Transition cow health and immune function

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Introduction

• Transition cow health:
  – Transition from lactation to dry
  – Transition form dry to lactation
  – Transition from healthy to diseased
  – Transition from diseased to healthy

• Resilience
  – Mammary microbiome
  – Immune responsiveness
Longitudinal metagenomic profiling of bovine milk to assess the impact of intramammary treatment using a third-generation cephalosporin

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Antimicrobial usage in food animals has a direct impact on human health, and approximately 80% of the antibiotics prescribed in the dairy industry are used to treat bovine mastitis. Here we provide a longitudinal description of the changes in the microbiome of milk that are associated with mastitis and antimicrobial therapy. Next-generation sequencing, 16S rRNA gene quantitative real-time PCR, and aerobically culturing were applied to assess the effect of disease and antibiotic therapy on the milk microbiome. Cows diagnosed with clinical mastitis associated with Gram-negative pathogens or resistant aerobic culture were randomly allocated into four treatment groups.
Mammary microbiota of dairy ruminants: fact or fiction?

Pascal Rainard

Abstract
Explorations of how the complex microbial communities that inhabit different body sites might contribute to health and disease have prompted research on the ways the harmonious relationship between a host and its microbiota could be used to keep animals healthy in their production conditions. In particular, there is a growing interest in the bacterial signatures that can be found in the milk of healthy or mastitic dairy cows. The concept of sterility of the healthy mammary gland of dairy ruminants has been challenged by the results of studies using bacterial DNA-based methodology. The newly obtained data have led to the concept of the intramammary microbiota composed of a complex community of diverse bacteria. Accordingly, mammary gland infections are not mere infections by a bacterial pathogen, but the consequence of mammary dysbiosis. This article develops the logical implications of this paradigm shift and shows how this concept is incompatible with current knowledge concerning the innate and adaptive immune system of the mammary gland of dairy ruminants. It also highlights how the concept of mammary microbiota clashes with results of experimental infections induced under controlled conditions or large field experiments that demonstrated the efficacy of the current mastitis control measures.
What is milk microbiome?

• Microbial populations as found using next generation sequencing or pyro-sequencing.

• Population of bacterial species identified using 16S sequencing of bacterial DNA isolated from milk

• Approximately 7000 bacterial 16S sequences corresponding to ~1000 bacterial species per milk sample (1 ml), and likely more in milk from healthy quarters.
Mammary microbiome

Oikonomou et al. 2014
T. pyogenes+ samples

Oikonomou et al. 2016
SCC <20 aerobic culture negative

% of identified sequences

Oikonomou et al. 2016
S. uberis+ samples

% of identified sequences

Streptococcus
Genera level results: Culture-negative mastitis samples

% of identified sequences

Asticcacaulus

Fusobacterium
Culture Negative CM samples versus SCC <10K

% of identified sequences

- Enterococcus
- unclassified_Carnobacteriaceae
- Asticcacaulis
- Lactobacillus
- Fusobacterium
- unclassified_Bacteria
- Streptococcus
- Staphylococcus
- Bacteroides
- unclassified_Clostridiales
- Anaerococcus
- unclassified_Xanthomonadaceae
- unclassified_Ruminococcaceae
- Faecalibacterium
- Porphyromonas
- unclassified_Bacteroidales
- unclassified_Lachnospiraceae
- Comamonas
- Propionibacterium
- Aeribacillus
Self vs non-self

Gensollen et al. 2016
Farm specific mammary eco system

Contributing: anaerococcus, streptococcus, propionibacter,..
Is there a resilient microbiome?

