An update on Marek’s disease

Isabel M. Gimeno
Content

• Marek’s disease (MD) and Marek’s disease virus (MDV) evolution
• MDV-IS: the newest challenge of MDV infection
• MDV-induced tumors:
  • Analysis of a MD outbreak- what to do
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Minor Disease

**Inflammatory** in nature
Only peripheral nerves
Low mortality
No economical relevance

Major disease
Neoplastic in nature (tumors)
Peripheral nerves, viscera, skin
High mortality
Major economical impact

- **Tumors** in vaccinated chickens
- Tumors in genetically resistant chickens
- Non neoplastic syndromes
  - Neurological (transient paralysis)
  - Vascular (arteriosclerosis)
  - Panophtalmis
- **Immunosuppression**
MDV evolution and consequences on MD

Clinical manifestation index vs. MDV pathotype

- Inflammation
- Tumors
- Immunosuppression

MDV pathotypes: m, v, vv, vv+
Causes for MDV evolution

• Changes in the production system
  • Backyard – Large integration
• Vaccines do not protect against superinfection or against MDV transmission
• Recent studies
  • Chicken genetics
  • Inmunization against MDV
Vaccines and MDV evolution

Relative virulence

1940 1960 1980 2000 2020

HVT HVT+SB-1 Rispens v v+ m

R.L. Witter
Features of highly virulent MDV

- Overpass vaccine immunity
- Induce tumors in other species (turkeys)
- Induce tumors in resistant lines of chickens
- Induce a variety of syndromes both neoplastic and non-neoplastic (very complex disease)
Syndromes associated with highly virulent MDVs

- Neuritis
- Arteriosclerosis
- Panophtalmitis
- Transient immunosuppression
- Transient paralysis
- Tumors (Lymphomas)
- Permanent Immunosuppression
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Immune system dysregulation

1) Early cytolytic infection
2) Immune system dysregulation
   Permanent MDV-IS (pMDV-IS)
3) Tumors

Maternal antibodies

0 dpi  5-6 dpi  10 dpi  21 dpi
Experimental model to evaluate pMDV-IS

MDV + CEO + LTV → Laryngotracheitis

Indirect evaluation of pMDV-IS
Permanent immunosuppression (Mab)
Cell-mediated immunity (CEO protection)
Experimental model

Day -15
Shedders hatch

Day 0
Comingle shedders with experimental chickens

Day 15
CEO vaccination

Day 30 – Day 37: Monitoring ILT clinical signs

Day 34 & Day 37: Sampling trachea/Termination

Day 30
LTV Challenge

MDV

CEO

LTV

NC STATE Veterinary Medicine
### Experimental design

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>Dose/Age</th>
<th>Vaccine</th>
<th>Dose/Age</th>
<th>Strain</th>
<th>Dose/Age</th>
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<tbody>
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<td>MR/15d</td>
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<tr>
<td>4</td>
<td>648A</td>
<td>Contact/1d</td>
<td>CEO</td>
<td>MR/15d</td>
<td>Illinois</td>
<td>4000/30d</td>
</tr>
</tbody>
</table>
Late-MDV-IS model (results)

% chickens showing clinical signs

-/-/ILTV

-648A/CEO/ILTV

-/-/CEO/ILTV

D1 D2 D3 D4 D5 D6
pMDV-IS using ILT model

• Pathotype effect
  • vv+MDV (648A, 686) but not vvMDV (Md5) or vMDV (GA, 617A)

• Protection conferred by vaccines
  • HVT, HVT+SB-1, CVI988 administered at 1d do not protect against pMDV-IS (648A 1d)
  • Combination of HVT and CVI988 in ovo y in revaccination protocols do not protect against pMDV-IS (686 1d)
  • Experimental vaccine rMd5ΔMeq protect against both MDV-induced tumors and pMDV-IS

• pMDV-IS occurs in chickens without tumors and with no lymphoid organ atrophy
Effect of pathotype on MDV-IS
Protection of MD vaccines against late-MDV-IS

<table>
<thead>
<tr>
<th>Vaccination at day of age</th>
<th>Various vaccine protocols (revac, IO..)</th>
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<tbody>
<tr>
<td>MD (%)</td>
<td>50</td>
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</table>

