



A Study of Chicken Astrovirus Pathobiology in Emerging White Chick Hatchery Disease

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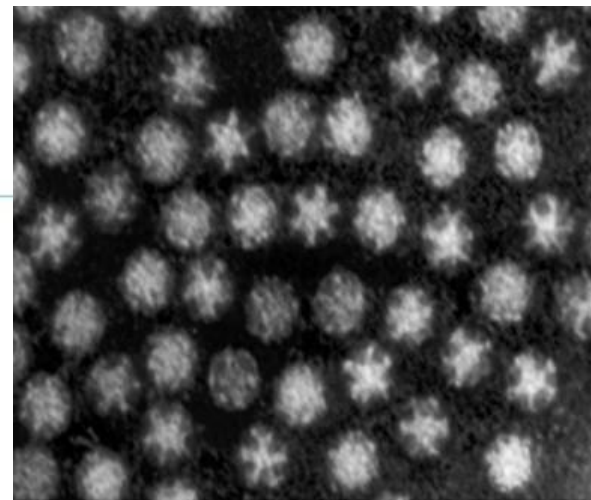
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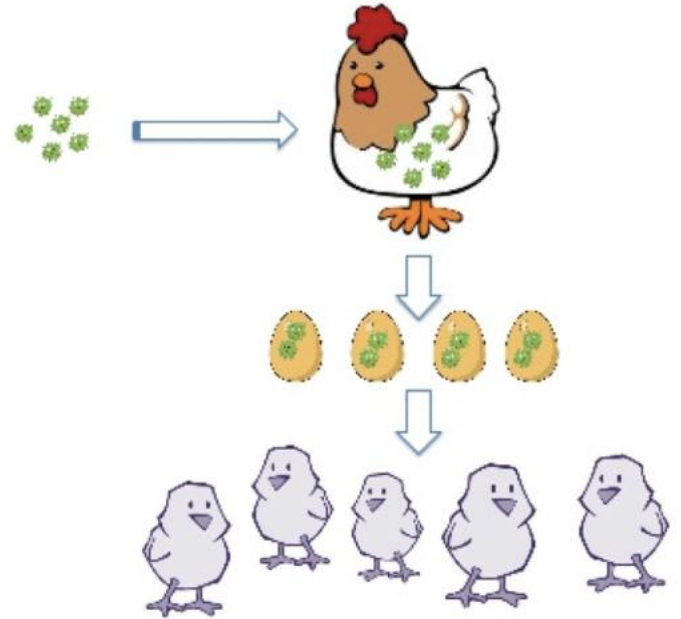
White Chicks Hatchery Disease

- Chicken Astrovirus
- Mid to late embryo deaths
- Weak chicks with white down
- Runting
- Discoloured livers and enlarged kidneys
- Substantial economic loss



Vertical Transmission

- 1-day old eggs
- Flocks are seronegative before infection
- Unsure of the mechanism of VT
- Generate a virus expressing a reporter gene (GFP)



Aims and Objectives

Uncover the mechanism of vertical transmission using the green fluorescent protein to track CAstV infection

