

## Supplementary Information

# Reproducing the Rift Valley fever virus mosquito-lamb-mosquito transmission cycle

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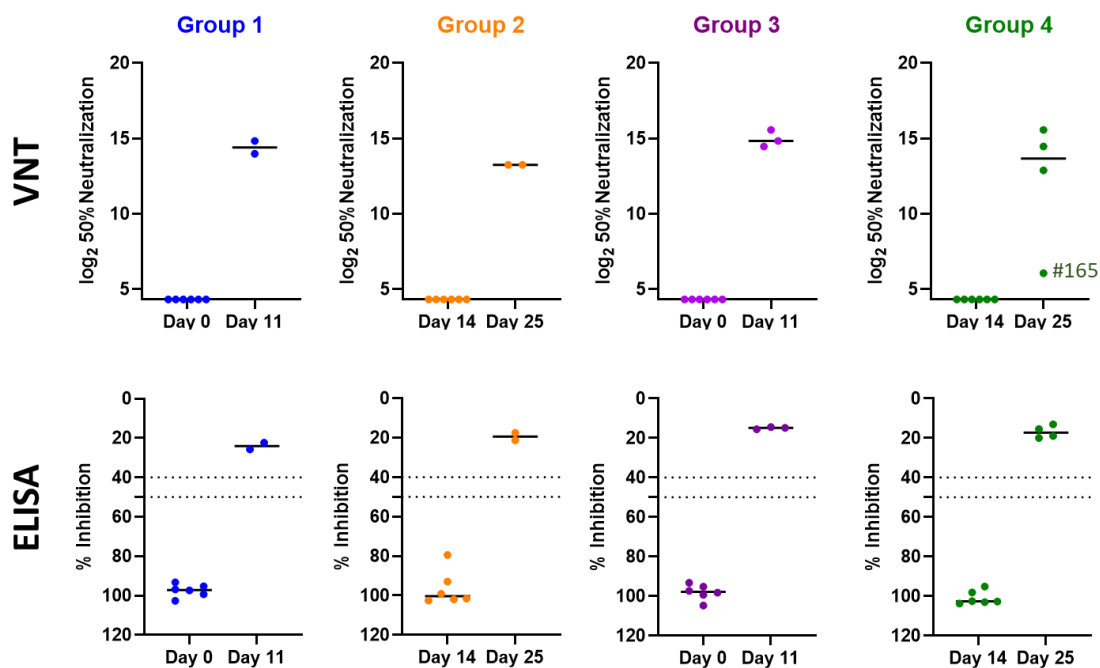
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**Supplementary Figure S1. Antibody responses after RVFV infection following needle inoculation or exposure to RVFV-positive mosquitoes.** Sera collected from lambs before IV inoculation or mosquito exposure (day 0 for Groups 1 and 3; day 14 for Groups 2 and 4) and 11 days after infection via needle inoculation (Group 1) or mosquito exposure (Groups 2, 3, 4) were analyzed for the presence of anti-nucleocapsid (N) antibodies by the ID Screen® Rift Valley Fever Competition Multi-species ELISA (ID-VET) and neutralizing antibodies by virus-neutralization test<sup>26</sup>.

**Supplementary Table S1: Experimental details**

Animals	
Species	Sheep
Breed and/or strain	Texel cross-breed
Source	Conventional sheep farm Netherlands
Sex	Male and female
Developmental stage	Between 8-16 weeks of age
Health status	Healthy upon enrolment
Weight	12-30 kg
Identification	By ear tag and by non-irritating coloured spray on the back of the animal
Housing and Husbandry	
Type of facility	BSL-3 facility
Type of housing	Stables (18 m <sup>2</sup> )
Bedding material	Wood shavings
Number of animals per stable	12
Light/dark cycle	12/12
Temperature	20°C
Quality of water	Tap water, quality checked daily
Type of food	Hay, grass pellets and sheep grain
Access to water and food	<i>Ad libitum</i>
Access to food	Once per day
Environmental enrichment	None
Acclimatization time	7 days
Experimental Procedure	
Number experimental groups	4
Number of animals per group	6
Randomisation procedure	Animals were randomly allocated to groups by a veterinarian.
Experimental unit	Group
Route of administration	Intramuscular injection in jugular vein or exposure to mosquitoes in cardboard containers.
Anaesthesia and analgesia	Sedator and atipamezol
Method of euthanasia	Intravenous injection with sodium pentobarbital.
Humane endpoints	-The animal is recumbent and does not rise even after stimulation -The animal is unable to drink -The animal is lethargic (listless, apathic, non-responsive to stimuli)
Observations	The animals were observed for clinical signs once per day before, and twice per day after inoculation or mosquito exposure.

**Supplementary Table S2. Mean and peak viremia measured in RVFV infected lambs<sup>a</sup>**

Group	Area Under the Curve (AUC)		Peak viremia	
	Mean	SD	Mean	SD
1	6.28	0.52	6.21	0.53
2	6.41	0.30	6.33	0.32
3	5.53	1.40	5.47	1.52
4	5.71	1.97	5.59	2.02

<sup>a</sup>Numbers represent TCID<sub>50</sub>/ml.