Infection dynamics of novel influenza A viruses isolated from Australian pigs

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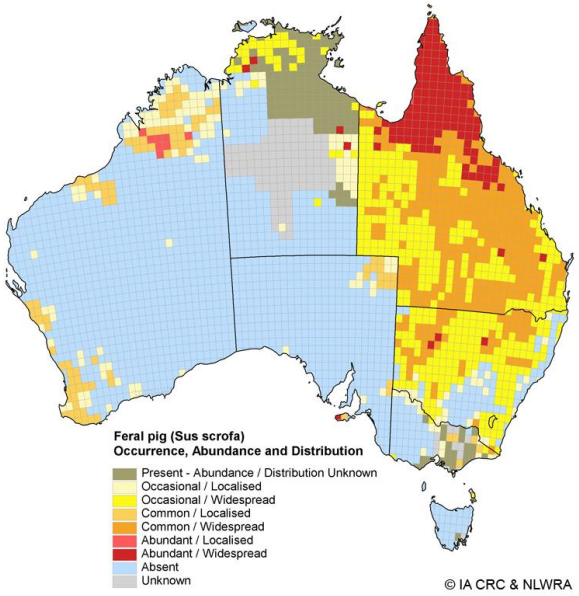
AUSTRALIAN ANIMAL HEALTH LABORATORY AND UNIVERSITY OF QUEENSLAND

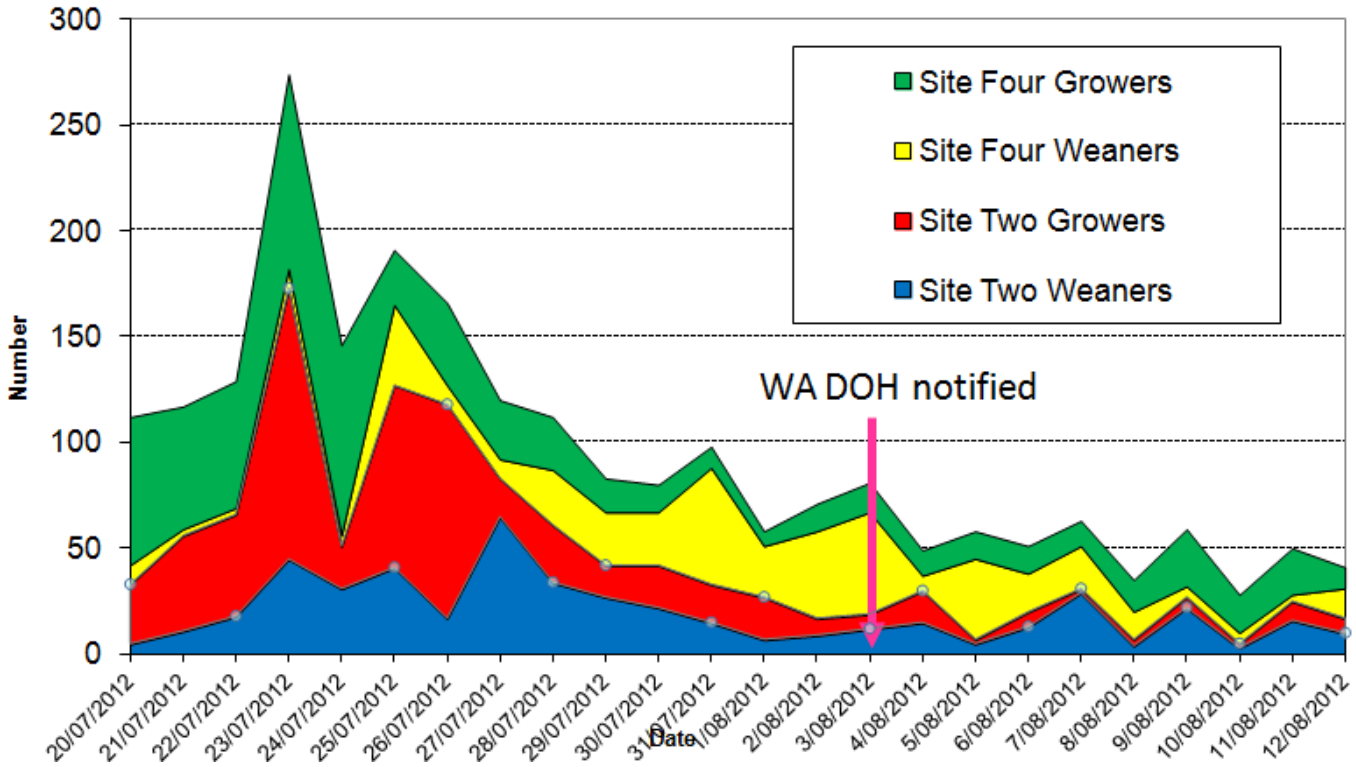
Oceania surveillance update

- Overall, SIV surveillance in Oceania has been limited
- In 2013, AAHL performed limited opportunistic surveillance for SIV in pig slaughterhouses in Laos.
- At least three reviews have been published from the Pacific islands in the past year outlining that influenza in pigs is acknowledged as an issue, but that surveillance is limited
- In Australia, serosurvey is being carried out in feral pigs in South Australia and there is potential for expansion for surveillance in feral pigs in Northern Australia



Piggery outbreak in Western Australia, July 2012

On 14 July 2012, an outbreak of respiratory disease occurred in a large scale piggery operation in Western Australia.



Smith et al 2012

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Piggery outbreak in Western Australia, July 2012

- All age groups (weaners, growers, finishers, sows and suckers)
- Post mortem: Fibrinous peritonitis, pleurisy, pericarditis and bronchopneumonia
- *Haemophilus parasuis, Pasteurella aerogenes* and *Streptococcus suis* were isolated from necropsy
- Immunohistochemical Influenza A antigen staining was positive in consolidated lung tissue
- All were serologically positive for IgG antibodies against influenza A

Effler et al 2012

Outbreak in Western Australia : Genome Sequencing

Genome constellations of reassortant Influenza A viruses found in Australian pigs:

	GENE SEGMENT							
INFLUENZA ISOLATE	**PB2**	**PB1**	**PA**	**HA**	**NP**	**NA**	**MP**	**NS**
A/swine/WA/2111/2012(H1N2)	pH1N1	pH1N1	pH1N1	huH1N1 (1983)	pH1N1	huH3N2 (1971)	pH1N1	pH1N1
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A/swine/WA/2577766G/2012 (H3N2)	huH3N2 (1970+)	huH3N2 (1970+)	huH1N1 (1983)	huH3N2 (1970+)	huH3N2 (1970+)	huH3N2 (1970+)	huH3N2 (1970+)	huH3N2 (1970+)

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Investigation by Public Health Authorities

- At the time of sero-survey only 9 piggery workers had not received the seasonal influenza vaccine 2 weeks prior.

- Thus inferences of seroconversion to the isolates could not be confirmed.

- Of vaccinated workers,

43% had positive titres to rH1N2

77% had positive titres to rH3N2

Effler et al 2012

Piggery outbreak in Queensland, August 2012

Genome constellations of reassortant Influenza A viruses found in Australian pigs

	GENE SEGMENT							
INFLUENZA ISOLATE	**PB2**	**PB1**	**PA**	**HA**	**NP**	**NA**	**MP**	**NS**
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A/swine/QLD/2476-6/2012 (H1N2)	pH1N1	pH1N1	pH1N1	huH1N1 (1996)	pH1N1	H3N2 (2003)	pH1N1	pH1N1

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Aims of this project

1. Determine **infectivity and growth characteristics** of the novel Australian SIV isolates *in vitro*

2. Characterise the **extent of disease** caused by novel Australian influenza A viruses in **ferrets**; as a human analogue

3. Characterise **the extent of disease** caused by novel Australian influenza A viruses in **pigs**

4. Investigate **molecular determinants of infectivity and pathogenicity** of novel Australian influenza A viruses

Research Question 1:

What are the infectivity and growth characteristics of the novel Australian SIV isolates?

