A POLYMERASE CHAIN REACTION BASED ASSAY

FOR

THE DETECTION OF *Salmonella* IN RAW GROUND BEEF

by

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Abstract

Food-borne transmission of salmonellosis is of increasing concern to many countries. The control of the infection depends increasingly on the availability of rapid and specific diagnostic tests for monitoring the primary animal production as well as final food products. Standard culture methods are time consuming (4-5 days), costly and labour intensive. Alternative methods including PCR assays have been developed in other countries for the detection of *Salmonella.* However, in Sri Lanka, detection of *Salmonella* in food using PCR has not been evaluated and established. Therefore, this study was initiated towards the development of a rapid and sensitive PCR assay for the detection of *Salmonella* in raw beef.

A number of *Salmonella* and Non- *Salmonella* pure cultures were obtained from 7 institutions and a preliminary market survey was carried out using market beef samples to isolate *Salmonella* to be used in the PCR amplification and all were identified and confirmed morphologically, biochemically and serologically.

Salmonella specific primers were designed from previously sequenced *ompC* gene to amplify a 159 bp region. The optimum conditions for PCR were found to be 0.2 μM of each primer, 100 μM of dNTPs (dCTP, dGTP, dATP & dTTP) and 2 μM of $MgCl_2$. Thermal cycling profile; denaturation for 30 seconds at 95° C, annealing for 45 seconds at 56° C, elongation for 30 seconds at 72° C and final extension for 15 minutes at 72° C was used for 30 cycles. All *Salmonella* isolates tested (n=33) successfully amplified the 159 bp fragment whereas; non-*Salmonella* isolates (n=24) did not yield this fragment. Under optimal conditions the PCR assay was sensitive enough to detect 10 pg of *Salmonella typhimurium* genomic DNA and less than 600 cells of *Salmonella typhimurium* in pure culture, when the whole bacterial cells were processed with O.45 % Nonidet NP 40 prior to PCR. The PCR assay could also detect less than 4 cells of *Salmonella typhimurium* in artificially contaminated raw beef following a 6 hour-enrichment step in trypticase soy broth. In addition, 25 cells could be detected after 4 hours of enrichment while 172 cells could be detected after 2 hours. The sensitivity and the specificity of this assay in detecting *Salmonella* in market samples were in total agreement with the culture method. However, the culture method requires 4-5 days for detection whereas, the PCR assay is rapid and can be carried out after a short enrichment period.