Haptoglobin in milk – immunologic biomarker for monitoring health status by on-farm analysis

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**Introduction**

- **Acute Phase Response (APR)** plays a central role in the action of the non-specific innate immune system (systemic response)

- Is triggered when an animal is **subjected to challenges**, such as infection, inflammation, trauma or stress

- **Haptoglobin (Hp)** is a major Acute Phase Proteins (APP) - one of the most sensitive bovine APP → low concentration in normal animals + rapid increase during inflammation + rapid decrease with the resolution of the disease

- Hp acts in plasma as a **scavenger molecule** and has **antioxidant activities**

- Hp in plasma is **clinically** a useful parameter for measuring the occurrence and severity of inflammatory responses

- Several studies showed: **Hp is secreted in bovine milk** during clinical mastitis

- Different hypothetical pathways for the presence of bovine Hp in milk (exported to the mammary gland or extrahepatic production)
Objectives

- Investigate relations between Hp in milk and systemic inflammatory reactions of the body
  - Based on a new on-farm technique to measure Hp in milk (ELISA)
  - How I have to collect the samples
  - How stable is the test after repeated measures
  - What is the sensitivity and the specificity of the test as an early indicator
  - Which thresholds and expected values could be assumed during the lactation

- Can Hp in milk with the new on-farm test be used ...
  - ... as a early indicator of systemic inflammatory reactions of the body?
  - ... as a biomarker to monitor the health status of dairy cows?
Materials and Methods

On-farm test system for Hp in milk

- On farm device eProCheck 2.0 was used (test version of FrimTec GmbH [www.frimtec.de])
- Sandwich-ELISA
- Only 50 µl of milk samples must be pipetted in prepared testwells
- 22 samples and 2 standards per analysis
- 60 – 80 minutes per cycle
- Color reaction is analyzed – values in µg/ml in a range of 0 to about 30 with one decimal place
Test design

- Investigations under **field conditions**
  - In a dairy cattle farm with 1350 cows / 2 x 40 side by side milking parlour
  - Average milk yield 9.500 kg / a

- 30.07.2012 – 05.10.2012 / 100 cows / 2nd day p.p. up to the 44 d p.p. in an 2 or 3 - day - rhythm (Mo – We – Fr)

- Composite sample (mix from all quarters)

- Documentation of veterinary diagnoses and treatments

- Test-day records, calving ease
Expected inflammatory reaction as a reference

We assume that the cow has an inflammatory reaction around an incidence detected:

- **Calving ease / dystocia (KV)**
  - Status 1: 7 d
  - Status 2 and 3: 14 d

- **Diagnoses**
  - EU: -3’ +3 d
  - SO: -3’ +5 d
  - BW: -5’ +7 d
  - ZH: -3’ +5 d

- **Test-day records**
  - Somatic cells > 150.000: -5’ +2 d
  - Two times consecutively (-5’ + 14 d - including time between)

EU = udder diseases / mastitis
KV = dystocia
SO = fever, digestive and respiratory diseases
BW = lameness and hoof disorders
ZH = puerperal diseases
Materials and Methods

Haptoglobin in milk

- After data preparation **1552 values** were usable
- 0 – 30 (mean 3.1, median 1.1, s 4.3) - extreme skew distribution
- Logarithmic transformation (mean 0.06, median 0.04, s 0.64)
Materials and Methods

Days with an expected inflammatory reaction

<table>
<thead>
<tr>
<th>Disease</th>
<th>Expected</th>
<th>Not Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU (udder diseases/mastitis)</td>
<td>1600</td>
<td>1200</td>
</tr>
<tr>
<td>KV (dystocia)</td>
<td>1300</td>
<td>1000</td>
</tr>
<tr>
<td>SO (fever, digestive and respiratory diseases)</td>
<td>1200</td>
<td>900</td>
</tr>
<tr>
<td>BW (lameness and hoof disorders)</td>
<td>1300</td>
<td>1000</td>
</tr>
<tr>
<td>ZH (puerperal diseases)</td>
<td>1500</td>
<td>1100</td>
</tr>
</tbody>
</table>

EU = udder diseases / mastitis  
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Results

Results of some preliminary investigations

1. **What samples** should be used?
   - **Starting milk** (a sample immediately after udder cleaning and forestripping) or **collection milk** (like milk recording): \( u = 0.85 \rightarrow \) both is possible
   - **Quarter sample** (sample from each quarter) or **composite sample**: 
     - Hp content shows a high variation between quarters
     - All quarters must be includes in a composite sample

2. How large is the **repeatability** of the Hp analysis of comparable samples for starting milk, collection milk and the composite sample
   - \( u = 0.96 - 0.99 \)
   - Test shows a high repeatability
Results

Test of influences on Hp in milk

\[ y = \mu + \ln + \lw + \text{diag} + \text{animal} + e \]

- \( y \) - lg of daily Hp in composite milk in µg/ml
- \( \ln \) - lactation number
- \( \lw \) - lactation week
- \( \text{diag} \) - complex of diagnoses

- **Lactation number** has no significant influence
- **Lactation week** has significant influence – first two weeks show higher Hp-concentration

**Diagnoses**

- Alle Erkrankungen \( 0,000*** \)
- EU \( 0,000*** \)
- SO \( 0,001*** \)
- ZH \( 0,029* \)
- BW \( 0,056 \)

**EU** = udder diseases / mastitis
**SO** = fever, digestive and respiratory diseases
**ZH** = puerperal diseases
**BW** = lameness and hoof disorders
Results

Receiver Operating Characteristic (ROC)

All expected inflammatory reactions

- Specificity = 0.790
- Sensitivity = 0.659
- Cutpoint 2.55 µg/ml Hp

AUC: 0.785

All expected inflammatory reactions DIM > 14

- Specificity = 0.749
- Sensitivity = 0.748
- Cutpoint 1.85 µg/ml Hp

AUC: 0.798
Results

Receiver Operating Characteristic (ROC)

Expected inflammatory reactions from udder diseases

- Specificity = 0.681
- Sensitivity = 0.787
- Cutpoint **1.85 µg/ml Hp**

Expected inflammatory reactions from udder diseases DIM > 14

- Specificity = 0.714
- Sensitivity = 0.837
- Cutpoint **1.65 µg/ml Hp**
Results

Example for mastitis

![Graph showing Haptoglobin levels over DIM for animal 7162. The graph includes symbols for Haptoglobin, test day recording, and expected inflammation.]
Results

Example for hoof disorders

![Graph showing hoof disorders data]

- Haptoglobin
- Test day recording
- Expected inflammation

*Animal: 9582*

**Graph Details:**
- Y-axis: Haptoglobin [μg/mL]
- Data points indicating health status and events over time.
Hyp assay in milk can be performed on-farm with eProCheck 2.0 device using a Sandwich-ELISA (but costs and effort must be have in mind)

Test system yields a high repeatability

Healthy cows had undetectable levels of Hp | increased levels varied markedly

Beside the diseases only the lactation week shows a significant influence on Hp-concentration (especially during the first two weeks of lactation)

Sensitivity and specificity is about 0.75, Hp-threshold about 1.85

From this value a inflammatory reactions seems to proceed

It seems that Hp in milk can be used as an early indicator for systemic inflammatory reactions of the body- not only for mastitis

For different disease complexes

Up to 3-5 day before first visible symptoms

The test should be used mainly in sensitive situations: Start of lactation, dry cow treatment, before first insemination

Could be useful in diagnosis, prognosis and in monitoring response to therapy

If the test system is able to monitor the health status of dairy herd (general health screening) needs further investigation
Acknowledgements

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