Executive summary

Project title: Cost Effective Treatments to Minimize *Listeria monocytogenes* Cross Contamination of Ready-To-Eat Meats by the In-Store Deli Meat Slicer, Phase II

Principal investigators:
Phil Crandall, Ph.D.
John Marcy, Ph.D.
Steve Ricke, Ph.D.
Corliss O'Bryan, Ph.D.
Betty Martin, Ph.D.
Research Institution:
University of Arkansas, Fayetteville, Arkansas 72704.

Final report submitted: 27 May 2011

Objectives:

a) Use a simple, enhanced visual verification system to insure that food contact surfaces of deli slicers are "clean to sight and touch" using a food grade or fluorescent dye.

b) Evaluate commercial cleaning and sanitizing compounds and cloths against strains of Lm recovered from the actual operating environment of retail delis.

c) Optimize lethal treatments to achieve a 5 log reduction of Lm on actual deli slicers' food contact surfaces.

d) Evaluate the "Best Practices" for cleaning and exposing deli meat slicers to a lethal treatment step under commercial deli conditions and in cooperation with commercial deli managers.

Conclusions:

a) Red food dye was effective in locating potential contamination of meat and fat on the deli food surfaces. Glo Germ™ could be used as tool in training deli employees on the ease of spreading contaminants throughout the deli environment.

b) A common 100% terry cloth towel gave the greatest log reduction of *Listeria* contamination when using Barrier II as the sanitizer. Commonly used wipes containing disinfectant were not as effective. Additional testing is under way with industrial support.

c) We are continuing to interact with local deli managers to develop a "mock deli" training center and develop training modules for managers and employees.

Deliverable: Effective measures to reduce or eliminate cross contamination at the retail level to protect consumers from foodborne listeriosis.

Technical abstract:

We hired an engineer to support our efforts on maximizing the transfer into the deli meat slicer. We have two reports from Ready-to-Eat luncheon meat manufacturers that this is definitely the direction we need to go. We have a vision of how to deliver a lethal treatment to residual *Listeria* in harborages on the slicer while protecting the electrical components. We have leveraged our AMIF funding with the submission of a USDA NIFSI grant to train minority deli employees and expand the research we are currently conducting in this AMIF funded Phase II research and seeking additional funding to take these findings to market.

Objectives:

Objective a:

Develop a visual verification system to insure that food contact surfaces are "clean to sight and touch". Our aim was to develop a simple visual system to indicate to users that the food contact surfaces are "clean to sight and touch"

During Phase I of this project, it was determined that an inexpensive food grade red food dye could verify contamination on the deli slicer and its components. For phase 2 we have chosen a food-safe fluorescent dye (Glo Germ).

In cooperation with Elliot Ryser, MSU, using a fluorescent indicator, FI, it was possible to identify 5 product contact surfaces on the deli slicer that were cross-contaminated following the slicing of Glo Germ contaminated cooked, RTE turkey chubs (Vorst et al, 2004).

Conclusions:

We have tested the potential transfer of pathogens and other contaminants using Glo Germ™. A ready-to-eat (RTE) bologna log was coated with Glo Germ. The transfer of Glo Germ™ from the log to gloves and different parts of deli slicer was obvious, using a black light to illuminate the presence of the Glo Germ™ fluorescence. Additional testing will leverage these results as part of our NIFSI project.

Deliverable:

Glo Germ™ could be used as tool in training deli employees on the ease of spreading contaminants (possibly pathogenic bacteria) throughout the deli environment. The visual effects of the Glo Germ™ presence are an important training tool, not only in determining the possible transfer of deli contaminants but also in determining the thoroughness of equipment cleaning and personal hygiene. Products such as Glo Germ™ and similar products would be very important in the training and retraining of all deli personnel.

Objective b.

Improve cleaning and sanitation methods to insure the effectiveness in removing *Listeria* and *Listeria* biofilms.

