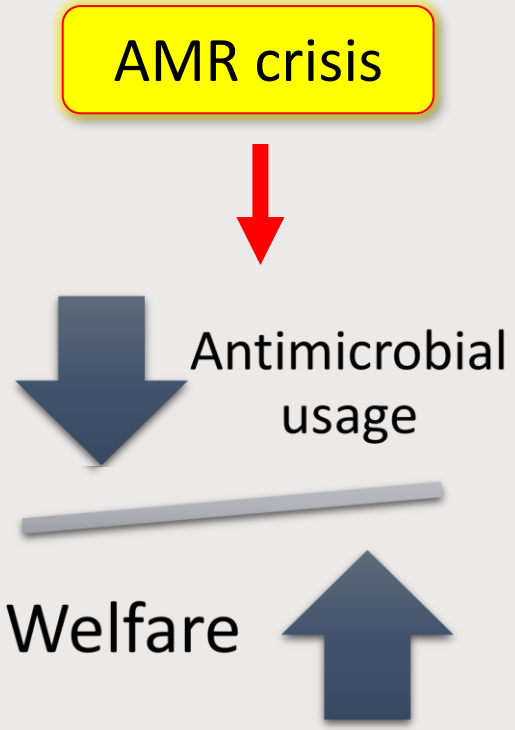
Biomarkers for mastitis diagnostics

- Exploring acute phase proteins (APPs) as biomarkers for selective dry cow therapy
- Biomarkers for differentiating pathogens and directing antimicrobial therapies for clinical mastitis
- What's needed to bridge the gap between what diagnostics are required and what's available?

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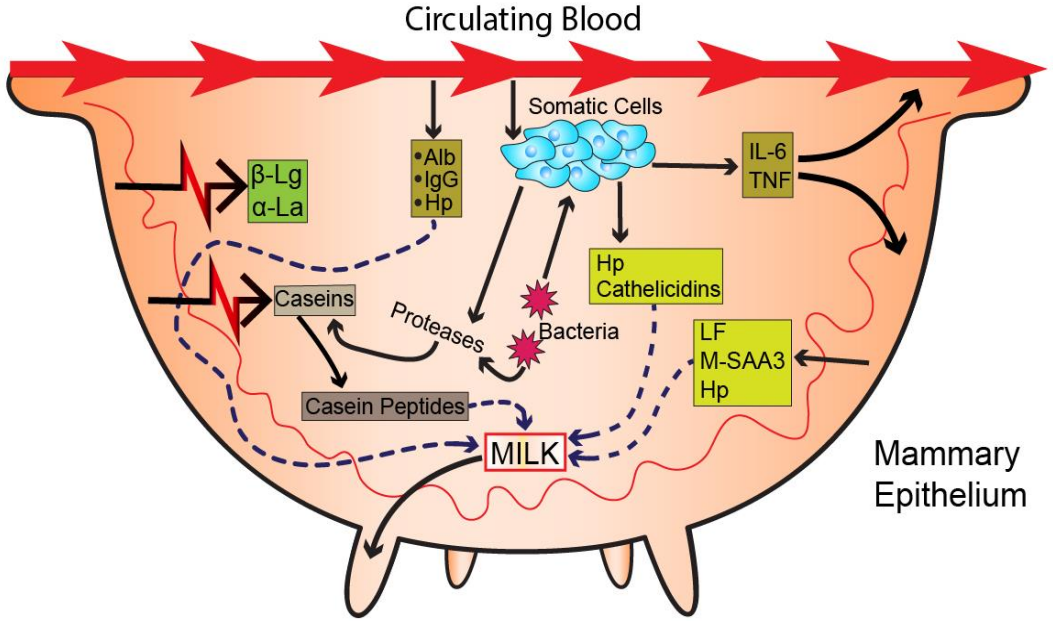
Focus on endogenous biomarkers for mastitis



Targeted approach

1. Focus on known biomarkers for mastitis – Acute phase proteins (APPs) for **selective dry cow therapy**
2. (a) Focus on known biomarkers for mastitis – Acute phase proteins (APPs) for **distinguishing pathogens in clinical mastitis**

