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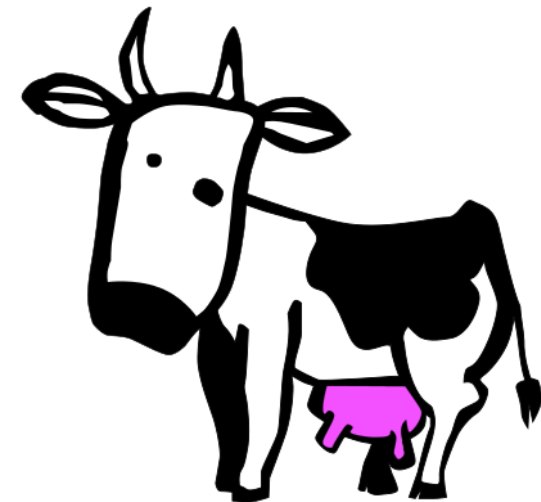


# Haptoglobin: A biomarker for selective dry cow therapy

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## Study Aims

### Dry cows

- Routinely treated with long acting infusions of antimicrobials to rid the udder of bacteria that could cause mastitis and to treat any SM present.
- In the UK, blanket use of antimicrobials is extremely high: 99% of dairy cows receiving antimicrobials at this point\*
- AMR concerns

### Haptoglobin

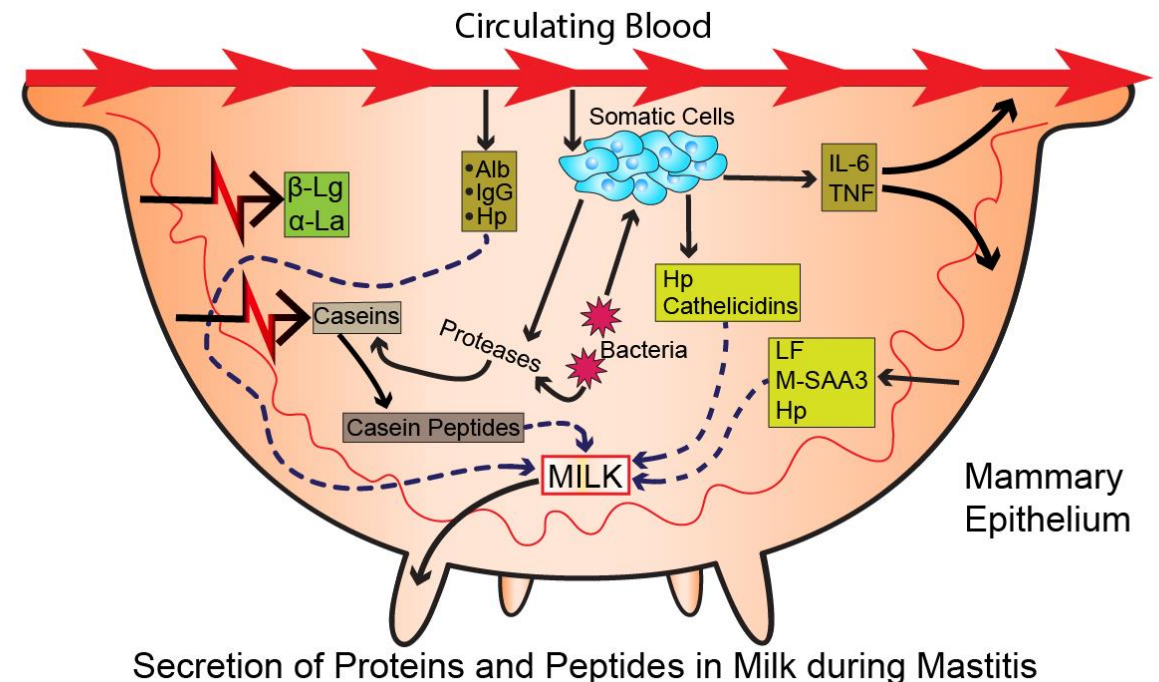
- Suitable biomarker for selective dry cow therapy?
- Compare to SCC & bacteriology

# Acute phase proteins

- Synthesised and released from the liver during inflammatory (acute-phase) response
- Measured in the serum in human and veterinary medicine and in various veterinary research areas

