

# High and Low QTL associated resistance to *E. coli* mastitis

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**A EU (SABRE) and AU-funded project  
WP5 – Mammary Function**



# Quantitative trait locus (QTL)

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- A region of DNA associated with a measurable trait (haplotype)
- Multiple genes determine the trait
- DNA and Pedigree information
- Recordings of SCC, clinical mastitis and bacteria in the Danish National Cattle Data Base
- QTL affecting clinical mastitis and SCS in Nordic red cattle breeds (Sahana et. al, 2008)

# E. coli mastitis QTL

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- Pathogen associated mastitis QTLs (Sørensen et al. 2008, JDS)
- E. coli QTL in Danish Holstein Friesian, 2 lactation
- QTL located on chromosome BTA 9
- QTL+ high resistance (position 42,43, 54, 55)
- QTL- low resistance (position 4, 6, 7, 8)
- **E. coli model:** "Proof of concept" and identification of candidate genes/pathways in QTL

# Animal experiment permit

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- Permit from the Danish Animal Experiments Inspectorate to conduct E. coli infections combined with blood sampling, fixation and biopsies...
- Comitee visits during the acute stage of the disease
- Guidelines - how far can we go?
- **Medicin cabinet:**
  - E. coli "sensitive" and "resistent" antibiotic
  - Pain and fever treatment (NSAID)
  - Water- and salt balance treatment
  - Ca-borogluconate
  - Local anaesthetics
  - Sedation



# Selection and purchase of heifers for trial

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QTL testing of bulls (sire and grand sire)



## Danish National Cattle Database

Daughters with potential high and low M-QTL in pedigree



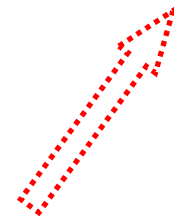
Matching of heifers QTL, expected calving dates, age, recorded health status (~1000)



Health status of herds **No**: S. dublin, BVD, B-streptococcus, (IBR)  
**"Low ParaTB status"**



Contact farmers



Blood sampling for testing of QTL (~200)




Purchase and transport of 42 pregnant heifers to AU experimental dairy barn (1.5-2 months prior to expected calving date)

1 batch of 20 heifers (winter)

1 batch of 22 heifers (summer)

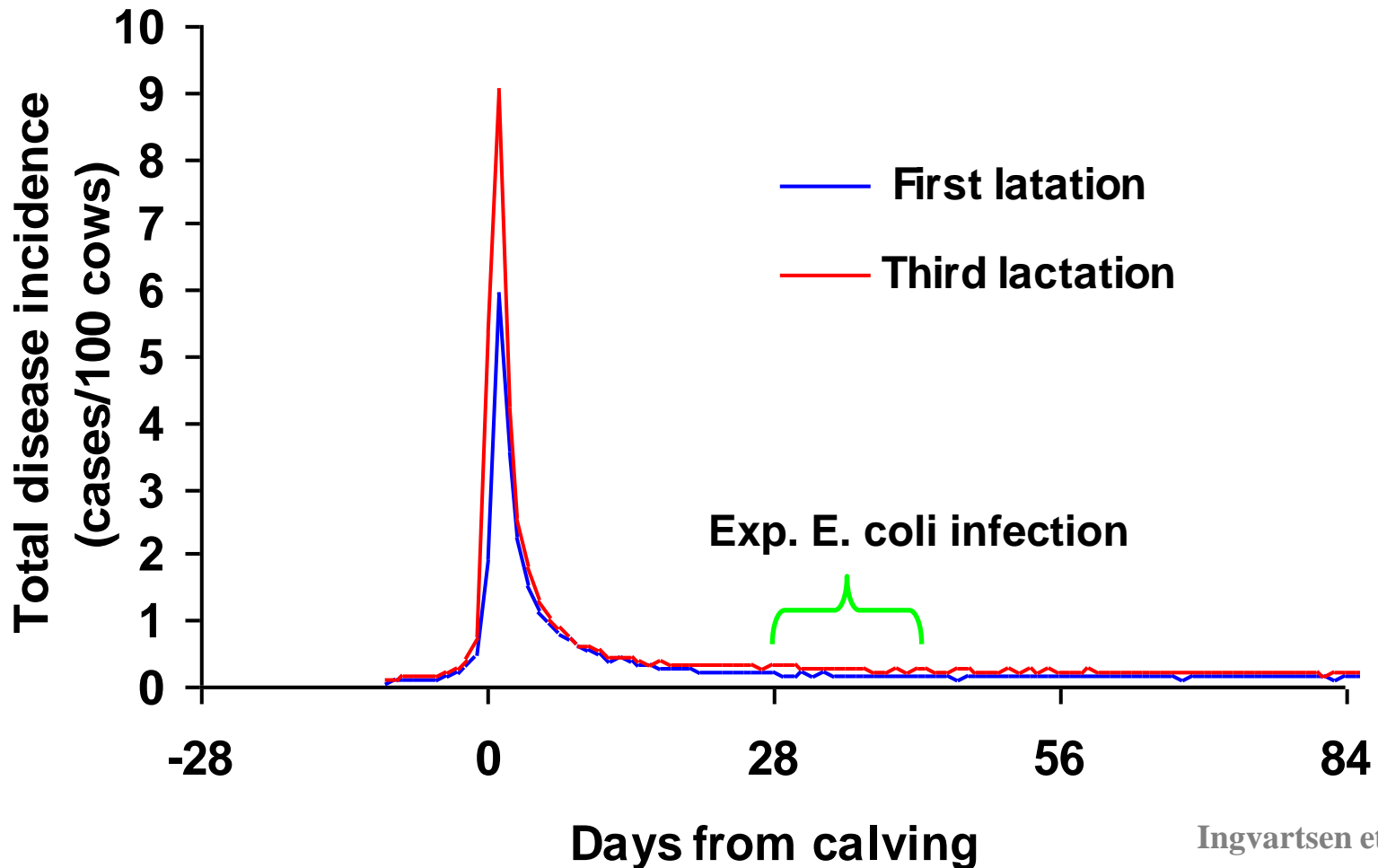
# Experimental Design

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- 42 heifers (10 reserves), 6 homozygotes
- 4 rounds with 8 "most healthy" cows
- 32 cows in E. coli trial (18 QTL+, 14 QTL-)
- Biopsies of 16 cows (8 QTL+, 8QTL-)
- Challenge 4-6 weeks after calving
- E. coli strain and dose  Low dose model
- How and when: clinical recordings and sampling
- Pilot study, 6 other AU cows

# Disease risk in periparturient cows

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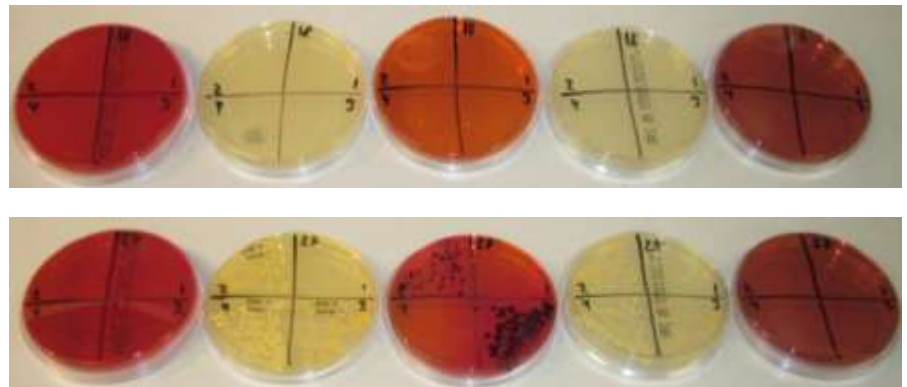


# Screening of cows for udder health prior to E. coli infection

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- One week prior to challenge
- At least 2x per cow
- CMT test (1 or 2)
- Testing of quarter milk for mastitis bacteria
- SCC, DeLaval DCC
- **Quarter free of bacteria and  $SCC < 100.000/ml$  was chosen**





