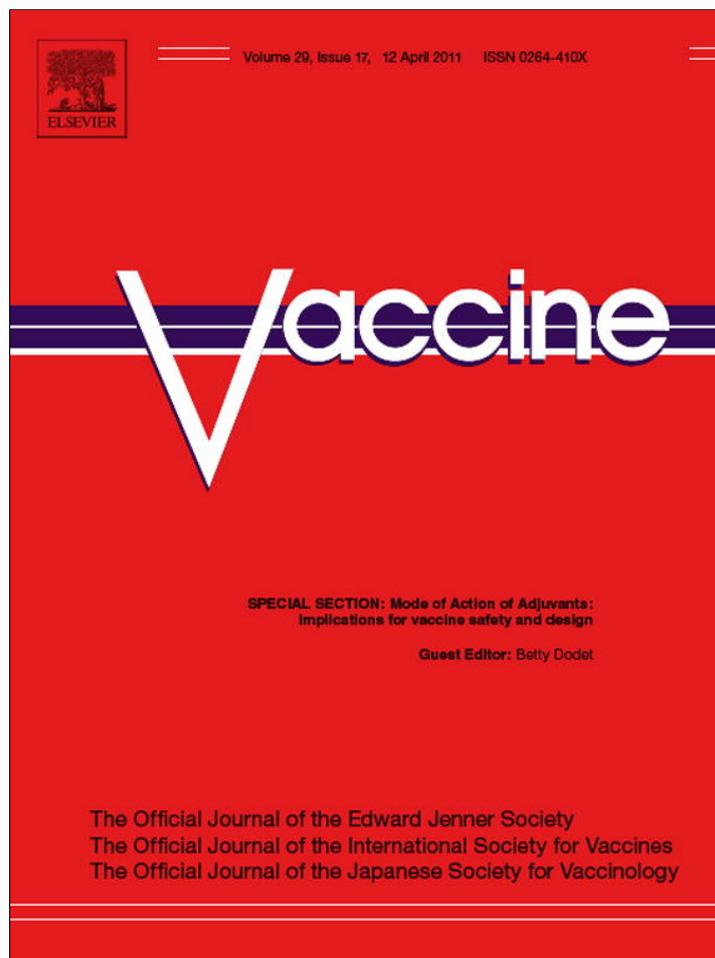


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Immunogenicity and safety of an inactivated Rift Valley fever vaccine in a 19-year study[☆]

Janice M. Rusnak^{a,*}, Paul Gibbs^b, Ellen Boudreau^c, Denise P. Clizbe^c, Phillip Pittman^c

^a *Clinical Research Management, Inc., Special Immunizations Program, United States Army Medical Research Institute of Infectious Diseases (USAMRIID), 1425 Porter Street, Fort Detrick, MD 21702, United States*

^b *Department of Statistics, USAMRIID, 1425 Porter Street, Fort Detrick, MD 21702, United States*

^c *Medical Division, USAMRIID, 1425 Porter Street, Fort Detrick, MD 21702, United States*

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ABSTRACT

An investigational, formalin-inactivated Rift Valley fever (RVF) vaccine, known as The Salk Institute-Government Services Division (TSI-GSD) 200 vaccine, was administered to 1860 at-risk subjects (5954 doses) between 1986 and 2004 as a three-dose primary series (days 0, 7, and 28) followed by booster doses as needed for declining titers. An initial positive serological response (PRNT₈₀ ≥ 1:40) to the primary series was observed in 90% of subjects. Estimate of the PRNT₈₀ response half-life in initial responders to the primary series by Kaplan–Meier plot was 315 days after the primary series dose 3. Differences in a serological response were observed at 2 weeks after dose 3 of the primary series between vaccine lots and for gender (women > men); a trend was observed for age (<40 years). When response to the primary series was measured by PRNT₅₀ titer ≥ 1:40, nearly all subjects (99.1%) responded. In individuals not initially responding to the primary series (PRNT₈₀ < 1:40), a response was observed in most subjects after receiving only one booster dose. Immune response (all subjects) to subsequent booster doses for a declining titer (PRNT₈₀ < 1:40) was 98.4%. The vaccine was well-tolerated; vaccine-related adverse reactions were generally mild and self-limited. Differences in adverse events were observed with vaccine lot and sex. The data support the safety and immunogenicity of the inactivated RVF vaccine, and may serve as a standard of comparison for immunogenicity and safety for future RVF vaccines.

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1. Introduction

Rift Valley fever virus was initially recognized in the Rift Valley of Kenya in 1931 and is endemic to many areas of sub-Saharan Africa [1–4]. After initial introduction to Egypt in 1977 that resulted in an extensive epidemic in humans and domestic animals, the virus has since emerged in Madagascar, Yemen, and Saudi Arabia [4,5]. While illness from RVF virus most commonly presents as an undifferentiated febrile illness, severe disease in humans may cause retinitis (that may result in permanent loss of vision), a hemorrhagic syndrome associated with gastrointestinal hemorrhage and

hepatitis, or meningo-encephalitis [5–9]. Infection in humans is generally acquired from close contact with the blood of infected livestock or the bite of an infected mosquito. As the *Aedes* mosquito vector resides in many areas of the world, introduction of virus to these areas could have a significant public health and agricultural impact during epizootics [10].

