PRIFYSGOL ABERYSTWYTH UNIVERSITY

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Institute of Biological, Environmental and Rural Sciences

Metabolites and *Mycobacterium bovis*- the hunt for novel biomarkers at Aberystwyth University

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Project Aims

Bovine tuberculosis (bTB), caused by *M. bovis* is undoubtedly one of the greatest challenges affecting the UK livestock industry today. Currently, accurate and conclusive diagnosis of the disease is a challenge.

This project investigates the metabolome, which consists of thousands of small molecules, typically <1500Da in size, whose composition in a sample can alter as a result of genetic, transcriptional and post-transcriptional changes. A significant advantage of metabolites over other "omic" techniques it the metabolome can be considered the closest representation of the phenotype. This can provide novel insights into host-pathogen interactions, along with the potential to identify novel diagnostic markers.

Figure 1: A selection of the equipment available in the IBERS High Resolution Mass Spectrometry Laboratory

Methodology and Results

Through collaboration with the APHA, both pre- and post-infection plasma samples have been collected from 61 experimentally infected calves across three independent experiments (n=22,19,20). Presence of active disease was confirmed in all cases by post-mortem examination. Plasma metabolites initially were profiled using flow infusion electrospray mass spectrometry, tandem mass spectrometry was then used to better identify specific metabolites of interest.

Significant changes in the metabolite signature were detected following each infection (Figure 2), these were reproducible across the three independent experiments.

Interestingly, similarities appear to exist between metabolites identified in this study and those associated with human tuberculosis. Encouragingly, many of these metabolites appear to have good discriminatory capability individually and plotting receiver operating characteristic (ROC) curves for various combinations of between three and six metabolites produces area under curve (AUC) values in the region of 0.96 as early as four weeks post infection (Figure 3).

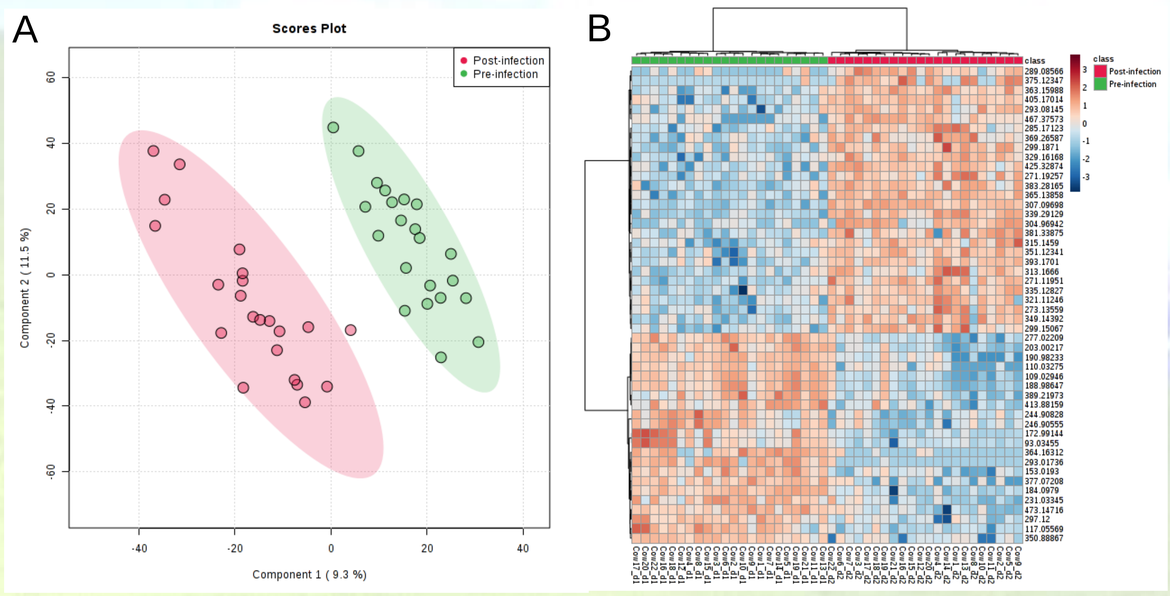

Figure 2A. Partial Least Squares- Discriminant Analysis indicating variation between pre-infection and post-infection plasma samples from a single experimental infection.
Figure 2B. Heatmap showing normalised abundance of top 50 explanatory features from Flow Infusion Electrospray Mass Spectrometry based on a single experiment.

