

Efficacy of a Rift Valley fever virus vaccine against an aerosol infection in rats

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The formalin-inactivated Rift Valley fever virus (RVFV) vaccine, TSI-GSD-200, was administered subcutaneously to highly susceptible adult Wistar-Furth rats (LD₅₀-1 p.f.u., ZH501 strain). Vaccine was administered on days 0, 7 and 28, the same time course used for at-risk personnel. Six months postimmunization, when the serum plaque-reduction neutralization titre (PRNT)₈₀ had declined to low or undetectable levels, rats were challenged with 4.4 log₁₀ p.f.u. of the virulent ZH501 strain in a nose-only dynamic aerosol apparatus. Ninety-seven per cent (33/34) of the non-vaccinated control rats died. In contrast, only 32% (33/105) of the vaccinated animals died. In vaccinated rats that succumbed, there was a doubling of the mean time to death and the cause of death shifted from hepatitis to encephalitis. Rats with a PRNT₈₀ of $\geq 1:40$ were protected from clinical disease and histological evidence of hepatic or encephalitic lesions. While the precise mechanisms of immunity against aerosol challenge remain unresolved, here the serum PRNT titre correlated with protection.

Keywords: Rift Valley fever virus; aerosol; vaccine; rat; *Phlebovirus*

INTRODUCTION

Rift Valley fever virus (RVFV) is a member of the *Phlebovirus* genus, Bunyaviridae family. The virus is endemic to sub-Saharan Africa and has caused several epizootics since its isolation in the 1930s¹. Persons working with cattle and sheep, which act as amplifiers, are at the greatest natural risk of acquiring the disease. This virus is usually transmitted by arthropods. Experience in the laboratory and in the field suggest that the virus is also infectious through aerosol exposure²⁻⁶. Infection causes several disease manifestations in humans, including acute self-limiting febrile disease, hepatitis, encephalitis, haemorrhagic fever, and ocular sequelae¹.

Inactivated Rift Valley fever (RVF) virus vaccines have been used to protect at-risk laboratory and field workers from RVF since the late sixties^{7,8}. No clinical cases have been reported in the vaccinees; however, a few subclinical infections have been detected by an active screening programme to monitor serum neutralizing antibody titres⁷. The current immunization schedule consists of three doses given subcutaneously (s.c.) on days 0, 7 and 28 with a serum sample taken on day 42 to monitor

antibody response. An 80% plaque-reduction neutralization titre (PRNT₈₀) of $\geq 1:40$ is currently recommended for at-risk personnel.

Several studies in laboratory animals have shown serum neutralizing antibody to be efficacious against a peripheral RVFV challenge⁹⁻¹². Further, passive immune therapy, which protects against fatal hepatitis, can be insufficient to protect against encephalitis^{9,10,12}.

A recent report¹³ has raised concern about the ability of formalin-inactivated RVF vaccines to protect against aerosol exposure. The vaccine was efficacious in protecting mice against a parenteral, but not an aerosol exposure. However, the immunization schedule and dosage were different from that received by at-risk personnel. A similar finding has been shown previously for the formaldehyde-inactivated Venezuelan equine encephalitis virus vaccine¹⁴. Although current practices minimize the exposure of laboratory workers to aerosol infection, it is important to be able to predict the efficacy of the RVFV vaccine against an aerosol exposure.

Inbred rats have been used as a laboratory model for RVF because they mimic the disease patterns seen in humans¹⁵. Wistar-Furth rats were chosen for this study because they appear to develop many of the clinical manifestations found in humans, are highly sensitive to challenge, and neutralizing antibody has been shown to be protective against a peripheral RVFV challenge⁹. In this study, the serum PRNT was compared with protection after aerosol exposure and differences in pathogenesis between vaccinated and non-vaccinated controls were examined.

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MATERIALS AND METHODS

Animals and immunization

Inbred male Wistar-Furth rats (*Rattus norvegicus*) weighing ≈ 200 g were obtained from Harlan Sprague Dawley, Inc., Indianapolis, IN. Water and rodent chow were provided *ad libitum*. The animal room was maintained at 22–23°C and 40–50% humidity with a 12 h photo period.