There is currently no Food and Drug Administration (FDA)-approved vaccine for RVF infection. The Salk Institute-Government Services Division (TSI-GSD) 200 vaccine is an investigational, formalin-inactivated vaccine that was manufactured in 1979 from a plaque-cloned version of the seed virus and cellular substrate of diploid fetal rhesus lung cells [11]. The vaccine development was based on an earlier investigational formalin-inactivated RVF vaccine developed from monkey kidney cells infected with a pantropic strain of the virus, known as the National Drug Biological Research Company (NDBR) 103 vaccine. The NDBR 103 vaccine had demonstrated (1) immunogenicity and efficacy in animal models and (2) safety and immunogenicity in over 2000 at-risk individuals [12–16].

Although reports of the TSI-GSD 200 RVF vaccine were previously published, the earlier reports involved smaller cohorts with inadequate numbers to address demographical differences (age

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* Corresponding author. Tel.: +1 301 619 4156; fax: +1 301 619 2511.

E-mail address: Janice.Rusnak@amedd.army.mil (J.M. Rusnak).

and sex) in immune response and adverse events as observed in this study report [11,17–19]. The experience of the RVF TSI-GSD 200 vaccine administered to at-risk individuals (mainly laboratory workers) from 1984 to 2004 was reviewed to better define the safety and immunogenicity of the vaccine. In addition to differences in immunogenicity related to gender, age, and lot (only interlot differences in immunogenicity previously reported with this vaccine), this study noted differences in adverse events related to gender, age, and vaccine lot [11,18]. Also, earlier publications reported data only by 80% plaque-reduction neutralization (PRNT₈₀) assay response and not by PRNT₅₀ results, which has since become a standard in vaccine development. With the emergence within the past year of several RVF vaccine candidates demonstrating immunogenicity in animal models, the information on the inactivated TSI-GSD 200 RVF vaccine may provide insight into immunogenicity and safety issues for clinical trials with future recombinant vaccine candidates [20].

2. Methods

2.1. Vaccine

The formalin-inactivated, TSI-GSD-200 RVF vaccine was developed in 1979, using a master seed made from passage of the mouse serum seed into diploid fetal rhesus monkey lung cells, DBS 103. Details of the vaccine have been described in previous publications [11,17]. The lyophilized vaccine product (lots 1–16, and lot 18) was stored at $-20^{\circ} \pm 10^{\circ} \text{C}$ and reconstituted with 5 ml of sterile water before injection. A total of 31 lots and runs of the vaccine were administered during the study.

2.2. Serology

Immunological response of volunteers was assessed using an 80% plaque reduction neutralization (PRNT₈₀) assay, as described in previous publications [18,19]. RVF virus was diluted to approximately 100 plaque-forming units (PFU)/0.2 ml and mixed with sera in serial twofold dilutions. After incubation overnight at 4 °C, the mixtures were placed into 23-mm wells containing confluent monolayers of VERO cells (0.1 ml/well). After incubation at 37 °C for 1 h with 5% CO₂, the inoculated cells were overlaid with nutrient medium containing 1% agar, 5% fetal bovine serum, 200 U of penicillin/ml, and 200 mg of streptomycin/ml, and reincubated again at 37 °C with 5% CO₂. The cells were then overlaid again with the nutrient medium to which a 1:7500 dilution of neutral red solution had been added. The highest dilution of serum that inhibited 80% or more of the plaques (compared to virus control titration) was defined as the PRNT₈₀ titer. PRNT₅₀ titers were calculated but were not used for clinical decisions in the protocol.

2.3. Vaccination

Subjects were randomized to receive a specific vaccine lot until 1993. After 1993, only the vaccine lot(s) currently in use at the time was administered to all subjects (same lot always used to complete the primary series). The primary series of the vaccine consisted of three 1.0-ml subcutaneous injections in the triceps region of the arm (given at day 0, day 14, and day 28). If the subject's PRNT₈₀ titer was $\geq 1:40$ after the primary series (referred to as an initial responder to the primary series), PRNT₈₀ titers were subsequently obtained at month 2, 5, 8, and 11 after dose 3 of the primary series, and then at 6-month intervals. If the subject's PRNT₈₀ was $< 1:40$ after the primary series (referred to as an initial nonresponder), the subject was given a booster dose (maximum of four booster doses in a 1 year) until a PRNT₈₀ titer $\geq 1:40$ was achieved. In 2001, the protocol was amended to extend the windows of time for vaccine

doses to day 0, days 7–14, and days 28–42, and to only obtain titers at days 21–35 after vaccine doses and then annually in responders with a PRNT₈₀ titer $\geq 1:40$.

2.4. Study recruitment

From 1984 to 2000, at-risk individuals for exposure to RVF virus were recruited and vaccinated under informed consent both in the Special Immunizations Program (SIP) at USAMRIID and 59 external sites (39 domestic and 21 nondomestic sites). Beginning in May 2000, all vaccinations were performed only at USAMRIID. Study volunteers were evaluated with a baseline history and physical examination, complete blood count (CBC), serum chemistries, urinalysis, hepatitis panel, human immunodeficiency virus (HIV), enzyme-linked immunosorbent assay (ELISA), electrocardiogram (EKG), and chest X-ray. Enrollment criteria required individuals to be at-risk for exposure to RVF virus, and to be ≥ 18 years of age and in general good health. Women of childbearing potential were required to have a negative beta subunit human chorionic gonadotropin (hCG) pregnancy test. Individuals were excluded for a history of an allergy to a vaccine component (formaldehyde, neomycin sulfate, and streptomycin), a previous severe reaction to the vaccine, or evidence of immunodeficiency.