# Screening of the cows general health condition prior to E. coli infection

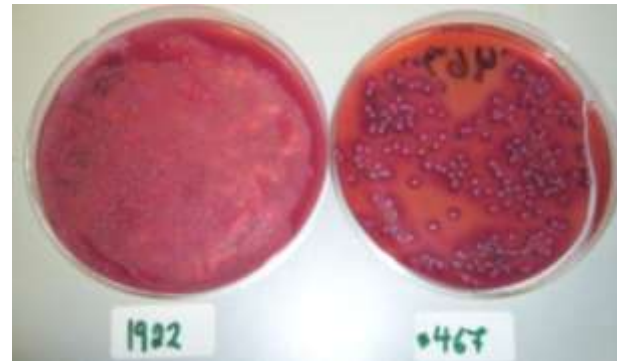
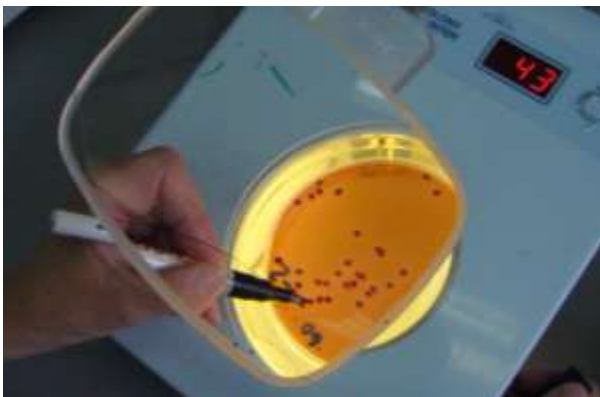
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- General health, appetite, milk yield, uterus, udder, legs and claws
- Body temperature (normal 38.4 C)
- Blood: White blood cell count (WBC) x 2, Glutaraldehyde test (Quick test for acute and chronic inflammatory conditions)
- Temper and behaviour

# E. coli




- Danish field strain, clinical acute mastitis (Helle D. Larsen, former DVI, DK)
- **20-40 CFU/10 ml** 0.9% endotoxin-free NaCl in **one front quarter per cow**
- CFU counted on MacConkey agar, 10-folds dilutions
- Purity test on blood and TSA agar.



# Registration of clinical symptoms and production data

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- Body temperature
  - Udder inflammatory condition (score 1-4)
  - Respiratory rate
  - Pulse
  - Daily milk yield (true tester)
  - Daily feed intake
  - Appetite (score 1-4)
  - Rumen motility (conc. per 2 min)
  - Faeces condition (score 1-4)
  - Standing- and lying activity
- 
- 1. Data for disease degree and length**
  - 2. Guidelines for medical treatment**

# Collection of samples

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- Milk (E. coli, SCC, APP and cytokines)
- Blood/plasma (WBC, APP and cytokines)
- Milk leukocytes (SC)
- Blood leukocytes (PBL)
- Liver tissue
- Udder tissue

**Gene expression  
and/or proteom**

# Collection of fore milk for CMT og bacteria

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# Milk samples and yield

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- CMT (all quarters)
- Colour (score)
- Filter(score clumps,homogeneity)
- SCC/ml (DCC DeLaval)
- Diff. count of selected SC samples
- 200-500 ml milk isolation of cells

to mRNA and proteom



- Milk yield – true tester

# Californien Mastitis test (CMT)

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- Simple cow side test
- Semi quantitative cell count
- **Principle:** SDS-detergent dissolves cells that release DNA + pH-analysis
- DNA gel formation and colour change
- Scoring 1 – 5 of gel viscosity
- Normal milk CMT 1(-2)
- Mastitic milk alkaline (purple) + strong gel formation (score 4-5)

# Scoring of milk colour and clumps

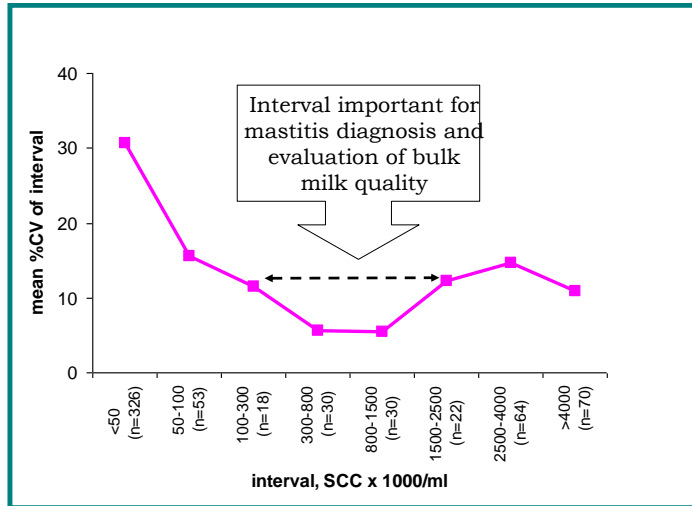
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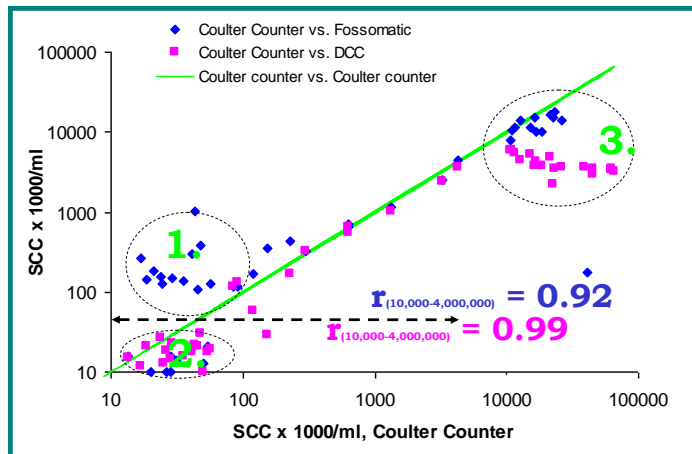
# Evaluation of DeLaval DCC

Røntved et al., IDF, Maastricht, 2005 (poster presentation)



## Measure SCC/ul

- **Principle:** Propidium iodide staining of cellular DNA in milk measured in a portable "mini-fluorometer"
- Handy on farm instrument for cow side testing and bulk milk



# Differential cell count of milk SCC by microscopy

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- Cytospin preparation
- May-Grünwald-Giemsa stain, methanol fixated cytospin of somatic cells isolated from milk

# Collection of blood samples using a vene catheter

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- Installed one day prior to infection in sedated cows
- Flushed with endotoxin-free 0.9% NaCl and heparin
- Repeated sampling minimize stress.
- IV medical treatment



