Response of Primary Mammary Epithelial Cells to Pathogen Challenge in Dairy Cows

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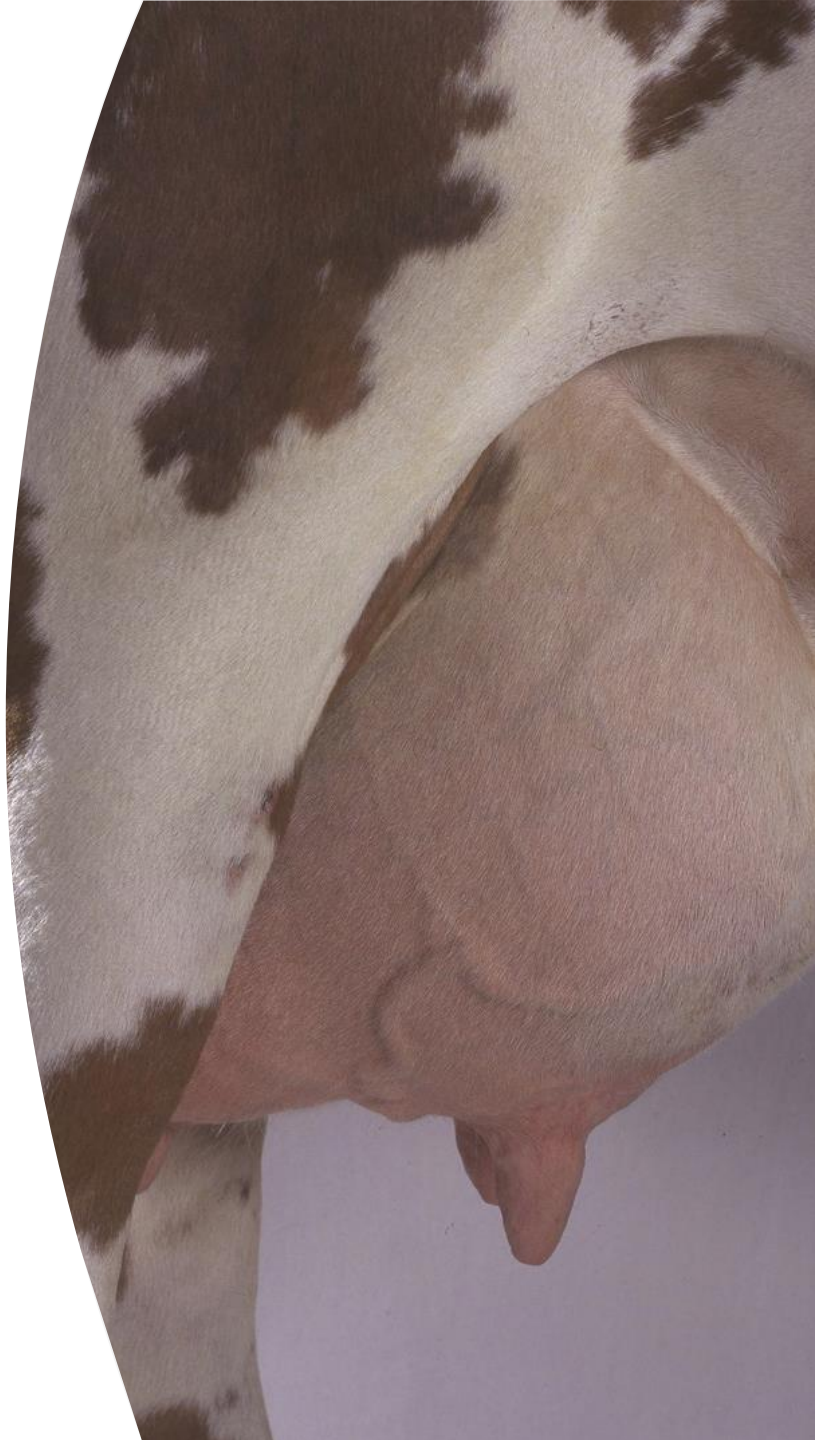
One Health and One Welfare in Dairy and Beef Cattle, ICPD, 9.7.2025

Background

Mastitis

- An inflammation of the mammary gland commonly caused by bacterial infection
- Economical and animal welfare issue
- most common cause for antibiotic use in dairy cattle
  - concern for antimicrobial resistance
- Has been studied intensively
- Better understanding of biological background still needed for improved breeding and/or management



Aim

To use an *in vitro* functional approach to identify genomic features underlying mastitis resistance.