## Extra Hepatic acute phase proteins

- Increasing body of evidence that detail extra-hepatic synthesis (adipose tissue, lung...)
- Mammary acute phase synthesis well studied owing to presence of APPs in milk



# Milk acute phase proteins

- Haptoglobin
- Mammary associated Serum amyloid A
- C-reactive protein

## An Automated Biochemical Assay for Haptoglobin: Prevention of Interference from Albumin

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<sup>4</sup>Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK;

<sup>5</sup>Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland

J. Dairy Sci. 89:1488–1501

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## Acute Phase Proteins in Bovine Milk in an Experimental Model of *Staphylococcus aureus* Subclinical Mastitis

P. D. Eckersall,<sup>\*</sup> F. J. Young,<sup>\*</sup> A. M. Nolan,<sup>†</sup> C. H. Knight,<sup>‡</sup> C. McComb,<sup>\*</sup> M. M. Waterston,<sup>\*</sup> C. J. Hogarth,<sup>\*</sup> E. M. Scott,<sup>§</sup> and J. L. Fitzpatrick<sup>#</sup>

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Thomas et al. BMC Veterinary Research (2015) 11:207  
DOI 10.1186/s12917-015-0533-3



RESEARCH ARTICLE

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## The major acute phase proteins of bovine milk in a commercial dairy herd

Funmilola Clara Thomas<sup>1</sup>, Mary Waterston<sup>2</sup>, Peter Hastie<sup>3</sup>, Timothy Parkin<sup>3</sup>, Hayley Haining<sup>3</sup> and Peter David Eckersall<sup>1\*</sup>

## Haptoglobin and serum amyloid A in milk and serum during acute and chronic experimentally induced *Staphylococcus aureus* mastitis

Ulrika Grönlund<sup>1</sup>, Cecilia Hultén<sup>2</sup>, Peter D. Eckersall<sup>3</sup>, Caroline Hogarth<sup>3</sup> and Karin Persson Waller<sup>4\*</sup>

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Received 1 October 2002 and accepted for publication 19 December 2002

## Acute phase proteins in serum and milk from dairy cows with clinical mastitis

P. D. Eckersall, F. J. Young, C. McComb, C. J. Hogarth, S. Safi, A. Weber, T. McDonald, A. M. Nolan, J. L. Fitzpatrick

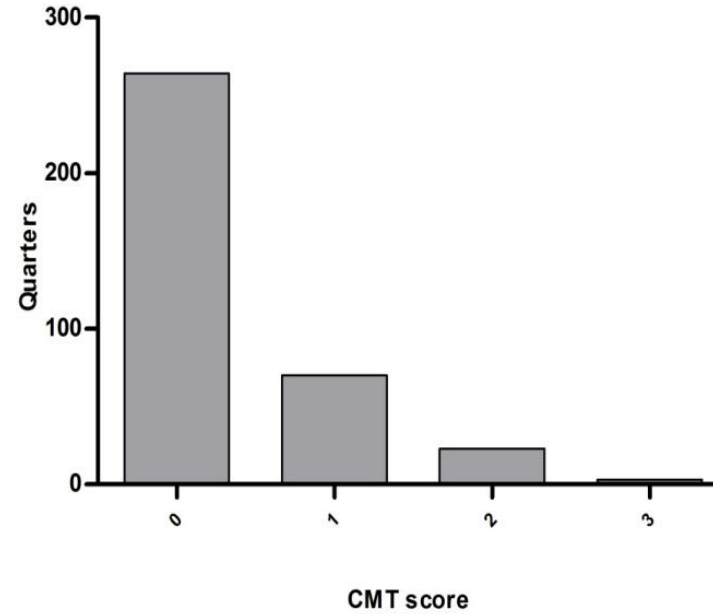
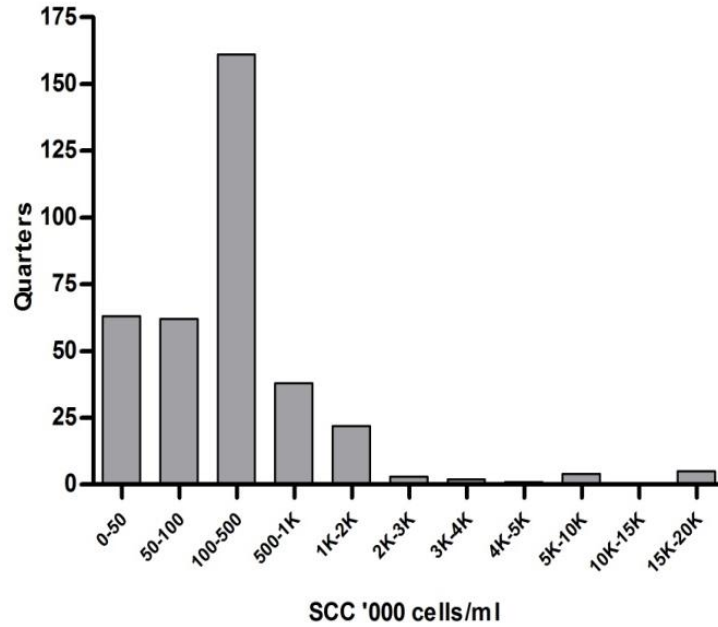
## Study design