2.5. Adverse events

Adverse events were collected by passive reporting until May 29, 2000, when the study was amended to actively collect adverse events on day 1 postvaccination and then weekly through day 28 after a vaccine dose. Serious adverse events were collected for the duration of the study.

2.6. Statistical analysis

2.6.1. Immunogenicity

Serological analysis assessed the (1) percentage of initial responders to the primary series (PRNT₈₀ titer $\geq 1:40$ after dose 3 of the primary series) and (2) the number of booster doses in initial nonresponders required to achieve a PRNT₈₀ $\geq 1:40$. Persistence of immunogenicity in initial responders to the primary series was assessed by both the percentage of subjects with a PRNT₈₀ titer $\geq 1:40$ and by the geometric mean titer (GMT) at time points from 2 weeks to 11 months after dose 3 of the primary series, and by the estimated numbers of days until the PRNT₈₀ titer fell below 1:40 using a Kaplan–Meier plot. The serological response of subjects was also determined for PRNT₅₀ titers. For the most frequently used vaccine lots, immunogenicity was compared at time points from 2 weeks to 11 months after dose 3 of the primary series by logistic regression for lot, sex, age, and race.

2.6.2. Adverse events

Analysis of adverse events data was performed primarily on vaccine doses given May 29, 2000 through 2004, when adverse event data were actively collected (the absence of adverse event data for a significant number of vaccine doses given before May 29, 2000 prohibited meaningful analysis of these adverse events other than descriptive analysis). Only adverse events assessed to be definitely, probably, or possibly related to the vaccine were included in the analysis. The percentage of subjects with related adverse reactions was compared for vaccine lot, shot series (primary versus booster doses), sex, age, and race (Caucasian versus non-Caucasian) by multiple logistic regression analysis.

3. Results

3.1. Demographics

Between 1986 and 2004, 1860 subjects were enrolled in the study and received at least one dose of the RVF vaccine. While subjects were enrolled at 60 sites (39 U.S. sites; 21 sites outside the U.S.), the majority of subjects (59.4%) were enrolled at USAMRIID. Demographics of the 1860 vaccinees were predominantly male (66%) with most subjects between the ages of 20–59 years of age (Table 1).

3.2. Vaccinations

The 1860 subjects (mainly subjects working in research laboratories) received 5954 vaccine doses (4431 primary and 1523 booster doses) during the study (Table 2). Of the 1860 subjects, 929 persons received primary vaccine doses only, 584 received both primary and booster doses of the vaccine, and 347 received boosters only (subjects had received the primary series on an earlier RVF vaccine protocol). A total of 31 lot runs were used during this study (nearly all vaccinations in the last 4 years of the study were with lots runs 7-1 and 7-2).

3.3. Immunology

3.3.1. Primary series serological response (PRNT₈₀ titers)

The overall response rate to the primary series (a PRNT₈₀ titer $\geq 1:40$ at any time after receiving the primary series of three doses, but before the first booster dose) was 90% (1180 of 1314 subjects with available titers for analysis). After an initial response rate

Table 1
Demographics of 1860 subjects receiving the formalin-inactivated TSI GSD 200 RVF vaccine.

	N (%)
Sex	
Males	1229 (66%)
Females	520 (28%)
No data	111 (6%)
Race	
Caucasian	1238 (66.6%)
Black	123 (6.6%)
Asian	47 (2.5%)
Hispanic	44 (2.4%)
Indian	5 (0.3%)
Other	104 (5.6%)
No data	299 (16.1%)
Age range	
16–39	1241 (66.7%)
40–59	501 (26.9%)
60–80	38 (2.0%)
No data	80 (4.3%)
Study site	
USAMRIID	1104 (59.4%)
Off-site	748 (40.2%)
Unknown	8 (0.4%)

Table 2
Number of vaccinated subjects and number of vaccine doses administered.

Subjects	N	Vaccine doses	N
Primary doses only	929	Primary doses	4431
Booster doses only ^a	347	Booster doses ^b	1523
Both primary and booster doses	584		
Total subjects	1860	Total doses	5954

^a 47 subjects received primary series on earlier RVF vaccine study (received total of 87 of the 347 booster doses).

^b Subjects in study received a range of 0–22 booster doses.

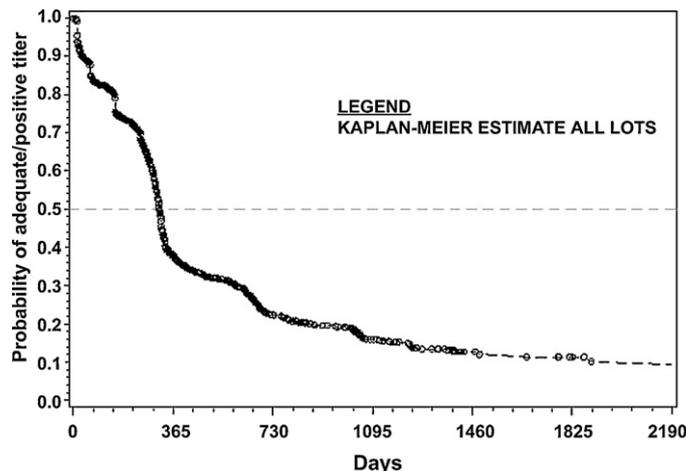


Fig. 1. Kaplan–Meier analysis of RVF loss of titer (PRNT₈₀ < 1:40 after primary dose 3 for subjects negative at baseline. Kaplan–Meier estimate for half-life of the PRNT₈₀ was 315 days (95% CI 308–321 days) from dose 3 of the primary series.

at 2 weeks of 90% (745/827), PRNT₈₀ titers in subjects steadily declined, with PRNT₈₀ $\geq 1:40$ persisting in 35% (108/311) of subjects at month 11 after dose 3 of the primary series (titers at month 11 were comprised of subjects not removed from the analysis for a booster dose due to a waning PRNT₈₀ titer). Kaplan–Meier estimates for half-life of the PRNT₈₀ (titer loss to <1:40) was 315 days from dose 3 of the primary series (Fig. 1).