- Growth curve kinetics
- Comparison of continuous cell line cultures for the diagnostic evaluation of Australian SIVs
- Solid phase receptor binding assay

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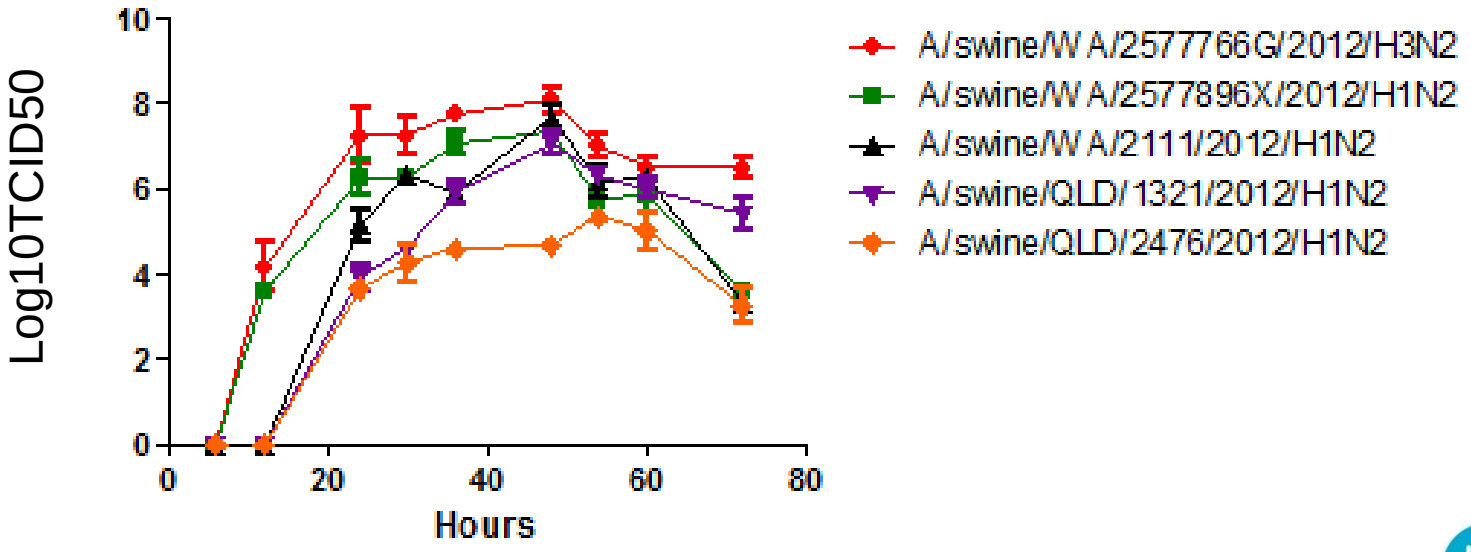
Growth curve kinetics of novel Australian SIVs

- MDCK cells were infected with Multiplicity of Infectivity (MOI) 0.001 of each virus

- TCID50 titration in MDCK cells carried out at time points 6, 12, 24, 30, 36, 48, 54, 60, 72hr



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Comparison of continuous cell line cultures for diagnostic evaluation of novel Australian Swine Influenza A viruses

Mammalian Cell lines used:					
Cell line	**Species**	**Cell type**	**Cell line**	**Species**	**Cell type**
LLC-PK1	Porcine	Kidney	ST	Porcine	Testicle
3D4/21	Porcine	Lung macrophage	CACO-2	Human	Colon
PK15a	Porcine	Kidney	MDCK	Canine	Kidney
DF1	Chicken	Embryo-fibroblast			

Measurement outcomes:

1. Cytopathic effect scoring daily

2. Haemagglutination assay titres using washed 0.5% chicken erythrocytes

3. Live virus titres (TCID50)

4. Quantitative RT-PCR

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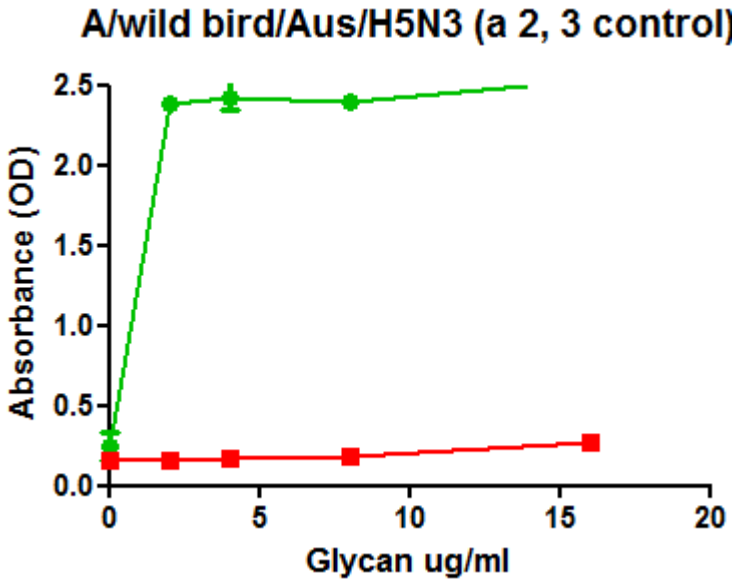
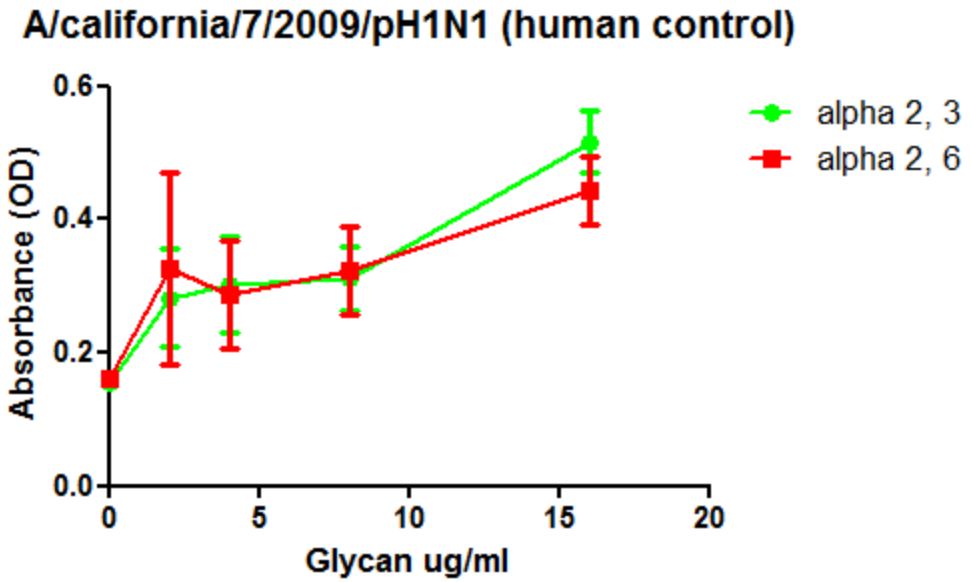
Summary of results

Swine origin influenza virus	Cell line: LLCPK1 (Porcine)	PK15a (Porcine)	ST (Porcine)	C/3D4 (Porcine)	DF1 (Chicken)	CACO2 (Human)	MDCK (Canine)
A/swine/WA/2111/2012 (H1N2)							
A/swine/WA/2012/2577896X (H1N2)							
A/swine/WA/2577766G/2012 (H3N2)							
A/swine/QLD/1321/2012 (H1N2)							
A/swine/QLD/2476-6/2012 (H1N2)							

