SAFETY ASSESSMENT OF RIFT VALLEY FEVER VACCINE CANDIDATE 40FP8 REASSORTANTS RESCUED BY REVERSE GENETICS

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INTRODUCTION AND AIM

The Rift Valley Fever Virus (RVFV) is a mosquito-borne zoonotic phlebovirus that affects livestock and humans, causing significant outbreaks with major economic and health repercussions. Currently, there is no licensed vaccine. We developed the attenuated 40Fp8 strain through random mutagenesis of the virulent South African 56/74 strain. This variant showed a hyper-attenuated phenotype and an optimal safety profile in both mice and pregnant sheep, making it a promising candidate for a live attenuated vaccine. In this study, we used reverse genetics to generate recombinant viruses carrying individual segments of the 40Fp8 genome, combined with 56/74 segments, to determine their contribution to attenuation. The aims of this work are to identify which segments of 40Fp8 are responsible for attenuation, evaluate phenotypic changes in vitro, and assess protective capacity and virulence in vivo.

GENERATION OF RECOMBINANT VIRUSES

Recombinant RVFV was generated using a reverse genetics system based on plasmids encoding the complete cDNA of the viral segments from the virulent 56/74 strain and the attenuated 40Fp8 strain. BSR-T7 cells were transfected with combinations of plasmids containing two segments from 56/74 strain and one from 40Fp8 strain. After several passages in Vero E6 cells, we successfully rescued four recombinant viruses (L 40Fp8, M1 40Fp8, M2 40Fp8, S 40Fp8), as well as the recombinant 56/74 virus used as a control.