# Collection of liver biopsies

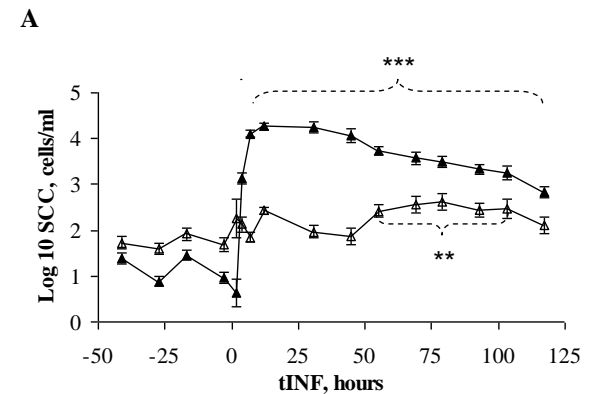
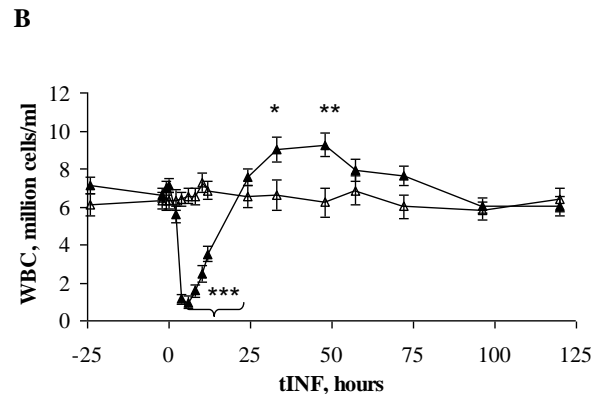
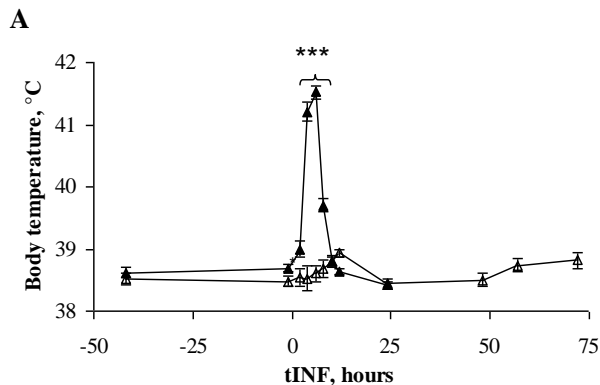
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# Repeated liver biopsies in dairy cows with *E. coli* LPS-induced mastitis (Vels et al., 2009, JDS)

## Liver biopsies

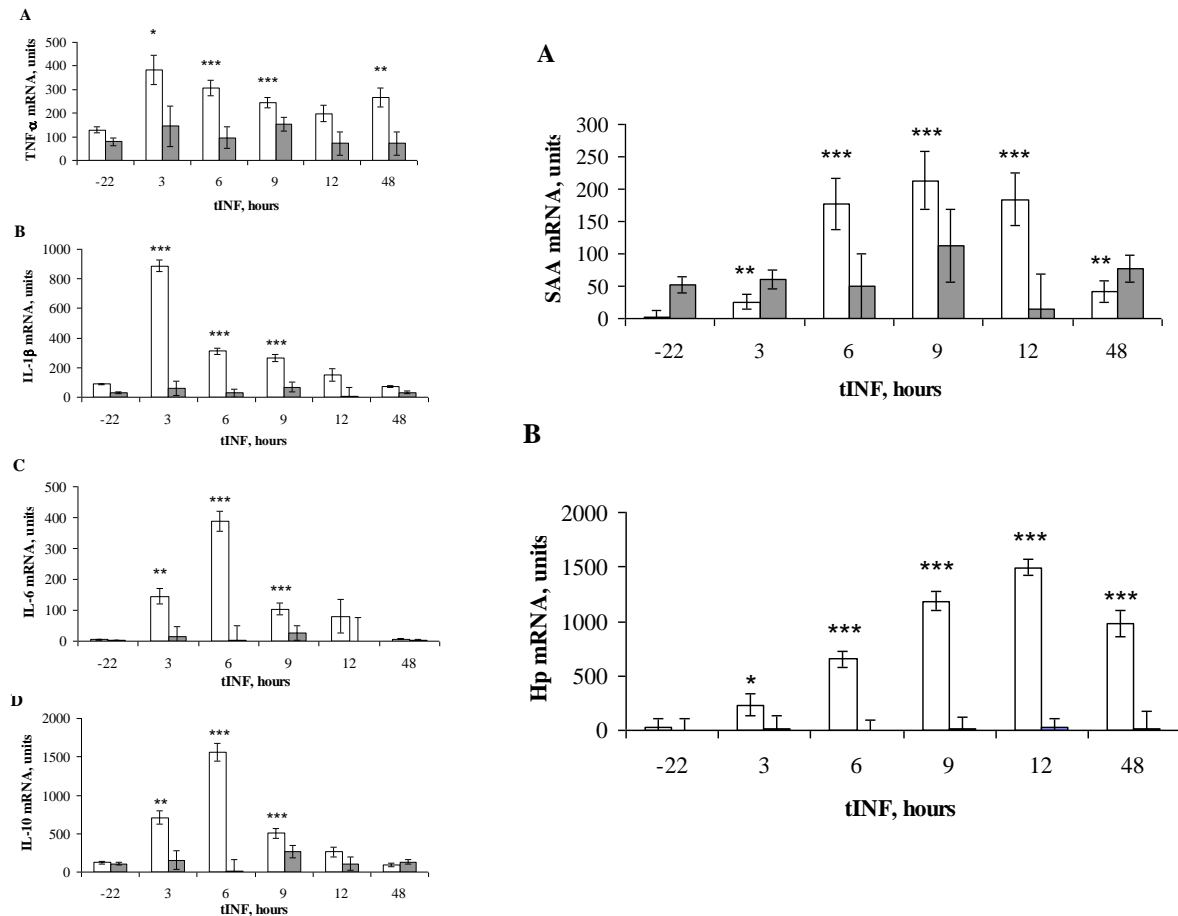
- “Gentle” invasive technique, minimal bleeding
- Repeated biopsies within hours
- No effect on inflammatory response (cytokines and APP) hence suitable for studying the hepatic APR in cows



# Hepatic APR in dairy cows with *E. coli* LPS-induced mastitis using RT-PCR

(Vels et al., 2009, JDS)

Figure 4.



# Collection of udder biopsies

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- Severe bleeding and hematomas
- Alter milk composition and may reduce milk yield
- Risk of secondary G+ bacteria infection
- 7 days between sampling



# Sampling over time

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Blood samples /PBL: -6,0,3,6,12,18,24,36\*,60\*, 84\*,144\*, 192, 240\* h

Milk samples /SC: -12, -6,0,3,6,12,18,24,36\*,48,60\*,72, 84\*,120,144\*,168, 192,216, 240\*,312 h

