

Regulated Genes in the Bovine Mammary Gland During *E. coli* and *S. aureus* Infection

Kirsty Jensen, Liz Glass (Roslin Institute)

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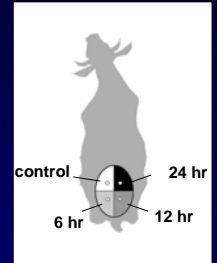
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In vivo Infection Study

- 12 Holsteins in middle of first lactation.
- Individual udder quarters sequentially infected with:
 - 500 cfu *E. coli*
 - 10,000 cfu *S. aureus*
- 48 samples
 - 4 animals infected with *E. coli*
 - Quarters infected for 0, 6, 12 & 24 hours
 - 4 animals infected with *S. aureus*
 - Quarters infected for 0, 6, 12 & 24 hours
 - 4 animals infected with *S. aureus*
 - Quarters infected for 0, 12 & 72 hours
- ARK-Genomics Bov20K cDNA microarray
- Two colour microarrays
- Common reference design
 - Multifactorial analysis
 - Reference = Mixed pool of all RNA samples
 - Cy5 = common reference sample
 - 48 slides



Genes Exhibiting Differentiation Expression During *S. aureus* and *E. coli* Infection

(using the uninfected quarters from the 72hr *S. aureus* infections as a baseline)

Time (hours post infection)	<i>S. aureus</i>	<i>E. coli</i>
0 hours	164	359
6 hours	153	330
12 hours	181	537
24 hours	265	1189
72 hours	96	-

E. coli FDR<0.05
S. aureus P<0.05, >1.5 fold change

Top 10 Up-Regulated Genes 24 Hours Post *E. coli* Infection

- S100 calcium binding protein A12 (calgranulin C) 31.7X
- S100 calcium binding protein A9 (calgranulin B) 26.4X
- S100 calcium binding protein A8 (calgranulin A) 25.7X
- Chemokine (C-X-C motif) ligand 1 23.9X
- Serum/glucocorticoid regulated kinase 23.0X
- Superoxide dismutase 2 (SOD2) 20.9X
- Chemokine (C-X-C motif) ligand 2 19.8X
- CD244 molecule, natural killer cell receptor 2B4 18.2X
- Colony stimulating factor 3 (G-CSF) 16.7X
- Interleukin 8 14.3X

Ten 10 genes indicate neutrophil recruitment

Top KEGG Pathways Affected at 24 Hours Post *E. coli* Infection

	No. Genes	
	↑	↓
• Cytokine-cytokine receptor interaction	24	6
• MAPK signaling pathway	17	4
• Toll-like receptor signaling pathway	20	0
• Focal adhesion	9	10
• Pathogenic <i>Escherichia coli</i> infection - EHEC	18	0
• Pathogenic <i>Escherichia coli</i> infection - EPEC	18	0
• Cell adhesion molecules (CAMs)	9	8
• Hematopoietic cell lineage	11	5
• Regulation of actin cytoskeleton	13	3
• ECM-receptor interaction	5	10
• Leukocyte transendothelial migration	10	5
• Proteasome	14	0
• Apoptosis	12	2
• Jak-STAT signaling pathway	12	2

Kyoto encyclopaedia of genes & genomes (KEGG) www.genome.jp/kegg/pathway.html

Top KEGG Pathways Affected By *E. coli* Infection of Neighbouring Mammary Gland Quarters

	No. Genes	
	↑	↓
• MAPK signaling pathway	7	3
• Regulation of actin cytoskeleton	4	3
• Focal adhesion	1	4
• Glycan structures - biosynthesis 1	1	4
• Cysteine metabolism	4	0
• Cytokine-cytokine receptor interaction	3	1
• ECM-receptor interaction	0	4
• Purine metabolism	3	1
• Valine, leucine and isoleucine degradation	4	0

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Transcriptional Response Indicative of Pathogenic Agent

- 76 genes exhibit a similar transcriptome profile during *E. coli* & *S. aureus* infection.
- *S. aureus*
 - Lactotransferrin (LTF) ↑
 - Cathepsin G (CTSG) ↑
 - Acyl-CoA synthetase long-chain family member 6 (ACSL6) ↑
 - Leucine-rich repeat kinase 2 (LRRK2) ↓
 - EST (AJ814901) ↓
- *E. coli*
 - 102 genes affected at all 3 time points, but not during *S. aureus* infection
 - B-cell translocation gene 1, anti-proliferative (BTG1) ↑
 - cathepsin C (CTSC) ↑
 - FK506 binding protein 5 (FKBP5) ↑
 - ras homolog gene family, member F (RHOF) ↓



Conclusions

- The uninfected quarters respond to the infection of neighbouring quarters with *E. coli* and *S. aureus*.
- There is cross-talk between the udder quarters.
- The uninfected quarters from the 72 hour *S. aureus* infection are the samples most representative of an uninfected/unaffected control.
- A large number of genes are differentially expressed during *E. coli* infection.
- There is some overlap in the transcriptional response of the bovine mammary gland to *E. coli* and *S. aureus* infection.
- The transcriptional response of several proteins are indicative of the pathogenic agent.
- The statistical power of the *S. aureus* infection experiment is low, mainly due to variation between the biological replicates.
- Unfortunately, the transcriptional response observed at each time point will be the combined response to infection and the interaction with neighbouring quarters.



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