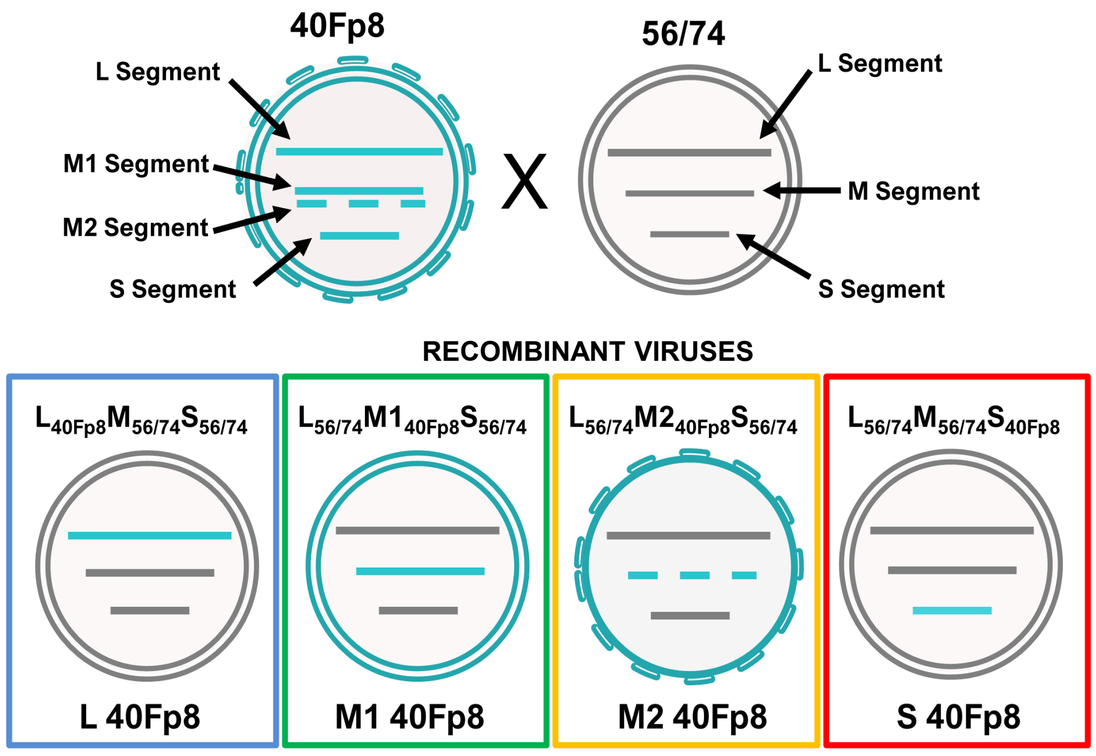

Figure 1. Scheme representation of recombinant viruses

CHARACTERIZATION OF REASSORTANT rRVFVs *IN VITRO*

The rRVFV were characterized by their growth kinetics and plaque size in Vero cells. (A) Growth curves indicate that viruses carrying M segments 40Fp8 replicate similarly to those of the attenuated strain 40Fp8, reaching higher titers than those with an M segment 56/74 and WT 56/74. (B) Plaque assays show that recombinants viruses with an M 40Fp8 segment exhibit a small plaque phenotype similar to 40Fp8 strain, while viruses carrying an M 56/74 segment show large plaques like those of the virulent 56/74 strain.

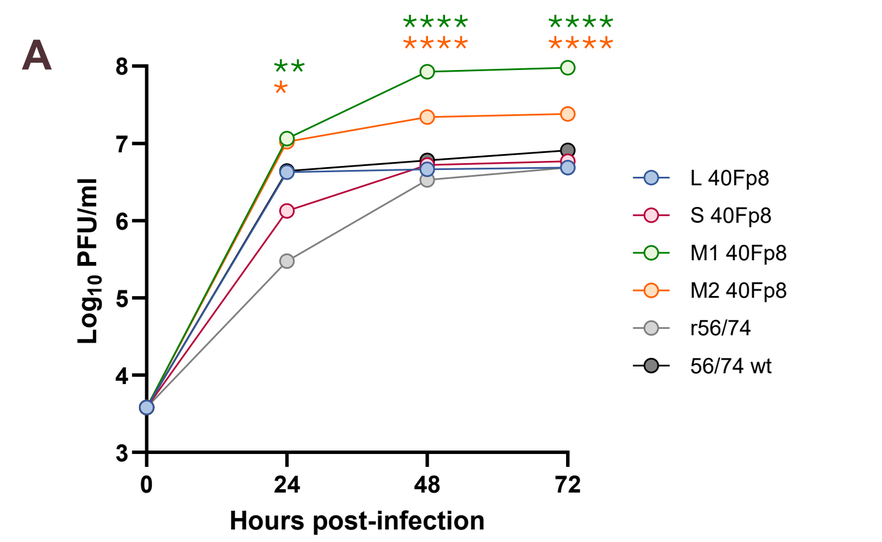

Figure 2.- (A) Growth kinetics of recombinant RVFVs in Vero cells at 24, 48, and 72 h post-infection (two-way ANOVA, Dunnett's test for multiple comparisons; P < 0.05, P < 0.002, **P < 0.0001). (B) Plaque phenotype evaluated in infected Vero cells by immunohistochemical staining at 5 days post-infection.

CHARACTERIZATION OF REASSORTANT rRVFVs *IN VIVO*

We evaluated the virulence and pathogenicity of the rescued recombinant viruses in 129sv WT mice. (A) Groups of four females and four males were inoculated with 10^3 or 10^2 PFU of each reassortant, using r56/74 as a control. Mice were monitored daily for clinical signs and weight loss, and blood and serum samples were collected to assess viremia and seroconversion. (B.1) Female mice exhibited low susceptibility to recombinant viruses carrying any 40Fp8 segment, with very low or undetectable viremia, unlike the r56/74 control, in which all mice succumbed to infection. (B.2) Male mice showed greater susceptibility at the highest dose, particularly to reassortants carrying M1 or M2 segments from 40Fp8, while L40Fp8 resulted in partial attenuation and S40Fp8 in complete attenuation. At the lower dose, all reassortants showed varying degrees of attenuation compared to r56/74. Notably, S40Fp8 exhibited complete attenuation, with 100% survival and almost undetectable viremia regardless of dose and sex.

