

I would like to begin by acknowledging the Wadawurrung people as the Traditional Owners of the land that I'm presenting from today, and pay my respect to their Elders past and present.





Understanding the biosecurity risk posed by Australian-lineage H5 LPAIVs and their potential genesis to highly pathogenic forms.

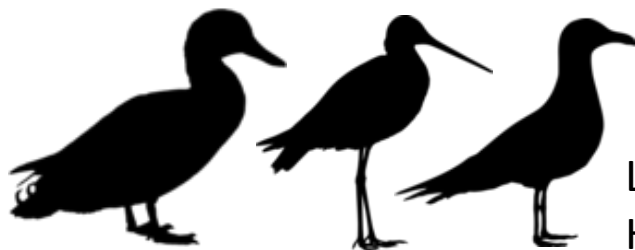
Dr Jasmina Luczo

Australian Centre for Disease Preparedness

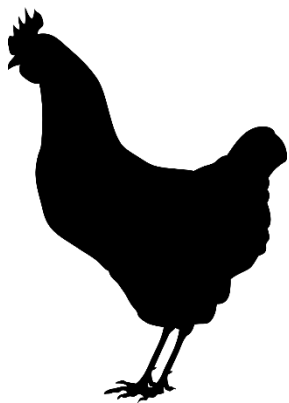
Australia's National Science Agency



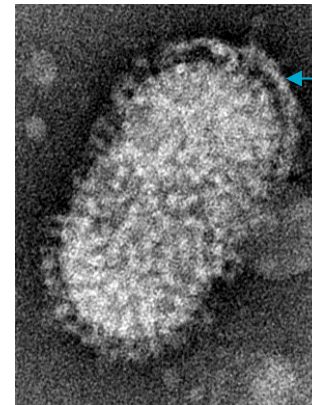
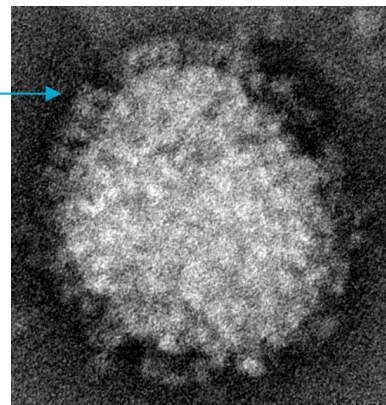
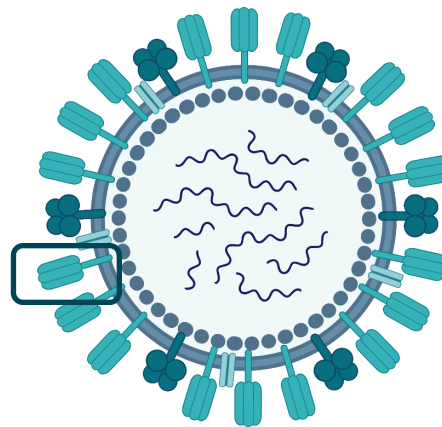
Avian influenza virus



Low pathogenicity
H1-H16/H19



High pathogenicity
H5, H7



Adam Costin (ACDP)

Avian Influenza Virus

Low pathogenicity (LPAI)

H1-H16, H19

Mild disease



High pathogenicity (HPAI)

H5 & H7

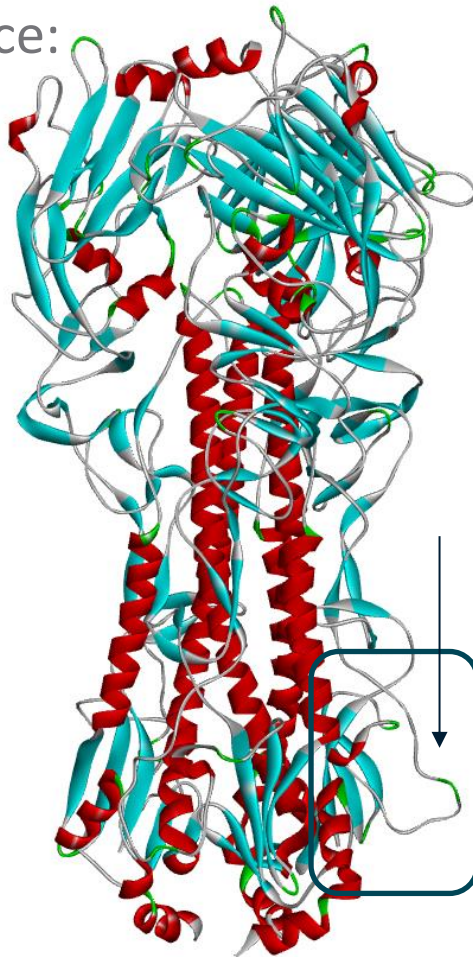
Severe disease



HPAI emerges from low pathogenic precursors

Avian influenza virus virulence: HA cleavage site

OFFICIAL: Sensitive



H5 low pathogenicity
Monobasic HA cleavage site:

RETR/G

H5 high pathogenicity
HA cleavage site:

RERRRKKR/G



OFFICIAL: Sensitive

H5 HPAIV – a global threat

Year	Subtype	Lineage	Location	Cleavage site
1959	H5N1	EA-non-gs/Gd	Scotland	PQ_RKKR/G
1961	H5N3	EA-non-gs/Gd	South Africa	PQ_RETRRQKR/G
1966	H5N9	Am-non-gs/Gd	Canada	PQ_RRKKR/G
1983-84	H5N2	Am-non-gs/Gd	USA	PQ_KKKR/G
1983	H5N8	Am-non-gs/Gd	Ireland	PQ_RKRKKR/G
1991	H5N1	EA-non-gs/Gd	England	PQ_RKRKTR/G
1994-95	H5N2	Am-non-gs/Gd	Mexico	PQ_RKRKTR/G
1996-present	H5N1/x	gs/Gd	Global (except Oceania)	PQ_RERRRKKR/G
1997-98	H5N2	EA-non-gs/Gd	Italy	PQ_RRRKKR/G
2004	H5N2	Am-non-gs/Gd	USA	PQ_RKKR/G
2004	H5N2	EA-non-gs/Gd	South Africa	PQ_REKRRKKR/G
2006	H5N2	EA-non-gs/Gd	South Africa	PQ_RRKKR/G
2011	H5N2	EA-non-gs/Gd	South Africa	PQ_RRKKR/G
2012-13	H5N2	Am-non-gs/Gd	Taiwan	PQ_RKKR/G
2015-16	H5N1/x	EA-non-gs/Gd	France	HQ_RRKR/G

High pathogenicity has emerged in every H5 lineage *except* the Australian lineage.

Since 1994, 7/9 (non-gs/Gd) H5 outbreaks caused by H5N2 HPAIVs.

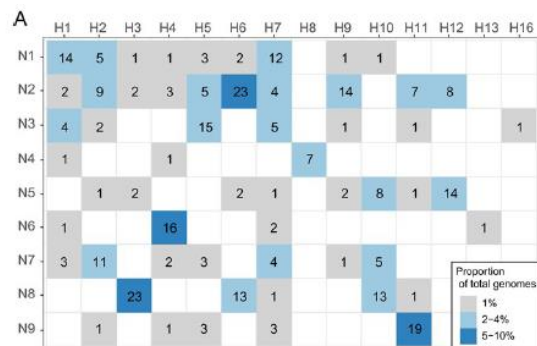
HPAIV in Australia

To date, Australia has had twelve H7 HPAIV outbreaks, but **no** H5 HPAIV outbreaks.

- The reason for this is **unknown**.

H5 and H7 LPAIVs are detected in Australian wild birds in approximate equal proportion.

Year	Subtype	Location	Cleavage site
1976	H7N7	Victoria	PE_IPKKREKR/G
1985	H7N7	Victoria	PE_IPKKREKR/G
1992	H7N3	Victoria	PE_IPKKKKR/G
1994	H7N3	Queensland	PE_IPRKRKR/G
1997	H7N4	New South Wales	PE_IPRKRKR/G
2012	H7N7	New South Wales	PE_IPRKRKR/G
2013	H7N2	New South Wales	PE_IPRKRKR/G
2020	H7N7	Victoria	PE_IPGKREKR/G
2024	H7N3	Victoria	PE_IPGKRERR/G
	H7N9	Victoria	PE_IPGKREKR/G
	H7N8	New South Wales	PE_IPGKREKR/G
2025	H7N8	Victoria	PE_IPHGVSPARRKR/G