- Over 11 weeks **409** quarter milk samples were obtained at dry off from **104** cows.
- August 2016 – November 2017
- 409 quarter milk samples collected from 104 cows during drying off

The following were determined on all *quarter* milk samples:

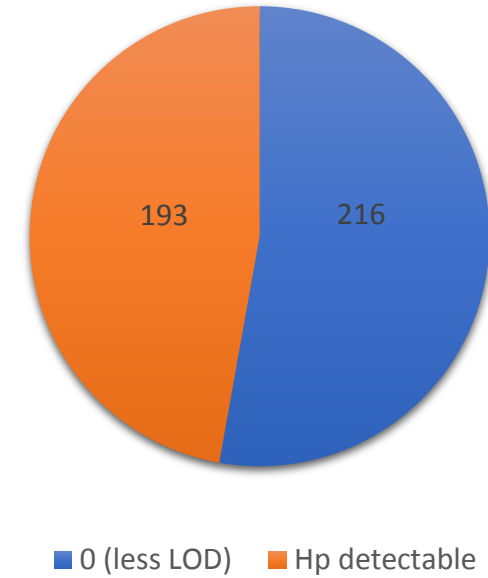
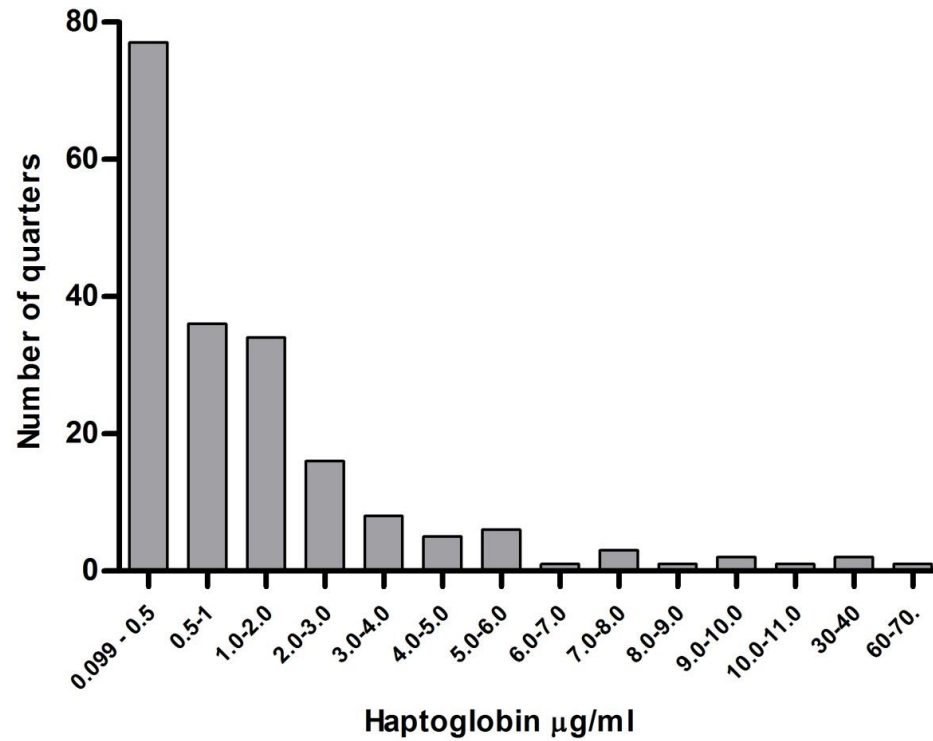
- California mastitis test (CMT)
- Bacteriology
- Somatic cell counts (SCC)
- Haptoglobin (Hp) (ELISA\*)

