Evaluation of two recombinant viral-vectored vaccines against orf virus in sheep

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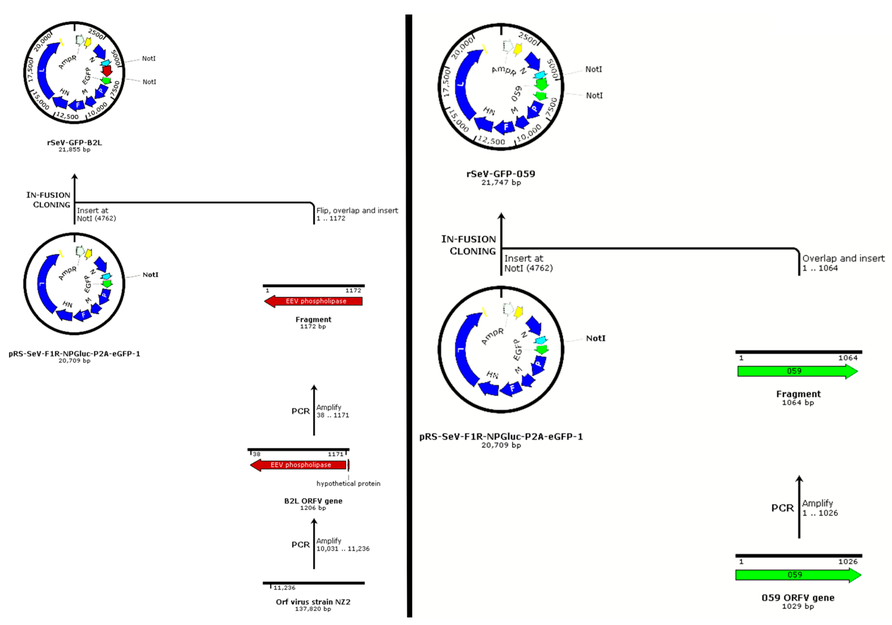
Introduction

Orf virus (ORFV), a member of the genus *Parapoxvirus*, is the causative agent of contagious ecthyma, a disease responsible for severe economic losses in small ruminants worldwide. Currently, commercial vaccines are based on attenuated strains of ORFV that elicit short-lived protection and can reverse to virulence, highlighting the need for new vaccine prototypes. Sendai virus (SeV) has obtained promising safety and protection potency results as a viral vector in the development of vaccine prototypes for many viral pathogens. In sheep, SeV has shown efficient transgene expression and robust innate immune response activation *in vitro*. SeV-based vaccines could be utilized as an alternative strategy to develop a new generation of safe and efficacious vaccine against ORFV infection in small ruminants. This study evaluated the safety and protective efficacy of two recombinant Sendai virus (rSeV-GFP)-based vectors expressing proteins ORFV B2L (rSeV-GFP-B2L) and ORFV 059 (rSeV-GFP-059) in sheep, after challenge with wildtype ORFV.

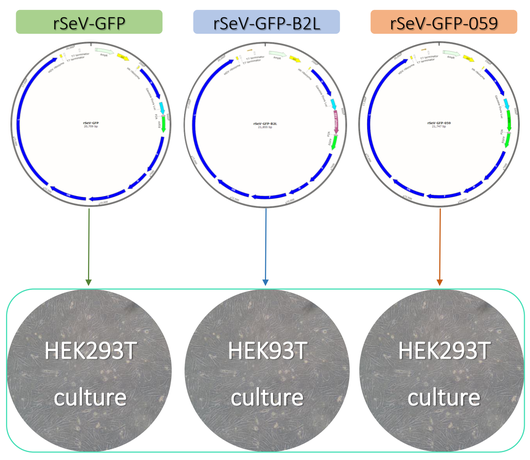
Material and methods

1. Construction of rSeV-GFP-B2L and rSeV-GFP-059 plasmids



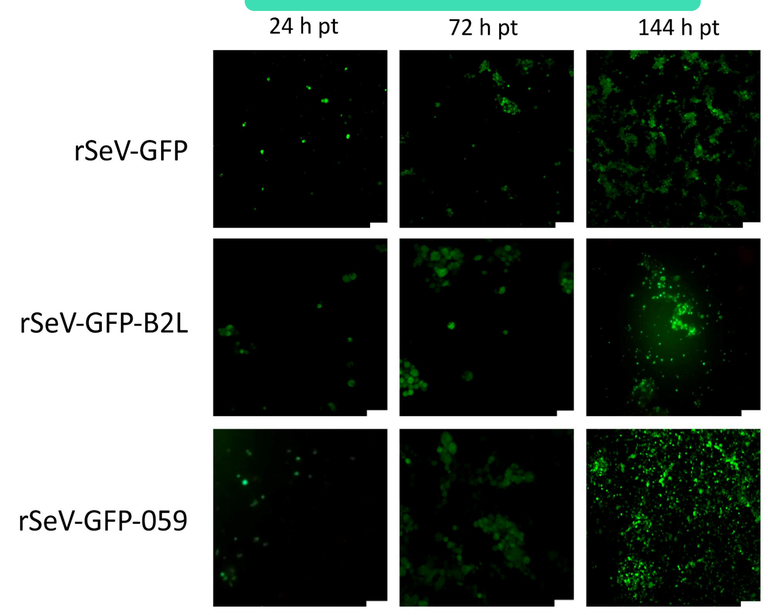
ORFV B2L and ORFV 059 gene sequences were amplified from wyldtype ORFV and cloned into rSeV-GFP plasmid by In-FUSION cloning generating recombinant plasmids rSeV-GFP-B2L and rSeV-GFP-059.