3.3.1.1. Serological analysis primary series of most prevalent lots utilized. When immune response analysis was restricted to the most prevalent lots (4-2, 5-1, 6-2, 7-1, 7-2, and 9-1), differences in response rates were noted at 2 weeks due to sex and vaccine lot, and a trend in a difference was observed for age (no titers at 2 weeks performed for lot 7-2). Women at 2 weeks had a greater immune response rate than men (initial immune response observed in 93.6% (213/228) females versus 88.2% (365/415) males; $p = 0.0199$), with women also having an equal or greater immune response within the individual lot analyses. Subjects less than 40 years of age had a trend for a greater immune response than subjects 40 years of age or older (91.5% (456/498) versus 85.2% (122/145); $p = 0.0637$). A greater immune response rate was observed at week 2 after the primary series with lots 5-1, 6-2 and 7-1 (>90% subjects with a PRNT₈₀ $\geq 1:40$ compared to lots 9-1 and 4-2 (77% subjects); $p < 0.0001$). However, differences in immunogenicity by lot were not noted at later time points after month 2, possibly due to the weakest responders being removed from the analysis due to boosting for waning PRNT₈₀ titers. Analysis of geometric mean titers (GMTs) at week 2 demonstrated lots 5-1, 6-2, and 7-1 with higher mean titers than lots 4-2 and 9-1 ($p < 0.0001$). No imbalances in sex ($p = 0.4476$) or age ($p = 0.5855$) between the groups of vaccine lots were noted to explain the difference in GMTs between lots. Kaplan–Meier estimates for half-life of the PRNT₈₀ (titer loss to <1:40) for lots 5-1, 6-2, and 7-1 ranged from 314 to 348 days after dose 3 of the primary series, and was longer than observed with lot 4-2 (246 days) (Fig. 2). The smaller sample sizes for lots 7-2 and 9-1 limited comparison of the estimated half-life to the other lots (estimated half-life of 286 days and 310 days, respectively) (Fig. 2).

3.3.2. Booster dose serological response (PRNT₈₀ titers)

The median number of days from dose 3 of the primary series to the first booster in initial responders was 354 days. Of the 437 initial responders who subsequently required a booster dose due to a waning PRNT₈₀ titer <1:40, the response rate to the initial booster was 98.4% (430 of 437 subjects). The percentage of initial

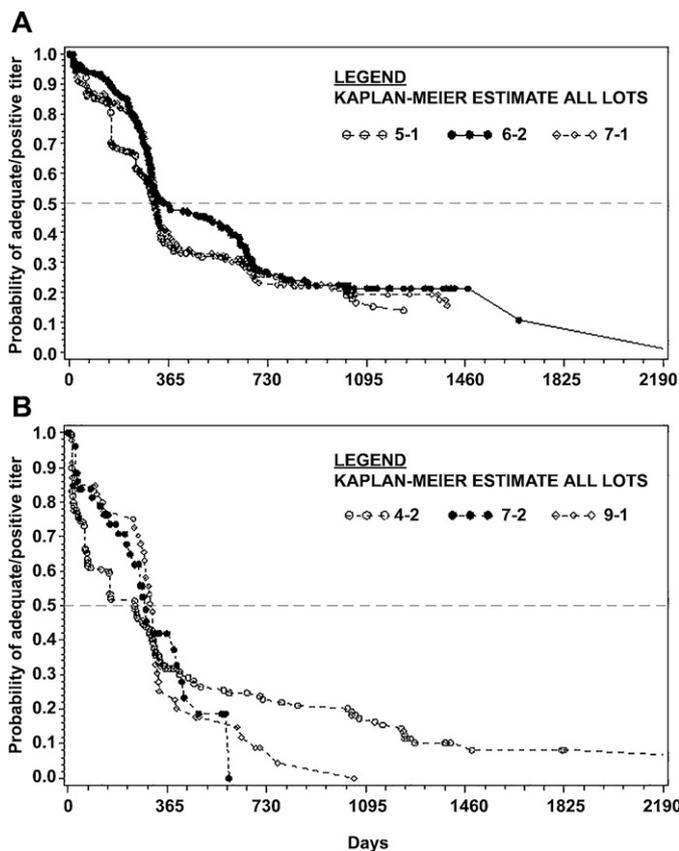


Fig. 2. Kaplan–Meier analysis of RVF loss of titer ($PRNT_{80} < 1:40$) for vaccine lots 5-1, 6-3 and 7-1 (A) and lots 4-2, 7-2 and 9-1 (B). Kaplan–Meier estimate for half-life of the $PRNT_{80}$ from dose 3 of the primary series in (A) was lot 5-1: 314 days (95% CI: 393–330 days), lot 6-2: 348 days (95% CI: 308–554), and lot 7-1: 322 days (CI: 312–335 days). Kaplan–Meier estimate for half-life of the $PRNT_{80}$ in (B) was lot 4-2: 246 days (95% CI: 154–300 days), lot 7-2: 286 days (95% CI: 229–399 days), and lot 9-1: 310 days (95% CI: 279–321 days).

