

Determining the optimal number of individual samples to pool for quantification of average herd levels of antimicrobial resistance genes in Danish pig herds using high-throughput qPCR - DTU Orbit (08/11/2017)

Determining the optimal number of individual samples to pool for quantification of average herd levels of antimicrobial resistance genes in Danish pig herds using high-throughput qPCR

The primary objective of this study was to determine the minimum number of individual fecal samples to pool together in order to obtain a representative sample for herd level quantification of antimicrobial resistance (AMR) genes in a Danish pig herd, using a novel high-throughput qPCR assay. The secondary objective was to assess the agreement between different methods of sample pooling. Quantification of AMR was achieved using a high-throughput qPCR method to quantify the levels of seven AMR genes (*ermB*, *ermF*, *sulI*, *sulII*, *tet(M)*, *tet(O)* and *tet(W)*). A large variation in the levels of AMR genes was found between individual samples. As the number of samples in a pool increased, a decrease in sample variation was observed. It was concluded that the optimal pooling size is five samples, as an almost steady state in the variation was observed when pooling this number of samples. Good agreement between different pooling methods was found and the least time-consuming method of pooling, by transferring feces from each individual sample to a tube using a 10 µl inoculation loop and adding 3.5 ml of PBS, approximating a 10% solution, can therefore be used in future studies.

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