


Milking the cow udder transcriptome: microarray analysis of the host response to mastitis
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ROSLIN

Mastitis Workshop: Biology and Meta-Analysis


Milking the cow udder transcriptome: microarray analysis of the host response to mastitis

Kirsty Jensen



Mastitis **ROSLIN**


- Important disease in dairy cattle
 - Economic losses
 - Animal health
- Major pathogens
 - *Staphylococcus aureus*
 - *Escherichia coli*
- Treatment
 - Antibiotics
 - Vaccination
- Development of new control methods
 - Suitable vaccines
 - Genetically resistant animals
 - Knowledge of host-pathogen interactions



Infection Study **ROSLIN**

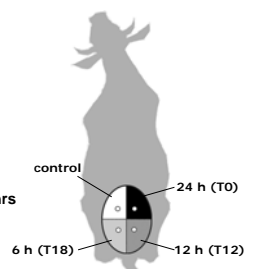
- EADGENE Mastitis Project
 - Dummerstorf
 - Munich
 - Hannover
 - Edinburgh
- Investigate host response to early infection
 - *E. coli* & *S. aureus*
- Strictly defined infection model

• Petzl et al., 2008 Vet. Res. 39:18




Infection Study **ROSLIN**

- Experimental Design
 - 12 Holsteins-Friesians in middle of first lactation
 - Individual udder quarters infected with:
 - 500 cfu *E. coli*
 - 10,000 cfu *S. aureus*
 - Sequential infection of udder quarters
 - *E. coli* infection for 0, 6, 12 & 24 hrs
 - *S. aureus* infection for 0, 6, 12 & 24 hrs
 - *S. aureus* infection for 0, 12 & 72 hrs
 - Total of 48 samples




• Petzl et al., 2008 Vet. Res. 39:18



Microarray Experiment **ROSLIN**

- Microarray experiment carried out at ARK-Genomics facility
- Bov20K cDNA microarray
- Reference design
 - Multifactorial analysis
 - Mixed pool of all RNA samples
 - 48 slides
- Quantify spots using BlueFuse
- Data analyzed using Bioconductor/limma package (R) with modifications
 - Normalization
 - ANOVA
 - Modified t-tests
 - False discovery rates
 - Gene lists

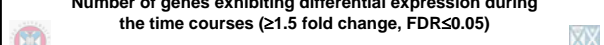


Microarray Experiment **ROSLIN**


Initial analysis:
 within animal comparison of infected quarters to control quarters

	6 hours	12 hours	24 hours	72 hours
<i>E. coli</i>	0	6	1048	-
<i>S. aureus</i>	1	4	0	0

Number of genes exhibiting differential expression during the time courses (≥ 1.5 fold change, $FDR \leq 0.05$)



Microarray Experiment ROSLIN

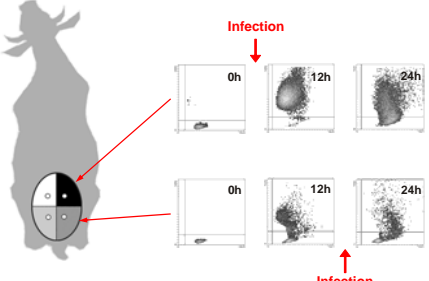


12 hr *E. coli* infection
 6 differentially expressed genes

Microarray Experiment ROSLIN

- Reasons for low number of significantly differentially expressed genes
 - Genuine result
 - Design of microarray experiment
 - Small number of biological replicates
 - Variation in biological replicates:
 - Large tissue samples resulting in immune status variability
 - Variation in the response of animals to infection
 - Status of the uninfected quarters
 - Bacteriologically negative

FACS Analysis of Milk Cells ROSLIN



Infection

0h 12h 24h

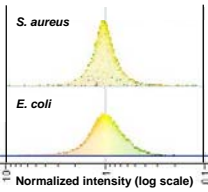
0h 12h 24h

Infection

Infection alters the cellular response of adjacent quarters

•Petzl et al., 2008 Vet. Res. 39:18

Microarray Experiment ROSLIN



S. aureus

E. coli

Normalized intensity (log scale)

- Preliminary analysis using GeneSpring
- Intensity plots (sample/reference)
- Distribution differs for control quarters
- Greater response in control quarters from *E. coli* infected animals

Microarray Experiment ROSLIN

Reanalysis of microarray data:
 comparison of quarters to *S. aureus* control quarters

	0 hours	6 hours	12 hours	24 hours
<i>E. coli</i>	359	330	537	1189

Number of genes exhibiting differential expression during the time courses (≥ 1.5 fold change, $FDR \leq 0.05$)

Uninfected Quarters ROSLIN

- Comparison of gene lists:
 - Uninfected quarters shared many transcripts in common with *E. coli* infected quarters:
 - 6 hrs 233 (64.9%)
 - 12 hrs 222 (61.8%)
 - 24 hrs 180 (50.1%)

