The influenza A viruses of swine in Japan, Thailand, and Vietnam

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IAV-S surveillance in Japan in 2015

1. Active surveillance has just been started in Sep. 2015.

<table>
<thead>
<tr>
<th>Province</th>
<th>Number of farm</th>
<th>Number of nasal swab</th>
<th>Virus isolation</th>
<th>Sampling date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hokkaido</td>
<td>4</td>
<td>214</td>
<td>1</td>
<td>23rd ~ 29th Oct.</td>
</tr>
<tr>
<td>Gunma</td>
<td>7</td>
<td>210</td>
<td>6</td>
<td>10th ~ 30th Nov.</td>
</tr>
<tr>
<td>Chiba</td>
<td>1</td>
<td>80</td>
<td>2</td>
<td>29th Sep ~ 24th Nov.</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12</strong></td>
<td><strong>504</strong></td>
<td><strong>9</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Virus isolation and sequencing analysis are in progress.

2. Passive surveillance

<table>
<thead>
<tr>
<th>Province</th>
<th>Number of sample submitted</th>
<th>Month</th>
<th>Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kagoshima</td>
<td>1</td>
<td>May</td>
<td>H1N1(pdm09v)</td>
</tr>
</tbody>
</table>

Provinces under survey in 2015

IAV-S occurrence in 2015

Genetic analysis
## Genetic characterization of the IAV-S in Kagoshima prefecture in Japan in 2015

<table>
<thead>
<tr>
<th>Gene segment</th>
<th>Highest identity with</th>
<th>Identities</th>
<th>Lineage</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>A/swine/Japan/KU-YG5/2013(H1N1)</td>
<td>1663/1701(98%)</td>
<td>pdm09</td>
</tr>
<tr>
<td>NA</td>
<td>A/swine/Japan/KU-YG5/2013(H1N1)</td>
<td>1390/1410(99%)</td>
<td>pdm09</td>
</tr>
<tr>
<td>PB2</td>
<td>A/Mexico/24024/2009(H1N1)</td>
<td>2234/2280(98%)</td>
<td>pdm09</td>
</tr>
<tr>
<td>PB1</td>
<td>A/Maryland/NHRC0001/2009(H1N1)</td>
<td>2230/2272(98%)</td>
<td>pdm09</td>
</tr>
<tr>
<td>PA</td>
<td>A/Singapore/DMS29/2009(H1N1)</td>
<td>2110/2151(98%)</td>
<td>pdm09</td>
</tr>
<tr>
<td>NP</td>
<td>A/swine/Thailand/UD402/2009(H1N1)</td>
<td>1490/1515(98%)</td>
<td>pdm09</td>
</tr>
<tr>
<td>MP</td>
<td>A/Mexico/24015/2009(H1N1)</td>
<td>972/982(99%)</td>
<td>pdm09</td>
</tr>
<tr>
<td>NS</td>
<td>A/Singapore/GP3021/2009(H1N1)</td>
<td>831/844(98%)</td>
<td>pdm09</td>
</tr>
</tbody>
</table>
Genetic characterization of the swine pdm09 viruses in Japan

Phylogenetic trees of H1 pandemic genes (532 taxa selected by CD-Hit)

1. Human-to-pig direct transmission of pdm09
Genetic characterization of the swine pdm09 viruses in Japan

Phylogenetic trees of H1 pandemic genes (532 taxa selected by CD-Hit)

2. Possibility of a maintenance of the A(H1N1)pdm09v among pigs for a certain period

A/Hungary/40/2009_H1N1_4_(HA)2009/10/26
  A/swine/Hiroshima/8/2011_H1N1_4_(HA)2011/01/15
  A/swine/Nanchang/F9/2010_H1N1_4_(HA)2010/01/13
  A/Athens/INS339/2009_H1N1_4_(HA)2009/12/30
  A/Maine/04/2011_H1N1_4_(HA)2011/02/17
    A/swine/Kagoshima/65/2012_H1N1_4_(HA)2012/01/15
    A/swine/Hong_Kong/4140/2011_H1N1_4_(HA)2011/10/20
  A/swine/Arkansas/SG1321/2009_H1N1_4_(HA)2009/12/28
  A/New_York/03/2010_H1N1_4_(HA)2010/02/09

Japanese swine pdm09v
• **Active surveillance** of the IAV-S in Japan has been started since September 2015.

• **Sporadic introductions** of A(H1N1)pdm09 viruses from human to pig have occurred since 2009.