Green = Haemagglutination activity, TCID50 positive result, CT score <25

Sialic acid receptor binding preferences of Australian SIVs





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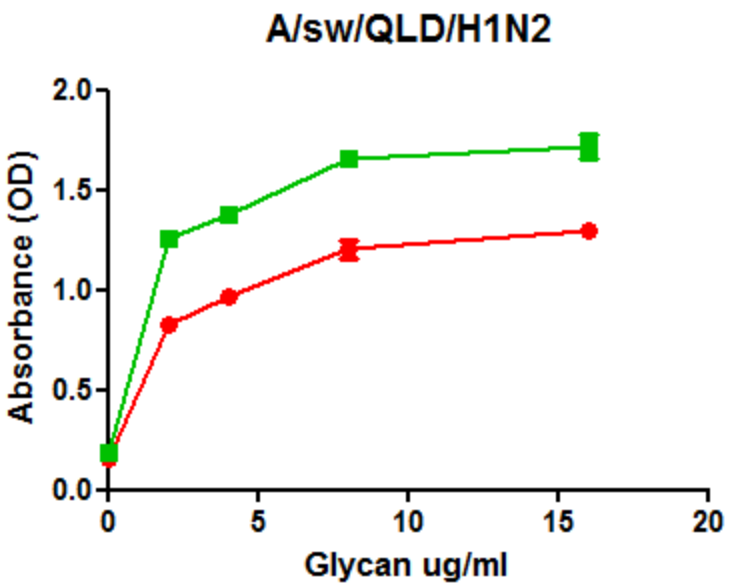

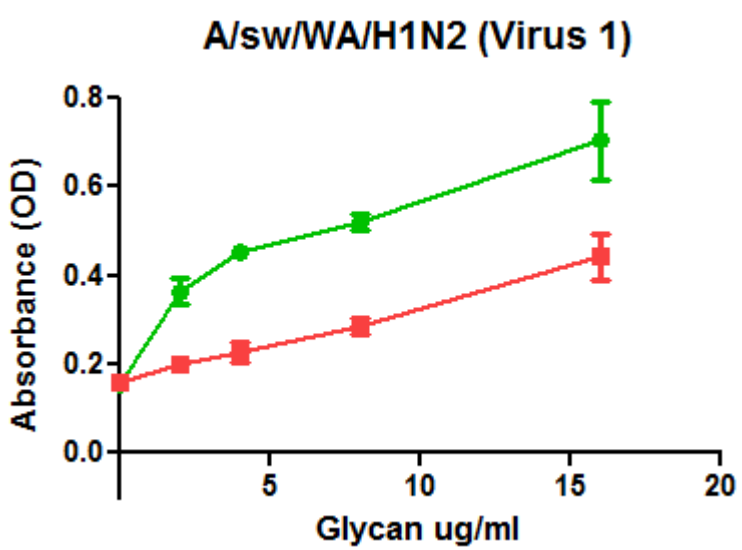

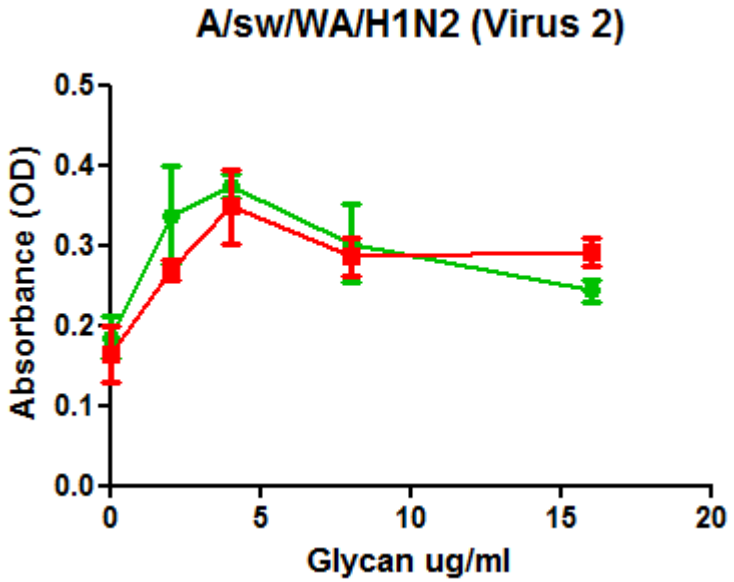

alpha 2, 3

alpha 2, 6

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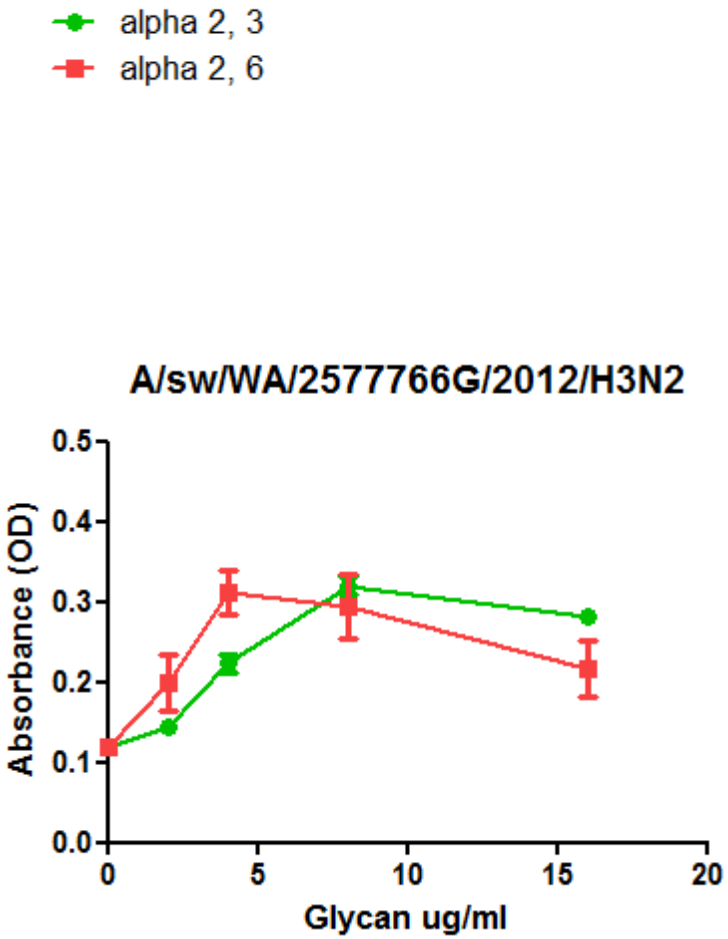
Sialic acid receptor binding preferences of Australian SIVs



Characterisation of two novel Australian Influenza A viruses :

A/swine/Western Australia/2012/H3N2

A/swine/Queensland/2012/H1N2

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Research question 2: Are the novel Australian swine influenza A viruses a risk to humans?

Objectives:

- Determine whether experimentally infected ferrets **clinical signs** such as weight loss and dyspnoea
- Determine **virus shedding** over days 1-10 post infection
- Determine **gross and histopathological change** in infected ferrets at days 1, 3 and 5 post infection
- Determine **tissue tropism of virus** at days 1, 3, 5 post infection using immunohistochemistry and isolation

In FERRETS, what is the extent of disease caused by novel Australian swine influenza A viruses?