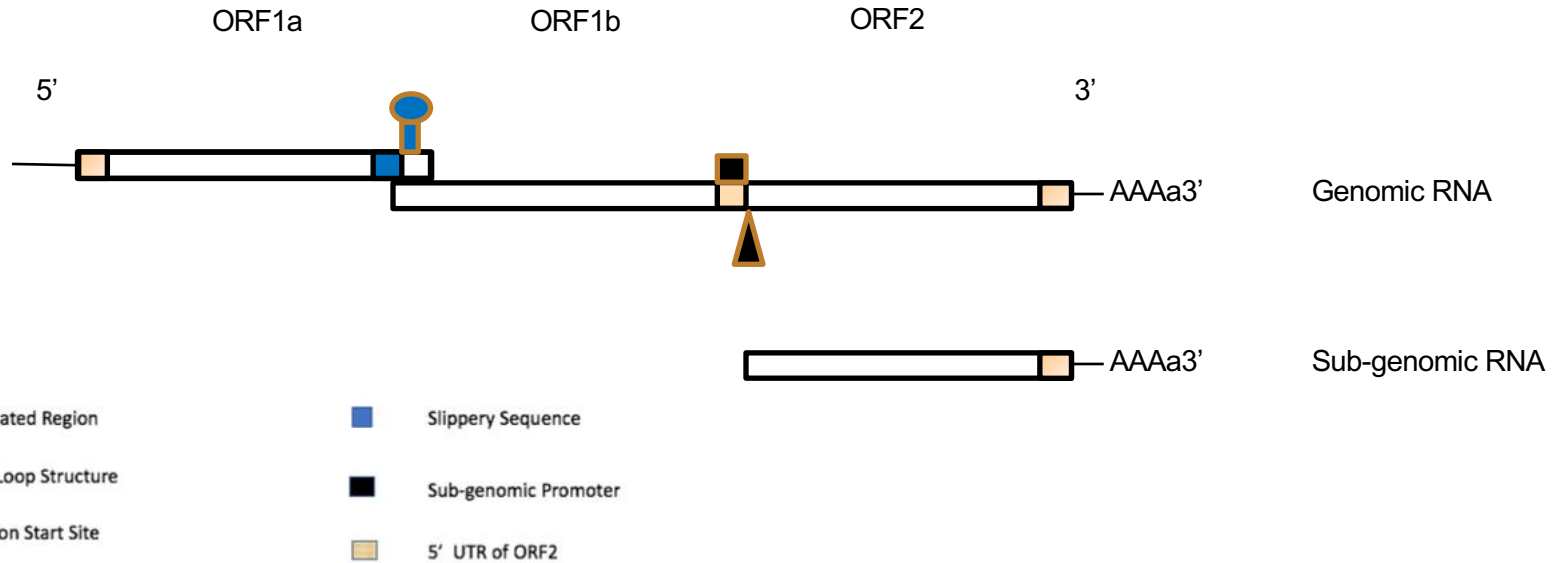


Identify organs infected in adult and the transmission route to embryo

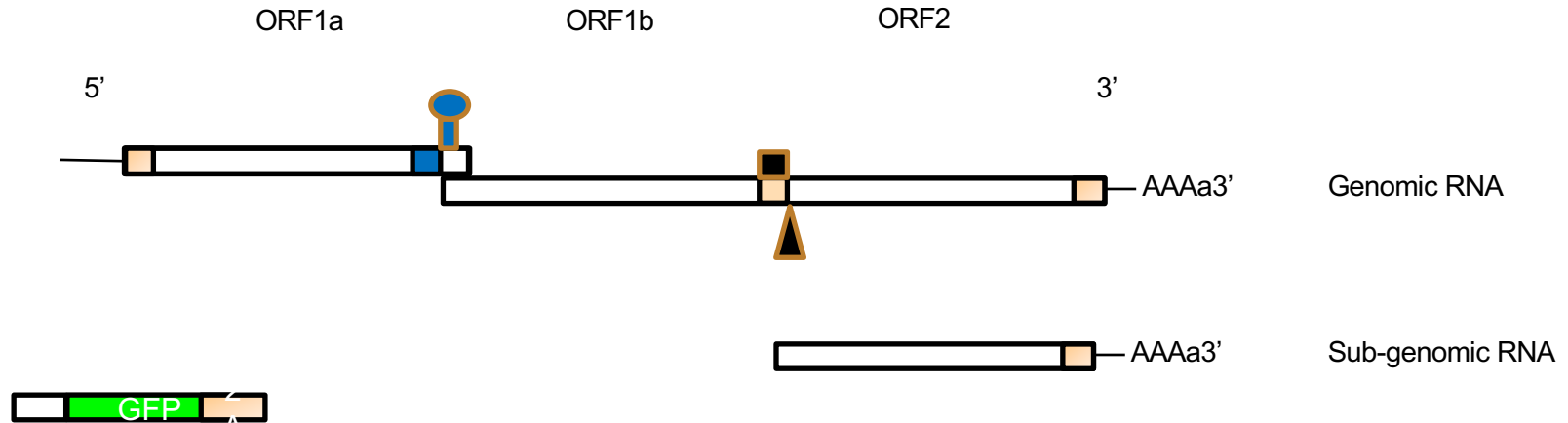


Determine maternal antibody titres, types and their duration in the adult and embryo