Liver biopsies: -144

12 24

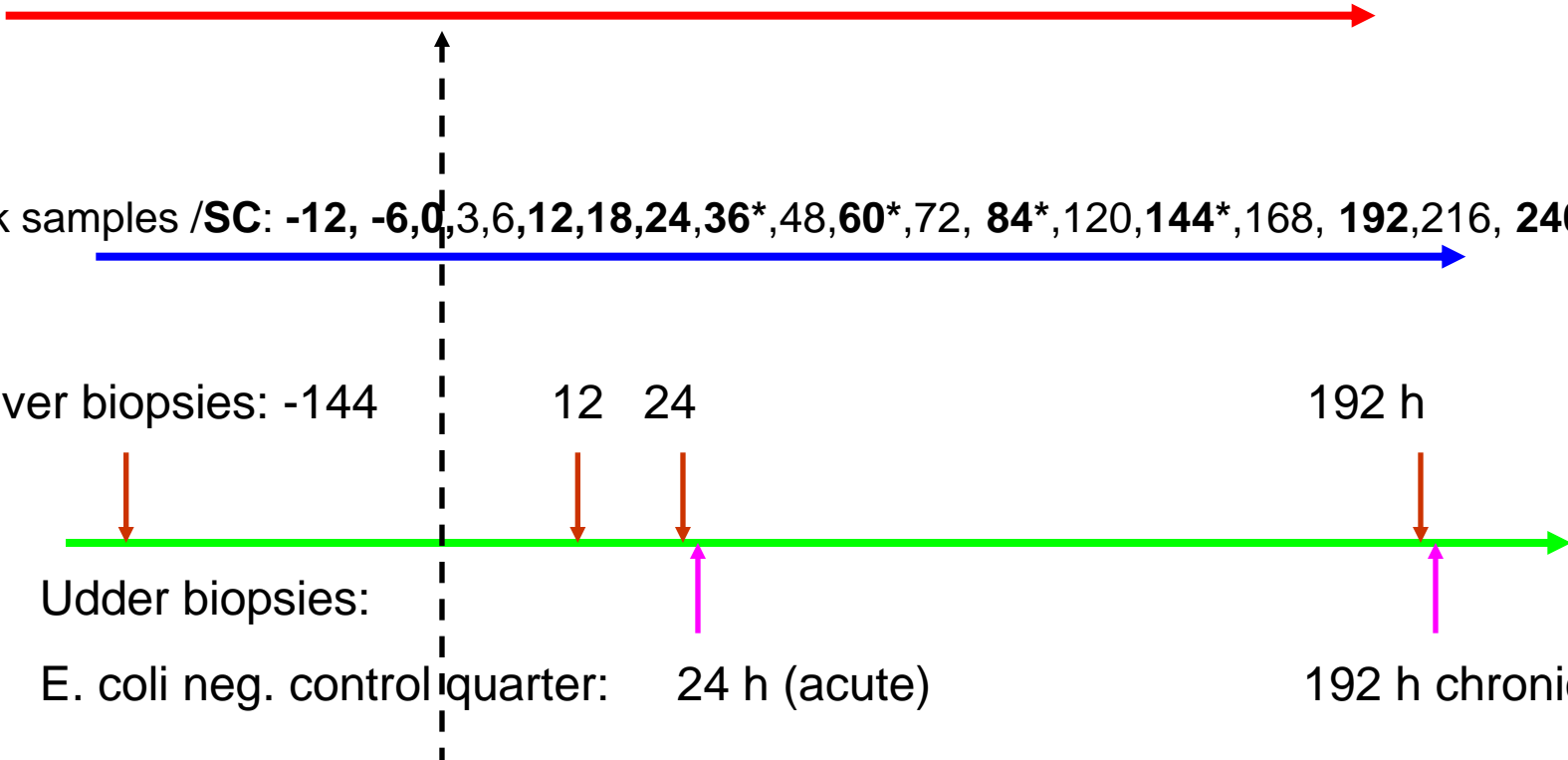
192 h

Udder biopsies:

E. coli neg. control quarter: 24 h (acute)

192 h chronic

**E. coli inoculation: 0 h**





# Labour demanding and good planning!!!

Exp 615, E. coli trial, Round 4										cmr 08.08.07							
Date	Week day	m	b	k	bi	hour code	day code	Barn+lab	Blood	Barn:	Blood:	Milk	Barn:	LAB:	mRNA-proteom		Cyto-spin, SCC
								BU, CMT screening	ex vivo	Clinical records	in vivo	BU, DCC, CMT	Liver biopsi	Udder biopsi	WBC, Plasma	LYSIS, PBL	
03-sep-07	Monday	6				-192	-8	X									
04-sep-07	Tuesday	6	8			-168	-7	X	X					X	X		
05-sep-07	Wednes				16	144	-6		X				X/6	X	X		
06-sep-07	Thursda					120	-5										
07-sep-07	Friday					84	-4			test							
08-sep-07	Saturda					60	-3										
09-sep-07	Sunday					48	-2	E. coli prep									
10-sep-07	Monday	6		16		-36	-1	E. coli prep		test						(X)	
10-sep-07	Monday	9				-24	-1	E. coli prep		catheters							
11-sep-07	Tuesday	6				-12	0	E. coli prep								(X)	
11-sep-07	Tuesday	12	11	13		-6	0	E. coli prep		X	X	X		X	X		
11-sep-07	Tuesday	18	17	16		0	0	E. coli inoc		X	X	X		X	X	X	X
11-sep-07	Tuesday	21	22	23		3	0			X	X	X		X	X	**	
11-sep-07	Tuesday	24	1	2		6	0			X	X	X		X	X	**	
12-sep-07	Wednes	6	7	9	7	12	1			X	X	X	X/4	X	X	X	X
12-sep-07	Wednes	12	11	13		18	1			X	X	X		X	X	X	X
12-sep-07	Wednes	17	16	18	19	24	1			X	X	X	X/4	X/K/4	X	X	X
13-sep-07	Thursda	6	7	10		36	2			X	X***	X		X	X***	X***	X
13-sep-07	Thursda	17				48	2			(X)		(X)					
14-sep-07	Friday	6	7	10		60	3			X	X***	X		X	X***	X***	X
14-sep-07	Friday	17				72	3			(X)		(X)					
15-sep-07	Saturda	6	7	10		84	4			X	X***	X		X	X***		
16-sep-07	Sunday	6				120	5			(X)		(X)					
17-sep-07	Monda	6	7	10		144	6			X	X***	X		X	X***	X***	
18-sep-07	Tuesday	6				168	7			(X)		(X)					
19-sep-07	Wednes	6	7	10	15	192	8			X	X	X	X/4	X/K/4	X	X	X
20-sep-07	Thursda	6				216	9			(X)		(X)					
21-sep-07	Friday	6	8	10		240	10			X	X***	X		X	X***	X***	
22-sep-07	Saturda					264	11										
23-sep-07	Sunday					288	12										
24-sep-07	Monday	6	8	10		312	13			(X)		(X)					

# Results

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- 30/32 cows were infected with E. coli
- 1 cow – no detection of E. coli
- 1 cow was left out 1 h prior to challenge due to spontaneous mastitis
- 1 cow was so sick of E. coli, that she had to be given medical treatment
- 2 outliers removed in the statistics (ill precalving, oxytocin-treated)

# Results

(raw, mean values of non-transformed data)

		E. coli hours	E. coli max peak h	Temp hours	Temp max	Temp peak h	SCC hours	SCC max	SCC peak	Yield drop	Mean Yield/l pd	
T-test	p-val	0,98	0,31	0,87	0,48	0,98	0,91	0,087	0,56	0,39	0,45	0,57
F-test	p-val	0,21	0,000000007	0,59	0,90	0,31	0,85	0,38	0,29	0,87	0,40	0,55
	Varian	3931	4762929765	38	222	1	19	15941	704738	1084	51	37
	Varian	1906	193367599092	50	204	1	16	9554	1259061	1172	29	26

5x lower E. coli count  
E. coli count var 36x >

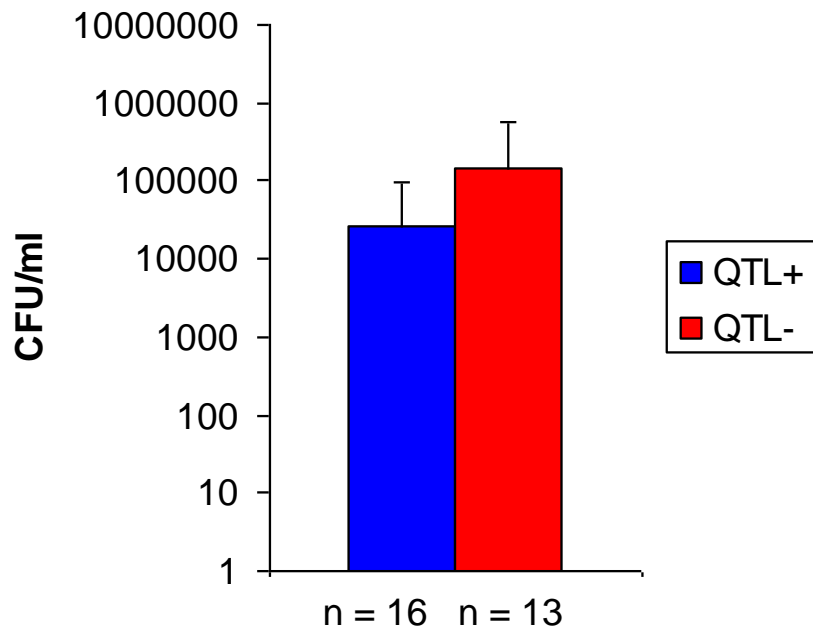
QTL1: 3 days earlier return to normal SCC  
Defined by SCC <150.000 cells/ml

