Using Omic Approaches to Improve the Detection of Paratuberulosis in Dairy Cattle

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Introduction – Current Diagnostic Techniques

- Paratuberculosis (MAP) UK herd prevalence is 27.5 - 42.5%[1]
- Costs UK economy approximately £13 million anually[2]
- On-farm loss of £122.89/MAP-infected cow, £60.57 via milk yield loss and £51.19 via voluntary culling[2]
- 15.4% of pasteurized milk samples and 22.8% of cheese samples from 7 major retailers across 5 countries tested MAP-positive via PCR[3]

- Only 10 – 15% of MAP-infected cattle display clinical signs[4]
- Performance of diagnostic test is dependent on the stage of infection[5,6]

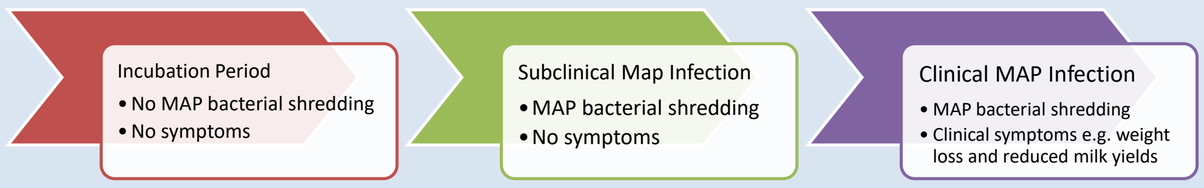

Figure 1 - The progression of MAP bacterial shredding and symptoms[7]

- Current MAP diagnostic tests are unable to detect the bacterium until bacterial shredding occurs[8] and subclinical cattle frequently shred MAP in insufficient quantities to be detected[9]
- Despite high specificity, current MAP diagnostic tests detect less than half of MAP-infected cattle, allowing MAP-infected cattle to potentially infect other cattle[5]

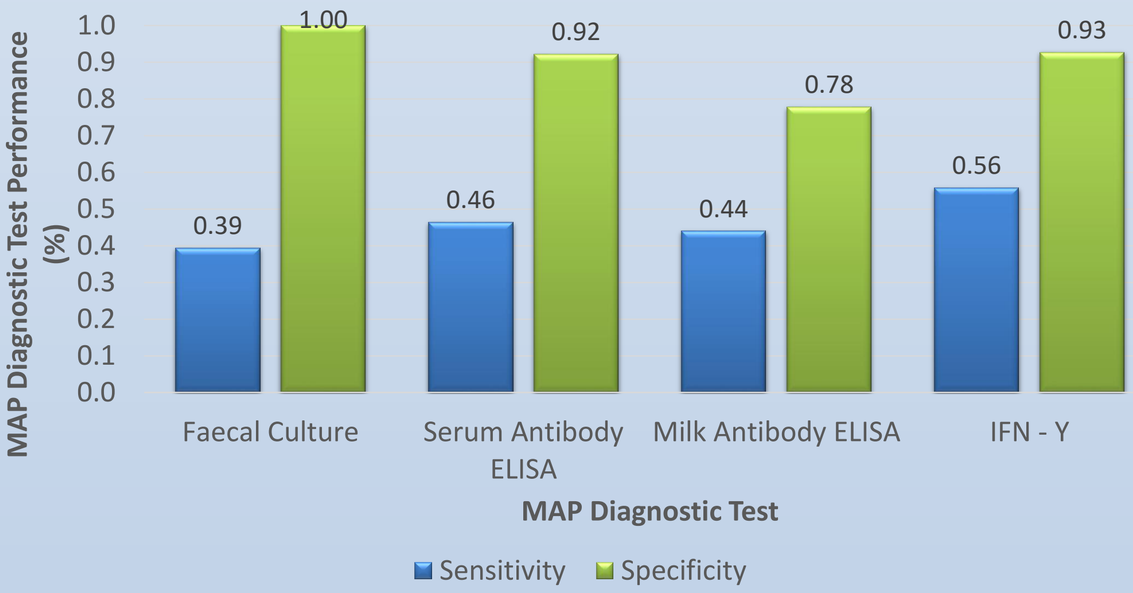

Figure 2 - Sensitivity and specificity values (%) from a meta-analysis of MAP diagnostic tests[4].

