Technical Presentations (in alphabetical order by title)

Behavior of* Bacillus cereus *under Conditions Simulating the Proximal Gut

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Introduction: *Bacillus cereus* is a foodborne pathogen that can cause diarrhea through production of pore-forming enterotoxins in the ileum of the host. The mechanism of the toxico-infection is not evident, because *B. cereus* vegetative cells cannot survive gastric passage and the resistant spores cannot easily germinate and proliferate in the unfavorable intestinal biotic and abiotic environment. Even when growth occurs, the involved toxins are susceptible to the proteolytic enzymes secreted by the host or the competitive microbiota.

Purpose: We hypothesized that adhesion of *B. cereus* on the ileal mucosa may protect cells from the toxic effect of the intestinal slurry and preserve or enhance the activity of enterotoxins due to the direct and close contact of *B. cereus* with the eukaryotic site.

Methods: We have used an in vitro system to mimic the proximal gut conditions in order to investigate the behavior of *B. cereus* NVH 0500/00 in the stomach and small intestine. The total incubation period lasted 8 hours.

Results: A stepwise pH gradient from 5 to 2 for 2 hours (1 pH unit reduction per half an hour) representing the stomach resulted in a 2.5 log decrease in *B. cereus* concentration due to the death of most vegetative cells. pH values lower than 3 were those that severely influenced the resistance of the strain in the simulated stomach. The upper small intestinal phase (duodenum/jejunum) that started with the addition of bile and pancreatine (intestinal juice) at neutral pH initially did not have an effect on the bacterial counts probably because the spores that survived the acid stress were also resistant to these chemicals. After 2 hours, luminal bacteria started proliferating and completely recovered reaching the initial inoculum size within the next 4 hours of incubation at pH 7 (ileal lumen). The presence of mucin beads (ileal mucus) during this incubation stage did not influence the number of suspended cells but resulted in an overall 10% increase in the total *B. cereus* population due to the presence of adhered cells. Surprisingly, no enterotoxins could be detected in the lumen during the last ileal phase.

Significance: With this setup, it was not possible to offer any further insight in the mechanisms that rules *B. cereus* diarrhea. For this purpose, further optimization of the system is required involving the increase of the mucin to lumen ratio and the investigation of the possibility that enterotoxins produced by *B. cereus* adhered cells are actually trapped in the mucin mesh preventing potential denaturation.

Construction of the Analytical Platform for Food Safety Information in China

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Introduction: Food safety issues are becoming more frequent and the amount of related media reports have increased exponentially with the globalization of the food supply chain. In Mainland China, the massive media reports are chaotic and some of them are malicious. Customers are frightened by these sometimes groundless media reports and their panicky behaviors cause sizable economic loss. It is necessary to develop analytical tools for revealing the patterns of media publications in food safety on the internet for China.

Purpose: The primary purpose of this project is to collect and analyze Chinese food safety information scattered on the internet to reveal the related publication patterns in China. As a free information analytical platform, it will also facilitate the research in food safety communication.

Methods: A list of food safety sources in China was generated and a catalog of food safety keywords in Chinese was constructed. The techniques of web crawlers and Solr, an open-source software, were used to extract and index information from these sources. The number of keywords appearing in one piece of information is collated, calculated and stored with the information content in a database. An inquiry-web was built as the user interface using Java script . A series of functional plug-ins were developed based on the results of keyword statistics for data analysis.

Results: This analytical platform can be visited at this link: http://kwanlab.bio.cuhk.edu.hk/FS/. Three million pieces of food safety information has been collected and data can be searched by entering or selecting keywords. The line chart of the resulting information number is automatically generated and displayed in years. The frequencies of keywords in all resulting information are ranked for topic separation and further content analysis. The resulting information can also be sorted in terms of geography and source site.

Significance: It is an analytical platform for understanding the publication patterns of Chinese food safety information. Based on the results of subsequent research, the delivery efficiency of credible information will be increased in food safety incidents to relieve the unnecessary anxiety of the public.

Contamination of Bivalve Molluscs and Vegetables by the Protozoan Parasites* Cryptosporidium*, Giardia and Toxoplasma: Development and Validation of a Standardized Strategy of Detection and Characterization

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Introduction: The protozoa *Giardia intestinalis*, *Cryptosporidium* spp. and *Toxoplasma gondii* are major parasitic pathogens of humans and animals. *Giardia* cysts and *Cryptosporidium* and *Toxoplasma* oocysts are excreted in high quantities in the infected hosts' feces and may retain their infectivity for several months from land to sea. Raw materials in contact with water or submitted to intensive irrigation, such as vegetables and bivalve molluscs, are particularly at high risk of contamination. If data concerning the resistance capacities of these parasites in the environment are available, their

prevalence and behavior in complex food matrices are poorly known because of the lack of a standardized strategy of detection and characterization.

Purpose: In this context, we have recently set up the Protofood consortium in order to develop a complete strategy allowing the extraction, detection, and characterization of *Giardia*, *Cryptosporidium* and *Toxoplasma* parasites in bivalve molluscs and vegetables. This consortium has been developed in the framework of a national program of research (ANR-09-ALIA-2009).

Methods: We developed rapid tools for the simultaneous extraction, detection and characterization of the three parasites by using available or innovative immunomagnetic separation (IMS) techniques, or flotation methods, coupled to fluorescence microscopy and/or specific quantitative PCR assays.

Results: IMS methods proved to be particularly useful to extract parasites from relatively simple matrices, with detection limits of 5 *Toxoplasma* oocysts, 50 *Cryptosporidium* oocysts and 50 *Giardia* cysts achieved by qPCR. In case of mollusc homogenates, delipidation, proteolysis and qPCR allowed the detection of at least 25 *Cryptosporidium* oocysts and 250 *Toxoplasma* oocysts. In parallel, multiplex qPCR and RT-qPCR assays were developed and validated in order to detect and characterize the three parasites from experimentally contaminated raspberries and bivalve molluscs, and to evaluate their viability following inactivation treatment compared to gold standard bioassays.

Significance: These tools and methods will allow us (i) to study the bioaccumulation and depuration of parasitic protozoa in molluscs and their persistence at the surface of vegetables following exposure with contaminated water, and (ii) to characterize the efficiency of domestic cooking treatments applied to prepare bivalve molluscs. This work is supported by the French National Research Agency (grant ANR-09-ALIA-009), and the AQUIMER and PEIFL research clusters.

Detection of Enterotoxins Produced by Bacillus cereus Strains Involved in Food Poisoning Using MALDI-TOF Mass Spectrometry

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Introduction: *Bacillus cereus* is a foodborne pathogen that can cause diarrhea through secretion of enterotoxins, such as NHE and CytK, in the small intestine of the host.

Purpose: Detection of these enterotoxins is critical for successful microbial food safety risk assessment. Among the existing detection methods, serological assays appear to be the most promising, but their application is limited by the availability and the appropriate selection of antibodies. Available polyclonal kits for verification of NHE production lack specificity and frequently result in false negatives. At this moment, there are no commercial serological assays for detection of CytK. Therefore, alternative approaches need to be developed.

Methods: We have used MALDI-TOF MS and MS/MS to detect enterotoxins in four pure *B. cereus* cultures using tryptic digests of proteins separated by SDS-PAGE. The selected strains have been involved in foodborne outbreaks.

Results: CytK1, a rare but lethal toxin, was detected in *B. cereus* NVH 0391/98, and the fully cleaved tryptic peptides ANPTLSDAPVDGYPIPGASVTLR and TYPHETDAR are selected as MS biomarkers due to their specificity and abundance in the mass spectra. Forty-five different peptides from all tested strains were matched to the NheA component of the NHE complex, a toxin genetically present in all *B. cereus* strains. Only three of them were common and abundant in the four strains, i.e., QKELLPLIQK, EWIDEYNPK and LIDLNQEMMR, but they were either incompletely digested or susceptible to modification. These characteristics obscured their application as universal markers, thus detection of NheA in unknown samples should be based on the simultaneous identification of other enterotoxin specific peptides. Our results demonstrated that NheA from *B. cereus* NVH 0391/98 was very heterogeneous compared to that of the other strains, explaining the failed previous attempts to detect this toxin through antibodies or PCR primers. Our method was comparable with the commercial immunological assay in respect to detection of NheA produced by *B. cereus* NVH 0075/95, but the latter technique is based on polyclonal antibodies which can also react with other proteins, hence limiting its specificity.

Significance: We showed that MALDI-TOF mass spectrometry is a powerful tool for detection of enterotoxins because its application is not restricted by toxin sequence diversity among different strains. The recommended MS biomarkers will be used for the screening of environmental and food associated strains that can be involved in food poisoning.

Development and Application of Immuno-tools for the Analysis of Non-O157 Shiga Toxin-producing* Escherichia coli *(STEC) in Raw Beef

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Introduction: Worldwide, *Escherichia coli* O157:H7 is the most common Shiga toxin-producing *E. coli* (STEC) associated with human illness. However, non-O157 STEC are becoming increasingly important and recently, the U.S. Department of Agriculture-FSIS has begun regulating serogroups O26, O45, O103, O111, O121, and O145 in raw beef as adulterants. Current methods for non-O157 STEC detection in raw beef are complex and expensive and ultimately require bacterial isolation and colony confirmation. In order to simplify this, we have developed multiplexed lateral flow devices (LFDs) and immune-magnetic separation (IMS) reagents for sample screening, isolation, and confirmation.

Purpose: The purpose of this study was to develop and apply new antibody-based tools including multiplexed LFDs and IMS reagents for the detection of non-O157 STEC in raw beef.

Methods: Affinity-purified polyclonal antibodies were developed against *E. coli* serogroups O26, O45, O103, O111, O121, and O145 and incorporated into multiplexed LFDs and IMS reagents. The LFDs were tested against a panel of 29 STECs and 34 non-STEC bacteria for sensitivity and specificity. The IMS reagents were tested both in mixed culture and beef enrichments for recovery of the respective non-O157 STEC. The methods were applied to the analysis of raw beef either as a screening method or for isolation and confirmation.

Results: The LFDs demonstrated 100% sensitivity and 100% specificity for the detection of non-O157 STECs. The IMS reagents showed near 100% recovery of the respective STEC in spike-recovery studies in complex matrices. Serogroup screening of raw beef samples (100) showed that over 50% of the samples were positive for at least one of the non-O157 STEC. Serogroups O45, O103, and O145 were the most prevalent found in the samples tested.

Significance: Application of these immuno-tools to the analysis of non-O157 STECs in raw beef products should streamline process and regulatory monitoring. Therefore, by increasing sample analysis throughput, the risk of contaminated product entering into commerce will be minimized.

Development and Validation of Antibody-based Test Kits for the Detection of Allergens in Wine

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Introduction: Today's industrial-scale wine production demands for high standards regarding food safety in the production process. For instance, the process of fining constitutes a potential risk to introduce allergens into wine. There, proteins like albumin from egg white, milk casein and gelatin are added to remove undesirable phenolic compounds and at the same time reduce bitterness and astringency. New legislation implemented 1st July 2012 (EC Regulation 579/2012) requires the labeling of egg and milk protein residues that may be used in wine fining procedures and that are still present at a detection level of 0.25 mg/L (ppm) or greater.

Purpose: In this study ELISA test kits and lateral flow devices were challenged to see if they meet these specifications and are therefore suitable tools for allergen management in wine production.

Methods: Recovery was determined by spiking experiments on four different wines.

Results: It could be shown that both ELISA kits for ovalbumin and lysozyme are capable of detecting allergens down to 0.04 ppm. Furthermore, due to a small adaption in the protocol, limit of detection of both ELISA kits for casein and egg could be improved to levels down to 0.1 ppm and thus are now suitable for detection of low levels allergens in wine as well. If lateral flow tests for casein and egg are used in conjunction with their respective modified extraction buffers they both meet the above specifications for analysis of allergenic proteins in wine. Down to 0.2 ppm casein or egg could be recovered from various wine samples.

Significance: Summarizing the Allergen test kits validated meet the strict thresholds defined by the European Union and are therefore suitable tools detecting allergens in wine samples at low concentrations.

Diagnosing and Improving Food Safety Culture in Food Businesses

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Introduction: This work was commissioned by the UK's Food Standards Agency (the Agency). Food safety culture has been given greater attention recently due to increased interest in the role of business attitudes in achieving compliance and avoiding food poisoning. An outbreak of *Escherichia coli* O157 in South Wales in 2005 highlighted the issue of cultures and behaviours in businesses and enforcement bodies and its role in influencing compliance with food hygiene legislation. The outbreak – the largest ever incidence of *E. coli* O157 in Wales and the second largest in the UK – affected more than 150 people, most of whom were children in 44 schools. 31 people were admitted to hospital and a five-year-old boy died. Similar lessons have been learned from incidents overseas. For example, in the USA a peanut corporation was responsible for a *Salmonella* outbreak, which affected 3,000 companies and resulted in 9 deaths and 4,000 recalls. They had been audited and given a high rating. The failure was attributed in part to its food safety culture. Food safety culture is viewed as how and what the employees in an organization think about food safety and the food safety behaviours that they demonstrate. From a cultural perspective, employees will learn these thoughts and behaviours from other people in the organization. These thoughts and behaviours cascade throughout the organization and thereby have a sustained influence on people's performance – whether this is for better or worse.

Purpose: This work developed a tool for use in identifying aspects of good/poor safety cultures in food businesses, for use with all sizes of businesses, and linking this to advice on how to improve food safety culture in food businesses.

Methods: The first stage of work reviewed existing safety culture assessment tools. A total of 169 questionnaires and tools were identified. A large number of these were variations of safety climate questionnaires and had been used in safety culture research. Fifteen toolkits/questionnaires were shortlisted for potential inclusion in the detailed review. The review of the current tools noted that: a) Many of the existing safety culture tools have some form of validation, most notably construct validity; b) None of the tools had been developed specifically to assess food safety culture or specifically for application to micro or small firms; c) A large majority of the tools are diagnostic in nature. These tools also exemplify a way of categorizing businesses' safety culture in a way that can be mapped on advice. Food safety culture research and the review of existing safety culture assessment techniques was used to develop an initial version of a food safety culture toolkit. The initial draft of the toolkit was reviewed in workshops by environmental health officers and food business operators.

Results: The final version of the toolkit included 5 categories of safety culture including calculative non-compliers, doubting compliers, dependent compliers, proactive compliers and leaders, and 8 elements:

1. Priorities and attitudes;
2. Food hygiene risk perceptions and knowledge;
3. Confidence in food hygiene systems;
4. Business ownership of food hygiene;
5. Competence, learning, training, knowledge etc.;
6. Leadership on food hygiene;
7. Employee engagement in review and development of food hygiene practices;
8. Communications and trust to engage in food hygiene and report issues.

The categories and elements were presented in a matrix, along with a body of guidance for inspectors on how to improve

food safety culture in businesses. The results of the assessment link to guidance, within the toolkit, on how to improve food safety culture within organizations.

Significance: This is the first ever food safety culture assessment tool. Future work may include piloting and testing of the tool.

Direct Detection of Foodborne Pathogens Using SPRi System during Enrichment Step

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Introduction: Foodborne pathogens caused many serious epidemics and highlighted the importance of better understanding of how pathogens can be detected under food processing conditions (salt addition and low temperature conservation notably). Furthermore, it's essential to dispose of sensitive (able to detect few bacteria) and reliable detection techniques to obtain results before shelving the food concerned.

Purpose: The aim of our study is to evaluate the SPRi system for detection of enterohemorrhagic *Escherichia coli* O157:H7 in food.

Methods: In order to detect *E. coli* O157:H7, we propose to use the surface plasmon resonance imaging (SPRi) carried out on an antibodies biochip. Our direct detection method consists in monitoring bacterial growth during enrichment step. It's a simple, label free, qualitative and quantitative technique based on the immuno-detection of bacteria. It's a good alternative method to time consuming actual reference techniques (culture or PCR) which need an enrichment step before analysis.

Results: SPRi process allows the specific detection of 8 CFU/ml in less than 7 h. Furthermore, bacterial pathogens are also reliably detected in food processing conditions and directly in food (milk and meat).

Significance: The SPRi used during the bacterial growth is a suitable detection tool for *E. coli* detection in food at different steps of processing and during storage, it represent a promising detection system to warn against bacterial pathogens. We acknowledge the Pasteur Institute (Centre de Ressources Biologiques de l'Institut Pasteur - Paris) for providing the *E. coli* O157:H7 CIP105917 strain. This work was supported by the "Nucléaire, Radiologique, Biologique et Chimique" program (Colitrack project) of the CEA.

Efficacy of Atmospheric Gas Plasma Treatment for the Control of* Listeria monocytogenes *on Salad Vegetables

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Introduction: *Listeria monocytogenes* has been frequently isolated from fresh vegetables which are consumed with minimally-processed packaging. Although those vegetables are kept at refrigerator temperatures, *L. monocytogenes* can grow and pose threats to human health. To control foodborne pathogens on minimally-processed vegetables, Atmospheric Gas Plasma (AGP) can be applied because AGP can damage cell walls and chromosomes of pathogens without thermal damage to the vegetables.

Purpose: This study was conducted to investigate the efficacy of AGP treatment for the control of *L. monocytogenes* on salad vegetables.

Methods: The cut leaves (40 mm × 40 mm square) of lettuce (*Luctica sativa*) and cabbage (*Brassica oleracea* var. capitata) were experimentally contaminated with *L. monocytogenes* NCTC11994 inocula (5.6 log CFU/g ± 0.1), before the AGP treatment. The applied voltage and the argon gas flow rate for plasma generation were kept constant at about 8.0 kV and 3.0 L/min. After treatment, the enumeration of *L. monocytogenes* was performed with quantitative real-time PCR.

Results: When the distance from the plasma generator to leaf-pieces was 60 mm, the 9 minute treatment by AGP was required to cause significant reduction in the *L. monocytogenes* count (1.4 log CFU/g). A 5-minute treatment could cause only a 0.3 log CFU/g reduction. When the treatment time was 9 minutes, with an increasing distance from 60 to 80 mm, the observed reductions decreased from 1.4 to 0.5 log CFU/g. No significant reduction was observed at 100 mm distance. On the other hand, decreasing the distance caused surface damage to leaves.

Significance: These results indicate that AGP at the appropriate time and distance is effective for the control of *L. monocytogenes* on salad vegetables. This study was supported by the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

Efficacy of Peroxyacetic Acid and Lactic Acid Washes on Removal of* Toxoplasma gondii *Oocysts from Blueberries

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Introduction: Protozoan parasite *Toxoplasma gondii* is an emerging food safety threat. One-third of the world's population is infected with *T. gondii*. Produce has been recognized as one of the vehicles for transmission of *T. gondii* oocysts. The oocyst is the most infectious stage of *T. gondii's* life cycle; therefore, an intervention targeting the oocyst is crucial in reducing the incidence of infection.

Purpose: This study was conducted to determine the effectiveness of using peroxyacetic acid and lactic acid washes to remove *T. gondii* oocysts inoculated on the surface of blueberries.

Methods: Blueberries (10 g, n = 5) were spot-inoculated with 1.2×10^5 *T. gondii* oocysts and votexed in 10 ml of 1% peroxyacetic acid, 2% lactic acid, or water for 2 minutes to simulate washing process. The oocyst removal rate was determined by dividing the number of removed oocysts with the number of inoculated oocysts.

Results: There were significant differences ($P < 0.05$) in the removal rates of *T. gondii* oocysts among the wash treatments. The peroxyacetic acid wash removed 69.6 ± 2.9% of the inoculated oocysts, followed by the lactic acid wash

with a removal rate of 53.7 ± 2.2%, while the water wash removed only 32.4 ± 2.4% of the oocysts. The appearance of blueberries was not altered following 1% peroxyacetic acid or 2% lactic acid washing treatment.