Bottom up proteomic approach

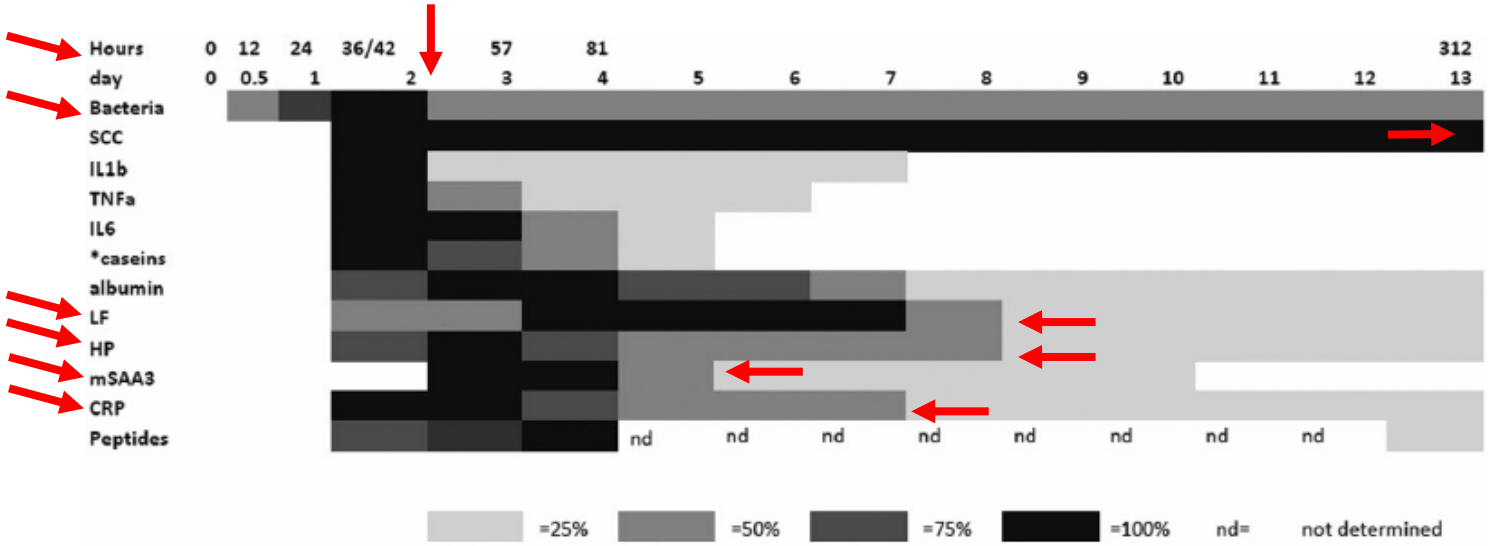
2. (b) Look at *all* differentially abundant proteins between mastitic groups by pathogen to identify biomarker targets for **distinguishing pathogens in clinical mastitis**

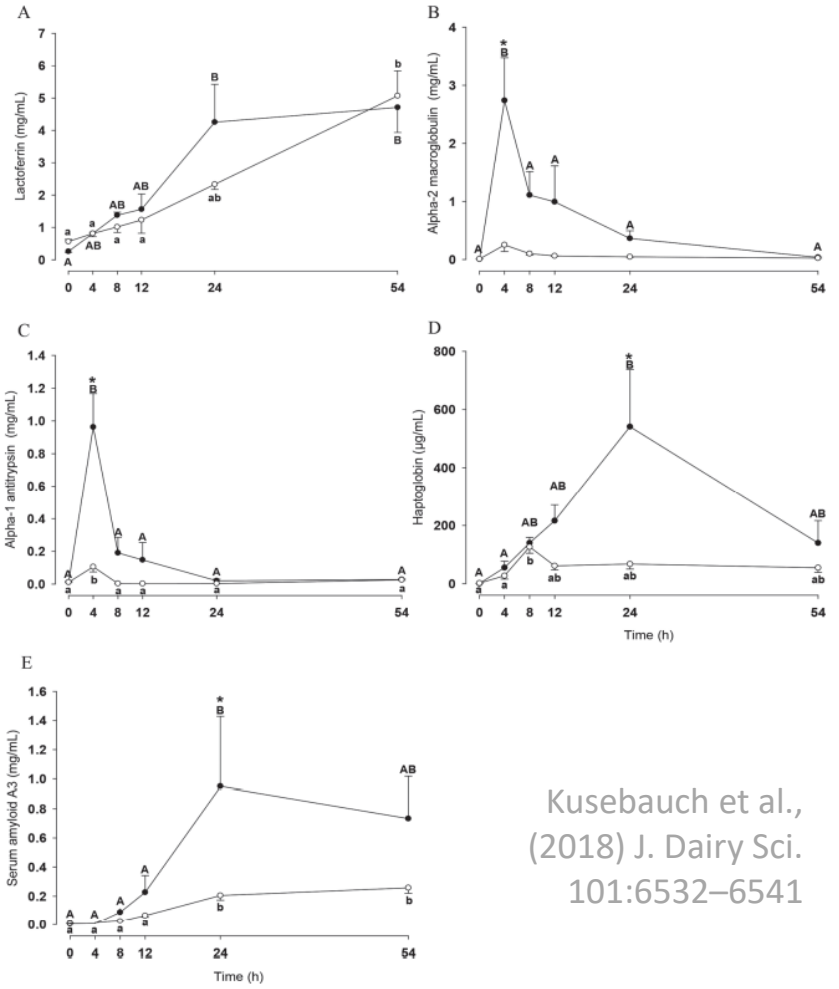
Challenges with bacterial culture
- *Turn-around time*
- *Contamination*
- *Skills*
- *Cost*

Viora et al., (2014) *The Veterinary Record*, *175*: 89.