**MIXED MODEL in SAS, repeated measurements**

# E. coli max counts at 24 h

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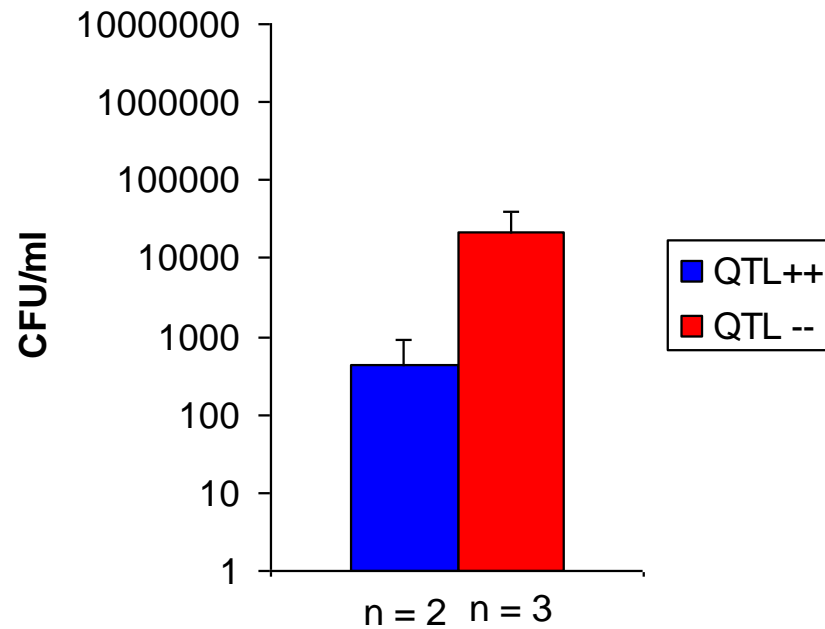
## All cows



t-test,  $P = 0.31$

F-test,  $P < 0.00001$

## Homozygotes

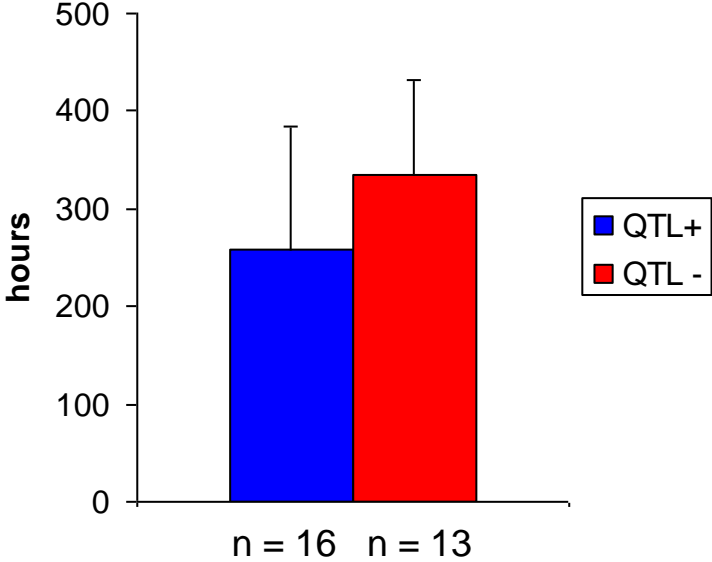


t-test,  $P = 0.11$

# Hours for return to SCC < 150.000/ml

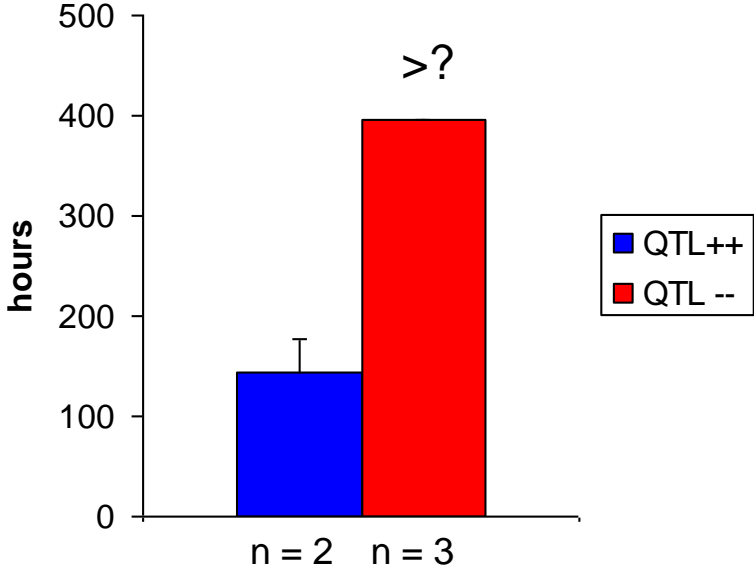
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### All cows



t-test, P = 0.09

### Homozygotes

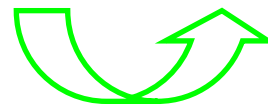
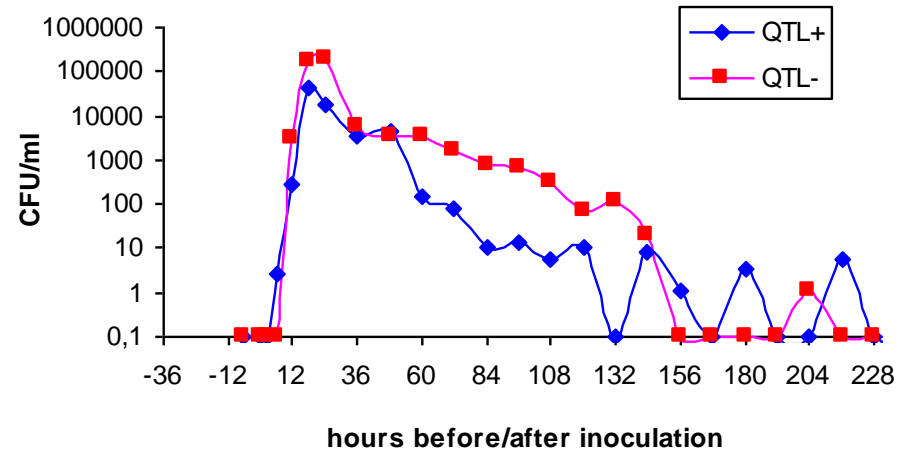
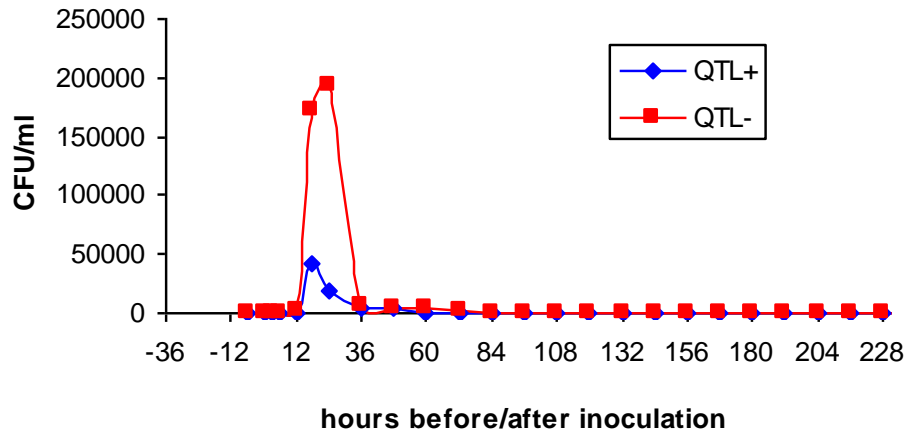