Significance: The results suggest that the 1% peroxyacetic acid wash is more effective than 2% lactic acid and water in removing *T. gondii* oocysts from the surfaces of blueberries. Peroxyacetic acid may be selected for the further procedure development in order to completely remove the parasites attached to the blueberry surfaces.

Endospore Inactivation in Liquid Foods by Pulsed Electric Fields – An Innovative Ultra-high Temperature Process

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Introduction: The intense process needed to inactivate spores in liquid foods causes a high loss of nutrients and sensorial properties. Thus, a novel method to inactivate spores is needed. Pulsed Electric Fields (PEF) has already been proposed as an alternative method for killing spores, but so far no proper process conditions have been found.

Purpose: The intention of this study was to investigate the inactivation of endospores by a combined thermal and PEF treatment.

Methods: Self-cultivated spores of *Bacillus subtilis* and commercial *Geobacillus stearothermophilus* spores with certified heat resistance were utilized. Both spore strains were suspended in saline water (5.3 mS/cm), skim milk (0.3%, fat; 5.3 mS/cm) and fresh prepared carrot juice (7.73 mS/cm). The combination of moderate preheating (70 to 90°C) and an insulated PEF-chamber combined with a holding tube (65 cm) and a heat exchanger for cooling, enabled a rapid heat up to 105 to 140°C (measured above the PEF chamber) within 92.2–368.9 µs. To compare the PEF process with a pure thermal inactivation, each spore suspension was heat treated in thin glass capillaries and D-values from 90 to 130°C and its corresponding z-values were calculated. For a comparison of the inactivation data, F-values for the temperature fields of both processes were calculated by using Comsol Multiphysics combined with a Matlab routine.

Results: A preheating of saline water to 70°C with a flow rate of 5 l/h, a frequency of 150 Hz and an energy input of 226.5 kJ/kg, resulted in a measured outlet temperature of 117°C and a 4.67 $\log_{10}$ inactivation of *Bacillus subtilis*. The thermal process with identical F-value caused only a 3.71 $\log_{10}$ inactivation. This synergism of moderate preheating and PEF was even more pronounced for *Geobacillus stearothermophilus* spores in saline water. A preheating to 95°C and an energy input of 144 kJ/kg resulted in an outlet temperature of 126°C and a 3.28 $\log_{10}$ inactivation, whereas nearly no inactivation (0.2 $\log_{10}$) was achieved during the thermal treatment.

Significance: The presented investigation suggests combination of heat and PEF as an alternative method for inactivating highly thermal resistant bacterial spores, such as the sterilization indicator spore former *Geobacillus stearothermophilus*, in liquid foods at continuous conditions. The advantage of the PEF treatment is besides the rapid heat up in µs that no dilution of the liquid product with e.g,. steam like in the UHT-process occurs. However, for an industrial scale application of this process for sterilization, optimization of the treatment chamber design is needed to reduce the occurring inhomogeneous temperature fields.

Evaluation of Resistance and Adaptability of Mono-species and Dual-species Biofilms of* Pseudomonas putida *and* Listeria monocytogenes *against Sublethal Concentration of Benzalkonium Chloride

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Introduction: In the majority of natural and man-made environments, microorganisms are usually associated with surfaces in complex multi-species biofilm communities. Microbial interactions are believed to play a key role in cell attachment and detachment from biofilms and the resistance of biofilm community members against antimicrobial treatments.

Purpose: The aim of this study was to evaluate the resistance and adaptability of mixed-culture biofilms of *Pseudomonas putida* and *Listeria monocytogenes* against a common industrial disinfectant (benzalkonium chloride, BAC) used in sub-lethal concentration.

Methods: 3 strains from each species were selected and left to develop biofilms on stainless steel (SS) coupons incubated at 18^{o}C for 10 days in periodically renewable Tryptone Soy Broth (TSB), under either mono- or dual-species conditions. Each day, SS coupons were exposed to disinfection treatment (50 ppm of BAC).

Results: The simultaneous presence of *L. monocytogenes* strongly increased resistance of *P. putida* biofilm cells to BAC. Interestingly, BAC mainly killed *L. monocytogenes* cells when this was applied against the dual-species sessile community and as thus, following disinfection with BAC, this community was mainly composed of *P. putida* cells. No adaptation to BAC was observed in either *P. putida* or *L. monocytogenes* biofilm cells. Interestingly, pulsed field gel electrophoresis (PFGE) analysis clearly showed that the three *L. monocytogenes* strains did not contribute to the same level neither at the formation of the mixed culture sessile communities (mono-/dual-species), nor at their antimicrobial recalcitrance.

Significance: The results presented highlight the impact of microbial interactions taking place inside a dual species sessile community on both its population dynamics and chemical disinfection resistance. Knowledge on the physiological behaviour of multi-species biofilm communities formed by bacterial pathogens on abiotic surfaces in food processing environments could provide the information necessary to prevent their formation and therefore reduce the contamination of food products and transmission of diseases. This work was partly financially supported by the European Union project (ProSafeBeef) within the 6th Framework Programme (ref. Food-CT-2006-36241).

Exposure of* Escherichia coli *O157:H7 to Soil, Manure or Water Influences Persistence of that Pathogen on Plants and Initiation of Plant Defense

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Introduction: Bacteria used in challenge studies to determine behavior on plants are cultured using a wide range of conditions which can influence the outcome of the study. In this study, we investigated whether growth medium or exposure conditions influence the bacterial capsular polysaccharides (CPS) of *Escherichia coli* O157:H7, and whether changes in CPS impact plant defense responses, consequently affecting survival on plants.

Purpose: The objectives of this study were to investigate whether growth medium or exposure to carrier vehicle environments influence the bacterial CPS, and whether that precipitates changes in plant defense responses, consequently affecting bacterial survival on plants.

Methods: Bacteria were cultured in Luria-Bertani broth supplemented with manure or soil extracts. In other experiments bacteria were exposed to soil or manure extracts. Arabidopsis thaliana ecotype Columbia (Col-0) wild-type (CS 70000) and Col-0 transgenic line (BGL2::GUS) were used. BGL2-GUS transgenic plants contain a β-glucuronidase reporter gene (GUS) driven by the β-1,3-glucanase (BGL2) promoter. Plants were challenged with the bacteria and change or differences in bacterial population determined by plating.

Results: *Escherichia coli* O157:H7 grown in Luria-Bertani (LB) broth supplemented with manure extracts showed an approximately 58% increase in CPS production compared to cells grown in LB medium alone. Levels of CPS were significantly higher for *E. coli* O157:H7 cells exposed to soil or manure extracts as compared to the non-exposed LB cultured control. Arabidopsis thaliana plants expressing β-glucuronidase (GUS) under the control of the β-1,3-glucanase (BGL2) promoter were used to investigate whether *E. coli* O157:H7 induces defense-related gene expression. Plants inoculated with *E. coli* O157:H7 grown in LB containing manure extracts or cells exposed to manure extracts exhibited 3-fold and 2-fold lower GUS activity, respectively, suggesting a limited plant defense response compared to plants inoculated with cells grown in LB. On day 5 post inoculation, the population of *E. coli* O157:H7 grown in LB supplemented with manure on plants was significantly greater than the population of *E. coli* O157:H7 grown in LB medium alone. *E. coli* O157:H7 cells exposed to soil or manure exhibited greater survival on plants compared to LB-grown *E. coli* O157:H7.

Significance: In this study, we showed that capsular polysaccharide production of *E. coli* O157:H7 was significantly affected by the growth medium. These findings suggest that CPS of *E. coli* O157:H7 may enable the human pathogen to evade the plant defense responses by possibly masking the PAMPs, and resulting in increased survival of the enteric pathogen on plants. This has important implications in the safety of crops intended for human consumption. Results also underscore how bacterial culture conditions can impact experimental results, ultimately leading to conflicting results between two seemingly similar studies. Research on bacterial CPS and the plant defense response is needed to better understand human pathogen-plant interaction(s) to enhance the microbial safety of produce.

Flow Cytometric Study of the Sanitizer-Induced Viable But Non-culturable State in* Escherichia coli *in Orange Juice

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Introduction: Acidic fruit juices have been implicated in outbreaks of highly acid-resistant *Escherichia coli* O157:H7, hence effective sanitization of fruit surfaces is crucial for reducing the risks imposed by this pathogen. However, it has been shown that treating *E. coli* with sanitizers could induce viable but non-culturable (VBNC) state, which plating techniques are unable to detect. In contrast, flow cytometry (FCM) can be used not only for monitoring the viability and morphology of cells but also for detection of VBNC state when used in conjunction with plating.

Purpose: The main objectives were to use FCM for detection of sanitizer-induced VBNC *E. coli* in orange juice (OJ) and investigating the antimicrobial potential of commonly used sanitizers.

Methods: Log-phase *E. coli* K-12 MG1655 was washed with different concentrations of six food-grade sanitizers after which 2×10^8 CFU mL^{-1} was added to 1.2 μm filtered OJ. The viability and culturability of cells were subsequently investigated during 14 days storage at 4°C using FCM (propidium iodide/bis-oxonol viability staining) and plating (recovery on nutrient agar plates) techniques respectively. Results were statistically analyzed using the Mann-Whitney test.

Results: FCM results demonstrated an inverse relationship between concentration of sanitizers (e.g. hydrogen peroxide or sodium hypochlorite) and viability of *E. coli* in OJ. Nevertheless, higher concentrations of sanitizer resulted in a significantly greater number of VBNC cells (both $P < 0.001$). For instance, while FCM and plating results for cells treated with 1 or 2.5% hydrogen peroxide were comparatively similar (0.23 ± 0.20 Log_{10}), increasing the concentration to 5% significantly increased the number of VBNC cells (2.11 ± 0.54 Log_{10}) ($P < 0.001$).

Significance: The results confirmed the hypothesis that consecutive subjection of *E. coli* to maximum legally permitted concentrations of sanitizers and OJ induces VBNC state. Furthermore, the data demonstrated successful application of FCM for monitoring the efficacy of washing procedures. BBSRC funding is acknowledged.

Foodborne Viruses: Integration of the Viral Risk in the HACCP Plan of a Food Company

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Introduction: Foodborne viruses are recognized as the major cause of foodborne diseases. A large number of outbreaks have implicated soft fruits.

Purpose: Following several alerts and to face this food safety issue, a fruit company has decided to integrate viruses in its HACCP plan.

Methods: To set up the HACPP plan, several steps has been included:
- theoretical education of food safety managers.
- working with food safety managers to identify the critical points of the production and transformation processes.
- visits on the field with agricultural and food safety manager to confirm the identified critical points.
- analyses on fruits and water samples to set up the initial level of contamination.

Results: The potential sources of contamination were: food handlers and environment (irrigation water, phytosanitary treatments and process water). Identified sources of contamination were confirmed by norovirus analyses:

level of contamination ranging from 104 to 107 genome copies/L in water and from 100 to 104 genome copies/25 g in fruits. The complete details of corrective actions put in place will be detailed. Briefly, to educate operators, actions have been implemented, such as the creation of a fact sheet detailing the problem and the importance of good hygiene practices. Restrooms near the fields were installed and wearing gloves set for manipulators. A control plan has been set up to evaluate the persistence of norovirus in environmental samples before use in production and on the fruits after harvest and before transformation. In case of European alert, a communication plan has been established to inform the company and track the potential origin of contamination.

Significance: Implementing actions has helped to reduce effectively norovirus risk as no contamination has been found in the products since that time. These data demonstrate that by introduction of simple actions, it is possible to integrate the viral risk in a HACCP plan to ensure consumer safety.

Food Safety Knowledge among Persons Living with AIDS and Design of an Educational Intervention

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Introduction: Persons living with AIDS who have CD4 T-lymphocyte counts below 200 cells per cubic mL are highly vulnerable to foodborne enteric infections with the potential for substantial morbidity and mortality.

Purpose: Little is known about the food safety knowledge of this highly vulnerable population. The objectives of this study were to determine food safety knowledge among persons living with AIDS and create an educational comic book that targets their knowledge gaps.

Methods: 268 AIDS patients in Chicago, New Orleans and Bayamon (Puerto Rico) were interviewed using a food safety survey in English and Spanish during April 2010 through July 2011. A response of "Not sure" was categorized as an incorrect response because it represented a lack of knowledge.

Results: The mean age was 44.7 years; 63 percent were male. The overall food safety score was 63 percent (range 28 to 93 percent). Among the findings, 38 percent of patients believed it was okay to eat eggs served loose or runny, 27 percent believed it was okay to eat store-bought hot dogs without heating them first, 40 percent did not know that eating unpasteurized cheese may get germs inside their body that could cause hospitalization and possibly death, and 40 percent would not throw away salad that had been splashed with raw chicken juice. Among the 260 patients that said that they eat at home, they were often responsible for cooking some or all of their food (37 percent all, 28 percent most, 18 percent sometimes, 7 percent rarely, and 8 percent never). An 18-page comic book was created derived from these data that brightly illustrated key food safety concepts sensitive to the low literacy level of many of the target population. With abundant illustrations, the comic book emphasized basic health literacy including the definition of pasteurization, the steps of hand hygiene, the definition and rationale for hepatitis A vaccination, and the importance of adherence to antiretroviral medication to preserve or improve immune system health, best food handling and cooking practices, circumstances to avoid, food preparations to avoid and what the health consequences may be.

Significance: These data demonstrate substantial knowledge gaps and behavioral risk related to acquisition of foodborne disease among AIDS patients. The data support the creation of educational material directed towards reducing foodborne illness risk by providing practical prevention information.

Friday 13th Risk Modelling: A New Risk Model of UV Irradiation for Potable Water

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Introduction: Ultraviolet (UV) irradiation for potable water is an alternative to widespread disinfection methods using chlorine. However, sudden and unexpected failure of UV irradiation can lead to enduring public health effects, with or without fatalities. Here a new risk analysis of UV irradiation for potable water is presented using Friday 13th risk modelling (Fr 13) and a comparison made with current risk methods.

Purpose: The aim was to gain an understanding of possible effects of stochastic (random) changes in plant parameters on plant behaviour. Failure is defined as unexpected survival of pathogenic *Escherichia coli*.

Methods: The analysis is based on a unit-operations model and experimental data derived from Ye (2007). A failure factor (p) is defined in terms of a design reduction and actual reduction in viable *E. coli* as affected by stochastic change. UV irradiation is simulated using a refined Monte Carlo sampling of plant parameters.

Results: Results show that with an overtreatment tolerance of 15% on the design reduction some 2.8% of all UV operations can unexpectedly fail. This translates, on average, to a failure nearly each month of continuous operation. This insight is not available from current risk methods, with or without sensitivity analyses.

Significance: The Fr 13 analysis is a significant advance on current risk methods because it produces all possible practical UV operations and outcomes. This quantitative insight can be used to assess re-design and targeted physical changes to UV plant for improved safety in operation.

Growth of* Listeria monocytogenes *in Presence of* Listeria innocua *during Traditional Detection Method

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Introduction: Numbers of published data suggest that detection of *Listeria monocytogenes* is interfered by occurrence of other *Listeria* species in food. In this study the problems of traditional standard detection method of *L. monocytogenes* (ISO 11290-1:1996) were investigated, focusing on the enrichment steps in Fraser broth.

Purpose: The growth of different mixtures of *L. monocytogenes* strains and *L. innocua* strain in enrichment steps was investigated.

Methods: Growth of *L. monocytogenes* co-cultured with *L. innocua* in Fraser enrichment broths was examined. Growth parameters were determined by DMFit software using Baranyi-model.

Results: When initial cell concentration of *L. innocua* was higher than that of *L. monocytogenes*, inhibition of the latter was observed. Based on the results it was concluded that one strain of *L. monocytogenes* was overgrown by *L. innocua* in Fraser enrichment steps. When their growth was monitored during co-culturing in hFB (half Fraser broth) the lag phase of one strain of *L. monocytogenes* was prolonged by 3.4 hours. *L. monocytogenes* grew in the presence of *L. innocua* in 1:1 ratio three log cycle difference was observed at the end of Fraser enrichment step.

Significance: It was demonstrated that *L. monocytogenes* could be inhibited during Fraser enrichment steps. All these problems might lead to false negative results. The work of A. Belak was supported by the European Union and Hungary in frame of TÁMOP 4.2.4.A/1-11-1-2012-0001 project.

High-pressure Sterilization (HPST) of Baby Food Puree and the Possible Reduction of Food Processing Contaminants

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Introduction: Due to the growing consumer demand for minimally processed low acid foods in recent years, HPST may offer an alternative to retort processing, and means by which safe high quality foods are achieved with lowered energy inputs. Until now, there has been nearly no data about a possible formation or reduction of unwanted and sometimes carcinogenic food processing contaminants (FPCs) such as furan and HMF by HPST has been published. It can be postulated that the lower temperatures and shorter dwell times, which result in reduced thermal load applied to the product, used in HPST compared to conventional retorting could give a lower formation of FPCs.

Purpose: Since the consumer group of this tested food system are infants. Therefore, it is important to reduce the risk of exposure to FPCs as much as possible.

Methods: To test this and to gain a deeper insight in the formation of FPC, the vegetable puree (suitable for baby food, recipe from Nestlé) was HPST treated. To establish suitable process conditions in the vegetable puree, and an ACES-buffer (pH 7, 0.05 M) were inoculated with two different spore strains, *Bacillus amyloliquefaciens* and *Geobacillus stearothermophilus*. The samples were treated at 600 MPa at 90°C to 121°C in a U111 high pressure system (Unipress Warsaw) for up to 30 minutes.

Results: The formation of furan was present in the baby food puree, whereby depending on the temperature, a reduction in comparison to the retort process of 80–99 % was possible. The most pressure resistant strain of the tested strains was *B. amyloliquefaciens.* This could become the target microorganism for the HPST and in this way help to implement this promising technology in the food industry.

Significance: The results of this study show that a reduction of FPCs is possible by this emerging technology. A reduction of the process time and temperature is also possible to achieve a high quality sterile vegetable puree by using HPST in comparison to the retorting process.

High-pressure Inactivation of the Shiga Toxin-producing* Escherichia coli *O104:H4 and O157:H7 Outbreak Strains

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Introduction: Each year, 75,000 cases of illnesses in the United States are associated with the enterohemorrhagic *Escherichia coli* (EHEC) strain O157:H7. In some cases EHEC infections are accompanied by the hemolytic uremic syndrome, like in the 2011 outbreak in Germany, which was caused by an unusual Shiga toxin-producing *E. coli* O104:H4 associated with the consumption of contaminated produce presumably sprouts.

Purpose: To inactivate EHEC on the surface of food, a mild heat treatment is the common method, which causes changes in color, flavor and texture of the processed product. To avoid these kinds of changes and to guarantee the consumers safety without chemical preservation, the application of high pressure is a potential alternative.

Methods: Within this study, we tested the pressure and temperature stability of an O157:H7 strain and the 2011 outbreak strain O104:H4 in pressure stable ACES buffer solution (pH7, 0.05 M) and carrot juice (pH 4.2) under isothermal isobaric conditions during the pressure dwell time. For all strains the heat (50 to 80°C) as well as the pressure resistance up to 500 MPa (20 to 60°C) was determined in triplicates. From the inactivation kinetics isorate inactivation diagrams in the pressure-temperature landscape were calculated by the Weibullian power law.

Results: Pure thermal inactivation data showed, that at least 60°C and 8 min are needed to inactivate 3.94 $\log_{10}$ (O157:H7) and 4.3 $\log_{10}$ (O104:H4) in ACES buffer. Pressures $\leq$ 200 MPa and temperatures $\leq$ 50°C caused nearly no inactivation. A pressure increase continuously accelerated the inactivation and decreased the minimum inactivation temperature, resulting in a 5 $\log_{10}$ inactivation after 60 to 90s at 500 MPa and 40°C in ACES buffer. Comparing the heat (-3.01 $\log_{10}$ after 10 min at 60°C) and pressure resistance (-4.8 $\log_{10}$ at 500 MPa, 40°C and 3 min) for strain O104:H4, it was higher in carrot juice than in ACES buffer. This may be attributed to different membrane properties of the pre-culture, which was cultivated at pH 5.5 for carrot juice compared to pH 7 for the inoculation in ACES buffer.