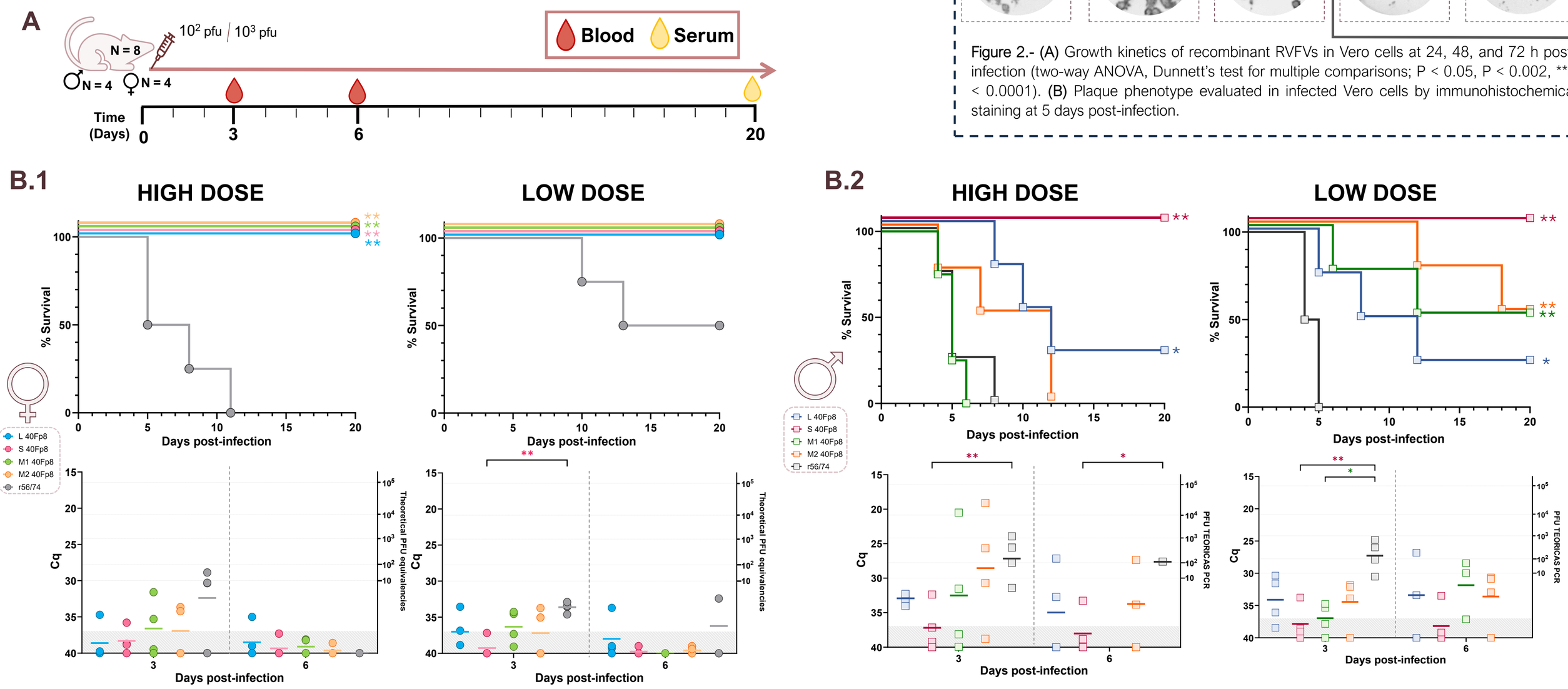

Figure 3.- (A) Schematic of experimental design. (B.1) Groups of female mice. (B.2) Groups of male mice. On the top represents the survival rate post-infection. (Log-rank test. * P value < 0.05, ** P value < 0.002, **** P value < 0.0001). At the bottom is the RT-qPCR analysis of viremia at day 3 and 6 post-infection. Results were expressed as Cq (left y-axis) and PFU/mL equivalents (right y-axis and dotted horizontal lines). Cut-off Cq ≥ 37(Grey shaded area) Points represent individual Cq for each mouse and lines of the corresponding color represent the mean Cq value of each group (ANOVA (Šidák's multiple comparisons test. * P value < 0.05, ** P value < 0.002)).

CONCLUSIONS & FUTURE PERSPECTIVES

- Exchange of the 40Fp8 M-segment in WT 56/74 increases replication and produces a small-plaque phenotype in vitro.
- The 40Fp8 S-segment confers complete attenuation in immunocompetent mice.
- All reassortant viruses showed markedly reduced pathogenicity compared to r56/74.
- Female mice consistently showed lower susceptibility to RVFV infection, highlighting the need to include both sexes in pathogenesis studies.
- Further work is required to deepen in vivo characterization of reassortants in mice and natural hosts.

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