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Short time variation in daily shedding of *Strep. agalactiae* and *Staph. aureus* determined by bacterial culture and PCR test



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AIM OF STUDY

The aim of our study was to evaluate the daily shedding pattern of *Streptococcus agalactiae* and *Staphylococcus aureus* determined with a qPCR test (Mastit 4, DNA Diagnostic), bacterial culture (BC) and somatic cell count (SCC) over a 21 day period.

MATERIALS AND METHODS

Selection criteria herds

- Positive bulk tank PCR test for *Strep. agalactiae* and *Staph. aureus* (Ct value ≤ 37)
- Herd size ≥ 180 Holstein cows
- Milking parlor, 2 daily milkings
- Within 50 km distance to the laboratory and willingness to participate in the study.

Selection of infected quarters

- Infected cows (PCR positive cows):
 - Screening all cows (aseptic composite foremilk samples)
 - Ct value <40 for *Strep. agalactiae*
 - Ct value ≤ 37 for *Staph. aureus*
- Infected quarters (PCR positive quarters):
 - Aseptic quarter foremilk sample from infected cows
 - Ct value <40 for *Strep. agalactiae*
 - Ct value ≤ 37 for *Staph. aureus*
- Quarters positive at screening but negative in PCR and BC the subsequent 3 days were omitted.

Tests during 21 day sampling period

Aseptic quarter foremilk samples at one daily milking following NMC guidelines:

- Bronopol preserved sample for PCR test (Mastit 4)
- Bronopol preserved sample for SCC test (Fossomatic)
- Sample for culturing (BC, NMC guidelines, approximate CFU count, detection limit 100 CFU/ml)

Table 1: Number of cows and quarters in the study

	Screening total number (cow)	Positive at screening (cow)*	Positive at screening (quarter)	Negative at subsequent 3 days (quarter)
Herd 1				
<i>S.aureus</i>	112	9	11	2
<i>S.agalactiae</i>		5	9	1
Herd 2				
<i>S.aureus</i>	487	14	16	4
<i>S.agalactiae</i>		6	7	1
Total	589	34	43	8

* Herd 1: n=12 cows, 3 cows with both pathogens in different glands. Herd 2: 19 cows, 1 cow with both pathogens

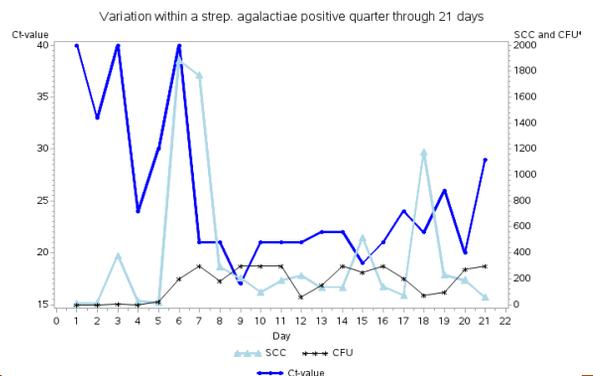
RESULTS

- Ct-values, approximate CFU counts and SCC varied greatly between and within quarters during the 21 day period
- 77% of quarters were consistently PCR positive:
 - *Staph. aureus* 90%
 - *Strep. agalactiae* 57%
- 71% of quarters were consistently BC positive
 - *Staph. aureus* 80%
 - *Strep. agalactiae* 64%
- 7 of 8 inconsistently PCR positive quarters had a higher number of PCR test positive days than BC positive days.

Table 2: Results based on PCR test

	Followed for 21 days (quarter)	Positive all 21 days (quarter)	Not consistent (quarter)
Herd 1			
<i>S.aureus</i>	9	7	2
<i>S.agalactiae</i>	8	5	3
Herd 2			
<i>S.aureus</i>	12	12	0
<i>S.agalactiae</i>	6	3	3
Total	35	27	8

Figure 1: An example of the 21 days variation in Ct-values, CFU and SCC in an inconsistently *Strep. agalactiae* positive quarter



*CFU is approximate counts up to 300

TAKE HOME MESSAGE

The consistent PCR test positive results for the majority of quarters indicates that less frequent sampling may be required to identify and confirm positive cows within the herd, while BC results show greater variation from day to day and may require repeated sampling to obtain the same sensitivity in identifying positive cows. There is no visual correlation between SCC and Ct-values.

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