Chicken Astrovirus Genome



Introduction of GFP into the CAstV genome



Untranslated Region



Slippery Sequence



Sub-genomic Promoter



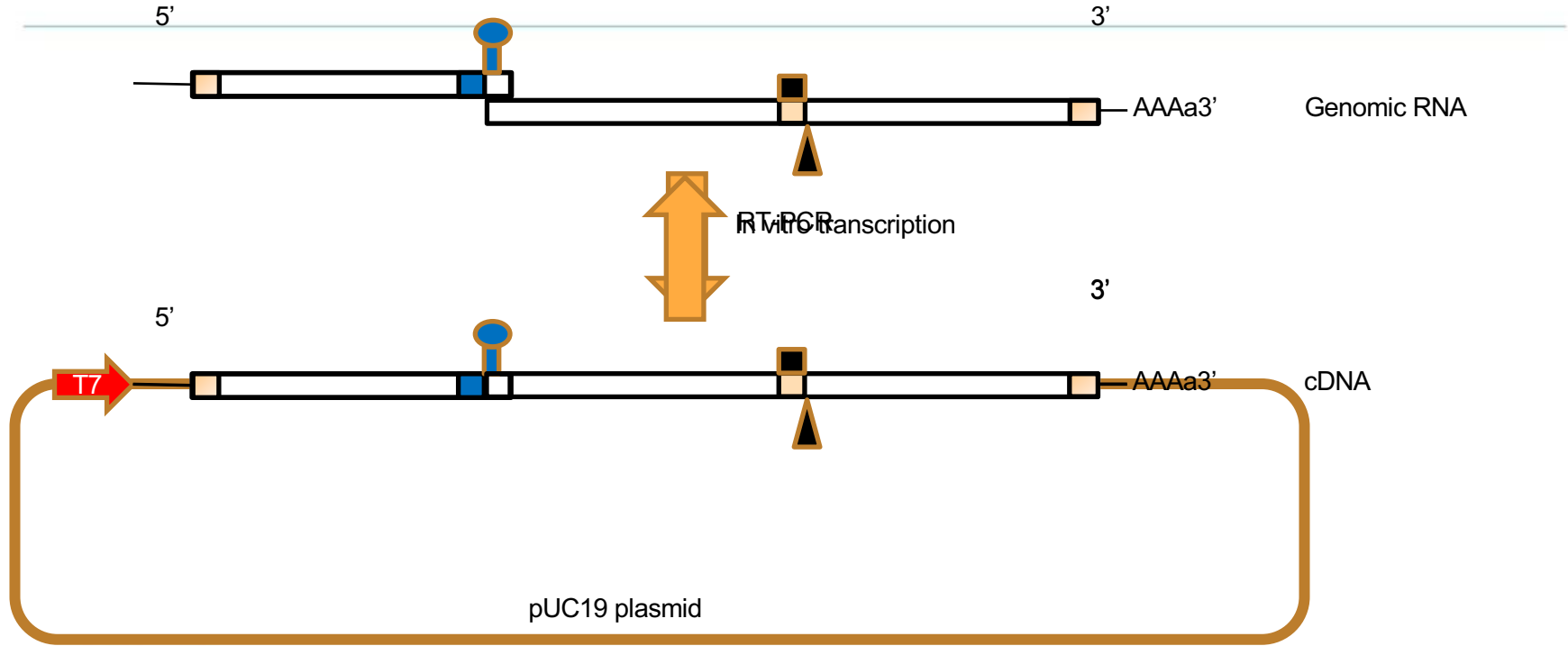
Translation Start Site



5' UTR of ORF2

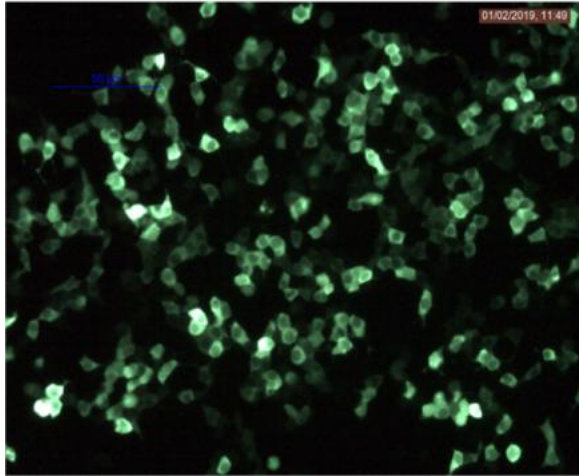


CAstV reverse genetics

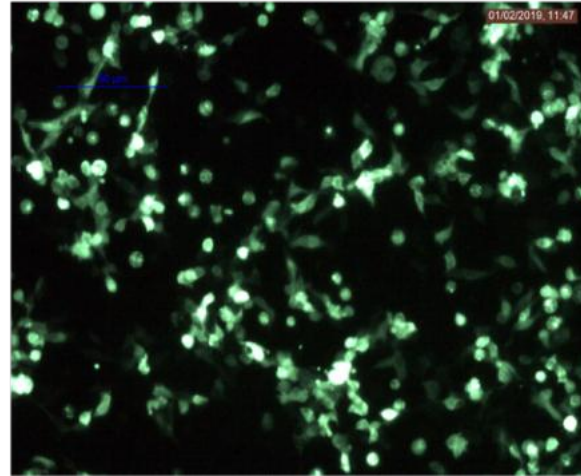


Cell Culture and Cell transfection using pc-DNA-eGFP plasmid

HEK 293T cells – Positive Control

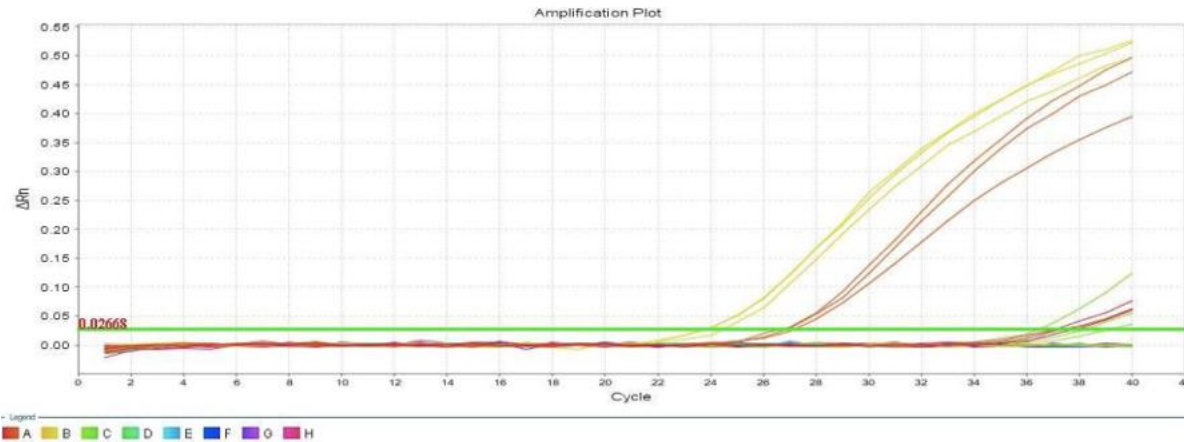


LMH cells – Test sample



LMH Cell Transfections

Transfection	Cells transfected	Media	Lipofectamine 2000 or 3000	Transfect fresh cells using previous transfection	Virus present based on CPE on images	Viral RNA present based on qPCR results
1	LMH	OPTI-MEM and LMH Media	3000	No - newly extracted	No	N/A
2	LMH	LMH Media	2000	Yes- from transfection 1	No	Yes - minimal amounts
3	LMH	LMH Media	2000 and 3000	No - newly extracted	No	No - results were negative



