PhD Project Advertisement

Project title: Evaluation and validation of LAP-MALDI mass spectrometry profiling for the detection of bovine tuberculosis (bTB)

Project No: FBS2024-085-Cramer-ra

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Project description:

This project will investigate and validate a novel method for rapid and cost-effective analysis of animal (livestock) diseases, focused on bovine tuberculosis. The analytical technique employed is mass spectrometry (MS), which is one of the most powerful molecular analysis techniques and already well-established in clinical (NHS) laboratory for the diagnosis of infectious diseases (biotyping) and metabolic diseases of newborns. Here, we will use 'LAP-MALDI' mass spectrometry, which has been developed in our labs, and exploit its advances in sample classification for the detection of bovine diseases and characterising/validating disease biomarkers that have been identified by this new analytical method.

Bovine tuberculosis (bTB) is a world-wide disease with serious economic impact for dairy farming and significant risks to the human population through zoonotic transmission. bTB costs the UK ~£100 million per year, with over 27,000 cattle being slaughtered for disease control in 2021. In 2013, the UK Government launched various bTB eradication strategies, with the aim of declaring the UK bTB-free by 2038, showing the importance for UK agriculture and one health.

There are currently two bTB diagnostic tests approved for use in the UK. The primary test is the tuberculin skin test and the secondary test is the interferon (IFN)-y blood test. The IFN-y test is used to supplement the tuberculin skin test to detect bTB infections that may not have been detected simply with the skin test. Both tests are invasive, time-consuming and depend on specific reagents. Therefore, there is the need for faster, less invasive and more cost-effective tests at the same or higher level of detection accuracy.

Earlier BBSRC-funded research demonstrated that LAP-MALDI MS analysis of cow milk allowed rapid detection of mastitis two days before clinical manifestation (see Piras et al.: https://doi.org/10.1039/D1SC05171G). Recent DEFRA-funded research showed that nasal swab samples from bTB cattle can be distinguished from samples of healthy cattle and cows with mastitis. Samples were collected non-invasively, prepared within 4 hours and analysed by LAP-MALDI MS within minutes. The obtained 3-class LDA model of the MS data provided a classification accuracy of 85.7% (see Lellman et al.: https://doi.org/10.1021/acs.jafc.3c01879). S100-A12 was putatively identified as protein biomarker for bTB-specific detection.

Objectives:

The project is expected to provide further evidence on the potential of LAP-MALDI mass spectrometry in veterinary diagnostics, in particular demonstrating that:

1. LAP-MALDI MS profiling enables the detection of bTB at a level of accuracy that is similar to current methods but faster, less-invasive and more cost-effective.
2. LAP-MALDI MS enables disease-specific biomarker discovery and multiplex disease detection.
3. S100-A12 is a biomarker for bTB detection.

The main work packages will be:

- Optimisation of LAP-MALDI MS profiling for bTB detection. Optimal sample preparation, including best MS-compatible sample collection by nasal swabs, optimal analyte extraction methods and timeframes for extraction and, if needed, limited digestion will be systematically investigated. These parameters and instrument settings will be evaluated and optimised.
- Multiplexed LAP-MALDI MS profiling analysis. As shown in the Piras and Lellman articles, LAP-MALDI MS profiling has the potential for multiplexing. Thus, samples from cattle displaying other diseases but focused on diseases closely related to bTB will be collected from the Animal & Plant Health Agency (APHA) and farms around Reading and Wales, and analysed, using machine learning for prediction model building.
- Validation of S100-A12 as a bTB-specific disease marker. One extremely promising result from the DEFRA-funded research (see Lellman article) was the identification of the S100-A12 protein as a potential bTB-specific disease marker in nasal fluids. It is planned to use commercial ELISA tests (e.g. https://www.abbexa.com/cow-s100a12-elisa-kit) to validate this result on cattle samples from APHA and UK farms (Wales, Berkshire, Scotland).
- Identification of novel bovine disease markers. The classification algorithms obtained in Objective 2 will also allow us to identify specific molecular marker for other diseases, e.g. through their loading plots. These will be selected and further characterised by tandem mass spectrometry and analysed by other biochemical means such as ELISA or nucleic acid amplification (cf. Objective 3).

In summary, this project is expected to provide the evidence for a potential step-change in veterinary diagnostics through a faster, more cost-effective and less invasive test that is based on novel mass spectrometry.

Training opportunities:

Experimental methods:

- LAP-MALDI MS and analytical sample preparation for MS analysis,
- Assaying liquid biopsies using ELISA-based methods,
- Biomolecular characterization using MS data,
- Collection, handling and preparation of farm animal samples, including CL-2/3 lab work, GLP and containment procedures,
- Laser safety training,
- Prediction model building using machine learning and multi-omics bioinformatics.

General Training:

- Lab training: The successful candidate will be trained in lab-based analytical and microbiological research, incldluing SOPs and instrument training, provided by technical services/senior researchers. He/she will also be trained in H&S (risk assessments) and research ethics.
- Research/data analysis: Mandatory attendance of weekly group meetings, discussing day-to-day running of research labs and work data with journal club presentations. Weekly 1-2-1 meetings with the lead supervisor, discussing data and learning relevant data mining techniques by using their own data.
- Transferable skills: A wide range of transferable skills courses are available in teaching, leadership, mathematical/statistical skills, science communication/writing and management courses, e.g. >50 RRDP courses run by the Doctoral and Researcher College. The successful candidate is also required to further their studies by attending some taught UG courses.

Student profile:

Applicants should hold or expect to gain a minimum of a 2:1 Bachelor Degree or equivalent in the physical, life or medical/veterinary sciences. Hands-on experience in the field of mass spectrometry (e.g. through a final year project) is desirable but not essential as is basic knowledge in animal sciences or microbiology.

The ideal candidate would have a strong interest in one or more of the following areas:

- Bioanalytical sciences
- Livestock/Animal research
- Microbiology
- Diagnostics

Stipend (Salary):

FoodBioSystems DTP students receive an annual tax free stipend (salary) that is paid in instalments throughout the year. For 2023/24 this is £18,622 and it will increase slightly each year at rate set by UKRI.

Equality Diversity and Inclusion:

The FoodBioSystems DTP is committed to equality, diversity and inclusion (EDI), to building a doctoral researcher(DR) and staff body that reflects the diversity of society, and to encourage applications from under-represented and disadvantaged groups. Our actions to promote diversity and inclusion are detailed on the FoodBioSystems DTP website.

In accordance with UKRI guidelines, our studentships are offered on a part time basis in addition to full time registration. The minimum registration is 50% FT and the studentship end date will be extended to reflect the part-time registration.

References:

Piras et al. Chem. Sci. 2022; https://doi.org/10.1039/D1SC05171G
Lellman et al. J. Agric. Food Chem. 2023; https://doi.org/10.1021/acs.jafc.3c01879

For up to date information on funding eligibility, studentship rates and part time registration, please visit the FoodBioSystems website.