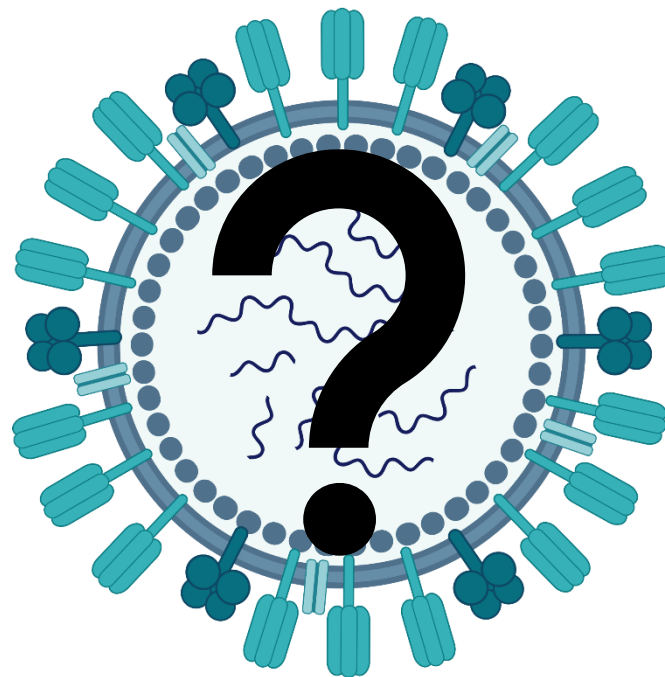
Wille *et al.* 2022 PLOS Pathogens

What is the biosecurity risk of Australian H5 LPAIVs?

Why has Australia only had H7 HPAIVs outbreaks, but no H5 outbreaks?

What is the biosecurity risk posed by Australian H5 LPAIVs?

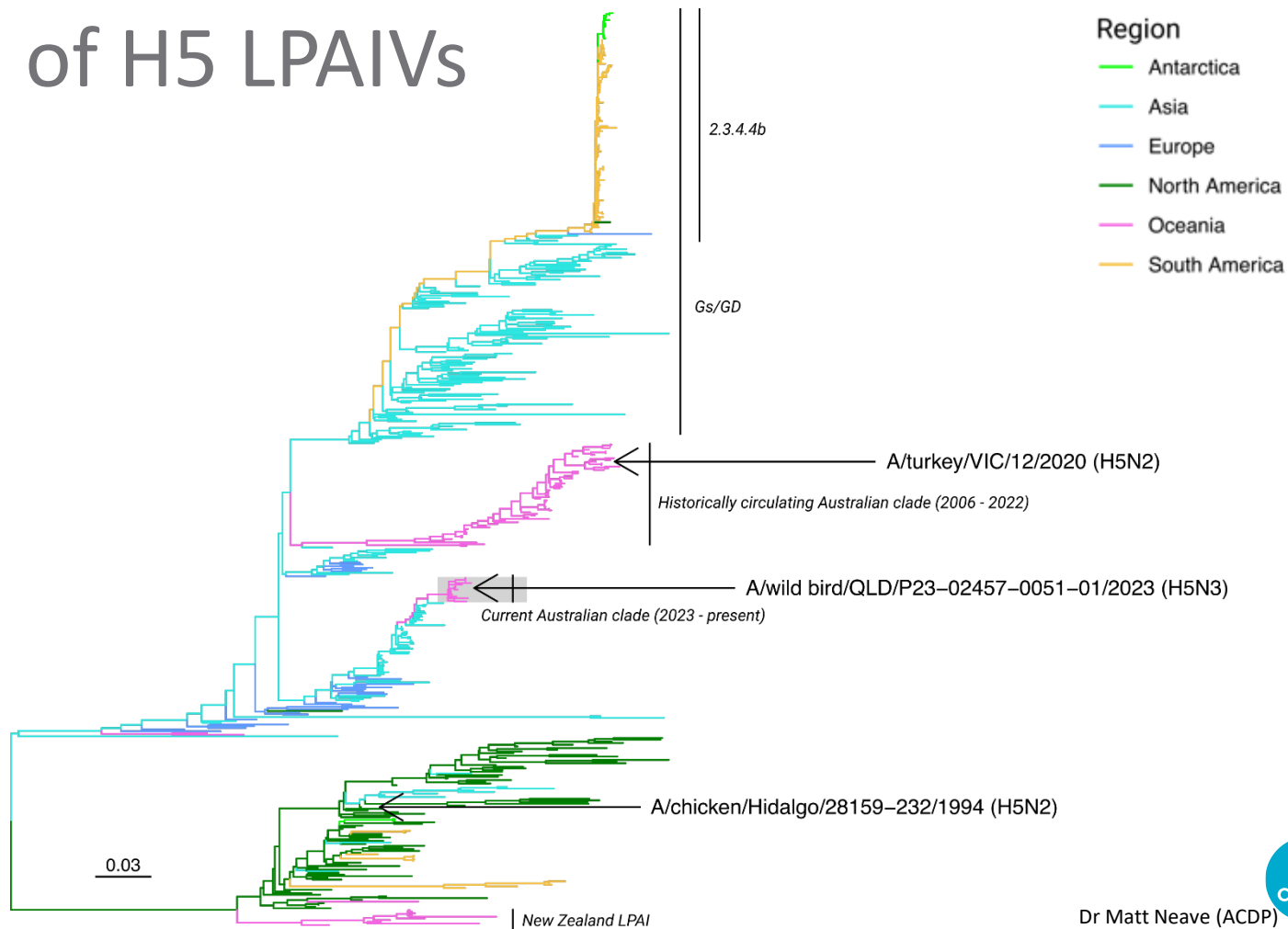
Can Australian H5's convert to highly pathogenic forms?



