Short time variation in daily shedding of contagious mastitis pathogens

CPH Cattle - Up-to-date with Cattle Research

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Aim of the project

- To make recommendations on relevant milk sampling for detection of *Staphylococcus aureus* and *Streptococcus agalactiae* (B-streptococci)
- Investigate & understand shedding patterns
- Investigate the relationship between
  - Bacterial Culture (BC)
  - Polymerase Chain Reaction (PCR)
  - Somatic Cell Count (SCC)

**My Ph.D. project:** Characteristics of *Staph. aureus* and *Strep. agalactiae* for improved mastitis diagnosis and control

**STOPMAST:** Funded by the Danish milk levy foundation, participants from DTU, AU and SEGES
Background

- Subclinical mastitis
- Diagnostic tool for control or eradication
- Culture - higher sensitivity with PCR

**Staph. aureus**
- Widespread mastitis pathogen
- Cyclic shedding patterns expected (Studer et al., 2008, Sears et al., 1990)

**Strep. agalactiae**
- Increasing herd prevalence in DK
- Re-emergence in Nordic countries
- Single study (Thieme und Haasmann, 1978)
Materials and Methods

- 2 herds with repeatedly PCR positive bulk tank (Ct<30)

- Screening on cow level - PCR test foremilk samples all cows (n=589)

- Positive cows: PCR test on quarter milk samples
  - *Staph. aureus* (Ct≤37)
  - *Strep. agalactiae* (Ct<40)

- Positive quarters (n=43) followed for 21 days
- Quarters negative ‘first’ 8 days omitted (n=8)
Materials and Methods

- 35 quarters
  - 21 *Staph. aureus* quarters
  - 14 *Strep. agalactiae* quarters
- 1 daily milking in 21 days
- Aseptic foremilk samples:
  - Routine preparation by staff
  - New gloves between cows
  - Cotton with 70% alcohol
  - Discard 3 strips
  - 50 mL
Materials and Methods

- **SCC:**
  - Bronopol preserved fresh sample

- **PCR:**
  - Bronopol preserved fresh sample
  - Non-preserved frozen sample
  - Mastit4BD PCR kit (DNA Diagnostic)
  - *S. aureus*, *S. agalactiae*, *S. uberis*, *S. dysgalactiae*, CNS, *Myc. bovis*, *Myc. sup.*, Beta-lactamase

- **BC:**
  - Non-preserved fresh sample
  - 10uL (loop), Esculin blood agar
  - Approximate CFU < 300/10uL after 24 hours
Preliminary Results...
Consistent shedding
False negative?
17/21 days positive

Low shedding
Variation within a strep. agalactiae positive quarter through 21 days

K0_NR=2962 KIRTEL=HF

Day

Ct-value

 SCC and CFU

SCCx1000/mL

* CFU is approximate counts up to 300

New infection?
Summary...

Great variation in SCC, CFU and Ct-values between and within quarters over 21 days

Inconsistent shedding of both pathogens

Better chance to find *Strep. agalactiae* with PCR

*Staph. aureus*:
- Mean PCR positive days = 18 of 21
- Mean BC positive days = 19 of 21

*Strep. agalactiae*:
- Mean PCR positive days = 15 of 21
- Mean BC positive days = 10 of 21

Further analysis 2017
- Tendencies in shedding patterns
- Diagnostic test evaluation/test properties
Take home message

A single negative sample is no warranty of freedom from udder infections with Staph. aureus and Strep. agalactiae

- False negatives (test sensitivity)
- Low shedding (repeated testing or test sensitivity)
- Biological inconsistent shedding (repeated sampling)