Investigating the ovine mammary microbiome using PCR-DGGE

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Introduction

- The development and composition of colonising bacteria in animal-associated microbiomes is an important determinant of animal health.
- Intramammary infections (IMI) in ewes present as clinical mastitis or subclinical infection detectable by a raised somatic cell count (SCC).

Clinical mastitis

Subclinical infection?

- IMI have a major economic impact as they can result in reduced milk quality and production, premature culling and death.
- To date, published literature has only focused on the interaction between individual bacterial species, their molecular attributes and the host's response.
- However, over 130 bacterial species have been associated with IMI in cattle[1] and there is no reason to suggest that a similar number of bacterial species cannot infect the ovine mammary gland.

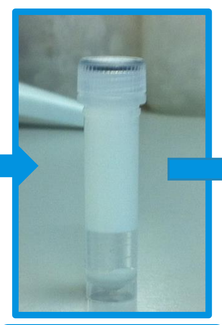
Research Hypothesis

The ovine mammary gland is a site where bacteria live as part of a natural microbial community and changes in this community lead to the development of disease.

Materials and Methods

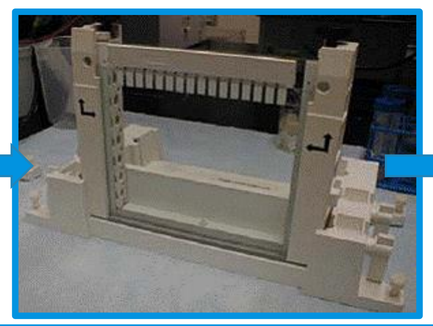
- Milk samples were collected from both halves of the mammary gland of 30 ewes in five different age groups over eight weeks.
- 380 milk samples were processed via DNA extraction[2], PCR amplification of bacterial DNA using16S rRNA primers[3] and Denaturing Gradient Gel Electrophoresis (DGGE)[4]. DGGE images were then analysed using the software Gel Compar II and MLwiN.

Collect milk samples



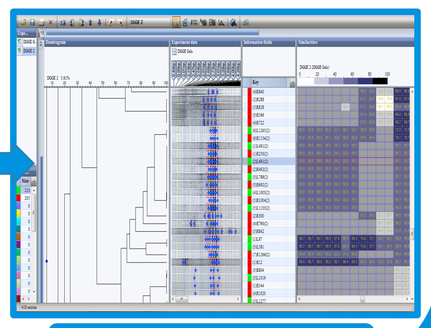
Extract DNA

Amplify bacterial DNA



Load PCR products onto DGGE gel

Run DGGE gel



Analyse DGGE images

Results

- 2,068 bands were identified in 380 milk samples.
- The average number of DGGE bands was similar across ages suggesting a bacterial community is consistently present throughout a ewes' lifetime.
- There were similarities and differences within and between mammary gland halves and time points (Figure 2).
- Comparisons between DGGE fingerprints using a fixed effects regression model indicated that 15 DGGE bands (6 circled in Figure 2) are significantly associated with a change in SCC (8 linked to an increase and 7 a decrease).

Figure 1: Average number of bands in DGGE analysis grouped by sheep parity (age).

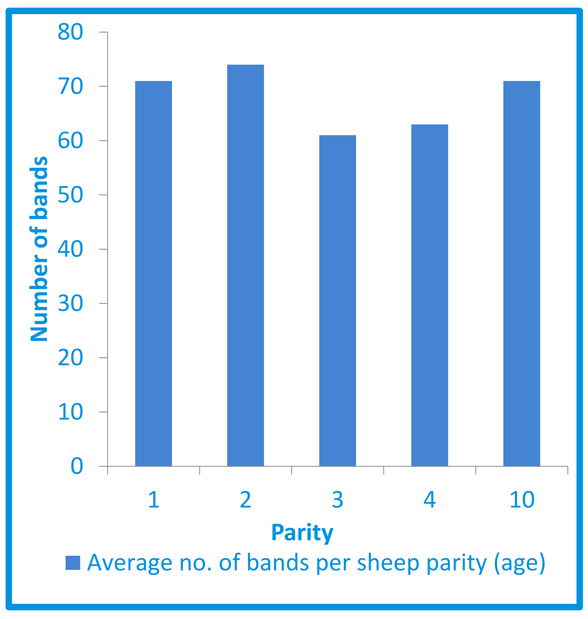
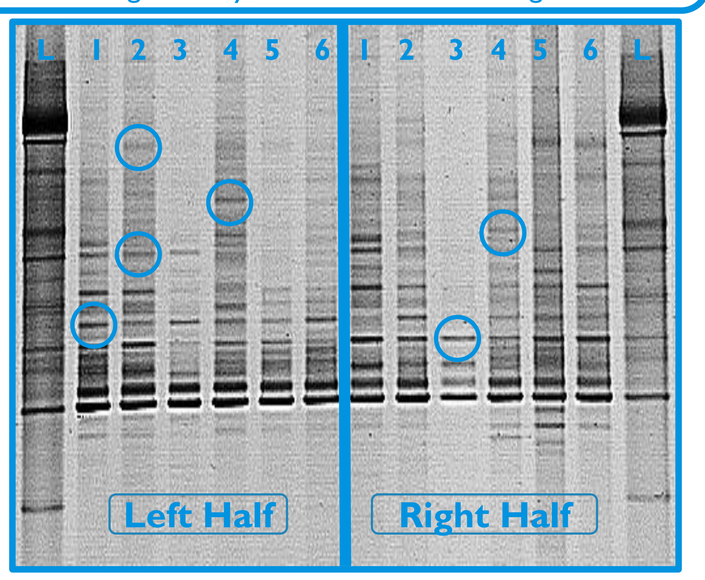


Figure 2: Banding patterns produced by milk samples processed for one ewe. 'L'; reference ladder. Numbers 1-6; weeks 1-6 for each half of the mammary gland. Circled bands are significantly associated with a change in SCC.



Conclusions and Future Work

- A complex and dynamic bacterial community is present in the mammary glands of sheep.
- Change in SCC is associated with bacterial community composition.
- Bands from the PCR-DGGE analysis significantly associated with a change in SCC will be sequenced to identify the bacterial species.
- All 380 longitudinal study samples will undergo high-throughput sequencing. This will identify bacterial strains to facilitate sophisticated epidemiological analyses that will improve our understanding of the interactions between sheep and their mammary gland microbiome.
- The aforementioned techniques will provide a unique insight into the mammary gland microbiome and its role in sheep health.

References

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[3] Hunt, K.M et al (2011). *PLoS ONE* **6**(6), pp. e21313. [4] Muyzer, G. and Schäfer, H. (2001). *Method Microbiol* **30** pp.425-468.

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