Research on Vaccines: Key Topics and New applications

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Key Issues

Avian Influenza as an Example

- Genetic and antigenic change of field viruses away from vaccine strains (‘drift’)
- Developing interchangable cassettes for vectored vaccines with quicker approval process
- Mass immunization vectors and application systems
- More effective vaccination protocols for field
- Differentiating infected animals in vaccinated population
Avian Influenza Virus (AIV)

- Small RNA virus – no proof reading
- Surface spikes – hemagglutinin (H1-16) & neuraminidase (N1-9)
- Hemagglutinin prone to genetic & antigenic change (“drift”)
- Protection: primarily antibody-mediated immunity against hemagglutinin
- Issue: Reports of inconsistent field protection by AI vaccines
1. Genetic and Antigenic Drift

Homosubtypic HA Protection by AI Vaccines

Chickens vaccinated SQ 1d with rFP-H5-Ire/83 and IN challenged at 3 wks with $10^{5-6}$ EID$_{50}$ of HPAIV:

Different challenge viruses (87.3-100% amino acid sequence similarity)

<table>
<thead>
<tr>
<th>Challenge Virus</th>
<th>Subtype</th>
<th>HA Similarity to Tk/Ire/83</th>
<th>Mortality (MDT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ck/Scotland/59</td>
<td>H5N1</td>
<td>92</td>
<td>0/10</td>
</tr>
<tr>
<td>Tern/S. Afr/61</td>
<td>H5N3</td>
<td>93.1</td>
<td>0/10</td>
</tr>
<tr>
<td>Tk/Ontario/66</td>
<td>H5N9</td>
<td>89.1</td>
<td>0/10</td>
</tr>
<tr>
<td>Ck/PA/83</td>
<td>H5N2</td>
<td>87.3</td>
<td>0/10</td>
</tr>
<tr>
<td>Tk/Ireland/83</td>
<td>H5N8</td>
<td>100</td>
<td>0/10</td>
</tr>
<tr>
<td>Tk/England/91</td>
<td>H5N1</td>
<td>94.2</td>
<td>0/10</td>
</tr>
<tr>
<td>Ck/Queretaro/95</td>
<td>H5N2</td>
<td>89.3</td>
<td>0/10</td>
</tr>
<tr>
<td>HK/156/97</td>
<td>H5N1</td>
<td>90.2</td>
<td>0/10</td>
</tr>
<tr>
<td>Ck/S. Korea/03</td>
<td>H5N1</td>
<td>89.9</td>
<td>0/10</td>
</tr>
<tr>
<td>ck/Vietnam/04</td>
<td>H5N1</td>
<td>88.4 est</td>
<td>0/22</td>
</tr>
<tr>
<td>WS/Mongolia/05</td>
<td>H5N1</td>
<td>89.7</td>
<td>1/8</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>80-100%</td>
</tr>
</tbody>
</table>

- Excellent, broad, homosubtypic protection from mortality by classic H5 AIV vaccine strain from 1983
1. Genetic and Antigenic Drift

rFP-H5 vaccines, pre-2005 challenge HPAI viruses

- Higher HA identify between vax and challenge AIV
  - Respiratory shedding – significant reduction
  - Alimentary shedding – no association in reduction

(Swayne et al., Vaccine 18:1088-1095. 2000)
1. Genetic and Antigenic Drift

- H5 AI Vaccine in Mexico since 1/1995

<table>
<thead>
<tr>
<th>Challenge virus</th>
<th>DPI&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Vaccinated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine strain</td>
<td>3DPI</td>
<td>1.66 (5/10)</td>
<td>4.5 (5/5)</td>
</tr>
<tr>
<td>Jalisco Lineage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK/AG/124-3705/98</td>
<td>3DPI</td>
<td>4.44 (10/10)</td>
<td>4.2 (5/5)</td>
</tr>
<tr>
<td>Lineage A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK/Guatemala/194573/02</td>
<td>3DPI</td>
<td>4.86 (10/10)</td>
<td>4.9 (5/5)</td>
</tr>
<tr>
<td>Lineage B</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 1998 and 2002 Central American H5N2 LPAI viruses have drifted away from inactivated vaccine viruses and vaccine seed stock is no longer protective.