<table>
<thead>
<tr>
<th>Protocol</th>
<th>PI CEO relative to -/CEO/LTV group</th>
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</thead>
<tbody>
<tr>
<td>648A/CEO/LTV</td>
<td>*</td>
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<tr>
<td>HVT/648A/CEO/LTV</td>
<td>*</td>
</tr>
<tr>
<td>HVT + SB1/648A/CEO/LTV</td>
<td>*</td>
</tr>
<tr>
<td>CV1988-B/648A/CEO/LTV</td>
<td>*</td>
</tr>
<tr>
<td>686/CEO/LTV</td>
<td>*</td>
</tr>
<tr>
<td>HVT (IO) CV1988-B (D1)/686/CEO/LTV</td>
<td>*</td>
</tr>
<tr>
<td>HVT + CV1988-B (IO)/686/CEO/LTV</td>
<td>*</td>
</tr>
<tr>
<td>Md5-BAC/CEO/686/CEO/LTV</td>
<td>*</td>
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</tbody>
</table>
Implications of pMDV-IS in the field

- It can occur under field conditions
- As of today cannot be detected
- The methods for controlling tumors are not valid to protect against pMDV-IS
- It affects cell-mediated responses – protection against other diseases could be seriously compromised.
Future studies

• Mechanisms involved in pMDV-IS
• Detection methods
• Better characterization of protection conferred by experimental vaccine rMd5Δmeq
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Marek’s disease outbreak

• Higher economical losses due to MD (deaths, condemnations, decreased production) than expected
• Remember there will be some chickens developing MD (vaccine protection is not 100%)
What could have failed?

- Early MDV infection
- Inhibition by maternal antibodies
- Suppression of immune system
- Highly virulent MDV field strains

Inappropriate storage & reconstitution
Incorrect administration
Administration of other vaccines
Immune response to vaccine
Establishment of immunity
Auditing at the hatchery

- Proper storage of vaccines
- Protocols for thawing
- Protocols for vaccination
- Check for factors that could reduce vaccine dose
  - Higher dilution than recommended
  - Delay in administration of reconstituted vaccine
  - Insufficient mixing of the vaccine
  - Addition of antibiotics
- Live cell counts vs. vaccine titers
Time and titers

Room temperature: reduced to 55% within 1 hour
Refrigeration: reduced to 76% within 1 hour
MD cell associated vaccines

Cell suspensions are unstable

Green cells indicate cells that are actually infected with MD vaccine virus
Mixing and dose uniformity

Vaccine A
(2000-7000 PFU/dose)

Vaccine B
(3500-6100 PFU/dose)
Time plus mixing..... and titers

2000-3500 PFU/dose
1500-3000 PFU/dose
500-1500 PFU/dose
Antibiotics and titers

![Graph showing the effect of antibiotics on titers over time.](image-url)
What else can we check?

- Immune response to vaccine
- Establishment of immunity
- Pathotyping of oncogenic virus
- Protection
  - Quantification of oncogenic virus
  - Vaccine replication in the chicken
  - Vaccine titers
Vaccine Titrations

• Measure live vaccine virus
  • PFU/dose

• How does this differ from live cell counting?
  • It might have low PFU to begin with

• When and how to titrate vaccines?
  • From the reconstituted vaccine
Important facts for vaccine titration

• Vaccines are cell suspensions and there is variability of doses within a vial – Range of PFU per dose
• Vaccine titration should be done in replicates (10-20X)
• Results can vary from lab to lab depending on cell culture protocols
• Requires laboratories with experience in Marek’s disease virus cell culture
Counting of live cells vs vaccine titration

Infectivity rate
- A: 5%
- B: 25%
- C: 25%

Viability Trypan blue
- A: 99%
- B: 99%
- C: 10%

Titration PFU/dose
- A: 1500 PFU/dose
- B: 7500 PFU/dose
- C: 200 PFU/dose
Vaccine Replication \textit{in vivo}

- Gives information if vaccine is properly replicating in the chicken
- Feather pulp (do not use blood)
- Real time PCR
- 1 week of age
Collection of FP samples on FTA® cards

