The 2019 Annual Meeting of the International Association for Food Protection (IAFP) took place in Louisville, Kentucky, USA from 21-24th July. International Food & Meat Topics takes a look at some of the current research being undertaken.

Each year the IAFP hosts a meeting which provides attendees with information on current and emerging food safety issues, the latest science, innovative solutions to new and recurring problems, and the opportunity to network with thousands of food safety professionals from around the globe. Held in various locations throughout North America, this meeting has grown over the years to become the leading food safety conference worldwide.

Isolation and assessment of poultry-derived LAB

Alyxandra Reed, Amy Mann and Henk den Bakker
Center for Food Safety, University of Georgia, Griffin, GA, USA.

Lactic acid bacteria (LAB)-based probiotics have been in high demand within the past few years due to their potential positive effects on host health and ability to reduce foodborne pathogens in final products (poultry meat). Research on probiotics is key to understanding if and how probiotics can increase food safety.

● **Purpose:**
The purpose of this study is to identify poultry-derived, host-specific LABs for use as probiotics in the poultry industry.

● **Methods:**
Caecal and ileal samples were taken from 15 production broilers from both 'Conventional' and 'No-Antibiotics-Ever' farms on day zero and 20 of the grow-out cycle (120 birds total). LABs were cultured using two different CO_2 levels (aerobic and elevated/5% CO_2) using MRS media. One LAB isolate was chosen per plate (278 total), Gram stained, and sequenced (16S rRNA and Whole Genome Sequencing).

Phylogenetic analyses of our WGS data and publicly available strains were used to test potential host specificity, and our WGS data was also used for identification of genomic elements (bacteriocins) within strains known to suppress the growth of foodborne pathogens.

● **Results:**
16S rRNA results from our isolates (n=100) identified 40 Lactobacilli (40%), specifically L. salivarius (37; 92.5%) and L. johnsonii (3; 7.5%) with the remaining 60 identified as Enterococcus faecalis (38), E. faecium (16), E. hirae (3), E. durans (2), and E. villorum (1).

Phylogenetic analysis of five of our L. salivarius strains and 88 L. salivarius strains in GenBank (n=93) determined that our strains were genetically diverse, and nested in a clade of poultry-associated isolates, suggesting host specificity. Two L. salivarius strains contain genes encoding bacteriocin-like peptides.

● **Significance:**
These initial results indicate that a small number of L. salivarius strains show promising characteristics for poultry-derived, host-specific probiotics. ■

Prevalence of top seven shiga toxin-producing E. coli in beef

Seong-san Kang, Joshua T. Ravensdale, Ranil Coorey, Gary A. Dykes and Robert Barlow, School of Public Health, Curtin University, Bentley, WA, and CSIRO, Agriculture & Food, Brisbane, QLD, Australia.

Australian beef processors must ensure that manufacturing beef being exported to North American markets are deemed free of top seven shiga toxin-producing Escherichia coli (STEC) serogroups. A better understanding of the microbiota of beef carcases, the abattoir environment and the dissemination of STEC in the abattoir could assist in determining the sites of cross-contamination of STEC through slaughter.

● **Purpose:**
Investigate changes in the microbiota and STEC presence throughout slaughter in an integrated and a fragmented Australian beef export abattoir.

● **Methods:**
Abattoir A (integrated) and B (fragmented) were each visited twice. At each visit 90 samples consisting of 10 faecal samples, 15 hides, 15 post-hide pull carcases, 15 post-evisceration carcases, 15 pre-chill carcases and 10 environmental samples were collected. Samples were assessed for total viable count (TVC), E. coli/coliforms, and traditional (stx, eae and O-antigens) and novel (espK and/or espV) STEC markers.

Culture confirmation was conducted on potential positive (PP) samples (stx+, eae+ and O-antigen+). In addition, samples were processed and analysed for 16S rRNA metagenomics.

● **Results:**
Potential positives were identified in 64-81% of samples across all visits. Additional novel STEC markers did not significantly ($P<0.05$) reduce the number of PPs. STEC were isolated from both abattoirs and were typically from hide and faecal samples.