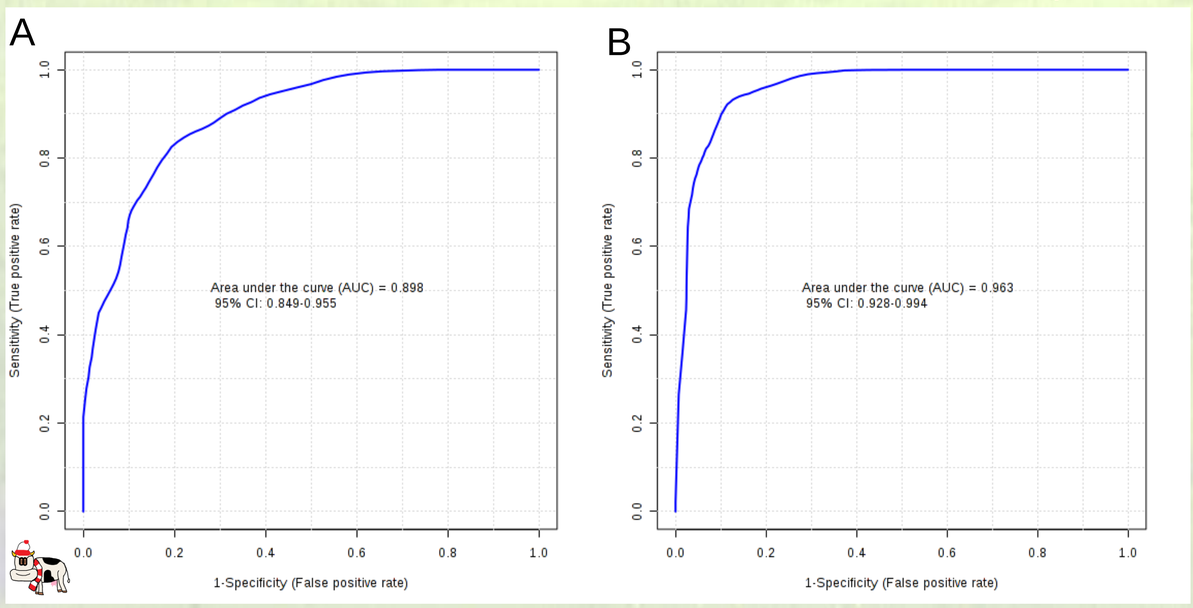

Figure 3A. ROC Curve for differentiation pre- and post-infection animals using a single metabolite. Figure 3B. ROC Curve generated using three explanatory metabolites. Data based on samples from 61 animals.

Discussion

Based on experimental infections, these identified features appear to have excellent diagnostic potential. However, how real-world performance of these markers has yet to be ascertained and could be impacted by concurrent disease, co-infections or similar pathologies.

The ability of a diagnostic to differentiate infected from vaccinated animals (DIVA) could pave the way for the use of vaccination against *M. bovis* in cattle. Upcoming work, using further experimental samples from the APHA will investigate whether metabolomics can be used to accurately differentiate BCG vaccinated and infected cattle.

However, significant further work is required to assess the performance of these biomarkers in identifying infected animals in field situations.

Parallel research

Based on the initial promise shown by metabolomics in relation to bTB, work is just beginning to assess whether metabolome and microbiome changes could have diagnostic potential for *M. bovis* infection of badgers, in order to assist in decision making with regards to the management of wildlife. Work is underway to investigate this in conjunction with the APHA and Wales Veterinary Science Centre.

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