Acute phase proteins

- Experimental studies comparing LPS and PGN (Kusebauch et al., 2018)
- APP concentrations compared with bacteria in clinical mastitis:
  - Pyörälä et al., (2011), Kalmus et al., (2013), Jaeger et al., (2017)
- Mastitomics series (2016): Thomas, *et a*l., 2016 / Mudaliar, *et al*., 2016.





Kusebauch et al., (2018) J. Dairy Sci. 101:6532–6541

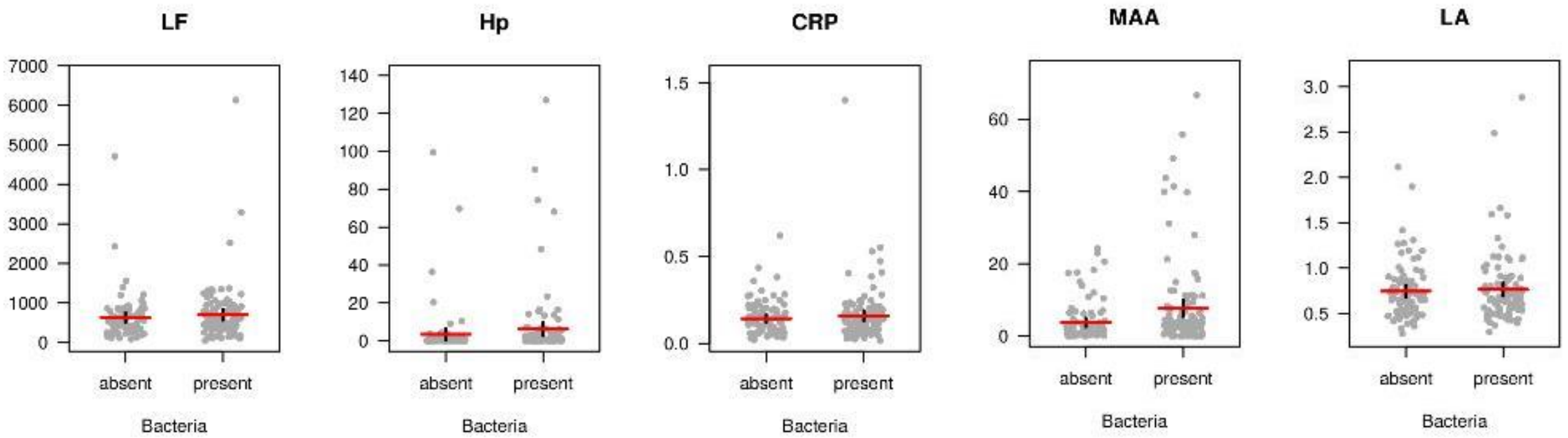
1. Acute phase proteins: Biomarkers for selective dry cow therapy?

Pilot study & large scale targeted study

- Compare APPs with SCC & bacteriology in cows at dry off
- Arrive on farm on day of dry off
- Target cows using CMT and match controls (within cowQ + CMT0 cows)
- 209 targeted quarter milk samples
- Measure APPs (Life Diagnostics Sparcl Immunoassay & ELISA)
- SCC (milk recording)
- Bacteriology: culture + MALDI ToF
- Classification Tree Model (cross-validated)
- Biomarker classification compared to rest utilising McNemar test / bacteriology as gold standard

Haptoglobin (Hp)
α-lactalbumin (LA)
Lactoferrin (LF)
C-reactive protein (CRP)
Mammary Amyloid A (MAA)