Do not mix samples from different chickens in one circle

Extract FP samples in a clean area (outside the chicken house)

Let the samples air dry before closing FTA cards
Collection of samples 1wk vs 3wk

HVT (1d)

% positive chickens

HVT d freq

HVT f freq

Modified from Gimeno et al. Avian Dis. 2011, 55:263-72
Factors influencing % positive chickens for vaccine virus at 1 week

- Vaccine used
  - Serotype
  - Origin of the vaccine
- Route/Age at vaccination
- Vaccine dose
- Combination of vaccines used
Factors influencing CVI988 replication

Dose and vaccine strain

Modified from Gimeno et al, Avian Dis. 2015, 59:400-9
Effect of route and vaccine combination: **CVI988**

- **In ovo with HVT+SB-1**
- **In ovo alone**
- **At day of age**

Modified from Gimeno et al, Avian Dis. 2015, 59:400-9
Effect of route and vaccine combination: HVT and SB-1

Modified from Gimeno et al, Avian Dis. 2015, 59:400-9
Vaccine replication is not correlated with protection

CVI988: $r = 0.427$ p>0.05  
SB-1: $r = 0.421$ p>0.05  
HVT: $r = 0.309$ p>0.05
Quantification of oncogenic virus
Early diagnosis

Immune response to vaccine
Establishment of immunity
Protection
Quantification of oncogenic virus
Rationale for early diagnosis

• Tumor cells have more copies of the virus than latently infected cells

• Chickens can be divided into three categories based on the amount of viral DNA:
  - Negative
  - Latently infected
  - Tumors

• Tumor cells circulate in the blood of the chicken before gross tumors are detected

• Feather pulp is a suitable sample for early diagnosis (as early as 3 weeks)
Correlation between PI (tumors) and % T FP 3wks

\% T FP 3wk = 80.338 - .7638 \times PI

Correlation: \( r = -0.9 \)  \( p<0.05 \)
Quantification of oncogenic virus

Pathotyping

Establishment of immunity

Immune response to vaccine
Pathotyping MDV

• Rationale
  – Protection conferred by various vaccines

• Limitations of the pathotyping assays
  – Cost, labor, difficult to do in most laboratories
  – USDA-ARS ADOL (Dr. John Dunn)

• Alternative to pathotyping
  – Neuropathotyping
  – Lymphoid organ atrophy
  – Viral replication
  – Molecular markers
Outbreak of Marek’s disease

Auditing at the hatchery

OK

Not OK

Replication of the vaccine in the chicken
Real time PCR - feather pulp 1 week

OK

Not OK

Improve storing and handling of the vaccine

Poor titer of vaccine
Inadequate storing and handling of vaccine
Interaction with other vaccines administered simultaneously

Early infection
More virulent virus

Evaluate vaccine protocols
Strategies to increase vaccine protection

• Vaccine used
  • HVT < HVT+SB-1 < CVI988

• Revaccination
  – The second vaccine should be more efficient than the first vaccine administered
  – In ovo follow by subcutaneous at day of age

• In ovo vaccination
  – Better protection against MD
  – Hasten immune system development in the embryo

• Others
  – Protective synergism
  – Adjuvants (TLR agonist, cytokines….)


Summary

• MD is a complex disease. MDV induces a variety of syndromes both neoplastic and non-neoplastic

• MDV-IS is the non-neoplastic syndrome of highest relevance. It is not associated with lymphoid organ atrophy or with tumors.

• Only vv+MDVs induce pMDV-IS

• As of today there are not methods for the diagnosis or control of pMDV-IS (only one experimental vaccine protects)
Summary

• Proper control of MD requires further investigation of MD outbreaks
• Auditing at the hatchery is critical as MD vaccine management is complex
• Evaluation of vaccine replication (1 wk) can confirm that vaccines were properly administered but it is not related to protection
• Early MD diagnosis is possible by evaluating oncogenic MDV DNA load in feather pulp at 3 weeks of age
• Pathotyping is expensive and labor intensive but it is the only way to confirm MDV virulence (m, v, vv, vv+)