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Comparison of high and low virulence serotypes of *Actinobacillus pleuropneumoniae* by quantitative real-time PCR

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. Three previously validated reference genes, *glyA*, *pykA* and *tpiA* were included for normalization of the qPCR data. Among the eight genes compared, three appeared to be significantly differently expressed. In serotype 6, the genes *cpxB* and *kdsB*, both involved in capsule formation, were upregulated compared to serotype 2. In serotype 2, the production of *pgaB*, involved in biofilm formation, seem negligible in comparison to serotype 6. However, more data is needed on this particular gene. Still, this result is in agreement with observations in the laboratory, where biofilm formation is observed for some strains of serotype 6. Interestingly, in serotype 2, the toxin gene *apxII* appears to be significantly upregulated compared to serotype 6, which is in accordance with the observed difference in pathogenicity. However, further investigations will be needed to establish whether these results have any relevance to the difference in 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*.