Lee, C.W., et. al, J. Virol. 78:8372-8381, 2004
• **H5 viruses are diverse** – N. Amer. and Eurasian lineages

• **H5N1 HPAI is not a single virus, but a family of viruses with 10 lineages and many sublineages**

• **Virus is drifting as other influenza A viruses have done** – from natural infections and vaccine pressure
1. Genetic and Antigenic Drift

Prior 2006 - Inactivated AIV vaccine in chickens against
H5N1 HPAI – clade 2.1, 2.2 and 2.3

- Prevented illness and death
- Reduced challenge virus replication and shedding from
  respiratory and intestinal tracts by $10^4$ EID$_{50}$ of virus

2006 – one unique 2.1 clade virus was identified

- Completely resistant to vaccine strains: ck/Mexico/94,
  Tk/WI/68, tk/Eng/73
- Marginal protection – ck/Legok/03, dk/VN/04
- Best protection – rFP-H5 plus killed ck/Legok/03

Emphasizes the need to look for AI variant field strains
and improved application
Drift of Indonesian H5N1 HPAI Virus

- Efficacy: Challenge Virus Selection
- CSIRO phylogenetics
- 3 viruses – phylogenetically diverse and most recent
  - A/ck/WJ/PWT-WIJ/06 9/06
  - A/ck/WJ/SMI-HAMD/06 5/06
  - A/ck/Papua/TAS/06 7/06

Peter Daniels and Frank Wong
1. Genetic and Antigenic Drift

Antigenic Cartography

- Historic H5 Vaccines – Similar antigenicity
- Drifting of HA away from root
  - Acceptable protection – Ck/HK/220/97, Ck/Legok/03, VN/1203/04, Ck/WJ/HAMD/06
  - Partial protection – Ck/Papua/06
  - Poor protection – PWT/06
- Indicates need for closer matching of vaccine seed strain(s) to field virus(es)

Swayne, Smith and Fouchier, 2008
1. Genetic and Antigenic Drift

Challenge Study

• Inactivated vaccine seed strain: PWT-WIJ HPAIV

• Inactivated vaccine seed strain: rgPWT-WIJ (HA) x 7 gene segments PR8 LPAIV

![Graphs showing survivor percentages for HPAI Challenge Virus with strains PWT-WIJ HPAIV and rgPWT-WIJ (HA) x PR8 LPAIV.](image-url)
2. Cassette Application

Cassette concept for licensing and use: replace only the gene that codes protein that elicits protective immune response

- Reverse genetics produced inactivated AIV strain: change HA in vaccine virus backbone
- Replaceable cassette for virus and bacterial vectors as live or killed vaccine – rFP, etc.
- Streamline and accelerate the licensing process
3. Mass Immunization: Respiratory Application

Recombinant NDV-AI-H7 Vaccine: Eyedrop

Chickens vaccinated at 2 and 4 weeks-of-age with rNDV-AI-H7 or B1 parent virus vaccines (eyedrop), and IN challenge at 6 weeks-of-age with 10^5 EID50’s of vvNDV (Fontana/73) or A/Steele/59 (H7N7)

<table>
<thead>
<tr>
<th>Vaccine Group (10 birds/group)</th>
<th>Challenge</th>
<th>Morbidity (&gt;/= 3+)</th>
<th>Mortality (MDT)</th>
<th>Survivors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1H7, 1x</td>
<td>Fontana</td>
<td>0/10</td>
<td>0/10</td>
<td>100</td>
</tr>
<tr>
<td>B1H7, 1x</td>
<td>Steele</td>
<td>1/10</td>
<td>1/10 (4.0)</td>
<td>90</td>
</tr>
<tr>
<td>SEP B1, 2x</td>
<td>Fontana</td>
<td>0/10</td>
<td>0/10</td>
<td>100</td>
</tr>
<tr>
<td>SEP B1, 2x</td>
<td>Steele</td>
<td>10/10</td>
<td>10/10 (3.3)</td>
<td>0</td>
</tr>
<tr>
<td>Sham, 2x</td>
<td>Fontana</td>
<td>10/10</td>
<td>10/10 (4.0)</td>
<td>0</td>
</tr>
<tr>
<td>Sham, 2x</td>
<td>Steele</td>
<td>7/10</td>
<td>7/10 (2.3)</td>
<td>30</td>
</tr>
</tbody>
</table>