Distributions of the 5 biomarkers

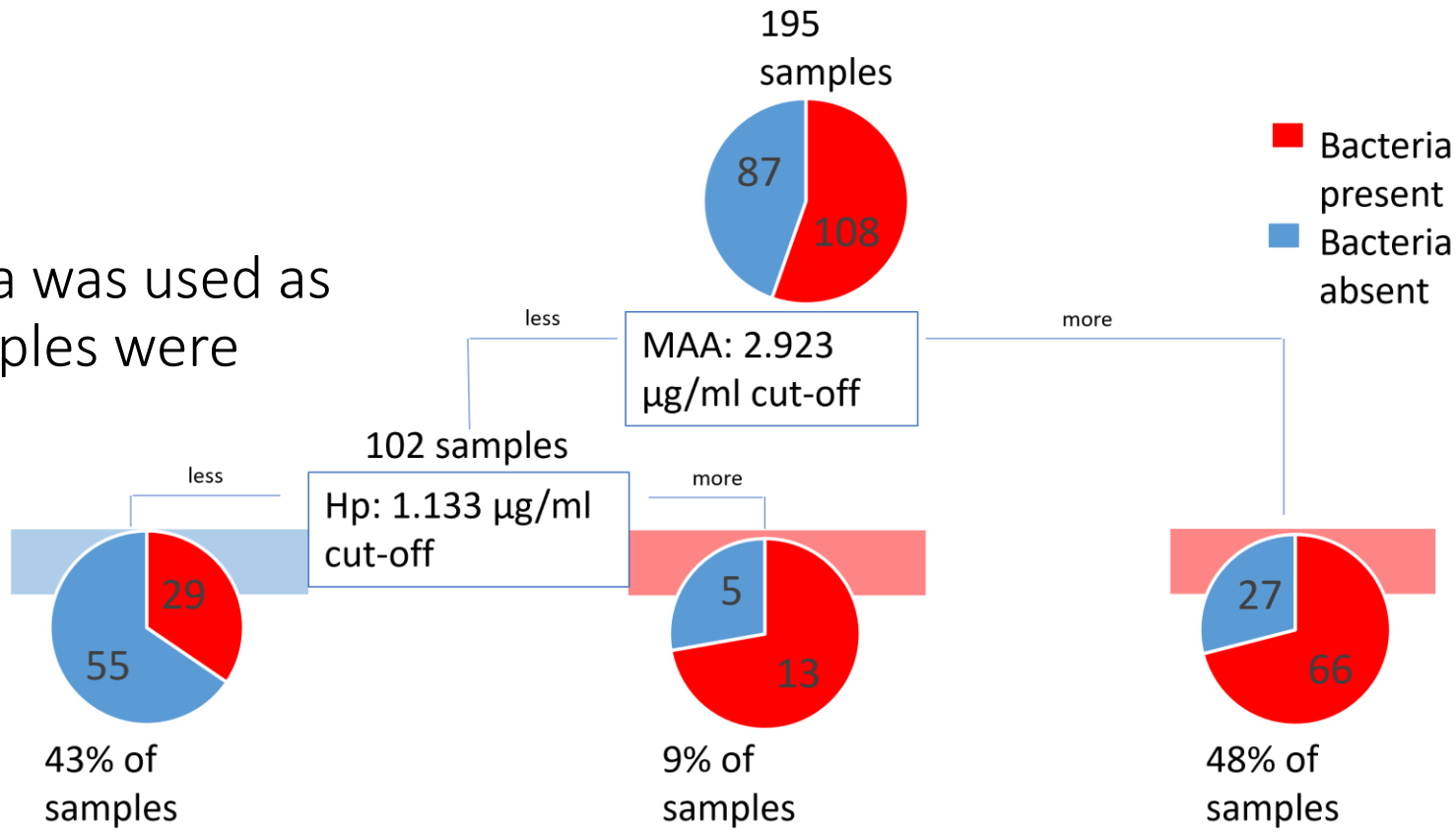


Haptoglobin (Hp)
Mammary associated Serum amyloid A (MAA)
C-reactive protein (CRP)
α-lactoglobin
Lactotransferrin (LF)

Classification Tree Model (cross-validated)

- 5 biomarkers (Hp, LA, LF, CRP, MAA) from the 195 samples were combined using a Classification Tree Model
  - 10-fold Cross Validation (MAA, Hp)

- Bacteriology (presence of bacteria was used as gold standard; contaminated samples were excluded)



Diagnostic performance (195 samples)

	Se	Sp	Accuracy	PPV	NPV
SCC over 199	**79%** (70.1; 85.4)	39% (29.5; 49.6)	**61%** (54.0; 67.6)	**62%** (53.3; 69.3)	**60%** (46.7; 71.4)
CMT over 0	**90%** (82.7; 94.2)	25% (17.3; 35.3)	**61%** (54.0; 67.6)	**60%** (52.2; 67.1)	**67%** (49.6; 80.2)
Biomarker tree (MAA + Hp)	73% (64.1; 80.6)	**63%** (52.7; 72.6)	**69%** (61.9; 74.8)	**71%** (62.1; 78.8)	**65%** (54.8; 74.8)

Biomarker decision tree diagnostic performance:

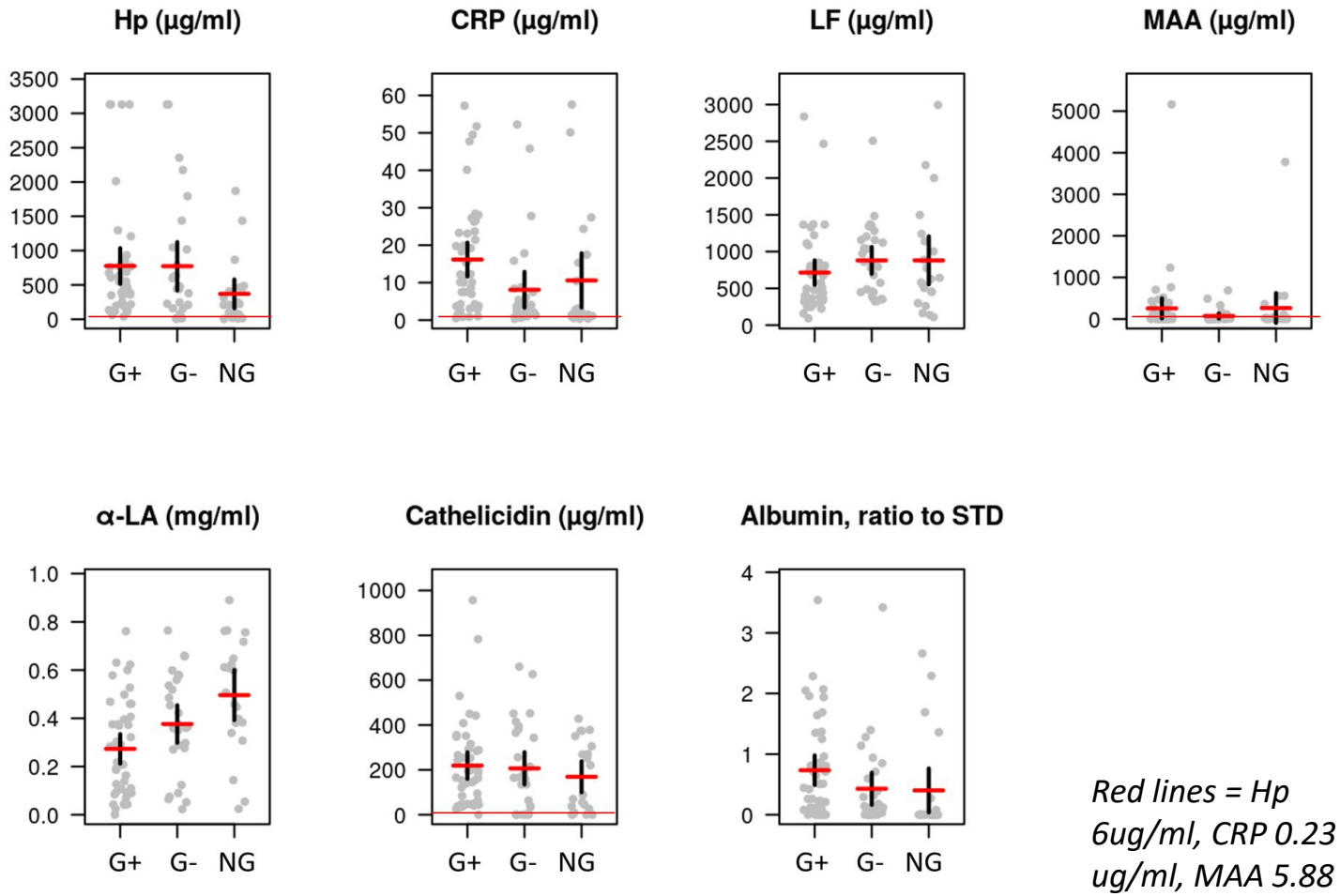
- Specificity higher: fewer animals treated unnecessarily (AM reduction)
- Sensitivity lower: possible welfare concern

Bacteriology (presence of bacteria was used as gold standard)

2 (a) Biomarkers for **clinical mastitis**: Can APPs be used to differentiate pathogenic cause?

Compare clinically mastitis samples:

- Clinically diagnosed mastitis with associated severity score (1-3)
- Measured the aforementioned APPs plus Alb ratio and cathelicidin
  - Albumin: the overlooked APP, currently doing a lot of work on milk Alb
  - Cathelicidin: underused APP despite significant potential (Addis et al., 2016)
- Compare G+, G- & no growth (NG)



Maximum biomarker concentration in dry cow samples with SCC<200x10^3 cells/ml (n=20)

Red lines = Hp 6ug/ml, CRP 0.23 ug/ml, MAA 5.88 ug/ml Cathelicidin 2.11 ug/ml

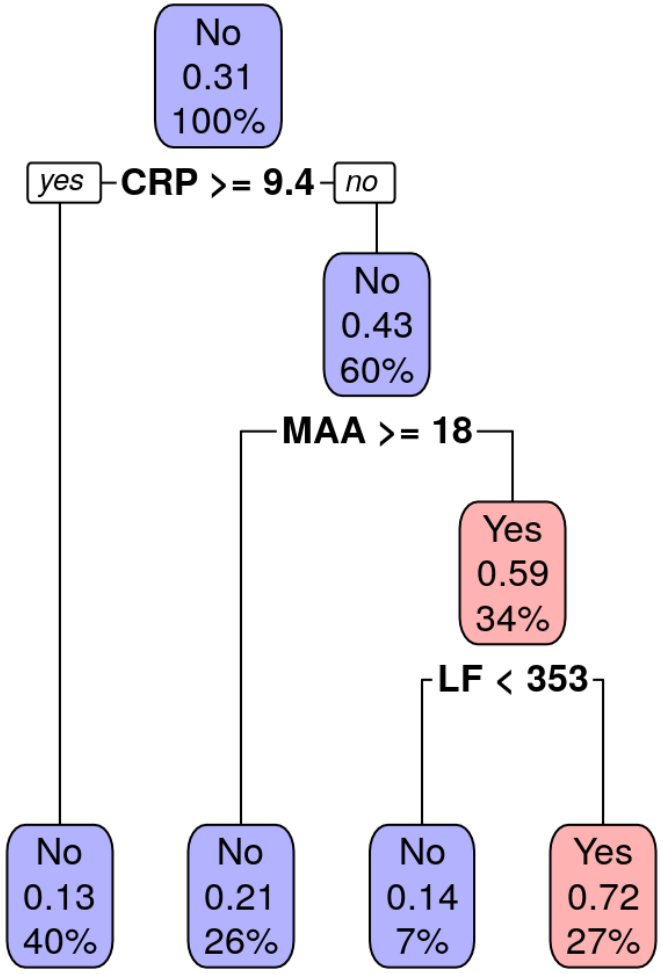
* redness, tenderness, swelling +/- clots in milk