Project objectives

1. Characterise the ability of wild-type Australian H5 low pathogenicity avian influenza viruses to infect and productively replicate in chickens.
2. Monitor viral genetic sequences in virally infected tissues for changes associated with virulence.
3. Using reverse genetics, determine whether Australian H5 LPAIVs support the genesis of highly pathogenic avian influenza viruses.

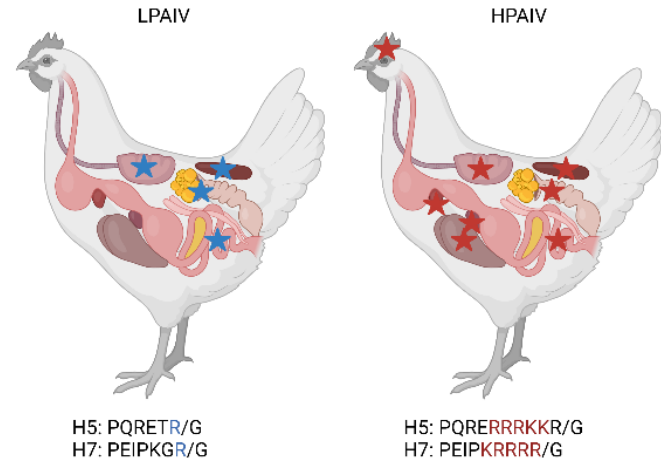
Selection of H5 LPAIVs



In ovo and in vitro
characterisation of Australian-
lineage H5 LPAIVs

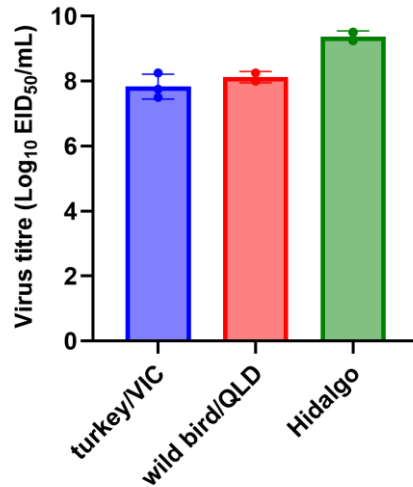
In ovo / *in vitro* characterisation of H5 LPAIVs

- 1) Virus growth in embryonated chicken eggs.
- 2) Virus growth in cell culture in the presence or absence of trypsin.
- 3) Virus growth in chicken neural cultures ('brain in a dish').
- 4) Tropism of H5 LPAIVs *in ovo*.



In ovo / in vitro characterisation of H5 LPAIVs

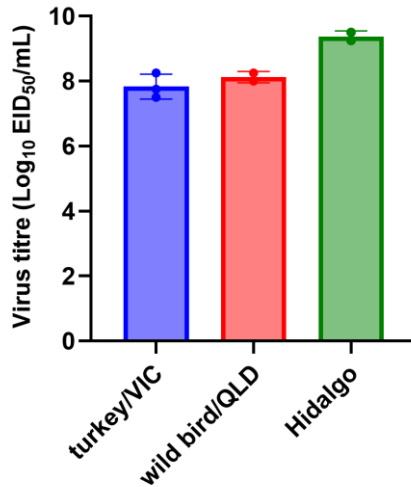
1) Growth of H5 LPAIVs in embryonated chicken eggs



turkey/VIC – AUS HA (historical)
wild bird/QLD – EA HA (current)
ckn/Hidalgo – positive control

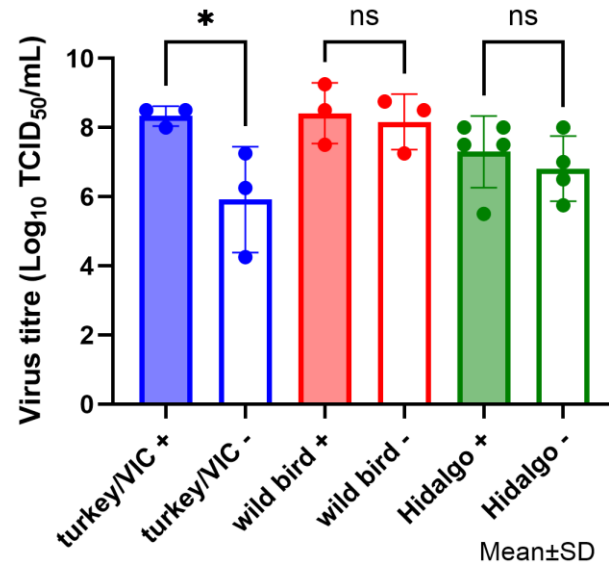
In ovo / in vitro characterisation of H5 LPAIVs

