Part 3:

Variant swine H3N2 and novel reassortant H3N1 viruses with A(H1N1)pdm09-like segments, Republic of Korea
Spread of North American-like TRSw and European swine influenza viruses in Asia
**pH1N1 2009 virus**
- Human infection Sw-like H1 virus
- TRSw H1N2 virus in Korea
- TRSw H1N1 virus in Korea
- Human seasonal H1N1 virus

**H1**

**δ -H1 cluster**

**α -H1 cluster**

**β -H1 cluster**

**γ -H1 cluster**

**Classical Swine-like**

**Human Seasonal-like**

**A/Sw/Korea/1130/09**

**A/Sw/Korea/1204/09**
Sero-group I (US H3N2)

A/Sw/Korea/CAS09/06
A/Sw/Korea/CAS05/04

Sero-group II (US H3N2)

A/Sw/Korea/CY05/07
A/Sw/Korea/CY07/07

Sero-group III (US H3N2)

A/Sw/Korea/JNS06/04
A/Sw/MN/22057/02
A/Tk/MN/674-2/03
A/Tk/NC/12344/03

A/Paris/896/97
A/Texas/9/96

A/Sw/Korea/CY07/07
A/Sw/Korea/CY10/07

A/Sw/IL/21587/99
A/Sw/WI/14094/99
A/Sw/OK/18717/99
A/Sw/OK/18089/99

A/Sw/Korea/JNS06/04
A/Sw/Korea/CY09/07
A/Sw/ON/41848/97
A/Nagasaka/97/95
A/Finnland/339/95
A/Sw/NC/35922/98

A/Sw/Korea/CAS09/06
A/Sw/Korea/CAS07/05
A/Sw/Korea/CAN04/05

A/Sw/Korea/CY04/07
A/Sw/Korea/CY05/07
A/Sw/Korea/CY07/07

A/New York/622/96
A/New York/647/95

A/Sw/Korea/CAS05/04
A/Canterbury/01/05
A/Brisbane/3/05
A/Wisconsin/67/05
A/Aichi/133/05

A/Sw/Korea/PZ72-1/06
A/Sw/Korea/CAS05/04
A/Sw/Korea/CY04/07
A/Sw/Korea/CY05/07
A/Sw/Korea/CY07/07

A/Wuhan/359/95
A/New York/521/98
A/Sydney/5/97
A/Memphis/98
A/Sw/CO/23619/99
A/Panama/2007/99

A/Sw/Korea/CAS06/04
A/Canterbury/01/05
A/Brisbane/3/05
A/Wisconsin/67/05
A/Aichi/133/05

A/Sw/Korea/CAS09/06
A/Sw/Korea/CAS07/05
A/Sw/Korea/CAN04/05

A/Sw/Korea/CY04/07
A/Sw/Korea/CY05/07
A/Sw/Korea/CY07/07
Swine influenza viruses in Korea

Appearance of strain with changes in pre-existing ones; *violet* entries designate isolation of virus strains atypical in swine.
Epidemiologic surveillance (2011-2012)
### Genotypes of identified reassortant H3 swine influenza viruses.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Subtype</th>
<th>No. of virus</th>
<th>Genotype</th>
<th>Stock titers (TCID&lt;sub&gt;50&lt;/sub&gt;/ml)</th>
<th>MLD&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sw/Korea/CY02-07/12</td>
<td>H3N1</td>
<td>1</td>
<td>H3</td>
<td>pH1N1, pH1N1 TRSw, pH1N1 TRSw</td>
<td>6.30E+07</td>
</tr>
<tr>
<td>Sw/Korea/CY02-08/12</td>
<td>H3N1</td>
<td>1</td>
<td>H3</td>
<td>pH1N1, pH1N1 TRSw, pH1N1 pH1N1 pH1N1</td>
<td>6.30E+07</td>
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<tr>
<td>Sw/Korea/CY03-12/12</td>
<td>H3N1</td>
<td>1</td>
<td>H3</td>
<td>pH1N1, pH1N1 pH1N1 pH1N1 pH1N1 pH1N1</td>
<td>1.00E+08</td>
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<tr>
<td>Sw/Korea/CY03-13/12</td>
<td>H3N1</td>
<td>1</td>
<td>H3</td>
<td>pH1N1, pH1N1 TRSw, pH1N1 pH1N1 pH1N1</td>
<td>4.00E+07</td>
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<tr>
<td>Sw/Korea/CY02-10/12</td>
<td>H3N2</td>
<td>8</td>
<td>H3</td>
<td>N2, TRSw, TRSw, TRSw, TRSw, TRSw</td>
<td>2.00E+06</td>
</tr>
</tbody>
</table>