2. Transfection



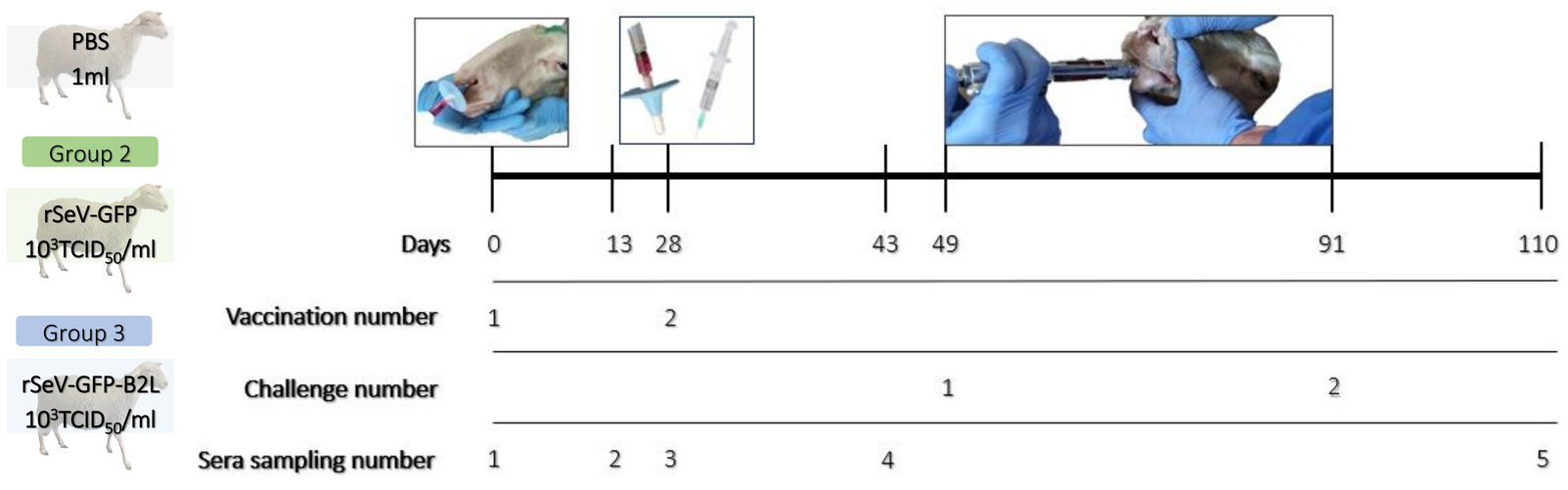
SeV reverse genetics system was used for recombinant viral vector production. Briefly, antigenomic rSeV-GFP, rSeV-GFP-B2L or rSeV-GFP-059 and accessory plasmids (T7-SeV- N, T7-SeV-P, T7-SeV-L, T7opt) were co-tranfected in 60-70% confluent HEK293T cells using Jet Prime transfection reagent (1:2 ratio).

3. Viral rescue and titration



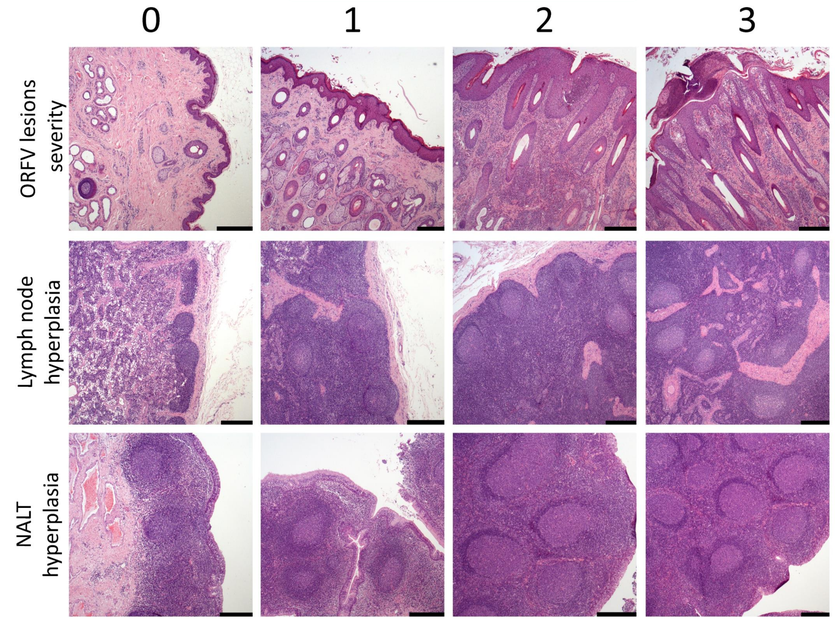
Cell transfection efficiency was monitored by fluorescence microscopy and GFP positive cell culture supernatants collected at 96-144 h, clarified by centrifugation at 2500 rpm for 5 min and stored at -80ºC. rSeV-GFP, rSeV-GFP-B2L and rSEV-GFP-059 viruses were titrated achieving 10^6 $TCID_{50}$/mL.