nonresponders with a $PRNT_{80} \geq 1:40$ response to the first booster dose was lower (70 of 91 (76.9%) subjects) than observed in initial responders. A second booster dose was given to 14 individuals, with 8 of 14 (57%) subjects responding to the second booster. GMTs after the first booster dose were much lower for initial nonresponders compared to initial responders (GMT 143 versus GMT 763; $p < 0.0001$). Of the 567 individuals who received only booster doses in this protocol, postbooster $PRNT_{80}$ titers were $\geq 1:40$ in 533 (94%) subjects.

3.3.3. Analysis of immunological response rate to primary series comparing $PRNT_{50}$ titers and $PRNT_{80}$ titers

A regression line demonstrated correlation between the $PRNT_{80}$ and $PRNT_{50}$ titers (0.899) in the 786 subjects who had both $PRNT_{50}$ and $PRNT_{80}$ titers available at 2 weeks after completing the primary series (Fig. 3). Discordancy in baseline titers (negative by $PRNT_{80}$ but positive by $PRNT_{50}$) was observed in 13 subjects (12 of the 13 subjects resided in RVF endemic areas in Africa); the 13 subjects were not included in the $PRNT_{50}$ seroconversion analysis.

The response rate in subjects with both $PRNT_{50}$ and $PRNT_{80}$ data at 2 weeks after completion of the primary series was 99.1% (766 of 773 subjects) by $PRNT_{50}$ compared to 89.9% (706 of 786 subjects) by $PRNT_{80}$. At month 11, 72.5% of subjects had a $PRNT_{50}$ titer $\geq 1:40$ compared to only 33.7% subjects with a $PRNT_{80}$ titer $\geq 1:40$ (Table 3a). GMTs were considerably higher for $PRNT_{50}$ than $PRNT_{80}$ at 2 weeks (1320 versus 287) and at 11 months (115 versus 78) after dose 3 of the primary series, respectively (Table 3b).

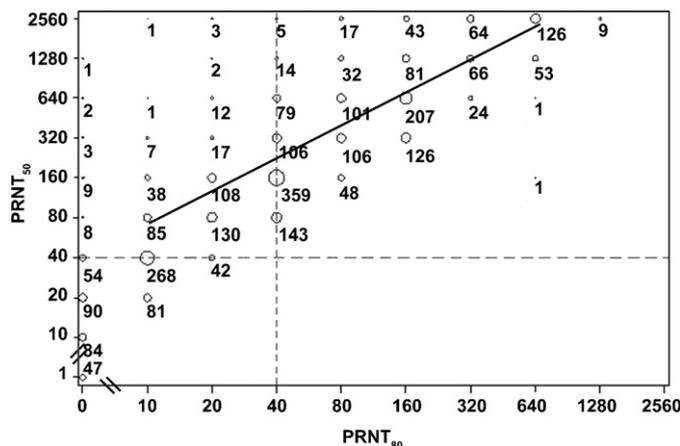


Fig. 3. Log $PRNT_{50}$ versus log $PRNT_{80}$ with weighted least squares regression line. Log $PRNT_{50} = 0.9486 + 0.8695$ Log $PRNT_{80}$; $r = 0.899$; $Rsq = 0.809$; $RMSE = 0.3518$.

Table 3a

Immune response of $PRNT_{80} \geq 1:40$ and $PRNT_{50} \geq 1:40$ after primary series dose 3 for subjects with both $PRNT_{80}$ and $PRNT_{50}$ assays and with negative baseline $PRNT_{80}$ and $PRNT_{50}$ using all vaccine lots.^a

Time post dose 3	Subjects (%) with $PRNT_{80} \geq 1:40$	Subjects (%) with $PRNT_{50} \geq 1:40$
In week 2	706/786 (89.9%)	766/773 (99.1%)
In week 4	115/129 (89.1%)	122/128 (95.3%)
Month 2	150/224 (67.0%)	200/209 (95.7%)
Month 5	96/215 (44.7%)	176/199 (88.4%)
Month 8	88/221 (39.8%)	166/205 (81.0%)
Month 11	101/300 (33.7%)	211/291 (72.5%)

^a Subjects who subsequently had a $PRNT_{80} < 1:40$ were removed from subsequent analysis after week 2 due to boosting.

Table 3b

Geometric mean titer (GMT) after primary series dose 3 for subjects with both $PRNT_{80}$ and $PRNT_{50}$ assays and with negative baseline $PRNT_{80}$ and $PRNT_{50}$ using all vaccine lots.^a

Time post dose 3	GMT $PRNT_{80}$	N^b	CV (%) ^c	GMT $PRNT_{50}$	N	CV (%) ^c
In week 2	287	706	23	1320	766	19
In week 4	180	115	23	745	122	20
Month 2	125	150	28	247	200	25
Month 5	101	96	29	126	176	25
Month 8	101	88	30	135	166	27
Month 11	78	101	26	115	211	24

^a Subjects who subsequently had a $PRNT_{80} < 1:40$ were removed from subsequent analysis after week 2 due to boosting.

