Nanopore Identification of SNPs in Serially Passaged BUNV

don whitley scientific
culturing innovation

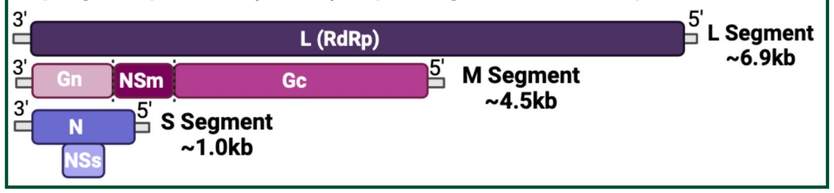
UNIVERSITY OF LEEDS

ABSTRACT

- **Bunyamwera virus (BUNV)** is a prototype bunyavirus
- BUNV is transmitted by **mosquitoes**; many bunyaviruses are arboviruses and pose a significant public health risk
- Currently**, no vaccines or antivirals** are available to treat **bunyavirus infection in humans**
- Improving our limited understanding of **bunyavirus-vector interactions** could aid therapeutic developments

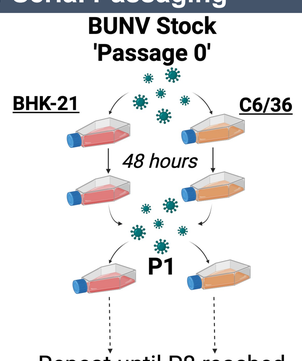
1. INTRODUCTION

- ***Bunyavirales*** order comprises over 500 enveloped, segmented RNA viruses with five families containing human-infecting species
- **BUNV** infection in **mosquito cells: persistent & non-cytopathic**
- **BUNV** infection in **mammalian cells: highly cytopathic**[1]
- Here, **BUNV** infection of mosquito and mammalian cells was compared utilising a **serial passaging-based approach**
- **Nanopore sequencing** identified **single nucleotide polymorphisms (SNPs)** in passaged **BUNV** samples

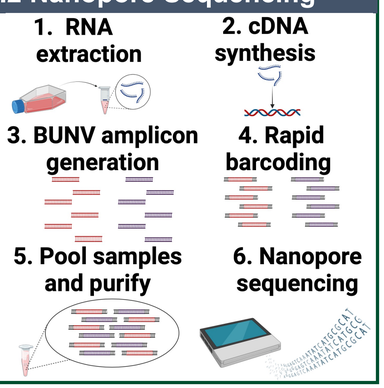


2. METHODS

2.1 Serial Passaging

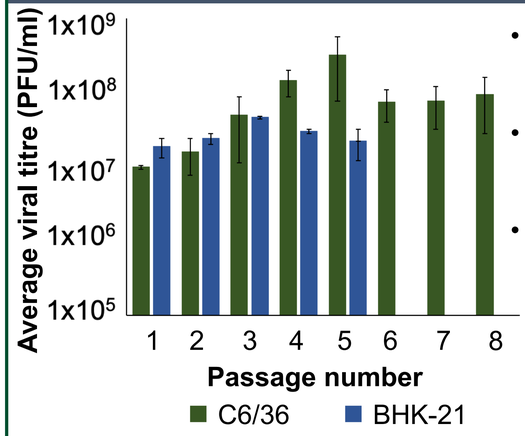


2.2 Nanopore Sequencing



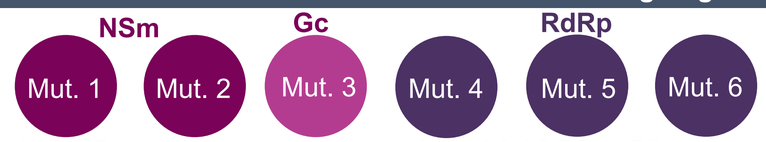
3. RESULTS

3.1 BUNV Successfully Serially Passaged in Both BHK-21 and C6/36 Cells



- Viral titres were determined via **plaque assay**
- BUNV was passaged to reach **P8 in C6/36** cells and **P5 in BHK-21** cells
- **Successful serial passaging** of **BUNV** in both cell lines enabled downstream analysis via **Nanopore sequencing**

