H5N1 in Egypt: Situation, OIE twinning, and escape mutants

Christian Grund, El-Sayed M. Abdelwhab, Martin Beer, Timm Harder
Praxis of O.I.E. Laboratory Twinning

Reference laboratories for avian influenza viruses
NLQP (Egypt) and FLI (Germany)

H.M. Hafez
Director, Poultry Clinics,
FU Berlin, Germany

M.M. Aly
Director, AHRI, Giza, Egypt

C. Grund, T. Harder
OIE/NRL, FLI, Riems,
Germany
Putatively matching twins to re-inforce AI diagnostic and management powers in Northern Africa and the Near East

**Twin-1.** Animal Health Research Institute, National Laboratory for Quality Control of Poultry Production (NLQNP), Giza, Egypt

**Twin-2.** Friedrich-Loeffler-Institute, O.I.E. and National Reference Laboratories for Avian Influenza and Newcastle Disease, Isle of Riems, Germany
Starting the first meeting

Prof. M.M. Aly, Director, AHRI, Giza, Egypt
Overcoming prejudices and clichés
Overcoming prejudices and clichés
Another interpretation of twinning?

Dr. C. Grund,
head OIE/NRL ND,
FLI, Riems
The Egyptian tree of life: It's all about birds
Egypt today: It's all about HPAIV H5N1 infected birds

Cartoon education series from NLQP public awareness campaign
Egypt today: It's all about HPAIV H5N1 infected birds

- Introduction of HPAIV H5N1 clade 2.2 into Egyptian poultry in 2006
- Introduction into Northern Africa by migratory birds (late 2005)
- Rapid spread along the Nile valley
- Highly fissured poultry-human interface leads to spill-over infections into humans (pandemic risks)
- Serious and frequent outbreaks in poultry on-going despite massive intervention by cull/control and vaccination campaigns
- Virus established endemicity
- Vaccine escape mutants emerged and started to circulate
Impact of AI on Egyptian poultry industry

Poultry industry in Egypt
• Commercial sectors ~850 Mio birds,
• Backyard sectors ~ 250 Mio birds
• around 15,892 retail shops / Live bird markets
  Abdelwhab et al., 2010

• estimated loss after the first emergence of HPAI H5N1 in February 2006 was 1 billion US$
• affected income of 1.5 million people whose livelihoods depended on poultry (Meleigy, 2007).
• About 30 million birds were culled or depopulated in Egypt in the first wave of 2006.
  Hafez et al., 2010
### HPAIV H5N1 detection on live bird markets

<table>
<thead>
<tr>
<th>Month</th>
<th>No. positive LBMs</th>
<th>% positive LBMs/total tested</th>
<th>Positive LBMs/total positive (%)&lt;sup&gt;A&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>7</td>
<td>14</td>
<td>7/71 (9.9%)</td>
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<tr>
<td>February</td>
<td>29</td>
<td>15</td>
<td>29/71 (40.8%)</td>
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<td>March</td>
<td>23</td>
<td>12</td>
<td>23/71 (32.4%)</td>
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<tr>
<td>April</td>
<td>12</td>
<td>8</td>
<td>12/71 (16.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>12.4</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Egyptian National Laboratory for Veterinary Quality Control on Poultry Production*

Abdelwhab et al., 2010
## HPAIV H5N1 detection in Egypt

<table>
<thead>
<tr>
<th>Season (^1)</th>
<th>Commercial farms</th>
<th>Backyard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>20/2,989 (0.7%)</td>
<td>66/832 (7.9%)</td>
</tr>
<tr>
<td>Spring</td>
<td>6/1,785 (0.3%)</td>
<td>11/421 (2.6%)</td>
</tr>
<tr>
<td>Summer</td>
<td>1/1,932 (0.05%)</td>
<td>6/103 (5.8%)</td>
</tr>
<tr>
<td>Autumn</td>
<td>0/1,976 (0%)</td>
<td>6/367 (1.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>27/8,682 (0.31%)</td>
<td>89/1,723 (5.2%)</td>
</tr>
</tbody>
</table>

\(^1\) Season

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Egyptian National Laboratory for Veterinary Quality Control on Poultry Production

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Hafez et al., 2010
Evidence for improved sensitivity and clade-specific differentiation of Egyptian H5N1 by multiplex H5 RT-qPCR

Analytical sensitivity (sample not detected)

Ct values

2.2.1 (FAM) n=16
2.2 (HEX) n=34
EGY (ROX) n=50
H5 (Slomka) n=50
M (Spackman) n=50--
Live bird market – Giza, Pyramids Road
Fresh poultry must be kept fresh
Butchering on the spot(s)
Gulp!
Biosecurity measures: Practical training in overpressurized suits (BSL3+)
AI vaccination strategy in household poultry

• 2 campaigns per year, each one lasting 3 months
• Door-to-door vaccination protocol
• one dose of vaccine is administered for all birds (no booster)
• Chicks and ducklings are vaccinated
• In some Governorates, double or more volume dose of vaccine
• is given to ducks and geese
## AIV H5N1 detection in vaccinated flocks

### Vaccines used
- A/Goose/Guangdong/1/1996 H5N1
- A/chicken/Mexiko/232/CPA/1994 H5N2, etc.

<table>
<thead>
<tr>
<th>Year</th>
<th>Vaccinated</th>
<th>Mixed</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>2007</td>
<td>32 (94.1)</td>
<td>2 (5.9)</td>
<td>34 (100)</td>
</tr>
<tr>
<td>2008</td>
<td>34 (97.1)</td>
<td>1 (2.9)</td>
<td>35 (100)</td>
</tr>
</tbody>
</table>

Egyptian National Laboratory for Veterinary Quality Control on Poultry Production

Hafez et al., 2010
AIV H5N1 detection in vaccinated flocks

Vaccines used
- A/Goose/Guangdong/1/1996 H5N1
- A/chicken/Mexiko/232/CPA/1994 H5N2, etc.

