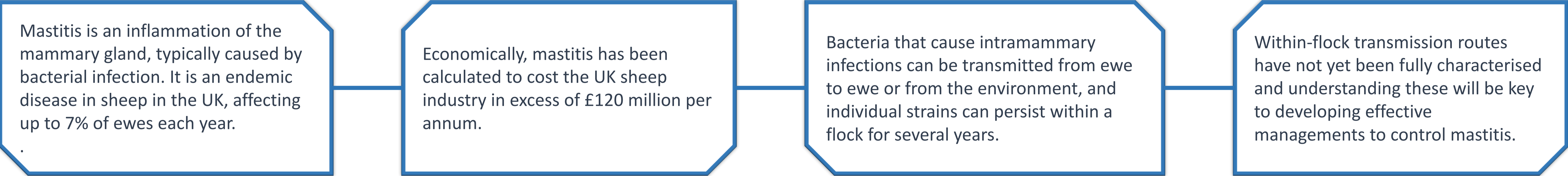


Using MALDI-ToF MS to investigate transmission of intramammary pathogens within sheep flocks

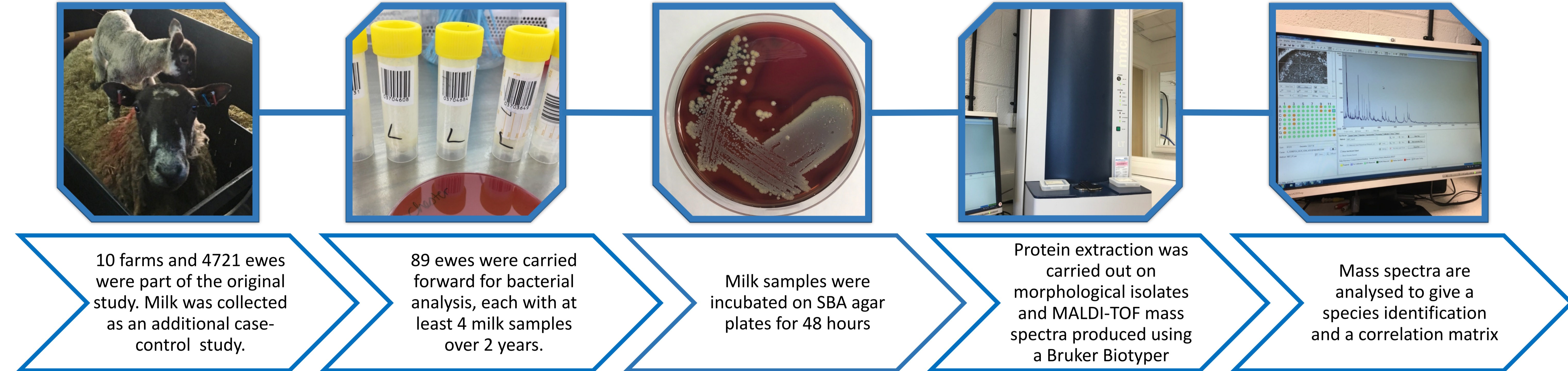
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Background



Materials and Methods



Preliminary Results

Choosing a threshold – When are two bacteria the same?

Once mass spectra have been created using MALDI-ToF, individual spectra must be compared and a value used to determine when two bacteria are sufficiently similar to indicate transmission.

In order to investigate how this might be achieved, technical and biological replicates were isolated and mass spectra produced so that they could be compared. Four different *Staphylococcus* species were chosen, including *S. aureus*.

Two methods of generating the similarity threshold have been investigated; these are explained below.