^b N , number of titers.

^c Coefficient of variation on the log scale.

3.4. Safety analysis

Vaccine-related adverse events were reported (passive surveillance) with 4% (241/5954) of vaccine doses given from 1986 to 2004 (Table 4). As adverse event data were absent for 83% of injections given before May 29, 2000 (mainly from external sites), only descriptive analysis of vaccine-related adverse events by body systems is presented for vaccine doses with safety data available given before May 29, 2000 (Table 5). None of the 27 serious adverse events (including one death) documented over the 19 years of the study were determined to be related to the RVF vaccine, and were not included in the analysis of adverse events. Five subjects reported 11 severe reactions events during the study. All severe reactions resolved; four subjects with severe reactions were reassessed as not vaccine-related reactions and received subsequent vaccine doses with no or only mild adverse reactions.

Table 4
Vaccine-related adverse events (AEs) associated with vaccine doses.^a

	Total doses (%)	Number (%) vaccine doses	
		Before May 29, 2000 ^b	After May 29, 2000 ^b
Vaccine related AE	241 (4%)	171 (3%)	70 (11.5%)
Vaccine unrelated AE	117 (2%)	103 (2%)	14 (2%)
No AE	1133 (19%)	646 (12%)	487 (81%)
No data	4463 (75%)	4430 (83%)	33 (5.5%)
Total vaccine doses	5954 (100%)	5350 (90% total)	604 (10% total)

^a 27 serious adverse events reported, including one death, were determined to not be vaccine-related and are not included in the table.

^b May 29, 2000 and later was a period of increased compliance in collection of adverse event data that is more representative of safety data, with all vaccines administered at USAMRIID and active collection of adverse events.

Table 5
Vaccine injections associated with vaccine-related adverse events by body system for primary and booster doses with available data for adverse event analysis before May 29, 2000 and May 29, 2000 and later.

Shot series	Adverse event	Pre May 29, 2000 N (%) 718 injections	May 29, 2000 and later N (%) 407 injections
Primary	No adverse event	573 (79.8%)	355 (87.2%)
	Local adverse events	104 (14.5%)	31 (7.6%)
	Systemic	50 (7.0%)	21 (5.2%)
	Musculoskeletal	13 (1.8%)	8 (2.0%)
	Nervous system	4 (0.6%)	0 (0%)
	Ears, nose, throat	7 (1.0%)	9 (2.2%)
	Lymphatics	1 (0.1%)	1 (0.2%)
	Respiratory	3 (0.4%)	4 (1.0%)
	Gastrointestinal	11 (1.5%)	6 (1.5%)
Shot series	Adverse event	Pre May 29, 2000 N (%) 99 injections	May 29, 2000 and later N (%) 150 injections
Booster	No adverse event	73 (73.7%)	132 (88.0%)
	Local adverse events	21 (21.2%)	15 (10.0%)
	Systemic	8 (8.1%)	5 (3.3%)
	Musculoskeletal	5 (5.1%)	3 (2.0%)
	Nervous system	1 (1.0%)	1 (0.7%)
	Ears, nose, throat	0	2 (1.3%)
	Lymphatics	0	1 (0.7%)

3.4.1. Adverse events analysis (May 29, 2000 and later)

In the period of active surveillance for collecting adverse event data (May 29, 2000 and later), vaccine-related adverse events were associated with 11.5% (70/604) of vaccine doses administered to 243 subjects (Table 4). Of these 604 doses, safety data were available on 557 vaccine doses (407 primary and 150 booster injections) administered to 226 subjects. A total of 487 of 557 doses (72%) were not associated with a vaccine-related adverse event. Seventy (11.5%) of the 557 vaccine doses were associated with 186 vaccine-related adverse events and were reported by 57 subjects (25.2%). Most vaccine-related adverse events generally occurred within the first 3 days after vaccination and were self-limiting (majority resolving within 7 days). The 186 vaccine-related adverse events were judged as 46 mild (6.8%), 19 moderate (2.8%), six severe (0.9%), and 115 not determined (17.1%) reactions. The relationship of the vaccine-related adverse events was assessed as 97 (14.4%) definitely related, 7 (1%) probably related, 82 (12.2%) possibly related, and 115 (17.1%) unknown.

For the 557 injections with adverse event data available, differences in rates of adverse events were observed using multiple logistic regression for gender, age group (<30 years and ≥30 years), and vaccine lot, but not for shot series (primary versus booster dose) [$p = 0.8376$]. Adverse events were noted on Chi square analysis to occur more commonly in females (22.5%) compared to males (7.6%) [$p < 0.0001$] (Table 6), in individuals ≥30 years old (14.2%) com-

Table 6
Vaccine-related adverse events in 57 of 226 subjects and 557 analyzed doses (407 primary and 150 boosters) in study period May 29, 2000 or later.

Injections	Subjects with related AEs	Doses with related AEs
All		
Females	32/73 (43.8%)	42/187 (22.5%)
Males	25/153 (16.3%)	28/370 (7.6%)
Total	57/226 (25.2%)	70/557 (12.5%)
Primary series		
Primary 1	14/135 (10.5%)	14/135 (10.5%)
Primary 2	21/136 (15.6%)	21/136 (15.6%)
Primary 3	17/136 (12.7%)	17/136 (12.7%)
Females	23/52 (44%)	32/147 (21.8%)
Males	18/95 (19%)	20/260 (7.7%)
Total	41/147 (28%)	52/407 (13%)
Booster series		
Females	10/37 (27%)	10/40 (25.0%)
Males	8/93 (9%)	8/110 (7.3%)
Total	18/130 (14%)	18/150 (12%)

pared to <30 years (10.3%) [$p = 0.0039$], and with lot 6-2 (20.2%) compared to lot 7-1 (7.8%) and lot 7-2 (7.9%) [$p = 0.0004$]. Fisher exact test did not support the differences in adverse events between the lots due to imbalances of gender ($p = 0.2155$) or age ($p = 0.3184$).