t-test, P = 0.03

# E. coli CFU/ml in milk of biopsy cows (n =8 in each group)

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t-test,  $P < 0.10$

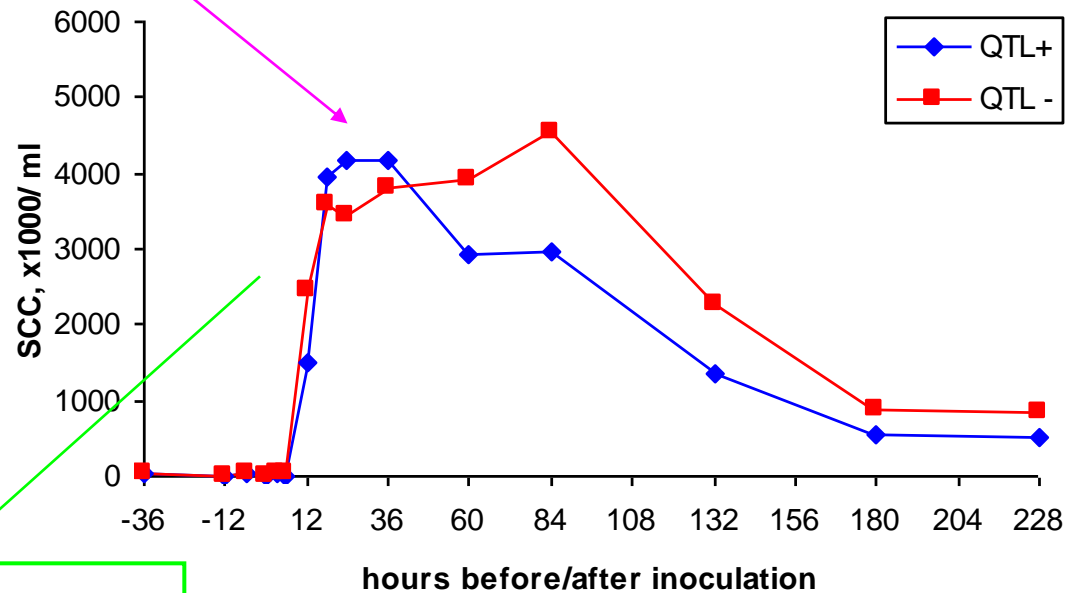


Log scale

# SCC measured by DeLaval DCC E. coli mastitis in biopsy cows (n =8 in each group)

DCC upper limit of detection range

t-test (60-228 h),  $P < 0.05$

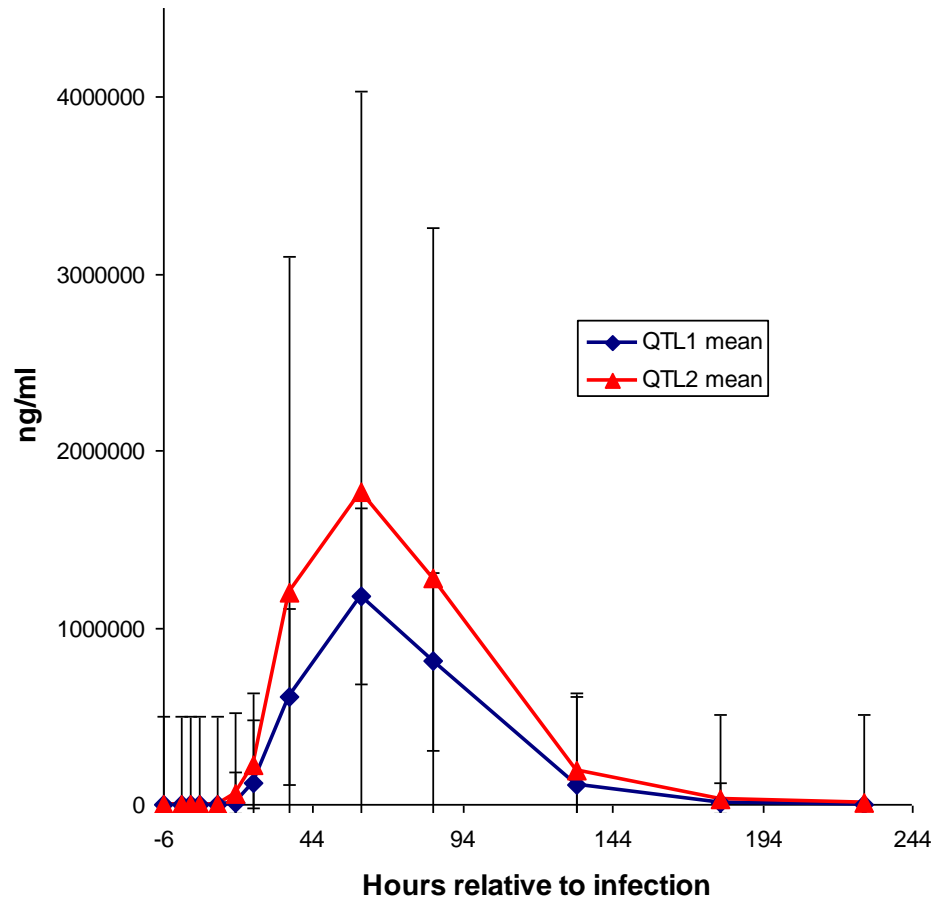


Enzyme: LDH

Acute phase protein: MAA

# Preliminary non-transformed MAA ELISA results in milk of all QTL cows with induced E. coli mastitis

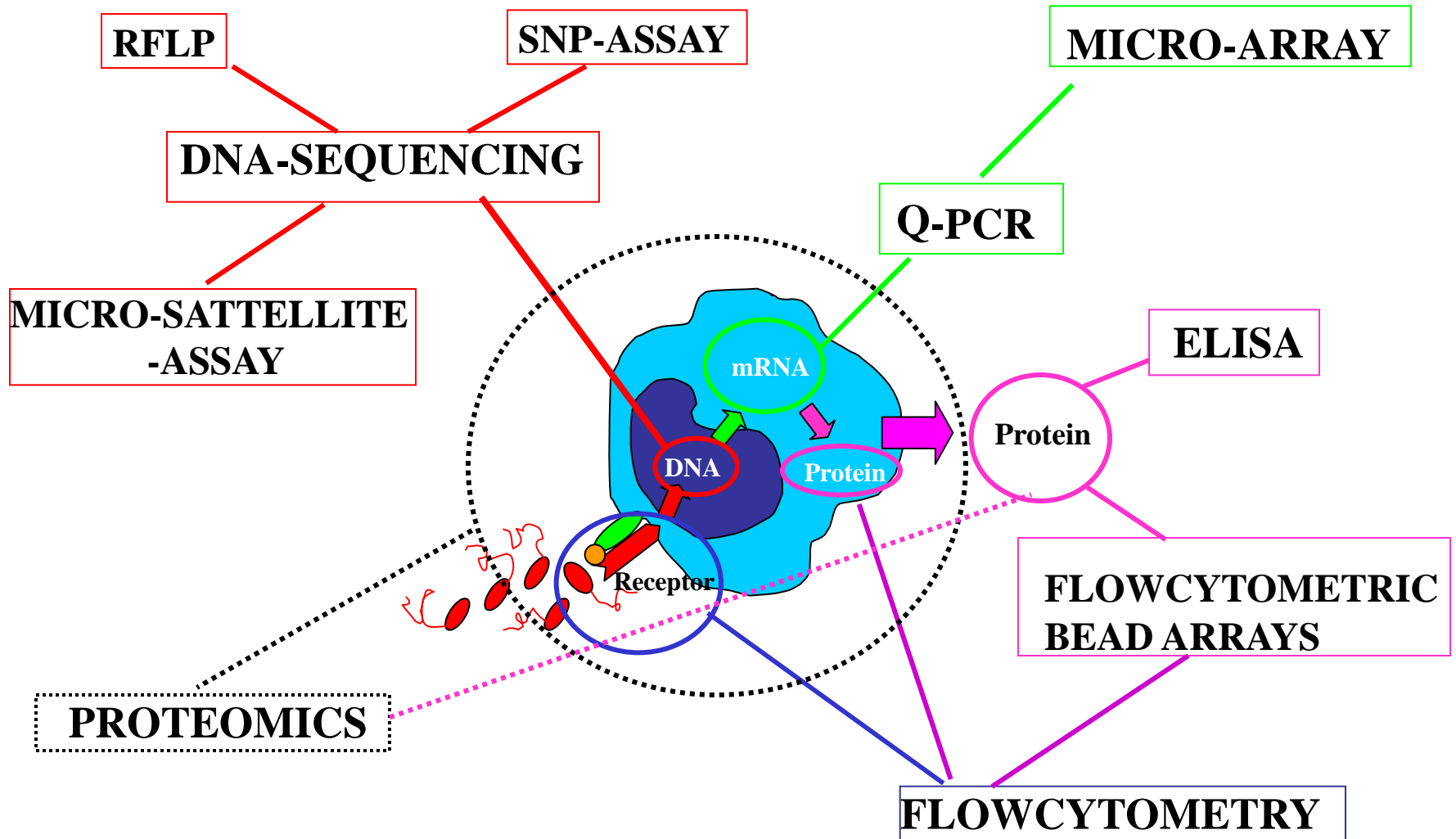
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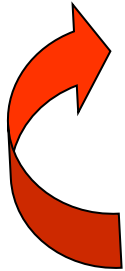
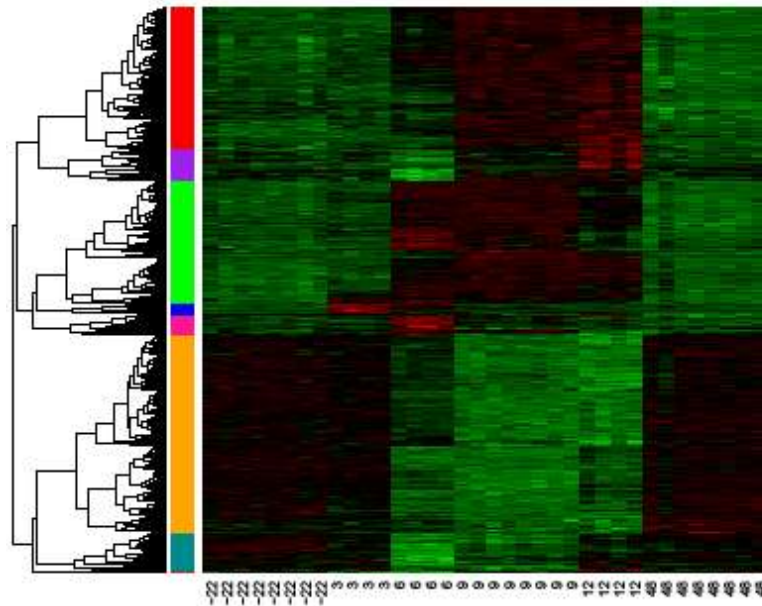