Other vaccines can be used as mass immunizing vectors: adenoviruses, HVT, in ovo admin live or killed vaccines
4. Vaccine Combinations and Vaccination Protocols for AIV

- Optimal time to use killed vaccine and number of doses
  - Most situations, single immunization is not adequate for field protection: i.e. 2 or more needed
  - Before live IBDV to provide better immune response
- Negative impact of maternal antibody on some live vaccines: may require monitoring and adjusting vaccination time
- Prime-boost advantage of live and inactivated vaccine combinations
Prime-boost in MDA+ chicks: Study 1

- **2x Inact.**: 2 log2 lower with H5N1 antigen
- **Prime-boost**: High titers against both antigens

Clear priming effect of rFP-AIV-H5 in MDA+ birds

Bublot & van den Berg, 2008
Potency:
Pre-Challenge Serology

- Classic vaccines can be very potent, but individual vaccines vary (Goal: min. 1:40 titer (no death), but min 1:120 ↓ respiratory shedding)
- Lowest serological titers with all Legok/03 & VN/04, some Tk/Eng vaccines
- Prime-Boost effect with rFPV+Legok/03
4. Vaccine Protection: Challenge Model

- Sham group – no protection

- Vaccine protection varied with vaccine
  - PWT/06:
    - Poor – Tk/WI, Ck/MX, Tk/Eng, GS/GD;
    - Intermediate – Legok/03, VN/04;
    - Good – ExpPWT/06, rFP+Indo/03
5. Identification of infected animals in vaccinated population

- Must be able to distinguish infected from non-infected flocks in vaccinated populations - detect “silent” infections and eliminate immediately

<table>
<thead>
<tr>
<th>Vaccinated Flocks</th>
<th>Non-vaccinated sentinels</th>
<th>Vaccinated Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Serology</td>
<td>Virological Exam – Dead Bird</td>
<td>Special Serology</td>
</tr>
<tr>
<td>H5 HI, AGID, ELISA</td>
<td>VI, RT-PCR, RRT-PCR</td>
<td></td>
</tr>
</tbody>
</table>
## 5. Identification of infected animals in vaccinated population

<table>
<thead>
<tr>
<th>Serological Test</th>
<th>Homo. Hetero.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NP/M (AGP/ELISA)</td>
</tr>
<tr>
<td>AI Field Virus</td>
<td>X</td>
</tr>
<tr>
<td>Homologous NA</td>
<td></td>
</tr>
<tr>
<td>inactivated AIV vaccine</td>
<td>X</td>
</tr>
<tr>
<td>Heterologous NA</td>
<td></td>
</tr>
<tr>
<td>inactivated AIV vaccine</td>
<td>X</td>
</tr>
<tr>
<td>Recombinant Fowlpox,</td>
<td>(-)</td>
</tr>
<tr>
<td>subunit HA &amp; DNA HA vaccines</td>
<td></td>
</tr>
<tr>
<td>Unvaccinated sentinels</td>
<td>-</td>
</tr>
</tbody>
</table>
Conclusions

1. Genetic and antigenic change of field viruses away from vaccine seeds has decreased or eliminated protection in some situations for AIV

2. Changeable cassettes in recombinant vaccines can rapidly be used to replace ineffective drift vaccines

3. Mass application vectors and application systems are needed to provide efficacious vaccines to large segments of at risk populations
Conclusions

4. New application protocols are needed to optimize the use of conventional and recombinant vaccines to maximize field protection.

5. Strategies should be developed to identify infected animals in vaccinated populations.
Thank You For Your Attention!