3.4.1.1. Adverse events related to primary series and booster doses. A total of 41 of 147 subjects (28%) reported vaccine-related adverse events (133 recorded vaccine-related reactions) that were associated with 13% (52 of 407) of primary injections (Table 6). The percentage of subjects reporting a vaccine-related reaction was similar for each primary dose evaluation. Vaccine-related adverse events were mainly local reactions at the vaccine site (i.e., primarily erythema, pruritis, and tenderness at site) and general body reactions (i.e., headache, fatigue, malaise, and mild fever). No difference in vaccine-related reactions for age ($p = 0.1867$) or race ($p = 0.5301$) was detected. Only gender was statistically different by multiple logistic regression analysis (44% (23/52) females and 19% (18/95) males; $p = 0.0011$). Although sample size prohibited statistical comparisons, tables suggest the differences of adverse events between females and males were due to local reactions (primarily tenderness, erythema, and pruritis), comprising 48.2% female versus 16% male of the total vaccine-related reactions, respectively.

Vaccine-related adverse events were reported by 14% (18/130) of subjects who received a booster dose and associated with 12% (18/150) of booster dose injections (Table 6). The most common adverse events were local reactions at the vaccine site and general body adverse reactions. As in the primary series, sex was the only distinguishing factor by multiple logistic regression comparing percentages of subjects with one or more related reactions to a booster dose injection (27% females and 9% males; $p = 0.0027$) (Table 6). A trend for increased adverse events with age ≥40 years was detected ($p = 0.056$), but not for race ($p = 0.1879$).

4. Discussion

RVF virus had remained endemic to sub-Saharan Africa, until an RVF epidemic occurred in Egypt in 1977 [1–4] and the virus subsequently emerged in the Mideast [4,5]. Introduction of RVF virus to areas of the world where the *Aedes* mosquito vector resides could have a significant agricultural and public health impact due to epizootics and endemnicity of the virus (even more so than observed with West Nile fever virus). While control of RVF virus during outbreaks may be achieved with vaccination of livestock, sustaining vaccination programs in animals between outbreaks has proved difficult.

There is no licensed RVF vaccine for humans. In 1978, Swedish civilian and military authorities recommended vaccination of

Swedish United Nations soldiers deployed to the Sinai Peninsula with an investigational RVF vaccine (NDBR 103 RVF vaccine), based on serological studies in soldiers suggesting the presence of RVF virus in the peninsula [13]. Many at-risk laboratory and field workers to RVF virus have also been vaccinated with the NDBR 103 or TSI-GSD 200 RVF vaccines as an adjunct to personal protective equipment and environmental controls when working with the virus. Safety analysis of the TSI-GSD 200 RVF vaccine reported the vaccine to be well-tolerated. While an increase in adverse events was associated with female gender, adverse events were generally mild and self-limited in both sexes.

The inactivated TSI-GSD 200 RVF vaccine demonstrated immunogenicity after a three-dose primary series, using a $\text{PRNT}_{80} \geq 1:40$ criterion (>90% subjects). The PRNT_{80} value of 1:40 is considered a conservative measure for immunity, as protection has been demonstrated in animal studies with lower PRNT_{80} values of 1:10 and 1:20 [21–25]. The PRNT_{50} in recent years has replaced the PRNT_{80} as a standard in vaccine development. Studies of new RVF vaccine candidates have reported protection in animals with a PRNT_{50} of 1:40 or lower [26–28]. Using this less restrictive $\text{PRNT}_{50} \geq 1:40$ criteria as an acceptable vaccine immune response, nearly all subjects (99%) receiving the TSI-GSD 200 RVF vaccine in this study developed a serological response after the primary series.

Most initial responders to the TSI-GSD 200 vaccine required an initial booster dose (due to a PRNT_{80} decline to below 1:40) within 6–12 months after completing the primary series. Pittman et al. reported a prolonged immune response (mean estimated half-life of 8 years) after the initial booster dose in initial responders, using the more stringent PRNT_{80} criteria for immunogenicity [19]. In 2004, because of the PRNT_{80} decline at 6–12 months after the primary series in most initial responders, the dosing regimen was modified to require a 6-month booster dose for initial responders. An interim analysis of the study revealed long-term persistence of PRNT_{80} titers $\geq 1:40$ after the 6-month booster dose in most initial responders (follow-up as long as 4 years after the 6-month booster) [unpublished data, USAMRIID].