• A(H1N1)pdm09 viruses could be maintained among pig populations for a certain.
IAV-S surveillance in Thailand

### Active surveillance in 2015

<table>
<thead>
<tr>
<th>Province</th>
<th>Number of farm</th>
<th>Number of nasal swab</th>
<th>Virus isolation</th>
<th>Subtype</th>
<th>Sampling date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chonburi</td>
<td>2</td>
<td>202</td>
<td>17</td>
<td>H3N2 (15 strains), A(H1N1)pdm09 (2 strains)</td>
<td>1\textsuperscript{st} July</td>
</tr>
<tr>
<td>Chachoengsao</td>
<td>2</td>
<td>202</td>
<td>1</td>
<td>H3N2</td>
<td>2\textsuperscript{nd} July</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4</strong></td>
<td><strong>404</strong></td>
<td><strong>18</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sequencing analysis is in progress.*
Genetic characterization of the IAV-S in Thailand

Phylogenetic trees of H1 pandemic genes (532 taxa selected by CD-Hit)

1. Establishment of three lineages of Thai swine pdm09 viruses

- After 2009

- Thai swine pdm09v
Phylogenetic trees of H3 genes (seasonal human–lineage)

After 1968

Thai H3N2 strains 2012-2014

Thai swine lineage

Thai swine strains 2015

Thai H3N2 strains 2005-2009

Triple reassortant H3

Thai H3N2 strains 2015

Swine Vietnam and China

A/Mexico/VER60/2011(H3N2)
A/California/16/2011(H3N2)
A/Bethesda/NIH12/2011(H3N2)
A/Nanjing/1655/2010(H3N2)
A/Florida/22/2009(H3N2)
A/Quebec/26-031205/2005(H3N2)
A/Mexico/DIF2662/2003(H3N2)
A/TW/872/02(H3N2)
A/Panama/2007/1999(H3N2)
A/swine/Iowa/H03LDH5/2003(H3N2)
A/TW/3446/02(H3N2)
A/Moscow/10/1999(H3N2)
A/swine/Tochigi/14/2013(H3N2)
A/swine/Brazil/365-11-7/2011(H3N2)
A/swine/Thailand/CU-S14252N/2014(H3N2)

Thai H3N2 strains 2012-2014

Thai swine lineage

Thai swine strains

Triple reassortant H3

Thai H3N2 strains 2005-2009

Swine Vietnam and China

A/Mexico/VER60/2011(H3N2)
A/California/16/2011(H3N2)
A/Bethesda/NIH12/2011(H3N2)
A/Nanjing/1655/2010(H3N2)
A/Florida/22/2009(H3N2)
A/Quebec/26-031205/2005(H3N2)
A/Mexico/DIF2662/2003(H3N2)
A/TW/872/02(H3N2)
A/Panama/2007/1999(H3N2)
A/swine/Iowa/H03LDH5/2003(H3N2)
A/TW/3446/02(H3N2)
A/Moscow/10/1999(H3N2)
A/swine/Tochigi/14/2013(H3N2)
A/swine/Brazil/365-11-7/2011(H3N2)
A/swine/Thailand/CU-S14252N/2014(H3N2)
• At least **three lineages of A(H1N1)pdm09 viruses** have been circulating in Thai pig population.

• **Thai H3N2 lineage possessing seasonal human-like surface antigens** has been circulating in Thai pig population since 2005.
Genetic and antigenic analysis of Vietnamese IAV–S
IAV–S surveillance in Vietnam

9584 nasal swabs were collected from clinically healthy pigs in 257 pig farms

Virus isolation using the floating MDCK cells and/or primary cultures of porcine alveolar epithelial cells

Genetic analysis: To determine the genetic origins of the HA genes.

Antigenic analysis (HI test): To examine the cross-reactivity between the Vietnamese IAV–S and human strains.
HA genes were genetically divided into 5 groups

Subtype:

- Pdm(H1N1)09v (47)
- H1N2 (75)
- H3N2 (153)

<table>
<thead>
<tr>
<th>Pdm09v H1</th>
<th>Pre-pandemic human-like H1</th>
<th>Classical swine H1</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>15</td>
<td>54</td>
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<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seasonal human-like H3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triple reassortant H3</td>
</tr>
</tbody>
</table>

Legends: Pdm09v H1, Pre-pandemic human-like H1, Classical swine H1, Seasonal human-like H3, Triple reassortant H3
Genetic characterization of the IAV-S in Vietnam

Phylogenetic trees of pandemic H1 genes

After 2009

H1N2 viruses
Genetic characterization of the IAV-S in Vietnam

Phylogenetic trees of H1 genes

Pre-pandemic human H1

Classical swine H1 (H1α)

