DEVELOPMENT OF A NEW SWINE MYCOPLASMA MULTIPLEX QPCR

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Background & Objectives

In swine 3 Mycoplasma species are deemed to be important, *Mycoplasma hyopneumoniae, Mycoplasma hyosynoviae* and *Mycoplasma hyorhinis*. This paper is describing the development of a new multiplex Mycoplasma qPCR which detects all 3 species.

Materials and methods

Primer/Probe

Specific primers for *M.hyopneumoniae, M.hyosynoviae* and *M.hyorhinis* were designed. To these specific primers probes were designed for detection of qPCR products. An internal control primer/probe set was used for excluding inhibition.

Samples

For evaluation of sensitivity and specificity, reference strains for the specific Mycoplasma's, other Mycoplasma species, other common agents in swine and field samples with a known qPCR status were used. Specific plasmids were used to determine the Lower Limit Of Detection (LLOD).

Methods

DNA extraction was done by using a spin column based method. qPCR was performed according to BioChek's protocol. Data was quantified by using Cq values.

Results

In total 516 tests were performed to check specificity, no false positive results were obtained.

68 Field positive samples (oral fluids) were used for evaluating sensitivity. Based on these results sensitivity for *M.hyopneumoniae* was 98.2%, for *M.hyosynoviae* 100% and for *M.hyorhinis* 82.4% respectively. For the LLOD plasmids were used and LLOD's were for *M.hyopneumoniae* <100 copies/reaction, for *M.hyosynoviae* <50 copies/reaction and for *M.hyorhinis* <50 copies/reaction.

Discussion and Conclusion

The new developed multiplex qPCR makes it possible to detect all 3 relevant Mycoplasma species in one single run. Both specificity and sensitivity are high. Sensitivity itself will depend on extraction methods used. With the low LLOD values the qPCR will start detection below clinical relevant levels. Combining the 3 Mycoplasma species in one qPCR will save time and effort for evaluation of Mycoplasma in swine.