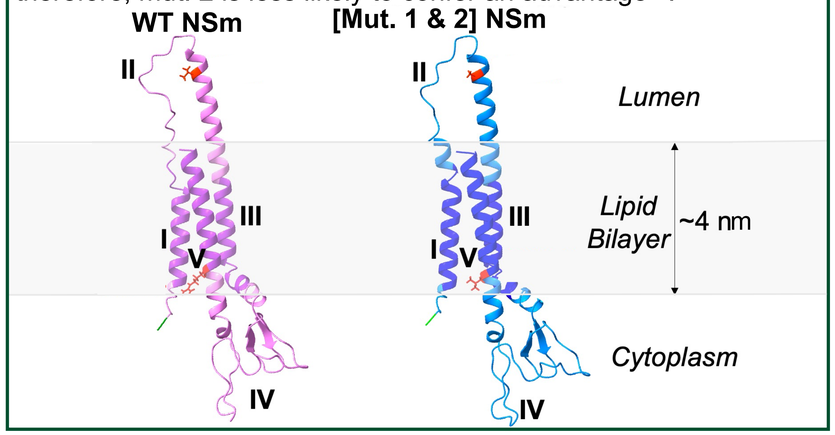
3.2 Nanopore Sequencing of Passaged BUNV Samples Identified Six Distinct SNPs within the BUNV Coding Region



Mut. 1 (NSm): increasing frequency from C6/36 P6 to P8
Mut. 2 (NSm): high frequency in various BHK-21 & C6/36 passages
Mut. 3 (Gc): low frequency in sporadic C6/36 passages
Mut. 4 (RdRp): low frequency in BHK-21 P5
Mut. 5 (RdRp): medium frequency in BHK-21 P5 & C6/36 P1
Mut. 6 (RdRp): low frequency in various BHK-21 & C6/36 passages
Mutations 1 and 2 were prioritised for further characterisation via modelling their effects in **AlphaFold** due to their presence in **C6/36 P6-P8**. Nanopore sequencing identified SNPs, but coverage across BUNV was inconsistent and requires improvements.

4. DISCUSSION

The role of **NSm** in bunyavirus infections remains largely uncharacterised; it has been thought to have roles in BUNV maturation and establishing mosquito infections[2]. **AlphaFold** was used to model NSm as the structure has not been solved. **Mut. 1** would sit within the theoretical **lumen domain** and speculatively may promote **viral maturation** in mosquito cells, however this requires further exploration. **Mut. 2** would sit within domain IV (shown to be less important in contributing to BUNV assembly) therefore, mut. 2 is less likely to confer an advantage[3].



5. CONCLUSION

An effective workflow was established which requires **optimisation** via improving **BUNV genome coverage**, particularly within the S segment. Identified SNPs require further characterisation to inform on their contribution to infection mechanisms. **Mut. 1** specifically warrants **further exploration**: site-directed mutagenesis of BUNV to introduce mut. 1 would enable infectivity, replication and assembly to be investigated and compared against WT-BUNV.

REFERENCES & ACKNOWLEDGEMENTS

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[1] Kohl, A., Hart, T.J., Noonan, C., Royall, E., Roberts, L.O. and Elliott, R.M. 2004. A Bunyamwera Virus Minireplicon System in Mosquito Cells. *Journal of Virology*. **78**(11), pp.5679–5685.
[2] Crabtree, M.B., Kent Crockett, R.J., Bird, B.H., Nichol, S.T., Erickson, B.R., Biggerstaff, B.J., Horiuchi, K. and Miller, B.R. 2012. Infection and Transmission of Rift Valley Fever Viruses Lacking the NSs and/or NSm Genes in Mosquitoes: Potential Role for NSm in Mosquito Infection. *PLoS Neglected Tropical Diseases*. **6**(5), p.e1639.
[3] Shi, X., Kohl, A., Léonard, V.H.J., Li, P., McLees, A. and Elliott, R.M. 2006a. Requirement of the N-Terminal Region of Orthobunyavirus Nonstructural Protein NSm for Virus Assembly and Morphogenesis. *Journal of Virology*. **80**(16), pp.8089–8099.

Name: Teagwen Cameron Supervisor: Dr Samantha Hover Poster No. 12