<table>
<thead>
<tr>
<th>Year</th>
<th>Grandparent</th>
<th>Breeders</th>
<th>Layers</th>
<th>Broilers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>1 (2.8)³</td>
<td>5 (14.3)</td>
<td>15 (42.9)</td>
<td>14 (40)</td>
<td>35 (100)</td>
</tr>
<tr>
<td>2008</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>10 (37)</td>
<td>17 (63)</td>
<td>27 (100)</td>
</tr>
</tbody>
</table>

Egyptian National Laboratory for Veterinary Quality Control on Poultry Production

Hafez et al., 2010
Phylogenetic analysis of Egyptian HPAIV H5N1 viruses

1 – A/chicken/Mexico/232/94 (vac, Mexico/H5N2),
2 – A/duck/Potsdam/1402-6/1986 (vac, Potsdam/H5N2),
3 – A/chicken/Anhui/1/2006 (vac, Re-5/H5N1),
4 – rec A/Vietnam/1194/2004(H5N1) (NIBRG-14),
5 – A/duck/Egypt/NLQP-0827/2009 (EGYext;HI),
6 – A/chicken/Egypt/NLQP-0918/2009 (chg, EGYcls/H5N1),
7 – A/chicken/Egypt/0879/2008 (chg, EGYvar/H5N1).
3D prediction model of the hemagglutinin

antigenic sites (Duvvuri et al. 2009)
A – red,
B – green
C – blue
D – magenta
E – yellow)
receptor binding domain (cyan)

mutations in relation to the 2006 index virus
3
23
**Cross reactivity of HPAIV H5N1 and vaccine strains**

- Sera from chickens vaccinated once with inactivated adjuvanted subtype H5 vaccines, three weeks post vaccination
- Tested by hemagglutination inhibition test (HI)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Serum</th>
<th>Antigen</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Potsdam/ H5N2</td>
</tr>
<tr>
<td>Potsdam/ H5N2</td>
<td>#1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>8</td>
</tr>
<tr>
<td>Mexico/ H5N2</td>
<td>#1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>#2</td>
<td>7</td>
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<td></td>
<td>#3</td>
<td>6</td>
</tr>
<tr>
<td>EGYvar</td>
<td>#1</td>
<td>6</td>
</tr>
<tr>
<td>A/ck/EGY/0879/08</td>
<td>#2</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>#3</td>
<td>2</td>
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</table>

**Notes:**
- bold: homologous antiserum / antigen
- 1: HP AIV /H5N1-isolates from NLQP
- "classic" (2.2. proper)
- extinkt clade
- "variant" (clade 2.2.1)
HI antibody responses of vaccinated chickens

Grund et al., Vaccine 2011
Survival after challenge

Potsdam/H5N2 (○), Mexico/H5N2 (●), EGYvar/H5N1 (▼) or Re-5/H5N1 (△)

Grund et al., Vaccine 2011
Virus shedding after challenge
Summary

• Available inactivated commercial H5N2 virus vaccines induce protection against “classic” (2.2. proper) but not “variant” (clade 2.2.1) HPAIV H5N1

• H5N1 vaccines, representing antigenic sites of “variant” (clade 2.2.1) HPAIV H5N1, recognized by HI (R5 and EGYvar) are inducing protection against “classic” and “variant” HPAIV H5N1

• Indication that antigenic drift appears to be vaccine driven (cls <-> var)
Conclusion

• In case of ongoing vaccination, surveillance of the circulating HPAIV H5N1 viruses in Egypt and their antigenic characterization is needed
• AIV vaccines for poultry have to be optimised for the current / local situation
• Vaccination of poultry has to be paralleled by concerted efforts for efficient AI control
Thank you for your attention!
Prolonged until 11/2011!!
more than 80% (24 million USD) of the available budget for HPAI control has been devoted to vaccination since the programme was launched (GOVS, 2009).
Thank you …

Mario Ziller
Martin Beer

Friedrich-Loeffler Institute
Greifswald-Insel Riems

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Hafez M. Hafez

Institute for Poultry Diseases
Free University Berlin, Germany

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Diversifying evolution of Egyptian H5N1 Lineage 2.2.1
## Amino acid changes in the hemagglutinin

<table>
<thead>
<tr>
<th>Amino acid position</th>
<th>Virus strains</th>
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<tbody>
<tr>
<td></td>
<td>“Parent”¹</td>
<td>“Classic”²</td>
<td>Study (30)³</td>
<td>“Variant”⁴</td>
<td>Epitope⁵</td>
<td>RBD⁶</td>
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<tr>
<td>43</td>
<td>D</td>
<td>N</td>
<td></td>
<td></td>
<td>E</td>
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<tr>
<td>74</td>
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</table>
Aim of the vaccination study

• Are available inactivated commercial and experimental whole H5 virus vaccines able to protect against HP AIV H5N1 viruses which are currently co-circulating in Egypt?

• Testing two antigenically widely distinct HPAIV H5N1 “variant” (clade 2.2.1) and “classic” (2.2. proper) lineages
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