Mastitis cows and immunization

In Spain, the antibiotherapy against mastitis moves €12,000,000 with an interannual growth of 10.2%. Only 4 of these millions are drying antibiotherapy.

**Conclusion:** farmers spend a lot of money on mastitis treatment during lactation (injectables and intramammary antibiotics, NSAIDs, fluidtherapy, etc.).

The main costs for a farm are mastitis treatments followed by vaccine protocols.
Mastitis cows and immunization
Mastitis cows and immunization

MAIN CHARACTERS
Mastitis cows and immunization

Vaccine:
Inactivated vaccine against bovine mastitis

Composition per dosage (2ml):
Active substances:

- *E.coli* (J5) (inactivated)
- *Staphylococcus aureus* (Sp8) (inactivated), Slime Associated Antigenic Complex (SAAC)
Mastitis cows and immunization

Dosage and administration route:
• Bovine: 2ml / animal
• Intramuscular administration in the neck.

Recommended administration protocol:
• 1st. administration: 45 days before calving
• 2nd. administration: 10 days before calving
• 3rd. administration: 52 days post-partum
Mastitis cows and immunization

KEY POINTS
Mastitis cows and immunization

1.- Action mechanism of the components in vaccine

Vaccine activates immunity in cows and heifers, and prevents intramammary infections, reducing clinical and sub-clinical mastitis against *S. aureus*, *E. coli* and CNS

**E. coli:**

- **Does not adhere** to the ducts and alveoli of the mammary gland; they grow quickly in milk, producing **toxic substances** that end up in the bloodstream.
- **Vaccination** destroys cell wall development and enables humoral immunity activity.

**S. aureus** and **CNS:**

- Colonization due to the **Slime or Biofilm**, which facilitates adhesion between the bacteria and, simultaneously, does not allow the penetration of antibiotic treatments.
- **Vaccine** stops the development of micro-colonies
Some general features of *Escherichia coli*

*E. coli* J5 is a strain that lacks the enzyme Uridin Diphosphate Galactose 4-Epimerase, which is responsible for binding the somatic antigen (O-Antigen of polysaccharide) to the LPS molecule of the cell wall.

The vaccine was developed with the concept of the exposure of the core antigen common to Gram-negative organisms in the mutant J5 strain (rough strain).
Mastitis cows and immunization
Mastitis cows and immunization
The Biofilm as survival mechanism

Biofilms are survival mechanisms of clinically relevant microorganisms.

The production of this extracellular matrix defines the ability of bacterial strains to colonize host tissues and biomaterials, participating in the intercellular adhesion among bacterial cells and subsequent development of a BIOFILM, leading to chronic infections and bacterial resistance to phagocytosis and antibiotic treatments.

Scanning electron micrograph of a biofilm on a metal surface from an industrial water system.
Mastitis cows and immunization
The Biofilm as survival mechanism

• Bactericidal effect of the antibiotics on the biofilm:

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Antibiotic</th>
<th>Reference Organism Antibiotic MIC or MBC of planktonic phenotype (µg/ml)</th>
<th>Concentration effective against biofilm phenotype (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus NCTC 8325-4</td>
<td>Vancomycin</td>
<td>2 (MBC)</td>
<td>20</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Imipenem</td>
<td>1 (MIC)</td>
<td>1,024</td>
</tr>
<tr>
<td>ATCC 27853</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25922</td>
<td>Ampicillin</td>
<td>2 (MIC)</td>
<td>512</td>
</tr>
<tr>
<td><em>P. pseudomallei</em></td>
<td>Ceftazidime</td>
<td>8 (MBC)</td>
<td>800</td>
</tr>
<tr>
<td><em>Streptococcus sanguis</em></td>
<td>Doxycycline</td>
<td>0.063 (MIC)</td>
<td>3.15</td>
</tr>
</tbody>
</table>

MIC: Minimum Inhibitory Concentration
MBC: Minimum Bactericidal Concentration

From antigens to protection

Vaccine - ANTIGENS

- E. Coli J5
- Core LPS
- SA–SP140 (CP8)
- SAAC

CLINICAL RESULTS

- Coliforms
- SA + CNS
Vaccination: Mechanism of protection

Vaccination

Induction of antibodies against core epitopes of LPS common to gram-negative bacteria

Induction of antibodies against SAAC components present in the biofilm matrix that produce staphylococcal bacteria during the initial phase of infection.