Shaded segments indicate TRSw H3N2 virus origin. The MLD50 (expressed as log<sub>10</sub> TCID<sub>50</sub>) was determined by inoculating groups of 10 mice with 10<sup>5</sup> TCID50 of virus in 30 µl of sterile PBS.
Growth properties in cell lines

**Virus titers (log\textsubscript{10} TCID\textsubscript{50}/0.1 ml)**

- MDCK
- HTBE
- A549

**Cell Lines:**
- CY02-07
- CY02-08
- CY03-12
- CY03-13
- CY02-10
Virulence in mice

Virus titers (log_{10} TCID_{50}/g)

Day post-inoculation

Survival rate (%)

Day post-inoculation

CY02-07  CY02-08  CY03-12  CY03-13  CY02-10

Sw/Korea/CY02-07/12  Sw/Korea/CY02-08/12  Sw/Korea/CY03-12/12  Sw/Korea/CY03-13/12  Sw/Korea/CY02-10/12
Experimental set-up in ferrets

Intranasal and intra-tracheal inoculation with $10^{5.5}$ TCID$_{50}$/ml of respective viruses.
Ferret to ferret transmission

Virus titers (log_{10} TCID_{50}/0.1 ml)

Sw/Korea/CY02-07/12 (rH3N1p)

Sw/Korea/CY02-08/12 (rH3N1p)

Sw/Korea/CY03-12/12 (rH3N1p)

Sw/Korea/CY03-13/12 (rH3N1p)

Sw/Korea/CY02-10/12 (rH3N2p)

Days post-infection/exposure
Viral titers in tissues

- Virus titers (log_{10} TCID_{50}/g)

- Viral titers in tissues
  - CY02-07
  - CY02-08
  - CY03-12
  - CY03-13
  - CY02-10

- Graphs showing viral titers in different tissues for various viruses:
  - A: Sw/Korea/CY02-07/12
  - B: Sw/Korea/CY02-08/12
  - C: Sw/Korea/CY03-12/12
  - D: Sw/Korea/CY03-13/12
  - E: Sw/Korea/CY02-10/12

- Tissues: Trachea, Lung, Spleen, Intestine, Brain

- Comparison of viral titers in different tissues for different viruses.
Evaluation of clinical disease in experimentally inoculated ferrets.

<table>
<thead>
<tr>
<th>Virus exposure</th>
<th>No. with sneezing</th>
<th>No. with weight loss (%)*</th>
<th>No. with fever (°C)†</th>
<th>No. with virus detection ((\log_{10} \text{TCID}_{50}/\text{ml}))‡</th>
<th>No. with seroconversion§</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sw/Korea/CY02-07/12</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Inoculated</td>
<td>2/2</td>
<td>2/2 (8.5)</td>
<td>2/2 (2)</td>
<td>2/2 (6.4)</td>
<td>2/2 (2560)</td>
</tr>
<tr>
<td>RD contact</td>
<td>2/2</td>
<td>2/2 (5.7)</td>
<td>2/2 (1.2)</td>
<td>2/2 (5.5)</td>
<td>2/2 (2560)</td>
</tr>
<tr>
<td><strong>Sw/Korea/CY02-08/12</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>2/2</td>
<td>2/2 (7.3)</td>
<td>2/2 (1.6)</td>
<td>2/2 (5.9)</td>
<td>CY03-12 2/2 (2560)</td>
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<tr>
<td>RD contact</td>
<td>0/2</td>
<td>2/2 (1.8)</td>
<td>2/2 (2.2)</td>
<td>0/2</td>
<td>2/2 (320)</td>
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<tr>
<td><strong>Sw/Korea/CY03-12/12</strong></td>
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<td></td>
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<tr>
<td>Inoculated</td>
<td>1/2</td>
<td>2/2 (6.2)</td>
<td>2/2 (1.7)</td>
<td>2/2 (5.4)</td>
<td>2/2 (1280)</td>
</tr>
<tr>
<td>RD contact</td>
<td>0/2</td>
<td>0/2</td>
<td>1/2 (0.8)</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td><strong>Sw/Korea/CY03-13/12</strong></td>
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<tr>
<td>Inoculated</td>
<td>2/2</td>
<td>2/2 (7.9)</td>
<td>2/2 (1.9)</td>
<td>2/2 (5.8)</td>
<td>2/2 (2560)</td>
</tr>
<tr>
<td>RD contact</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2 (1)</td>
<td>1/2 (5.3)</td>
<td>2/2 (1920)</td>
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<td><strong>Sw/Korea/CY02-10/12</strong></td>
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<td></td>
</tr>
<tr>
<td>Inoculated</td>
<td>2/2</td>
<td>2/2 (6.8)</td>
<td>2/2 (1.2)</td>
<td>2/2 (4.2)</td>
<td>2/2 (640)</td>
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<tr>
<td>RD contact</td>
<td>2/2</td>
<td>2/2 (2.1)</td>
<td>2/2 (1)</td>
<td>2/2 (4.8)</td>
<td>2/2 (480)</td>
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</table>