Re-analysis of Microarray Data

- Comparison of control quarters from animals infected with *E. coli* or *S. aureus* for 24 hours
 - 187 annotated genes are differentially expressed (FDR \leq 0.05, FC \geq 2)
 - 77 SA24T0 > EC24T0
 - 110 EC24T0 > SA24T0

SA24T0 V EC24T0

- Database of Annotation, Visualization & Integrated Discovery (DAVID) analysis
 - Gene enrichment of Gene Ontology classifications:
 - Cellular compartment organization & biogenesis
 - Response to stress
 - Cell death
 - Cell activation

Meta-Analysis

- Mastitis infection
 - 84 genes indicative of mastitis infection
 - 14 (17%) genes are present in EC24T0 v SA24T0 gene list
- Early mastitis infection
 - 79 genes indicative of early mastitis infection
 - 33 (42%) genes are present in EC24T0 v SA24T0 gene list

SA24T0 > EC24T0


- Selection of genes exhibiting greater expression in control quarters from *S. aureus* infected animals
- ATP-binding cassette, sub-family G (WHITE), member 2 (ABCG2) 3.5X
- Chemokine (C-X-C motif) ligand 12 (CXCL12) 3.2X
- Cathepsin D 2.8X
- Colony stimulating factor 2 (CSF2/GM-CSF) 2.6X
- Interferon induced transmembrane protein 3 (IFITM3) 2.5X
- CD74 molecule, major histocompatibility complex, class II invariant chain (CD74) 2.4X
- Calreticulin (CALR) 2.4X
- Transforming growth factor beta 1 (TGFB1) 2.3X
- Complement factor D (CFD) 2.3X
- Fc fragment of IgG, low affinity IIa, receptor for (CD32/FCGR2A) 2.3X

EC24T0 > SA24T0



- Selection of genes exhibiting greater expression in control quarters from *E. coli* infected animals
- FK506 binding protein 5 (FKBP5) 6.6X
- Metallothionein 2A (MT2A) 5.2X
- Metallothionein 1E (MT1E) 4.6X
- Lipocalin 2 (LCN2) 3.8X
- Tribbles homolog 3 (TRIB3) 3.0X
- B-cell translocation gene 1, anti-proliferative (BTG1) 2.8X
- Lipopolysaccharide binding protein 2.8X
- Signal transducer and activator of transcription 3 (STAT3) 2.2X
- Chemokine (C-X-X motif) ligand 2 (CXCL2) 2.0X
- BCL2-associated X protein (BAX) 2.0X


qRT-PCR Validation

* = P<0.02
 error bars = standard errors



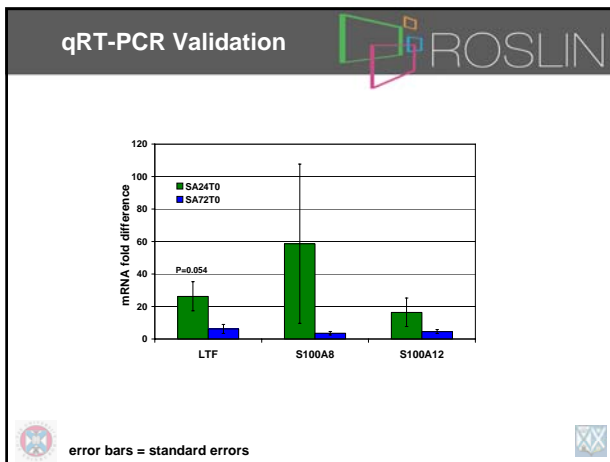
S. aureus Control Quarters 


- **Question:**
 - Is *S. aureus* infection of neighbouring quarters contributing to the differential expression in the control quarters?
- **Re-analysis of SA24T0 v SA72T0:**
 - 0 genes differentially expressed ($FDR \leq 0.05$)



S. aureus Control Quarters 

- **Less stringent analysis**
 - 73 genes differentially expressed ($P \leq 0.05$)
- **SA24T0 > SA72T0**
 - Lactotransferrin 3.5X
 - Superoxide dismutase 2, mitochondrial 2.3X
 - S100 calcium binding protein A8 (calgranulin A) 2.3X
 - S100 calcium binding protein A12 (calgranulin C) 2.2X
- **Indicative of response in control quarters from animals infected for 24 hours with *S. aureus***

Conclusions 

- *E. coli* infection in one mammary gland quarter affects the transcriptional profile of the neighbouring quarters
- Neutrophil influx into uninfected mammary gland quarters
- *S. aureus* infection appears to have a similar effect, which is greater at 24 hours post infection than 72 hours post infection
- There is cross-talk between bovine mammary gland quarters
- Implications for design of experiments using within animal controls

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