Clinical signs included:

- Fever (>39.7°C)
- Lethargy
- Sneezing
- Nasal discharge
- Open mouth breathing
- Abdominal effort when breathing
- Puffy eyes
- Weight loss

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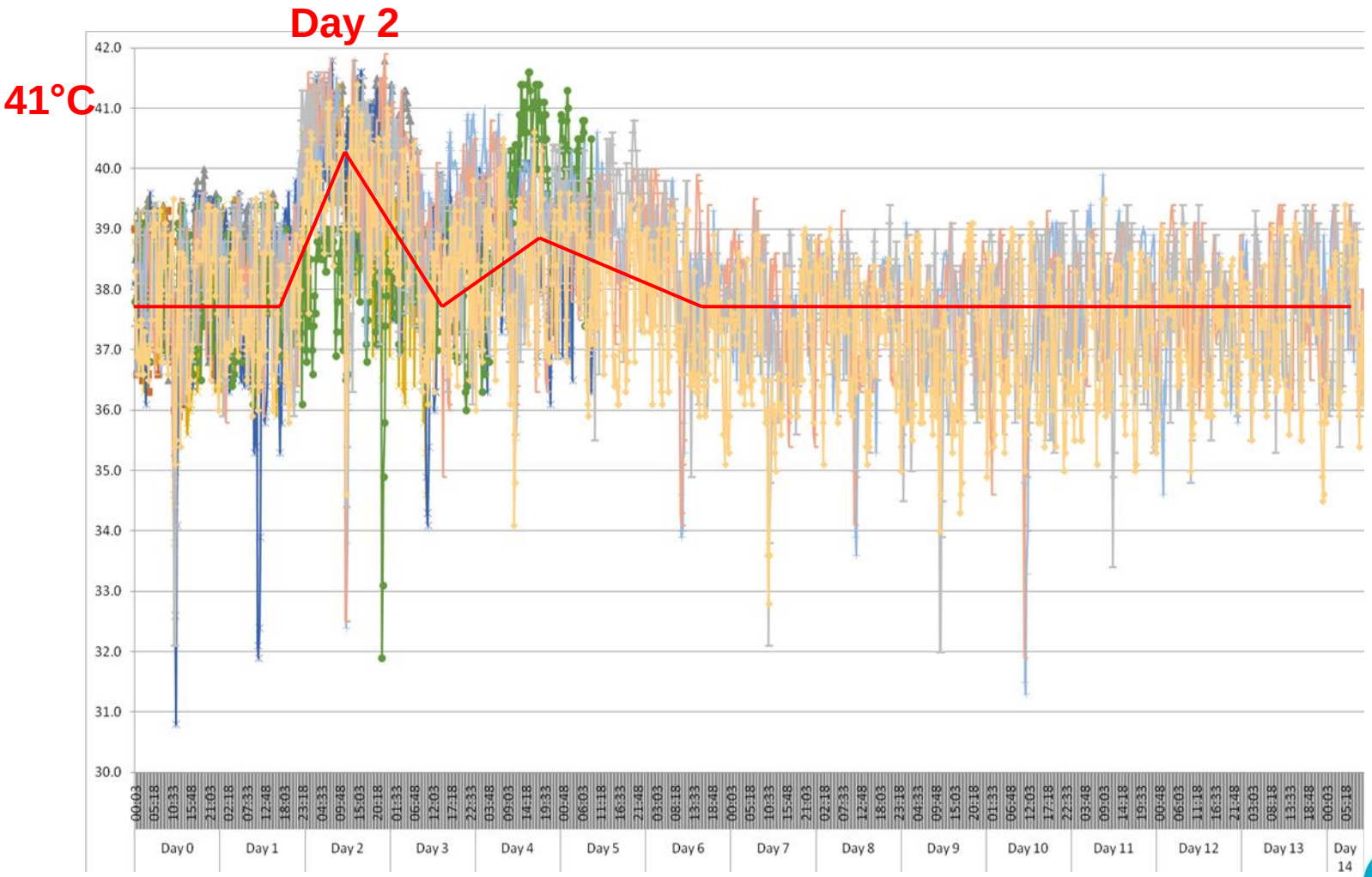
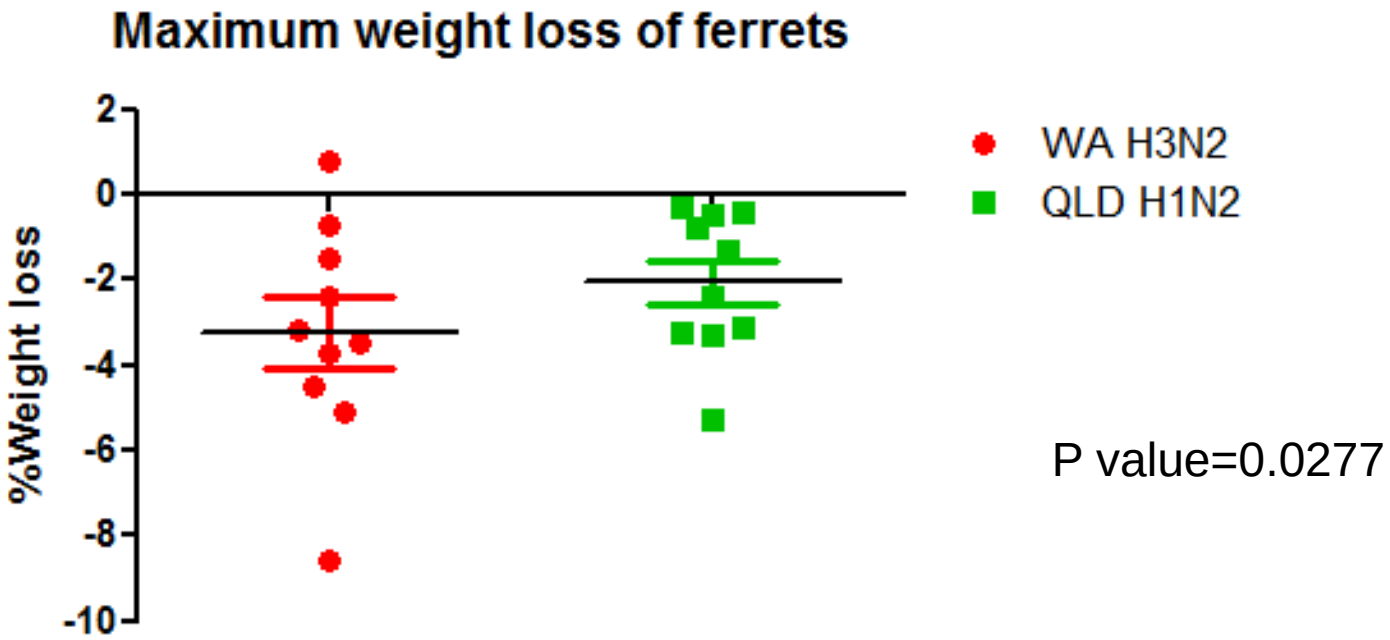
Clinical signs: Fever

