

WP5 - Mammary Function

Experimental E. coli mastitis in dairy cows with high and low QTL resistance to E. coli mastitis:
 Design and Results

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E. coli mastitis QTL

- Recordings of clinical mastitis and bacteria in the Danish National Cattle Data Base
- Pathogen associated mastitis QTLs (Sørensen et al. 2008, JDS)
- E. coli QTLs in Holstein Friesian
- QTL+ high resistance (position 42,43, 54, 55)
- QTL- low resistance (position 4, 6, 7, 8)

Animal experiment permit

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



Selection and purchase of heifers for trial

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 QTLs, expected calving dates, age, recorded health status (~1000)

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

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)

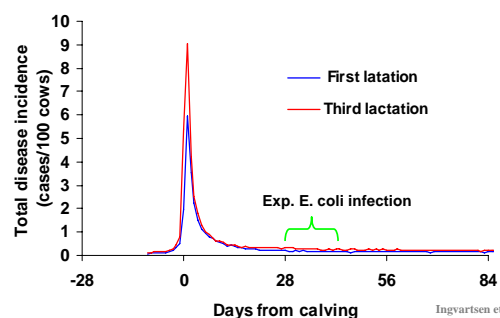
Blood sampling for testing of QTLs (~200)

Contact farmers

Experimental Design

- 2 batches of 42 heifers (10 reserves)
- 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
- How and when: clinical recordings and sampling
- Pilot study, 6 cows

Disease risk in periparturient cows

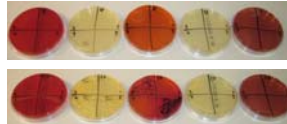


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Screening of cows for udder health prior to E. coli infection



- 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



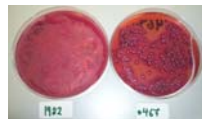
Screening of the cows general health condition prior to E. coli infection

- 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)
- Temperament 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

- 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

- 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



Milk samples and yield

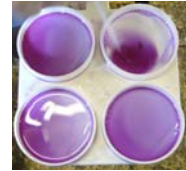


- CMT (all quarters)
- Colour (score)
- Filter(score clumps, homogeneity)
- SCC/ml (DCC DeLaval)
- Diff. count of selected SC samples
- 200-500 ml milk isolation of cells to mRNA and proteom



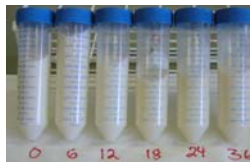
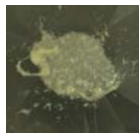
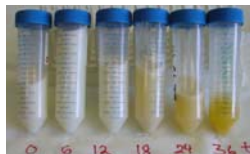
- Milk yield – true tester

Californien Mastitis test (CMT)



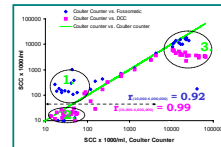
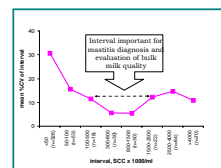
- Simple cow side test
- Semi quantitative cell count
- **Principle:** SDS-detergent dissolves cells that release DNA + pH-analysis
- DNA gel formation and colour change
- Scoring 1 – 5 of gel viscosity
- Normal milk CMT 1(-2)
- Mastitic milk alkaline (purple) + strong gel formation (score 4-5)

Scoring of milk colour and clumps



Evaluation of DeLaval DCC

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

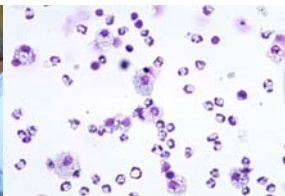


Measure SCC/ul

- **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



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

Collection of blood samples using a vene catheter



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

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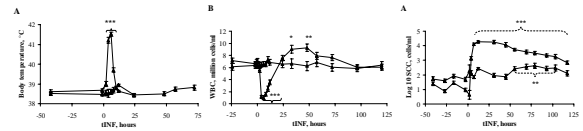
Collection of liver biopsies



Repeated liver biopsies in dairy cows with E. coli LPS-induced mastitis (Vels et al., 2008, JDS, acc.)

