Use of real time PCR for Marek’s disease diagnosis and for monitoring vaccination

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Content

• Introduction to real time PCR
• Use of real time PCR for monitoring MD vaccination
  • Differentiation among serotypes
  • Differentiation between CVI988 and oncogenic MDVs
• Use of real time PCR for diagnosis of MD
  • Confirming etiology of tumors
  • Early diagnosis (3 weeks of age-onwards)
• Collaboration with Merial
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Real time PCR

• It allows to identify the presence of a particular gene and **quantify** how much of the gene is in the sample
• It is highly specific
  - Differentiate among MDV serotypes
  - Differentiate between CVI988 (Rispens) and oncogenic MDVs
• Useful technique for
  • Monitoring MD vaccination
  • Diagnosis of MD
    • Tumors
    • Early diagnosis
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Real time PCR for monitoring MD vaccination

- Gives information if vaccine is properly replicating in the chicken
- Specific for each serotype and for CVI988
- Feather pulp or spleens (do not use blood)
- 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
Monitoring HVT vaccination
(Vaccination at 1d)

% positive chickens (FP)

1 3 8 weeks

HVT diluted dose
HVT full dose

Modified from Gimeno et al. Avian Dis. 2011, 55:263-72
Monitoring CVI988 vaccination

• Limitations of the technique
  - Based on one single nucleotide difference in the sequence of pp38
  - Limitations to detect CVI988
    - Specificity decreases when oncogenic virus load is high
      (viral DNA load in tumors ~10^3 copies)

• Applications of the technique
  - Detection of CVI988 at 1 week – oncogenic viral DNA load is never at tumor levels
Monitoring CVI988 vaccination
CVI988 (in ovo)

% positive chickens (FP) at 7d

- CVI988A-HD
- CVI988A-LD
- CVI988B-HD
- CVI988B-LD

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

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

<table>
<thead>
<tr>
<th></th>
<th>In ovo with CVI988</th>
<th>At day of age</th>
<th>In ovo alone</th>
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</thead>
<tbody>
<tr>
<td><strong>HVT</strong></td>
<td><img src="image1.png" alt="Graph" /></td>
<td><img src="image2.png" alt="Graph" /></td>
<td><img src="image3.png" alt="Graph" /></td>
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<tr>
<td><strong>SB-1</strong></td>
<td><img src="image4.png" alt="Graph" /></td>
<td><img src="image5.png" alt="Graph" /></td>
<td><img src="image6.png" alt="Graph" /></td>
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</tbody>
</table>

Modified from Gimeno et al, Avian Dis. 2015, 59:400-9
Factors influencing the percentage of chickens with detectable levels of vaccine virus at 1 week

• Vaccine used
  - Serotype
  - Origin of the vaccine
• Vaccine dose
  • Over-dilution, use of antibiotics, improper mixing…..
• Route/Age at vaccination
• Combination of vaccines used
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
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• Collaboration with Merial
Use of real time PCR for MD diagnosis

• Tumor cells have more copies of the virus than latently infected cells
  • Tumors induced by MDV has high amounts of MDV DNA
• Early diagnosis
  • 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)
• Chickens can be divided into three categories based on the amount of viral DNA:
  - Negative
  - Latently infected
  - Tumors
Early diagnosis
Monitoring oncogenic MDV replication at 3 wks

Modified from Gimeno et al, Avian Dis. 2015, 59:400-9
Correlation between PI (tumors at 8 weeks) and % T FP (percentage of chickens with high load of MDV DNA in the FP at 3wks)

\[ \text{% T FP 3wk} = 80.338 - 0.7638 \times \text{PI} \]

Correlation: \( r = -0.9 \) \( \text{p}<0.05 \)
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  • Differentiation among serotypes
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• Use of real time PCR for diagnosis of MD (oncogenic virus)
  • Confirming etiology of tumors
  • Early diagnosis (3 weeks of age-onwards)

➢ Collaboration with Merial
Collaboration with Merial

• Optimization of real time PCR techniques for the diagnosis of MD and monitoring vaccination
  ▪ Cortes et al., Avian Dis. 2009, 53:510-516 (FTA cards)
  ▪ Cortes et al., Avian Dis. 2011, 55:302-310 (Sample type)

• Monitoring vaccination for experimental trials conducted at Merial
• Monitoring vaccination for commercial flocks
• Confirmation of MD diagnosis for commercial flocks
Summary

• Monitoring MD vaccination
  • Feather pulp or spleen (never blood) in FTA cards
  • 1 wk of age
  • Confirms that vaccines were properly administered but it is not related to protection
  • Results depends on vaccine, vaccine dose, age/route and vaccine protocols

• MD diagnosis
  • Imprint of tumors
  • Early diagnosis: Feather pulp or blood as early as 3 weeks of age
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Thank you for your attention!!

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