Intramammary infection (IMI)

Antibodies anti-SAAC bind to the initial exopolysaccharide matrix production before the biofilm is established.

Antibodies anti-\textit{E. coli} J5 bind to the core antigens during the multiplication of invading bacterial cells, exposed just before the synthesis of LPS is completed.

Phagocytosis of the opsonized bacterial cells by neutrophils

Clearance of IMI due to \textit{S. aureus} and CNS

Clearance of IMI due to \textit{E. coli} and coliforms
Mastitis cows and immunization

REGISTRATION PROGRAM EMA
Mastitis cows and immunization

Register Program EMEA (European Medicines Agency):

- Producer
- Dossier of register
- EMA
  - Experts (rapporter & co-r)
    - (Evaluation Inform)
    - CVMP (Experts Group-27)
  - Questions
  - Answer
- Firm
- European Commission Decision
  - Opinion positive
- Register
Mastitis cows and immunization

Vaccine

**EMA register conclusions:**

- EMA is the most exigent system to register at this moment in the world.
- It is the first mastitis vaccine registered by this kind of method.
- It has been registered simultaneously in 30 European countries.
Mastitis cows and immunization

EMEA FIELD TRIALS
# Mastitis cows and immunization

<table>
<thead>
<tr>
<th>Variable</th>
<th>STARTVAC Group</th>
<th>PLACEBO Group</th>
<th>STATISTICAL SIGNIFICANT DIFFERENCES BETWEEN STARTVAC AND PLACEBO (α = 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Incidence of intramammary infection clinical or subclinical until day 130</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>1,18%</td>
<td>10,34%</td>
<td>0,001</td>
</tr>
<tr>
<td>E. coli</td>
<td>4,14%</td>
<td>17,82%</td>
<td>0,001</td>
</tr>
<tr>
<td>CNS</td>
<td>16,57%</td>
<td>32,18%</td>
<td>0,001</td>
</tr>
<tr>
<td><strong>Incidence of intramammary infection clinical until day 130</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>0,00%</td>
<td>2,87%</td>
<td>0,032</td>
</tr>
<tr>
<td>E. coli</td>
<td>1,78%</td>
<td>6,90%</td>
<td>0,02</td>
</tr>
<tr>
<td>CNS</td>
<td>2,37%</td>
<td>6,90%</td>
<td>0,047</td>
</tr>
<tr>
<td><strong>Incidence of intramammary infection subclinical until day 130</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>1,18%</td>
<td>9,77%</td>
<td>0,001</td>
</tr>
<tr>
<td>E. coli</td>
<td>2,37%</td>
<td>13,22%</td>
<td>0,001</td>
</tr>
<tr>
<td>CNS</td>
<td>15,98%</td>
<td>39,98%</td>
<td>0,002</td>
</tr>
<tr>
<td><strong>Spontaneous Cure Rate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiparous</td>
<td>44,19%</td>
<td>20,45%</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Primiparous</td>
<td>53,33%</td>
<td>50,00%</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Total</td>
<td>51,43%</td>
<td>32,18%</td>
<td>&lt; 0,05</td>
</tr>
</tbody>
</table>
## Mastitis cows and immunization