Significance: The consumers demand minimally processed high quality foods. This demand has already generated an impressive number of commercially available high pressure pasteurized products. Especially for the growing market of pressure treated smoothies as well as fruit and vegetable juices, the presented data are of high industrial relevance. Further, recent data show a shift from meat related EHEC outbreaks to produce related infection and the sources of contamination during cultivation and in postharvest handling can be manifold. Consequently, an effective and gentle decontamination/preservation step within the postharvest chain of fruit and vegetables is essential to guarantee the consumers safety.

Impact of Climate Change on the Microbial Safety of Pre-harvest Leafy Green Vegetables

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Introduction: The likelihood of leafy green vegetables (LGVs) contamination and the associated pathogen growth and survival are strongly related to climatic conditions. Particularly, temperature increase and precipitation pattern changes have a close relationship not only with the fate and transport of enteric bacteria, but also with their growth and survival.

Purpose: This study aims to review and synthesizes major impacts of climate change (temperature increases and precipitation pattern changes) on contamination sources (manure, soil, surface water, sewage and wildlife) and pathways of foodborne pathogens (focusing on *Escherichia coli* O157 and *Salmonella*) on pre-harvested LGVs.

Methods: Relevant literature, including peer review scientific papers and grey literature, on LGVs but limited to *E. coli* O157 and *Salmonella* spp., has been studied for each contamination source and pathway, and for their relationship to different climate variables. Each contamination source has been searched in combination with each of these two pathogens, and with temperature and precipitation.

Results: Whether climate change increases their prevalence depends not only on the resulting local balance of the positive and negative impacts but also on the selected regional climate change scenarios. However, the contamination risks likely increase.This study gives an extensive overview of the impacts of climate change on the contamination of pre-harvested LGVs and shows that climate change should not be ignored in food safety management and research.

Significance: This review shows the need for quantitative modelling approaches with scenario analyses and additional laboratory experiments.

Impact of Probiotic Bio-compounds on Virulence of Foodborne and Zoonotic Pathogens

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Introduction: The ability of probiotics to inhibit virulence gene expression in pathogenic bacteria offers potential novel therapeutic approaches to combat pathogens in the food chain. The study of antivirulence factors produced by probiotics is an alternative strategy studied in our laboratory for human and animal pathogen control. Antivirulence agents can circumvent antimicrobial peptide resistance and restricted inhibitory spectra observed with other probiotic compounds proposed as pathogen control agents.

Purpose: The objective of the current study is to assess the effect of probiotic compounds on the virulence factors of different foodborne pathogens.

Methods: A cell free spent media (CFSM) was concentrated by freeze-drying following growth of *Lactobacillus acidophilus* La-5 in a whey-protein based medium. The CFSM was co-cultured with *Clostridium perfringens* CP-1, *Salmonella* Typhimurium DT104 and *Escherichia coli* R08-O149:H7:K88ac to determine its effect on virulence of each pathogen. The effect on gene expression was analyzed by real-time PCR. The influence of CFSM on the presence of *E. coli* K88 in 2-week old piglets was also studied.

Results: Downregulation of *netB*, *cpa* and *virA* was observed for *C. perfringens*, the *hilA*, *rpoD*, *ssrB* and *sopD* genes of *S.* Typhimurium were also down-regulated by the bioactive fraction as were the LT, STb and EAST for *E. coli* K88. Mild infection was found in pigs that were not treated with CFSM and symptoms were not apparent in the treated pigs. In addition, there was a low prevalence of *E. coli* K88 in the large intestine of treated animals.

Significance: The use of probiotic bioactives for pathogen control will lead to a reduction in cases of foodborne diseases and associated economic losses. The financial support of the Government of Canada through the Federal Development Program, MicroSintesis Inc., Dairy Farmers of Ontario, Dairy Farmers of Canada and the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

***Influence of Isolation Procedures on Official Monitoring Data of Pathogenic* Yersinia enterocolitica**

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Introduction: Recovery of pathogenic *Yersinia enterocolitica* from naturally contaminated samples is time consuming and difficult. For monitoring of pathogenic *Y. enterocolitica* on pig carcasses and minced meat in Belgium, the International Standard Organization method (ISO 10273:2003) is used.

Purpose: This study compares different isolation methods, including ISO 10273:2003, for the recovery of pathogenic *Y. enterocolitica* from pig carcasses and minced meat.

Methods: Pig carcass swabs (n = 254) and minced meat samples (n = 82) were collected in the frame of the official monitoring program of the Belgian Food Agency. Samples were enriched in three different broths: irgasan-ticarcillin-potassium chlorate (ITC) broth at 25°C for 2 days, peptone-sorbitol-bile (PSB) broth at 25°C for 2 and 5 days, and phosphate-buffered saline supplemented with 1% mannitol and 0.15% bile salts (PMB) broth at 4°C for 7 and 14 days.

Results: In total, 28 carcasses (11.0%) were contaminated with *Y. enterocolitica* bioserotype 4/O:3 and one (0.4%) with bioserotype 2/O:9. Four (4.9%) minced meat samples tested positive for *Y. enterocolitica* bioserotype 4/O:3. Fifty-nine out of the 72 *Y. enterocolitica* isolates carried the virulence plasmid. Isolation by ISO 10273:2003 detected 8 out of 29 positive carcasses and none of the positive minced meat samples. Reducing the enrichment time in PSB from 5 to 2 days significantly increased the number of positive samples ($P < 0.001$).

Significance: Isolation of pathogenic *Y. enterocolitica* from lowly contaminated samples is difficult and requires experience in the identification of typical colonies. As the exclusive use of the proposed ISO 10273:2003 method results in a strong underestimation of *Y. enterocolitica* positive carcasses and minced meats, efforts are needed to optimize current

version of the ISO method. Data of official monitoring programs from different countries should be compared with caution as the applied isolation methods significantly influence results.

Inter- and Intra-serovar Variation in* In-Vitro *Pathogenicity of* Salmonella *spp.

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Introduction: Food may contain numerous bacteria whick are harmless, beneficial or pathogenic. Health risks due to a combination of food products and a pathogenic bacterial species can be assessed with a Microbial Risk Assessment (MRA). An important part of a risk assessment is hazard characterization, also referred to as Dose Response (DR) relation, describing the relation between the dose of ingested pathogens and the probability of infection. Besides the limitations due to the current methods for obtaining DR-data, DR-relations usually refer to one serotype/serovar or even one specific strain thus lacking data on strain-variation within and between serotypes/serovars.

Purpose: To assess the intra- and interserovar variability in pathogenicity of various *Salmonella* serovars (10 strains each).

Methods: Human and animal strains of *Salmonella* Typhimurium DT104, human and animal strains of monophasic *S.* Typhimurium, and animal strains of *S.* Derby and *S.* Rissen were compared using a simulated gastrointestinal (GI) passage system including the interaction with cells mimicking human small intestinal epithelial cells (Caco-2 cells). The survival of *Salmonella* through the simulated GI-passage and the final levels of attachment and invasion to Caco-2 cells were determined. The probability of one cell resulting in invasion, considered as a measure for virulence, was calculated and used to construct exponential dose-response curves.

Results: *S.* Rissen exhibited on average the lowest virulence followed by monophasic *S.* Typhimurium (human and animal), *S.* Derby (animal) and *S.* Typhimurium DT104 (human and animal). *S.* Typhimurium DT104 isolates from animals showed the highest virulence. Animal isolates of monophasic *S.* Typhimurium were more virulent compared to human isolates of the same serovar.

Significance: The virulence of human monophasic *S.* Typhimurium strains was comparable to that of *S.* Typhimurium DT104, thus endorsing the opinion of the EFSA BIOHAZ panel (2010) on the virulence of monophasic Typhimurium.

ISO 22000-based Food Safety Management in a Dairy Farm

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Introduction: Primary production has important influence on the safety of milk products. Microbiological hazards can be introduced both from the farm environment and from the milking animals themselves. Potential also exists for the contamination of milk with residues of veterinary drugs, pesticides and other chemical contaminants. ISO 22000:2005 (Food Safety Management Systems – Requirements for any Organization in the Food Chain) provides an internationally recognized framework for establishing a food safety management system that combines prerequisite programs (PRPs), HACCP principles, system management and interactive communication.

Purpose: The purpose of our study was to evaluate whether a dairy farm can establish a food safety management system compliant with the requirements of ISO 22000, and have this system certified by a third-party auditing company.

Methods: The study took place in a Mexican dairy farm producing about 60,000 liters a day with 1,700 cows. Under the commitment of the top management, a food safety leader was appointed and a food safety team was set up. Its role was to establish the management system according to the requirements of ISO 22000:2005 and ensure its effective implementation in the field by the 47 workers.

Results: The establishment of the ISO-22000 based food safety management system took approximately 1 year and led to successful certification in the course of 2012. In addition to identifying and implementing new control measures related to personnel hygiene, this approach has strengthened workers' adherence to hygienic practices and promoted continual improvement.

Significance: We show that ISO 22000 is a food safety standard applicable not only to food manufacturing, but also to other steps of the food chain, including farming. To ensure food safety at all stages of the food chain, it is important that clear communication and interactions exist between all parties within an integrated food safety management system. This can be facilitated by ISO 22000 standard.

Isolation of Environmental Bacteriophages against* Listeria monocytogenes *to be used as Bio-decontaminants in Food Productions

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Introduction: *Listeria monocytogenes* is a big issue in some ready-to-eat food productions, especially cheese, fish and meat-products. Recently, new decontamination processes have been developed to assure healthy and quality food products, and novel substances called "biodecontaminants" are taken into serious consideration because of their activity against bacteria and because they are naturally present in the environment. Bacteriophages can be identified as good biodecontaminants, thanks to their ability to selectively and actively kill germs. They play a fundamental role in bacterial control and are naturally abundant in the environment, estimated to be more than 10^31. In this work we report some preliminary data regarding the isolation and partial characterization of two listeriophages.

Purpose: Isolation of lytic phages for *Listeria monocytogenes* in order to assess their safety and efficacy against the pathogen for their use in food productions.

Methods: More than one-hundred samples were screened for phage isolation. They were represented by animal faeces, silages and sewages. Phage isolation and host range activity was carried out by double-agar layer techniques (Spot and Plaque Assays). Bacteriophage partial characterization was assessed by Transmission Electron Microscope.

Results: Two lytic bacteriophages active against *Listeria monocytogenes* were isolated from sewage waters collected from one cheese plant with *Listeria* problems of contaminations during productions. They were differentiated on the bases of their plaque forming shapes and propagation rates. They are active against twenty different *L. monocytogenes* strains including reference strains serotypes ½a, ½b, ½c , 4a, 4b and 4c.

Significance: The successful isolation and characterization of lytic phages active against the foodborne pathogen *Listeria monocytogenes* would enable their use during food productions in addition or with reduction of the actual detergents. Differently from chemicals, in fact, they are selectively active against the target bacteria, are safe and their effect is long lasting. They can be easily applied on surfaces and on food products, are completely innocuous for human health and are cost effective.

Mechanical and Adhesive Properties of the Toxoplasma gondii Oocyst Wall

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Introduction: The environmental impact of the protozoan parasite *Toxoplasma gondii* is closely related to the extended survival of its oocyst form to contrasting climatic conditions and disinfection processes. The oocyst wall plays a key role by forming a highly resistant barrier to physical and chemical attacks. In this context, addressing the structure and biochemistry of the *Toxoplasma* oocyst wall is a crucial prerequisite to better understand the environmental dynamics of the parasite.

Purpose: We addressed the structure and chemistry of the *Toxoplasma oocyst* wall in terms of mechanical and adhesive properties.

Methods: The mechanical and adhesive characteristics of the *Toxoplasma oocyst* wall were investigated by combining wall surface treatments, fluorescence imaging, electron microscopy and Atomic Force Microscopy (AFM) techniques.

Results: Elasticity and indentation measurements indicated that the oocyst wall resembles hard plastic materials, based on the Young moduli evaluated by AFM. Our study demonstrates that the inner wall layer is as robust as the bilayered oocyst wall itself. Besides wall mechanics, important differences regarding the non-specific adhesive properties of each oocyst wall layer support possible differences in their biochemical content.

Significance: All together, these findings suggest a key biological role for the oocyst wall mechanics in maintaining the integrity of the *T. gondii* parasites in the environment or after exposure to disinfectants, and therefore their potential infectivity to humans and animals. This work was supported by the French National Research Agency (grant ANR-09-ALIA-009) and Aix-Marseille University (Preciput 2011 program). P.-H.P. is supported by the ANR JCJC DissecTion program.

Methylcellulose Films Containing Natural Extracts: Antibacterial Properties

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Introduction: Food contamination is a widespread problem in our society. Food is exposed to foodborne pathogens, which can be developed by the food itself. Both processes lead to degradation/spoilage. Food packaging is an essential device to avoid and/or minimise this process, as the food can be protected from external contamination. The incorporation of antimicrobial agents into the packaging matrix has been widely explored in recent years; indeed, antibacterial food packaging could not just create a barrier for external agents but also inactivate possible bacterial strains which could affect food properties. Lately, the use of natural antimicrobial agents by the food industry has been a tendency, but also a challenge.

Purpose: The aim of this study was to develop and test edible food packaging coatings enriched with natural extracts obtained from bitter cherry by-products showing antibacterial activity.

Methods: The extracts of Ginja cherry (native Portuguese cherry, variety Prunus cerasus L., Rosaceae) stems were used as antibacterial agents. They were incorporated into matrices of methylcellulose. The film structure and properties were studied. Their antibacterial properties were tested with both Gram positive and Gram negative strains.

Results: The microbiological tests showed the antimicrobial efficiency of these films in the inhibition of several microorganisms, such as Methicillin Sensitive *Staphylococcus aureus* (MSSA), Methicillin Resistant *Staphylococcus aureus* (MRSA), *Listeria innocua* and *Salmonella enteritis*. Additionally, the inclusion of natural antimicrobial extract in the film showed a continuous structure, making the film effective as a barrier from external contamination.

Significance: The development of antibacterial coatings can help preventing food spoilage, improving food safety and consumers health. This works highlights the potential of natural extracts obtained from by-products and therefore not exploited.

Microbiology of New Zealand Bulk Tank Milk

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Introduction: Bovine milk is rich in proteins, lipids, carbohydrates, vitamins and minerals essential for human health and well-being. However, consumption of unpasteurised milk can pose a substantial risk to human health due to

potential presence of pathogenic microorganisms in milk. Growing levels of raw milk consumption in New Zealand increased a demand for research on quality and safety of unpasteurised milk.

Purpose: The purporses of this study were 1) to determine the occurrence of *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter* spp., *Salmonella* spp. and *Escherichia coli* O157 in bulk raw milk collected from farm vats; 2) to estimate the effect of on-farm control measures on the level of hazards in raw milk.

Methods: Samples were collected 5 times throughout 2011–2012 milking season from each of 80 randomly selected dairy farms. All samples were refrigerated and analysed within 2 days of sampling. In addition, the information was collected to characterise the hygienic quality of the milk (Total Bacteria Count, Somatic Cell Count and coliforms), herd sizes and farming practices at surveyed farms.

Results: *Salmonella* was not detected in this study. *Campylobacter* was isolated twice. *E. coli* O157:H7 was detected once and one sample contained non-pathogenic strain of *E. coli* O157. In comparison with a similar survey conducted in 2007–2008, there is evidence of a decrease of *S. aureus* and an increase of *Listeria* spp. (including *L. monocytogenes*). Average total bacterial count (TBC) was below 104 cells/ml annually with the lowest numbers in November- February and the highest in May-August. Statistical analysis of TBCs revealed a mixture-distribution with approximately 7% of bulk tanks belonging to a high count distribution that is likely to be attributed to faecal contamination from infected cows. Additional models describe pathogen concentrations in New Zealand bulk milk and investigate impacts of on-farm hygiene and animal health (sub-clinical mastitis).

Significance: While the survey showed good hygienic quality of New Zealand bulk milk, the presence of pathogens poses a potential health risk if the milk is consumed prior to pasteurisation.

A Model and Software for Quantitative Microbial Risk Assessment

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Introduction: Assessing microbial risk in a population of consumers requires modelling consumer impact via exposure assessment and/or dose response. In order to assess consumer exposure, both the level of pathogen in the food and the amount of food consumed must be known. National dietary databases provide a profile of food consumption in consumer populations, typically surveying over 1,000 consumers and several thousand foods. At the same time, laboratories routinely analyse foods for various pathogens at different points in the food chain. It is possible to integrate these data sources into one food safety system for microbial risk assessment.

Purpose: To develop a model and software for estimating consumer exposure to foodborne pathogens.

Methods: Databases of food consumption surveys were integrated into a web-based software program, as well as interfaces for integrating concentration data of pathogens in foods. A probabilistic dietary exposure model was developed to estimate the distribution of pathogen exposure in a population of consumers, which in turn is fed into a dose-response model for that pathogen. Monte Carlo simulations are used to simulate a large amount of consumers to capture sources of variability in the model. A cloud-based server was used to support the computational requirements of the system.

Results: The software can be used for both rapid-response and routine risk assessment, as much of the required data is already installed in the system which is supported using cloud computing. The system also supports probabilistic inputs, enabling distributions of pathogen levels to be generated from multiple measurements to assess their consequences in one assessment.

Significance: Quantitative estimates of consumer exposure coupled with dose-response provide more detailed information on consumer risk, allowing risk assessor to fully exploit the consequences of food safety analysis.

Molecular and Phenotypic Characterization of STEC in The Netherlands

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Introduction: The incidence of STEC O157 disease and the distribution of the three LPSA6 lineages involved differ considerably between The Netherlands and the United States (Franz 2012). The reasons for this are unclear. Besides O157, there is a large group of STEC belonging to other serotypes (non-O157 STEC), displaying a high diversity in the capacity to cause disease. Cuurently, it is not possible to efficiently discriminate STEC posing a high risk from STEC characterized by lower risk of disease and/or outbreaks.

Purpose: This study had two major goals. First, the genomic comparison of STEC O157 isolates belonging to similar LSPA6 lineages from The Netherlands and the U.S. Secondly, developing a molecular risk assessment (MRA) approach to discriminate between STEC of high and lower human health risk.

Methods: Eighteen Dutch human STEC O157 isolates (5 LSPA6 lineage I, 8 lineage I/II, 5 lineage II) were subjected to whole genome sequencing using the Illumina MiSeq platform and compaired with STEC O157 Sakai as a reference strain. Based on a published single nucleotide polymorphism (SNPs) list from sequenced U.S. isolates (Bono 2012) phylogenetic comparisons were made. Additionally, isolates from different LSPA lineages were phenotypically characterized with respect to adherence to human and bovine epithelial cells. A set of 225 non-O157 strains isolated from humans, food and cattle were screened for a large number of virulence genes. In addition, these strains were phylotyped and grouped in different seropathotypes (Karmali 2003).

Results: Phylogenetic analysis of SNP lists generated from the genome sequences revealed that Dutch and U.S. isolates belonging to the same LSPA6 lineage cluster, suggesting no major differences between isolates from both geographic locations. Further analysis of the genome sequences and SNPs of the Dutch strains is ongoing. Dutch LSPA I isolates adhered significanty better to human epithelial cells compared to LSPA II isolates, which adhered in turn better to bovine epithelial cells. Lineage I/II produced the highest level of Shigatoxin. With respect to the non-O157 STEC, cluster

analysis based on the presence/absence of virulence genes showed that STEC seropathoype A and B (including the top 5 relevant STEC serotypes in the EU) clustered together and were separated from seropathotype D and E. The majority of the isolates belonged to *Escherichia coli* phylogroup B1 (60%) and A (20%). Genes responsible for the differentiation between the seropathotypes include *eae*, *efa1*, and *nleB*. The latter determinant was strongly associated with isolates from hospitalized patients.

