EXPERIMENTAL RESULTS WITH MHYO ID ONCE

SYSTEMIC AND LOCAL IMMUNE RESPONSE IN PIGS INTRADERMALLY AND INTRAMUSCULARLY INJECTED WITH INACTIVATED Mycoplasma hyopneumoniae VACCINES

Paolo Martelli
DVM, Diplomate ECPHM
Full Professor of Veterinary Clinical Medicine
Department of Veterinary Sciences
Parma University – Italy
www.unipr.it
Content

1. Immunological basis of the ID administration
   – Overview of the immune system
   – Immune cells in the dermis
   – Advantages & disadvantages

2. Intradermal vaccination. Do the vaccines work?

3. Systemic and local immune response in pigs intradermally and intramuscularly injected with inactivated *Mycoplasma hyopneumoniae* vaccines

4. Take home messages
The first vaccine by Edward Jenner applied the intradermal delivery in 1796: scratching techniques for application of cowpox vaccines.
Overview of the immune system

Innate immunity

Adaptive immunity

Microbe

Epithelial barriers

Phagocytes

Dendritic cells

Complement

NK cells

B lymphocytes

T lymphocytes

Effector T cells

Antibodies

Hours

Days

Time after infection

0

6

12

1

4

7

Abbas, Lichtman, Pillai. 7th Edition. Cellular and Molecular Immunology. Elsevier Saunders
Recognition of danger by Pattern recognition receptors

Pattern:
black clothes and boots, aggressive, in group, etc.
Adaptive immune system...

Recognition of persons: precise and requires a clear memory effort
Many lymph and blood vessels
Summary of essential immunological components of ID vaccination

1. Danger recognition and alerting
   - Bystander cells in tissue are activated by PAMPs and DAMPs
   - Fibroblasts
   - Tissue resident macrophage
   - Type I IFNs, pro-inflammatory cytokines and chemokines

2. Maturation and Migration
   - DC maturation and migration to lymph node
   - Cells damage at injection site releases DAMPs
   - DCs can be activated by PAMPs
   - Type I IFNs, pro-inflammatory cytokines and chemokines

3. Antigen presentation and initiation of immune responses
   - Naive CD8+ T-cell
   - Bystander cell
   - Plasma cell
   - Memory B cell differentiation
   - IL-4-dependent antibodies
   - IFNγ-dependent antibodies
   - IL-2, IL-12, type I IFNs, IL-21
   - Effector and memory CD8+ T cell differentiation
   - IL-2, IL-12, IL-17, IFNγ
   - Co-stimulatory molecules
   - MHC class I
   - TCR
   - IL-17, IFNγ
   - IL-4, IL-13

Desmet and Ishii, 2012, Nat. Immunol 12:479
Sequence of events after intradermal vaccination

**0.5**
- Free antigen accessing LN through lymphatic vessels

**0.5-2**
- Inflammatory response
  - Influx of more DC

**4**
- First DC with Ag in LN

**16-24**
- Second wave of DC with Ag in LN

**24-48**
- Third wave of DC with Ag in LN

**72**
- First T-cell response detected

**120**
- First antibody response detected

(Time (hours))
Delivery options for intradermal vaccination (2012)

Kis et al., 2012 Vaccine 30:523
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4. Take home messages
1. Immune response to Aujeszky’s Disease vaccination
2. Immune response and protection induced by PRRS vaccination
3. Local and systemic immune response to *Mycoplasma hyopneumoniae* vaccination
Immune response to ADV vaccination

Ferrari L. et al., RVSC 90 (2011) 64-71.

IFN-γ secreting cells (PBMC)

anti-ADV VN antibodies (serum)

IFN-γ gene expression (PBMC)
PRRS vaccination + Experimental infection in laboratory conditions