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>VACCINATED GROUP</th>
<th>PLACEBO GROUP</th>
<th>STATISTICAL SIGNIFICANT DIFFERENCES BETWEEN VACCINATED AND PLACEBO ($\alpha = 0.05$)</th>
<th>OBSERVATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatic cell count ($\text{mean SSC} \times 10^3$)</td>
<td>328.2</td>
<td>548.6</td>
<td>YES ($p&lt;0.05$)</td>
<td>Internationally recognized indicator for mastitis and milk quality.</td>
</tr>
<tr>
<td>Milk aspect ($&gt;1$)</td>
<td>11.42 %</td>
<td>19.79 %</td>
<td>YES ($p&lt;0.05$)</td>
<td>Implies less economic losses due to lost quarters, discarded milk and replacement cows.</td>
</tr>
<tr>
<td>Mammary gland aspect ($&gt;1$)</td>
<td>14.44 %</td>
<td>24.03 %</td>
<td>YES ($p&lt;0.05$)</td>
<td>Implies less economic losses due to treatments and reduces the risk of residues in milk.</td>
</tr>
<tr>
<td>Treatment with pharmacological products</td>
<td>34 treatm 22 cows</td>
<td>93 treatm 40 cows</td>
<td>YES ($p&lt;0.05$)</td>
<td></td>
</tr>
<tr>
<td>Death of cows due to mastitis</td>
<td>0</td>
<td>3</td>
<td>NO ($p&gt;0.05$)</td>
<td>Low number of deaths. Deaths due to mastitis only occurred in the placebo group.</td>
</tr>
</tbody>
</table>
Mastitis cows and immunization

Figure 2. Serological response anti-\textit{E. coli} J5 in serum at days post-vaccination (EC-2005-CB-001)

- Vaccinated group (n=44)
- Placebo group (n=41)
Figure 1. Serological response anti-slime in serum at days post-vaccination (EC-2005-CB-001)
Mastitis cows and immunization

PROTOCOLS
Mastitis cows and immunization

SEGMENT:

- Highly skilled farmers and veterinarians
- Farmers with computer
Mastitis cows and immunization

COMPLEMENTARY WITH DRY ANTIBIOTICS

Infection risk during the first 3 weeks of dry period is **6 times higher** than during previous lactation.

High infection risk during the **10 latest days before** partum.

**STARTVAC** start its action at this moment (13 d after 1st application) is necessary dry antibiotics action.
Mastitis cows and immunization

COMPLEMENTARY WITH DRY ANTIBIOTICS

More than 50% of mastitis observed during first 100 DIM are originated in dry period
Mastitis cows and immunization

Economic advantages with this protocol

- Distribution of the average of the economic loss due to clinical mastitis in the beginning (1-3 postpartum months) and the end (4-9 postpartum months) of the lactation

Origin: mastitis is an economic problem (Henk Hogeveen, The Netherlands)
Mastitis cows and immunization

**ADVANTAGE:**

- Colimastitis:
  - it prevents when there is more risk and more mastitis cases
  - Combination with T-Sealant increase the benefit
  - Bibliography J5 vaccines in USA
  - Reduction mastitis treatment cost
  - Increase milk production
- S.Aureus and CNS:
  - Tendency to decrease new infections and tendency to decrease SCC
  - Increase the immunity in farms that separate s.aureus animals

**WEAKNESSES:**

- It’s necessary to know the calving date
- Results are not immediate (only 15% of dry-off cows each month)
- Mistakes with protocol’s dates very frequently (Study Francis Sérieys)
- Colimastitis
  - Does not decrease SCC in bull tank (5-15% of clinical case, and not all in same moment)
  - Immunity: short time, does not protect all lactation (130days)
  - Does not protect seasonal mastitis
- S.Aureus /CNS

Difficult to fit with others standard S.aureus control measures
Mastitis cows and immunization

Economic advantages with this protocol

Conclusion:

• The most important consequence of the mastitis affection is the milk reduction, due to its effect during lactation

• If we prevent mastitis during postpartum, we can obtain more benefits than if we make it during lactation
### STARTVAC® - Data from a study on 6 farms

**Results (0-130 DIM)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discarded milk (days)</td>
<td>1.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Daily production (kg/day)</td>
<td>30</td>
<td>302</td>
</tr>
<tr>
<td>SCC (cells/ml)</td>
<td>548,000</td>
<td>328,000</td>
</tr>
<tr>
<td>Risk of clinical mastitis</td>
<td>15%</td>
<td>4%</td>
</tr>
<tr>
<td>Risk of sub-clinical mastitis</td>
<td>46%</td>
<td>18%</td>
</tr>
<tr>
<td>Elimination</td>
<td>9%</td>
<td>5%</td>
</tr>
</tbody>
</table>
### Economic Evaluation of the Intervention