Briefly, the rats were vaccinated on days 0, 7 and 28 with either 0.3 or 0.6 ml s.c. of lot 10 (run 2) or 15 (run 1) of the formalin-inactivated RVFV vaccine (TSI-GSD-200) manufactured at The Salk Institute, Government Services Division, Swiftwater, PA (Table 1). The vaccine was made from the Entebbe strain of RVFV. Each rat was bled on days 42 and 177 for serum PRNT_{50 and 80}. Vaccinated ($n=105$) and non-vaccinated control ($n=34$) rats were challenged by aerosol exposure 187 days postimmunization. Animals were observed twice daily for clinical signs of disease over a 28-day observation period. An ophthalmic examination with an indirect ophthalmoscope was conducted approximately every other day, starting 6 days postchallenge.

Virus and virus assay

The origin, propagation and assay of the ZH501 strain of RVFV have been previously described⁹. Brain, liver, spleen and serum samples were obtained from moribund, dead, and rats surviving the observation period and assayed for infectious virus. All procedures utilizing infectious virus were carried out by immunized personnel conforming to the guidelines of the Centers for Disease Control, National Institutes of Health¹⁶, and the Subcommittee on Arbovirus Laboratory Safety of the American Committee on Arthropod-borne Viruses¹⁷.

Aerosol exposure

Rats were infected in a nose-only exposure chamber with a dynamic aerosol containing infectious RVFV. The aerosol was generated with a Collision atomizer¹⁸, mixed in a Henderson-type aerosol mixing tube¹⁹, which was attached to a plexiglass chamber with 13 ports suitable for exposure of adult rats. The virus dose was $4.3 \log_{10}$ p.f.u. The concentration of virus in the exposure chamber was determined by collecting a 5-min sample at the midpoint of the trial with an all-glass impinger (AGI)²⁰. The AGI contained 20 ml Eagle's minimum essential medium containing 10% fetal bovine serum (FBS), gentamicin, and Antifoam Y-30 (Dow-Corning, Midland, MI) as the collection fluid. Infectious virus in the

collection fluid was quantified by plaque assay on Vero cells⁹. The inhaled viral dose was then calculated by the formula of Guyton²¹. The exposure apparatus was housed in a class III biological safety cabinet. The temperature was 27°C with a 43–48% relative humidity in the aerosol line.

Groups of eight female rats were exposed to decimal dilutions of the ZH501 strain of RVFV by both the nose-only and whole-body²² method of exposure to determine LD₅₀. Rats were 6 months old at the time of challenge.

Pathology

Necropsies were performed on all vaccinated and non-vaccinated rats in the study when found dead. Moribund animals which would be expected to die within hours were killed humanely in CO₂ atmosphere, and necropsied. Animals surviving the infection were killed on days 27–28 postchallenge for histopathology. The liver, brain, lungs and eyes were fixed by immersion in 10% neutral-buffered formalin and stained with haematoxylin and eosin.

Serum PRNT

These assays were carried out with RVFV (ZH501 strain) as previously described⁹. Briefly, dilutions of serum were mixed with 50–100 p.f.u. of the ZH501 strain of RVFV and residual infectivity measured by plaque assay on Vero cells. The values are expressed as the highest serum dilution required in the absence of complement to reduce the plaque number by 50 or 80%.

Statistical methods

Analysis of the survival data was divided into two parts, each utilizing a stepwise logistic regression computer program²³. To enter a model, an independent variable had to produce an improvement in the goodness of fit to the model at the $p \leq 0.1$ level. The first model was constructed to determine the order of entry of each of the PRNT_{50 and 80} versus survival of each of the animals at day 42 or 177 postimmunization. In the second model, the PRNT_{50 and 80} and the lot/dose group were ordered by the same technique. The LD₅₀ values for the groups exposed by the nose-only or whole-body method were estimated by linear regression after applying the arcsine transformation²⁴ to the proportion of animals surviving.

RESULTS

Efficacy

The mortality curves for the different combinations are presented in Figure 1. Survival ranged from 59–77% among the four groups of rats receiving different lot/dose combinations (Table 1). Overall survival 6 months postimmunization was 69% (72/105) when the results of all four lot/dose groups were combined. Serum PRNT₈₀ ranged from $\leq 1:10$ –1:80. There was little difference in mean time to death (MTD) among the vaccinated groups, although they survived approximately twice as long as non-vaccinated infected controls (2.9 ± 3.3 days).

