Comparison of high and low virulence serotypes of Actinobacillus pleuropneumoniae by quantitative real-time PCR - DTU Orbit (28/12/2018)

Until now, 15 different serotypes of Actinobacillus pleuropneumoniae (Ap) have been described based upon differences in the capsular polysaccharides of the bacterium. The virulence of different serotypes of Ap has been experimentally determined and the differences in mortality and morbidity are considerable. The genetic mechanisms behind these variations in virulence are largely unknown, and for bacteria in general, the non-virulent strains often contain many of the virulence genes required for an infection. In Denmark, serotype 2 and serotype 6 are the most commonly found, with serotype 2 being of high virulence while serotype 6 strains are normally found to be less pathogenic. To gain an understanding of the differential virulence of serotype 2 and 6, the expression of a panel of Ap genes during infection of porcine epithelial lung cells (SJPL) were examined by quantitative real-time PCR (qPCR). Flasks of SJPL cells were inoculated with equal amounts of Ap serotype 2 and 6, respectively. After two hours, the supernatant was discarded, the cells and attached bacteria harvested, and total RNA isolated. After an enrichment step for bacterial RNA, the expression of a number of Ap genes believed to be important for early establishment of the bacteria in the host were examined by qPCR. The genes examined were apfA, coding for a subunit of Type IV pili, kdsB coding for a gene involved in lippopolysacceride biosynthesis, and pgaB which is involved in biofilm formation, all three believed to be important with respect to host cells adhesion. Also included in the analysis were the capsular gene, cpxB, the RTX toxin genes apxII, and apxIV and the gene exbD, involved in binding of iron from host cells. Finally, three previously validated reference genes, glyA, pykA and tpiA were included for normalization of the qPCR data. Preliminary results showed that in both serotype 2 and serotype 6, the toxin producing gene apxIV was the most highly expressed of the investigated genes. The major difference observed between the two serotypes was that apfA, involved in type IV vili production, was significantly upregulated in serotype 2 compared to serotype 6. Further investigations will be needed to establish whether this result has any relevance with respect to virulence. Results from this study will be used as the basis for a microarray approach to examine the overall gene expression variation between Ap serotypes in vitro and in vivo.