Efficacy of a commercial rHVT-H5 vaccine against the 2014 European HP H5N8 AIV

M. Steensels\textsuperscript{a}, S. Marché\textsuperscript{a}, F. Rauw\textsuperscript{a}, T. Harder\textsuperscript{b}, M. Beer\textsuperscript{b}, T. van den Berg\textsuperscript{a}, Y. Gardin\textsuperscript{c}, V. Palya\textsuperscript{d}, B. Lambrecht\textsuperscript{a},

\textsuperscript{a} Veterans and Agrochemical Research Centre, Belgium.
\textsuperscript{b} Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Germany.
\textsuperscript{c} Ceva Animal Health, Libourne, France.
\textsuperscript{d} Ceva Animal Health, Budapest, Hungary
Asian H5 evolution
rHVT-H5 vaccine

Clade 2.2 A/swan/Hungary/4999/2006, HP cleavage site: GERRRKKR*GLF
H5-gene with modified cleavage site: RETR*GLF

Inserted into a non-essential gene of the FC126 HVT strain
rHVT-H5 vaccine
# Vaccination and Challenge Schedule

<table>
<thead>
<tr>
<th>Group</th>
<th>Nr of SPF Chicken</th>
<th>Vaccine / Route / Dose / Age</th>
<th>ON Challenge D28 H5N8 HP Ge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>No</td>
<td>$10^5$ EID$_{50}$/dose</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>No</td>
<td>$10^6$ EID$_{50}$/dose</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>rHVT-H5 / sc / 1 dose / D1</td>
<td>$10^5$ EID$_{50}$/dose</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>rHVT-H5 / sc / 1 dose / D1</td>
<td>$10^6$ EID$_{50}$/dose</td>
</tr>
</tbody>
</table>

sc: subcutaneous vaccination, dose 4000 pfu/100 µl
ON: oculonasal challenge
Protection against mortality

![Graph showing survival rates after different doses of H5N8 GE virus.]

**Vaccinated SPF chicken**
- No dose-effect of challenge
- No clinical symptoms/No mortality

**Challenge controls**
- Clear dose-effect of the challenge
- No typical HPAI clinical signs
- Depression – apathy – loss of activity
- No neurological/respiratory signs, conjunctivitis

**Results**
- No vaccination - $10^5$ EID50/dose H5N8 GE
  - MDT: $3.89 \pm 2.26$
- No vaccination - $10^6$ EID50/dose H5N8 GE
  - MDT: $3.00 \pm 0.67$
- rHVT-H5 - $10^5$ EID50/dose H5N8 GE
- rHVT-H5 - $10^6$ EID50/dose H5N8 GE
Protection against viral excretion

<table>
<thead>
<tr>
<th></th>
<th>OROPHARYNGEAL</th>
<th></th>
<th></th>
<th></th>
<th>CLOACAL</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpi</td>
<td>2</td>
<td>5</td>
<td>9</td>
<td>14</td>
<td>2</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Vacc rHVT-H5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive AVG+STDEV</td>
<td>2,57±0,49</td>
<td>2,21±0,32</td>
<td>2,00</td>
<td>1,29±0,12</td>
<td></td>
<td>2,23±0,09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ratio of excreting birds</td>
<td>9/10</td>
<td>5/10</td>
<td>1/10</td>
<td>2/10</td>
<td></td>
<td>3/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total AVG+STDEV</td>
<td>2,35±0,85</td>
<td>1,27±1,02</td>
<td>0,50</td>
<td>0,52±0,41</td>
<td></td>
<td>0,90±0,92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not vaccinated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive AVG+STDEV</td>
<td>5,66±1,17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3,71±0,73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ratio of excreting birds</td>
<td>9/9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9/9</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>3,71±0,73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.M.: specific mortality</td>
<td>All unvaccinated birds-died by 4 dpi</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

✓ oropharyngeal > cloacal

✓ 2 dpi
oropharyngeal and cloacal: vaccinated < non-vaccinated (P<0,05)
oropharyngeal: significant difference in the amount of virus excreted
cloacal: significant difference in the amount and number of birds excreting