Conclusions:

We have shown that Barrier II sanitizer (which is currently used in our cooperating delis) was the most effective sanitizer for use in the deli. These tests involved using strains of *Listeria monocytogenes* that were implicated in listeriosis outbreaks, and also *Listeria innocua* M1, that is known for its persistence. Barrier II was used along with various wiping and sanitizing cloths that are in use in the delis, including 100% terry cloth, and two microfiber cloths manufactured in Germany, Tectronic microfiber cloth and Softronic microfiber cloth. From that study it was determined that the 100% terry cloth gave the greatest log reduction of *Listeria* contamination when using Barrier II as the sanitizer.

In a continuation of this objective we are continuing this research with a commercial manufacture of cellulosic/cotton towels. In these studies, the *Listeria* "cocktail" that has been described was used to inoculate Formica and/or stainless steel surfaces. The cloths studied were cellulosic/cotton, non-woven, terry, and microfiber, used slightly dampened by sterile water. Results from these studies indicated that the cellulosic/cotton cloths and the microfiber cloths gave the highest log reduction of *Listeria* from the inoculated surfaces. We are continuing with such projects that will involve incorporating sanitizers. This AMI grant helped in developing the proper protocol for these studies. Manuscripts are being written and will be submitted to refereed journals for publication.

Objective c.

It was determined that cleaning and sanitizing the deli components along with a lethal treatment significantly reduced the Lm/Li contamination by greater than 5 logs. We hypothesized that the combination of cleaning/sanitizing along with a lethal treatment would aid in the elimination of Lm/Li. From this study it was shown that with initial inoculum levels on the coupons was 5.7 log/cm^2, that after lethal treatment there levels of Lm were below the detection limit.

Bacterial Strains

One strain of *Listeria innocua*, M1 (Li), a known heat resistant strain and six strains of *L. monocytogenes*, obtained from Cornell University: LM27 (4b), LM 98 (1/2 c), LM187 (4b), LM 189 (1/2 a), LM 190 (1/2 a), and LM 191 (1/2 a) were used in the testing. A cocktail for inoculation was prepared by placing 1mL of each culture in a single sterile tube and vortexing to mix the cocktail.

Intact deli component

An aluminum deli slicer chute was marked off into 2 cm^2 gridded areas using permanent markers.

Aluminum and stainless steel coupons

The stainless steel blade of a Hobart heavy duty slicer was cut into 2 X 2.5 cm coupons, using a Flow Waterjet Cutting System. Cast aluminum coupons (2 X 2 X 0.5 cm) were cut using a Milwaukee Heavy-Duty metal cold-cutting metal saw and a Wellsaw metal-cutting band saw.