1) Growth of H5 LPAIVs in embryonated chicken eggs



turkey/VIC – AUS HA (historical)
 wild bird/QLD – EA HA (current)
 ckn/Hidalgo – positive control

2) Growth of H5 LPAIVs±trypsin in cell culture



H5 low pathogenicity
 Monobasic HA cleavage site:
 R/KXXR/S



In ovo characterisation of H5 LPAIVs

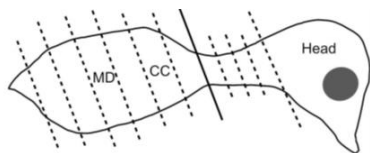
4) Tropism of H5 LPAIVs *in ovo*

Embryos and CAMs from H5 inoculated ECEs were harvested and fixed.

Histopathology and antigen distribution assessed:

- H&E, IHC nucleoprotein (NP) antigen

Histology



Smallridge *et al.* PMID: 40168291

Virus	NP antigen CAM	NP antigen Embryo
turkey/VIC AUS-lineage HA	Yes – ectoderm	Yes – feather and nasal epithelium
wild bird/QLD EA-lineage HA	Yes - ectoderm	Yes – feather and nasal epithelium
ckn/Hidalgo (+ve control) North American-lineage	Yes - ectoderm	Yes – oesophagus, trachea, nasal, GIT epithelium.

Antigen distribution consistent with LPAIVs.

In ovo and *in vitro* characterisation

- Conclusions and summary:
 - Australian H5 LPAIVs grow to similar titres in embryonated chicken eggs.
 - Australian H5 LPAIVs possessing an AUS HA, but not Australian LPAIVs with EA HA, require exogenous trypsin for efficient replication *in vitro*.
 - May indicate and enhanced ability of Australian H5 LPAIVs with EA HA to replicate in chickens.
 - Australian H5 LPAIVs antigen distribution in embryos and CAM was consistent with LPAIVs.

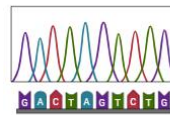
In vivo characterisation of Australian-lineage H5 LPAIVs

Can Australian H5 LPAIVs infect and productively replicate in chickens?

A critical first step in the potential emergence of high pathogenicity

- What tissues do the viruses replicate in and to what levels?
- Clinical disease signs.
- Monitor viral sequence .
- Viral shedding.
- Serum antibody response.
- Histopathology.

