Development of a Mucosal Virus-Vectored Vaccine for Bovine Parainfluenza Virus type 3

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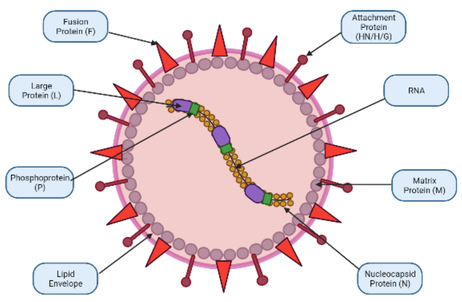
Background

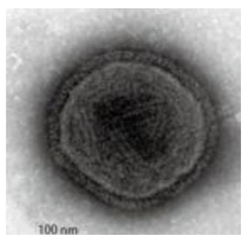
- bPIV3 - major component of Bovine Respiratory Disease Complex (BRDC)
- Respiratory disease- morbidity and mortality
- No efficient vaccine
- Virus-vectored vaccine approach

ANTIGEN- bPIV3 Structural Proteins



VECTOR- SeV

Non-pathogenic in cows and humans
Tissue tropism - respiratory tract
Reverse genetics established



Hypothesis and Aims

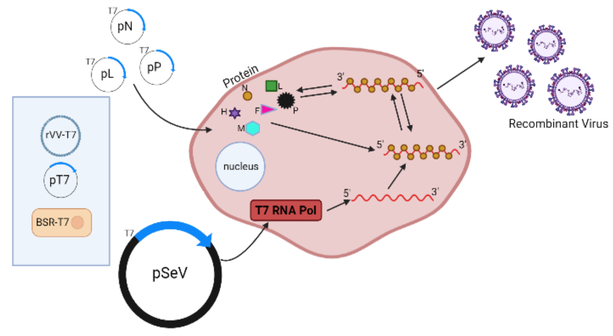
Sendai virus (SeV) recombinant vaccine vector expressing bPIV3 antigens should:

- induce protective immune responses
- be effective in presence of maternal antibodies
- exhibit high levels of biosafety
- be serologically distinguishable from wild type bPIV3

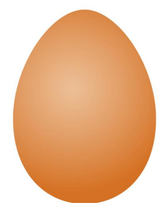
Aim - generation of a safe and effective mucosal virus-vectored vaccine for bPIV3

Methods

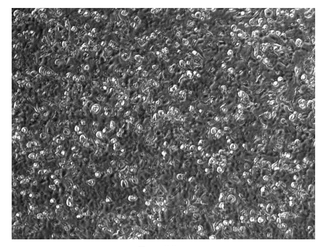
1.Plasmid Generation and Virus Rescue



2. Virus Amplification

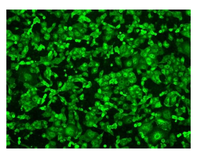


Embryonated eggs

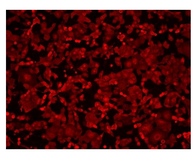


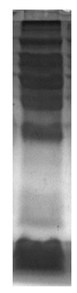
Cells

3. In Vitro Characterisation



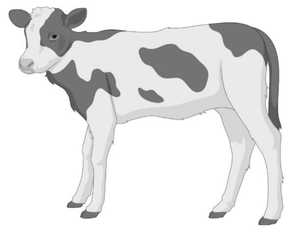
Immuno-fluorescence





SDS-PAGE

4. In Vivo Studies



Results

1. Successful generation of SeV infectious clones

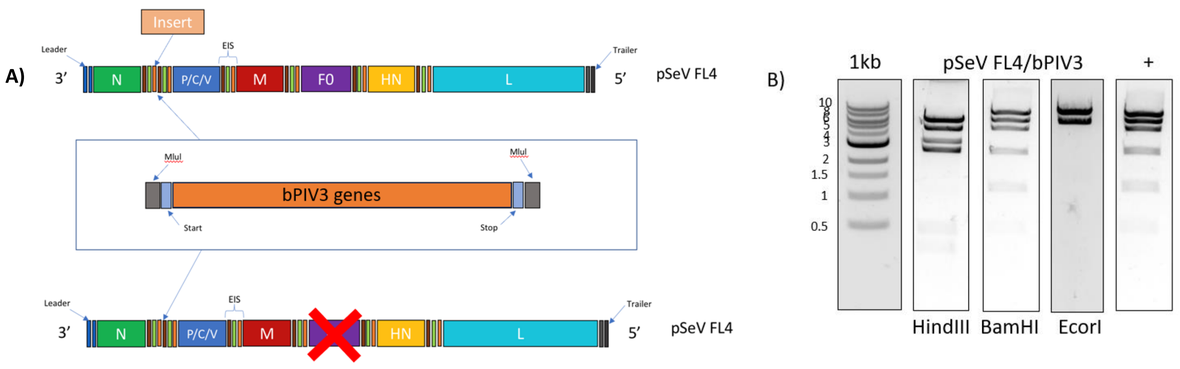


Figure 1. Generation and screening of SeV infectious clones. (A). Diagrammatic representation of insertion of bPIV3 genes into replication competent and incompetent SeV infectious clone plasmid; (B) Restriction maps of rSeV/bPIV3 infectious clones using restriction enzymes HindIII, BamHI and EcorI.

2. Rescue of recombinant SeV expressing bPIV3 antigens

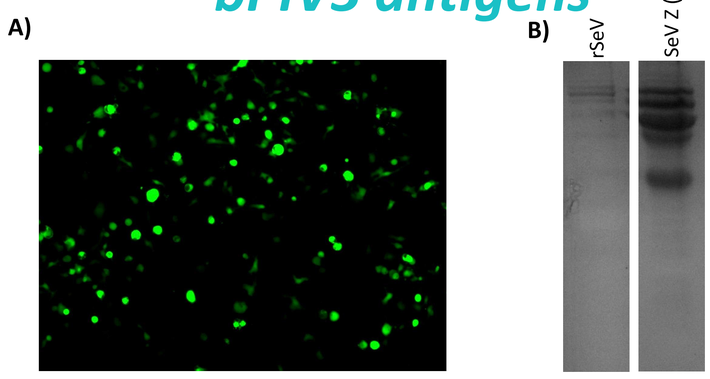


Figure 3. Rescue of rSeVs from SeV infectious clones. Infectious clones and helper plasmids were into A549 cells pre-infected with rVV-T7. An infectious clone encoding eGFP was used as a positive control to confirm that rescue conditions were capable of producing infectious virus (A). After 5 days, transfected cells were lysed by two cycles of freeze-thawing and inoculated into embryonated hens eggs. Allantoic fluid was recovered at 3 days post inoculation and clarified by centrifugation. One mL was added to a 250 µL 25% glycerol/NTE cushion and centrifuged at high speed. Pellets were resuspended and proteins were resolved by SDS-PAGE gel to identify SeV proteins. Purified SeV Z was used as a positive control (B).

Conclusions

Sendai virus (SeV) infectious clone plasmids encoding bPIV3 vaccine antigens were successfully generated

Rescues of recombinant Sendai virus vectored bPIV3 vaccines are underway.
Rescued viruses will be validate *in vitro* before progressing to *in vivo* characterisation.

Acknowledgements

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