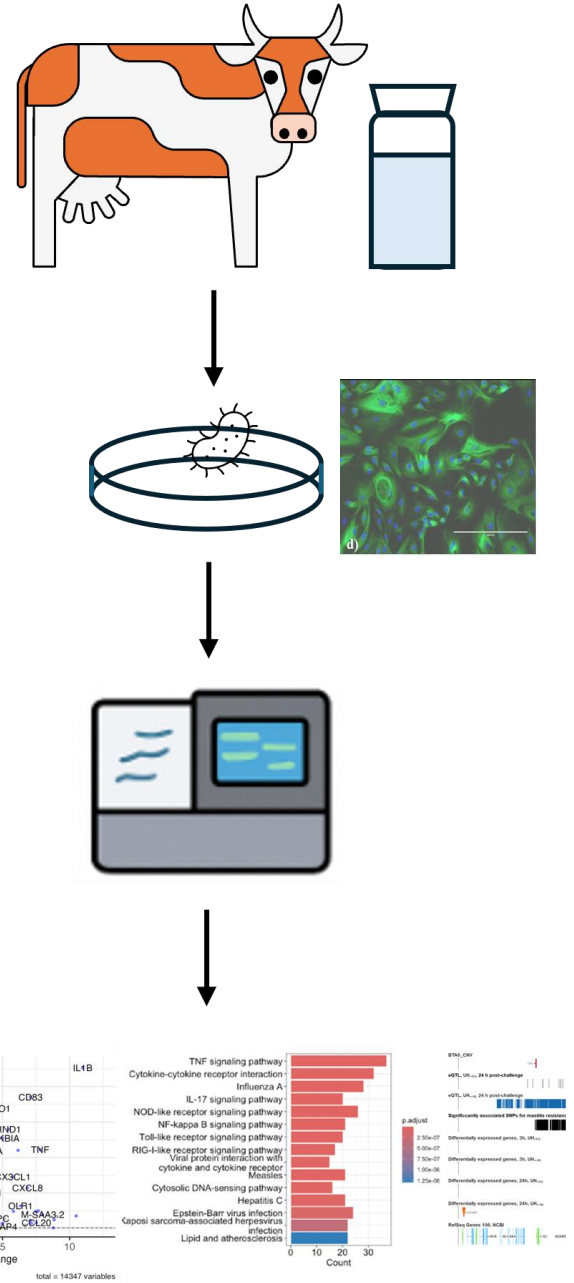
To achieve this, we chose dairy cows genetically divergent for udder health index and focused on **the host initial cellular response** differences to *Escherichia coli* in the mammary gland.

Methods

1. The milk samples were collected from whole genome sequenced donor Nordic Red cows with high (n=3) or low (n=3) genomic index value for udder health

2. primary bovine mammary epithelial cells (pbMECs) were extracted from milk and cultured (Iso-Touru *et al.* Vet Res. 2024