2 (a) Biomarkers for **clinical mastitis***:
Can APPs be used to differentiate pathogenic cause?

Compare Gram positive with G-/No growth:

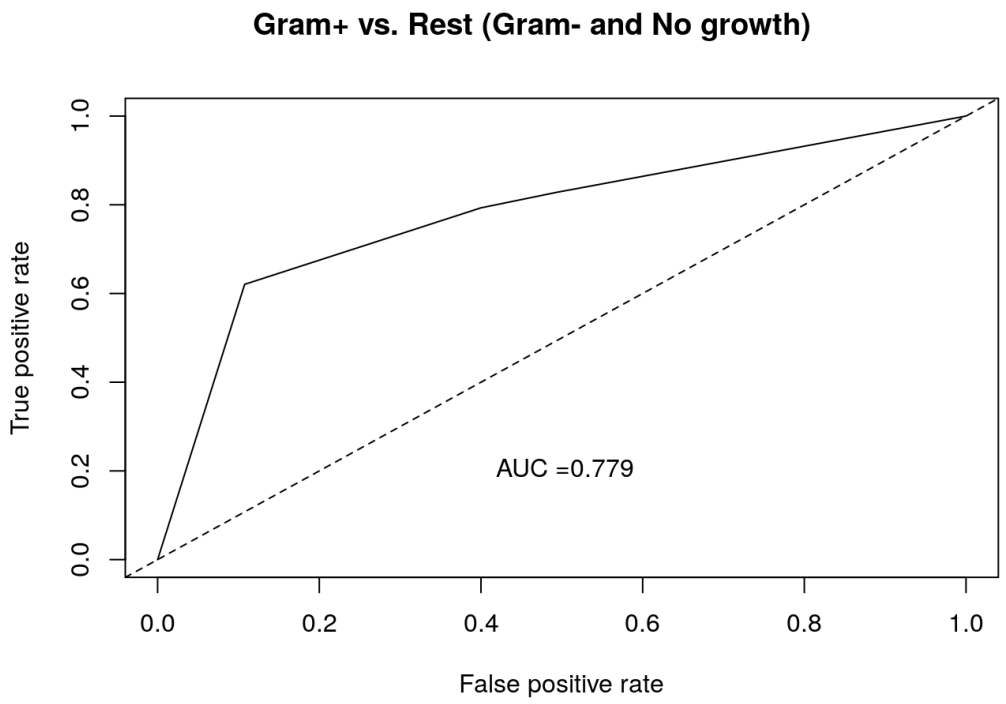
- G+ associated with a combination of:
  - low CRP (< 9.4)
  - low MAA (< 18)
  - high LF (>= 353)

- 62% of the G+ samples had this combination of biomarkers, compared to 11% of the other samples (G- and No growth).