# IMMUNOLOGISTS' TOOLBOX

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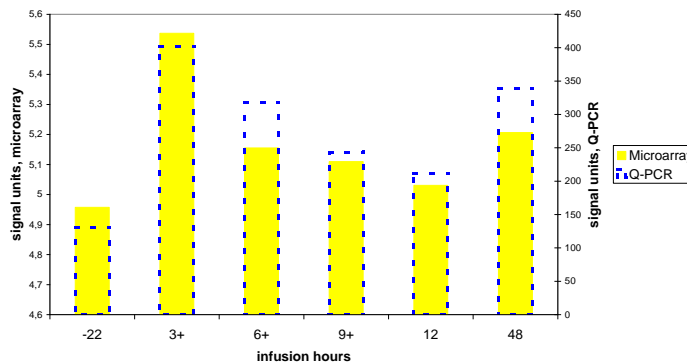
# Hepatic APR gene expression in *E. coli* LPS-induced mastitis of dairy cows using gene array

(Jiang et al., 2008, BMC genome)



20% of genes differentially expressed

Time Series  
TNF $\alpha$

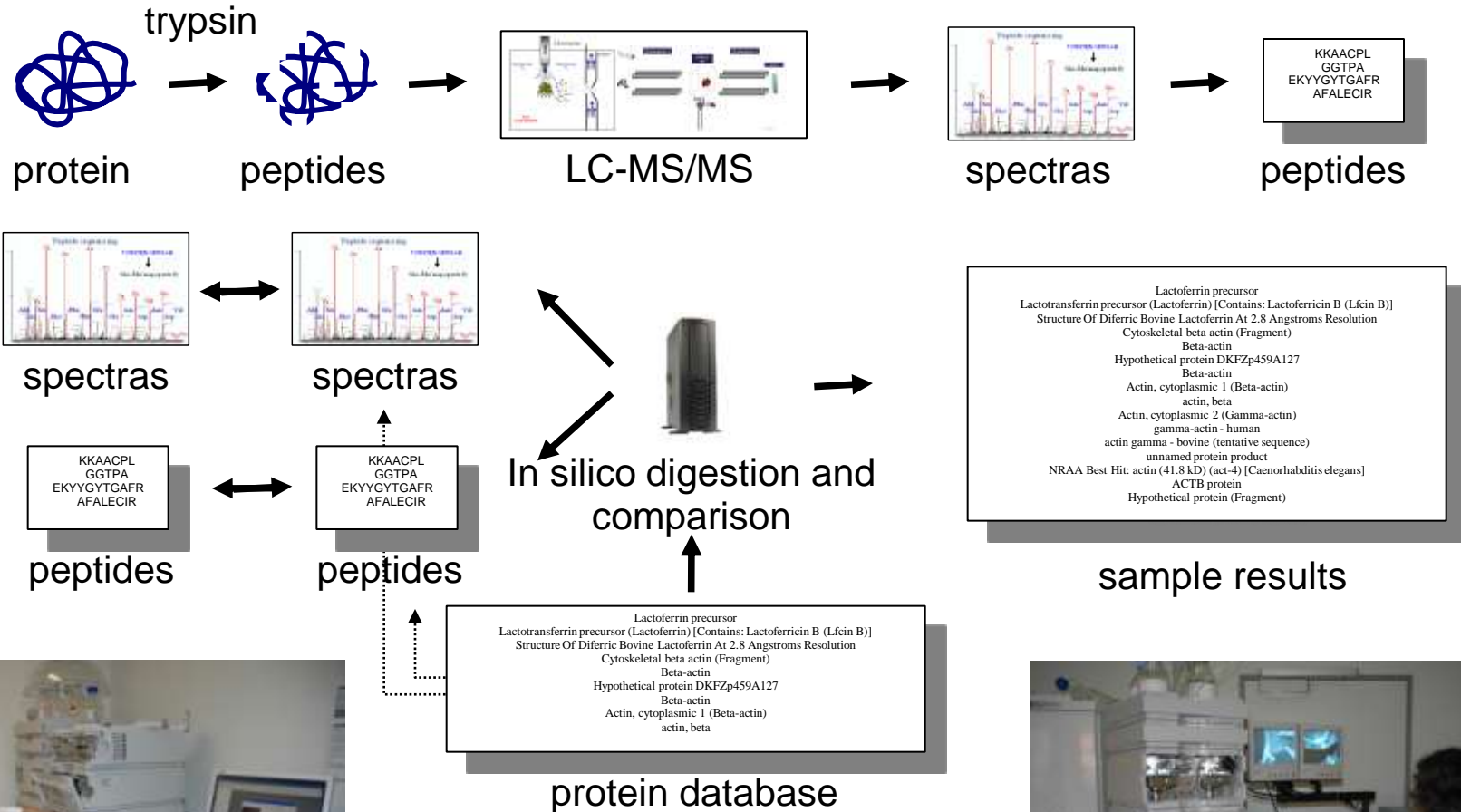


- Oligo nucleotide array - bovine Affymetrix array (24128 target transcripts)
- Measurement of multiple gene transcriptions → clusters
- Up and down regulated genes
- No amplification of mRNA
- Comparison of two samples sick vs. healthy
- Database search
- Evaluation by Q-PCR
- Major tool for pathway analysis during disease
- Demands collaborative work between scientists with different skills