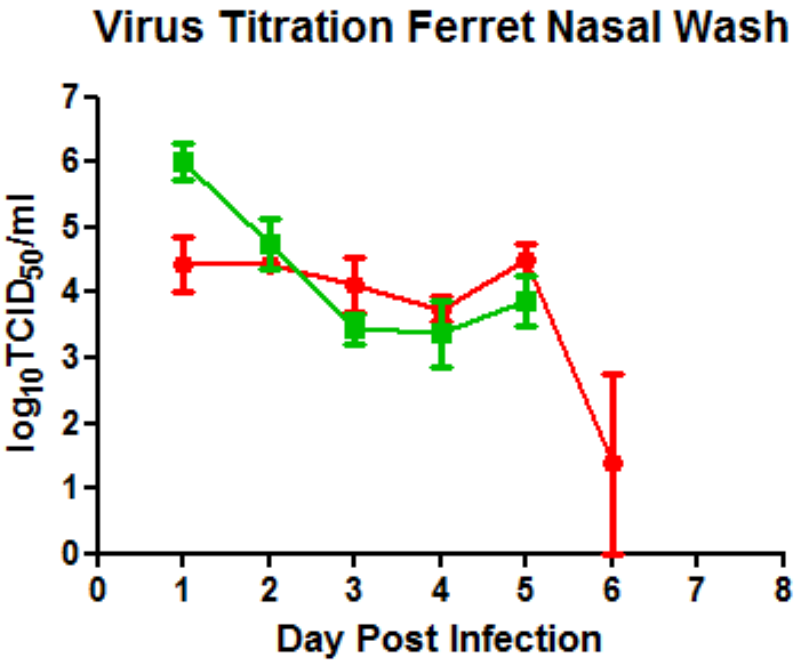


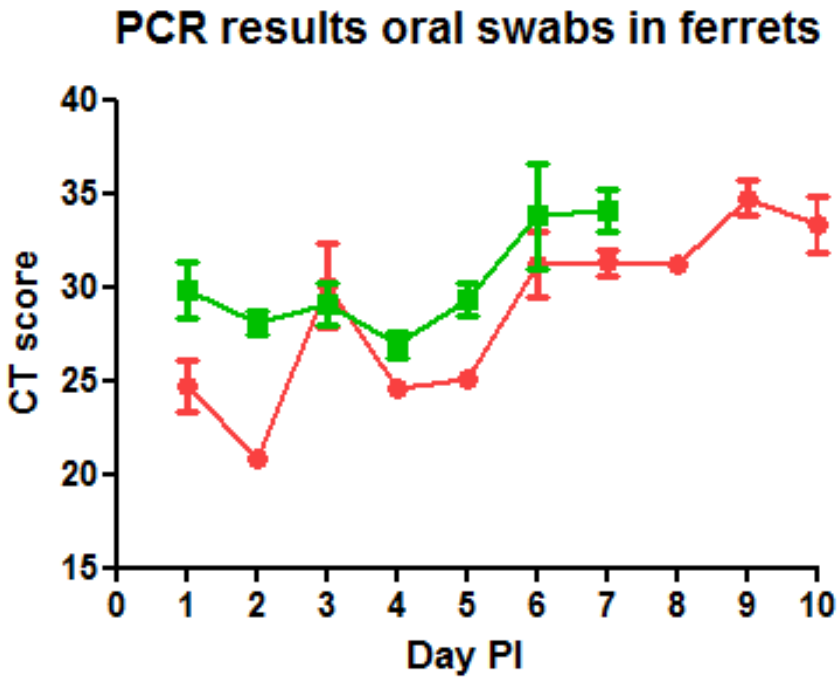
Clinical signs: Weight loss



Virus Shedding

A/sw/WA/H3N2
A/sw/QLD/H1N2





Summary of ferret trial virology results:

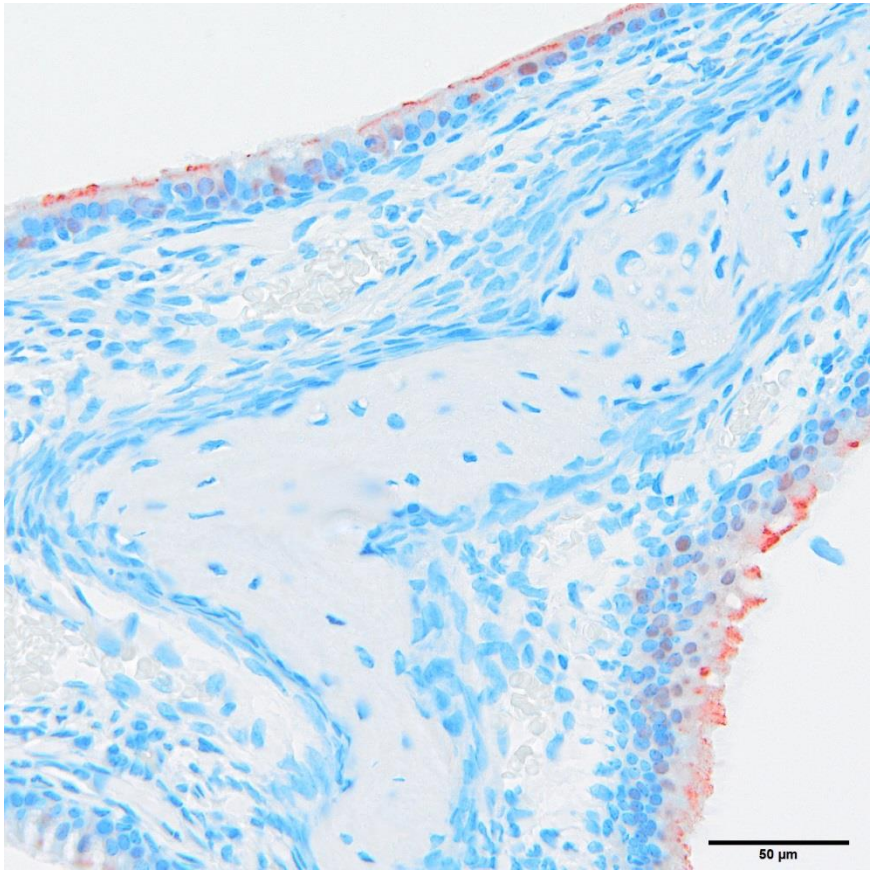
- Shedding results in nasal, oral and rectal swabs very similar to pigs, for both **H1N2 and H3N2**, with positive virus isolation from nasal and oral swabs

- **H3N2** viral RNA was detected by RT-PCR in cerebrum, heart, liver, kidney, lung, trachea and retropharangeal lymph nodes from days 1 to 5

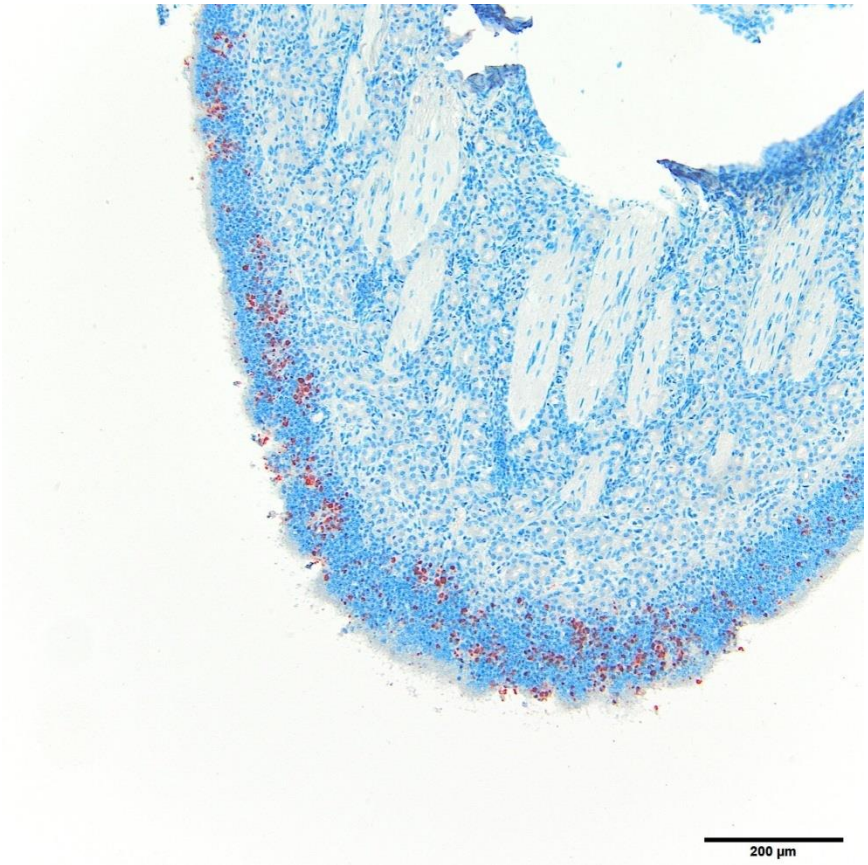
- **H1N2** viral RNA was only detected in tissues from the respiratory tract