Table 1 Response of rats to four different dose/lot combinations of formalin-inactivated Rift Valley fever virus vaccine (TSI-GSD-200) administered subcutaneously

	Lot 10		Lot 15		Control
	0.3 ml	0.6 ml	0.3 ml	0.6 ml	
Survival rate	17/29	16/24	22/30	17/22	1/34
Time to death (days)					
Mean \pm s.d.	6.3 \pm 4.2	7.9 \pm 7.3	7.5 \pm 6.0	6.6 \pm 3.4	2.9 \pm 3.3
Range	3–6	3–25	3–18	3–11	3–7
Run no.	2a	2b	1a	1b	Control

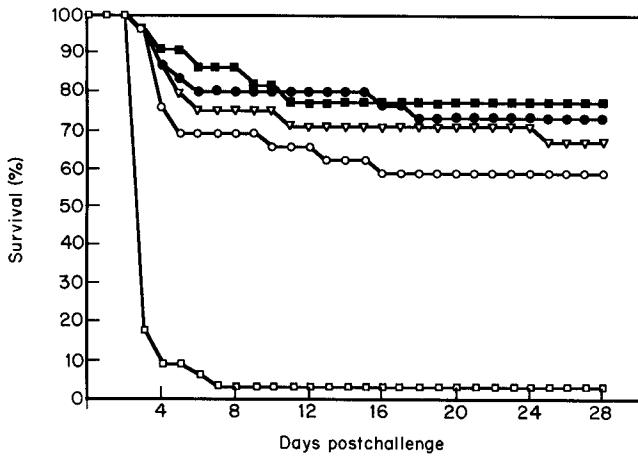


Figure 1 Survival of immunized Wistar-Furth rats to a nose-only aerosol challenge (\log_{10} 4.4 p.f.u.) of RVFV (ZH501) strain. Male rats (200 g) were vaccinated s.c. with a formalin-inactivated RVFV vaccine on days 0, 7 and 28 and were challenged 187 days postimmunization. ●, Run 1a; ■, run 1b; ○, run 2a; ▽, run 2b; □, control (see Table 1 for details)

Correlation of PRNT and lot/dose with survival

Survival of 105 vaccinated-challenged rats versus their \log_{10} PRNT_{50 and 80} at days 42 and 177 postimmunization is depicted in Figure 2a-d. When these values were modelled by stepwise logistic analysis, the day 42 PRNT₅₀ showed the best correlation to the model, $p < 0.001$. This was followed by the day 177 PRNT₅₀, $p \leq 0.038$; and finally, the day 42 PRNT₈₀, $p \leq 0.094$. The day 177 PRNT₈₀ did not enter the model as the probability of any improvement of the goodness of fit was $p > 0.015$. Overall, the data for the day 42 PRNT₅₀ showed a significant degree of non-fit, $p \leq 0.03$. This non-fit can be explained by the few entries and variability at the 1:20 and 1:5120 titres.

If vaccine lot/dose groups were added to the model, the day 42 PRNT₅₀ still entered the model first, $p < 0.001$. This was followed by the lot/dose group, $p \leq 0.016$, and day 180 PRNT₅₀, $p \leq 0.033$. The day 42 PRNT₈₀ and the day 180 PRNT₈₀ did not enter the model, as the probability of any improvement of the goodness of fit was $p > 0.15$.

Currently a PRNT₈₀ of $\geq 1:40$ is recommended for at-risk personnel who work with RVFV²⁵. There was a significant difference (Fisher exact test, $p \leq 0.046$) in survival 6 months postimmunization between all immunized animals who had a PRNT₈₀ of $\geq 1:40$ (9/9, 100% survival) and those with a PRNT₈₀ $\leq 1:20$ (63/96, 66% survival) prior to aerosol exposure.

Histopathology

The frequency of histological lesions is presented in Figure 3. Animals dying early (<7 days postchallenge) had severe necrotizing hepatitis, while late deaths were due to encephalitis, with evidence of perivascular cuffing in a few cases. Encephalitis was detected in addition to hepatitis in a few of the animals dying early. This correlated with the virus burden detected in these tissues. Rats dying with histological signs of hepatitis or encephalitis generally had virus titres of $\approx 7-9 \log_{10}$ and $5-7 \log_{10}$ p.f.u. g⁻¹ tissue, respectively. Eleven out of 72 vaccinated-challenged animals that survived the observation period without signs of clinical disease had

