

## Plasmid DNA-based reverse genetics as a platform for manufacturing of Bluetongue

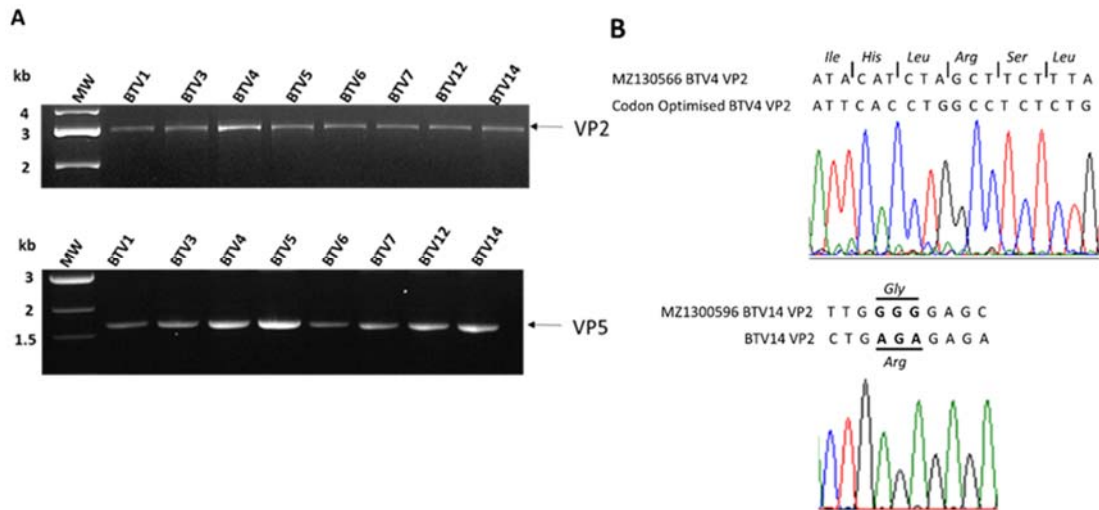
### Vaccine: Supplementary Tables and Figures

**Table S1** Animal Welfare Monitoring Sheet for BTV Disease

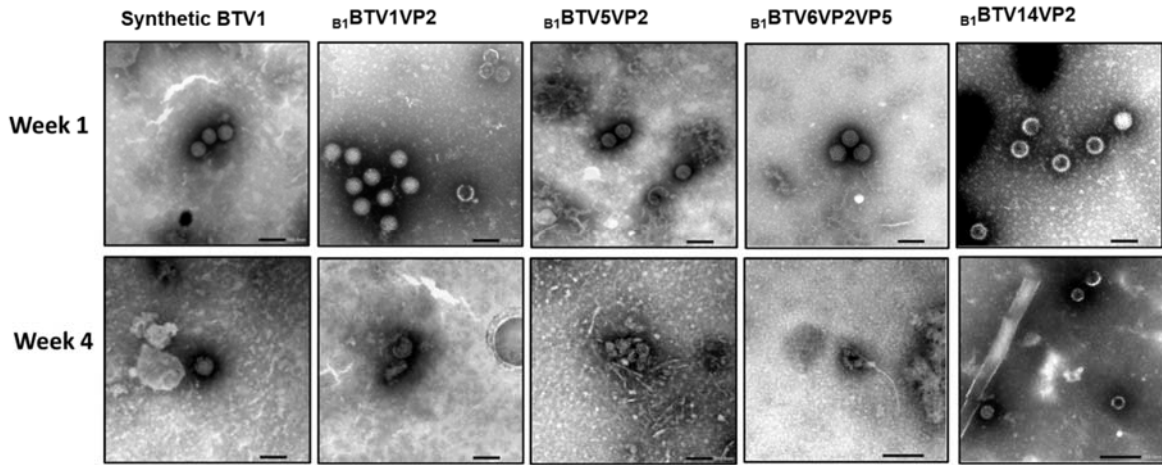
<b>Numerical Score</b>	<b>Nose</b>	<b>Mouth</b>	<b>Hooves</b>
0	Normal	Normal	Normal
1	Slight hyperaemia	Slight hyperaemia	Slight hyperaemia of the coronary band.
2	Hyperaemia & slight erosion	Slight hyperaemia & erosions at mucocutaneous junction of upper lip and swelling of mouth	Slight hyperaemia & erosions at mucocutaneous junction of upper lip and swelling of mouth
3	Erosion & petechiae	Hyperaemia, erosion and slight cyanosis of the mucosa	Hyperaemia of the coronary band with petechiae
4	Severe erosion, hyperaemia & haemorrhages	Severe erosion and cyanosis	Severe hyperaemia of the coronary band with petechiae (might become streaky)

