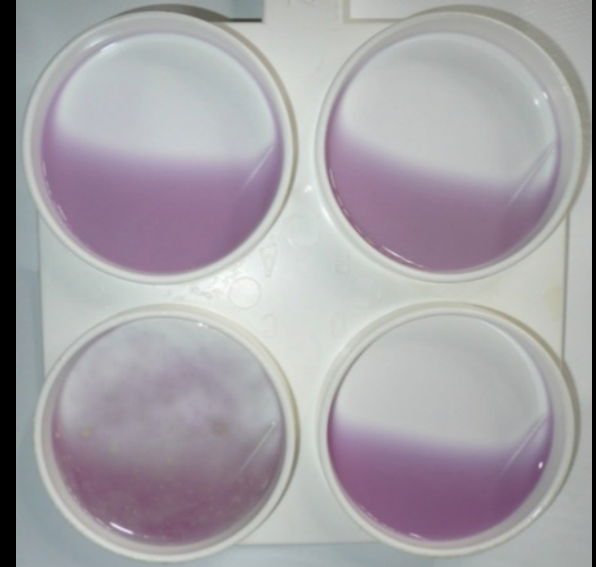


# Detection of *Staphylococcus aureus* genotype B in routine milk recording samples and in bulk tank milk by qPCR analysis - a field study

C. Syring, R. Boss, M. Reist, M. Bodmer, J. Hummerjohann, P. Gehrig, H. Graber



**Mastitis: No panacea against cow disease number one**

# Introduction - qPCR

- qPCR: up to 2000 x more sensitive and highly specific, result in 1 day

(H. Graber et al., 2007)

- Subtyping: 17 genotypes of *S. aureus*

→ *S. aureus* GTB (Fournier et al., 2008)

- qPCR: specific detection of *S. aureus* GTB in bulk tank milk (BTM): 1 GTB-positive cow among 138 cows

(R. Boss et al., 2011)

# Introduction - Epidemiology

- Prevalence of *S. aureus* in a herd is associated with the incidence of specific subtypes
- *S. aureus* GTB
  - Causes subclinical mastitis
  - Is udder-associated
  - Is highly contagious → herd problem, economical loss

(H. Graber et al., 2009, A. Michel et al., 2010)

# Aims of the study

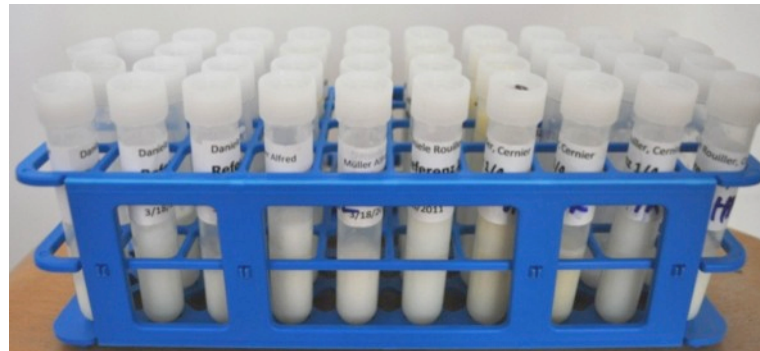
Evaluation of the diagnostic sensitivity and specificity of a newly developed bulk tank milk analysis procedure

(Boss et al., 2010)

# Material and Methods

- Herds: at least 10 lactating cows
- More than 20% of the cows with a SCC > 150,000 cells per milliliter
- Visits of 54 farms within a year
- Different breeds, housing- and milking systems

# Material and Methods



pooling



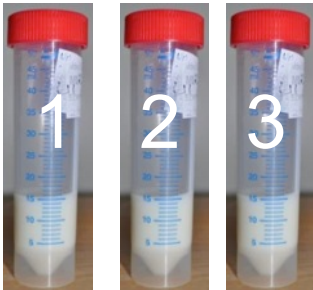
# Material and Methods

During one milking time

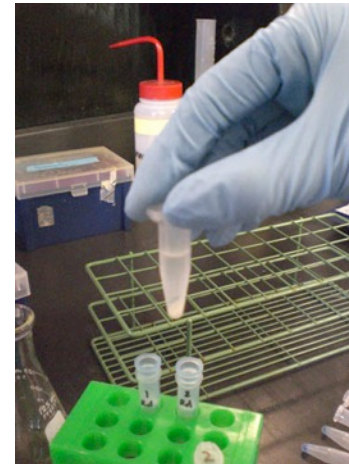
- 1) Quarter milk samples = references
  - 2) Individual cow milk without Bronopol
  - 3) Bulk tank milk (BTM)
  - 4) Individual cow milk containing Bronopol for determination SCC
- } Pooling

