RESEARCH ABSTRACT
PORK SAFETY

**Title**: Novel molecular approaches for the rapid detection of *Salmonella* from pork products and the pork processing environment- **NPB# 08-007**

**Investigator:** Doris H. D'Souza;

**Institution**: The University of Tennessee-Knoxville

**Co- Investigator:** F. Ann Draughon

**Date Submitted:** August 31, 2009

**Scientific Abstract:**

Novel, rapid, and sensitive detection assays that can be routinely used to prevent the transmission of *Salmonella* in the pork industry remain in high-demand. Our objectives were to utilize novel assays such as reverse-transcriptase Loop-mediated Isothermal Amplification (RT-LAMP) and real-time reverse-transcriptase Polymerase Chain Reaction (RT-PCR) that have improved speed for comparison to traditional cultural methods for *Salmonella* Typhimurium detection from the pork environment. The RT-LAMP assay has advantages over RT-PCR in that it does not require expensive equipment such as a real-time PCR machine as the reaction occurs in a waterbath at one temperature and detection is by turbidity or fluorescence after 2 h of amplification. Both, RT-LAMP and RT-PCR assays are based on the chromosomally located *inv*A (invasion A) gene specific for the detection of *Salmonella* enterica species and are based on mRNA detection, rather than DNA, that have increased potential of detecting viable cells. These assays were optimized and applied to pork products and the pork environment. Twenty-five gram pork chop, pork sausage, and ground pork samples were spiked with *S.* Typhimurium at high ($10^8$ to $10^6$ CFU) and low ($10^5$ to $10^0$ CFU) inocula levels. Carcass rinse samples (500 ml; after concentration through sequential filtration) and carcass swabs (100 $cm^2$) were spiked with only high inocula levels. Samples were stomached in 225 ml of Tetrathionate broth (TTB) and portions were serially diluted and plated on XLT4 agar for traditional cultural assays. RNA was extracted from 10 ml samples using the TRIzol® method and Qiagen Mini-kit, treated with DNAse I to remove any carryover DNA, and assayed.

The Trizol® method provided better RNA quality and yields than the Qiagen method. The RT-PCR assay using the SYBR Green I one-step RT-PCR Invitrogen kit gave the expected amplification product of 347 bp with a melt temperature (Tm) of 87.5°C, with detection sensitivities of $10^2$ CFU/ml for pure culture *S.* Typhimurium. Spiking of pork products with high inocula *S.* Typhimurium showed detection of $10^6$ CFU/25g *S.* Typhimurium within one day. For the low inocula levels, selective enrichment in TTB for 10 h was necessary to obtain detection of $10^1$CFU/25g for pork chop and pork sausage, which required 2 work shifts. To further ensure the absence of false negatives due to product inhibition, enzyme failure or instrument malfunction, each RT-PCR assay had an internal amplification control (IAC) that gave a product of 154 bp and a Tm of 82°C, with the same primers in the

assay. Un-inoculated pork products and sterile water (negative controls) did not show the amplified product by real-time PCR or gel electrophoresis, as expected. Background flora and autoclaved *Salmonella* cultures did not show any positive results from inoculated pork samples. Pork products spiked with cold stressed cells to simulate conditions of storage and transport, required pre-enrichment in buffered peptone water for 3 h followed by selective enrichment in TTB for 10 h to detect up to $10^1$CFU/ 25g.

Using previously described primers, the LAMP assay was developed into a RT-LAMP assay that gave detection sensitivities of $10^1$CFU/ml after gel electrophoresis for *S*. Typhimurium in pure culture. Spiked pork chop and pork sausage without enrichment gave detection sensitivities of $10^6$ CFU/25g similar to traditional plating and RT-PCR. However, even though this assay is faster, the lower inocula levels required selective enrichment for 10 h, to obtain detection limits of $10^2$ CFU/25g which was 1-log less sensitive than RT-PCR and traditional plating. Negative controls, autoclaved cells and background flora from inoculated samples did not show any positive results, eliminating false positives. Samples spiked with cold stressed cells gave detection limits of ~$10^2$ CFU/25g, after pre-enrichment and selective enrichment. Screening of 57 natural samples from pork processing facilities and grocery stores resulted in 9 positives by traditional cultural plating, 5 positives by RT-PCR and 10 positives RT-LAMP assays. These results indicate that RT-LAMP has potential to be applied in routine testing schemes for the detection of *Salmonella* in pork products within two 8 h work shifts, being faster and simpler than RT-PCR and traditional cultural assays, but less sensitive by 1-log CFU. Further work using fluorescence dyes is necessary to improve detection sensitivity and to convert the RT-LAMP to a quantitative assay by using automated portable devices, which will further simplify this assay for routine testing by the pork industry.