Milk haptoglobin as an indicator of udder health in dairy heifers after calving

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Intramammary infection in dairy heifers

Impact:
- Udder Health
  (↑ Risk mastitis: clinical; subclinical)
- Production
- Welfare
- Longevity

Economic losses

(Fox, 2009, Vet Micro 134, 82-89;
Piepers et al., 2009, J Dairy Sci 93, 2014-2024;
De Vliegher et al., 2012, J Dairy Sci 95, 1025-1040)
Intramammary infection in dairy heifers

Risk factors:
- Individual factors
- Management
- Environmental (e.g. hygiene)

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  (↑ Risk mastitis: clinical; subclinical)
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Unknown Udder Health Status at milking herd entry

Economic losses

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Intramammary infection in dairy heifers

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- Individual factors
- Management
- Environmental (e.g., hygiene)

Unknown Udder Health Status at milking herd entry

Early detection e.g., APP 

Impact:
- Udder Health
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- Production
- Welfare
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Decrease

Lack of udder health screening pre-calving

Economic losses

(Fox, 2009, Vet Micro 134, 82-89; Piepers et al., 2009, J Dairy Sci 93, 2014-2024; De Vliegher et al., 2012, J Dairy Sci 95, 1025-1040)
Acute phase proteins

External and internal challenges
- e.g. Injury, infection

Systemic reaction to inflammation

Local production of Hpt in mammary gland tissue

APP concentration changes
- Blood proteins produced by liver

e.g. blood Hpt

Udder health biomarker

Aim of the study

- Investigate the value of haptoglobin (Hpt) concentration in milk from dairy heifers in the first week postpartum

- Indicator of their udder health status at calving
  - IMI
Material & Methods

Population in study

- 1 commercial dairy farm (700 lactating animals)
- 24 Holstein-Friesian heifers
Material & Methods

Population in study
- 1 commercial dairy farm (700 lactating animals)
- 24 Holstein-Friesian heifers

Sampling
- Quarter level sampling
- Colostrum and milk

Animal recruited
2 months Prepartum
Calving event
3 DIM
Colostrum sampling
Bacteriology
SCC
Hpt
Milk sampling
SCC
Hpt
5 DIM
Milk sampling
SCC
Hpt

Pre-partum part
Post-partum part
Material & Methods – milk sample analysis

- SCC
  - Fossomatic – NML (National Milk Laboratory)

- Milk Hpt
  - Veterinary Genes and Proteins Laboratory – GUVS
    Thomas et al., 2015

- Bacteriological culture
  - Microbiology Laboratory - GUVS
Material & Methods – milk sample analysis

- Bacteriological culture

- Definitions:
  - **Culture-negative** = no growth
  - **Contaminated** = ≥ 3 morphotypes/plate
  - **IMI = lenient** (all culture-positive non-contaminated samples)
    &
  - **strict** (≥ 1000 CFU/ml)

(Andersen et al., 2010, J. Dairy Sci. 93, 2966–2975; Dohoo et al., 2011, J. Dairy Sci. 94, 250–61)
Material & Methods

**Population in study**
- 1 commercial dairy farm (700 lactating animals)
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**Sampling**
- Quarter level sampling
- Colostrum and milk

**Statistical methods**
- SPSS version 22
- Spearman’s rank correlation test
- Kruskal-Wallis H test and post-hoc test
- Friedman test and post-hoc test
- ROC curve
## Results & Discussion – Hpt and SCC

<table>
<thead>
<tr>
<th>Day in milk</th>
<th>1 (at calving)</th>
<th>3</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haptoglobin (µg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive samples</td>
<td>76 (79%)</td>
<td>95 (99%)</td>
<td>72 (75%)</td>
</tr>
<tr>
<td>Mean</td>
<td>7.7 ± 1.2</td>
<td>8.2 ± 1</td>
<td>5.6 ± 0.8</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>3.4</td>
<td>5.8</td>
<td>2.7</td>
</tr>
<tr>
<td>Q1</td>
<td>1.9</td>
<td>3.1</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>3.5</td>
<td>5.5**</td>
<td>3**</td>
</tr>
<tr>
<td>Q3</td>
<td>7.1</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>IQR</td>
<td>5.2</td>
<td>5.8</td>
<td>5.9</td>
</tr>
</tbody>
</table>

| **Somatic cell count** (x1000 cells/mL) | | | |
| LnSCC mean | 6 ± 0.3 | 5.5 ± 0.1 | 4.8 ± 0.1 |
| Geometric mean | 381 | 255 | 124 |
| Q1 | 112 | 90 | 58 |
| Median | 721 | 200* | 85** |
| Q3 | 1706 | 558 | 197 |
| IQR | 1594 | 468 | 139 |

Q1 – first quartile; Q3 – third quartile; IQR – interquartile range

*Values are mean ± standard error of mean

*Significant differences with day of calving (Friedman test, post hoc Dunn-Bonferroni test) **P < 0.01; ***P < 0.001, with day 3 on milk *P < 0.001.

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### Early post parturient changes in milk acute phase proteins

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Results & Discussion – Hpt vs. SCC

Day 1 (at calving)

\[ r_s = 0.21; \quad p<0.001; \quad n=94 \]

Day 3

\[ r_s = 0.68; \quad p<0.001; \quad n=96 \]

Day 5

\[ r_s = 0.18; \quad p>0.05; \quad n=96 \]
Results & Discussion – Hpt by IMI definition

Lenient (all culture-positive non-contaminated)

Strict (≥ 1000 CFU/ml)

** p<0.05; *** p<0.01
Results & Discussion – ROC curves

Lenient (all culture-positive non-contaminated)
Strict (≥ 1000 CFU/ml)

Lenient definition

Cut-off: 4.46 μg/ml
Se= 57% ; Sp= 61%

Strict definition

Cut-off: 4.17 μg/ml
Se= 60% ; Sp= 63%
Conclusions

- Limited potential of milk haptoglobin as diagnostic test for CNS intramammary infection

Further work would be necessary to explore:

- the value of milk Hpt as an indicator of heifers IMI with major pathogens

- the differences found between the Hpt pattern in colostrum/milk from heifers and multiparous cows during the first week postpartum
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