# Material and Methods

- 1) Pool of reference samples
- 2) Pool of individual cow milk
- 3) BTM



→  
*Enrichment in  
selective medium*



*DNA-  
extraction*

*DNA analysis  
by qPCR*



# Material and Methods

Based on detection of

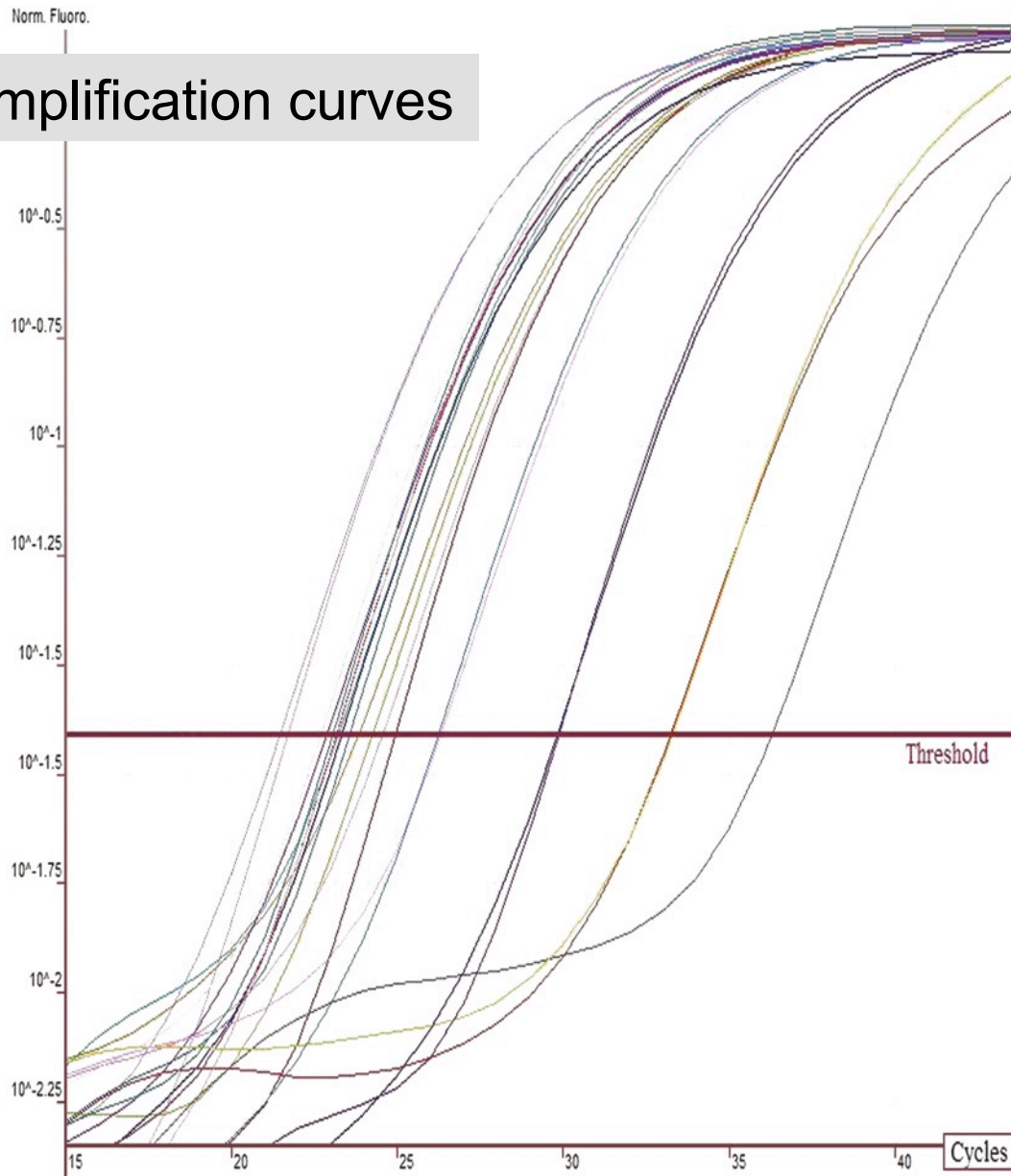
- 2 enterotoxin genes (*sea*, *sed*)
- Polymorphism within Leukotoxin-gene (*lukE*)  
by a real-time quantitative PCR (qPCR)