## Results: SCC & CMT



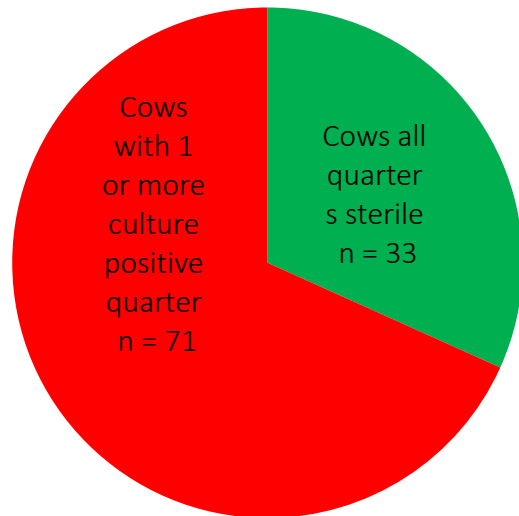


## Results: Haptoglobin

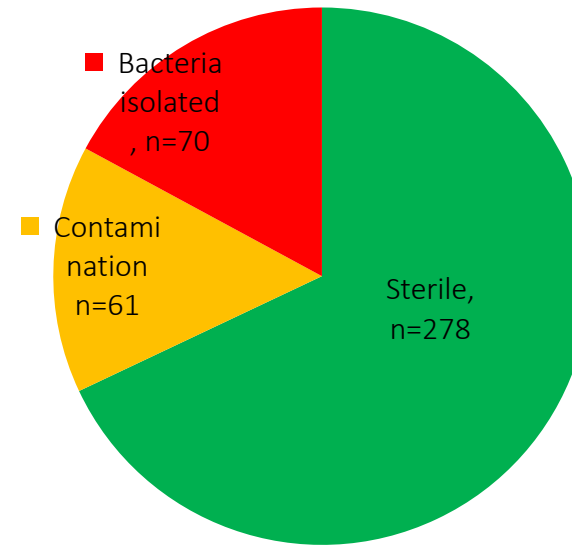


## Results: Bacteriology

At cow level



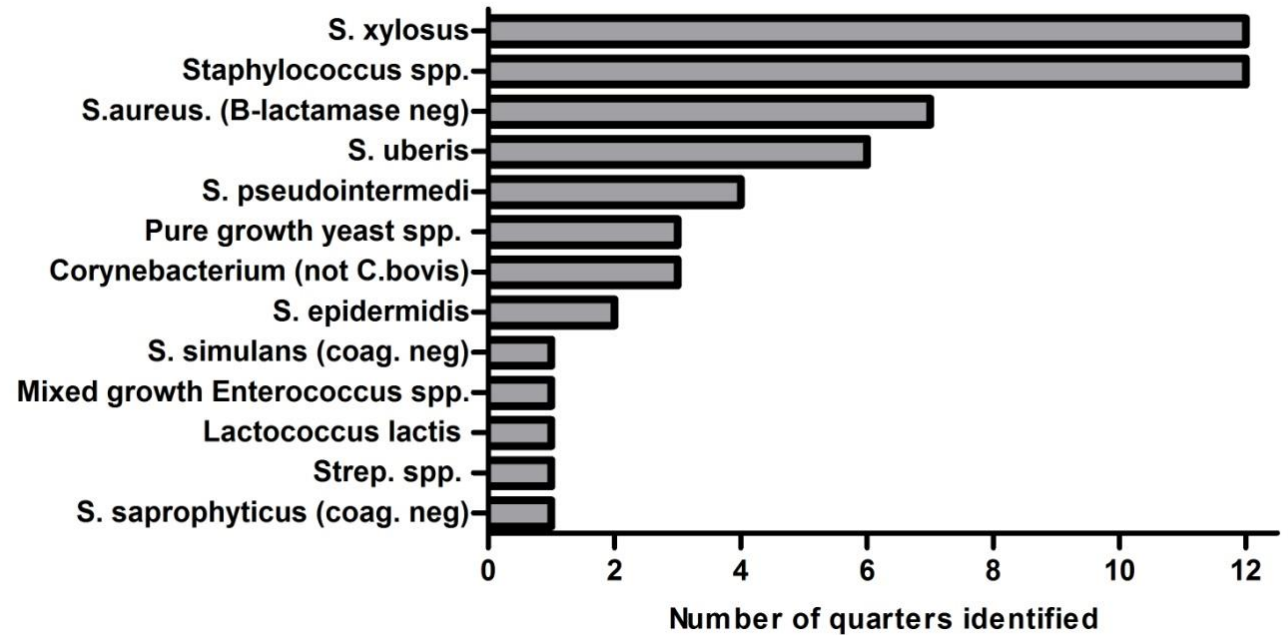