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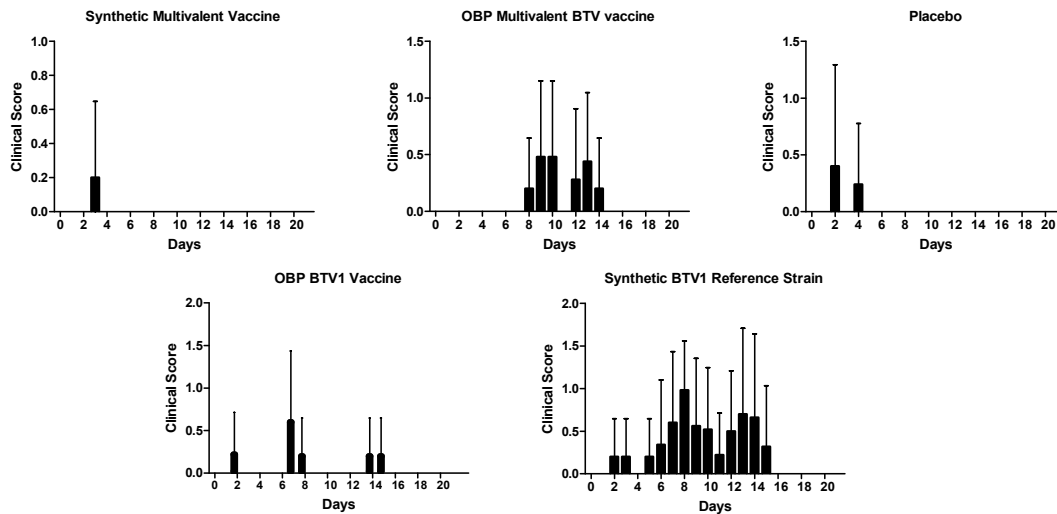
## Vaccine: Supplementary Figures



**Figure S1.** Construction of reverse genetics plasmids for recovery of synthetic viruses. (A) Reverse genetics plasmids expressing the BTV outer capsid proteins were PCR-amplified from cDNA or optimised synthetic genes. Gel-purified amplicons were separated by electrophoresis on ethidium bromide-stained 1% (w/v) agarose gels. MW represents the DNA 1Kb marker (New England Biolabs) used. (B) Amino acid sequence confirmation of the constructs gave the expected sequences for the codon-optimised segment 2 constructs. The chromatogram of BTV4 VP2 of the codon-optimised sequence is shown. A single non-synonymous mutation was identified on segment 2 of BTV14. The amino acid of the parental vaccine and construct sequence is shown in italics. Constructs with expected sequences were selected for virus rescue.



**Figure S2.** Stability assessment of synthetic viral particles using transmission electron microscopy. Viral particles were viewed weekly for four weeks. Fully assembled viruses were observed in high concentrations in week 1. Particles at varying degrees of disassembly were visualised in increasing quantities in the fourth week period of analysis.



**Figure S3.** Average clinical scores of vaccinated sheep. The average clinical score of sheep vaccinated with synthetic or licensed BTV1 monovalent or multivalent vaccine was calculated following an assessment of the body temperature, nose, mouth and hooves. The clinical scores observed 21 days following vaccination are depicted. The error bars indicate the standard deviation.