Reference method

- Genotyping by Fournier et al., 2008

# qPCR amplification curves

fluorescence



cycles

G1	BTM 1
G2	BTM 2
G3	BTM 3
G4	BTM 4
G5	BTM 5
G6	BTM 6
G7	BTM 7
G8	BTM 8
H1	BTM 9
H2	BTM 10
H3	BTM 11
H4	BTM 12
H5	NTC
H6	NTC
H7	luKEB Standard 1
H8	luKEB Standard 1
I1	luKEB Standard 2
I2	luKEB Standard 2
I3	luKEB Standard 3
I4	luKEB Standard 3
I5	luKEB Standard 4
I6	luKEB Standard 4
I7	luKEB Standard 5
I8	luKEB Standard 5

Bank On Bank Off  
Named On All On All Off  
Edit Samples...

**CT Calculation**  
 Invert Raw Data  
Threshold: 0.03  
Eliminate Cycles before: 1  
Auto-Find Threshold

**Standard Curve**  
conc= 10<sup>-1</sup>(-0.298\*CT + 11.902)  
CT = -3.351\*log(conc) + 39.883  
Type: Floating

# Results

- for GTB positive and negative herds

Herd ID	Herd size	SCC *10 <sup>3</sup> /mL	Genotypes found	QPCR Assay		
				1	2	3
<b><i>S. aureus</i> GTB herds (n = 21)</b>						
P 1	22	102	B, B''	+	+	+
P 2	36	56	B, R	+	+	+
P 3	16	74	B	+	+	+
P 4	54	114	B	+	+	+
P 5	19	100	B'	+	+	-
P 6	20	154	B	+	+	+
P 7	12	178	B, C, I'	+	+	+
P 8	22	245	B	+	+	+
P 9	22	259	B	+	+	+
P 10	22	144	B, B'''	+	+	+
P 11	14	128	B''	+	+	+
P 12	12	66	B'''	+	+	+
P 13	23	110	B	+	+	+
P 14	15	107	B	+	+	+
P 15	11	324	B, C''	+	+	-
P 16	27	59	B	+	+	+
P 17	16	112	B	+	+	+
P 18	27	70	B'	+	+	+
P 19	30	229	B	+	+	+
P 20	16	35	B	+	+	+
P 21	23	73	B	+	+	+

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P 19	30	229	B	+	+	+
P 20	16	35	B	+	+	+
P 21	23	73	B	+	+	+

## Results of control herd

- 33 herds tested negative for *S. aureus* GTB in all samples types
  - 17 herds with no evidence *S. aureus*
  - 16 herds with other genotypes of *S. aureus*

# Definitions

## Diagnostic sensitivity

- Probability towards 1 that a herd tested **positive** for *S. aureus* GTB is actually **infected** by this pathogen

## Diagnostic specificity

- Probability towards 1 that a herd tested **negative** for *S. aureus* GTB is actually **not infected** by this pathogen

# Results for reference and individual cow milk samples

- Diagnostic sensitivity  $> 0.95$ 
  - 19 of 19 GTB positive herds
- Diagnostic specificity  $> 0.97$ 
  - 33 of 33 GTB negative herds

## Results for SCC

- *S. aureus* *GTB* positive herds are associated with a increased somatic cell count  
(Michel et al., 2010)

# Discussion

- The novel analytics is powerful and cost-efficient
- BTM and related milk samples are suitable
- Collection of clean milk sample sufficient
- relevant simplification to control and monitor herds for *S. aureus* GTB
- CAVE: milk of all cows in BTM, no antibiotics or additives in samples

# Conclusion

- Very high diagnostic sensitivity and specificity of the new qPCR
- Assay highly cost-efficient, fast, robust and suitable for routine analysis

# Thank you for your attention



# Discussion

- Molecular biological methods allow new diagnostic possibilities
- GTB-positive herds showed increased SCC (Michel et al. 2010)

b	Herd ID	Herd size	SCC <sup>1</sup> *10 <sup>3</sup> / mL	Genotypes found	QPCR Assay <sup>2</sup>	
					MLP	BTM

*S. aureus* GTB<sup>6</sup> herds (n = 21)

P 1	22	102	B, B''	+	+	+
P 2	36	56	B, R	+	+	+
P 3	16	74	B	+	+	+
P 4	54	114	B	+	+	+
P 5	19	100	B'	+	-	+
P 6	20	154	B	+		+
P 7	12	178	B, C, I'	+		+
P 8	22	245	B		+	+
P 9	22	259			+	+
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P 12		66	B'''	+	+	+
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P 21	23	73	B	+	+	+

3 new subtypes of *S. aureus* GTB