Risk Assessment of H3N2 Avian Origin Canine Influenza Viruses

Henry Wan, Ph.D.
Department of Basic Sciences
College of Veterinary Medicine
Mississippi State University

E-mail: wan@cvm.msstate.edu
Phone: (662) 325-3559
Lab: http://sysbio.cvm.msstate.edu
One Health One Flu

Human
H1N1, H3N2, H1N2, H2N2;
H5N1, H7N2, H7N3, H7N7, H9N2, H7N9, H10N8

Pig
H1N1; H3N2;
H1N2; H3N1

Equine
H3N8; H7N7

Canine
H3N8; H3N2

Aquatic birds
H1-16; N1-9

Terrestrial poultry
H subtypes 4,5,6,7,9,10;
N subtypes 1,2,4,7
e.g. H9N2; H6N1.

Sea mammals (seals; whales)
H7N7; H4N5; H4N6; H3N3
H3N2; H13N9

Poster 31, H6N6
Poster 33, H10N8
144 Subtypes!

- Mutation
- Reassortment
- Recombination
- Reported in South Korea and China
- 6-100% sera positive in dog population

(Li, et al. 2010. IGE)
Influenza Risk of H3N2 canine influenza virus

- Risk of H3N2 CIV to public health
  - CDC IRAT tool

- Risk of emergence of novel influenza viruses by co-circulation of H3N2 CIV and 2009 H1N1 virus at the animal-human interface
  - Reassortment and sparse learning
Influenza Risk of H3N2 canine influenza virus

- Risk of H3N2 CIV to public health
  - CDC IRAT tool

- Risk of emergence of novel influenza viruses by co-circulation of H3N2 CIV and 2009 H1N1 virus at the animal-human interface
  - Reassortment and sparse learning
Influenza Risk Assessment Tool (IRAT) by CDC

**Public Health Impact**

- Disease severity and pathogenesis
- Existing population immunity
- Human Infections
- Antiviral treatment susceptibility/resistance
- Antigenic relationship to vaccine candidates
- Receptor binding
- Genomic variation
- Transmission in lab animals
- Global distribution (animals)
- Infection in animal species

(Trock et al. 2012. Avian Diseases, 56(4s1):1058-1061.)
Cross reactions among H3 CIVs and contemporary human H3N2 seasonal IAVs

<table>
<thead>
<tr>
<th>Virus</th>
<th>H3N2</th>
<th>H3N8</th>
<th>A/Bangkok/1/79(H3N2)</th>
<th>A/Caen/1/84(H3N2)</th>
<th>A/Johannesburg/33/94(H3N2)</th>
<th>A/Wisconsin/67/05(H3N2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3N2 CIV</td>
<td>1280</td>
<td>40</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>H3N8 CIV</td>
<td>67</td>
<td>320</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>A/Bangkok/1/79(H3N2)</td>
<td>ND</td>
<td>ND</td>
<td>2560</td>
<td>640</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>A/Caen/1/84(H3N2)</td>
<td>ND</td>
<td>ND</td>
<td>10</td>
<td>1280</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>A/Johannesburg/33/94(H3N2)</td>
<td>ND</td>
<td>ND</td>
<td>&lt;10</td>
<td>40</td>
<td>640</td>
<td>&lt;10</td>
</tr>
<tr>
<td>A/Wisconsin/67/05(H3N2)</td>
<td>ND</td>
<td>ND</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>1280</td>
</tr>
</tbody>
</table>
Human tracheal epithelium cells infected by H3N2 CIV

CIV H3N2 WT MOI=1

CIV H3N2 WT MOI=10

(Magnification, 400X)

Anti-NP/FITC merged with DAPI
Human tracheal epithelium cells infected by H3N2 CIV

MAA, Maackia amurensis Lectin, $\alpha$-2,3 linkage
SNA, Sambucus nigra lectin, $\alpha$-2,6 linkage
Infectivity of H3N2 CIV in ferret

Nasal washings after H3N2 inoculation

Negative control
CIV H3N2 infected

Rhinitis caused by H3N2 canine influenza virus
Influenza Risk Assessment Tool (IRAT) by CDC

Public Health Impact

- Disease severity and pathogenesis (1)
- Existing population immunity (10)
- Human Infections (1)
- Antiviral treatment susceptibility/resistance (7)
- Antigenic relationship to vaccine candidates (10)
- Receptor binding (3)
- Genomic variation (5)
- Transmission in lab animals (3)
- Global distribution (animals) (7)
- Infection in animal species (8)

(Trock et al. 2012. Avian Diseases, 56(4s1):1058-1061.)
Influenza Risk of H3N2 canine influenza virus