However, greater frequency and diversity of STEC was observed in abattoir B. In abattoir B, TVC in post-hide pull carcases was significantly higher ($P<0.05$), though TVC in pre-chill carcases was significantly lower ($P<0.05$). The microbiota of carcases shared the greatest similarity with hide samples, however, carcases from abattoir A were notably influenced by environmental contaminants.

● **Significance:**
The microbiota and associated Top 7 STEC prevalence are linked to the hide contamination of incoming animals. Reducing the level of contamination on hides and the transfer onto carcases will result in safety and quality improvements. ■

Detection and prevalence of listeria in produce packing

Genevieve Sullivan and Martin Wiedmann
Cornell University, Ithaca, NY, USA.

Listeria monocytogenes contamination of produce can often be traced back to environmental sources in packing and fresh-cut operations. Environmental monitoring programs (EMP) are of increasing importance, as they are a key strategy for identifying environmental L. monocytogenes sources that could lead to finished product contamination.

● **Purpose:**
The goal of this project is to work with production operations to develop listeria EMPs in order to gather baseline data on listeria prevalence and distribution over one year.

● **Methods:**
We developed and implemented sampling plans in eight produce operations and collected samples (n=2000) over one year, a minimum of six times per facility. Each operation was visited at the end of year one for a validation sampling by an outside expert that had never seen the operation previously. Samples were tested using the FDA BAM method. SigB sequencing was used as a preliminary typing method.

● **Results:**
Among the sponge samples collected, there was a statistically significant ($P<0.05$) difference between positives observed during routine (43 of 2000) versus validation (26 of 392) samplings.

Additionally, 24 of 85 samples with more than one listeria isolate sequenced had more than one allelic type present.

We also found that 35 of 114 samples were found only to be positive at one enrichment time (24 versus 48 h). L. monocytogenes prevalence overall varied, from zero (<0.40%) of 249 to 11 (6.9%) of 159.

● **Significance:**
Our data indicate that the classical culture-based methods that plate after two different enrichment times provide enhanced sensitivity, and that collection of multiple isolates is necessary to capture listeria diversity present.

The results also show the value of validation sampling by an outside party, which can both increase confidence in results or identify potentially problematic sampling schemes. While our data cannot be accurately extrapolated to other operations, they indicate overall a need for robust sampling techniques and testing procedures and individualised sampling plans. ■

Hydrogel patches and Vibrio parahaemolyticus in raw fish

Hyemin Oh and Yohan Yoon
Sookmyung Women's University, Seoul, South Korea.

In raw fish consumed for sushi and sashimi, non-thermal decontamination technology needs to be applied.

Hydrogels composed of edible compounds and antimicrobials should be appropriate as non-thermal decontamination.

● **Purpose:**
This study developed an antimicrobial hydrogel patch, composed of edible and non-toxic compounds, to reduce Vibrio parahaemolyticus cell counts on slices of raw fish.

● **Methods:**
The alginate-based hydrogel was prepared by dissolving 5% sodium alginate powder in 25ml of distilled water, and mixing with copolymers [1% agar (w/w), 40% glycerol (w/w) and a crosslinker ($CaCl_2$)).

The hydrogel was cut into 3.0 x 3.0cm squares, and they were placed in 10ml of 0.5% and 1.0% natural antimicrobials (grapefruit seed extract and citrus extract) for two hours, followed by drying at room temperature for 30 minutes.

A 100-μl mixture (OD620=0.1) of V. parahaemolyticus strains was inoculated on slices of raw fish (halibut). The antimicrobial hydrogels were placed on the inoculated samples and stored at 4°C for 1, 20, 40, and 60 minutes. After storage, V. parahaemolyticus cell counts in the samples were enumerated on thiosulphate-citrate-bile salts-sucrose agar.

● **Results:**
V. parahaemolyticus cell counts reduced by 1.5 to 2.5 log CFU/cm^2 after the hydrogel application, regardless of antimicrobials. Among the natural antimicrobials, the antimicrobial hydrogel formulated with 0.5% and 1.0% grapefruit seed extract reduced the bacteria by 2.0 and 2.3 log CFU/cm^2 on slices of raw fish, respectively.

The antimicrobial hydrogel formulated with 0.5% and 1.0% citrus extract decreased the cell counts by 2.0 and 2.1 log CFU/cm^2, respectively.