At quarter level





# Bacteriology

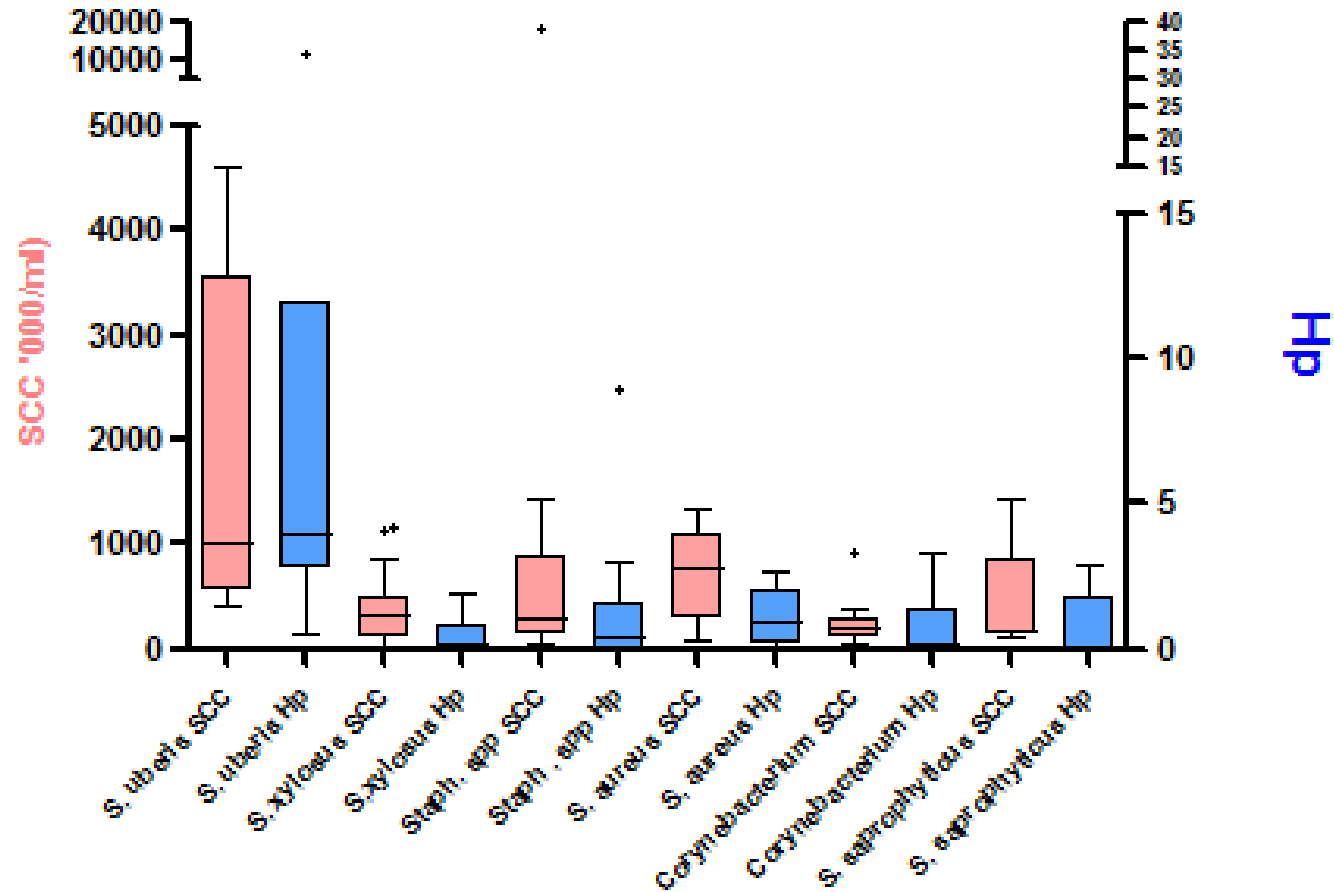
- Bacteria was cultured from 107 quarters
- From 53 quarters the following species were identified:



*Significant associations between bacteria cultured (n=53) and Hp, SCC, CMT (P=<0.0001)*



# Bacteriology



## Discussion

Haptoglobin better biomarker for active inflammation and infection

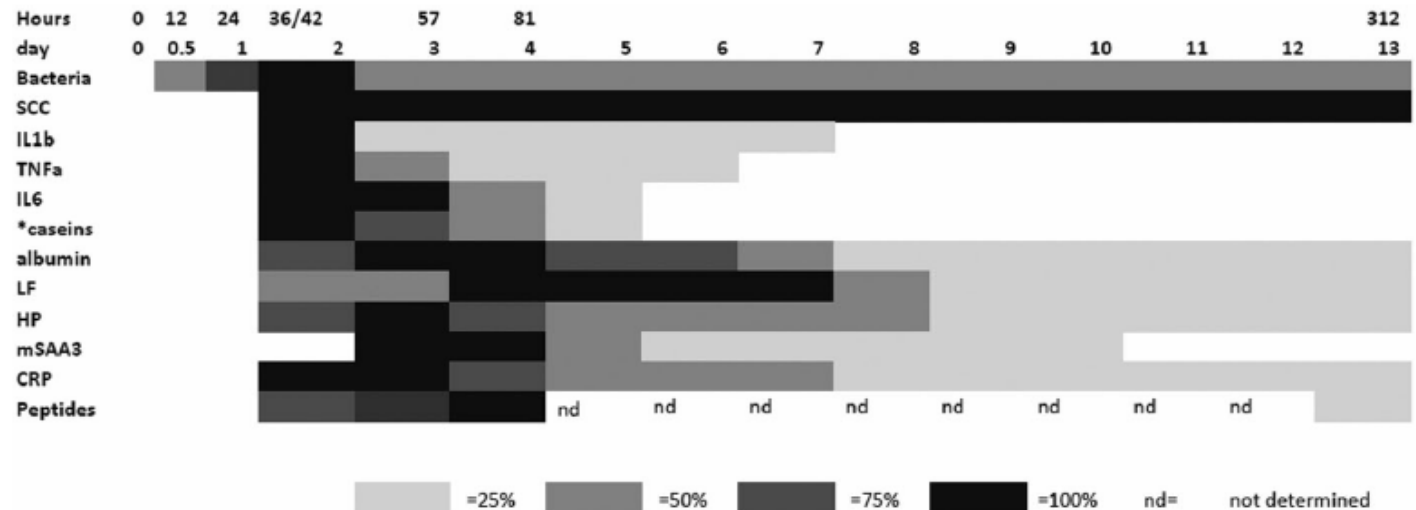
Sensitivity and Specificity for Hp, SCC and CMT with bacteriology as the 'gold standard' Higher sensitivity compared to SCC & CMT

	SCC	CMT	Hp
Sensitivity	0.69	0.46	0.76
Specificity	0.61	0.76	0.58

Other factors can effect levels of SCC in milk

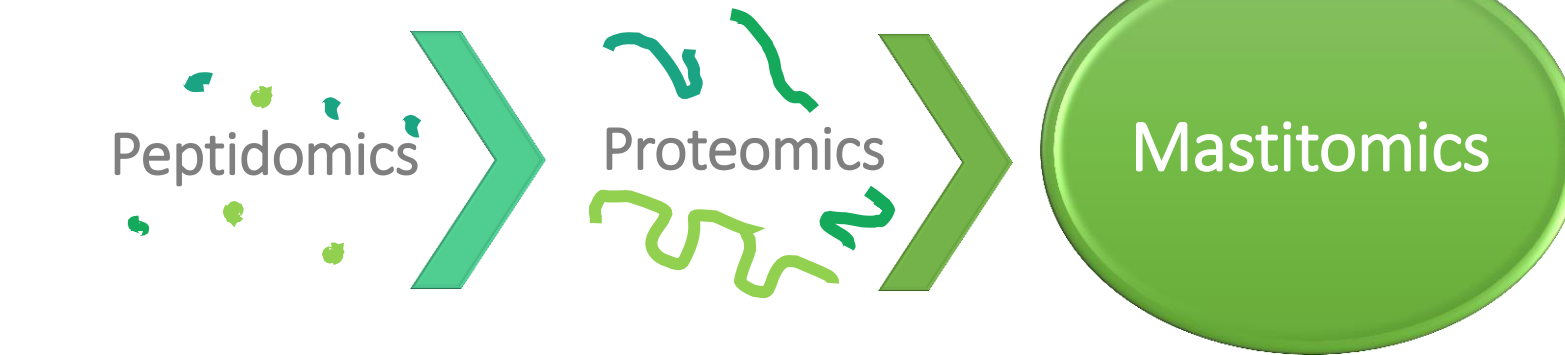
California mastitis test (CMT) → Subjective