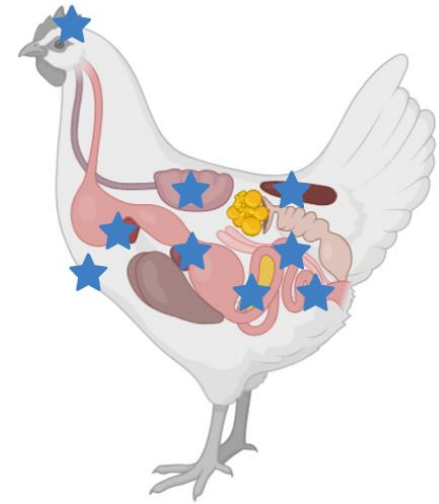
Sequencing



Virology



Histology

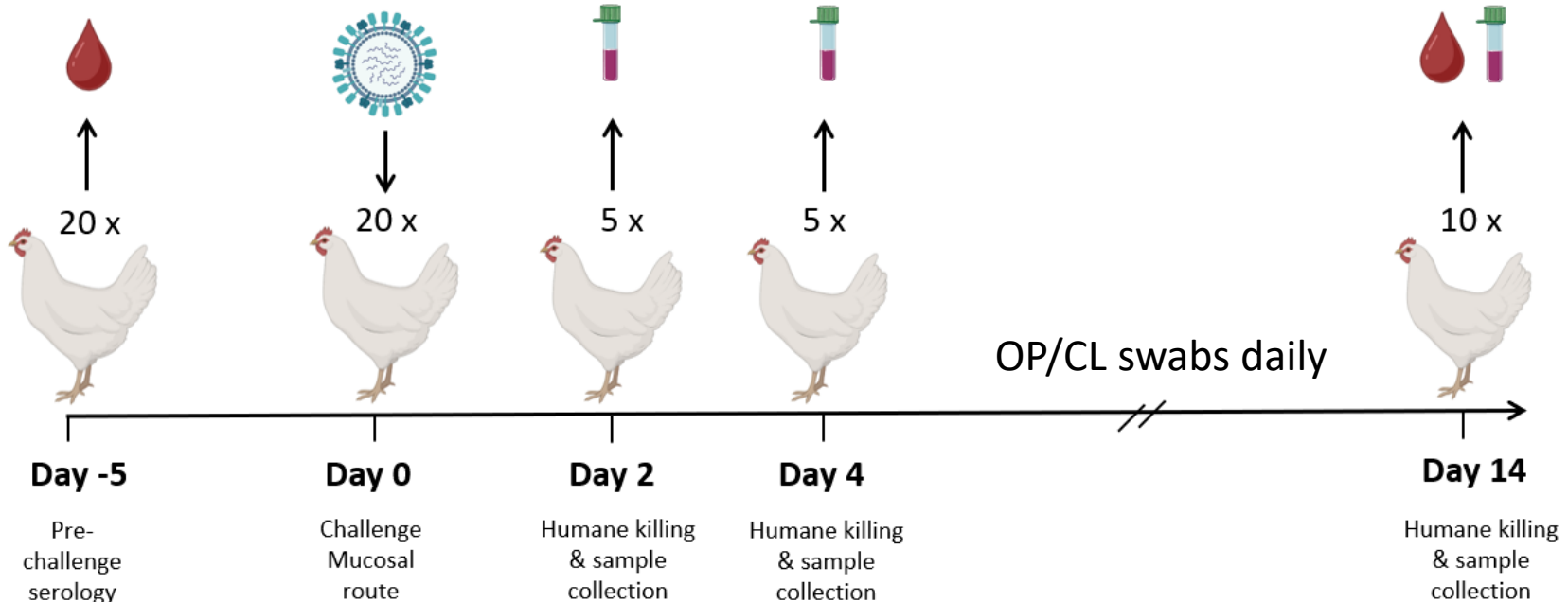


H5 LPAIV pathogenesis study

turkey/VIC – AUS HA (historical)
wild bird/QLD – EA HA (current)
ckn/Hidalgo – positive control

Can H5 LPAIVs in Australia productively replicate in chickens?

- Critical first step in the emergence of highly pathogenic avian influenza viruses.



H5 LPAIV clinical disease in chickens

ckn/Hidalgo – Positive control (swelling & oedema)

D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
	4	1	2	5	2								

Mild

turkey/VIC – HA-AUS (diarrhoea)

D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
		1							1				

Moderate

wild bird/QLD – HA-EA (diarrhoea)

D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
		4	1	1									

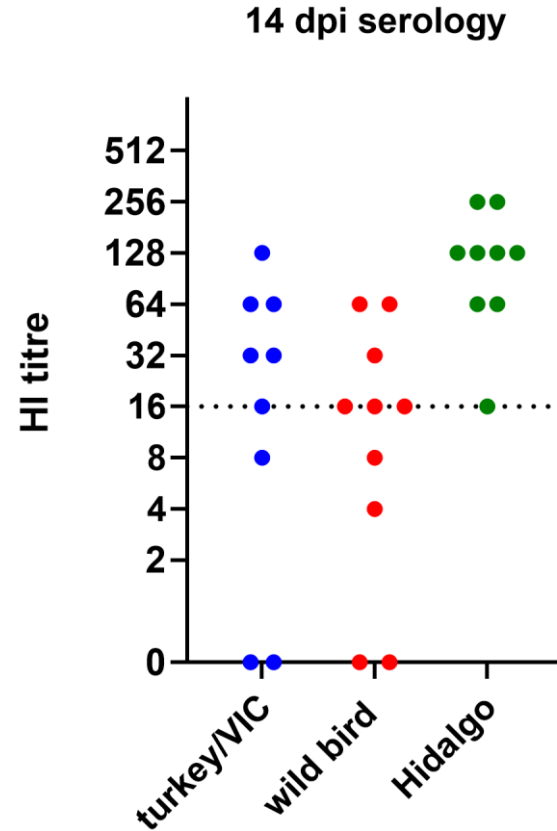
Moderate signs -
Humanely killed

Serological response

Hemagglutination inhibition assay

- **turkey/VIC:** 66% (6/9) birds seroconverted
- **wild bird/QLD:** 60% (6/10) birds seroconverted
- **ckn/Hidalgo:** All birds seroconverted

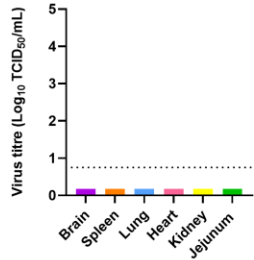
Australian H5 LPAIVs can infect and replicate in SPF chickens to a certain extent