- Positive **virus isolation** of both **H1N2 and H3N2** in trachea and lungs on days 1, 3 and 5, heart on day 3

Histopathology and immunohistochemistry



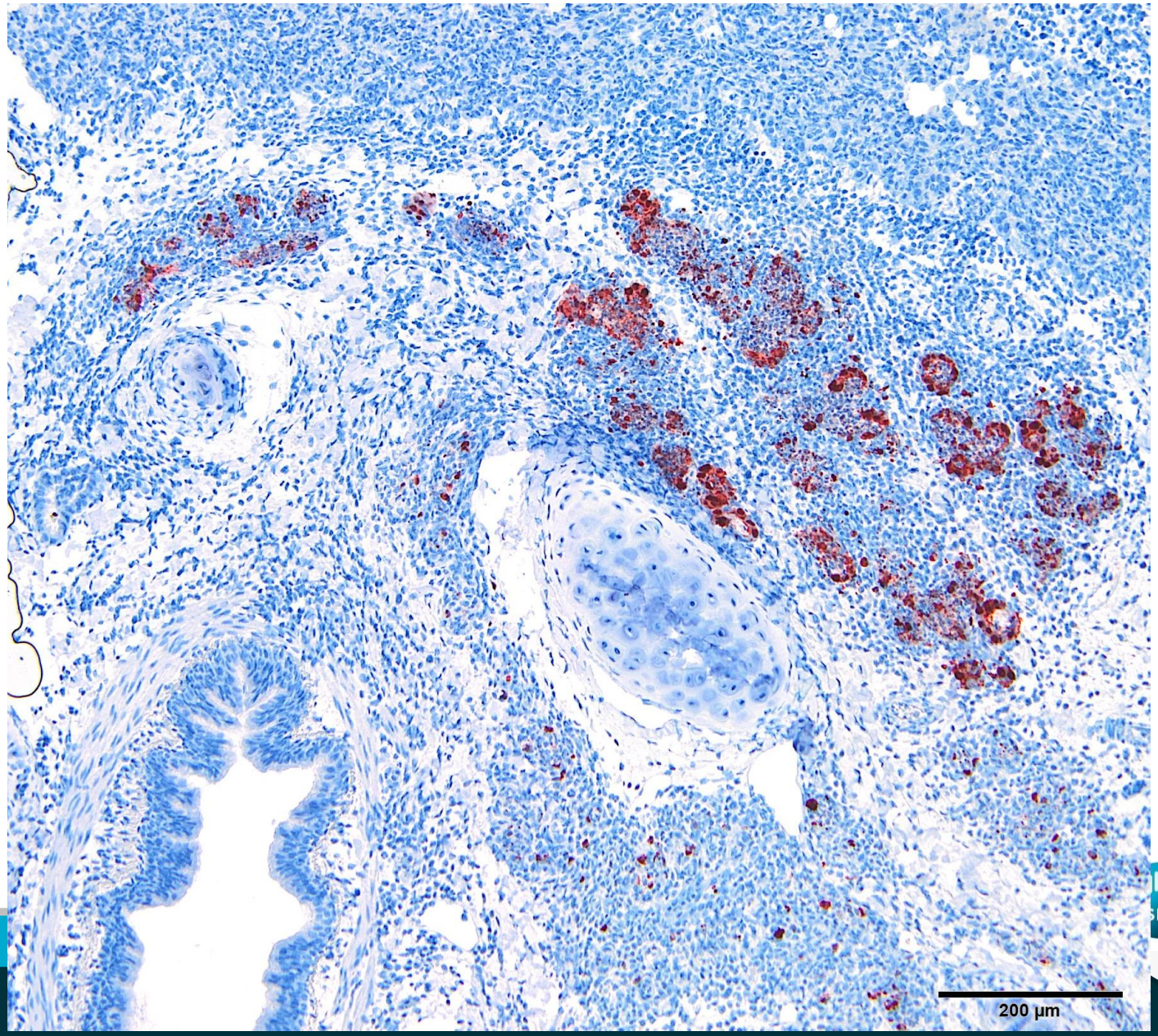
Nasal turbinates Ferret H3N2 infection Day 1