Significance: Insight in the molecular and phenotypic differences between STEC O157 from different geographic locations where O157 lineages are distributed differently among cattle and human isolates will provide basis for further control. A molecular risk assessment system for the entire STEC group will provide more risk-based surveillance, outbreak prevention and clinical management.

***A New Quantitative Microbiological Risk Assessment Model of* Listeria monocytogenes**

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Introduction: The quantitative risk assessment is becoming increasingly important for food processors. Quantitative risk assessment is primarily an approach dedicated to food safety to justify management options requested by regulatory bodies. Food business operators (FBO) can also use this approach to ensure consumer protection measures or food quality features.

Purpose: The study was aimed to develop a quantitative risk assessment model of *Listeria monocytogenes* in liver paté crostini. The model was performed combining experimental data of a challenge test at 12°C with the data extrapolated at 4°C and 8°C from predictive software. Model data were statistically assessed against a challenge test using the temperature gradient data.

Methods: The challenge test was conducted on 3 different batches of liver paté crostini stored at 12°C and inoculated with a mix of *L. monocytogenes* strains (1.6 Log CFU/g). Plate counts of *Lactobacillus* spp. (by ISO15214:1998) and *L. monocytogenes* (by ISO11290-02:2005) were performed daily on each sample until the stationary phase was reached by both populations. Challenge test results (dates and plate counts) were input in the Combase DMfit software to determine the growth parameters of *L. monocytogenes* and lactic flora which showed mutual interaction. Then, using the Combase Predictor and the SSSP software, the growth parameters of both populations were extrapolated at 4°C and 8°C. The growth parameters of *L. monocytogenes* and lactic flora at 4°C, 8°C and 12°C were then used to apply the quantitative risk assessment model in order to predict the maximum daily concentration of *L. monocytogenes*. Model results were assessed against the results of an additional challenge test conducted with the same strains mix inoculum in 1 batch stored for 4 days at 4°C, 4 days at 8°C and then 4 days at 12°C.

Results: The results obtained showed a limited underestimation of the real data (< 0.5 Log CFU/g) by the model versus the challenge test data.

Significance: A quantitative risk assessment based only on experimental studies is time-consuming and expensive. On the other hand, a predictive approach alone can't always provide accurate and realistic results. The proposed model represents a reliable quantitative risk evaluation which provides realistic results with limited cost as the result of a synergy between experimental and predictive data.

Novel Approach to Control Food Pathogens in Non-thermal Way: First Attempts to Decontaminate Strawberries by Photosensitization

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Introduction: Despite tremendous progress in food microbiology, the number of reported foodborne diseases continues to rise. Health experts estimate that every year foodborne illnesses in USA cost 5-6 billion U.S. dollars in direct medical expenses and lost productivity. Infections with the bacteria *Salmonella* alone account for 2.5 billion dollars yearly. Obviously, existing antibacterial technologies to decontaminate food or food-related surfaces are not enough effective. Photosensitization is a treatment involving the interaction of the two non-toxic factors, photosensitizer and visible light, which in the presence of oxygen results in the selective destruction of the target cell. Different microorganisms, such as multidrug-resistant bacteria, yeasts, microfungi and viruses, spores and biofilms are susceptible to this treatment and can be inactivated to undetectable level *in vitro*. After illumination by visible light, reactive oxygen species induce rapid disruption of the cell wall. Reactive ROS interacts with unsaturated fatty acids, amino acid residues, such as cystein, histidine, tryptophan, nucleic acid bases of DNA, particularly guanine and thymidine.

Purpose: The aim of this study was to evaluate susceptibility of food pathogens to Chlorophyllin-based photosensitization *in vitro* and when pathogens were inoculated on the surface of strawberries.

Methods: Food pathogens, mesophiles, yeasts and fungi in vitro and inoculated on the surface of strawberries were treated with 10-5 M Chlorophyllin solution (in PBS) for 5 minutes and afterwards illuminated with visible light (400nm, 10 mW/cm^2). Colony forming units indicated the rate of bacterial inactivation. Content of antocyanins, fenols and total antioxidant activity was evaluated in treated and control strawberries.

Results: High antimicrobial efficiency of chlorophyllin-based photosensitization has been used to inactivate harmful and pathogenic microorganisms on the surface of packaging material (4 log reduction) and decontaminating strawberries (2 log reduction). Decontamination of food-related surfaces by chlorophyllin-based photosensitization was 4 times more effective than conventional chemical treatment with hypochlorite. *Bacillus cereus*, yeasts/fungi and mesophiles, distributed on the surface of berry were inactivated by 2–2.5 log after chlorophyllin-based photosensitization treatment. No negative impact on fruit color, taste, antioxidant activity, fenolics or anthocyanins was detected after treatment.

Significance: Photosensitization might open a new avenue for the development of non-thermal, effective and ecologically friendly antimicrobial technology.

A Piezoelectic Immunosensor for Specific Capture and Enrichment of Viable Escherichia coli O157:H7 by Quartz Crystal Microbalance Sensor, Followed by Detection with Antibody-functionalized Gold Nanoparticles

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Introduction: Piezoelectric biosensor can detect targets based on frequency shifts caused by "mass changes" on the chip surface. It has been used for pathogen detection; however, its unique function has not been applied in the real-time enrichment and detection of viable bacteria.

Purpose: In this study, a simultaneous enrichment and detection system was developed for viable *Escherichia coli* O157:H7 on blueberries by a gold nanoparticles (AuNPs) functionalized quartz crystal microbalance (QCM).

Methods: In the circulating-flow QCM system, capture antibodies for *E. coli* O157:H7 were first immobilized onto the QCM chip. The sample containing *E. coli* O157:H7 was circulated through the system in the presence of 10 ml of brain heart infusion (BHI) broth for 18 h. The cells of *E. coli* O157:H7 specifically captured and enriched on the chip surface of the QCM were identified by QCM frequency changes. *Listeria monocytogenes* and *Salmonella* Typhimurium were used as negative controls. After bacterial enrichment, detection antibody-functionalized AuNPs were added to enhance the changes in detection signals.

Results: The use of BHI enrichment further enhanced the sensitivity of the developed system, achieving a detection limit of 0-1 log CFU/ml or g. The results indicated a 133 ± 35Hz decrease during BHI enrichment with the initial 2×10^1 CFU/ml of *E. coli* O157:H7 on blueberries whereas the negative controls showed only a 5 ± 8Hz decrease. In addition, a significant frequency decrease of 93 ± 24Hz ($P < 0.05$) was observed after the addition of antibody-conjugated AuNPs, indicating the enriched bacteria captured on the QCM chip surface were actual *E. coli* O157:H7.

Significance: The simultaneous enrichment and detection for *E. coli* O157:H7 established in the study could be used to detect viable bacteria at low concentration in food samples. The combination of both procedures in a nanoparticle-functionalized piezoelectric biosensor system indicates potential for the application in on-site screening of foodborne pathogen contamination.

Prevalence of the 7 Major Serogroups of Enterohemorragic* Escherichia coli *(EHEC) in Fresh Minced Beef in France: A Novel Real-time PCR Strategy for Their Early Detection in Food

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Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) are responsible for severe hemorrhagic colitis and life threatening hemolytic and uremic syndrome in humans. The serogroup O157 remains the most frequent worldwide, but non-O157 EHEC serogroups such as O45, O26, O103, O111, O121 and O145 have been increasingly reported as responsible for food poisoning. Our study aims at determining the prevalence in fresh minced beef in France of the 7 major EHEC serogroups.

Purpose: Our study aims at determining the prevalence in fresh minced beef in France of the 7 major EHEC serogroups and evaluating a new PCR-strategy for their early detection.

Methods: A total of 2,476 fresh minced beef samples, collected in supermarkets of 92 French departments from March to December 2010, were investigated by PCR for EHEC-associated genetic markers. Samples positive for *stx* and *eae* were further screened for the presence of the O group markers (ISO Technical Specification 13136), for the presence of the gene *nleB* (described as a genetic marker of virulent EHEC) and the four specific *eae* variants associated with the 7 EHEC serogroups tested. EHEC strains were recovered by immunomagnetic separation (IMS)-based isolations or by direct plating from all samples that tested positive for *stx* and one (or more) O group markers whatever the results obtained for *nleB* and *eae* variants. Serotypes and virulence profiles of strains isolated were then confirmed by PCR.

Results: The initial screening showed that *stx* and *eae* genes were simultaneously detected in 109 out of the 2476 samples (4.4%). Among these 109 positive samples, 80 were also positive for at least one O group marker associated with the 5 major EHEC serogroups (O26,O103, O111, O145 and O157) and 84, with the 7 major EHEC serogroups (top 5+, O45 and O121), which represented respectively 3.2% and 3.4% of the samples screened. After isolation, 6 samples were confirmed to be contaminated with an EHEC belonging to the top 5 serogroups, and 7 with an EHEC belonging to the top 7 serogroups which represent respectively 0.2% and 0.3% of the total samples. Of the 7 EHEC isolated, 4 were EHEC O26, 1 EHEC O157, 1 EHEC O145 and 1 EHEC O45. Notably, 13 *E. coli*, which could be EHEC derivatives were isolated from the samples: 7 EPEC O26, 4 EPEC O103 and 2 EPEC O157. Out of the 109 *stx* and *eae* positive samples, only 2.4% (60/2476) and 2.5% (62/2476) were simultaneously positive for the gene *nleB* and at least one combination of *eae* variant with O group marker of the top 5 or of the top 7, respectively. None of EHEC and EPEC strains were isolated in negative samples for this improved combination.

Significance: The five and seven major EHEC serogroups were detected in French minced meat with a low prevalence (0.2% and 0.3%). Interestingly, EHEC and EPEC O26 was the main isolated EHEC serogroup in meat. We also propose an interesting and reliable PCR-strategy for an early detection of major EHEC in meat.

proP is Required for the Survival of Desiccated* Salmonella *Typhimurium on a Stainless Steel Surface

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Introduction: Manufacturing facilities where intermediate and low moisture products are produced are generally maintained as a moisture-free environment, a feature designed to limit the risk of pathogen contamination. *Salmonella* species are an important biological hazard encountered by food producers and have been the cause of several foodborne

outbreaks linked to these products. Currently, our knowledge of the mechanisms that bacteria employ to ensure their survival in desiccated conditions is lacking. The effects of re-introduction of moisture to a desiccated cell are also largely unknown.

Purpose: The aim of this study was to investigate the response of *S*. Typhimurium ST4/74 to desiccation on an industrially important surface (stainless steel) at the transcriptional level, and to subsequent rehydration.

Methods: RNA was isolated from *Salmonella* following 4 h of desiccation on stainless steel. To examine the effects of rehydration desiccated cells were exposed to water for 30 min after which RNA was also isolated. Microarray analysis was carried out using the SALSIFY2 array, using RNA extracted from a liquid culture as a control. Deletion mutants were constructed to phenotypically confirm results obtained from the array.

Results: After 4 h of desiccation, 266 genes were differentially expressed, compared with a static broth culture. Osmoprotectant transporters proP, proU and osmU (STM1491-94) were highly up-regulated under desiccation. Deletion of any one of these systems resulted in a reduction in the long term viability of *S*. Typhimurium on a stainless steel food contact surface. The proP gene was critical for survival, as proP deletion mutants could not survive for long periods of desiccation. Following rehydration, 138 genes were differentially expressed, with up-regulation observed in genes such as proP, proU and phosphate transport (pstACS).

Significance: A number of features were identified that contributed to long-term desiccation survival. This investigation constitutes the first in-depth study of the processes occurring within a previously dried cell upon the re-introduction of moisture. These findings may aid manufacturers of low-moisture foods in the design of effective control strategies aimed at the elimination of desiccated *Salmonella* from the production environment, thereby improving food safety and protecting public health.

Purification and Characterization of Native Shiga Toxin 2F and Its Monoclonal Antibodies

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are a group of bacteria that can generate symptoms from common diarrhea to hemolytic-uremic syndrome in humans. They are responsible for many recent food illness outbreaks. STEC strains derive much of their virulence from the secretion of Shiga-like toxins (*Stxs*). There are two types of *Stxs* in *E. coli*, *Stx1* and *Stx2*. *Stx2* is associated more closely with severe human diseases. *Stx2f* is the most diverse of the seven known *Stx2* subtypes. It was first isolated in pigeons and initially thought to be uninvolved in human illness. However, recent studies have shown that the presence of *Stx2f* in human STEC is on the rise. There is no effective antibody against this subtype available currently. This emphasizes the need to have pure *Stx2f* for developing *Stx2f*-specific antibodies for immunodiagnostics.

Purpose: The objectives of this study are to purify the *Stx2f* subtype from bacterial cultures; compare the properties of *Stx2f* with the better understood prototype, *Stx2a*; develop *Stx2f*-specific monoclonal antibodies (mAbs) and immunoassays for *Stx2f*-expressing STEC.

Methods: *Stx2f* was purified using a four-step purification strategy, which includes cation exchange, hydrophobic interaction, anion exchange, and gel filtration.

Results: After the final purification step, 5.2 µg of pure *Stx2f* was obtained from 450 mL of bacterial culture supernatant. The *Stx2f* purified had a CD50 3.4 pg in Vero cells; bound to both the Gb3 and Gb4 receptors and its ability to bind the Gb4 receptor was much stronger than *Stx2a*; maintained its toxicity after a pH 2 or 72°C treatment significantly better than *Stx2a*, suggesting the toxin may survive harsher food preparation practices. A group of mAbs and a highly sensitive ELISA, capable of detecting 1 ng/mL *Stx2f* were developed using mAbs developed in this study.

Significance: Although *Stx2f*-encoding STEC strains are not yet considered a major health concern, it is becoming clearer that *Stx2f* infections are more common than we realize since most *Stx* immunoassays are poor at detecting *Stx2f.* The reagents developed in this study will be useful for identifying foodstuffs contaminated with *Stx2f*-producing STEC, and thus prompting implementation of control measures to prevent outbreaks.

Rapid Multiplex Detection of Norovirus in Food Samples

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Introduction: Foodborne disease caused by norovirus and hepatitis A virus are increasing every year. This underlines the need for simple, sensitive, fast, standardized and reliable methods of identifying viral contamination in order to offer analytical solutions to food industries. Theses methods must allow food industries to anticipate future guidelines of the Codex Alimentarius concerning the problem of enteric viruses.

Purpose: To simplify the detection of norovirus in food, a multiplex Q-RT-PCR was developed to identify simultaneously the 2 genogroups (I or II).

Methods: Primers and probes are those described in the European standard method for virus detection in food. Reagents and reporters have been optimized to reach a sensitivity and an amplification efficiency comparable to the one obtained for the detection of only one norovirus genogroup per reaction. A robustness study (reproducibility, repeatability) was conducted. An internal positive control was included. The detection method was validated on samples (50) previously found positive at various level of contamination for Nov GI or GII. Two detection kits were then produced and commercialized (ceeramTools).

Results: The specificity was validated for all genogroups. No cross reactivity was observed. A limit of detection of 10 genome copies/reaction was reached with a confidence level of 95%. The robustness study demonstrated standard deviations below 1.5 for inter and intra-assays and inter manipulator variations. All the tested samples were positive even those with a level of contamination inferior to 100 copies/25 g of food.

Significance: This method allows a rapid detection of norovirus and identification of the genogroup in one reaction. The analytical costs can therefore be reduced. As more analyses can be performed for lower costs, more data one norovirus circulation and prevalence can be generated leading to a better viral food safety management.

***Risk Mitigation in Reducing Thermal Processing with Hurdle Technologies: A Challenge Study in the Canning Industry with* Clostridium botulinum**

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Introduction: As an answer to continuous consumer demand for high quality foods, Agriculture Canada is challenging different techniques to provide microbiologically safe foods with minimal thermal processing damages in support to industries. Hurdle concept combining low pH, organic acids and thermal processing is one of those.

Purpose: Application of selected acidifiers was proposed to provide scientific data to food thermal processing authorities (Health Canada, CFIA and PHA) for new process approval. A full experimental program on selected processing parameters (pH, acidifiers, vegetables and temperature) was initially designed to evaluate the thermal resistance (D and z) of *Clostridium sporogenes* (PA3679) (as a surrogate to *C. botulinum*) but preliminary results showed a measured thermal resistance much lower than the one reported in literature for this strain. Therefore, a bio-validation objective with *Clostridium botulinum* was realized.

Methods: Three food matrices of cut green beans have been tested for survival of a cocktail of *C. botulinum* (62-a, PC0101AJ0 and 13983B): (1) un-acidified pH 5.8, (2) acidified with LA to pH 4.8, and (3) acidified with GDL to pH 4.8. A spoilage and thermoresistant microorganisms, *Geobacillus stearothermophilus* has also been tested for the same food matrices. Final validation (can-size scale) with a stock water-immersion pilot scale retort has been achieved.

Results: Main results indicated that a low acidification treatment is efficient to reduce the intensity of the thermal process needed to ensure safety and long term storage of thermally processed food. Predictive models are useful at mild treatment but are not well adapted at temperature higher than 107°C as the results are not following a linear distribution (D-z model).

Significance: D and z-value of *C. sporogenes* and *C. botulinum* could have been overestimated since a long time. Those have been used to design models which are supposed to be linears but our results are not supporting this. Multiples barriers technology shows another potential application in the canning industry combining a light acidification with mild thermal processing to increase the quality of processed vegetables.

Role of Food Lipids in Cold Adaptation of* Bacillus cereus *in Absence of Oxygen

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Introduction: Production of heat resistant spores combined with the ability of some strains to grow at temperatures of refrigeration, make *Bacillus cereus* a hazard in cooked chilled foods. Cooked chilled foods are mostly packaged anaerobically to reduce oxidative spoilage. In air, *B. cereus* adapts its membrane to cold through fatty acids desaturation, which is not possible in absence of oxygen.

Purpose: The impact of absence of oxygen on growth of *B. cereus* at low temperature, and the potential role of food lipids was studied.

Methods: *Bacillus cereus* was grown in cooked spinach and in synthetic media with spinach extracts. *B. cereus* lipids were extracted and characterized.

Results: Anaerobiosis inhibited growth of *Bacillus cereus* at low temperatures in synthetic medium but not in cooked spinach. Adding a lipid extract of cooked spinach to the synthetic medium restored growth of *B. cereus*, whereas an aqueous extract had no such effect. The lipid extract of spinach contained a high proportion of unsaturated, low melting point fatty acids, which were presumably integrated in *B. cereus* membrane, increased its fluidity, and permitted *B. cereus* growth at low temperature. When added to the synthetic medium, triglycerides and various phospholipids rich in unsaturated fatty acids restored growth of *B. cereus* at cold and anaerobiosis, but not free unsaturated fatty acids and phospholipids containing only saturated fatty acids. In the case of phospholipids rich in unsaturated fatty acids such as soy lecithin, 25 µg ml-1 in the growth medium were sufficient to observe an effect, and 125 µg ml-1 were sufficient for the maximal effect. Lipids from *B. cereus* membranes grown at low temperature anaerobically in presence of soy lecithin, were at least as fluid as those from membranes of *B. cereus* grown aerobically. Triglycerides were integrated unmodified in *B. cereus* membranes, whereas phospholipids were integrated in diverse ways, mostly as diacylglycerol. Presence of these very unusual lipids in its membrane did not prevent multiplication of *B. cereus*, but may explain the distorted morphology of the cells observed under electronic transmission microscopy.

Significance: Ability of *B. cereus* to grow at cold temperatures in absence of oxygen depends on the presence of lipids rich in unsaturated fatty acids. Lipids are widespread in foods and should not be overlooked when predicting the response of pathogenic bacteria to stress conditions.