* mean maximum weight loss;
† mean maximum temperature increase;
‡ mean peak titer;
§ geometric mean HI titer.
<table>
<thead>
<tr>
<th>Viruses</th>
<th>Subtype</th>
<th>Ferret antiserum</th>
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<tr>
<td>California/04/2009</td>
<td>H1N1</td>
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<tr>
<td>Sw/Korea/CY02-07/12</td>
<td>H3N1</td>
<td>2560</td>
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<tr>
<td>Sw/Korea/CY02-08/12</td>
<td>H3N1</td>
<td>2560</td>
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<tr>
<td>Sw/Korea/CY03-12/12</td>
<td>H3N1</td>
<td>2560</td>
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<td>Sw/Korea/CY03-13/12</td>
<td>H3N1</td>
<td>2560</td>
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<tr>
<td>Sw/Korea/CY02-10/12</td>
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<td>640</td>
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<tr>
<td>Brisbane/10/07†</td>
<td>H3N2</td>
<td>&lt;20</td>
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<tr>
<td>Perth/16/09†</td>
<td>H3N2</td>
<td>&lt;20</td>
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<td>Sw/Korea/CAS05/04‡</td>
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<tr>
<td>Sw/Korea/CY07/07§</td>
<td>H3N2</td>
<td>80</td>
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</tbody>
</table>

†Human seasonal H3N2 influenza A virus; *, prototype cluster I TRSw; ‡, prototype cluster II TRSw; §, prototype cluster III TRSw.
Are reassortant H3 swine viruses still circulating in Korea?

Surface genes: 
H1 Eurasian avian-like swine 
N2: Korean-like swine

Internal genes: 
H3N2pM-like

CY0423-12/13(H1N2)
**Title**: Characterization of the novel reassortant H1N2 swine influenza virus in South Korea and assessment of its transmissibility in ferrets

Table 1. Nucleotide homology (%) between a novel H1N2 SIV (A/swine/Korea/CY0423-12/2013) and reference strain obtained from GenBank using BLAST search.

<table>
<thead>
<tr>
<th>SEGMENT</th>
<th>A(H1N1)pdm09</th>
<th>Eurasian H1N1</th>
<th>Korean-like H1N2</th>
<th>H3N2pM-like</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>California/07/09</td>
<td>Korea/SCJ01/09</td>
<td>SW/HongKong/199/09</td>
<td>SW/Jiangsu/40/11</td>
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<tr>
<td>HA</td>
<td>70.9</td>
<td>71.1</td>
<td>97.3</td>
<td>97.2</td>
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<tr>
<td>NA</td>
<td>37.4</td>
<td>37.7</td>
<td>37.5</td>
<td>37.8</td>
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<tr>
<td>PB2</td>
<td>93.5</td>
<td>93.2</td>
<td>83.1</td>
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<td>PB1</td>
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<td>93.3</td>
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<tr>
<td>PA</td>
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<td>93.4</td>
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<tr>
<td>NP</td>
<td>94.6</td>
<td>94.1</td>
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<tr>
<td>M</td>
<td>98.8</td>
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<td>94.1</td>
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<tr>
<td>NS</td>
<td>94</td>
<td>93.6</td>
<td>78.6</td>
<td>78.2</td>
</tr>
</tbody>
</table>

Note: Highlighted in **bold** are the highest nucleotide homologies of reference strains with the corresponding gene of A/swine/Korea/CY0423-12/2013.