Thus, the antimicrobial hydrogel composed of 5% alginate, 0.2% $CaCl_2$, 1% agar, and 0.5% grapefruit seed extract was the most appropriate.

● **Significance:**
These results indicate that the developed hydrogel can be used to control V. parahaemolyticus on slices of raw fish by one-minute application on the surface. ■

Toxoplasma gondii survival while cooking and freezing fresh cut meats

Surabhi Rani, Jitender P. Dubey and Abani Pradhan
University of Maryland, US Department of Agriculture, Agricultural Research Service, Beltsville, MD, USA.

Toxoplasma gondii is a widespread zoonotic parasite with high seroprevalence in the human population. More than 40 million people carry this parasite in the United States.

Consumption of raw or undercooked meat containing T. gondii tissue cysts is a major source of infection in humans.

Cooking and freezing processes are the most important steps to inactivate T. gondii in meat.

● **Purpose:**
Current available data are not sufficient to suggest safe cooking or storage temperatures and holding time for fresh cut meats. Consumer preferences of cooking and storing meats introduce variability. The main objective of this study was to analyse uncertainty and variability in the survival pattern of T. gondii tissue cysts in fresh meat after cooking and freezing processes.

● **Methods:**
In the United States, there has been only two studies done on heat inactivation of T. gondii tissue cysts and one on its inactivation by freezing.

Data regarding survival time of tissue cysts and treatment temperature were collected from those studies and were resampled with a bootstrapping method in R software.

Monte Carlo simulation was used to quantitatively simulate uncertainty and variability associated with parameters.

● **Results:**
The results showed a negative correlation (r=-0.992) between cooking temperature and survival time for T. gondii, whereas positive correlation (r=0.9989) was observed between freezing temperature and time.

All correlations were highly significant at a P-value of 0.05.

Regression models were established with correlation coefficients and confidence intervals. The uncertainty level was further decreased by the jackknife-after-bootstrap method which identified outliers and narrowed down the bootstrap confidence interval by 16.7% for the cooking process.

● **Significance:**
This study could be helpful in validating the current USDA recommended minimum cooking temperature for fresh cut meats and establishing a survival module for T. gondii under a temperature gradient. ■

Evaluation of commercially available protective cultures

Catherine Gensler and Dennis D'Amico
University of Connecticut, Department of Animal Science, Storrs, CT, USA.

Listeria monocytogenes and Shiga toxin-producing Escherichia coli (STEC) are pathogens of concern in the production of cheese, including those manufactured from unpasteurised milk.

The use of protective cultures (PCs) capable of producing antimicrobial compounds in situ represents a potential biological control strategy compliant with current regulation.

● **Purpose:**
The purpose of this study was to evaluate two commercially available PCs of Lactococcus lactis and Hafnia alvei for the control of L. monocytogenes and STEC, respectively, in a surface mould-ripened soft cheese.

● **Methods:**
Lab scale soft-ripened cheeses were manufactured using unpasteurised milk inoculated with cocktails of either L. monocytogenes or STEC alone (control; two batches per pathogen) at ~two log CFU/mL or with one of two PCs at ~six log CFU/mL (two batches per treatment).

Cheese composition targets and culture selection allowed for an atypically long aging period without ripening prematurely. After de-hooping and dry salting, cheeses were ripened at 12°C and 93% relative humidity prior to cold storage. Samples were collected throughout cheesemaking, ripening, and storage. Pathogens in subsamples (two per cheese) were enumerated on CHROMagar Listeria or STEC.

● **Results:**
During manufacture L. monocytogenes counts did not increase during milk ripening, however, STEC counts increased by ~one log CFU/g. At day 21 mean counts in both STEC treatment and control cheeses decreased by ~one log CFU/g.

L. monocytogenes counts in cheese increased 1.43 and 0.65 log CFU/g in control and PC treated cheeses, respectively, from day one to 77; however, the change in counts over time was not significant (P=0.98).

● **Significance:**
These results identify the survival and growth of pertinent pathogens during the manufacture and aging of a soft-ripened cheese manufactured from unpasteurised milk.

The data suggest that some protective cultures available to producers may not provide an adequate level of pathogen control in this cheese type. Further work to validate the effectiveness of PCs in other cheeses of the differing composition is needed. ■