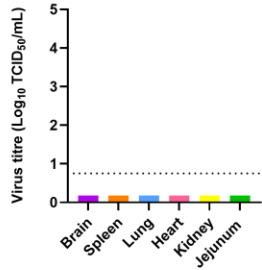
Virus replication – tissues and swabs

2 dpi

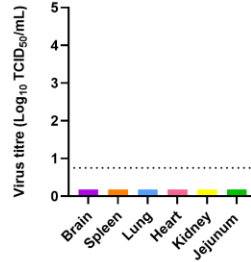
turkey/VIC tissues D2



wild bird/QLD tissues D2

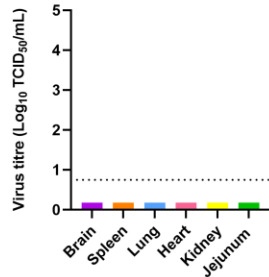


chicken/Hidalgo tissues D2

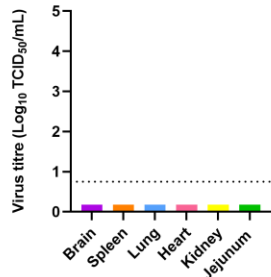


4 dpi

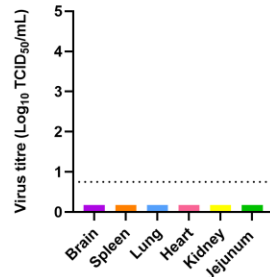
turkey/VIC tissues D4



wild bird/QLD tissues D4



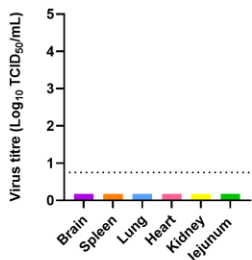
chicken/Hidalgo tissues D4



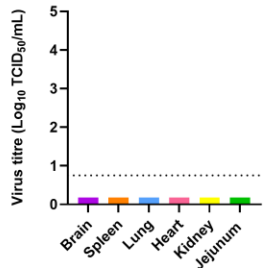
Virus replication – tissues and swabs

2 dpi

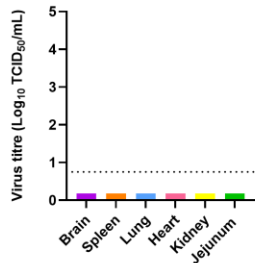
turkey/VIC tissues D2



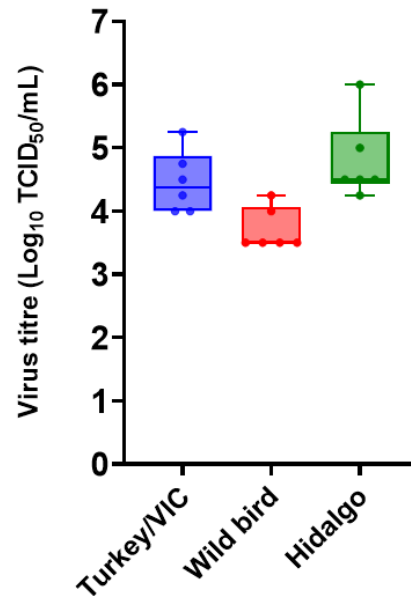
wild bird/QLD tissues D2



chicken/Hidalgo tissues D2

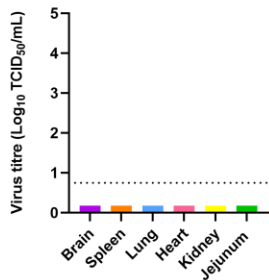


3 dpi cloacal swabs

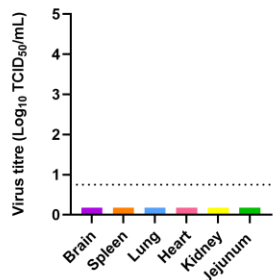


4 dpi

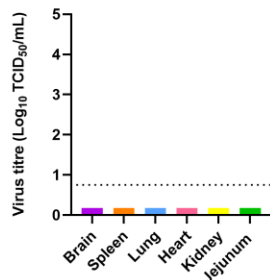
turkey/VIC tissues D4



wild bird/QLD tissues D4



chicken/Hidalgo tissues D4



In vivo characterisation

- Conclusions and summary:
 - Australian H5 LPAIVs can infect and productively replicate in chickens.
 - Most chickens seroconverted following challenge with Australian H5 LPAIVs.
 - Australian H5 LPAIVs can cause mild clinical disease in chickens.
 - Australian H5 LPAIV inoculated chickens can shed virus in the absence of clinical disease signs.
 - Preliminary assessment of virus replication in tissues indicate limited replication, with virus titres below the limit of assay detection in all tissues examined to date.
 - Histopathology and IHC of virally infected tissues pending & sequencing of virally infected tissues pending.