As the PRNT_{50} (in lieu of PRNT_{80}) has become a standard for assessing immunogenicity in vaccine development, PRNT_{50} analysis from this study may be useful for clinical trials of future RVF vaccine candidates [29–31]. PRNT_{50} results in this study were generally fourfold dilutions higher than the respective PRNT_{80} assay results (i.e., a 1:40 PRNT_{80} correlated approximately to a 1:160 PRNT_{50}) (Fig. 3). The discordancy in PRNT titers for RVF (positive PRNT_{50} but negative PRNT_{80}) observed at baseline in 13 of the 786 subjects may represent low antibody titers from unrecognized remote RVF infection (antibody reported to persist for decades after infection) as all but one of these 13 subjects resided in an RVF endemic area in Africa or possibly cross-reaction to other Bunyaviruses (i.e., phleboviruses) that has uncommonly been reported [32–36]. Also, while the regression line demonstrated correlation between the PRNT_{80} and PRNT_{50} titers (Fig. 3), a greater than two standard deviation increase in the difference of the PRNT_{50} compared to the PRNT_{80} titer was intermittently observed at a small number of time points (3%). The occurrence did not appear to be random as it was (1) disproportionately observed at two external sites (one research laboratory in the U.S. and the other in an RVF endemic area in Africa) and (2) most commonly after dose 3 of the primary series (82% of occurrences). It is unclear if this uncommon occurrence of greater variation between the PRNT_{50} and PRNT_{80} (observed equally with PRNT_{80} values $<1:40$ and $\geq 1:40$) represented a real immunological reaction specific to the RVF vaccine, a higher than expected PRNT_{50} titer due to priming from prior exposure to other Bunyaviridae (i.e., phleboviruses), or artifacts of the assay.

Variability in immunogenicity with lot and gender (trend noted for increase immune response for age <40 years) was observed with

the TSI-GSD RVF vaccine, and should be assessed with future vaccine candidates. Inter-lot variability in immunogenicity was also observed in the predecessor NDBR-103 RVF vaccine and attributed to the lack of an optimal preclinical test that would adequately predict immune response in humans and to the need to better standardize vaccine production [11,21]. Specific issues potentially related to inter-lot variability of the TSI-GSD 200 RVF vaccine were problems with the Reed-Muench statistical method for estimating the ED_{50} and LD_{50} , the number of animals or cell culture replicates used in assays, and/or the broad spaces in dilution series chosen for tests [18]. The female gender-associated increase in immune response (94% female versus 88% males) associated with this vaccine has also been observed with other vaccines (i.e., anthrax vaccine, hepatitis A and B vaccines, measles vaccine, influenza vaccines) [13,37–42]. The increased humoral immune response in females to vaccines has been postulated to be due to functional differences in CD4+ T cells and TH₂ responses, but the exact mechanism is unknown. While gonadal hormones may play a role, the occurrence in pre-pubertal subjects suggests other factors are also involved [41].

A criticism of the TSI-GSD 200 vaccine has been the need for a multiple three-dose primary series. “Early” immunogenicity was a factor in determining the primary series dosing regimen for many of the earlier biodefense vaccines, and was generally achieved with a three-dose primary series administered over 4 weeks (i.e., day 0, days 7–14, and day 28). Experience with other biodefense vaccines (particularly the recent dose change for the anthrax vaccine) suggests a two-dose primary series (i.e., days 0 and 28) may result in a decrease in immunogenicity before but not after the subsequent third vaccine dose (generally given between months 6–12) [43–46]. Based on experience with other viral recombinant vaccines, a recombinant RVF vaccine may likely require two to three vaccine doses in the initial 12 months (i.e., day 0 or days 0 and 28, followed by a third dose given at a time point between 6 and 12 months) to achieve a sustained immune response. Several RVF recombinant protein vaccine candidates have been developed in recent years; animal studies with these new RVF vaccine candidates may better define the protective PRNT_{50} for RVF virus by the various routes of exposure and if more stringent protective criteria should be used for higher risk populations (i.e., at-risk laboratory workers) [26–28,47–55]. A main advantage of a recombinant RVF vaccine (and also a live, attenuated RVF vaccine) over a formalin-inactivated vaccine is facilitation in vaccine production, as a high-level biocontainment facility would not be required.

Live, attenuated RVF vaccines may have an advantage of requiring only one dose to achieve long-lasting immunity, but potential disadvantages are (1) reversion (i.e., Smithburn strain used in South Africa is associated with reversion and cannot be used in countries where RVF virus has not yet been introduced) and (2) teratogenicity or abortion [56]. However, the investigational live, attenuated MP-12 RVF vaccine has not been associated with reversion. While teratogenicity was reported in one study with sheep that received MP-12 in the first trimester of pregnancy, other studies in ewes and in cattle showed no evidence of teratogenicity or abortogenicity from the MP-12 attenuated viral strain [56–60]. Also, increases in abortions or RVF seropositivity of women with abortions during RVF outbreaks have not been observed in humans [61,62]. A recent MP-12 vaccine clinical trial in 19 subjects demonstrated safety and immunogenicity, but no future studies with the MP-12 vaccine are currently planned.

5. Conclusion

The inactivated TSI-GSD 200 RVF vaccine has demonstrated both safety and immunogenicity, with 90% of subjects develop-

ing a PRNT₈₀ ≥ 1:40 after a three-dose primary series and most (77%) initial nonresponders to the primary series developing a PRNT₈₀ ≥ 1:40 after only one booster dose. The vaccine was well-tolerated; adverse events were generally mild and self-limiting. The TSI-GSD 200 RVF vaccine continues to be administered to at-risk individuals, as there is currently no FDA-approved RVF vaccine. The safety and immunogenicity data of the TSI-GSD 200 RVF vaccine, particularly the PRNT₅₀ analysis, may be useful in providing insight to the design of clinical trials for future recombinant vaccine candidates.

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