<table>
<thead>
<tr>
<th></th>
<th>Control (0-130 DIM)</th>
<th>STARVAC® (0-130 DIM)</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>days of discarded milk</td>
<td>1.6</td>
<td>0.9</td>
<td>2.5</td>
</tr>
<tr>
<td>average daily milk yield (kg)</td>
<td>32</td>
<td>30</td>
<td>62</td>
</tr>
<tr>
<td>average SCC (cells/ml)</td>
<td>559,000</td>
<td>431,000</td>
<td>989,000</td>
</tr>
<tr>
<td>clinical mastitis risk</td>
<td>15%</td>
<td>4%</td>
<td>19%</td>
</tr>
<tr>
<td>subclinical mastitis risk</td>
<td>46%</td>
<td>18%</td>
<td>64%</td>
</tr>
<tr>
<td>culling risk</td>
<td>9%</td>
<td>5%</td>
<td>14%</td>
</tr>
<tr>
<td>milk production NSD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC premiums - €</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced / extra costs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>days of discarded milk</td>
<td>€ 7</td>
<td></td>
<td>€ 7</td>
</tr>
<tr>
<td>clinical mastitis treatment</td>
<td>€ 5</td>
<td></td>
<td>€ 5</td>
</tr>
<tr>
<td>infection transmission</td>
<td>€ 22</td>
<td></td>
<td>€ 22</td>
</tr>
<tr>
<td>culling</td>
<td>€ 30</td>
<td></td>
<td>€ 30</td>
</tr>
<tr>
<td>marginal feed cost NSD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vaccination cost - €</td>
<td>€ (17)</td>
<td></td>
<td>€ (17)</td>
</tr>
<tr>
<td>STARVAC* net profit per cow (direct effects)</td>
<td>€ 25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STARVAC* net profit per cow (indirect effects)</td>
<td>€ 22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STARVAC® net profit per cow</td>
<td>€ 47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mastitis cows and immunization

3:3:3 PROTOCOL

0 D 21 D 180D 180D

STARTVAC IMMUNITY STARTVAC IMMUNITY

ALL ANIMALS (LACTATION, DRY-OFF COWS AND HEIFERS)

SEGMENT:

- Medium low skilled farmers and veterinarians
Mastitis cows and immunization

ADVANTAGE:

- Results in a short time
- Take advantage of seasonality
- Facilities management
- Benefit: good feeling by the farmer
- Appropriate response in variable situations
- **Colimastitis:**
  - To protect all lactation
  - Seasonal campaign
  - Reduces cost of treatments
  - Reduces SCC of subclinical cases
- **S.Aureus:**
  - Increase Immunity level
  - Reduce the possibility of contagious
  - Reduce new infections
  - Reduce SCC in milk tank (farms with prevalence > 20%)

WEAKNESSES:

- There aren’t officials results
- E.Schmitt (from France) next May
- A.Bradley (from UK) next November
Mastitis cows and immunization

**S.AUREUS MASTITIS (>20% Prevalence)**

**Classical Protocol** (high level farmers and veterinarians)
- SCC
  - Reduction Tendency
- Clinical mastitis
  - Reduction Tendency
- News Infections
  - Reduction Tendency

**3:3:3 Protocol** (Medium & small level farmers and vets)
- SCC
  - High reduction
- Clinical mastitis
  - Reduction Tendency
- New Infections
  - High reduction
Mastitis cows and immunization

**COLI MASTITIS**

**Classical Protocol** (high level farmers and veterinarians)

- SCC
  - Low reduction
- Clinical mastitis
  - High reduction
- Cost of treatment
  - Reduction Tendency

**3:3:3 Protocol** (Medium & small level farmers and vets)

- SCC
  - Low reduction
- Clinical mastitis
  - High reduction
- Cost of treatment
  - High reduction