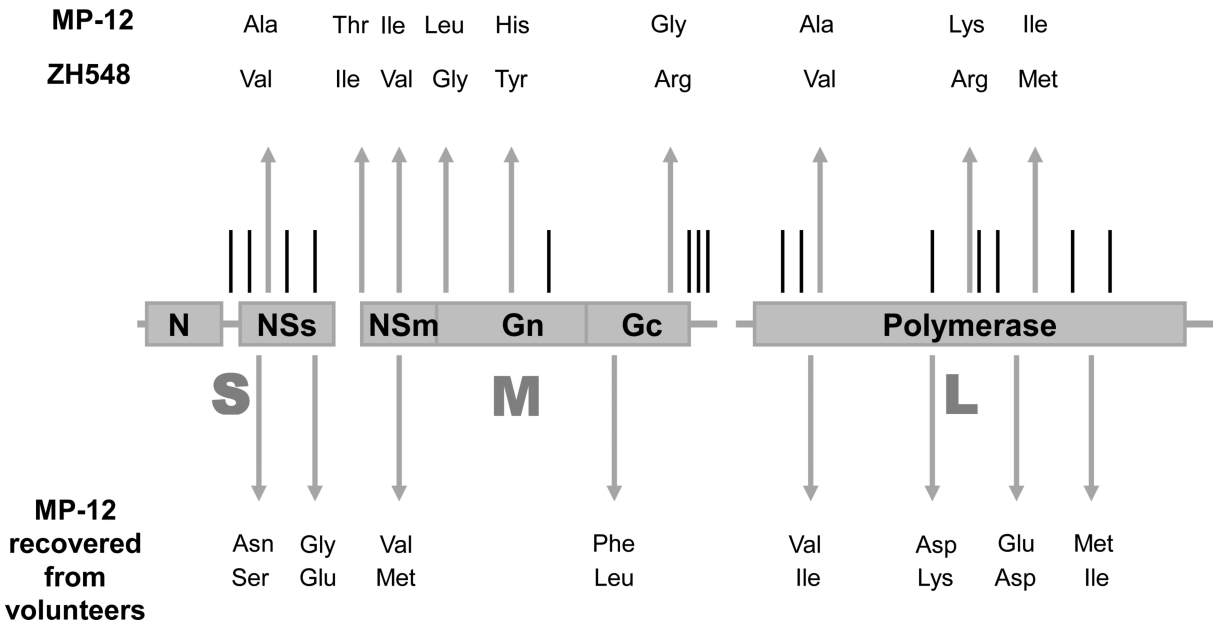


Figure 3.

RVFV genomic analysis. The upper half of the diagram shows the differences between the amino acid codons in the attenuated RVF MP-12 vaccine (top row) and ZH548 (second row). The lower half of the diagram shows the pooled differences between the amino acids of the MP-12 recovered from subjects and those in the vaccine. No reversions of amino acids to those of the virulent ZH548 parent virus were observed.

Table 1

Number of subjects experiencing adverse events after administration of the RVF MP-12 vaccine

	Total Subjects (N = 19)	
	N	**%**
Local Adverse Events		
Tenderness (vaccination site)	9	47.4
Muscle pain	4	21.1
Induration	3	15.8
Redness (vaccination site)	3	15.8
Bruise	2	10.5
Injection site warm	1	5.3
Systemic Adverse Events		
Headache	11	57.9
Fatigue	8	42.1
Nausea	5	26.3
Flu-like symptoms	4	21.1
Fever	3	15.8
Nasal congestion	2	10.5
Rhinorrhea	2	10.5
Blurred vision	1	5.3
Clammy skin (hands)	1	5.3
Cold sore	1	5.3
Diarrhea	1	5.3
Itching (left eye)	1	5.3
Muscle pain	1	5.3
Pain (joint-knee)	1	5.3
Spacey*	1	5.3
Stiff neck	1	5.3
Throat scratchy and sore	1	5.3
Any local adverse event	*13*	*68.4*
Any systemic adverse event	*15*	*78.9*
Any adverse event	*17*	*89.5*

Note: The table shows only those adverse events (a) with onset within 30 days of vaccination and (b) deemed possibly, probably, or definitely related to the vaccine.

*As reported by subject.

