Infection dynamics of novel influenza A viruses isolated from Australian pigs

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3rd December 2015 OFFLU Swine Influenza meeting

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

Smith et al 2012
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:

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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

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

### Mammalian Cell lines used:

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Species</th>
<th>Cell type</th>
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</tr>
</thead>
<tbody>
<tr>
<td>LLC-PK1</td>
<td>Porcine</td>
<td>Kidney</td>
<td>ST</td>
<td>Porcine</td>
<td>Testicle</td>
</tr>
<tr>
<td>3D4/21</td>
<td>Porcine</td>
<td>Lung macrophage</td>
<td>CACO-2</td>
<td>Human</td>
<td>Colon</td>
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<tr>
<td>PK15a</td>
<td>Porcine</td>
<td>Kidney</td>
<td>MDCK</td>
<td>Canine</td>
<td>Kidney</td>
</tr>
<tr>
<td>DF1</td>
<td>Chicken</td>
<td>Embryo-fibroblast</td>
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### 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
## Summary of results

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<th>Swine origin influenza virus</th>
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Green = Haemagglutination activity, TCID50 positive result, CT score <25
Sialic acid receptor binding preferences of Australian SIVs
Sialic acid receptor binding preferences of Australian SIVs

A/sw/QLD/H1N2

Absorbance (OD)

0.0 0.5 1.0 1.5 2.0

Glycan ug/ml

0 5 10 15 20

A/sw/WA/H1N2 (Virus 1)

Absorbance (OD)

0.0 0.2 0.4 0.6 0.8

Glycan ug/ml

0 5 10 15 20

A/sw/WA/H1N2 (Virus 2)

Absorbance (OD)

0.0 0.1 0.2 0.3 0.4 0.5

Glycan ug/ml

0 5 10 15 20

- green: alpha 2, 3
- red: alpha 2, 6
Sialic acid receptor binding preferences of Australian SIVs

- **alpha 2, 3**
- **alpha 2, 6**

**Graph:**
- **A/sw/WA/2577766G/2012/H3N2**

- **Absorbance (OD)**

- **Glycan ug/ml**

- **Graph Data Points:**
  - Beta 2, 3 (green line)
  - Beta 2, 6 (red line)

**Legend:**
- **Alpha 2, 3**
- **Alpha 2, 6**
Characterisation of two novel Australian Influenza A viruses:

A/swine/Western Australia/2012/H3N2
A/swine/Queensland/2012/H1N2
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
Clinical signs: Fever

Day 2

41°C
Clinical signs: Weight loss

Maximum weight loss of ferrets

- WA H3N2
- QLD H1N2

P value = 0.0277
Virus Shedding

Virus Titration Ferret Nasal Wash

PCR results oral swabs in ferrets
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
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
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?**
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

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