Nasal turbinates Ferret H1N2 infection Day 5

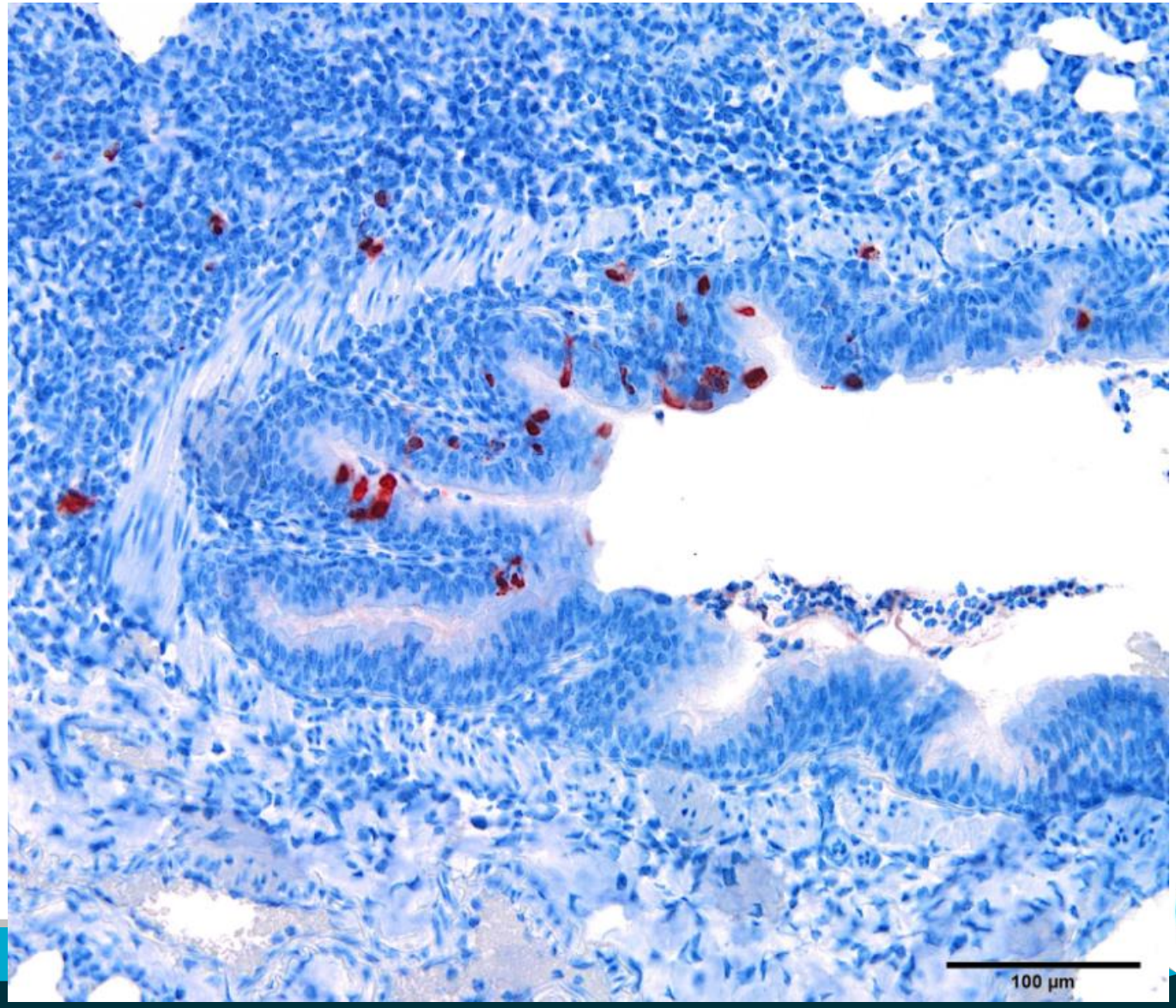
Histopathology and immunohistochemistry

H3N2 Day 5
Lung tissue



Histopathology and immunohistochemistry

H3N2 Day 5
Lung tissue



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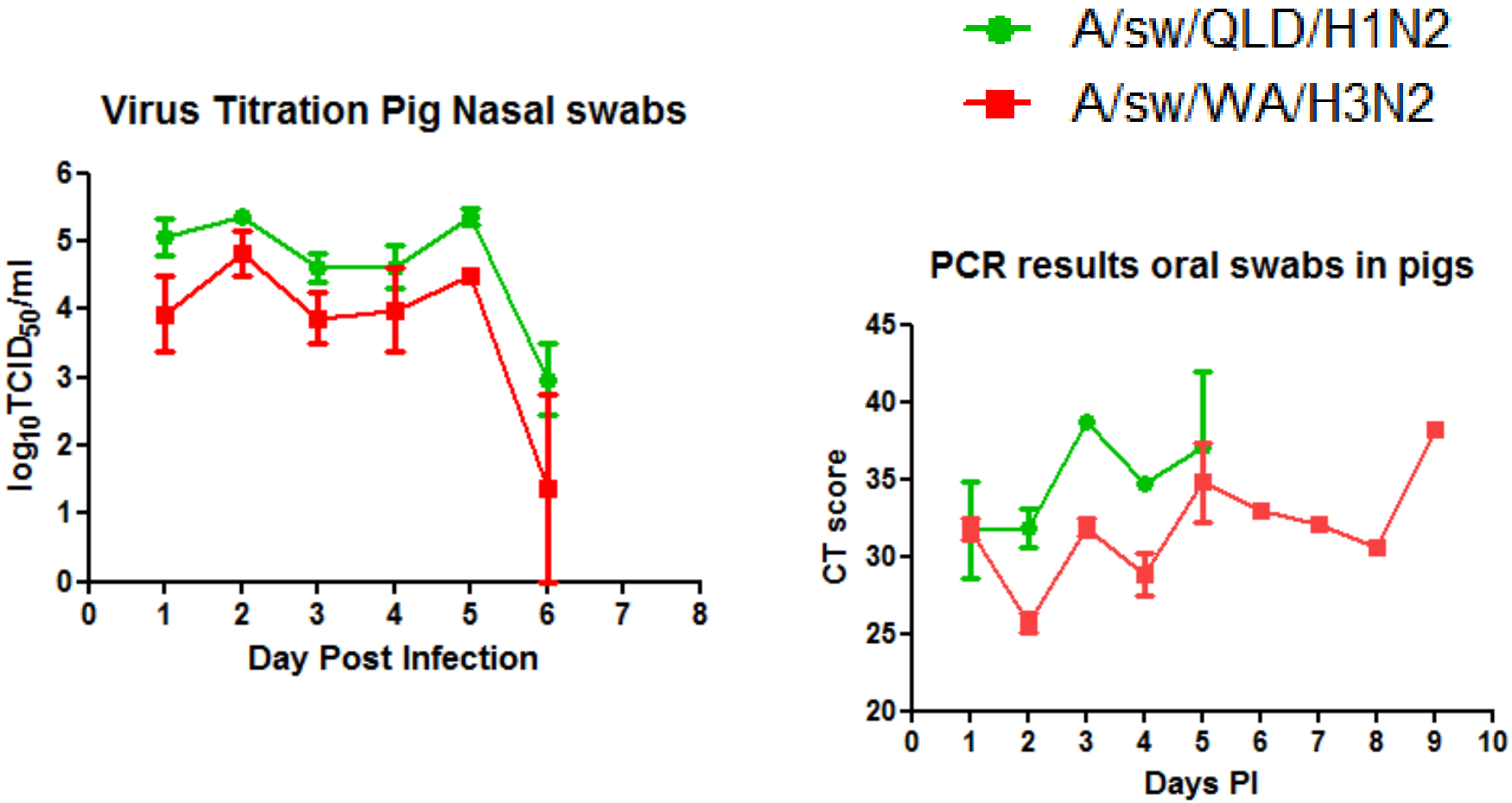
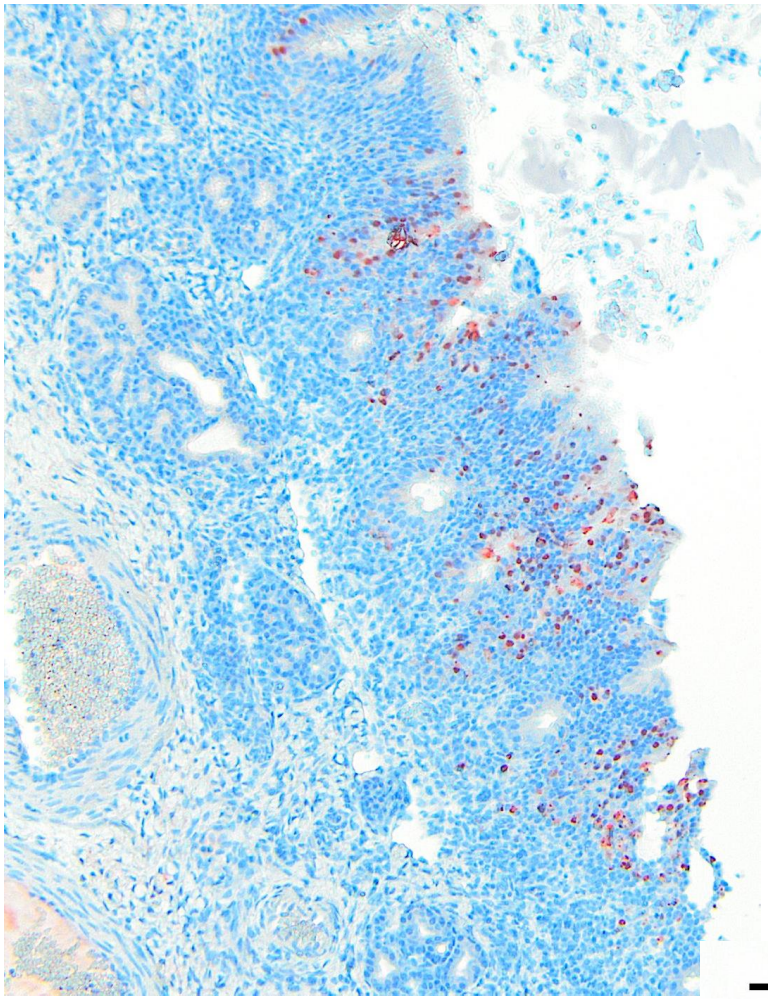
What about pigs?