Unique SIV Genetic Combination

- PB2
- PB1
- PA
- HA
- NP
- NA
- M
- NS

Legend:
- H3N2pM variants
- Eurasian avian-like swine
- North American/Korean lineage
Supported results obtained from the BLAST homology analysis
EA avian-like swine H1 viruses have never been reported in Korea

Carried out serological surveillance of pig sera from Chungcheong, Cheonbuk and Gyeonsang regions of South Korea

n=371 in 2012 and n=1185 in 2013
Total: 1556
<table>
<thead>
<tr>
<th>Seropositivity</th>
<th>Number (%) of sera positive</th>
<th>Subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2012</td>
<td>2013</td>
</tr>
<tr>
<td>None</td>
<td>338 (91.1)</td>
<td>669 (56.5)</td>
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<tr>
<td>A(H1N1)pdm09</td>
<td>24 (6.5)</td>
<td>126 (10.6)</td>
</tr>
<tr>
<td>Korean H1N2</td>
<td>5 (1.3)</td>
<td>47 (4)</td>
</tr>
<tr>
<td>EA avian-like swine H1</td>
<td>0</td>
<td>131 (11.1)</td>
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<tr>
<td>A(H1N1)pdm09, Korean H1N2</td>
<td>4 (1.1)</td>
<td>23 (1.9)</td>
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<tr>
<td>A(H1N1)pdm09, EA avian-like swine H1</td>
<td>0</td>
<td>157 (13.2)</td>
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<td>6 (0.5)</td>
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<td>A(H1N1)pdm09, Korean H1N2, EA avian-like swine H1</td>
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<tr>
<td><strong>Total sera positive</strong></td>
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<td>516 (43.5)</td>
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<td><strong>Total sera tested</strong></td>
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<td>1185</td>
</tr>
<tr>
<td>Seropositivity</td>
<td>Number (%) of sera positive</td>
<td>Subtotal</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------------------</td>
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</tr>
<tr>
<td></td>
<td>2012</td>
<td>2013</td>
</tr>
<tr>
<td>None</td>
<td>141 (38)</td>
<td>1035 (87)</td>
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<td>H3N2pM</td>
<td>109 (29)</td>
<td>115 (10)</td>
</tr>
<tr>
<td>CY05/07</td>
<td>16 (4)</td>
<td>7 (1)</td>
</tr>
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<td>CY07/07</td>
<td>11 (3)</td>
<td>11 (1)</td>
</tr>
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<td>H3N2pM, CY05/07</td>
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<td>CY05/07, CY07/07</td>
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<td>H3N2pM, CY05/07, CY07/07</td>
<td>61 (16)</td>
<td>13 (1)</td>
</tr>
<tr>
<td><strong>Total sera positive</strong></td>
<td><strong>230 (62)</strong></td>
<td>150 (13)</td>
</tr>
<tr>
<td><strong>Total sera tested</strong></td>
<td>371</td>
<td>1185</td>
</tr>
</tbody>
</table>

Abbreviations of H3N2 viruses tested:
- H3N2pM: A/swine/Korea/SwLu0306-4/2012
- CY05/07: A/swine/Korea/CY05/2007
- CY07/07: A/swine/Korea/CY07/2007
CELL GROWTH KINETICS

Log10 TCID50/ml

**

MDCK

NHBE

CY03-11/2012

CY0423-12/2013

hours post infection (hpi)
Nasal turbinates

Mice Lungs
HISTOPATHOLOGY

A. CY03-11/2012

B. CY423-12/2013
PATHOGENICITY AND TRANSMISSIBILITY IN FERRETS

A

CY0423-12/2013

B

CY03-11/2012

6.0 $\log_{10} \text{TCID}_{50}$/ml
Timeline of the genotype of viruses identified in surveillance program in China

Zhu et al., 2013. Curr Top Microbiol Immunol
Genotypes of swine influenza viruses detected in South Korea

Transcript variants and atypical viruses

- Avian
- TR H1N2
- TR H3N2
- TR H1N1
- Classical Swine H1
- A(H1N1)pdm09
- H3N2pM
- EA avian-like swine H1
Concluding remarks

- Overall findings underscore the possibility that influenza viruses in swine populations can become a threat to animal and human health.

- Reviving concerns over the capacity of pigs to potentially create future pandemic influenza viruses, continuous and efficient surveillance among swine herds worldwide remains vital and beneficial.