Viremia and humoral immunity


PRRSV ELISA antibodies

PRRSV viremia PE

PRRSV infection

Viremia and humoral immunity

PRRSV ELISA antibodies

PRRSV viremia PE

PRRSV - PCR positivity PV

<table>
<thead>
<tr>
<th>Time</th>
<th>A (IM)</th>
<th>B (ID)</th>
<th>C (ctrl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 = vaccination</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>T1 = 7 dpv</td>
<td>0/6</td>
<td>4/6</td>
<td>0/6</td>
</tr>
<tr>
<td>T2 = 14 dpv</td>
<td>1/6</td>
<td>2/6</td>
<td>0/6</td>
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<tr>
<td>T3 = 21 dpv</td>
<td>2/6</td>
<td>0/6</td>
<td>0/6</td>
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<tr>
<td>T4 = 28 dpv</td>
<td>1/6</td>
<td>1/6</td>
<td>0/6</td>
</tr>
<tr>
<td>D0 = 35 dpv/challenge</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>

PRRSV - PCR positivity PE

<table>
<thead>
<tr>
<th>Time</th>
<th>A (IM)</th>
<th>B (ID)</th>
<th>C (ctrl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1 = 3 dpc</td>
<td>5/6</td>
<td>5/6</td>
<td>6/6</td>
</tr>
<tr>
<td>D2 = 7 dpc</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>D3 = 10 dpc</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>D4 = 13 dpc</td>
<td>2/4</td>
<td>2/4</td>
<td>4/4</td>
</tr>
<tr>
<td>D5 = 17 dpc</td>
<td>0/4</td>
<td>1/4</td>
<td>1/4</td>
</tr>
</tbody>
</table>
**PRRS vaccination + Natural infection**

### Clinical Monitoring

<table>
<thead>
<tr>
<th></th>
<th>IM</th>
<th>ID</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Clinical Score (GCS)</td>
<td>0.74±1.16*</td>
<td>0.67±1.01*</td>
<td>2.13±1.67</td>
</tr>
<tr>
<td>Respiratory Clinical Score (RCS)</td>
<td>0.84±1.54*</td>
<td>0.64±1.30*</td>
<td>2.61±2.25</td>
</tr>
<tr>
<td>ADWG gr/day</td>
<td>510±77*</td>
<td>515±59*</td>
<td>346±105</td>
</tr>
</tbody>
</table>

Overall Clinical Score

\[ \text{OCS} = \text{GCS} + \text{RCS} \]
PRRS vaccination + Natural infection
Virology and Serology

PRRSV

→

PRRSV positivity (PCR)

Anti-PRRSV antibodies

→

S/P ratio (ELISA)

cut-off = 0.4
PRRS vaccination + Natural infection

Frequency of IFN-γ secreting cells

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4. Take home messages
What we know about Mycoplasma

- The chronic pathogenesis of *M. hyopneumoniae* is complex and consists of two separate mode of action:
  1. Adherence and colonization of the cilia with reduction in the ability of the mucociliary apparatus
  2. Alteration or modulation of the respiratory immune response
    - The microorganism develops mechanisms to circumvent the immune system
      - Immune system can be double edge sword
        » Control disease
        » Induce disease

- The immune system can be a double-edged sword:
  - Control disease
  - Induce disease
What we know about Mycoplasma

*Mycoplasma hyopneumoniae* diminishes mucociliary apparatus
Effect on the Respiratory Tract

- Influx of immune cells
  - lymphocytes and macrophages
    - primarily B cells
    - non-specific

- **Modulates** the ability of respiratory tract to respond to other pathogens
What we know about Mycoplasma control

• M. hyopneumoniae and EP
• Occurrence: worldwide

• Control is based on:
  • Optimization of housing
  • Management procedures
  • Antibiotic Strategic Medication
  • Vaccination (effective in reducing the clinical signs and the lung lesions associated with the infection)
Mechanisms of protection by vaccination

- Secretion of specific antibodies in serum and respiratory tract
  - IgG and IgA
  - No direct correlation between the amount of total ELISA antibodies and protection
- Generation of cell-mediated immunity (IFN-g SC) in the blood
- Mucosal immunity
- Protection
  - Local mucosal antibodies
  - Systemic CMI (no changes in Lymphocytes subpopulations- CD4⁺ & CD8⁺)
- The exact mechanism of protection induced by inactivated MHYO vaccines is not completely known
THE AIM OF THE STUDY

• To analyse and compare the **systemic and the local immune response** induced by **inactivated whole-cell vaccines** administered via
  • Intramuscular route (2 commercial vaccines)
  • Intradermal route (1 commercial vaccine)
  • Intradermal route of the adjuvant only

that have been shown to be effective in reducing the clinical signs and the specific lung lesions.
Materials and methods