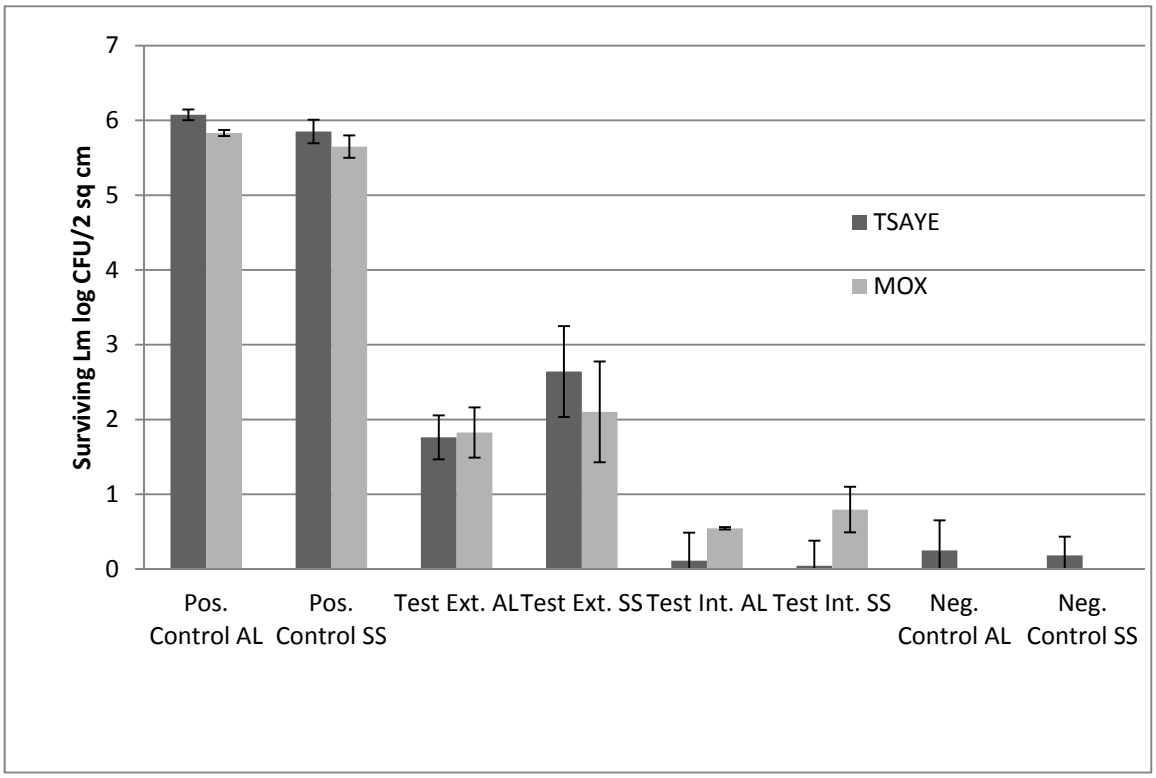
Inoculation of Components and Coupons

Gridded areas of the blade chute were inoculated with 100 µL of the Lm/Li cocktail. Inocula were allowed to dry for 2 h in a Biosafety cabinet.

Sterile coupons were placed in a Petri dish and inoculated with 100 µL of the concentrated cocktail using a pipette. The inocula were allowed to air dry for 2 h.

Sampling after Thermal Inactivation

After each lethal treatment, gridded areas on the chute component or coupons were swabbed with sterile cotton swabs saturated with phosphate buffered saline (PBS). Each saturated swab was placed in 9 mL of PBS then serially diluted and plated onto Tryptic soy agar and modified Oxford agar (MOX) and incubated at 37 °C for 24 h and 48 h respectively. Plates were enumerated and data was recorded and analyzed.



Thermal inactivation of *Listeria* on deli slicer aluminum (AL) and stainless steel (SS) coupons, inoculated with 100 µL concentrated cocktail (7 mL cocktail, centrifuged and reduced to 1.5 mL) Graph shows surviving Lm/Li colonies for positive controls (Pos. Controls), Tests samples positioned on shelves (Test Exterior), Test samples positioned inside the compartment (Test Internal) and Negative controls (Neg. Control). Down arrows indicate that no detection at the dilution.

Deliverable:

There is a need for procedures that go beyond the daily routines of cleaning and sanitizing the deli surfaces to aid in the reduction/elimination of Lm that might contaminate these surfaces. The results from this research indicate using lethal treatment resulted in a 4-5 log reduction of Lm/Li inoculated onto the deli slicer surfaces.

Conclusions:

We are continuing to work with deli managers in our cooperating stores to develop modules for training of deli workers. We had obtained additional support for this objective from USDA.

Presentations and Publications:

1. Thermal Inactivation of *Listeria innocua* as a surrogate for *Listeria monocytogenes* on Deli Slicer Components. Elizabeth M. Martin, Sean Pendleton, Erin Shannon, Sara R. Milillo, Philip G. Crandall, Steven C. Ricke, Michael G. Johnson, Corliss A. O'Bryan, and John A. Marcy. Poster presentation at annual American Society of Microbiology (ASM) in Philadelphia 2009.
2. Dry Heat Thermal Inactivation of *Listeria innocua* on Deli Slicer Components. Crandall, P.G., O'Bryan, C.A., Martin, E.M., Pendleton, S., Shannon, E., Marcy, J., Ricke, S. C. (2010) *Food Protection Trends, Vol. 30, No. 9, Pages 588–592*
3. Efficacy of cleaning and sanitizing agents against surface attached *Listeria monocytogenes* on deli meat slicer components. Crandall, P.G., O'Bryan, C.A., Martin, E.M., Kuefner, H.M., Pendleton, S., Shannon, E.M., Marcy, J.A., Ricke, S.C. Submitted to Food Protection Trends.