Efficacy of a rHVT-AI Vector Vaccine in broilers with passive immunity against HVT and AIV, against challenge with H5N1 HPAI

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Global vision of the situation

- Globally speaking, vaccination against HPAI (H5N1) is not a success, because of:
  - Poor application (general and constraints)
  - Poor efficacy of inactivated vaccines (type and quality)
- There is a need for more efficacious / convenient vaccines (mass vaccination)
- There is a race between vaccine and challenge
- There is interference of MDA
- There is a need for more adapted vaccines
Day old vaccination:
- Immaturity of immune system
- Poor uptake of “dead” antigens until 10-15 days of age
- Maturation of immune system by “live” antigens (virus)
- Interference of MDA with live vaccines

Investigate new live vaccine candidates:
- rFP-AI
- rNDV-AI
- r HVT – AI (VECTORMUNE HVT-AI)
Information on the HVT vector

- Herpes Virus of Turkey
- Serotype 3 in family Marek’s Disease Virus
- Used world wide to protect chickens against the Marek’s disease:
  - Almost all breeders and layers pullets are vaccinated world wide
  - Broilers vaccinated in some areas (Americas, Mediterranean countries, Eastern Europe countries)
- Large genome allowing genetic manipulation
Advantages of rHVT – AI vaccine

- Can be applied:
  - At day-old (or even “in-ovo”)
  - Whatever the MDV / HVT passive immunity status
  - In a much more “reliable” manner
  - To millions of chicks using semi-automatic or automatic injectors

- No post vaccination reactions

- Life long persistence (life long immunity?)

- Possibility for DIVA vaccination strategy:
  - Specific PCR for the vector on feather tips samples
  - HI titres but no NP ELISA titres

- Possibility to adapt the vaccine to field changes by changing the insert.
Characteristics of rHVT – AI vaccine

- The vaccine can be presented under:
  - **Cell associated (ca) / Deep frozen form**:  
    - no loss in titer
    - no interference with MDV passive immunity
    - need to be stored in liquid nitrogen
  - **Cell free (cf) / freeze dried (lyophilized) form**:  
    - titer loss due to freeze drying process
    - no need for liquid nitrogen logistic

- Cheaper than classical inactivated vaccines
### Design of the study

- Commercial broilers with or without MDA against H5N2
- SQ vaccination (2000 pfu) or not at day of age
- Challenge at 14 or 21 days with $10^6 \text{EID}_{50}$ of clade 2 H5N1 HPAIV of Hungarian origin (A/duck/Hungary/11804/2006)

<table>
<thead>
<tr>
<th>Group</th>
<th>#</th>
<th>MDA (H5N2)</th>
<th>Vaccine</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14d</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>+</td>
<td>rHVT-AI ca</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>+</td>
<td>rHVT-AI cf</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>+</td>
<td>No</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>-</td>
<td>rHVT-AI ca</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>-</td>
<td>rHVT-AI cf</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>-</td>
<td>No</td>
<td>+</td>
</tr>
</tbody>
</table>
Protection against mortality challenge at 14 days

**MDA = Maternally Derived Antibodies (against H5N2 Mexico)**

**ca = cell-associated = deep frozen vaccine**

**fd = freeze dried (lyophilized) = cf = cell free vaccine**
Protection against mortality challenge at 21 days
**Antibody response after vaccination and challenge**

**Challenge at 14-day-old**

<table>
<thead>
<tr>
<th>Group</th>
<th>Before challenge (14-day-old)</th>
<th>After challenge (28-day-old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 = MDA+ HVT ca</td>
<td>3/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Group 2 = MDA+ HVT cf</td>
<td>2/10</td>
<td>8/8</td>
</tr>
<tr>
<td>Group 3 = MDA +</td>
<td>10/10</td>
<td>8/8</td>
</tr>
</tbody>
</table>

**Challenge at 28-day-old**

<table>
<thead>
<tr>
<th>Group</th>
<th>Before challenge (28-day-old)</th>
<th>After challenge (36-day-old)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 = MDA+ HVT ca</td>
<td>8/8</td>
<td>9/9</td>
</tr>
<tr>
<td>Group 2 = MDA+ HVT cf</td>
<td>9/10</td>
<td>10/10</td>
</tr>
<tr>
<td>Group 3 = MDA +</td>
<td>8/8</td>
<td>8/8</td>
</tr>
</tbody>
</table>

**HI antigen**

- H5N1 clade 2 A/Duck/Hungary/11804/2006
- H5N2 Queretaro Mexico 1994

**HI titre (log2)**

- Group 1
- Group 2
- Group 3
- Group 4
- Group 5
- Group 6
Reduction of excretion
Challenge at 14 days

Figure 4: Challenge at 14 days:
**rHVT-AI: PROTECTION AGAINST SHEDDING**

- **% of shedders**
  - MDA+  rHVT-AI ca
  - MDA+  rHVT-AI cf
  - MDA+  No vaccine
  - MDA-  rHVT-AI ca
  - MDA-  rHVT-AI cf
  - MDA-  No vaccine

- **Days Post Challenge**
  - 2
  - 4
  - 7
  - 10
  - 14

Tracheal swabs - Results expressed in log(number of copies RNA / ml)
Reduction of excretion
Challenge at 21 days

Figure 5: Challenge at 21 days:
rHVT-AI: PROTECTION AGAINST SHEDDING

Tracheal swabs - Results expressed in log(number of copies RNA / ml)
Conclusions

- MDA alone (against H5N2 Queretaro) delayed and reduced mortality but did not prevent it.
- MDA (against H5N2 Queretaro) did not interfere with installation of protection by rHVT-AI.
- rHVT-AI vaccination provided complete (in case of the ca vaccine) or almost complete (in case of the cf vaccine) protection against challenge.
- Vaccine protection is better if chickens were vaccinated in presence of MDA (passive protection seemed to add to active vaccine protection) in case of early challenge.
- rHVT-H5 ca worked better than rHVT-H5 cf in case of early or late challenge.
- Vaccination with rHVT-H5 delayed (challenge at 21 days) or suppressed (challenge at 14 days) re-excretion.
From preliminary results, performance of protection induced by rHVT-AI vaccine are very promising (ca form), including when given in the presence of passive immunity.

These results need to be confirmed under both laboratory and field conditions.

Important aspects that need further investigations are:

- Prime-boost combination
- Duration of immunity
- Impact of antigenic variation