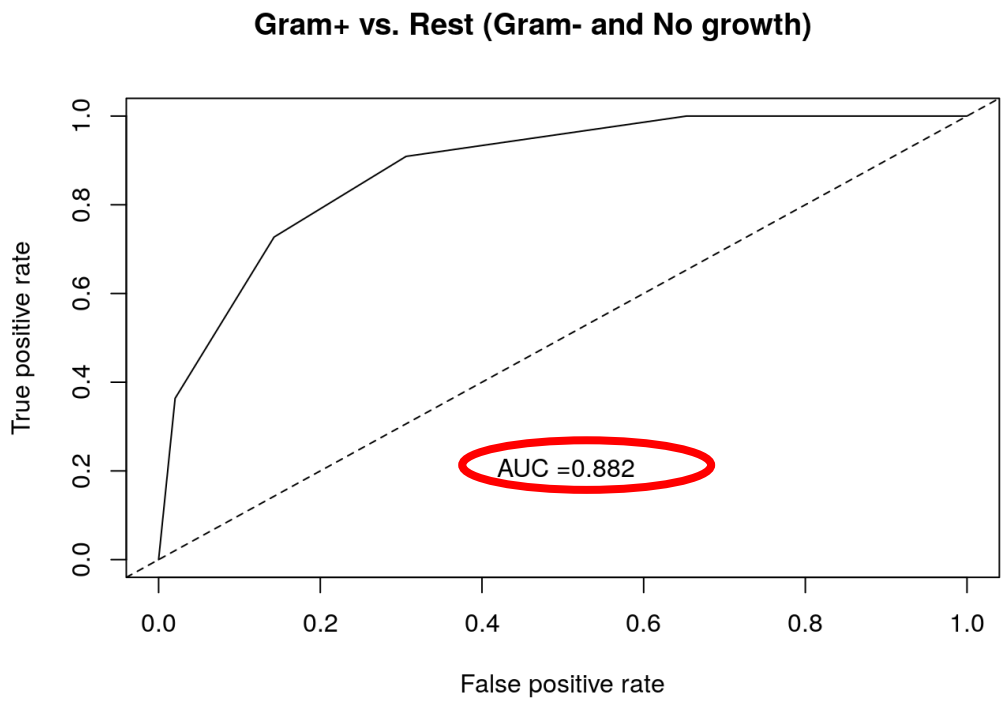


2 (a) Biomarkers for **clinical mastitis**:
Can APPs be used to differentiate pathogenic cause?

All clinical mastitis cases



Severity 1 & 2 clinical mastitis cases only

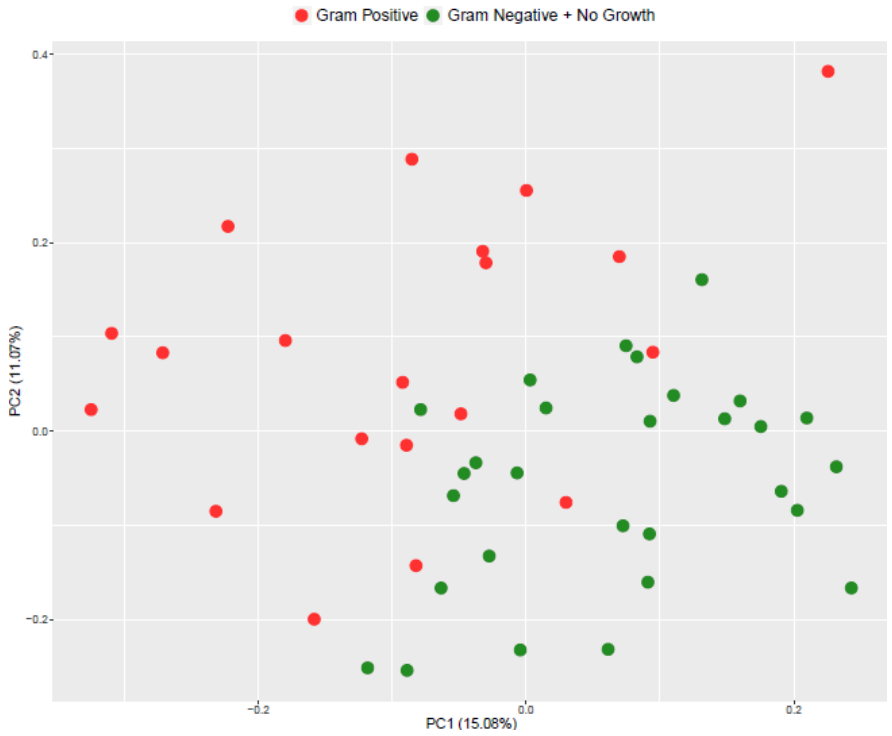


2 (a) Biomarkers for **clinical mastitis**:

A bottom up approach for differentiating pathogenic cause

Proteomic approach

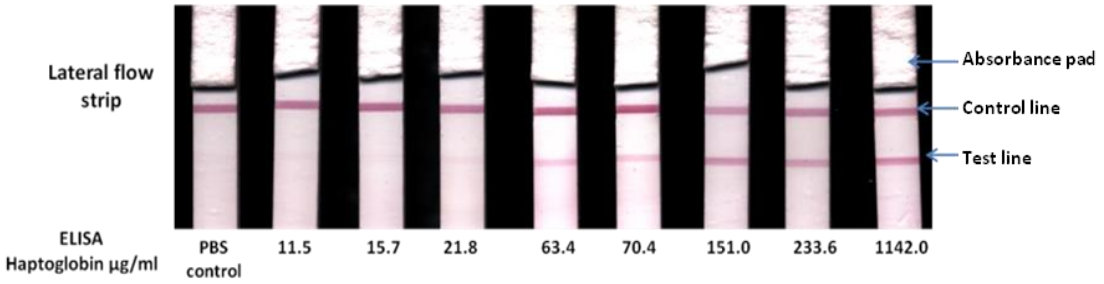
- Identify all differentially abundant proteins between groups



End point?