Around 2006

Bac Ninh H1N2 strains in 2012
Bac Ninh H1N2 strain in 2013
Bac Ninh strain H1N2 in 2013
Dong Nai strain H1N2 in 2014
Dong Nai H1N2 strains in 2015

A/Malaysia/32110/2005(H1N1) @2005-07-29
A/Malaysia/34291/2006(H1N1) @2006-04-14
A/Taiwan/10393/2005(H1N1) @2005-12-28
A/Taiwan/N86/2006(H1N1) @2006-01-09
A/Taiwan/192/2006(H1N1) @2006-06-30
A/Taiwan/0586/2006(H1N1) @2006-02-14
A/New Jersey/09/2007(H1N1) @2007-01-23
A/Taiwan/2823/2008(H1N1) @2008-09-19
A/Taiwan/5505/2006(H1N1) @2006-01-04
A/HoChiMinh/HCM639/2006(H1N1) @2006-06-13
A/TayNguyen/TN158/2006(H1N1) @2006-07-05
A/HoChiMinh/HCM554/2006(H1N1) @2006-01-04
A/HaNoi/Q555/2006(H1N1) @2006-08-02
A/Taiwan/2900/2006(H1N1) @2006-01-13
A/Philippines/WRAIR1736P/2006(H1N1) @2006-01-01
A/Solomon Islands/3/2006(H1N1) @2006-08-21
A/Malaysia/1652509/2006(H1N1) @2006-09-27
A/Malaysia/1706215/2007(H1N1) @2007-01-05
A/Malaysia/35405/2006(H1N1) @2006-10-05

A/Swine/Binh Doung/02-16/2010 (H1N2)
A/Taiwan/10393/2005(H1N1) @2005
A/Taiwan/0586/2006(H1N1) @2005
A/New Jersey/09/2007(H1N1) @2007
A/Solomon Islands/3/2006(H1N1) @2006
A/Malaysia/1652509/2006(H1N1) @2006
A/Malaysia/35405/2006(H1N1) @2006
A/Taiwan/2823/2008(H1N1) @2008
A/Taiwan/5505/2006(H1N1) @2006
A/HoChiMinh/HCM639/2006(H1N1) @2006
A/TayNguyen/TN158/2006(H1N1) @2006
A/HoChiMinh/HCM554/2006(H1N1) @2006
A/HaNoi/Q555/2006(H1N1) @2006
A/Philippines/WRAIR1736P/2006(H1N1) @2006
A/Solomon Islands/3/2006(H1N1) @2006
A/Malaysia/1652509/2006(H1N1) @2006
A/Malaysia/35405/2006(H1N1) @2006
## Antigenic analysis of Vietnamese H1 IAV-S

<table>
<thead>
<tr>
<th>Virus</th>
<th>Pre-pandemic human-lineage virus</th>
<th>A(H1N1)pdm09 virus</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>VN H1N2 (2010)</td>
<td>&lt;10</td>
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<tr>
<td></td>
<td></td>
<td>1280</td>
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<tr>
<td></td>
<td>'01~'07 vaccine strain</td>
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<td>'09~'10 vaccine strain</td>
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<td></td>
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<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>VN H1N2 (2011)</td>
<td>640</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;10</td>
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<td></td>
<td>VN H1N2 (2011)</td>
<td>640</td>
</tr>
<tr>
<td></td>
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<td>20</td>
</tr>
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<td></td>
<td></td>
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<td>20</td>
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<tr>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>VN H1N2 (2012)</td>
<td>640</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;10</td>
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<tr>
<td></td>
<td></td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

† Homologous HI titers are indicated in bold and underlined.
**Genetic characterization of the IAV-S in Vietnam**

### Phylogenetic trees of H3 genes

#### Seasonal human-like H3

- **Dong Nai H3N2 strains in 2012–2015**
  - A/swine/Hong_Kong/2503/2011(H3N2)
  - A/swine/Hong_Kong/NS2439/2011 (H3N2)
  - A/swine/Guangxi/NS2783/2010 (H3N2)
  - A/swine/Guangxi/NS3086/2011 (H3N2)
  - A/swine/Guangxi/NS3106/2011 (H3N2)
  - A/swine/Guangxi/NS3108/2011 (H3N2)
  - A/swine/Guangxi/2803/2011 (H3N2)

