A novel strategy to obtain quantitative data for modelling: Combined enrichment and real-time PCR for enumeration of salmonellae from pig carcasses

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Abstract

The primary sources for the major zoonotic pathogen Salmonella are food-producing animals such as pigs and poultry. For risk assessment and hazard analysis and critical control point (HACCP) concepts, it is essential to produce large amounts of quantitative data, which is currently not achievable with the standard cultural based methods for enumeration of Salmonella. As part of the European research project BIOTRACER, this study presents the development of a novel strategy to enumerate low numbers of Salmonella in cork borer samples taken from pig carcasses as a first concept and proof of principle for a new sensitive and rapid quantification method based on combined enrichment and real-time PCR. The novelty of the approach is in the short pre-enrichment step, where for most bacteria, growth is in the log phase. The method consists of an 8-h pre-enrichment of the cork borer sample diluted 1:10 in non-selective buffered peptone water, followed by DNA extraction, and Salmonella detection and quantification by real-time PCR. The limit of quantification was 1.4 colony forming units (CFU)/20 cm2 (approximately 10 g) of artificially contaminated sample with 95% confidence interval of ± 0.7 log CFU/sample. The precision was similar to the standard reference most probable number (MPN) method. A screening of 200 potentially naturally contaminated cork borer samples obtained over seven weeks in a slaughterhouse resulted in 25 Salmonella-positive samples. The analysis of salmonellae within these samples showed that the PCR method had a higher sensitivity for samples with a low contamination level (< 6.7 CFU/sample), where 15 of the samples negative with the MPN method was detected with the PCR method and 5 were found to be negative by both methods. For the samples with a higher contamination level (6.7-310 CFU/sample) a good agreement between the results obtained with the PCR and MPN methods was obtained.