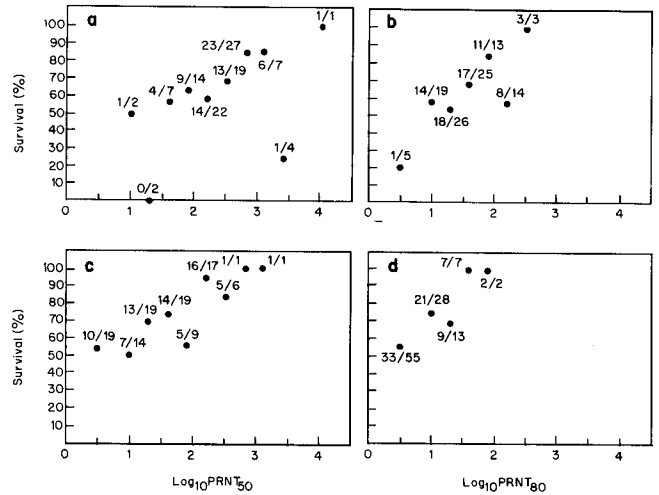


Figure 2 Survival versus serum PRNT of Wistar-Furth rats exposed to a nose-only aerosol of RVFV (ZH501 strain, \log_{10} 4.4 p.f.u.). Male rats (200 g) were vaccinated s.c. with a formalin-inactivated RVFV vaccine on days 0, 7 and 28 and were challenged 187 days postimmunization. Data from the four lot/dose groups were combined for this analysis. Serum PRNT assays were carried out on (a, b) day 42 and (c, d) day 177 postimmunization

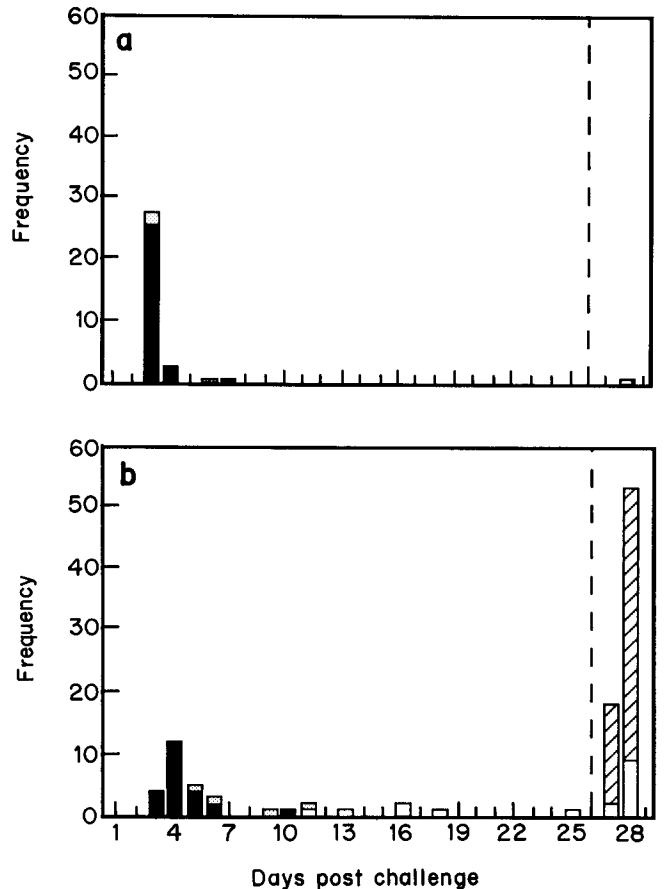


Figure 3 Frequency of histological lesions of (a), non-vaccinated and (b), vaccinated Wistar-Furth rats exposed to a nose-only aerosol (\log_{10} 4.4 p.f.u.). Necropsies taken prior to day 26 postchallenge (left of the dashed line) were from rats found dead, or found moribund and killed humanely before necropsy. Necropsies taken after day 26 were from rats that showed no clinical signs of disease before they were killed humanely. Male rats (200 g) were vaccinated s.c. with a formalin-inactivated RVFV vaccine on days 0, 7, 28 and were challenged 187 days postimmunization. Data from the four lot/dose groups were combined for this analysis. Lesions: ■, hepatitis; □, encephalitis; ▨, hepatitis and encephalitis; ▩, none

evidence of subclinical encephalitis. These 11 rats had a PRNT₈₀ of $\leq 1:10$ 10 days before challenge.