Option 1: Correlation Coefficient Indices

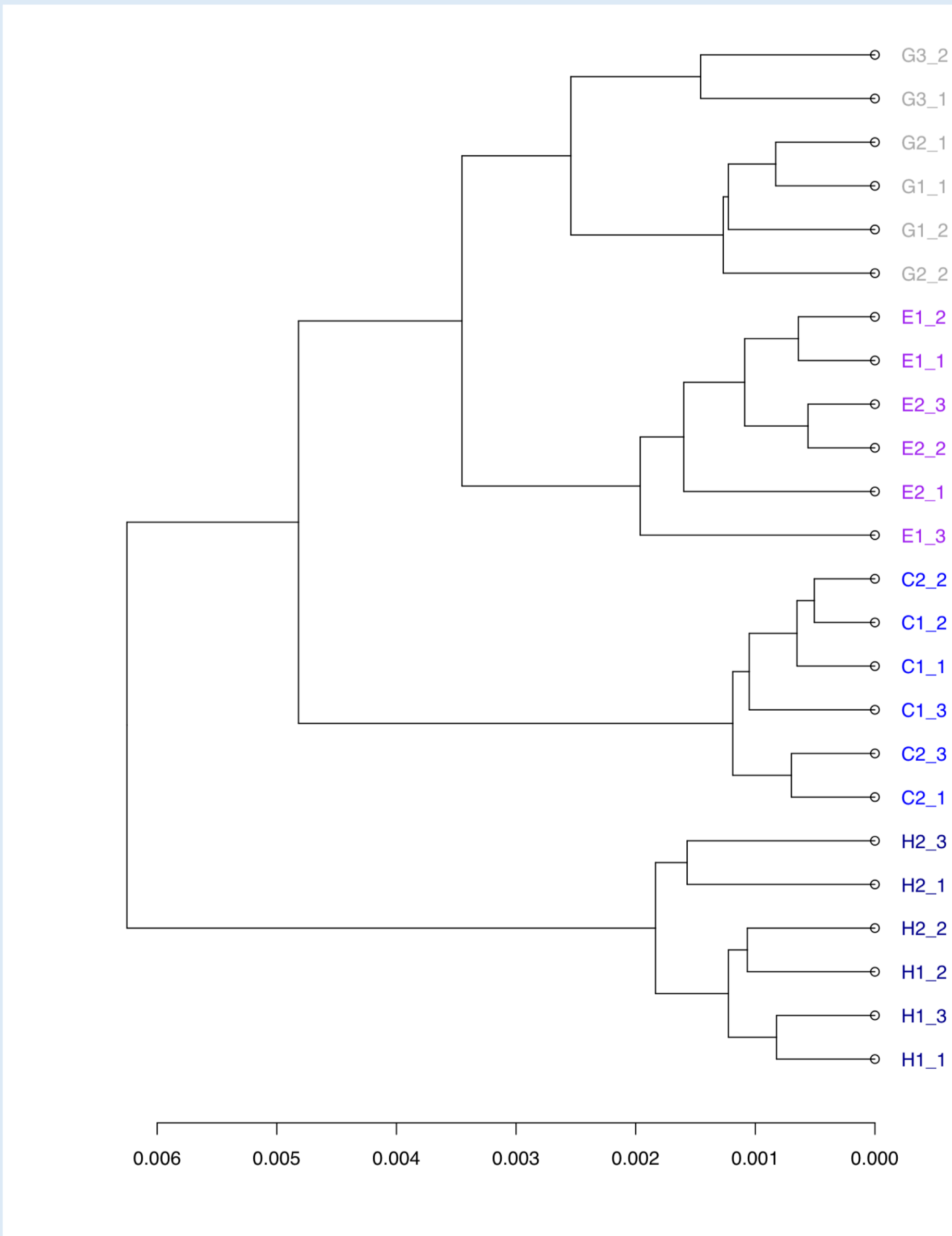
	C1-1	C1-2	C1-3	C2-1	C2-2	E1-1	E1-2	E1-3	E2-1	E2-2	E2-3	G1-1	G1-2	G2-1	G2-2	G3-1	G3-2	H1-1	H1-3	H2-1	H2-2
H2-2	0.6	0.5	0.5	0.7	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.4	0.2	1	0.9	0.8	
H2-1	0.5	0.4	0.4	0.5	0.5	0	0	0	0.2	0.1	0.1	0	0	0.2	0	0.4	0	0.9	0.9		
H1-3	0.6	0.4	0.5	0.6	0.6	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.3	0.2	0.4	0.2	0.9			
H1-1	0.6	0.5	0.5	0.6	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.4	0.2				
G3-2	0.4	0.2	0.3	0	0.4	0.4	0.5	0.4	0.6	0.5	0.5	0.9	0.9	0.9	0.9	0.7					
G3-1	0.5	0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.6	0.6	0.6	0.7	0.7	0.7	0.7						
G2-2	0.4	0.2	0.3	0	0.3	0.3	0.4	0.4	0.5	0.4	0.4	0.9	0.9	1							
G2-1	0.5	0.2	0.4	0.3	0.4	0.4	0.4	0.4	0.5	0.5	0.5	0.9	0.9								
G1-2	0.4	0.2	0.3	0	0.3	0.4	0.4	0.4	0.5	0.4	0.5	1									
G1-1	0.4	0.2	0.3	0	0.4	0.4	0.4	0.4	0.5	0.5	0.5										
E2-3	0.4	0.2	0.4	0	0.4	0.9	0.9	0.9	0.9	1											
E2-2	0.4	0.2	0.4	0	0.4	0.9	0.9	0.9	0.9												
E2-1	0.5	0.2	0.4	0.2	0.4	0.9	0.9	0.9													
E1-3	0.4	0.2	0.4	0	0.4	0.9	0.9														
E1-2	0.4	0.2	0.3	0	0.4	1															
E1-1	0.4	0.2	0.3	0	0.3																
C2-2	0.9	0.9	0.8	0.8																	
C2-1	0.8	0.7	0.7																		
C1-3	0.8	0.8																			
C1-2	0.8																				
C1-1																					

Bruker Biotyper 3.0 computes a correlation coefficient index (CCI), returning a value between 0 and 1, indicating the likelihood that two spectra are the same based on an algorithm created by Arnold and Reilly (1998).

The dark red areas show where replicates were compared and correlate to the higher scores on the heatmap.

Previous studies have used a CCI value < 0.99 to consider two isolates the same strain, however, in this study no replicate was given a value < 0.99, and some were as low as 0.7.

Option 2: Peak Lists - Hierarchical Clustering



The R package MALDIquant can be used to upload Bruker mass spectra, and then carry out quality control, pre-process and detect peaks from MALDI mass spectra.

Following pre-processing and peak detection, an Euclidean distance matrix was created using the mass spectra from the same replicates used for the CCI.

Each *Staphylococcus* species has a distinct cluster, suggesting that this method may be successful in determining similar bacteria within the dataset.

Future Work

In order to choose a similarity threshold some further work with bacterial replicates is required. Samples that are the same species but are known different strains also need to be compared, so that the threshold chosen is low enough to avoid false positives.

Once this has been carried out, isolates from each milk sample can be compared and transmission events identified within flocks.

Milk samples have been collected from the same ewe over a two year period, so the same method can be used to track persistence of strains within individuals.

Finally, this information can be used alongside disease state data, which may give some indication of important pathogens related to transmission and persistence of ovine mastitis.