Spoilt Rotten - The Impact of Spoilage in the No-preservative Foodscape

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Introduction: Food spoilage is and has always been a significant cost to the food industry, and many technologies have evolved since the 1950s to produce safe, wholesome food. In this new century, the consumer driven push against chemicals is systematically removing many of the tools from our food preservation toolbox, leading to significant increases in product losses. Three case studies, demonstrating the impact of 'preservative free' processing, will be presented across three industry segments, bakery, processed meats and packaged fresh produce. The impact of change, the disparate needs/wants of manufacturers, retailers and consumers, and the strategies used to implement change will be discussed.

Purpose: To re introduce the concepts of food spoilage to an audience that is often preoccupied with the more glamorous microbial pathogens, to gently introduce the concerns of consumer & marketing driven stigmatisation of food preservation techniques. To re introduce the concepts of hurdle technology and the need to fully understand details of pH,

a_W, and how small changes in basic food parameters can drastically alter food stability and shelf life. To keep all of the above entertaining and interesting enough so the core message is conveyed.

Methods: No methods, it's a review/case study presentation.

Results: None applicable.

Significance: Food spoilage, preservatives.

Transfer of* Campylobacter *from Chicken Legs to Cooked Slices via Domestic Cutting Board

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Introduction: *Campylobacter* represents the leading cause of gastroenteritis in Europe. Poultry meat is the main source of contamination, and cross-contaminations in the consumer's kitchen during meat preparation appear to be the important route for exposure. The characteristics of *Campylobacter* isolates able to transfer are currently not known.

Purpose: For the first time, an experimental design was used to demonstrate the risk for *Campylobacter* cross-contamination in the consumer's kitchen and to characterize the involved isolates.

Methods: The transfer of *Campylobacter* isolates from 94 naturally contaminated chicken legs to a ready-to-eat food product (cooked chicken slices) via a cutting board was studied. The isolates were characterized genetically by multiplex PCR and RFLP-PFGE, regarding their ability to adhere to inert surfaces by the BioFilm Ring Test method and their *in vitro* virulence properties.

Results: *Campylobacter* spp. was detected on 45 chicken legs and transfer from the chicken leg to the cooked chicken product via the cutting board occurred in 28.9 % of the cases (13 of the 45 positive samples). Moreover, samples with an initial contamination below the detection limit of 10 CFU/g have shown a possible transfer. The genetic characterization of isolates revealed that both species *C. jejuni* and *C. coli* were able to transfer. Transfer seems to be linked to specific isolates: some were able to transfer during separate trials while others were not. No correlation was found between transfer and adhesion to inert surfaces, but more than 90 % of the isolates (25/27) presented moderate or high adhesion ability. All tested isolates had the ability to adhere and invade Caco-2 cells, but presented high variability between isolates.

Significance: Our results highlighted the occurrence of *Campylobacter* cross-contamination via the cutting board in the kitchen. Moreover, they provided new interesting data to be considered in risk assessment studies.

Use of a Non-oxidising Disinfectant to Reduce Microbial Load in Produce Wash Water

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Introduction: Contaminated produce wash water can be responsible for the cross-contamination of produce and consequently, disinfectant is usually added. The most widely used disinfectant is chlorine in a mildly acidic solution. While chlorine can reduce the numbers of microbes, its efficacy is reduced by the presence of organic matter derived either from soil or the produce itself.

Purpose: A non-oxidizing disinfectant consisting of a synergistic combination of plant extracts and low concentrations of silver and copper ions (MicroPure Technologies LLC, (MPT)) was evaluated for its ability to reduce microbial load. The efficacy of the disinfectant as a produce wash was compared to free chlorine at 50 mg/L (pH 6.0) in over 60 experiments.

Methods: Mixtures of *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 (three strains per species) were used as inocula. Bacteriophage MS2 was tested, alone and in combination with the bacteria. The disinfectants were added to solutions of 5% sterile vegetable juice prior to inoculating with the bacteria or viruses. The suspensions were mixed thoroughly and constant agitation applied. Samples were removed after 1, 2 and 3 minutes of contact time. A solution of 5% juice with inoculum was used as a control.

Results: The chlorine rinse achieved typical reductions of <1 – 2 logs for bacteria and MS2. Conversely the non-oxidizing MPT disinfectant consistently achieved reductions of 4–6+ logs for all three bacteria and MS2 at both 4°C and 20°C. Repeated bacterial challenges of wash water containing a single dose of MPT disinfectant demonstrated that its disinfectant efficacy was largely unaffected, thus indicating the ability to reuse the disinfectant multiple times.

Significance: These results suggest that MPT was superior to chlorine in reducing the microbial burden in water, which suggests MPT will reduce the risk of cross contamination of produce during washing operations prior to packaging / distribution.

Use of Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) Sequence Polymorphisms for Specific Detection of Enterohemorrhagic* Escherichia coli *Strains of Serotypes O26:H11, O45:H2, O103:H2, O111:H8, O121:H19, O145:H28, and O157:H7 by Real-Time PCR

SABINE DELANNOY, ANSES, Maisons-Alfort, France, **Lothar Beutin**, Federal Institute for Risk Assessment, Berlin, Germany and **Patrick Fach**, ANSES, Maisons-Alfort, France

Introduction: Cumulative evidence from numerous countries indicates that a growing number of human enterohemorrhagic *Escherichia coli* (EHEC) infections are caused by non-O157 EHEC. However, detection of these non-O157 EHEC is particularly challenging because they have no phenotypical characteristics that distinguish them from the large number of non-STEC that share the same habitats.

Purpose: We explored the genetic diversity of the clustered regularly interspaced short palindromic repeats (CRISPR) regions of EHEC to design real-time PCR assays for each of the seven worldwide most important EHEC serotypes.

Methods: A panel of 958 *E. coli* was investigated for their CRISPR loci by high throughput real-time PCR.

Results: This study showed that CRISPR polymorphisms in *E. coli* strongly correlated with both O:H serotypes and presence of EHEC virulence factors (*stx* and *eae* genes). The CRISPR sequences chosen for real-time PCR amplification of EHEC strains belonging to the top7 EHEC serogroups differentiated clearly between EHEC and non-EHEC strains. Specificity estimates of the CRISPR PCR assays varied from 97.5% to 100%. Sensitivity estimates of the assays ranged from 95.7% to 100%. The assays targeting EHEC O145:[H28], O103:[H2], and O45:[H2] displayed 100% sensitivity. The combined usage of two simplex PCR assays targeting the O26 CRISPR locus allowed detection of EHEC O26:[H11] with 100% sensitivity. By combining two simplex PCR assays targeting the EHEC O157 CRISPR locus, EHEC O157:[H7] was detected with 99.6% sensitivity. EHEC O111:[H8] and EHEC O121:[H19] were detected with 95.9% and 95.7% sensitivity respectively.

Significance: This study demonstrates that the identification of EHEC serotype specific CRISPR sequences is more specific than the mere identification of O-antigen gene sequences as it is used in current PCR protocols for detection of EHEC strains. This approach could be used in EHEC surveillance and outbreak investigation and is likely to be of benefit to public health.

Poster Session 1 – Wednesday, 15 May 2013

Presenters will be at posters during coffee breaks to discuss with attendees

P1-01 Effects of Natural Phenolic Acids on Mycotoxin Biosynthesis by* Fusarium *spp. in Maize Grain

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Introduction: Mycotoxins are fungal secondary metabolites of great interest in agriculture and food safety. The main mycotoxins of concern produced by *Fusarium* species include deoxynivalenol (DON), T2 and HT2 toxins, fumonisins (FUM B1 and B2) and zearalenone (ZEA) (Glenn et al., 2007). Some strategies for controlling fungal growth and mycotoxin production could be natural antimicrobials, such as plant extracts (Soriano, 2007). Natural phenolic acids are found in the outer layers of grains and they have been reported as in vitro inhibitors of fungal growth and mycotoxin production (Samapundo et al., 2007; Boutigny et al., 2010). However, limited information is available on the effects of phenolic acids on the biosynthesis of *Fusarium* toxins in maize grains.

Purpose: To evaluate the effects of caffeic, chlorogenic, ferulic and p-coumaric phenolic acids on mycotoxin biosynthesis (DON, ZEA, FUM, T2+HT2) by five strains of *Fusarium* spp. in maize grain.

Methods: Single isolates of five strains of *Fusarium* were used in this study: *F. graminearum* (and its teleomorph *Gibberella zeae*), *F. proliferatum*, *F. sporotrichioides* and *F. verticillioides*. Sterilized rehydrated maize grains were treated with different concentrations of each phenolic acid, ranging from 1 mM to 10 mM plus a control group. Then, plates with a single layer of maize grains were inoculated with a conidial suspension of each strain and incubated at 25°C in the dark for 28 days. Mycotoxin analysis was carried out by quantitative lateral flow immunoassay (ROSA test by Charm Sciences Inc.).

Results: In general, the mycotoxin biosynthesis by *Fusarium* spp. in maize grains was not much affected by the phenolic acids, with the following exceptions: ZEA production by *G. zeae* was significantly reduced ($P < 0.05$) using chlorogenic at 5 mM, ferulic at 1-2.5 mM and p-coumaric at 2.5-3.5 mM; T2 and HT2 toxins production by *F. sporotrichioides* were significantly reduced ($P < 0.05$) with chlorogenic at 7–10 mM and p-coumaric at 7 mM. However, DON and ZEA levels by *F. graminearum* showed apparent increases with both caffeic and ferulic acids at 10 and 2.5 mM, respectively.

Significance: Even though phenolic acids may reduce the growth of toxigenic *Fusarium* species, the effects on mycotoxin production are often variable and the results conflicting. Therefore, there were a number of cases with inhibition, others of lack of inhibition and some cases which even showed the stimulation of mycotoxin production. This research was supported by the Spanish MINECO (Project AGL2011-26808), the Government of Aragón (Grupo de Investigación Consolidado A01), and the European Social Fund.

P1-02 In Vitro Inhibition of the Growth of Toxigenic* Fusarium *Species by Phenolic Acids

ELENA FERRUZ, **Isabel Gimenez**, **Marta Herrera**, **Susana Loran**, **Teresa Juan**, **Augustin Arino** and **Antonio Herrera**, University of Zaragoza, Zaragoza, Spain

Introduction: *Fusarium* species are pathogen fungi found in corn worldwide. These moulds are able to spoil corn and some species can produce mycotoxins, toxic secondary metabolites of known public and animal health significance (Samapundo et al., 2007). Some strategies for controlling fungal growth and mycotoxin production could be natural antimicrobials, such as plant extracts (Soriano, 2007). Natural phenolic acids represent the most common form of phenolic compounds in whole grains and they have been reported as inhibitors of fungal growth and mycotoxin production (Boutigny et al., 2008). Recent studies reported the *in vitro* ability of various phenolic compounds for the reduction in fungal growth of *Fusarium* spp. and *Aspergillus* spp. (Samapundo et al., 2007; Nesci et al., 2009; Boutigny et al., 2010). However, limited research has been conducted about the effects of phenolic acids on the growth of toxigenic *Fusarium* species.

Purpose: The aim of this study was to evaluate *in vitro* the effects of caffeic, chlorogenic, ferulic and p-coumaric acids at concentrations from 0.5 mM to 10 mM on the growth of six toxigenic *Fusarium* strains.

Methods: Single isolates of six strains of *Fusarium* were used in this study: *F. graminearum* (and its teleomorph *Giberella zeae*), *F. langsethiae*, *F. proliferatum*, *F. sporotrichioides* and *F. verticillioides*. *Fusarium* strains were grown in corn meal agar plates supplemented with different concentrations of each phenolic acid ranging from 0.5 mM to 10 mM plus a control group without phenolic acids. Plates were incubated at 25°C in the dark during seven days. The diameter of the colonies was measured daily to calculate the inhibition percentages of fungal growth.

Results: Inhibition percentages of fungal growth for the six strains studied were significantly higher when using greater phenolic acid doses (correlation coefficient r ranging from 0.82 to 0.96; $P < 0.05$). The most effective acid was ferulic acid, reaching a total inhibition of fungal growth for all the strains (except *F. langsethiae* and *F. sporotrichioides*) at the maximum concentration evaluated (10 mM).

Significance: *Fusarium* strains studied have shown to be sensitive to all phenolic acids concentrations assayed. Therefore, phenolic acids could be further explored as an alternative or complement to the use of synthetic fungicides for controlling *Fusarium* spp. infection in cereal crops. This research was supported by the Spanish MINECO (Project AGL2011-26808), the Government of Aragón (Grupo de Investigación Consolidado A01), and the European Social Fund. Author E. Ferruz acknowledges a grant from Fundación Cuenca Villoro.

P1-03 Determination of Aflatoxins in Nuts and Nuts Coated with Chocolate by an Optimized UPLC/FLD Method

Marta Herrera, **Laura Herrero**, **ELENA FERRUZ**, **Isabel Gimenez**, **Agustin Arino**, **Antonio Herrera**, **Susana Loran**, University of Zaragoza, Zaragoza, Spain

Introduction: Aflatoxins (AFs) are mycotoxins mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The major AFs of toxicological concern are designated B1, B2, G1 and G2, with AFB1 being the most toxic and

carcinogenic (Group 1 of IARC). Although aflatoxins have been found in a variety of foodstuffs, the most pronounced contamination has been encountered in nuts, corn, cottonseed, dried fruits and spices.

Purpose: The objective of this work was to determine the current levels of aflatoxins (B1, B2, G1 and G2) in different nuts and nuts coated with chocolate by a very sensitive UPLC/FLD method.

Methods: Thirty samples of nuts (n = 15) and nuts coated with chocolate (n = 15) were analyzed: almonds (6), hazelnuts (5), peanuts (14) and pistachios (5). All samples were provided by nut processing plants located in Spain. The extraction and chromatographic conditions were carried out based on the European Committee for Standardization method (EN 16050:2011). Briefly, a representative sample of 5 g was homogenized and extracted with 0.5 g of sodium chloride and 20 mL (methanol/water) using an homogenizer. The extract was filtered and diluted with distilled water and finally purified through immunoaffinity columns. The eluate was evaporated under a stream of nitrogen and reconstituted by mobile phase. Aliquots of 1 µL were injected into the optimized UPLC-FLD system.

Results: This report showed that most analyzed samples contained aflatoxins at low levels, being AF B1 present in 73% of samples with concentrations ranging between 0.10 and 2.39 µg/kg. The aflatoxins G1 and G2 were not detected in uncoated nuts, but they appeared in nuts coated with chocolate. Overall, total aflatoxin content was somewhat higher in uncoated (0.6 µg/kg) than in coated (0.4 µg/kg) nuts.

Significance: Aflatoxins occurred in many of the samples analyzed but their levels were generally low and never exceeded the maximum permitted levels established by the legislation (EC, 2010). This research was supported by the Government of Aragón (Grupo de Investigación Consolidado A01), the European Social Fund and the Technology Park "Aula Dei" (Zaragoza, Spain). Author E. Ferruz acknowledges a grant from Fundación Cuenca Villoro.

P1-04 Selective Removal and Inactivation of Bacteria by Nanoparticle Composites Prepared by Surface Modification of Montmorillonite with Quaternary Ammonium Compounds

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Introduction: Montmorillonite (MMT) nanoclays have exhibited great potential for industrial food packaging and food safety applications. Quaternary ammonium compounds (QACs) are cationic surface active agents with useful clinical purposes. Montmorillonites intercalated with QACs are known as 'organoclays', where the intercalation processes may induce modifications in clay surface structures mediating practical applications.

Purpose: The primary objective of this work is to prepare new nanocomposites with antibacterial activities by surface modification of montmorillonite using quaternary ammonium compounds that are widely applied as disinfectants and antiseptics against a number of foodborne pathogens in food-processing environments. Introducing new nanocomposites by using ammonium based organic modifiers possessing long term antibacterial effects, may lead to potential compounds that may present effective candidates with pharmacological properties and biological role in overcoming the increasingly resistance phenomena.

Methods: *Escherichia coli* O157:H7 (C7927, associated with apple cider outbreak), *Escherichia coli* (ATCC 6538), *Listeria monocytogenes* (ATCC 15313), *Pseudomonas aeroginosa* (ATCC 10145), *Salmonella* Tennessee (K4643), and *Staphylococcus aureus* (ATCC 13565) were used in the study. Relative changes in basal d-spacing values of modified variants induced by the cation exchange reaction were determined by X-ray diffraction analysis conducted at the Georgia X-Ray crystallography Laboratory (GXRCC) located at the Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA. Reduction of population counts was investigated by two processing conditions: low mMMT concentration (0.1 g), large volume of bacterial suspension (10 ml), 30 ml/min flow rate, and high mMMT concentration (1 g), small volume of bacterial suspension (1 ml), 10 ml/min flow rate. The ability of adsorbed bacterial cells to survive in 0.1 or 1 g of mMMT variants was studied as a function of variant concentration, suspension volume and flow rate. The ability of mMMT packed columns to maintain their inactivation capacity to the growth of two resistant strains (*S*. Tennessee and *S. aureus*) was evaluated as a function of storage time.

Results: The XRD was a successful technique to confirm that the prepared mMMT nanocomposites possessed different microcrystalline environments. All test mMMT variants exhibited different antibacterial activities against different volumes of strain suspensions eluted at different flow rates. Reduction in counts of microbial populations adsorbed to the new nanocomposites was substantially different from that in elution experiments, where interactions of nanocomposites with bacteria were specific and more complex than simple ability to inactivate. The capacity of the mMMT packed treatment columns to inactivate adsorbed populations of *S*. aureus and *S*. Tennessee was maintained over the course of 2 days, indicating a potential applicability of the novel mMMT nanocomposites.

Significance: New nanocomposites presented in this research may have potential applications in industrial scale as low cost substitution to QACs for the control of foodborne pathogens by their incorporation into high-performance filters in food processing plant environments where selectivity in removal and/or inactivation of species in fluid flow streams is desirable. Extensive in vitro and in vivo studies of these new nanocomposites is essential to outpace the understanding of their potential impacts and consequences on human health and the environment if they will make an appearance in commercialized food packaging and containment food materials in the future.

P1-05 Control of Foodborne Pathogens Using the Plant-derived Peptide Ib-AMP1

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Introduction: Consumer demand for use of fewer traditional antimicrobial agents in foods has driven research interest in development of plant based antimicrobial agents for use in food and food processing. Plant antimicrobial peptides, including Ib-AMP1, may have potential broad application from use in foods to personal care products. Previous studies on Ib-AMP1 did not investigate properties of the antimicrobial that would specially influence its use in food.

Purpose: The purpose of the present study was to investigate Ib-AMP1, a plant antimicrobial peptide (pAMP), isolated from seeds of *Impatiens balsamina*. Activity against foodborne pathogens, cytotoxicity to select human cells and residual activity were investigated.

Methods: The minimum inhibitory concentration (MIC) of Ib-AMP1 was determined using a standard assay. A broth based assay was used determine whether Ib-AMP1 was bactericidal. Cytotoxicity assays were conducted using human epithelial cell cultures.

Results: Results of these experiments aid in determining the feasibility of using Ib-AMP1 as an antimicrobial agent to control foodborne pathogens. Ib-AMP1 exhibited bactericidal activity against *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella enterica* serovar Newport, *Pseudomonas aeruginosa*, and *Bacillus cereus*. When tested using low (10^3 CFU mL^{-1}) and intermediate (10^6 CFU mL^{-1}) *E. coli* O157:H7 cell numbers, an approximately 1.46-2.69 log reduction in cell numbers occurred at the 1X and 2X MIC of Ib-AMP1. No residual activity of Ib-AMP1 was apparent following interaction of the peptide with bacteria. Results of the MTS cell proliferation assay indicated that Ib-AMP1 at 200, 400 and 600 µg mL-1 inhibited by 50% cell proliferation activity of Hep G2, FHs 74 Int and HT29 cells, respectively.

Significance: These results suggest that a concentration of Ib-AMP1 several fold greater than the MIC would be required in foods with high levels of commensal bacteria. Ib-AMP1 was not detrimental to survival of human cells. Taken together, these data suggest that Ib-AMP1 has potential application as an antimicrobial agent in food systems.