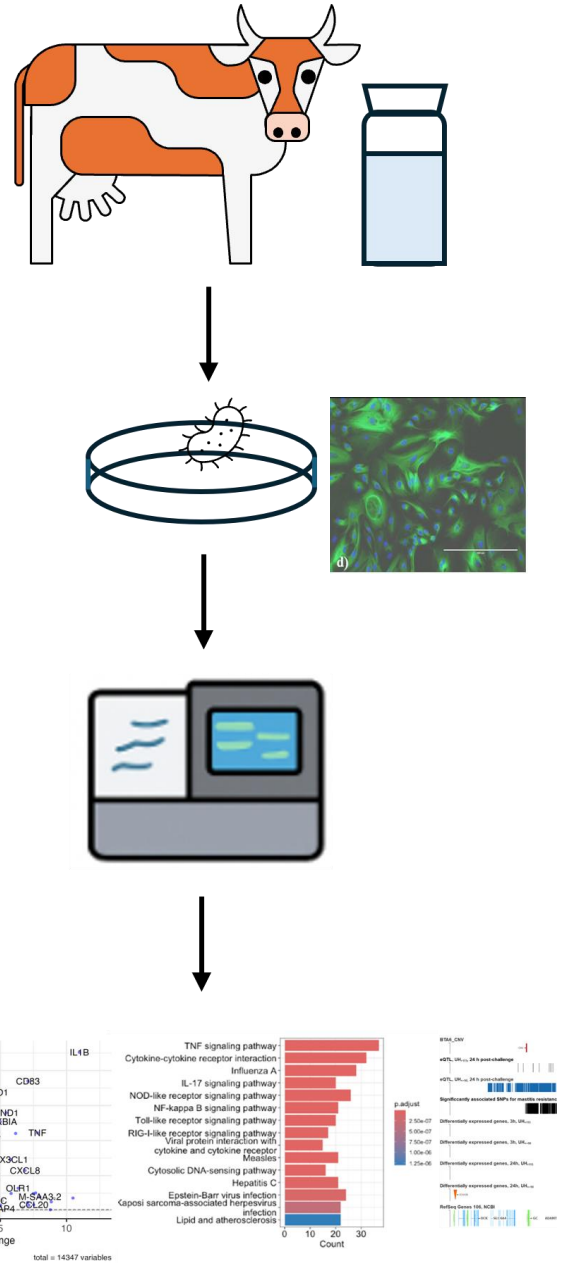
3. Cells were challenged with *E. coli* and harvested 3 h and 24 h after exposure
   - *E. coli* used was isolated from Nordic Red cow having mastitis



Methods

4. Total RNA was extracted and sequenced with 2 × 100 bp read length
   - Challenged and control cells

5. Transcriptomes were analyzed (DEGs, GO, KEGG, eQTL)
   - Differentially expressed genes (DEGs)
   - Gene ontology (GO) enrichment analysis
   - KEGG pathway enrichment analysis
   - Overlaps with GWAS results from the Nordic Red population (Cai *et al.* Genet Sel Evol. 2024)
   - eQTL analysis