- Double blinded controlled trial
- 40 piglets 3 week-old were moved from a Mycoplasma-free herd to the isolation facilities of the Department of Veterinary Sciences – Parma University
- After one week of acclimation, at 28 days of age, they were individually identified by ear-tags and randomly allocated to 4 different groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>TREATMENT</th>
<th>Dose</th>
<th># pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>MHYO ID ONCE (test product)</td>
<td>0.2 ml INTRADERMALLY in the neck by using IDAL device</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>Commercial MHYO VACCINE 1 – ONE DOSE</td>
<td>X ml INTRAMUSCULARLY in the neck</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>PLACEBO/CONTROL</td>
<td>0.2 ml INTRADERMALLY in the neck by using IDAL device</td>
<td>10</td>
</tr>
<tr>
<td>D</td>
<td>Commercial MHYO VACCINE 2 – ONE DOSE</td>
<td>X ml INTRAMUSCULARLY in the neck</td>
<td>10</td>
</tr>
</tbody>
</table>
Materials and methods

Weaning and arrival

Age (days) 21  28  56  84
1 week +4 weeks + 4 weeks

Blood sampling + Vaccination

Blood and BAL fluid* sampling

Blood and BAL fluid* sampling

TIMELINE OF THE EXPERIMENTAL PERIOD

Weaning and arrival

*BAL fluid samples were collected in vivo in immobilized, standing position
Materials and methods

**Investigations on blood samples**
- Specific antibodies to *M. hyopneumoniae* in serum by ELISA serology – specific IgG
- Amount IFN-γ SC specific to *M. hyopneumoniae* in PBMC (ELISpot)

**Investigations on BAL fluid**
- IgA level to *M. hyopneumoniae* by ELISA
- Cytokines from PBMC
- Nested and quantitative PCR (qPCR) for detection of *M. hyopneumoniae* DNA
Results

1. Nested and qPCR for detection of M. hyopneumoniae DNA

NONE OF THE BALF FLUID SAMPLES WERE PCR POSITIVE TO M. HYO
Detection of *M. hyopneumoniae* total antibodies in blood serum

![Graph showing antibody levels over time for different treatments](image)

- **Vacc**
  - +4 weeks
  - +8 weeks
  - 100%

Legend:
- **A**
- **B**
- **C**
- **D**

*Note: The graph illustrates the increase in antibody levels over time for different treatments.*
Levels of IgG specific to M. hyo in blood serum
IFN-γ SC specific to M. hyo in blood serum
M. hyo specific IgA in BAL fluid
Detection of cytokines gene expression in BAL fluid

(a) TNF-α

(b) IL-6

(c) IFN-γ

(d) IL-10
Detection of IL-10 gene expression in BAL fluid
Mucosal immunity

Dendritic cells are present in the derma
Local and systemic immune response to *Mycoplasma hyopneumoniae* vaccination

- **Immunity ≠ protection**
  - The trial is not aimed at assessing the efficacy of the vaccines

- **Immunological study**
  - **Focus on INTRADERMAL ADMINISTRATION**
    1. ID administration seems to stimulate the immune system at least as efficiently as IM route or even better
    2. ID administration engages a more efficient stimulation of the mucosal immunity (IgA response and proportion of positive animals + gene expression of cytokines correlated with the mucosal immunity)
    3. ID administration induces a more efficient stimulation of the cell mediated immunity (IFN-γ SC in the BAL fluid)
  - **Focus on INTRAMUSCULAR ADMINISTRATION**
    1. Different performance in term of immune stimulation comparing vaccines for IM administration
Take home messages

1. The dermis is an excellent “processor” of antigens – DC are abundantly present

2. The processing of the antigen after intradermal vaccination is fast and efficient

3. Advantages and disadvantages of ID administration

4. Intradermal vaccination to ADV and PRRSV induces an immune response and a clinical protection similar or even better than the IM administration

5. Mycoplasma vaccination via the ID route induces a humoral, local (mucosal) and cell mediated immune response as efficient as obtained by the more conventional IM administration
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Thank you for your attention