Diagnostic test properties of a Real-time PCR mastitis test of composite milk samples from milk recordings to identify intramammary infections with *Staphylococcus aureus* and *Streptococcus agalactiae*

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Backround

- ***PathoProof***™ Mastitis PCR-Assay offered on composite non-aseptically collected samples from milk recording
- Main use in DK:
  - Whole herd investigations, e.g. *Strep. agalactiae*
  - Cows at dry-off (test approved according to Danish law on dry cow treatments)
- Advantages: automatically ordered and taken, high analytical sensitivity (Se) and specificity (Sp)
- High Se for samples from clinical mastitis in comparison to bacteriological culturing (BC) (Taponen et al. 2009, Koskinen et al. 2009)

Research questions:

- What are the diagnostic test properties of the PCR tests taken at milk recording
  - Whole herd tests for diagnosis of *Strep. agalactiae*
  - Cows at dry-off for diagnosis of *S. aureus*
- Have presampling procedures prior to attaching the milking unit an effect on the PCR result?
- Is there a carryover effect between cows milked at the same milking unit?
- What are the practical implications?

Fieldstudy with focus on *Strep. agalactiae and S. aureus:* PhD-study Yasser Mahmmod

Aim:

- To investigate the diagnostic value of PCR tests from samples taken at milk recording

Study design:

- 6 herds with free-stall systems and milking parlour, 1389 cows
  - Bulk tank PCR-test Ct-value for *Strep. agalactiae* og *S. aureus* < 40
- PCR-tests
  - Composite samples taken at DHI (True-test milk meter) from all milking cows
  - Routine cow preparation/non-aseptic
- BC
  - Aseptically taken quarter foremilk samples from 50% of cows
  - All cows at every other milking unit selected
- Milking order registered in 4 herds to evaluate possible carryover

Se and Sp of PCR and BC at dry-off

Eliteproject in health promotion course, Ellinor Cederlöf 2011

- Aim:
  - To evaluate Se and Sp of PCR samples from DHI and BC from quarter foremilk samples to diagnose intramammary infections (IMI) with *S. aureus* at dry-off
  - To investigate the effect of choosing different Ct-value cut-offs (≤ 39, ≤ 37, ≤ 34, ≤ 32)

- Study design
  - 7 herds, including 3 with AMS
  - Varying selection criteria, mainly based on prior SCC and mastitis treatments
  - PCR at milk recording, quarter foremilk samples within 24 h
  - Positive BC: ≥ 1 CFU in ≥ 1 quarter
  - 140 cows with complete data

Latent class analysis to evaluate Se, Sp, and herd prevalences

- Bayesian formulation, Latent class model (Hui and Walter, 1980), which does not imply that one of the tests is the 'gold standard'
- Especially relevant if we hypothesize that the new test (PCR) has a higher Se than BC
- OpenBugs software
- Model *Strep. agalactiae*
  - Prevalence estimated for each herd
- Model *S. aureus*
  - Herds allocated in two populations according to geographic location

Sensitivity and specificity of PCR and BC to diagnose IMI with *Strep. agalactiae* at different Ct-value cut-offs in 6 herds

Positive BC: ≥ 1 CFU in ≥ 1 quarter

Test estimates	PCR Ct-value cut-off 39	37	34	32
Se_{PCR}%	96	92	87	74
Se_{BC}%	26	30	60	72
Sp_{PCR}%	97	97	97	97
Sp_{BC}%	100	99	99	99

Sensitivity and specificity of PCR and BC to diagnose IMI with *S. aureus* in cows pre dry-off

Positive BC: ≥ 1 CFU in ≥ 1 quarter

Test estimates	PCR Ct-value cut-off 39	37	34	32
Se_{PCR}%	93	93	81	61
Se_{BC}%	78	83	88	94
Sp_{PCR}%	93	95	96	99
Sp_{BC}%	97	97	94	90

So far…

- PCR-test seems to be the better choice
- What happens if applied in herds with different prevalence of truly infected cows?

  - Positive predictive value
    (% diseased out of test positive cows)
  - Negative predictive value
    (% healthy out of test negative cows)

Calculated for two scenarios:

A) Herd with 10% truly infected cows
B) Herd with 40% truly infected cows

Positive and negative predictive values for *Strep. agalactiae* at 10 og 40% true prevalence

Ct-value cut-off 37

	True prevalence %	PPV %	NPV i%
PCR (Se 92/ Sp 97)	10	77	99
	40	95	95
BC (Se 30/ Sp 99)	10	77	93
	40	95	68

Effect of low Se

Until know we only looked at test properties, but what about other factors influencing PCR test results?

Have presampling procedures prior to attaching the milking unit an effect on the PCR result?

Is there a carryover effect between cows milked at the same milking unit?

Have presampling procedures prior to attaching the milking unit an effect on the PCR result?

Yes, they do!

Teat disinfection + taking quarter milk sample reduced the odds for being PCR-test positive

S. aureus:	OR 0.75
Strep. agalactiae:	OR 0.63

Ct-value cut-off 37

Logistic regression model, R
herd as random effect

Is there a carryover effect between cows milked at the same milking unit?

Yes.

Dependent on the chosen Ct-value cut-off (34, 37, 39), a carryover effect between 9-13 % can be expected for *Strep. agalactiae* positive cows.

How did we estimate that?

- 4 herds with registration of milking order within milking unit
- Logistic regression model with generalized estimating equations (GEE), autoregressive correlation structure, SPSS

High values! – checked the carryover for fat, which was only 4%

If we do not have information on milking order, no other way than confirming test positive cows to reduce the number of false positive.

Discussion: Test results for *Strep. agalactiae* and *S. aureus*

- The changes of Se_{PCR} og Se_{BC} at different Ct-value cut-offs may imply that the underlying latent disease definition (=IMI) changes

- At cut-off 32 Se_{PCR} lowest and Se_{BC} highest:
  - High concentration of *Strep. agalactiae/S. aureus*
  - A heavily infected cow

- At cut-off 39:
  - Low concentration of bacteria
  - Cow positive (maybe from teat canal infections or contamination, teat skin, non-viable cells…)

- The choice of the cut-off depends on the aim of the testing.

  In a program to eradicate *Strep. agalactiae* we may be interested in high Se…

Discussion: contamination & carryover

- Teat preparation affects the odds of being positive in the PCR test
  - Thorough cleaning and disinfection of teats may reduce the risk of contamination
- Carryover effects for *Strep. agalactiae* maybe 2-3 times the size we expect for fat contents
- We need further studies to investigate factors that affect PCR results

Thank you

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References

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