Bacteriology → Expensive / preparation issues / contaminants



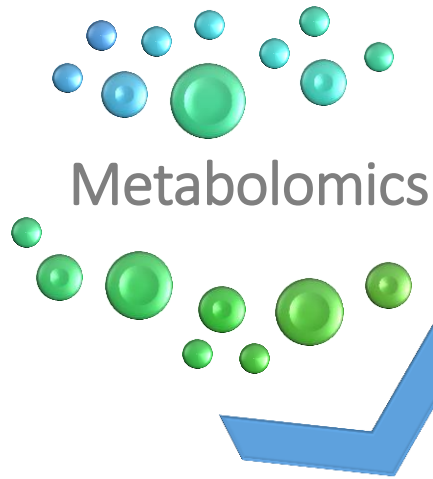
**Mastitomics, the integrated omics of bovine milk in an experimental model of *Streptococcus uberis* mastitis: 1. High abundance proteins, acute phase proteins and peptidomics†**

Funmilola Clara Thomas,<sup>a</sup> William Mullen,<sup>b</sup> Riccardo Tassi,<sup>c</sup> Adela Ramirez-Torres,<sup>d</sup> Manikhandan Mudaliar,<sup>ae</sup> Tom N. McNeilly,<sup>c</sup> Ruth N. Zadoks,<sup>ac</sup> Richard Burchmore<sup>e</sup> and P. David Eckersall<sup>\*a</sup>



**Mastitomics, the integrated omics of bovine milk in an experimental model of *Streptococcus uberis* mastitis: 2. Label-free relative quantitative proteomics†**

Manikhandan Mudaliar,<sup>ab</sup> Riccardo Tassi,<sup>c</sup> Funmilola C. Thomas,<sup>a</sup> Tom N. McNeilly,<sup>c</sup> Stefan K. Weidt,<sup>b</sup> Mark McLaughlin,<sup>d</sup> David Wilson,<sup>b</sup> Richard Burchmore,<sup>be</sup> Pawel Herzyk,<sup>bf</sup> P. David Eckersall<sup>a</sup> and Ruth N. Zadoks<sup>\*ac</sup>