# Proteomic Platform

Danielsen et al., 2010 acc. in "Proteomics" LPS mastitis study

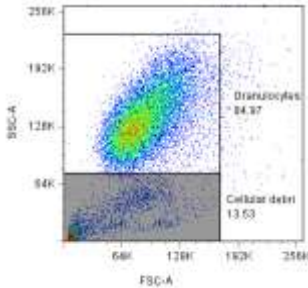
Ph.d. stud. Stine L. Christensen – targeted proteomics



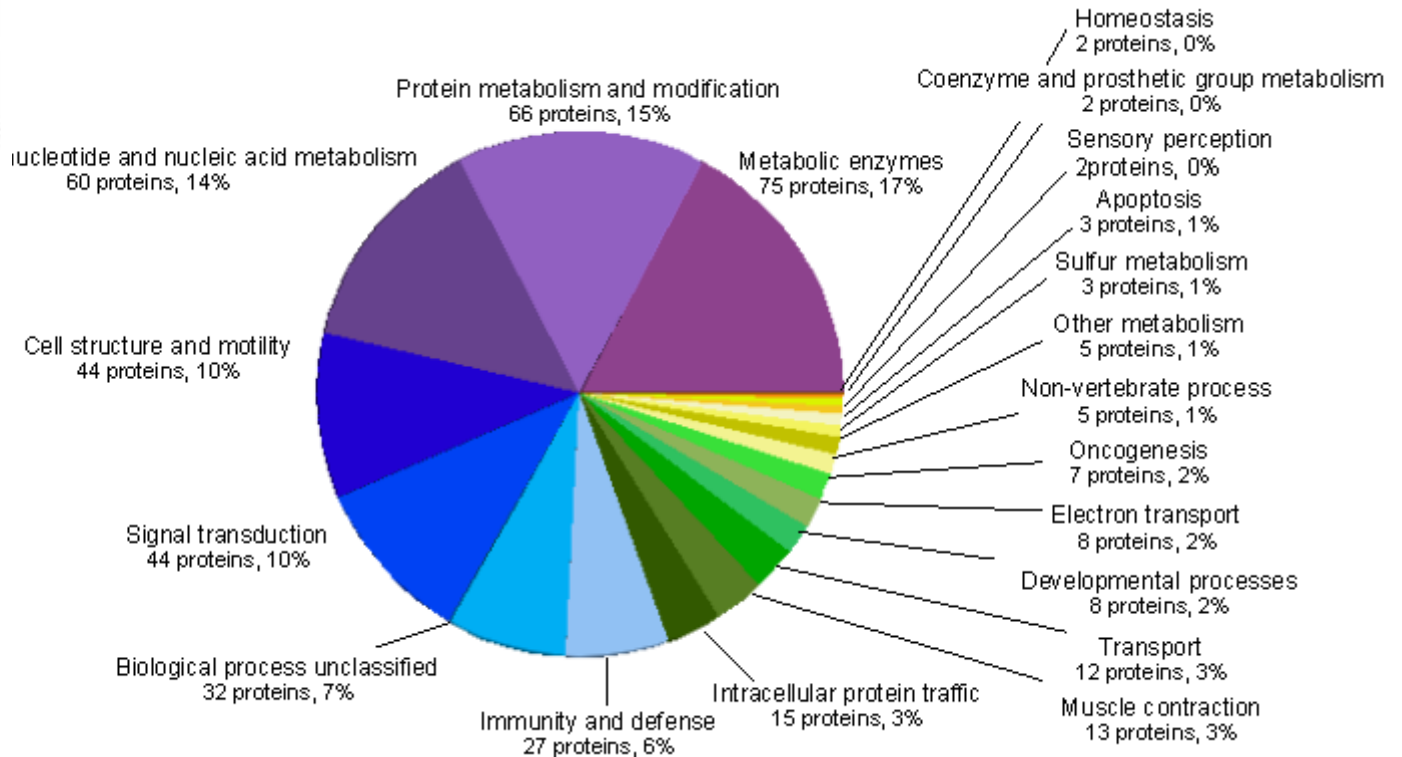
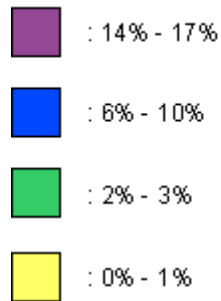
**Mass spectrometry (MS)**

# Proteom of PMNs in blood

(by Ph.d. stud. Asger Nissen, AU)

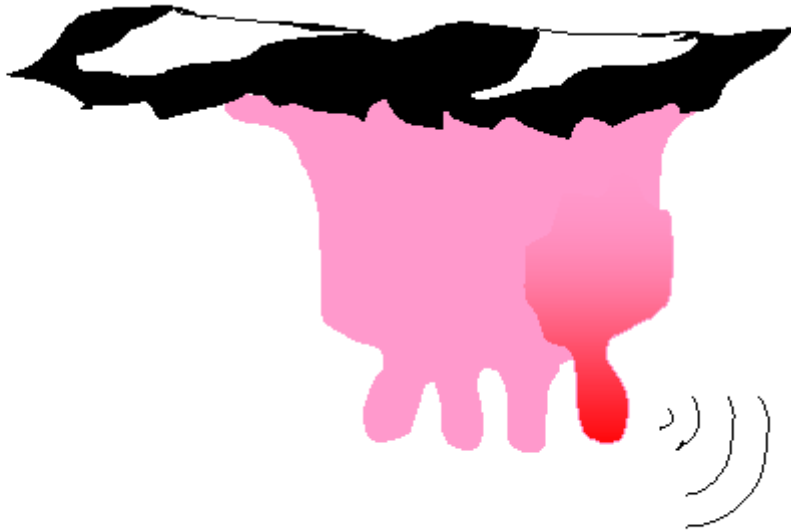


## 434 proteins found



# Ongoing .....

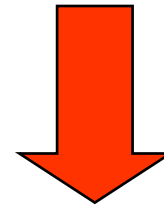
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Association of global gene  
expression in udder and liver  
of *E. coli* infected cows

&

Proteome analysis of milk....



Identification of up and downregulated  
genes/pathways in the QTL  
associated with *E. coli* mastitis

# Conclusion

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- **Low dose E. coli model worked**
- **Large variation in cows responsiveness to E. coli for both QTL groups**
- **Cows with QTL+ tended to have fewer E. coli CFU/ml than cows with QTL-**
- **Cows with QTL+ had a faster recovery (SCC) than cows with QTL-**
- **Effect of haplotypes can be "mimicked" to some degree**

# Acknowledgements

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- **QTL screening:** Johanna Vilkki, MTT, Agrifood Research, Finland
- **QTL and pathogen association:** Mogens Sandø Lund and Lars Peter Sørensen, AU, Denmark
- **Selection and purchase of heifers:** Mogens Sandø Lund, Lars Peter Sørensen, Jørn Rind, Peter Sørensen, Martin Bjerring, Christine Røntved, AU and Viking-genetics, Denmark
- **E. coli trial:** Dorte Agnholt, Hanne Purup Møller, Elisabeth Mark, Martin Bjerring, Jens og Britt Clausen, Klaus Lønne Ingvarsen, Lene Niklassen, Inger Marie Jepsen, Susanne Sommer, Pia Jensen, Jan Pedersen, Thomas Bitsch, Tina Hald, Ole Frank, Erik Moestrup, Christine Røntved, AU, Denmark

# THANK YOU