Metabolomics, Proteomics and Lipidomics

- **Omic approaches examine metabolites, proteins, mRNA and genetics [10]**

- Metabolites are intermediates and products of metabolism[11] which are produced from the hosts normal flora, infectious bacteria or the hosts. Composition is dependent on the age, sex, microbiome and lifestyle[12]
- > 20,000 biochemicals can be identified in 20 ul of sample in 2 min

- Proteomics explores the whole complement of proteins within a cell type or organism[13]
- > 1000 proteins can be identified in 200 ul of sample

- Lipidomics is a subfield of metabolomics which highlights lipid metabolites which may attribute to immunity, bacterial virulence and resistance[14]

Figure 3 – The process of producing metabolomic data. Following collection, samples are processed using the flow-infusion electrospray-mass spectrometry on the exactive orbitrap MS platform before being statistically analysed.

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Project Plan

Collaboration with UCD

- Aim: **Identify potential biomarkers** from serum aliquots

Location of sampling TBC

- Aim: Evaluate the effects of **metabolic stress** on novel MAP biomarker expression

Collaboration with Jersey Cattle Society

- Aim: Evaluate **inter-breed differences** between Jerseys and Holstein-Friesians

Identifying Novel MAP Biomarkers

- Holstein-Freisian bulls, 35 MAP-infected vs 20 age-matched controls
- Inoculated with the MAP strain CIT003 at 3.8X10[10] at 6 weeks old
- Cattle were sampled periodically over 33 months, diagnostic tests included; blood ELISA, IFN-Y, faecal MAP culture and tissue MAP culture

- Significant differences in cell mediated and humoral immune responses between MAP-infected and control cattle
- Limited positive faecal and tissue MAP culture
- During the trial, between 32%-94.44% of MAP exposed cattle and 11.11% - 44.44% of control cattle demonstrated a MAP-positive result[15]

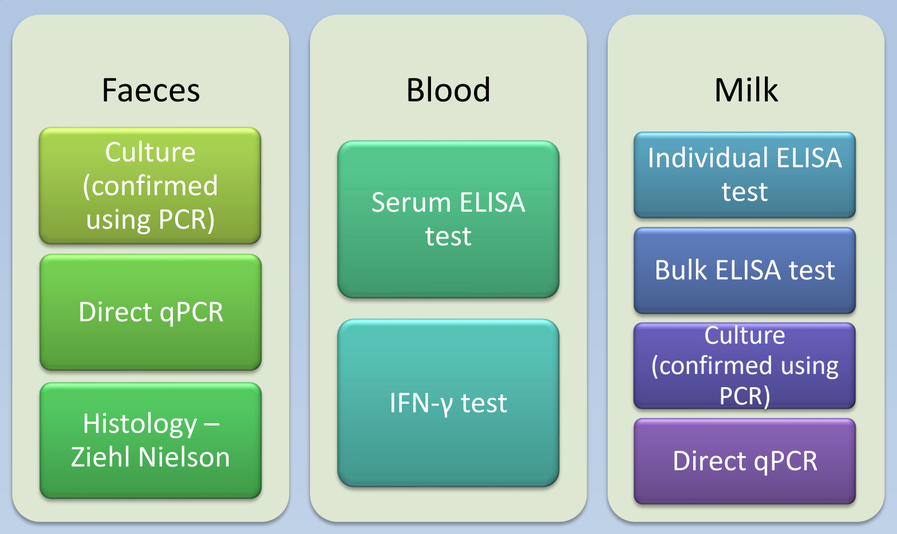
Figure 4 – (Left) healthy control cattle aged 33-months and (right) an intestinal biopsy from a MAP-infected cow showing subtle thickening despite negative MAP-culture[15]

Effect of Breed and Stress on MAP Biomarkers

- Stress has been shown to increase MAP shedding in sheep[16] and beef cattle[17]
- Cattle in the transition period demonstrate increased disease incidence and severity as their immune system is weaker[18]
- Sampling at three time points
  - Pre-calving
  - Post-calving
  - Mid lactation

- It is remains unclear if Jerseys are more susceptible to MAP, versus Holstein-Friesians (HF)[19,20]
- Cattle will be sampled once during mid-lactation and results compared to HF data

- Two groups: MAP-infected and MAP-free cattle
- Cattle must be 2nd lactation onwards to allow their MAP status to be determined using previous milk ELISA tests



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