Development of an improved species specific PCR test for detection of Haemophilus parasuis

A PCR test for identification of Haemophilus parasuis was optimized using the 16S rDNA sequences of the 15 serotype reference strains of H. parasuis. The test was evaluated on a collection of 218 Danish field isolates as well as on 81 representatives of 27 other species, including genetically affiliated species within Pasteurellaceae. In addition, DNA preparations from 56 H. parasuis isolates from North America were included. To obtain a test that was specific for H. parasuis, a multiplex PCR using 3 different primers was developed. The PCR test produced an amplicon of approximately 1090 bp only with representatives of H. parasuis. The test was further evaluated on 55 clinical samples from 16 Danish pigs suspected for being infected with H. parasuis, showing polyserositis or septicemia at autopsy as well as on 492 nasal swabs. The test was compared with the performance of a PCR test earlier published by Oliveira et al. [Oliveira, S., Galina, L., Pijoan, C., 2001. Development of a PCR test to diagnose Haemophilus parasuis infections. J. Vet. Diagn. Invest. 13, 495-501]. The sensitivity of the present PCR test was found to be slightly lower when applied on clinical samples from diseased pigs and 10-fold lower when tested on pure cultures of H. parasuis (5 CFU and 0.5 CFU/PCR reaction, respectively). Addition of 1.4 x 10^5 Escherichia coli to each PCR tube did not alter the sensitivity of the tests. No difference in sensitivity of the tests was observed when tested on purified DNA. On the other hand, the present PCR test was found to be 100% species specific for H. parasuis, in contrast to the PCR test of Oliveira et al., which also tested positive for strains belonging to A. indolicus, A. porcinus, and A. minor, species commonly occurring in the upper respiratory tract. However, when the PCR test of Oliveira et al. is used on samples from systemic locations the chances for false positive results are apparently low. The present PCR test represents a rapid and reliable method for genetically based identification of H. parasuis. The high species specificity of the test makes it suitable for detection of H. parasuis in clinical samples, regardless of the presence of affiliated species and contaminating flora. As the two PCR tests differ in sensitivity and specificity, the use of both PCR tests for different purposes is a possibility.