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KTN/BSAS Summer Vacation Scholarship Award – Report

Identifying the basic host defence processes in ovine polyarthritis ('joint ill'): *Streptococcus dysgalactiae* ssp. survival in ovine whole blood, joint fluid and joint cartilage explants

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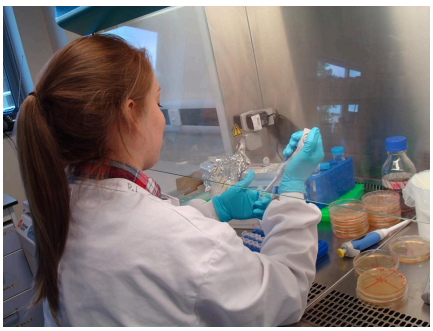

Introduction and aims

Joint ill is a significant economic problem and major animal welfare concern in the UK. *S. dysgalactiae* causes bacteraemia and localises in the joints of lambs causing lameness due to infection and subsequent inflammation of the joint.

The aim of the project was to develop an ovine joint explant model for *S. dysgalactiae* infection using joints of lambs post-slaughter and to investigate growth characteristics of the bacterium using whole blood and joint fluid survival assays. The overall aim was to gain a better understanding of which part of the joint was most susceptible to bacterial infection and how this leads to the clinical signs associated with joint-ill.

Methods

Ovine joint samples were collected fresh from the abattoir. A protocol was optimised in order to collect the joint fluid and 4mm cartilage biopsies whilst maintaining a high level of sterility.

Joint fluid and blood survival analysis

With the joint fluid, several growth curves were performed with two *S. dysgalactiae* isolates (1 & 5) both on the day of collection and 24 hours later, as well as with bacteria in different growth phases (stationary and logarithmic phase) in order to compare whether any of these factors affected the bacterial growth and survival in synovial fluid and fresh whole blood.

The preliminary results show there is evidence for growth of the selected bacterial strains in both synovial joint fluid and blood, however further repeats will be needed to provide reproducible results. The bacterial growth in fresh synovial fluid and 24-hour-old synovial fluid showed a significant difference in growth rate. Bacterial growth was similar between both log and stationary phases.

Cartilage explant models

Cartilage explant models were designed in which cartilage biopsies were collected from fresh abattoir samples. The joints were submerged in ethanol and scrubbed with HiBiSCRUB® to ensure sterility before opening up the joint capsule and collecting the biopsies. The cartilage biopsies were washed with antibiotics (penicillin & streptomycin) and anti-fungal agents (fungizome) in order to remove any contaminants and incubated overnight in DMEM at 37°C. Prior to bacterial infection, the cartilage pieces were changed into antibiotic-free medium. A bacterial overnight culture was diluted and grown to log growth phase. Bacteria were diluted in tissue culture medium and placed in the wells with the cartilage biopsies. At each time-point (2 hours and 24 hours) the media were removed from each well and the cartilage tissue pieces were gently washed with PBS, homogenised and plated onto BHI agar plates to enumerate the bacteria.

The uninfected controls for the cartilage explant models showed a lot of background, which we predicted to be either proteinaceous or bacterial contaminants that had survived the washing steps. To avoid this in future experiments, it has been suggested that the addition of another antibiotic (gentamycin) at the washing steps may help eliminate background growth. A low number *of S. dysagalactiae* were recovered.

Taking part in the summer studentship was an invaluable experience for me. Besides the obvious lab skills learned, I also discovered a whole new meaning to time-management and organisational skills, which I have now greatly improved. Having never worked in a research environment before, I had never considered it as a career. However, I thoroughly enjoyed the experience of working in a lab environment and will now seriously consider this pathway in my future career choice.