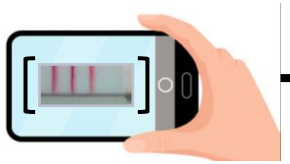
Lateral flow

- Fulfils the ASSURED criteria (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end users)

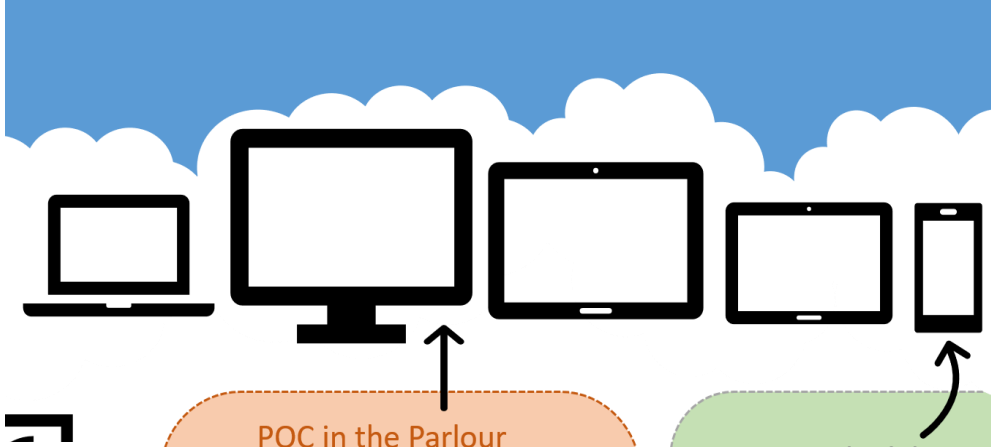


- Multiplex

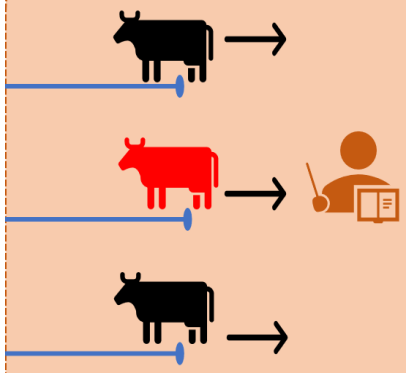
- Digital interface
  - Quantifiable result
  - Collective data



Treat / Don't treat



POC in the Parlour



What we have:
- Individual cows assessed by herds personal prior to milking
- Samples can be taken for on-farm culture
- Reliant on trained and motivated staff, thus prone to human error.

What we need:
- Individual cows sampled in parlour and results delivered within a timeframe that enables immediate, selective and targeted treatment

POC in the lab

Vet practice Lab

Farm Lab

What we have:
- Samples are processed in labs that are within the proximity to the animal.
- Able to differentiate pathogens

What we need:
- Improved ability to differentiate and target treatment in a more timely way.

Collective Data

What we have:
- Limited utilisation of collective data

What we need:
- Ability to synchronise, utilise and collate on farm production data and diagnostic data to improve diagnostic decision making.
- Utilise diagnostic data to direct AMR policies

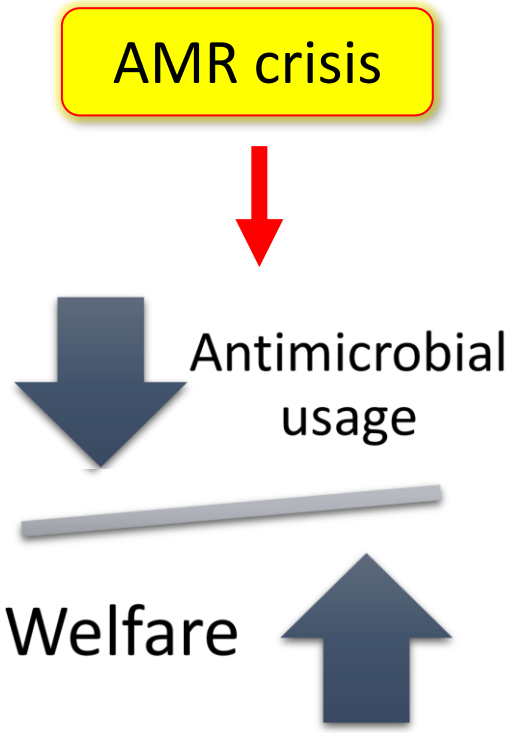
Malcata et al., (2020) Point-of-care tests for bovine clinical mastitis: what do we have and what do we need? J. Dairy Res 87: 60–66.

Summary

Exploring acute phase proteins (APPs) as biomarkers for selective dry cow therapy

Number of challenges:

- low concentrations of APPs
- Milk undiluted
- Significant potential



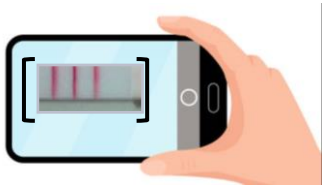
Biomarkers for differentiating pathogens and directing antimicrobial therapies for clinical mastitis

Targeted sampling:

- Exploring cathelicidins further (own Ab)
- Test more samples….

Proteomics:

- Ongoing work to validate the targets of interest
- Test on larger sample set



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