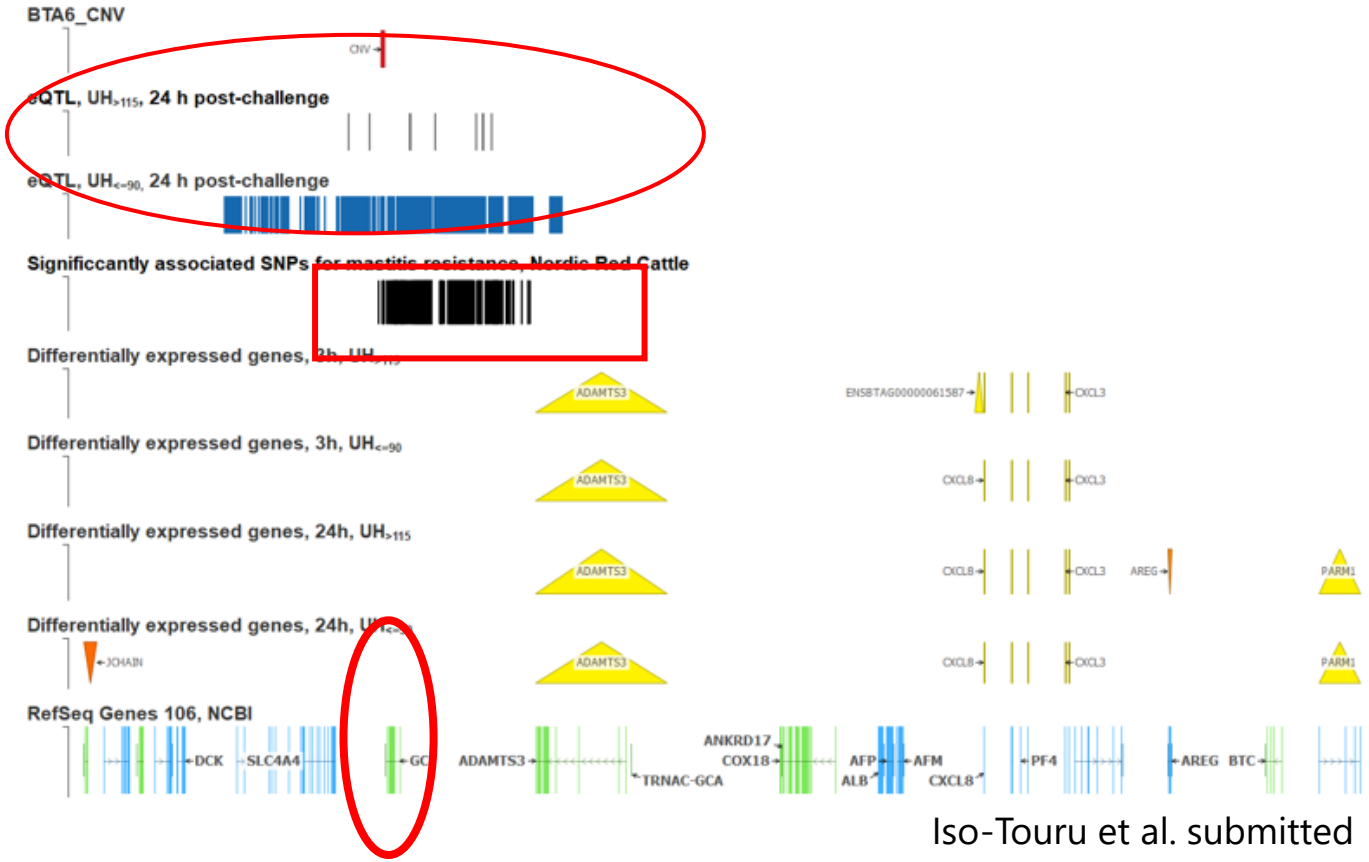


Results

- Markedly higher number of DEGs in the low mastitis resistance group after challenge compared to the high mastitis resistance group (3h: 414 vs 233 and 24h: 1192 vs 491)
- GO enrichment analyses of DEGs: adaptive immune response terms appeared enriched in the low mastitis resistance group later than in the high mastitis resistance group
- KEGG enrichment analyses: high mastitis resistance group shows earlier innate immune activity, while the low mastitis resistance group shifts toward adaptive immune responses later.

Results

- Genomic regions enriched with DEGs were found close to chromosomes (BTA6, BTA9) known to have mastitis QTL
- BTA6 region (88-89Mb) was both enriched with DEGs but also had eQTL



Iso-Touru et al. submitted

Conclusions

- Based on gene enrichment analyses, the low mastitis resistance group showed slightly **delayed adaptive immune response** and **early metabolic changes**.
  - Indicates possible **underlying genetic factors** influencing transcriptomic reactivity
- Key immune-related genes (e.g., **LTF, CXCL8, TLR2, BOLA-DRB3**) responded in both groups, but **without group-specific differences** at early infection stage
- No overlap between DEGs or cis-eQTLs and **GWAS-identified SNPs**, suggesting early epithelial immune response genes may not drive phenotypic mastitis resistance

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Funding

Research Council of Finland

HoloRuminant
Understanding microbiomes of the ruminant holobiont

The HoloRuminant project has received funding from European Union's Horizon 2020 research and innovation program under Grant Agreement No 101000213.

This publication reflects the views only of the author, and not the European Commission (EC). The EC is not liable for any use that may be made of them information contained herein.

Thank you!

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