in ovo Transfection



1) Inoculation



2) Candle eggs



3) Examine embryos

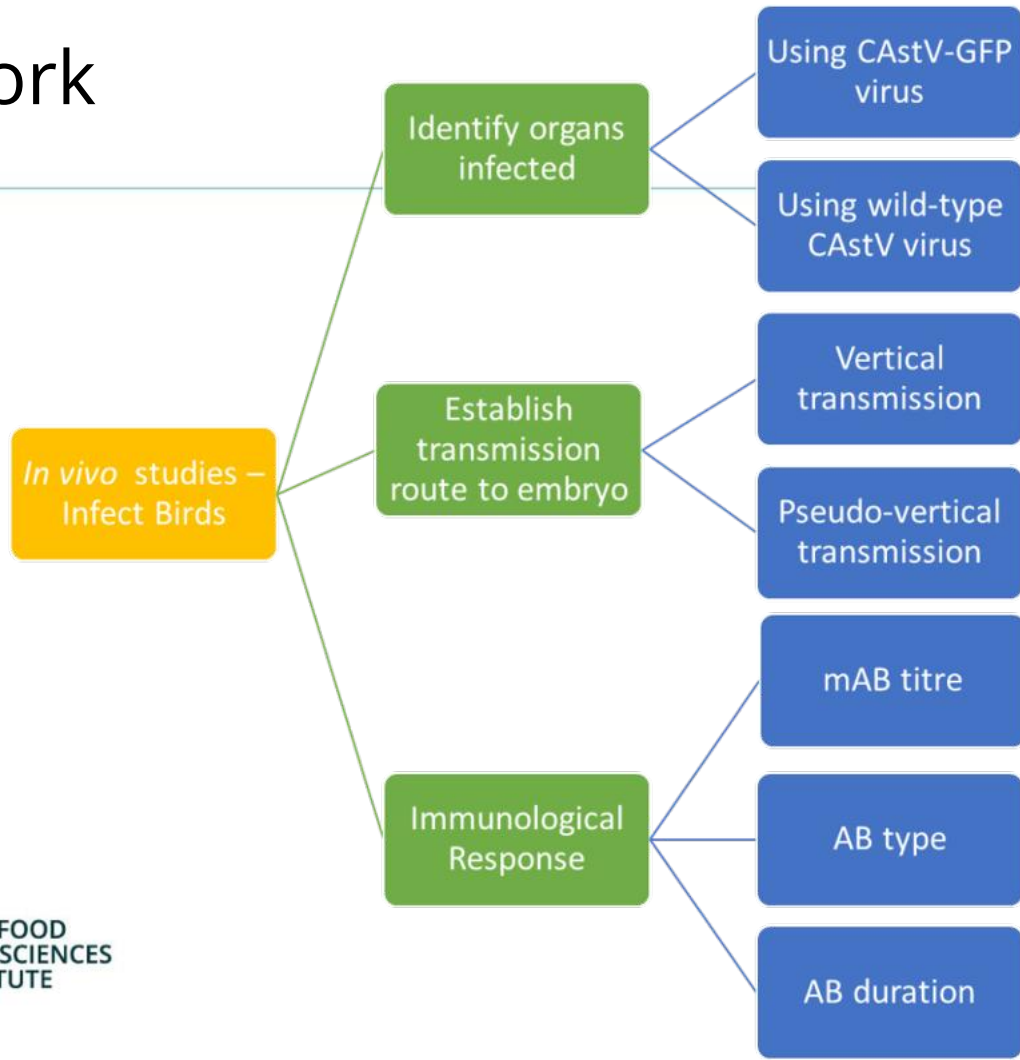


5µg transfection

Controls – not
inoculated at all

2µg transfection

Future Work



Thank you



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