Haptoglobin: A biomarker for selective dry cow therapy

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Study Aims

Dry cows

- Routinely treated with long acting infusions of antimicrobials to rid the udder of bacteria that could cause mastitis and to treat any SM present.
- In the UK, blanket use of antimicrobials is extremely high: 99% of dairy cows receiving antimicrobials at this point*
- AMR concerns

Haptoglobin

- Suitable biomarker for selective dry cow therapy?
- Compare to SCC & bacteriology

Acute phase proteins

- Synthesised and released from the liver during inflammatory (acute-phase) response
- Measured in the serum in human and veterinary medicine and in various veterinary research areas

Extra Hepatic acute phase proteins

- Increasing body of evidence that detail extra-hepatic synthesis (adipose tissue, lung...)
- Mammary acute phase synthesis well studied owing to presence of APPs in milk
Milk acute phase proteins

• Haptoglobin
• Mammary associated Serum amyloid A
• C-reactive protein
Study design

• Over 11 weeks 409 quarter milk samples were obtained at dry off from 104 cows.
• August 2016 – November 2017
• 409 quarter milk samples collected from 104 cows during drying off

The following were determined on all quarter milk samples:
• California mastitis test (CMT)
• Bacteriology
• Somatic cell counts (SCC)
• Haptoglobin (Hp) (ELISA*)

* Life Diagnostics COW HAPTOGLOBIN SPARCL™ ASSAY | HAPT-SP-11
Results: SCC & CMT
Results: Haptoglobin
Results: Bacteriology

At cow level
- Cows with 1 or more culture positive quarter, n = 71
- Cows all quarters sterile, n = 33

At quarter level
- Sterile, n=278
- Contamination, n=61
- Bacteria isolated, n=70
Bacteriology

- Bacteria was cultured from 107 quarters
- From 53 quarters the following species were identified:

Significant associations between bacteria cultured (n=53) and Hp, SCC, CMT (P=<0.0001)
Bacteriology
Discussion

Haptoglobin better biomarker for active inflammation and infection

Sensitivity and Specificity for \( \text{Hp} \), SCC and CMT with bacteriology as the ‘gold standard’ Higher sensitivity compared to SCC & CMT

<table>
<thead>
<tr>
<th></th>
<th>SCC</th>
<th>CMT</th>
<th>Hp</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>0.69</td>
<td>0.46</td>
<td>0.76</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.61</td>
<td>0.76</td>
<td>0.58</td>
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Other factors can effect levels of SCC in milk

California mastitis test (CMT) \( \rightarrow \) Subjective

Bacteriology \( \rightarrow \) Expensive / preparation issues / contaminants
Mastitomics, the integrated omics of bovine milk in an experimental model of *Streptococcus uberis* mastitis: 1. High abundance proteins, acute phase proteins and peptidomics†

Funmilola Clara Thomas, William Mullen, Riccardo Tassi, Adela Ramirez-Torres, Manikhandan Mudaliar, Tom N. McNeilly, Ruth N. Zadoks, Richard Burchmore and P. David Eckersall

Mastitomics, the integrated omics of bovine milk in an experimental model of *Streptococcus uberis* mastitis: 2. Label-free relative quantitative proteomics†

Manikhandan Mudaliar, Riccardo Tassi, Funmilola C. Thomas, Tom N. McNeilly, Stefan K. Weidt, Mark McLaughlin, David Wilson, Richard Burchmore, Pawel Herzyk, P. David Eckersall and Ruth N. Zadoks

Mastitomics, the integrated omics of bovine milk in an experimental model of *Streptococcus uberis* mastitis: 3. Untargeted metabolomics†

Funmilola Clara Thomas, Manikhandan Mudaliar, Riccardo Tassi, Tom N. McNeilly, Richard Burchmore, Karl Burgess, Pawel Herzyk and Ruth N. Zadoks and P. David Eckersall

- Panel of biomarkers
- Confirmed haptoglobin
- Highlighted novel biomarkers
- Systems biological approach to identifying biomarkers

Ingenuity Pathway Analysis

- 57 hours post challenge
- Highly significant proteins identified using repeated measures ANOVA
- Ingenuity Pathway Analysis (IPA)
- Increases in
  - Acute phase pathways
  - LXR= liver receptor pathways
  - RXR= retinoic acid receptor pathways
  - FXR = farenoid (bile acid) receptor pathway
Summary

- Haptoglobin suitable biomarker of active infection
- Haptoglobin – measure routinely as part of both bovine serum and milk profiles
- Greater impetus on exploring biomarkers beyond SCC
- Increasing evidence from mastitomics that acute phase pathways the most activated during infection
- Haptoglobin is useful biomarker for directing antimicrobial therapies at dry off
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