P1-06 WITHDRAWN

P1-07 Structure-activity Relationship of Synthetic Variants of the Milk-derived αs2-casein f(183-207) Antimicrobial Peptide

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Introduction: Milk proteins have been identified as a rich source of antimicrobial peptides, many of which have been extensively characterized. Fragment (183-207) of the bovine αs2-casein shows high antibacterial activity against both Gram-positive and Gram-negative bacteria, with minimum inhibitory concentrations (MIC) as low as 8-16 µM. However, the precise mechanism by which the αs2-casein f(183-207) peptide acts remains still unclear.

Purpose: The aim of this study was to assess the structure-activity relationship of αs2-casein f(183-207) peptide variants using *Listeria monocytogenes* LO28 and *Cronobacter sakazakii* DPC6440 as test organisms. The experimental strategy included downsizing the peptide, alanine scanning, performance of amino acid substitutions and hydrophobic end-tagging.

Methods: αs2-casein f(183-207) peptide variants were chemically synthesized by Metabion (Germany). The activity of these variants against L. monocytogenes LO28 and C. sakazakii DPC6440 was tested by means of the agar well diffusion assay and the broth dilution method. Minimum inhibitory concentrations (MICs) were calculated and arbitrary activity units (reflecting the MIC of the variant relative to the MIC of the wild-type peptide) were assigned.

Results: Downsizing of the αs2-casein f(183-207) peptide showed that the f(193-207) variant was the most active. Alanine scanning of this fifteen-amino acids peptide showed a considerable reduction in antibacterial activity when Arg at position 205 of the αs2-casein peptide was substituted. On the other hand, substitution of the Pro residues at positions 196 and 202 of the αs2-casein peptide resulted in an increase in antibacterial activity. Replacement of two or more positively charged amino acids (Lys, Arg) gave rise to a significant loss of activity. Hydrophobic end-tagging of αs2-casein f(193-203) and αs2-casein f(197-207) peptides with multiple Trp or Phe residues significantly increased their potency against *L. monocytogenes* LO28.

Significance: Template-based studies on peptide derivatives obtained through manipulation of the amino acid sequence are helpful to identify properties that are important for activity. This study sheds light on the importance of specific amino acids and residue positions to the activity of the milk-derived αs2-casein f(183-207) antimicrobial peptide.

P1-08 Development of a Biochip Array for the Simultaneous Detection of the Antimicrobials: Aminoglycosides, Lincosamides, Stretogramins and Macrolides

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Introduction: Aminoglycosides, lincosamides, streptogramins and macrolides are commonly used within the agricultural sector for the prevention of bacterial infection and as growth promoters. There has been some controversy surrounding the use of antibiotics for food animals due to the potential for development of resistant pathogens. The EU has implemented testing programmes to test for veterinary medicines. Maximum Residue Limits for these drugs have been set in some matrices. Controlling and detecting such drugs is vital for consumer protection and in this context the development of immunoassays capable of detecting a vast range of these analytes is relevant.

Purpose: This study aimed to develop simultaneous immunoassays on a biochip platform for multi-analytical detection of these antimicrobials from a single sample to increase the screening capacity. This biochip array enables the simultaneous detection of thirty-seven antimicrobials (including their associated isomers/analogues).

Methods: The simultaneous competitive chemiluminescent immunoassays defined 14 discrete test sites on the biochip surface. The detection of the immunoreactions takes place using digital imaging technology on the Evidence Investigator analyser. The system incorporates dedicated software to process and archive the multiple data generated.

Results: Initial analytical evaluation of the simultaneous biochip assays showed broad cross-reactivity profile with 37 analytes being detected over 14 assays. For example, the gentamicin assay can detect gentamicin C1, gentamicin C1a, gentamicin C2, G418, netilimicin and sisomicin (% cross-reactivity values: 229, 104, 180, 89, 38 and 32% respectively).The sensitivity values, expressed as the half maximal inhibitory concentration (IC50) ranged from < 0.2 ppb (neomycin) to < 5 ppb (gentamicin).The intra-assay precision (n = 6) was %CV < 12 for all the assays.

Significance: Data show that the developed biochip array allows the simultaneous detection of aminoglycosides, lincosamides, streptogramins and macrolides. This is advantageous for the rapid and simultaneous screening of these antimicrobials, which could be both incorporated into the food chain and the environment.

P1-09 Antimicrobial Compounds against Pathogenic Bacteria and Spoilage Yeasts in Medicinal Mushrooms
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Introduction: Medicinal mushrooms (MM) have been an important of eastern medicine for their immunomodulation, antitumor, and antioxidant activities. Mushrooms contain polysaccharides, β-glucans, and phenolic compounds that have pharmaceutical, nutraceutical, and functional properties. There has been little research reported on the antifungal and antibacterial activity of these mushrooms.

Purpose: Our objective was to determine if antimicrobial compounds were present in six species of medicinal mushrooms (*Agaricus blazei* Murill, *Inonotus obliquus*, *Grifola frondosa*, *Ganoderma lucidum*, *Phellinus linteus* and *Lentinula edodes*) against three pathogenic bacteria (*Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes*) and three spoilage yeasts (*Saccharomyces cerevisiae*, *Zygosaccharomyces bailii* and *Zygosaccharomyces bailii/bisporus*).

Methods: Extracts were prepared using ethanol. Antimicrobial activity in MM extract was assessed using disk diffusion assay with sorbic acid, trans-cinnamaldehyde, and ethanol as controls. In addition, 48-hour growth curves were determined using optical density obtained using a Bioscreen C. T-tests were used for statistical comparisons.

Results: In disk diffusion assay, *G. frondosa* and *P. linteus* extracts significantly inhibited all pathogens and spoilage yeasts. *A. blazei Murill* extract inhibited all but *E. coli*. *I. obliquus* only inhibited *S.* Typhimurium and *Z. bailii* growth. *L. edodes* extract inhibited all but *Z. bailii* and *E. coli*, and *G. lucidum* inhibited all but *S.* Typhimurium. The Bioscreen results were often in disagreement. Pathogens were not inhibited by the extracts in these results but there was some effect on *Z. bailii* and *Z. bailii/bisporus.*

Significance: The MM extracts have some inhibitory activities against food pathogens and yeasts, but the method of assessing inhibition affects the results. If the compounds that cause the antimicrobial activities can be identified and produced economically, they may have value in replacing artificial preservatives and improving the quality of consumer products.

P1-10 Assessment of Antimicrobial Surfaces for Use in Food Factories
COLETTE JERMANN, Campden BRI, Chipping Campden, United Kingdom

Introduction: The efficacy of antimicrobial surfaces in the food manufacturing environment has not been established. There is a need to develop appropriate assessment methods for these surfaces.

Purpose: The purpose of this study was to determine microbial load, temperature and relative humidity on factory surfaces. These data will be used in the project to develop appropriate assessment methods for antimicrobial efficacy.

Methods: Tests were undertaken in five food factories (vegetable, meat, brewery and two dairies). A hygrometer was used to measure relative humidity and temperature of surfaces. Microbiological analysis of surfaces was undertaken using pre-moistened swabs in neutralising buffer wiped over a 25cm2 surface. Enumeration of TVC was conducted by transferring the swab to a diluent, plating on PCA and incubating at 30°C +/-1°C for 48 hours. Enumeration of yeasts and moulds was conducted by transferring the swab to a diluent, plating on DRBC agar and incubating at 25°C +/-1°C for 5 days.

Results: Yeasts and moulds were highest on a surface in the brewery (240 CFU/cm^2 and 112 CFU/cm^2 respectively). TVC was highest in the vegetable processing plant (960 CFU/cm^2), followed by the brewery (>400 CFU/cm^2), the dairies (256 and 180 CFU/cm^2) and the meat factory (6 CFU/cm^2). All factories had some surfaces with microbial levels below detection (<5 for TVC and <10 for yeasts and moulds).

Significance: These data give an indication of the environmental conditions on food factory surfaces. It is of importance to develop a method to assess the efficacy of antimicrobial surfaces under conditions representative of food factory environments. Antimicrobial surfaces could provide enhanced food safety if they can be demonstrated to be effective. Campden BRI has developed considerable expertise and can help in the domain of hygienic design and evaluation of antimicrobial surfaces and disinfectants.

P1-11 Purification and Characterization of an Anti-*Listeria *Bacteriocin Produced by* Lactococcus lactis *KT2W2L
NORAPHAT HWANHLEM, Prince of Songkla University, Hat Yai, Thailand, **Yvan Choiset** and **Vanessa Biscola**, Institut National de la Recherche Agronomique , Nantes, France, **Aran H-Kittikun**, Prince of Songkla University, Hat Yai, Thailand, **Thomas Haertle** and **Jean-Marc Chobert**, Institut National de la Recherche Agronomique , Nantes, France

Introduction: Strain KT2W2L, isolated from mangrove water in southern Thailand and identified as *Lactococcus lactis* produced a component that inhibits the growth of Gram-positive bacteria, some foodborne pathogens, especially *Listeria monocytogenes*, a foodborne bacteria especially dangerous for pregnant women.

Purpose: To purify and characterize an anti-*Listeria* bacteriocin produced by *L. lactis* KT2W2L.

Methods: The active peptide from the cell-free supernatant of *L. lactis* KT2W2L was purified in 4 steps: (i) precipitation with 70% saturated ammonium sulfate, (ii) elution on a reversed phase C8 cartridge using different concentrations of acetonitrile, (iii) cation-exchange chromatography and (iv) final purification by reversed phase HPLC on a C8 column. The stability and sensitivity of the bacteriocin in different pH, temperature and proteolytic enzymes was determined. Tricine-SDS-PAGE was performed to estimate the molecular mass of the purified bacteriocin and PCR was carried out to detect nisin genes (A, F, Z).

Results: The purified bacteriocin was not affected by pH (2.0-10.0), heating (100°C) but sensitive to proteolytic enzymes. Tricine-SDS-PAGE of purified bacteriocin gave molecular weight ranging between 3.5 and 6.5 kDa. The fragment obtained after amplification of genomic DNA from *L. lactis* KT2W2L, with specific primers for bacteriocin genes, presented 100% homology to the nisin Z gene.

Significance: This bacteriocin appears potentially very useful to reduce *Listeria monocytogenes* in food product and could be used as a food preservative. This work was financially supported by the Office of the Higher Education

Commission, National Research University Project of Thailand and the Graduate School, Prince of Songkla University, and by the French Ministry of Foreign Affairs ("Bio-Asie" Project).

P1-12 Validation Data of the Pathatrix® Auto System Linked to the AB 7500 Fast PCR Platform Using MicroSEQ® Pathogen PCR Detection Kits, Demonstrating the Sensitivity of Post Enrichment Wet Sample Pooling

ADRIAN PARTON, **Ian Scheldrake**, **Cyril Dubuc**, **Silke Schneegans**, **Andrea Biserni**, **Katrien Vanhonacker**, LifeTechnologies, Ghent, Belgium

Introduction: A validation study has been set up with the Pathatrix® Auto System linked to the AB 7500 Fast PCR platform using MicroSEQ® pathogen PCR detection kits on a broad range of food samples, inclusive challenging matrices, to investigate its wet pooling capability.

Purpose: Pathatrix® Auto is a well established system which enhances the non-disruptive technique of Immuno-Magnetic Separation (IMS). The magnetic beads with specific antibodies, selectively bind and concentrate the target organism from many complex food matrices, prior to real-time PCR detection. This technology also enables wet pooling, a process where aliquots of up to 10 samples are wet pooled after enrichment.

MicroSEQ® pathogen PCR detection kits are built with lyophilized reagents into pre-formatted assay beads with an internal positive control. Universal PCR cycling conditions on the 7500 Fast PCR detection platform offer flexibility to run multiple pathogen assays simultaneously.

Methods: A validation study has been set up to demonstrate the performance of the wet pooling concept of the Auto System linked to the AB 7500 Fast PCR platform using pathogen PCR detection kits on a broad range of pooled food samples, like ham, raw beef, pastry, frozen meal, chocolate, milk, yoghurt and infant formula.

Results: Validation data have been collected over the broad range of food matrices. The optimal enrichment conditions have been determined for several food categories in line with the officials regulations.
Results from this study are showing:
-Pathatrix Auto System is successfully wet pooling food samples from different categories.
-Pathatrix Auto System is efficiently cleaning the sample from its matrix affects, making challenging samples, like chocolate, accessible to real-time PCR.
-Efficient concentration of the target organism during IMS on the Pathatrix system and the fast cycling conditions of the MicroSEQ PCR kits, reducing significantly the time to result.

Significance: This study demonstrates the combination of the Pathatrix® Auto System linked to the AB 7500 Fast PCR platform using MicroSEQ® pathogen detection kits is successfully wet pooling different food matrices, bringing clear benefits to the end user in the food testing laboratory.

P1-13 Enzymatic Food Analysis in a Microtiter Plate Format

LUKAS FRANK and **Elisabeth Halbmayr-Jech**, Romer Labs Division Holding GmbH, Tulln, Austria, **Andrea Klink** and **Tobias Hein**, ifp Institut für Produktqualität GmbH, Berlin, Germany

Introduction: For decades enzymatic methods have been part of routine analysis of sugars, acids and other metabolites. Due to the high selectivity of utilized enzymes these methods are highly specific and permit an accurate quantitative determination of metabolites. Common practice is a single photometric measurement in a cuvette. With a high sample throughput, these methods can be very labor intensive and require a lot of consumables.

Purpose: A validation of a new microtiter plate format enzymatic method, particularly with a kit for the determination of Lactose/D-Galactose, was performed.

Methods: To assay the precision of the new method, recovery rates from reference materials and food matrices with a declared nominal content of lactose were compared to the traditional cuvette method.

Results: Results obtained with the new microtiter plate format are in every respect comparable with the traditional cuvette method. The limit of detection (LOD) for lactose was determined as 0.005 g/100 g.

Significance: It can be stated that the new microtiter plate format enzymatic method has advantages compared to the traditional cuvette method without losing accuracy. Economic reasons as well as simple use indicate the efficiency of this new microtiter plate method.

P1-14 Comparative Evaluation of the Indirect Determination of 3-MCPD Esters in Vegetable Oils Using Alkaline Transesterification

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Introduction: The concern raised by the high levels of fatty acid esters of 3-monochloropropane-1,2-diol (3-MCPD esters) in refined vegetable oils prompted global investigations on the methods used to analyze these contaminants. Several indirect methods, performed under acid or alkaline conditions, have been proposed. The alkaline-catalysed transesterification has been considered more convenient due to its short duration. However, its reliability is still under discussion, especially regarding the presence of glycidyl esters that could lead to additional formation of 3-MCPD during the analysis.

Purpose: The objective of this work was to evaluate a procedure for 3-MCPD esters determination based on alkaline transesterification, and to compare it to a previously validated method based on acid transesterification.

Methods: Experiments were focused on the acid pre-treatment to remove glycidyl esters and on the use of different salts for salting-out. The analysis included the addition of 1,2-dipalmitoyl-3-MCPD-d5 solution to the sample, pre-treatment with sulfuric acid in methanol, transesterification with sodium methoxide, neutralization with glacial acetic acid, salting-out (sodium chloride, ammonium sulfate or potassium bromide), derivatization with phenylboronic acid and analysis by gas chromatography-mass spectrometry.

Results: The levels of 3-MCPD esters in a sample of soybean oil obtained with and without the acid pre-treatment were respectively: 11.3 and 18.4 mg/kg (sodium chloride), 8.3 and 7.7 mg/kg (ammonium sulfate), and 7.7 and 6.3 mg/kg (potassium bromide). A similar trend was observed for other samples. Even upon the acid pre-treatment, the use of sodium chloride resulted in higher levels in comparison to chloride-free salts, suggesting that the removal of glycidyl esters may have been incomplete. The use of chloride-free salts resulted in levels of 3-MCPD esters comparable to those obtained by acid transesterification, but showed poor precision and lower peak areas.

Significance: In the tested conditions, the alkaline procedure resulted in inconsistent and/or overestimated results as compared to a method based on acid transesterification. Acknowledgments: FAPESP (Proc. 2011/08936-0).

P1-15 ISO 16140 Validation Study of the VIDAS UP Salmonella Method for Detection of* Salmonella *in 25 to 375 g Food and Environmental Samples

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Introduction: The VIDAS® UP *Salmonella* assay (SPT) employs innovative technology involving recombinant phage proteins specific for detection of *Salmonella*. This new method utilizes a single primary enrichment supplemented with a proprietary additive to eliminate the need for secondary enrichments.

Purpose: The purpose of the study was to compare the new method to the ISO 6579 reference method for 25 g and 375 g samples as part of the AFNOR Certification validation process

Methods: 25 g samples (foods, feeds and environmental samples), 1/10 diluted in Buffered Peptone Water (BPW) plus a selective supplement were enriched for 18–24 hours at 41.5°C. 375 g samples, ¼ diluted in the selective BPW (raw beef, milk powder and ingredients), or in selective UHT milk (chocolate and cocoa), were enriched for 22–26 hours at 41.5°C. After incubation, samples were boiled for 5 minutes before performing the assay

Results: A comparative study was performed on 704 products, 452 for the 25 g samples and 252 for the 375 g samples. Overall, similar results were obtained by the two methods with 345 confirmed positive samples detected by the new method and 348 by the reference method.The 50% detection limit was found to be between 0.2 and 1.3 CFU/25 g for the alternative method and 0.2 and 1.1 for the reference method. A similar detection limit was found for both sample sizes

Significance: The results of this study demonstrated the reliability of the VIDAS® UP *Salmonella* method when compared to the traditional reference methods for the detection of *Salmonella* in 25 g and 375 g samples. The method offers a significant savings in time when compared to the traditional reference methods by producing presumptive results in less than 24 hours. For the 375 g samples the ¼ dilution of the matrix into the enrichment broth is also an advantage in term of cost, weight for the technician and space into the incubator.

P1-16 Validation study According to the ISO 16140 Standard of a Rapid Method for the Detection of* Listeria *spp. in Food products and Environmental Samples

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Introduction: The VIDAS® UP *Listeria* assay (LPT) employs innovative technology involving recombinant phage proteins specific for detection of *Listeria*. This new method utilizes a single primary proprietary selective enrichment to eliminate the need for secondary enrichments

Purpose: A study was conducted by the independent Expert Laboratory Eurofins IPL Nord, to validate this new method, as part of the NF Validation approval process

Methods: Samples were enriched at 30 ± 1°C for 26 hours (food samples) or 22 hours (environmental surfaces) in the ready to use proprietary broth, the LPT broth. After incubation, samples were boiled for 5 ± 1 minutes before performing the assay. All presumptive positive samples were further confirmed after streaking on PALCAM or on a chromogenic agar according to Ottaviani Agosti. This new method was compared to the ISO 11290-1/A1 reference method, according to the ISO 16140 standard

Results: A comparative study was performed on 349 products distributed over the 5 categories meat, dairy, vegetable, seafood and environmental samples of which 69% were naturally contaminated. The phage method detected 160 positive samples compared to 162 for the reference method. There was no statistical difference between the two methods using the Mc Nemar test at 5% level.The 50% detection limit was determined on 5 different products/strains combination tested at 4 contamination levels. Results were comparable between the two methods.In the inclusivity study, all the 51 *Listeria* spp. strains tested were detected and in the inclusivity study, none of the 30 non *Listeria* strains gave a positive result

Significance: The LPT method provides a simple, convenient and reliable method for detection of *Listeria* species in food and environmental samples, providing a presumptive result for the presence of *Listeria* in less than 23 hours for environmental surfaces and 27 hours for food samples.