Can Australian H5 LPAIVs
support the genesis of high
pathogenicity?

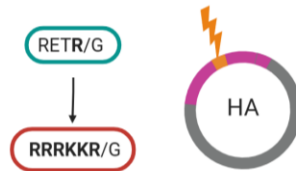
Can Australian H5 LPAIVs support the genesis of high pathogenicity?

Influenza reverse genetics:

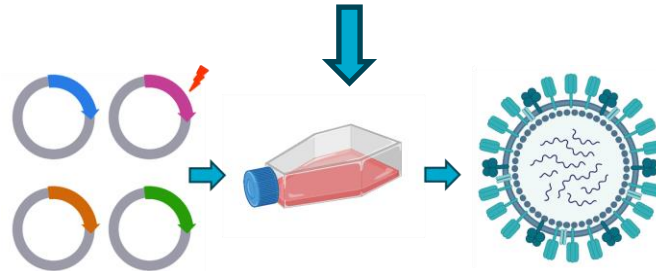
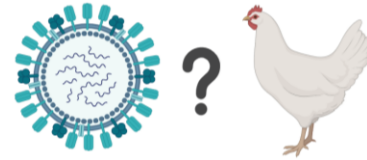
1. Generate reverse genetics viruses



2. Mutagenesis of cleavage site



3. Assess pathogenicity in chickens



Reverse genetics - molecular cloning of H5 LPAIVs

		PB2	PB1	PA	HA	NP	NA	M	NS
turkey/VIC H5N2 AUS-lineage HA	WT virus	✓	✓	✓	✓	✓	✓	✓	✓
	RKKR cleavage site mutant				RKKR cleavage site ✓				
	RRRKKR cleavage site mutant				RRRKKR cleavage site ✓				
wild bird/QLD H5N3 EA-lineage HA	WT virus	✓	✓	✓	✓	✓	✓	✓	✓
	RKKR cleavage site mutant				RKKR cleavage site ✓				
	RRRKKR cleavage site mutant				RRRKKR cleavage site ✓				

Can Australian H5 LPAIVs convert to HPAIV?

- Conclusions and summary:
 - Significant progress in the development of two Australian H5 LPAIV reverse genetics systems has been achieved.
 - Recombinant plasmids modified to encode the haemagglutinin cleavage site motif has been completed.
 - These systems will enable the evaluation of the potential for Australian H5 LPAIVs to convert to highly pathogenic forms.
 - Studies to commence second half of 2026.

Key findings on Australian H5 LPAIVs

- Australian H5 LPAIVs possessing an Australian HA-lineage, but not those with an EA-lineage HA, require exogenous trypsin for efficient replication *in vitro*.
- NP antigen present epithelium of embryos infected with H5 LPAIVs – consistent with LPAIVs.
- AUS HA-lineage and AUS-EA HA-lineage H5 LPAIVs are capable of infecting chickens.
- Australian H5 LPAIVs (AUS and EA haemagglutinin lineage) cause mild clinical disease in SPF chickens.
- Preliminary assessment of virus replication in tissues indicates limited replication, with virus titres remaining below the limit of assay detection.

Key findings on Australian H5 LPAIVs

- Australian H5 LPAIVs possessing an Australian HA-lineage, but not those with an EA-lineage HA, require exogenous trypsin for efficient replication *in vitro*.
- NP antigen present epithelium of embryos infected with H5 LPAIVs – consistent with LPAIVs.
- AUS HA-lineage and AUS-EA HA-lineage H5 LPAIVs are capable of infecting chickens.
- Australian H5 LPAIVs (AUS and EA haemagglutinin lineage) cause mild clinical disease in SPF chickens.
- Preliminary assessment of virus replication in tissues indicates limited replication, with virus titres remaining below the limit of assay detection.

Industry relevant outcomes

- Enhance poultry industry preparedness and resilience through a deeper understanding of the risk profile of Australian-lineage H5 LPAIVs.
- Guide optimal sampling strategies for early detection of H5 HPAIV.
- Enhanced understanding of biosecurity threats.

Acknowledgements

Ms Mia Campling

Research Technician – Avian Infectious Diseases Research

Dr Frank Wong

CSIRO ACDP WOAHA Avian Influenza Reference Laboratory Expert

NAIWB

Avian Infectious Diseases Research Team:

Maddy Belfrage, Matthew Smallridge

Animal Studies Team:

Teegan Allen, Tash Dollak, Grace Taylor, Elisha Soldani, Meg Stanley

Pathology and Pathogenesis Team:

Anjana Karawita, Richard Ploeg

Sequencing and Agent Characterisation:

Matt Neave, Vicky Stevens, Pat Mileto

Leanne McNabb – Diagnostic Serology

Rasan Mohamed Sathiqu