The major ovine mastitis pathogens *Mannheimia haemolytica, Staphylococcus aureus* and *Streptococcus uberis* selectively alter TLR transcription in ovine primary mammary epithelial cells

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Short title: **Toll like receptors in ovine mammary epithelial cells**

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Summary

Despite the importance of mastitis to sheep production worldwide, the pathogenesis and host response to bacterial infection of the ovine mammary gland are poorly characterized. Studies in cattle highlight the significance of the mammary epithelium in pathogen recognition and the subsequent host response. The objectives of this study were to determine the range of Toll like receptor (TLR) genes transcribed in ovine mammary epithelial cell (MEC) and, quantify the transcription of the TLR genes in MEC in response to culture with the principal mastitis pathogens of sheep. We generated a primary mammary epithelial cell (pMEC) line. Using quantitative reverse transcription PCR (RT-qPCR), we analysed the transcription of genes of the pattern recognition receptors family (TLRs) upon *in vitro* pathogen-pMEC co-culture. *TLR1*, *2*, *3*, *4*, *6* and *9* were shown to be constitutively transcribed by pMEC. *M. haemolytica* induced up regulation of transcription of *TLR1*, *2*, *3*, *4*. By contrast, *S. uberis* and *S. aureus* induced concentration-dependent transcription of TLR2 and TLR4 with a higher level of transcription in cells stimulated with the bacteria at a multiplicity of infection (MOI) of 200 compared to cells stimulated with a MOI of 20. These experiments define the range of *TLR* genes constitutively transcribed in sheep pMEC and show that bacterial infection has the capacity to regulate transcription in a species specific and concentration dependent manner.