4. Inmunization and challenge



Sheep were randomly divided into 4 groups and inoculated with 1 ml of PBS or a pseudo-virus suspension containing 10^3 $TCID_{50}$/ml. First immunization (day 0) was intranasally with a nebulizer. 28 days post-priming (dpi), booster was performed with the same dose per animal. However, half of animals in each group was inoculated intranasally and the rest subcutaneously. For challenging, 10^3 $TCID_{50}$/mL of wildtype ORFV was inoculated intradermally with Dermojet syringe in the lip. Challenge was performed twice, 21 and 63 days after booster.

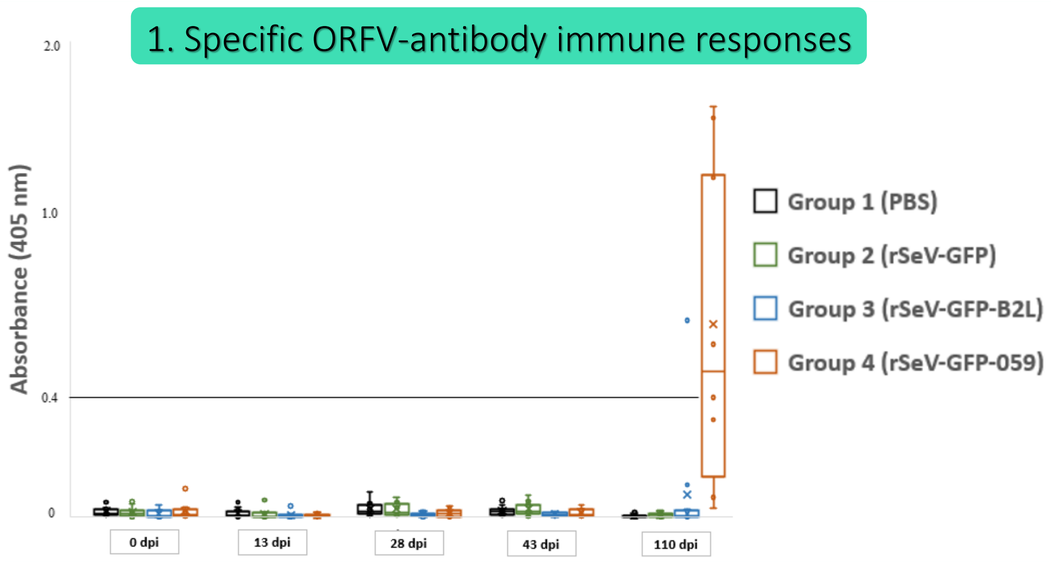
5. Postmortem study



Gross ORFV associated lesions were evaluated and scored from 0 (no lesions) to 1 (presence of lesions). Severity of microscopic lesions and retropharyngeal lymph nodes and NALT hyperplasia were scored from 0 to 3.

Results

1. Specific ORFV-antibody immune responses



Levels of anti-ORFV antibodies were evaluated by in-house indirect ELISA, based on the immunodominant ORFV envelope protein 109. ORFV specific antibodies were detected only in group 4 (rSeV-GFP-059) at 110 dpi.

2. Postmortem results

	Gross lesions		Histopathological severity			
	Absence	Presence	0	1	2	3
Group 1 (n=11)	54.5	45.5	0	0	60	40
Group 2 (n=11)	72.7	27.3	0	20	20	60
Group 3 (n=11)	27.3	72.7	0	20	0	80
Group 4 (n=12)	100	0	80	20	0	0

Group 4 all remained clinically healthy, not exhibiting any significant ORFV associated gross lesions during all experiment. No differences were observed between intranasally and intradermally boosted animals. Only one sheep of group 4 presented mild microscopic lesions Significant differences in retropharyngeal lymph nodes and NALT hyperplasia between vaccinated groups were not observed.

Conclusions

- rSeV-GFP-059 provides a complete protection to animals challenged with wildtype ORFV at high pathogenic dose, making it a promising candidate viral vector-based vaccine against ORFV infection
- rSeV-GFP-059 induces robust humoral immune response. To determinate if serum antibodies neutralize ORFV, a seroneutralization assay is in development
- rSeV-GFP-B2L might favor ORFV infection

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