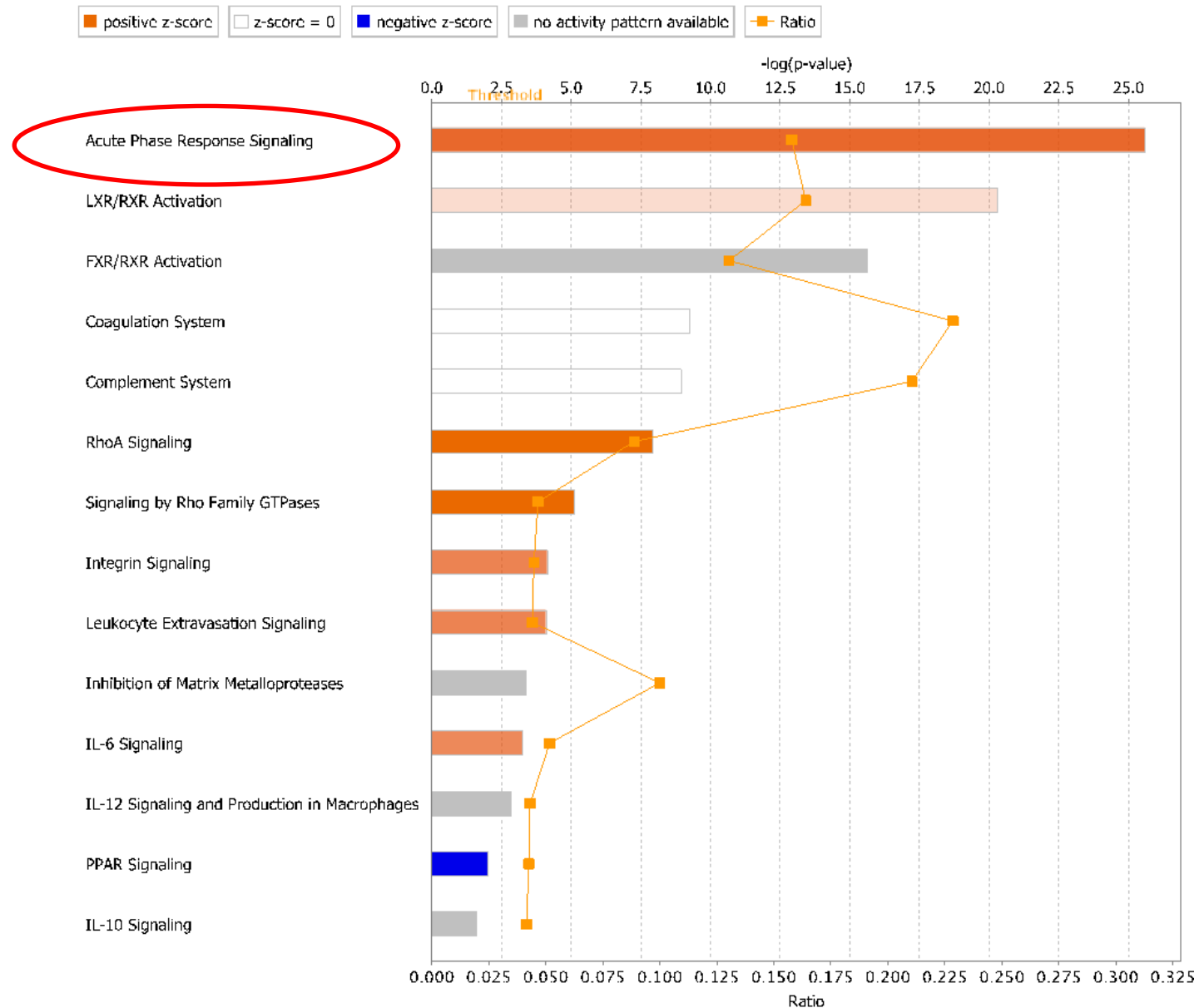
**Mastitomics, the integrated omics of bovine milk in an experimental model of *Streptococcus uberis* mastitis: 3. Untargeted metabolomics†**

Funmilola Clara Thomas,<sup>‡a</sup> Manikhandan Mudaliar,<sup>‡ab</sup> Riccardo Tassi,<sup>c</sup> Tom N. McNeilly,<sup>c</sup> Richard Burchmore,<sup>bd</sup> Karl Burgess,<sup>bd</sup> Pawel Herzyk,<sup>be</sup> Ruth N. Zadoks<sup>ac</sup> and P. David Eckersall<sup>\*a</sup>

- Panel of biomarkers
- Confirmed haptoglobin
- Highlighted novel biomarkers
- Systems biological approach to identifying biomarkers

# Ingenuity Pathway Analysis

- 57 hours post challenge
- Highly significant proteins identified using repeated measures ANOVA
- Ingenuity Pathway Analysis (IPA)
- Increases in
- Acute phase pathways
- LXR= liver receptor pathways
- RXR= retinoic acid receptor pathways
- FXR = farenoid (bile acid) receptor pathway



## Summary

- Haptoglobin suitable biomarker of active infection
- Haptoglobin – measure routinely as part of both bovine serum and milk profiles
- Greater impetus on exploring biomarkers beyond SCC
- Increasing evidence from mastitomics that acute phase pathways the most activated during infection
- Haptoglobin is useful biomarker for directing antimicrobial therapies at dry off

## Acknowledgements

Staff at Meldrum Dairy Farm, Stirling.

Life Diagnostics



BBSRC Follow on fund (BB/M022021/1)