Patho-gen
Non-gen
Physio-gen

Sphingobacterium
Streptococcus
Paenibacillus
Anaerococcus
Ecologic resilience

Scheffer et al. 1993
Challenge infection - *E. coli* -

Control

Challenge

Control

Antibiotics

LV

RV

LA

RA

Ganda et al. 2017
Challenge infection - *E. coli* -

Enterobacter (%)

*E. coli* challenge

Challenged, Non-Treated

Challenged, Treated

Treatments
Challenge infection - *E. coli* -

Shannon Index of diversity

*E. coli* challenge

- Green line: Challenged, Non-Treated
- Red line: Challenged, Treated

Treatments

- Vertical arrows indicate treatments at specific time points.
Antibiotics in healthy quarters

Shannon Index of Diversity

- Not challenged, not treated
- Not challenged, treated

Treatments
Intramammary resilience

Scheffer et al. 2001
Microbial eco-systems

Take home messages

• The mammary gland of dairy cows harbors a microbial ecosystem.
• Potential for a resilient ecosystem
• Intramammary challenge results in a loss of bacterial diversity.
• Some cows are more resilient to exotic bacterial growth compared to other more susceptible cows.
• Immediate appropriate antibacterial treatment results in a reduced loss of diversity.
Dry period immunobiology

Green et al. 2002
Challenge in late gestation: *E. coli*

Quesnell et al. 2012
Pro-inflammatory Cytokine IL-1β: no response

100 cfu E. coli

Clinical mastitis challenge strain

Quesnell et al. 2012
Anti-inflammatoire Cytokine response: IL-4

Gurjar et al. 2013
Pro-inflammatory response
Cytokine: IL-1β

Mid-lactation

Hours post challenge

100 cfu *E. coli*

Sipka et al. 2013
Th1:Th2 ratio

- **Mid-lactation**
- **Post-partum**
- **Late gestation**
Improve immune response

• Innate:
  – Teat-end quality
  – Lactoferrin
  – NEB
  – Immune modulation:
    • Selenium/vitamin E
    • Cytokines

• Adaptive:
  – Vaccination
    • *E. coli* & *S. aureus* & other
    • Imm immunostimulation
Bovine Granulocyte Colony Stimulating Factor (rbG-CSF)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N cows</th>
<th>N mastitis</th>
<th>% mastitis</th>
<th>Duration</th>
<th>Severity</th>
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<tr>
<td>Saline</td>
<td>53</td>
<td>18</td>
<td>34%</td>
<td>10.8</td>
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<tr>
<td>5 μg/kg</td>
<td>51</td>
<td>10</td>
<td>20%</td>
<td>5.3</td>
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<tr>
<td>10 μg/kg</td>
<td>54</td>
<td>9</td>
<td>17%</td>
<td>8.4</td>
<td>3.3</td>
</tr>
<tr>
<td>20 μg/kg</td>
<td>53</td>
<td>5</td>
<td>9%</td>
<td>6.9</td>
<td>3</td>
</tr>
</tbody>
</table>
**E. coli** vaccination reduces severity

- **E. coli** challenge study

~ 50% reduced production loss

*Wilson et al. 2006*
Intramammary immunization with UV IR *E. coli*

**Days -44, -30, -16**
- **Vaccine – J5 Bacterin**
  - N=5
- **Control**
  - N=5

**Day -30**
- LF
- RF
- LH
- RH

**Immunization with equivalent 100 cfu E. coli C1**

**Day 0**
- IMM challenge w/ 100 cfu/ml E. coli C1

Pomeroy et al. 2016
Intramammary immunization with UV irradiated *E. coli*

100 live CFU

P -10d

Parturition

\[ \text{Log}_{10}(\text{CFU}+1) \]

- Control
- Immunized

100 CFU
UV IR *E. coli*

Probability of having IMI

Pomeroy et al. 2016
Pregnancy and Immune response

• Immune response in late gestation:
  – anti-inflammatory IL-4 spike
  – No pro-inflammatory response after challenge

• Immune response changes from late gestation to early lactation:
  – From anti-inflammatory to pro-inflammatory

→ Infection in late gestation but clinical response in early lactation……..
Ecologic resilience in transitions

Scheffer et al. 1993
Take home message

• Mammary microbiome
  – High diversity is important
  – Resilient microbiome is likely
  – Farm specific
  – Treatment affects microbiome

• Immune responsiveness
  – Late gestation affects immune bias
  – Systemic and local immune response may be improved
  – Resilient system prevents infection and disease
Teaming up for animal health, in the interest of animals, their owners and society at large