Development and validation of a qPCR for the detection and quantification of *Actinobacillus pleuropneumoniae* DNA in pigs

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Actinobacillus pleuropneumoniae is a common pathogen of pigs, causing respiratory disease and economic losses. Detection of *A. pleuropneumoniae* antigen in live pigs is difficult and improvement of the current diagnostic procedures could contribute to better disease control. A common test for quantitative *A. pleuropneumoniae* detection in samples from live pigs is selective bacterial examination (SBE) using tonsillar or nasal swab samples, but differentiation between *A. pleuropneumoniae* and other members of the Pasteurellaceae family in the oropharyngeal flora is difficult. Therefore a quantitative real-time PCR (qPCR) protocol for detection of the apxIVA gene was developed based on primers apxIVANEST1-F and apxIVANEST1-R and a fluorescent probe. Validation studies were performed using pure cultures of *A. pleuropneumoniae* , as well as samples from both experimentally inoculated Caesarean-derived, colostrum-deprived (CDCD) piglets and conventional pigs. The analytical sensitivity was 5 colony forming units/ reaction. Diagnostic sensitivity was estimated at 0.98 in inoculated piglets, compared to SBE. Specificity was estimated at 1.0 in samples from 77 *A. pleuropneumoniae* free CDCD pigs and from 70 pigs from three SPF farms, free of *A. pleuropneumoniae*. A small-scale cohort study on two infected farms showed consistent results in repeatedly sampled pigs. No difference in *A. pleuropneumoniae* quantities between points in time or between farms was found, except at 24 weeks of age when incidence, as well as *A. pleuropneumoniae* quantities were significantly raised in one farm. Tonsillar brush samples and additional apxIVA qPCR analysis could therefore be a valuable additional tool in monitoring and epidemiological studies on *A. pleuropneumoniae*.