✓ oropharyngeal: 2 dpi: 90%, 5 dpi: 50%, 9 dpi: 10% and 14 dpi: 20% excreting birds
Cloacal: 2 dpi: 30%, 5 dpi: 0%, 9 dpi: 10% and 14 dpi: 0% excreting birds
SEROLOGICAL RESPONSE

1. HI Test

Clade 2.2.1. H5N1 A/swan/Hungary/4571/2006
Clade 2.3.4.4 H5N8 GE A/turkey/Germany-MV/R2472/2014

H5N8 clade 2.3.4.4 - clade

2.2. H5-vaccine insert

Distantly related

NT-identity: 0.906
AA-identity: 0.910
50 AA mutations – including antigenic sites

↔ Clinical protection
↔ Reduced viral excretion

<table>
<thead>
<tr>
<th>Antigens</th>
<th>4 weeks (age of challenge)</th>
<th>6 weeks (14 dpi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N1 clade 2.2</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>H5N8 GE</td>
<td>0/10</td>
<td>6/10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antigens</th>
<th>No Vacc</th>
<th>Vacc rHVT-H5</th>
</tr>
</thead>
<tbody>
<tr>
<td>H5N1 clade 2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H5N8 GE</td>
<td></td>
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</tr>
</tbody>
</table>

* indicates statistical significance.
SEROLOGICAL RESPONSE

2. NP-ELISA

Competitive ELISA: Detection of anti-NP antibodies against all Influenza A subtypes

By the use of a mAb against a conserved epitope of the NP

< challenge  
unvaccinated + vaccinated birds negative (DIVA)

14 days > challenge  
only 1 vaccinated-challenged birds seroconverted

⇒ Poor systemic H5N8 GE replication
SEROLOGICAL RESPONSE

3. M2-ELISA

Indirect ELISA: M2, an integral membrane protein

* Scarcely present on virus particles
* Abundantly expressed on virus-infected cells
⇒ Detection of viral replication

Evaluation of vaccinated birds 4 weeks after vaccination + at 14 dpi

- All remained negative
- Slower appearance due to reduced systemic viral replication?
SEROLOGICAL RESPONSE

4. H5-ELISA

**Commercial H5-ELISA**

- Vacc rHVT-H5/Challenge10^6 H5N8
- Day 27, 4 wpv
- Day 42, 14 dpi
- 8/10

**Prototype H5-ELISA**

- Vacc rHVT-H5/Challenge10^6 H5N8
- Day 27, 4 wpv
- Day 42, 14 dpi
- 7/10
- 10/10

*
CONCLUSION

rHVT-H5 vaccine – clade 2.3.4.4 HP H5N8 GE challenge

• Protection against morbidity and mortality

• Reduced systemic viral replication
  • Reduction of viral excretion measured by RRT-PCR
  • Weak humoral responses measured by HI and ELISA
  • Importance of cell-mediated immunity!

<table>
<thead>
<tr>
<th>Strain</th>
<th>Accession number</th>
<th>A/swan/Hungary/4999/2006</th>
<th>NT identity score</th>
<th>AA identity score</th>
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</thead>
<tbody>
<tr>
<td>A/swan/Hungary/4999/2006</td>
<td>KP969039</td>
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<td>A/swan/Hungary/4571/2006</td>
<td>FJ445234</td>
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<tr>
<td>A/turkey/Germany-MV/R2472/2014</td>
<td>EPI544756</td>
<td>0,906</td>
<td>0,910</td>
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</tr>
</tbody>
</table>
rHVT-vector vaccine: rHVT-F/NDV vaccine

Induction of humoral and cell-mediated immunity

Humoral immunity: NDV-specific IgG

Cell-mediated immunity: ChIFNg production after NDV-specific stimulation of splenocytes

= Necessary to obtain a larger spectrum of protection

Rauw et al. 2015, ‘Avian Pathology’
CONCLUSION

○ rHVT-H5 vaccine:
Timm Harder
Martin Beer

Yannick Gardin
Vilmos Palya

Bénédicte Lambrecht
Sylvie Marché
Fabienne Rauw
Anouar Nadi
Alexander Ausloos
Thierry van den Berg