P1-17 Detection of Allergenic Parvalbumin of Atlantic Herring (*Clupea harengus*) by Real-time PCR

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Introduction: Food allergies have become a serious health problem all over the world. In industrialized countries from 1 to 3% of adults and up to 8% of children and adolescents are affected by food allergies. Fish belong are a main sources of food allergens. The European Union (EU) Directives 2000/13/EC and 2003/89/EC and 2007/68/EC require the declaration of the exact allergenic food. Accurate and reliable labeling practices are important to enable the allergic consumer to prevent health difficulties.

Purpose: The purpose of this work was to develop a real-time PCR method for the detection of the major allergenic protein parvalbumin beta 2 of Atlantic herring (*Clupea harengus*) via targeting segments of pvalb 2 gene encoding this protein.

Methods: The real-time PCR method for the detection of the major allergenic protein parvalbumin beta 2 of Atlantic herring (*Clupea harengus*) has been developed. The specificity of designed primer pair and probe for pvalb 2 gene was tested on a spectrum of 23 fish species and no cross reactions were detected.Two fish product samples (No. 21 and 22) were negative in spite of the Atlantic herring declaration.

Results: The method was applied to the analysis of 22 commercial fish products. The specific DNA was detected in twenty samples.Two fish product samples were negative in spite of the Atlantic herring declaration. The internal amplification control 18S rRNA gene for eucaryotes was used.

Significance: The real-time PCR method is specific and sensitive enough and can be used as a fast, simple procedure for detection of Atlantic herring via pvalb 2 gene marker encoding the parvalbumin beta 2 protein. It can become a useful tool for routine food safety control of declared allergens of Atlantic herring in food and fish products. This work was supported by the Ministry of Agriculture of the Czech Republic (Grant MZe 00027 16202).

P1-18 Microbial Source Tracking: Tool to Identify the Fecal Contamination Origin

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Introduction: Fecal pollution of the environment is a major problem for human health and local economies. Such fecal contamination can result in shellfish areas or swimming areas closing. To date, no standard methods allow the identification of the fecal contamination sources. A Microbial Source Tracking (MST) kit was developed to determine sources of fecal pollution in environmental ecosystems.

Purpose: The objective of the study was to provide a MST kit in order to distinguish the origin of fecal contamination.

Methods: Two markers were selected, F-RNA bacteriophages and Bacteroides. Bacteriophages enable discrimination between human and animal origin as these viruses are classified in four genogroups. Genogroups I and IV are associated with animal contamination, and genogroups II and III with human contamination. Bacteroides, bacteria found in large quantities in stool and resistant in nature, are associated to the host, and therefore provide a more specific result on the origin of contamination. Primers and probes have been defined for the differentiation between GI, GII, GIII, GIV of F-RNA bacteriophages and human, ruminant or porcine Bacteroides. The detection of these markers is performed by multiplex real-time PCR. All the required controls have been included to ensure reliable results. Based on the developed method, a commercial kit was produced and commercialized under the trademark ceeramTools.

Results: As part of an internal study, 39 natural samples (seawater, river/lakes water and water treatment plant outflows) were tested. Using MST@ceeramTools kits, human contamination was detected in 87% of samples and contamination of animal origin (ruminants, swine or otherwise) was detected in 48% of samples. A mixed contamination (human and animal) accounts for 46% of samples.

Significance: These data show the importance of both human and animal fecal contamination in aquatic ecosystems. The determination of the fecal contamination origin is important to create the appropriate corrective actions in order to prevent the health risk.

P1-19 Validation of a New Real-Time PCR Method for Detection of **Listeria monocytogenes** *in Food and Environmental Samples*

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Introduction: The Thermo Scientific™ SureTect™ *Listeria monocytogenes* Assay is a new Real-Time PCR test for the detection of *L. monocytogenes* from food and environmental surfaces, which combines pre-dispensed lysis reagent and lyophilised, tableted PCR reagents to simplify and improve assay handling, along with dedicated software to interpret and display PCR results.

Purpose: The study was conducted according to the AOAC-RI Performance Test Method validation process to evaluate the SureTect Assay for use with a representative range of produce, meat, dairy and sea-food matrices as well as stainless steel surfaces.

Methods: Validation of the *Listeria monocytogenes* Assay was conducted by enriching 25 g samples of food matrices or surface sponges in supplemented Oxoid™ 24 LEB Broth for 22 hours, followed by PCR analysis according to method instructions. Cooked deli-ham, smoked salmon, salami, prawns, raw cod, ice-cream, American style cheese, brie, fresh spinach and lettuce, cantaloupe melon, frankfurters and stainless steel surfaces were evaluated in comparison to the ISO 11290-1:1998, Amd 1:2004 reference method. Foods were spiked at low (0.2–2 CFU/25 g) and high (2-5 CFU/25 g) levels, with low level spiking required to achieve fractional positive rates across 20 replicate samples. All PCR positive results were confirmed using the SureTect confirmation protocol (plating onto *Brilliance™Listeria* Agar) and by a shortened ISO confirmation procedure.

Results: Internal and external independent validation demonstrated that the SureTect *Listeria monocytogenes* Assay gave equivalent or better performance than ISO 11290-1 for all matrices studied. Results from the SureTect Assay were in agreement by probability of detection statistical analysis with ISO 11290-1. When compared with the reference method, the mean RLOD for all matrices was 1.370 CFU/25 g (0.701–2.506). Inclusivity testing detected all of 53 isolates of *L. monocytogenes* tested. None of the 38 exclusivity isolates were detected by the assay.

Significance: The SureTect PCR assay was shown to be an accurate and user-friendly method, due to the use of pre-dispensed lysis reagent, tableted PCR reagents and automatic interpretation of results. Results for a wide range of foods, including challenging matrices, demonstrated the assay was able to reliably detect the presence of *L. monocytogenes*.

P1-20 AOAC-RI Validation of a New Real-Time PCR Assay for Detection of* Salmonella *in Foods and Environmental Samples

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Introduction: The Thermo Scientific™ SureTect™ *Salmonella* species Assay is a new real-time PCR test for the detection of *Salmonella* from food and environmental surfaces, which combines pre-dispensed lysis reagent and lyophilised, tableted PCR reagents to simplify and improve assay handling, along with dedicated software to interpret and display PCR results.

Purpose: The study was conducted according to the AOAC-RI Performance Test Method℠ validation process to evaluate the Assay for use with a representative range of produce, raw meat, dairy, ready-to-eat meals, egg and sea-food matrices as well as stainless steel surfaces.

Methods: Validation of the *Salmonella* species Assay was conducted by enriching 25 g samples of food matrices or surface sponges in BPW (ISO) for 8, 18 or 20 hours, depending on the sample matrix, followed by PCR analysis according to method instructions. Raw ground beef, raw chicken, ready to eat meal, frankfurters, raw pork, shrimp, non-fat dried milk powder, lettuce, liquid egg and stainless steel surfaces were evaluated in comparison to the ISO 6579:2002 reference method. Foods were spiked at low (0.2–2 CFU/25 g) and high (2–5 CFU/25 g) levels, with low level spiking required to achieve fractional positive rates across 20 replicate samples. All PCR positive results were confirmed using the confirmation protocol and by a shortened ISO confirmation procedure.

Results: Internal and external independent validation demonstrated that the *Salmonella* species Assay gave equivalent or better performance than ISO 6579 for all matrices studied. Results from the Assay were in agreement by probability of detection statistical analysis with ISO 6579. Inclusivity testing detected all 117 different serotypes and species of Salmonellae tested. None of 36 exclusivity isolates were detected by the assay.

Significance: The SureTect PCR assay was shown to be an accurate and user-friendly method, due to the use of pre-dispensed lysis reagent, tableted PCR reagents and automatic interpretation of results. Results for a wide range of foods, including challenging matrices, demonstrated the assay was able to reliably detect the presence of *Salmonella*.

P1-21 Comparison of the New TEMPO® AC Method with the ISO 4833 and BAM Methods for Enumeration of Total Aerobic Count in Challenging Food Products

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Introduction: The TEMPO® AC (Aerobic Count) is a new automated test which allows the enumeration of viable aerobic mesophilic flora in food products and environmental samples in as little as 22–28 hours at 35°C (AOAC-RI validated) and also in 40–48 hours at 30°C.

Purpose: The purpose of the study was to evaluate the TEMPO® AC performance using a range of foods, particularly products with strong enzymatic activity (raw red offals, raw mollusks, walnuts, hazelnuts, almonds, flours such as raw crust and dough, ready-to-use cake mixes, dehydrated soups and sauces, and inhibitor products like spices). More than 120 naturally contaminated food matrices were tested. The AC was compared with the AOAC 966.23 method and the Standard Methods for the Examination of Dairy Products (SMEDP) at, respectively, 35°C and 32°C as whereas with the EN ISO 4833 method at 30°C.

Methods: The TEMPO system enumerates total aerobic microorganisms present in foods according to a principal based on the Most Probable Number (MPN) method.

Results: Statistical analysis was conducted using the regression analysis of TEMPO® AC versus the ISO 4833 method and the BAM 966.23/SMEDP method. TEMPO® AC results showed a good agreement with both the standard methods.

Significance: The TEMPO® AC method is reliable, rapid, and an automated alternative enumeration method of total viable count in foods and environmental samples. It provides results one day before the traditional methods (48h at 35°C and 72 hours at 30°C), in a wide range of food products.

P1-22 Rapid Detection of Yeast and Mold in Filterable Beverage Using BAX® System PCR Assay

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Introduction: Fungal spoilage of food accounts for 5%–10% of all losses in global food production. This spoilage is not only a quality issue, but also food safety concern since many common mold species are dangerously toxigenic. Due to this concern, it is imperative that manufacturers closely monitor their processes to prevent such contamination from occurring. While traditional colony count methods for yeasts and molds require 5–7 days to achieve a result, the BAX® System Yeast and Mold PCR Assay provides same day results for food samples containing >500 CFU/g (direct method), or in just 2 days for food samples with 50–500 CFU/g (enrichment method). This study was to validate the Yeast and Mold assay in filterable beverage for the detection of yeast and mold at very low level.

Purpose: The objectives of this study were to validate the System assay for detecting low levels (1 CFU/5mL) of yeast and mold in filterable beverage and to compare the efficiency of sample processing through both filtration and centrifugation to collect yeast and mold cells.

Methods: Two master samples of soda were spiked with *Saccharomyces cerevisiae* and *Aspergillus niger* at a level targeting 1 CFU/5mL. Samples were either filtered using in-line filter units or subjected to centrifugation, then all samples were incubated at 25°C for 44 hr. Samples were prepared for yeast and mold detection and a full process was run on the BAX® System instrument according to the procedures described in the System User Guide.

Results: All spiked samples regardless of the sample processing method used, returned positive results with the System for the target organism, and all non-spiked samples returned negative results. Actual spiking levels for inoculated samples was determined to be 0.6 CFU/mL for *S. cerevisiae* and 0.14 CFU/mL for *A. niger* after the 44-hour incubation.

Significance: This study demonstrates that the BAX® System assay can detect yeast and mold in soda within two days at only 1–3 CFU/5 mL. Sample processing using filtration is equivalent to centrifugation to collect cells from filterable beverages. This study also demonstrates that the System can detect yeast and mold in filterable beverages, provided that at least 1 CFU is retained by the filter.

P1-23 Evaluation of the Proteolytic Activity, Virulence Genes and Antibiotic Resistance of* Enterococcus faecalis *FT132, Isolated from Brazilian Raw Cow Milk

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Introduction: Milk is of great importance for human nutrition. However, it can be a problem for people allergic to its constituents, such as caseins and lactoserum proteins. The role of lactic acid bacteria in the modification of milk proteins during fermentation processes and their role in production of peptides with different biological activities and lower antigenicity have been highlighted.

Purpose: To evaluate the effect of temperature, pH and protease inhibitors on the proteolytic activity of *Enterococcus faecalis* FT132 on milk proteins, as well as to search for virulence genes and antibiotic resistance.

Methods: *E. faecalis* FT132 was isolated from Brazilian raw bovine milk, and identified by 16S rDNA sequencing. The proteolytic activity was initially detected in skim milk by cultivation at 37°C/24 h followed by tris-glycine SDS-PAGE. Caseins and whey protein hydrolysis were recorded at 3, 6, 9 and 24 h at 42°C and the influence of temperature, pH and protease inhibitors was also evaluated. Polymerase chain reaction (PCR) was used to detect virulence genes (*as*, *ace*, *cyl*A, *efa*A, *esp* and *gel*E) and antibiotic resistance was evaluated by Kirby-Bauer disk diffusion susceptibility test.

Results: Hydrolysis of caseins and whey proteins by *E. faecalis* FT132 was detected in the range of 30°C to 42°C/24 h with higher activity observed at 42°C, while the optimal pH for proteolysis was 6.5. Caseins and whey proteins hydrolysis started after 3 h and were inhibited by EDTA, suggesting a role for metalloproteases in the observed hydrolytic activity. Three virulence genes were detected in *E. faecalis* FT132 (*as*, *ace* and *gel*E) and it was sensitive to ampicillin, ciprofloxacin, chloramphenicol, penicillin, rifampicin and vancomycin, but resistant to erythromycin and tetracycline.

Significance: *E. faecalis* FT132 presented optimal proteolytic activity at 42°C and pH 6.5. In addition, only a few virulence genes were found in this strain and these genes are widely distributed among food isolates. It also presented a narrow antibiotic resistance profile, indicating its potential to be applied for reduction of milk protein antigenicity.

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P1-24 Improved Reagents for Rapid Hygiene Monitoring of Clean-in-Place Systems

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Introduction: Cleanliness is critical in breweries and dairies where automated equipment is cleaned using Clean in Place (CIP) systems to ensure consistent quality. Cleaning validation by traditional plate or membrane methods can take up to 7 days. In this time, vessels could be filled, emptied and cleaned again such that the testing is of little value in process control. A simpler, more pro-active approach is to use ATP rapid hygiene monitoring. Disposable all-in-one tests are available, and can be carried out in seconds, enabling technicians to test rinse water after each CIP cycle and to re-clean the automated equipment if 'fail' results occur. Tests can be easily performed by technicians with little training. Tests detect the presence of ATP from both microorganisms and product residues which, if present mean cleaning has not been effective.

Purpose: The study objective was to demonstrate improvements made due to reformulation of an ATP rapid hygiene monitoring test, 3M™ Clean-Trace™ Water Plus – Total ATP. Freeze-drying was replaced by innovative liquid stable chemistry.

Methods: ATP bioluminescence activity using test devices was measured according to the manufacturer's instructions, and using 10 ml of 5×10^{-9}M of ATP as sample, reading immediately in a 3M Clean-Trace NG Luminometer. Background light levels were measured by activating test devices without a water sample, reading immediately in a 3M Clean-Trace NG Luminometer.

Results: Results showed disposable test devices can be stored for 2 months at room temperature (21°C – 25°C) instead of 2 weeks, giving greater storage flexibility; allowing storage nearer test points, often located some distance from a refrigerator in CIP process areas. Test result accuracy has been improved, with a three fold reduction in mean background light levels from <35RLU to <10RLU, resulting in improved signal : noise ratios and avoidance of false positives. Additionally, the test can be used over a wider temperature range, increased to 15°C – 30°C from 15°C – 25°C.

Significance: The findings allow for improved temperature stability and test result accuracy. The lower backgrounds enable food and beverage producers to further improve their cleaning efficiencies as part of their process of continuous improvement.

P1-25 Two Sides to Every Food Safety Story: Factors Influencing Consumer Trust of Online Food Saftey Information

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Introduction: There is growing interest in the raw milk debate, with increasing numbers of websites dedicated to discussing the facts around this issue. However, little is known about how people use the Internet to seek trusted information about consuming dairy products.

Purpose: To carry out a psychological investigation to better understand how consumers search for information about milk on the Internet, and identify the factors which influence consumers' trust in websites.

Methods: An innovative Internet café-style research method was employed, whereby 7 ordinary (neither pro-pastuerised or pro-raw) milk consumers were invited to attend a 2.5 hour session. After a period of free Internet searching in which participants' Internet movements were logged, participants were directed to eight specific web sites dedicated to the milk debate - half pro-pasturisation and half pro-raw milk – which varied in terms of provider, content, and design features. Group discussions were held to explore trust and mistrust of the websites.

Results: The free search Internet logs showed 30 unique sites (6 were dairy industry sites, 9 were online media sites, and 5 were social media sites). Group discussions revealed two key factors: 1) website design, and 2) a balanced argument. Websites that were poorly designed were seen as "amateurish" and disliked by consumers. High visual appeal was important in generating credibility but could still be undermined by the presentation of a heavily biased perspective towards one side of the debate.

Significance: The results highlight that consumers are willing to explore information and evidence from a wide range of Internet sources, yet prefer and trust well-designed websites that present a balanced argument. Organisations that use websites to disseminate food safety information to the public should consider these factors to maximise consumer trust for the information and advice they advocate. This research was funded by the U.S. Department of Agriculture.

P1-26 Control Strategies for Food Safety of Traditional Dairy Products on Summer Alpine Pastures

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Introduction: In the framework of a regional legislation aimed to support traditional food production of the Friuli VG region (Northeastern Italy) and its socio-economic value, while ensuring their safety for consumers, a pilot study was implemented.

Purpose: The purpose was to identify control strategies to manage microbial risks in typical dairy products (DP) produced on summer alpine pastures, in particular coagulase-positive staphylococci (CoPS) risk. CoPS are recognized agents of mastitis, frequently subclinical and associated to intermittent shedding into the milk.

Methods: 21 mountain-premises, 9 fully complying with regulation requirements (FCR) and 12 used as control, were included in the project. Milk samples of individual animals from 104 farms were collected on two occasions and tested for CoPS before going to mountain premises. Bulk milk samples and typical DP (butter, fresh and smoked ricotta cheese) were collected on two occasions on each mountain-farm and analysed for CoPS, Staphylococcal enterotoxins (SE), *Escherichia coli*, *E. coli* O157, *L. monocytogens*, according to the potential risk of each type of product. Milk filters were also tested. Statystical analyses of CoPS counts were performed for bulk milk and DP.

Results: 306 of 1,314 (23.3%) individual milk samples were CoPS positive; these data helped the Official Veterinarians to manage positive animals, avoiding their presence in FCR farms. As a total, 700 analyses were performed on 301 bulk milk and DP samples. One butter sample of a non-FCR farm was positive for SE. The proportion of cattle with CoPS in milk and somatic cell counts were significantly lower in FCR premises as expected. Dividing CoPS counts of bulk milk in 3 classes (<10/10-1000/>1000 CFU/ml), FCR farms had a statistically significant probability of low or moderate contamination compared with moderate or high in non-FCR. The odds ratio indicated a probability of CoPS <10 CFU/ml 53 higher than in non-FCR farms. CoPS were always <10 CFU/g in cheese of FCR premises while the medium value in non-FCR was 1899 CFU/g.

Significance: This approach primarily focusing on health status of animals, along with farmers' education, good hygienic practices and management, was able to minimize the risk of CoPS milk contamination and thus of SE foodborne disease in DP of alpine pastures.

P1-27 Survival of* Escherichia coli *O157:H7 during the Manufacture and Storage of Brined White Cheese

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Introduction: White cheese is un-ripened rennet-coagulated type and is usually consumed fresh or after storing in brine. They may serve as an ideal medium for bacterial proliferation because of high water activity, protein and fat content. *Escherichia coli* O157H:7 is responsible for several outbreaks associated with cheese consumption, especially soft cheeses. *E. coli* O157:H7 is an emerging foodborne pathogen that presents serious food safety concerns to fresh white cheese types since these food products are consumed directly. Therefore, it is difficult to ensure that fresh white is safe for consumers.

Purpose: The objective of this project was to investigate the behavior of *E. coli* O157:H7 during the manufacture and storage of brined white cheese.

Methods: Pasteurized cheese milk was inoculated with a cocktail culture of *E. coli* O157:H7 (10^7 CFU/mL of milk), rennet and/or with starter culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The cheese samples were preserved in brine, 10 or 15% salt, and stored for 28 days at 10 or 21^{o}C. The populations of *E. coli* O157:H7 and lactic acid bacteria at each storage time/temperature were determined.