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



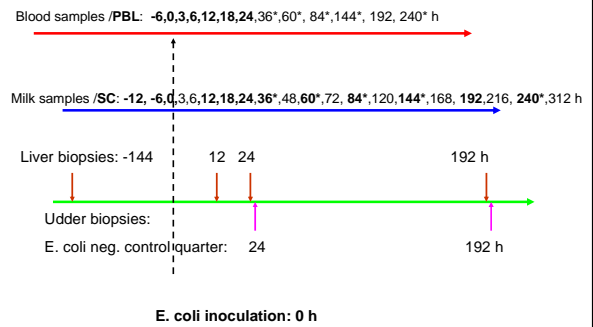
Collection of udder biopsies



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



Sampling over time



Labour demanding and good planning!!!

Exp 615, E. coli trial, Round 4										cont. 08.08.07											
Date	Week	m	b	k	bl	h	h	hour	day	Barn	lab	Blood	Milk	Barn:	Blood	Milk	Barn:	LAB:	mRNA	proteom	Cyto-
	day							code	code	BU, CMT	ex	Clinical	Udder	Barn:	WBC	Udder	LAB:	LYSIS	SCC	spn.	
										score	score	records	records	score	score	score	score	score	score	score	score
03-sep-07	Monday	6						192	7												
04-sep-07	Tuesday	6						168	7	X	X							X	X		
05-sep-07	Wednesday	6						144	6									X	X		
06-sep-07	Thursday	6						120	5												
07-sep-07	Friday	6						96	4												
08-sep-07	Saturday	6						60	3												
09-sep-07	Sunday	6						36	2												
10-sep-07	Monday	6						12	1												
11-sep-07	Tuesday	6						0	0												
12-sep-07	Wednesday	6						0	0												
13-sep-07	Thursday	6						0	0												
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23-sep-07	Sunday	6						0	0												
24-sep-07	Monday	6						0	0												

Results

- 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

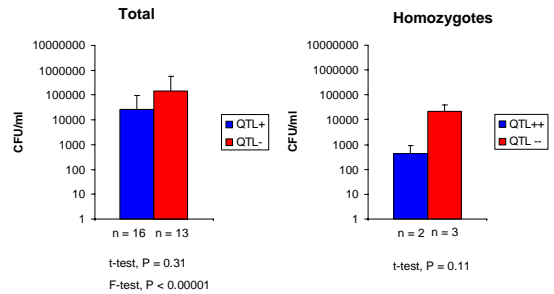
(raw, mean values of non-transformed data)

	E. coli hours	E. coli E. coli max peak h	E. coli peak h	Temp hours	Temp max	Temp peak h	SCC hours	SCC max	SCC peak	Yield drop	Mean Yield/l pd
T-test	0.98	0.31	0.87	0.48	0.38	0.91	0.087	0.58	0.39	0.45	0.57
F-test	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	28

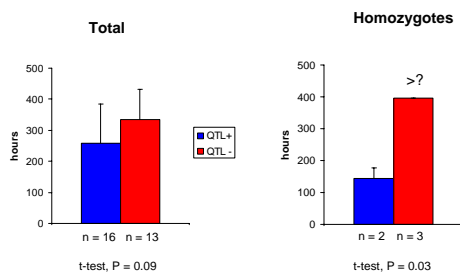
5x lower E. coli count
 E. coli count var 36x >

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

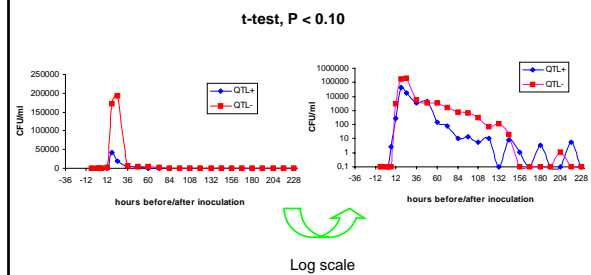
E. coli max counts



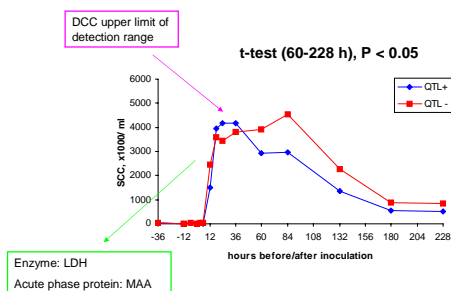
Hours for return to SCC < 150.000/ml



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



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



Conclusion

- Cows with QTL+ tended (so far) to have fewer E. coli CFU/ml than cows with QTL-
- Cows with QTL+ had a faster recovery (SCC) than cows with QTL-

Acknowledgements

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