Uveitis was noted in three of 82 rats by clinical ophthalmologic examination, but was not confirmed by histopathology in these vaccinated-infected rats. Lesions that appeared during the second week postchallenge resolved clinically by the time of necropsy.

Nose-only versus whole-body LD₅₀ determinations

Survival of female Wistar-Furth rats to decimal dilutions of RVFV by an aerosol route is shown in *Table 1*. The LD₅₀ (1 p.f.u.) was the same by either method of exposure, with little difference in the MTD.

Recovery of infectious virus from the spleen

Five out of 72 vaccinated-challenged rats had detectable infectious virus in their spleens on day 28 post aerosol exposure. Despite high levels of neutralizing antibody (PRNT₈₀ $\geq 1:10$, 240), virus was detected in the standard plaque assay on Vero cells. Virus titres ranged from log₁₀ 2.3 to 3.9 p.f.u. g⁻¹ wet tissue. These animals appeared clinically normal at the time of necropsy.

DISCUSSION

In this study, the pathogenesis of RVF in non-vaccinated rats exposed to a RVFV aerosol appeared similar to that reported by parenteral inoculation in Wistar-Furth rats⁹ or in natural human infections in Africa¹. The cause of death in the non-vaccinated rats was an early necrotizing hepatitis. The pathogenesis in the aerosol-challenged immunized rats was altered. In addition to early fatal hepatitis, there were late deaths attributable to necrotizing encephalitis with non-suppurative meningitis and some perivascular cuffing. The altered pathogenesis appears similar to parenteral infection of non-vaccinated rats passively treated with RVF immune serum⁹.

Kasahara and Koyama²⁶ showed that it was possible to recover infectious RVFV from the spleens of infected mice 62 days postinfection after parenteral inoculation, or day 42 in mice passively immunized prior to infection. In this study, 7% of the surviving rats had detectable infectious virus in their spleens 28 days post aerosol exposure, despite serum PRNT₈₀ of $\geq 1:10$, 240. This again shows similarities between aerosol and parenteral routes of exposure.

While serum neutralizing antibody titres have been presumed to indicate protection against parenteral exposure to RVFV, the relevance of serum antibodies in protection against an aerosol challenge has been unclear. This study is the first to model survival of a RVFV aerosol exposure with serum PRNT_{50 or 80}. The day 42 PRNT₅₀ provides the best fit of the serum neutralizing antibody response to survival. While the day 42 PRNT₅₀ appears to be a more robust test for protection, the PRNT₈₀ $\geq 1:40$ currently suggested for at-risk personnel seems reasonable, especially since no clinical disease or histological evidence of encephalitis were detected in rats with this titre. The PRNT titre provides a method of estimating efficacy, but we do not imply that serum neutralization antibody is the only possible protective mechanism from this study.

Two different lots of RVFV vaccine were used to examine lot variability. When lot/dose was entered as a categorical variable in the model, the early PRNT₅₀

was still most predictive, followed by lot/dose, then late PRNT₅₀. This suggests that there are significant differences between the two vaccine lots.

The LD₅₀ of the ZH501 strain of RVFV was determined to be 1 p.f.u. by nose-only or whole-body aerosol exposure, which approximates that of s.c. inoculation²⁷. The two aerosol exposure systems can yield different results²², but were shown to be comparable in this case. While the dose, 4×10^4 p.f.u., of virus used in the study may seem excessive, if the Wistar-Furth rat reflects the susceptible phenotype in humans, then this dose could reflect laboratory accidents or field exposures.

Histologically, there were no significant ocular lesions, even though there were clinical findings in 4% of the vaccinated-challenged rats that were examined. However, the rats were necropsied at a time when the clinical findings had resolved. To our knowledge, this is the first report suggestive of RVF ocular sequelae in a laboratory animal. Currently, a study is under way to address the susceptibility of inbred rats to RVFV-induced ocular disease.

The reason for the high degree of vaccine efficacy in this study compared with the lack of protection in a recent study in mice¹³ is unclear. However, these studies differed in many aspects: species, immunization schedule, and time of challenge after immunization. These variables are under current investigation. The importance of this study is that the immunization schedule used was modelled on that for at-risk laboratory personnel.

ACKNOWLEDGEMENTS

The authors are grateful for the guidance of Dr Louise Pitt and the technical assistance of Randy Turner, Rick High and Bill Geisbert in performing the aerosol exposures. In conducting the research described in this report, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals' as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission on Life Sciences-National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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