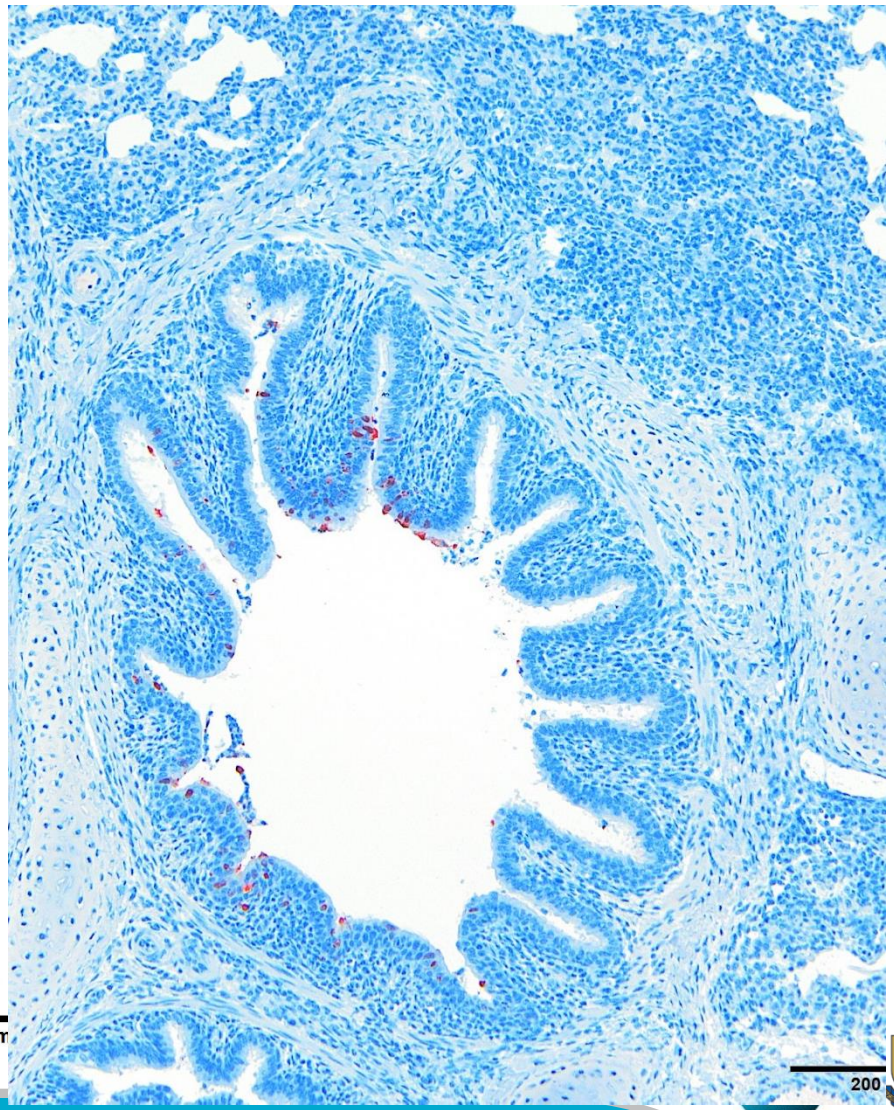
In PIGS, what is the extent of disease caused by novel Australian swine influenza A viruses?

- Pigs infected with **H1N2** virus did not show clinical signs
- Pigs infected with **H3N2** virus displayed clinical signs of varying severity from day 4, likely due to bacterial infection
- Both **H1N2 and H3N2** viruses **positive PCR detection** in lungs, trachea, heart, kidney, cerebrum, bronchial lymph nodes days 1-5
- Both **H1N2 and H3N2** viruses were **isolated** from lungs, trachea, tonsil, bronchial lymph nodes on days 3 and 5 using MDCK cells

Histopathology and immunohistochemistry

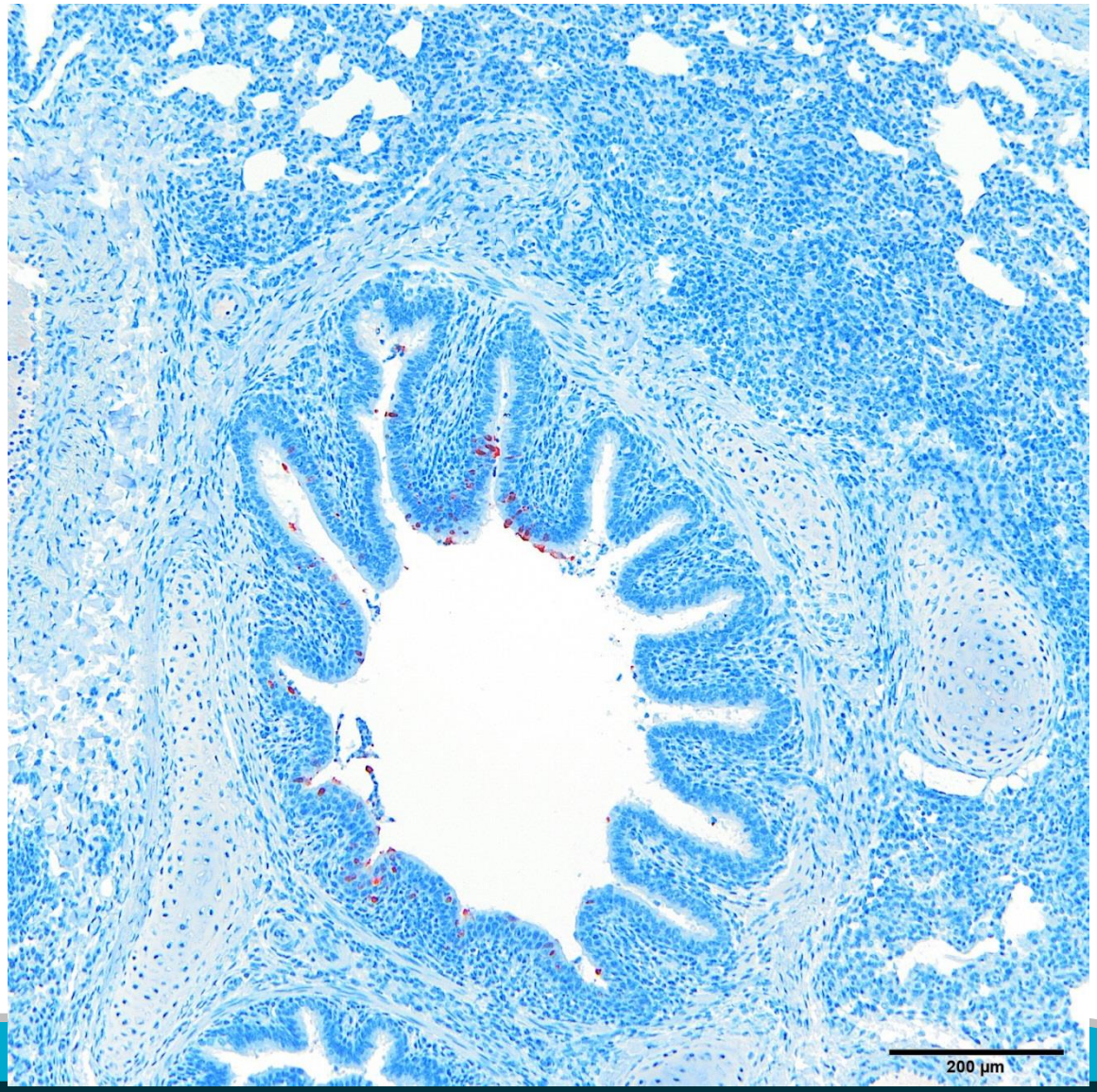


H3N2 Day 3 Nasal turbinates



Histopathology and immunohistochemistry

H3N2 Day 5
Lung tissue



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Evidence suggests that these viruses are zoonotic, and that Australia is at risk for commercial pigs to act as silent mixing vessels for human and animal influenza

Future Objectives:

- Assess the **immune response** of ferrets and pigs at days 1, 3, 5 and 14 post infection
- Comparison of growth in different continuous **cell lines** (human, porcine, avian, MDCK)
- **Molecular determinants of virus fitness in pigs?**

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Surveillance in Australia?

- There is no surveillance being carried out in domestic pigs in Australia

- Currently liaising with the Australian Animal Health Committee and Melbourne WHO centre for Influenza Collaboration to begin surveillance in domestic pigs and piggery workers

Thank you!

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Swine influenza outbreaks in Australia 2012

Genome constellations of reassortant Influenza A viruses found in Australian pigs:

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