- Risk of H3N2 CIV to public health
  - CDC IRAT tool

- Risk of emergence of novel influenza viruses by co-circulation of H3N2 CIV and 2009 H1N1 virus at the animal-human interface
  - Reassortment and sparse learning
<table>
<thead>
<tr>
<th>NUM</th>
<th>NAME</th>
<th>HA</th>
<th>NA</th>
<th>pB2</th>
<th>PB1</th>
<th>PA</th>
<th>NP</th>
<th>M</th>
<th>NS</th>
<th>Replication Efficiency (HA titer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>r1</td>
<td>CIV</td>
<td>CIV</td>
<td>PAN</td>
<td>CIV</td>
<td>CIV</td>
<td>CIV</td>
<td>CIV</td>
<td>CIV</td>
<td>&lt;2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>121</td>
<td>r14567</td>
<td>CIV</td>
<td>PAN</td>
<td>PAN</td>
<td>CIV</td>
<td>CIV</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>8</td>
</tr>
<tr>
<td>122</td>
<td>r24567</td>
<td>CIV</td>
<td>PAN</td>
<td>CIV</td>
<td>PAN</td>
<td>CIV</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>&lt;2</td>
</tr>
<tr>
<td>123</td>
<td>r124567</td>
<td>CIV</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>CIV</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>32</td>
</tr>
<tr>
<td>124</td>
<td>r34567</td>
<td>CIV</td>
<td>PAN</td>
<td>CIV</td>
<td>CIV</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>&lt;2</td>
</tr>
<tr>
<td>125</td>
<td>r134567</td>
<td>CIV</td>
<td>PAN</td>
<td>PAN</td>
<td>CIV</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>&lt;2</td>
</tr>
<tr>
<td>126</td>
<td>r234567</td>
<td>CIV</td>
<td>PAN</td>
<td>CIV</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>16</td>
</tr>
<tr>
<td>127</td>
<td>r1234567</td>
<td>CIV</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>PAN</td>
<td>1024</td>
</tr>
</tbody>
</table>
Genomic compatibility using sparse learning

\[ y = x \beta \]

Stock Trend and Sparse Learning
| Term                                      | Estimate | Std. Error | t value | Pr(>|t|) |
|-------------------------------------------|----------|------------|---------|----------|
| (Intercept)                               | -0.0470  | 0.0563     | -0.84   | 0.4054   |
| PAN.PAN.PAN.PAN.pB2.PB1.PA.NA.           | 2.2708   | 0.2819     | 8.05    | 0.0000   |
| PAN.PAN.PAN.PAN.pB2.PB1.NA..NS           | 1.9494   | 0.3303     | 5.90    | 0.0000   |
| PAN.CIV.PAN.PAN.pB2.PA.NP.NA.            | 1.1458   | 0.2677     | 4.28    | 0.0000   |
| PAN.CIV.PAN.PAN.pB2.PA.NA..M             | 0.9091   | 0.2684     | 3.39    | 0.0010   |
| PAN.CIV.PAN.PAN.pB2.PA.NA..NS            | 0.7403   | 0.2981     | 2.48    | 0.0144   |
| PAN.PAN.PAN.PAN.pB2.NP.NA..M             | 1.1155   | 0.2813     | 3.96    | 0.0001   |
| PAN.PAN.PAN.PAN.PB1.PA.NP.NA.            | 1.4459   | 0.2830     | 5.11    | 0.0000   |
| PAN.PAN.PAN.PAN.PB1.PA.NA..NS            | 1.0230   | 0.2899     | 3.53    | 0.0006   |
| PAN.PAN.PAN.PAN.PB1.NP.NA..M             | 1.0993   | 0.2688     | 4.09    | 0.0001   |
| PAN.PAN.CIV.PAN.PB1.NA..M.NS             | 1.0533   | 0.2638     | 3.99    | 0.0001   |
Summary

• H3N2 CIV is antigenically distinct from contemporary H3N2 human seasonal influenza viruses.
• Ferrets infected with H3N2 CIV developed rhinitis and bronchiolitis, a result consistent with tissue viral loads.
• Human human tracheal epithelium cells could be infected by H3N2 CIV, which preferentially infected cells with α2,3 sialic acid linked glycans.
• A new computational method was developed. The PA and MP genes of H3N2 CIV were shown to be compatible with the internal genes of the 2009 H1N1 virus.
• Reassortant virus H3N2xpH1N1(H3N2) replicated to significantly higher titers in A549 cells than the wild-type.
• Results demonstrate that H3N2 CIV is posing a moderate threat to public health and warrants continuous surveillance.
Acknowledgment

**Wan Systems Biology Lab**
Elizabeth Bailey
Sherry Blackmon
Lucas Ferguson
Liping Long
Bridgitte Martin
Alison Stokley
Lucy Wang
Feng Wen
Yifei Xu
Dr. Hailiang Sun
Dr. Jianli Xue
Dr. Alan Zhao
Dr. Yulong Zhao

**Previous students**
Dr. Jianqiang Ye
Guohua Yang

**Mississippi State University**
Dr. Jim Cooley
Dr. Larry Hanson

**Emory University**
Dr. Shoujun Li
Dr. Ming Liao

**St. Jude’s Children Research Hospital**
Richard Webby

**CDC**
Dr. Michael Shaw
Dr. Xiyang Xu

**Emory University**
Dr. David Steinhauer
Dr. Konrad C. Bradley
Dr. Dave Smith
Dr. Jamie Heimburg-Molinaro

**Funding Supports**
NIH NIAID R15AI107702A
NIH NCRR P20RR032694
NIH NIAID RC1AI086830