Table 2

Long-term immunogenicity of RVF MP-12, by subject

	Day 28		Month 12	Year 2	Year 3	Year 4	Year 5
Subject #	**$PRNT_{80}$**	**$PRNT_{50}$**	**$PRNT_{80}$**	**$PRNT_{80}$**	**$PRNT_{80}$**	**$PRNT_{80}$**	**$PRNT_{80}$**
001	1:120	1:320	1:60	1:30	1:20	1:30	1:15
002	1:240	1:640	1:120	—	—	—	—
003	1:480	1:960	1:240	1:120	1:320	1:120	1:60
004	1:10	1:30	< 1:10	—	—	—	—
005	1:640	1:960	1:640	1:240	1:320	1:120	1:120
007	1:480	1:1280	1:240	1:120	—	1:60	—
008	1:960	1:1280	1:960	—	—	—	—
009	1:1280	1:1920	1:320	1:240	1:320	—	1:120
011	1:960	1:1920	1:240	1:120	1:320	—	1:120
012	1:120	1:640	1:320	1:120	—	—	—
014	1:960	1:1920	1:120	1:60	1:80	1:60	1:60
015	1:240	1:640	1:60	1:60	1:80	1:60	1:30
016	1:60	1:480	1:40	—	—	—	—
019	1:1280	1:3840	1:240	1:120	—	—	—
020	1:240	1:640	1:80	1:60	1:20	1:30	1:30
021	1:1280	1:3840	1:240	—	—	—	—
023	1:240	1:640	1:80	—	—	—	—
026	1:1920	1:3840	1:1280	1:480	1:1280	1:480	1:480
029	1:960	1:2560	1:640	1:240	—	—	—

Notes: PRNT, plaque reduction neutralization titer (80% and 50%). $PRNT_{50}$ values are provided for day 28 for comparison to $PRNT_{80}$ values at the same time point. — denotes missing data for subjects who did not return for a follow-up visit.

Table 3

Recovery of RVF MP-12 isolates from plasma or buffy coat, by day

Day	Plasma					Buffy Coat				
	Negative		Positive		Total	Negative		Positive		Total
	N	%	N	%	N	N	%	N	%	N
0	18	*100*	0	*0*	18	18	*100*	0	*0*	18
1	19	*100*	0	*0*	19	19	*100*	0	*0*	19
2	19	*100*	0	*0*	19	19	*100*	0	*0*	19
3	19	*100*	0	*0*	19	19	*100*	0	*0*	19
4	18	*95*	1	*5*	19	19	*100*	0	*0*	19
5	17	*89*	2	*11*	19	19	*100*	0	*0*	19
6	17	*89*	2	*11*	19	19	*100*	0	*0*	19
7	18	*95*	1	*5*	19	18	*95*	1	*5*	19
8	18	*95*	1	*5*	19	18	*95*	1	*5*	19
9	17	*89*	2	*11*	19	19	*100*	0	*0*	19
10	19	*100*	0	*0*	19	19	*100*	0	*0*	19
11	19	*100*	0	*0*	19	19	*100*	0	*0*	19
12	19	*100*	0	*0*	19	19	*100*	0	*0*	19
13	18	*100*	0	*0*	18	18	*100*	0	*0*	18
14	19	*100*	0	*0*	19	19	*100*	0	*0*	19

Notes: Day 0 samples were collected prior to vaccination. The table indicates the number and percentage of subjects from whom RVF MP-12 virus isolates were recovered (positive) or not (negative) using blind, double passage of plasma or buffy coat on Vero cells.

Table 4

Sequence results of virus isolates

		Sequence results of virus isolates											
		Full sequence			Partial sequence of region where mutation found in isolate 1								
		Isolate 1			Isolate 2			Isolate 3			Isolate 4		
Subject no.	No. of isolates	Point of mutation Segment			Point of mutation Segment			Point of mutation Segment			Point of mutation Segment		
		S	M	L	S	M	L	S	M	L	S	M	L
005	1		M3773	L3465									
				L4749									
007	1		M2520	L2181									
014	1	S1469		L127									
021	4			L711			2 populations at 711			No mutation			No mutation
				L3879			2 populations at 3879			No mutation			No mutation
026	2	S1427											

Table 5

LD_{50} of MP-12 virus recovered from subjects

Plasma			Buffy Coat		
Subject no.	**Day Post Vaccination**	**LD_{50} (pfu/mL)**	**Subject no.**	**Day Post Vaccination**	**LD_{50} (pfu/mL)**
005	6	$10^{3.77}$	005	8	$10^{3.86}$
007	8	$10^{2.33}$	011	7	$10^{4.48}$
014	5	$>10^{5.0}$			
021	4	$10^{2.31}$			
021	5	$10^{2.1}$			
021	6	10^{5}			
021	9	$10^{4.82}$			
026	7	$>10^{5.0}$			
026	9	$10^{3.64}$			
			MP-12 Vaccine LD_{50} = $10^{1.88}$		

Note: Plasma and buffy coat specimens were collected from each subject on days 1–14. The table includes only those subjects from whose samples virus was recovered on the days indicated.