- **Tien Giang H3N2 strains in 2012**
  - Swine Hong Kong strains

- **Dong Nai H3N2 strains in 2015**
  - Ho Chi Minh H3N2 strains in 2011
    - A/swine/Binh_Duong/03-14/2010_(H3N2)_2010-03-01
    - A/swine/Binh_Duong/03-10/2010_(H3N2)_2010-03-01
    - A/swine/Binh_Duong/03-13/2010_(H3N2)_2010-03-01
    - A/swine/Binh_Duong/03-06/2010_(H3N2)_2010-03-01
    - A/swine/Binh_Duong/03-09/2010_(H3N2)_2010-03-01
    - A/swine/Binh_Duong/03-08/2010_(H3N2)_2010-03-01

- **A/New_York/359/20052005-01-17**

#### Triple reassortant H3 (Cluster IV)

- **A/swine/Korea/PL01/2012(H3N2)_2012-04-23**
- **A/swine/Wuhan/1597/2012(H3N2)_2012-06-17**
- **A/swine/Manitoba/SG1431/2008(H3N2)_2008-06-17**
- **A/swine/Manitoba/SG1432/2008(H3N2)_2008-06-17**
- **A/swine/Korea/PL01/2012(H3N2)_2012-04-23**
- **A/swine/Hanoi/422/2013(H3N2)_2013-08-13**
- **A/swine/Hanoi/415/2013(H3N2)_2013-08-13**

- **Bac Ninh H3N2 strains in 2012**
  - Swine Hong Kong strains

- **Bac Ninh H3N2 strains in 2013–2015**
  - Bac Ninh H3N2 strains in 2012

- **North American TR strains in 2004–2007**
  - A/swine/QC/2108-2/2009(H3N2)_2009-04-03
  - A/swine/North_Carolina/A01202766/2011(H3N2)_2011-10-20
  - A/swine/Virginia/A01203436/2012(H3N2)_2012-04-16

**Around 2005**

**A/New_York/359/20052005-01-17**

**Genetic characterization of the IAV-S in Vietnam**

**Phylogenetic trees of H3 genes**

- **Seasonal human-like H3**
  - Dong Nai H3N2 strains in 2012–2015
    - A/swine/Hong_Kong/2503/2011(H3N2)
    - A/swine/Hong_Kong/NS2439/2011 (H3N2)
    - A/swine/Guangxi/NS2783/2010 (H3N2)
    - A/swine/Guangxi/NS3086/2011 (H3N2)
    - A/swine/Guangxi/NS3106/2011 (H3N2)
    - A/swine/Guangxi/NS3108/2011 (H3N2)
    - A/swine/Guangxi/2803/2011 (H3N2)

- **Tien Giang H3N2 strains in 2012**
  - Swine Hong Kong strains

- **Dong Nai H3N2 strains in 2015**
  - Ho Chi Minh H3N2 strains in 2011
    - A/swine/Binh_Duong/03-14/2010_(H3N2)_2010-03-01
    - A/swine/Binh_Duong/03-10/2010_(H3N2)_2010-03-01
    - A/swine/Binh_Duong/03-13/2010_(H3N2)_2010-03-01
    - A/swine/Binh_Duong/03-06/2010_(H3N2)_2010-03-01
    - A/swine/Binh_Duong/03-09/2010_(H3N2)_2010-03-01
    - A/swine/Binh_Duong/03-08/2010_(H3N2)_2010-03-01

- **A/New_York/359/20052005-01-17**

**Triple reassortant H3 (Cluster IV)**

- **A/swine/Korea/PL01/2012(H3N2)_2012-04-23**
- **A/swine/Wuhan/1597/2012(H3N2)_2012-06-17**
- **A/swine/Manitoba/SG1431/2008(H3N2)_2008-06-17**
- **A/swine/Manitoba/SG1432/2008(H3N2)_2008-06-17**
- **A/swine/Korea/PL01/2012(H3N2)_2012-04-23**
- **A/swine/Hanoi/422/2013(H3N2)_2013-08-13**
- **A/swine/Hanoi/415/2013(H3N2)_2013-08-13**

- **Bac Ninh H3N2 strains in 2012**
  - Swine Hong Kong strains

- **Bac Ninh H3N2 strains in 2013–2015**
  - Bac Ninh H3N2 strains in 2012

- **North American TR strains in 2004–2007**
  - A/swine/QC/2108-2/2009(H3N2)_2009-04-03
  - A/swine/North_Carolina/A01202766/2011(H3N2)_2011-10-20
  - A/swine/Virginia/A01203436/2012(H3N2)_2012-04-16

**Around 2005**

**A/New_York/359/20052005-01-17**
## Antigenic analysis of Vietnamese H3 IAV-S

<table>
<thead>
<tr>
<th>Virus</th>
<th>VN strain</th>
<th>'96~'98</th>
<th>'98~'00</th>
<th>'00~'04</th>
<th>'06~'08</th>
<th>'10~'12</th>
<th>'11~'14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seasonal human-lineage virus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VN H3N2 (2010)</td>
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<td>&lt;10</td>
<td>40</td>
<td>320</td>
<td>320</td>
<td>320</td>
</tr>
<tr>
<td>VN H3N2 (2011)</td>
<td>160</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>40</td>
<td>80</td>
<td>160</td>
</tr>
<tr>
<td>VN H3N2 (2012)</td>
<td>160</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>20</td>
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<tr>
<td>'96~'98 vaccine strain</td>
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<td>320</td>
<td>160</td>
<td>20</td>
<td>&lt;10</td>
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<tr>
<td>'98~'00 vaccine strain</td>
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<td>2560</td>
<td>1280</td>
<td>160</td>
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</tr>
<tr>
<td>'00~'04 vaccine strain</td>
<td>&lt;10</td>
<td>10</td>
<td>160</td>
<td>1280</td>
<td>80</td>
<td>10</td>
<td>&lt;10</td>
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<tr>
<td>'06~'08 vaccine strain</td>
<td>&lt;10</td>
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<td>'10~'12 vaccine strain</td>
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<td>1280</td>
<td>1280</td>
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<tr>
<td>'11~'14 vaccine strain</td>
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<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
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<td>640</td>
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<tr>
<td><strong>Triple reassortant IAV-S</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>VN H3N2 (2012)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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</tr>
</tbody>
</table>

† Homologous HI titers are indicated in bold and underlined.
Sero-surveillance of IAV–S in Vietnam
Sero-surveillance in Vietnamese people using HI test

Serum
• 943 sera (Hanoi)
• 2–91 years old (Ave. 33)
• 2011 and 2012

Antigens
① A novel reassortant H3N2 IAV–S in Vietnam A/swine/Bing Duong/03–9/2010 (H3N2)
• HA and NA genes from a human H3N2 virus circulating around 2005.
• Internal genes from ‘Triple reassortant’ IAV–S circulating mainly in North America.
② Human seasonal vaccine strain A/Perth/16/2009 (H3N2)

(Risk for pig–to–human infection with IAV–S in Vietnam?)

(Collaboration with Dr. Juliet Bryant from Oxford Univ.)
Children may have a higher risk of being infected with Vietnamese IAV–S

Antigen:
- VN IAV–S
- Human vaccine strain A/Perth/16/2009 (H3N2)
Report: Human infection with IAV-S in Vietnam

- Less than 15 years old (unspecified)
- Respiratory disease
- Hospitalization

Seasonal human A/H3N2

Dong Nai H3N2 strains in 2012–2015
- A/swine/Hong_Kong/2503/2011 (H3N2)
- A/swine/Hong_Kong/NS2439/2011 (H3N2)
- A/swine/Guangxi/NS2783/2010 (H3N2)
- A/swine/Guangxi/NS3086/2011 (H3N2)
- A/swine/Guangxi/NS3106/2011 (H3N2)
- A/swine/Guangxi/NS3108/2011 (H3N2)
- A/swine/Guangxi/2803/2011 (H3N2)

Tien Giang H3N2 strains in 2012
- Swine Hong Kong strains
- A/Ho Chi Minh/459.6/2010 (H3N2)

Dong Nai H3N2 strains in 2015
- Ho Chi Minh H3N2 strains in 2011
- A/swine/Binh_Duong/03–14/2010 (H3N2) 2010–03–01
- A/swine/Binh_Duong/03–10/2010 (H3N2) 2010–03–01
- A/swine/Binh_Duong/03–13/2010 (H3N2) 2010–03–01
- A/swine/Binh_Duong/03–06/2010 (H3N2) 2010–03–01
- A/swine/Binh_Duong/03–09/2010 (H3N2) 2010–03–01
- A/swine/Binh_Duong/03–08/2010 (H3N2) 2010–03–01

A/New_York/359/20052005-01-17

Summary of Vietnamese IAV-S

- **Five distinct lineages** of IAV-S have been co-circulating in Vietnamese pig population.

- **Antigenicity of H1** HA proteins of pre-pandemic human-like and classical lineages **differs from** the contemporary human seasonal strains.

- **Antigenicity of H3** HA proteins of seasonal human and triple reassortant lineages **differs from** the contemporary human seasonal strains.

- **Pig to human infection** with IAV-S was evident in Vietnam.
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- **Dr. Nicola S. Lewis**, University of Cambridge, UK
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