Results: The results showed that *E. coli* O157:H7 in cheese kept in 10 and 15% salt solution and stored at 10 and 21^{o}C declined gradually to the end of 28 days of storage. The populations of *E. coli* O157:H7 in cheese brine, 10 and 15%

salt, decreased sharply during storage at 10 and 21°C. The presence of starter culture (lactic acid bacteria) did not have a major effect on the growth of *E. coli* O157:H7 during manufacture of cheese.

Significance: The results obtained from this study indicate that *E. coli* O157:H7 may have a great potential for survival in brined white cheese, depending on the salt concentration in brine which is used to preserve the cheese for a period of time. Control measures in the manufacture of brined white cheese are necessary to reduce the risk of contamination.

P1-28 Impact of the 'Knowledge Innovation Technology Exchange' (KITE) Project upon Dairy Sector Small and Medium-sized Enterprises (SMEs) in Wales, UK.

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Introduction: The KITE project is a Welsh-Government/European-Union study (2008-2015) implemented to facilitate the transfer/embedding of food science/technology knowledge in Welsh-food-sectorSMEs. The diverse Welsh dairy sector, including 45% cheese manufacturers, 22% ice-cream manufacturers and 13% milk/cream processors, contributes to 18% of food-sector turnover in Wales (predominately SMEs). Compliance to food safety regulations and obtaining 3rd party accreditations are essential for SME sustainability/growth, however a lack of technical/food safety knowledge required to achieve this has been reported.

Purpose: This study aims to evaluate the impact of KITE programmes implemented in dairy sector SMEs.

Methods: In-depth case studies of dairy sector KITE programmes include reviews of SME profiles, assessment of technical/food safety management documentation (including measured outputs/secondary outcomes) as well as internal audit non-conformances. In-depth interviews(n = 5) with KITE partners were undertaken to obtain perceptions of KITE implementation and identification of challenges/barriers encountered when applying technical controls in Welsh dairy SMEs.

Results: From 2009–2013, four KITE programmes were implemented in dairy-sector SMEs resulting in achievement of four 3rd party 'British Retail Consortium approvals, development/launch of 30 new products and creation/safeguarding of 59 quality assurance/manufacturing roles. Cumulatively, due to increased customer contracts resultant from 3rd party approvals and new product development, increased sales of £2.3 million(€2.6 million) in KITE-partner dairy companies have been reported. Case study analyses indicated increased pressures experienced by SME dairies due to global farming and the reduction of milk prices. These factors have led to the increased need for technical understanding and innovation on a local scale to enable business growth. KITE/SME challenges identified included isolation of *Listeria* in a processing environment; internal audits and reviews of on-site processing/cleaning practices indicated more regular cleaning created a moister environment for persistence of *Listeria*. Through KITE, cleaning practices were revised to reduce water usage and concise/limited cleaning times were applied, resulting in a verified reduction of *Listeria* evidenced by environmental swabbing.

Significance: Implementation of a knowledge transfer exchange programme in dairy-sector SMEs has demonstrated successful improvement of technical and food safety standards.

P1-29 Gentle Endospore Inactivation on the Surface of Whole Black Pepper by Direct and Indirect Plasma Treatment

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Introduction: The increasing consumer demand for minimally processed and ready-to-eat dishes cause new challenges with regard to the microbial safety of spices and herbs. Especially the surface of whole black pepper is often spoiled with a high load of bacterial endospores. The traditionally applied technologies to decontaminate these dry products often cause quality losses or are rarely accepted by the consumers.

Purpose: To ensure the microbial safety and preserving the quality of spices the application of cold atmospheric plasma is a promising non-thermal technology. The process enables a microbial multi-target inactivation process and the high diffusion rate of the plasma gas allows a treatment of non-uniformly shaped products. Hence, within this study the antimicrobial effect of direct and indirect plasma for the decontamination of whole black pepper was tested.

Methods: Whole black pepper seeds (purchased from JJ Albarracin s.a.) were inoculated with *Bacillus subtilis* and *Bacillus athrophaeus* spores. For the direct plasma treatment, the argon gas afterglow of a radio-frequency plasma jet was used. The plasma gas for the indirect treatment was generated by a microwave plasma setup with air as feed gas. During the treatments the surface temperature of the pepper remained constant at 22°C during the indirect plasma treatment and increased up to 55°C during the direct plasma application (after 15 min). To quantify quality changes, the color and the quantity of the main aroma compound of pepper, piperine, were measured. All trials were done in triplicates.

Results: The direct plasma treatment achieved a rapid inactivation of 1.61_{10} (*Bacillus subtilis*) within the first 5 min and 2.85 $\log_{10}$ after 15 min. All inactivation kinetics were biphasic, pointing towards a rapid UV light inactivation followed by slower inactivation caused by photodesorption and etching. Nearly no impact on the product color and the piperine content (-6.3%) was detectable, but a weight loss of 5.2%. The indirect plasma treatment rarely affect the pepper quality and reduced the colony counts for the natural pepper flora, *Bacillus subtilis* and *Bacillus athrophaeus* by 2.21 $\log_{10}$, 2.28 $\log_{10}$ and 3.18 $\log_{10}$ after 30 min exposure time. A treatment of slightly wet pepper further enhanced the inactivation, which was possibly caused by a reduction of the surface pH due the high amount of reactive NO_x species in the plasma gas.

Significance: Direct and indirect plasma treatments can inactivate highly resistant bacterial spores at moderate temperatures and preserve the quality of whole black pepper. Consequently it is a promising alternative for gentle decontamination of herbs and spices.

P1-30 Food Safety Behavioral Influences of Two Consumer Groups: Implications for Targeted Food Safety Education Strategies

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Introduction: Food safety education approaches tailored for relatively analogous audiences can enable variables affecting communication strategy focus to be targeted; this approach can potentially facilitate a more effective response. Previous food safety research suggests that young adults (18–25 years) attending university and older adults (≥ 60 years) implement more food safety malpractices during domestic food preparation than other consumer groups. However limited data detailing cognitive behavioural influences required for informing food safety education strategies is available concerning these consumer groups.

Purpose: The aim of this study was to determine food safety behavioural influences of two consumer groups: young adults (18–25 years) attending university and older adults (≥ 60 years).

Methods: Quantitative survey methods were used to ascertain food safety knowledge, self-reported practices and attitudes towards food safety, risk associated with food safety and specific food storage/handling behaviours, which involved young adults (YA) (n = 100) and older adults (OA) (n = 100).

Results: Cumulatively, a lack of food safety knowledge among both consumer groups has been determined. Although reported awareness of food poisoning bacteria was comparable between consumer groups for two of the most prevalent pathogens in the UK: *Escherichia coli* O157 (100% OA, 88% YA) and *Salmonella* (99% OA, 93% YA) however, reported awareness of *Listeria monocytogenes* was significantly different ($P < 0.05$) (OA 84%, YA 25%). Food safety behaviours that may result in cross-contamination were more frequently reported by young adults than older adults, such as 'always' washing raw meat/poultry before cooking (32% OA, 45% YA). A significant difference ($P < 0.05$) between consumer groups was determined for practices associated with failure to indicate the need for hand-washing prior to preparing ready-to-eat foods (4% OA, 42% YA). Reported safe refrigeration practices was more prevalent among young adults than older adults; knowledge of the recommended operating temperature (5°C) for domestic refrigeration was significantly different between groups ($P < 0.05$) (67% YA, 13% OA) and awareness of prolonged storage (> 4 hours) at non-refrigerated temperatures making leftover food unsafe to eat (90% YA, 40% OA). It can be suggested that young adults may be at increased risk of foodborne illness from failing to implement practices to safeguard from cross-contamination in the domestic kitchen; whereas, older adults may be at increased risk of foodborne illness from failing to implement safe storage of food products in the domestic kitchen.

Significance: Findings illustrate a diversity of knowledge and attitudes and the consequent need to target food safety education strategies for different groups of consumers in the population. Results suggest a need for educational strategies for young adults to focus upon cross-contamination behaviours and for older adults to increase awareness of safe refrigeration temperatures and prevention of prolonged food storage at ambient temperature. Overall, this study increases our understanding of food safety behavioural influences and provides important information to inform future food safety initiatives.

P1-31 Potential Influences of Initiative and Non-Initiative Sources on Young Adults' Food Safety Knowledge and Practices

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Introduction: Young adults are reported to implement unsafe food safety practices and behave with more food safety risks than other consumer groups. Food safety knowledge may influence domestic practices, therefore, there is a need to implement food safety education in order to improve food safety practices; to enable this, there is a need to determine what sources have influenced young adults' food safety knowledge and the sources young adults prefer to receive food safety information, delivery of which can include initiative or non-initiative sources such as in the media. In recent years the popularity of food media has increased in the UK with food television programs and online recipes; however little is known on the potential impact of such sources on young adults.

Purpose: Determine influences on young adults' (≤ 25 years) food safety knowledge and preferred sources for future food-safety information.

Methods: A quantitative self-complete questionnaire was administered to young adults (aged 18 – 25 years) (n = 100) to determine potentially influential sources of food safety information, food safety information recall and preferred delivery of food safety information initiative and non-initiative sources.

Results: From a selection of initiative and non-initiative sources, young adults reported that their food safety knowledge had been most influenced by three main sources which included: family and friends (43%), food safety campaign adverts (20%) and school (9%). The sources that were reported to have least influenced young adults' food safety knowledge were online recipes and food magazines. Preferences of young adults for receiving future food safety educational messages were reported to be initiative and non-initiative based, however both communicated via television by means of food safety campaign adverts (14%) as reported to have been influential, and inclusion of food safety information in television food programs (12%). This suggesting an opportunity for non-initiative food media sources to include information regarding key food safety practices. The potential of non-initiative food based media in providing food safety information was further investigated, with data indicating that the majority of young adults' frequently viewing food media sources including food television programs (69%) recipe books (33%) and online recipes (31%); if information on food safety practices were to become an integral part of such food media sources young adults indicated that television food programs (78%) recipe books (65%) and online recipes (62%) would be likely of influencing them, furthermore 76% reported that endorsement of food safety by celebrity chefs would be likely of influencing them.

Significance: Young adults should be specifically targeted through the combination of non-initiative food-media sources such as inclusion of food safety information in televisual food programs in combination with initiative lead food safety campaign adverts, communication of food safety information with credible celebrity-chef endorsement may increase

awareness and likelihood of adopting food safety practices. Such findings may be used to inform the future development of targeted food safety education.

P1-32 Older Adults (≥60 Years) Storage of Ready-to-Eat Foods; Knowledge, Attitudes, Self-Reported Practices and Actual Domestic Refrigerator Temperature Profiles

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Introduction: Unsafe food storage is a contributory factor in 39% of reported foodborne illnesses associated with domestic kitchens. Consumer demand for refrigerated ready-to-eat (RTE) foods has increased in recent years, particularly among older adults (aged ≥60 years) and effective temperature control of RTE foods in the domestic kitchen is critical for food safety. Given the significant increase of listeriosis among older adults in recent years, there is a need to determine cognitive behavioural influences and actual refrigerated storage practices in domestic kitchens to inform targeted risk-communication.

Purpose: This study aimed to evaluate older adults' knowledge and attitudes towards refrigerated storage of RTE-foods and determine actual domestic refrigerator temperature profiles and self-reported practices.

Methods: Time-temperature profiles of refrigerators (n = 43) in older adults' domestic-kitchens were determined using Signatrol SL52T self-contained button dataloggers (Range: -40°C – +85°C, accuracy: ±0.5°C) over 136 hours. Older adults (n = 43) documented self-reported refrigerator usage during profiling; quantitative survey techniques determined knowledge, attitudes and self-reported practices of refrigerated storage of RTE-foods associated with *L. monocytogenes*

Results: The majority of older adults (82%) did not know the maximum temperature (5°C) recommended for safe food storage; 70% did not know the temperature of their own refrigerator. 77% believed refrigeration was essential to ensure food safety; all respondents believed their refrigerator operated at sufficient temperature to achieve this. However, only 23% reportedly checked their refrigerator temperature on daily/weekly basis, majority (56%) 'never' checked. Time-temperature profiles determined 40% of refrigerators (door/central locations) operated at >5°C for whole profiling duration (136 hours). Although 9% operated at recommended temperatures for 75% of profiling, no refrigerator operated at <5°C for whole duration. A positive correlation ($r = 0.29$, $P < 0.05$) existed between self-reported door opening frequency and temperature changes. No significant differences ($P > 0.05$) were determined between refrigerator temperatures and participant demographic.

Significance: Although older adults are aware of the importance of refrigeration to ensure food safety, knowledge of implementing safe refrigeration practices is lacking. Temperature profiles indicate that majority of older adults store RTE foods at unsafe temperatures which may increase risk of foodborne illness. Findings highlight the need for older adult improvement of domestic kitchen refrigeration practices; data may be used to inform development of targeted food safety strategies.

P1-33 Social Media Coverage of the German Dioxin Crisis

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Introduction: Social media has evolved from a world where users searched and consulted information (Web 1.0) to a world where they are now able to generate and spread information themselves (Web 2.0). Thus, the ability to report or comment on news, i.e., practice journalism, is no longer solely the remit of professionally employed journalists. Should they now wish to, anyone can write and disseminate news as a 'citizen journalist.' The importance of 'citizen journalism' in food risk communication is therefore growing as are the inter-relationships between classical and social media channels.

Purpose: The purpose of this study was to examine media coverage of a food crisis (i.e., the 2010/2011 German Dioxin crisis in pork, chicken and eggs) and to focus in particular on the role played by social media in the communication process.

Methods: Media coverage of the crisis was monitored in seven European Countries using pre-selected terms and commercially available monitoring tools.

Results: Over 26,000 postings relating to this crisis were recorded in social media compared to 6,700 articles in traditional media. The top 3 social media channels were blogs, microblogs and online news sites. Blogs and microblogs represented 60% of all online conversations. Regarding speed of reporting, social media coverage surged/peaked quicker but also declined quicker than traditional media. Of particular interest is the length of time information remains prominent on-line in social media. This is known as the 'Echo-chamber Effect.'

Significance: This study highlights the significant role played by social media in food risk communication. In particular, due to the persistence of on-line communications after a food crisis has finished, it highlights the importance of social media monitoring so that any misleading or incorrect information is corrected as soon as possible. These findings should guide food risk communicators when developing their social media communication strategy. This research was conducted as part of the EU FP7 FoodRisC project.

P1-34 Reporting on Food Safety Risks in UK National Newspapers

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Introduction: Despite the attention to social media, traditional media such as newspapers still play a prominent role in daily communications. As consumers, we receive a lot of information about food, e.g., the benefits and risks associated with different foods and diets. This communication landscape should help us choose a nutritious and safe diet; however, research published in 2011 on dietary advice reported in UK national newspapers, found that misreporting is widespread.

Purpose: To investigate this further, research was conducted by the European Food Information Council (EUFIC) to examine the balance of 'positive benefit' and 'negative risk' reporting of food issues in UK national newspapers (broadsheets and tabloids).

Methods: Four national newspapers were selected based on circulation and readership figures. They were sampled over a four week period, i.e., 2 weeks in September 2011 and 2 weeks in March 2012. All content (e.g., articles, frequently asked questions) carrying statements/claims regarding 'positive benefits' and 'negative risks' were selected for inclusion in the study. Using a specially designed protocol the content of these statement/claims was coded so that further analysis could be conducted.

Results: 112 UK newspapers were analysed. Initial analysis has shown that over 600 'positive benefit' and 'negative risk' statements/claims were reported. The majority were published in the Daily Mail. Approximately 20% of all statements/claims were negative in tone (i.e., the statement/claim was seen to have a negative impact on health). The remainder were either positive or neutral in tone. Examples of food risk/safety topics reported included, 'Rhubarb is poisonous if eaten in large doses' and 'More than 100 people have been poisoned by wild mushrooms.'

Significance: This study which highlights the role played by UK national newspapers (in particular the tabloids) in the communication of food risks, should benefit food risk communicators when developing their communication strategy.

P1-35 Fuzzy-Trace Theory, Expertise and Risk Evaluations of Professional Food Handlers

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Introduction: Despite rigorous application of HACCP principles in the catering sector, foodborne illness outbreaks still occur. Official surveys underlined that food handlers' bad practices are often the cause. We know that novices' errors in reasoning under sanitary risks are mostly linked to a lack of knowledge. Does it mean that experts are immunized against reasoning fallacies? Recent data rather showed that experts' risk perceptions are also affected by specific cognitive biases (Reyna & Adam, 2003). Furthermore, in some case, experts seem to make more errors than novices do (Desaulty et al., 2012).

Purpose: This study first aims is to examine how the Fuzzy Trace Theory (FTT) could predict and explain differences between experts and novices biases in food-related risks evaluation. Thereby, our results could demonstrate that the FTT model of reasoning under risk, already used in other professional sectors, is relevant and useful for catering.

Methods: Seventy-three catering professionals (37 managers and 36 operators) answered a questionnaire consisting of various evaluations of food-related risks and a storage task.

Results: As predicted, errors in risk evaluations were linked to the four theoretically identified sources (all P are < .05). More precisely, errors linked both to retrieval failure and representational biases seem to increase with expertise, when novices are more affected by a lack of knowledge. Consistent with theory, the two groups were equally affected by the fourth source of error, named processing interference.

Significance: Although many foodborne illness outbreaks are due to errors in good hygiene practices application, models used for good practices trainings design mostly neglect cognitive aspects of reasoning and decision making. Knowledge dissemination is necessary, especially for novices, but insufficient to prevent experts' errors in reasoning. In addition to relevant knowledge, trainings should provide an effective support for knowledge retrieving, correct representation of risk and information processing. FTT model of reasoning under risk can be used by training designers to account for both novices and experts errors.

P1-36 The Epidemiology and Public Health Importance of Brucellosis in Pastoralist Production System in Karamoja Region, Uganda

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Introduction: Brucellosis is considered by the Food and Agriculture Organization (FAO), The World Health Organization (WHO) and The Office Internationale des Epizooties (OIE) as one of the wide-spread zoonosis in the world. Brucellosis in humans is caused by *Brucella melitensis, Brucella abortus* or *Brucella suis,* and animals are the exclusive source of infections for the humans. Livestock production losses are the consequence of abortions, reduced fertility, birth of weak offspring and decreased milk production. Furthermore, economic losses due to human public health hazards are caused by treatment costs and income losses for people infected with brucellosis.

Purpose: This paper discusses strategies that could be used to control Brucellosis given the enormous challenges associated with Normadic pastoralism and livestock rustling in the Karamoja region in Uganda

Methods: A cross-sectional study was performed in three districts of the Karamoja region to determine the prevalence of antibodies of *Brucella* spp. in pastoralist owned goats and sheep. 1,400 goats and sheep from 40 flocks were tested for Brucella antibodies using the Rose Bengal Test and Competitive ELISA. One hundred twenty herds boys, between 9–12 years of age, were sampled from the kraals were the goats and sheep originated from and screened for Brucellosis using the Rose Bengal Test and the Competive ELISA test. A logistic regression model was used to explore the association between seropositivity in goats/sheep, herds boys and risk factors for brucellosis in this pastoralist production system

Results: The seroprevalence ranged from 14% to 27% in the drier and wetter areas for the goats/sheep using the Rose Bengal Test and 17% to 28% in the drier and wetter areas using the competive ELISA test. A prevalence of 17% and 15% were recorded for the herds boys using the Rose Bengal Test and Competitive ELISA respectively

Significance: This paper discusses strategies that could be used to control Brucellosis given that most milk, meat and meat products in this pastoralist production system are contaminated right from infected animals in the kraals. The enormous challenges associated with normadic pastoralism and insecurity in the Karamoja region are responsible for poor control of the spread of brucellosis from the animal products and to the human population in the Karamoja region in Uganda.

***P1-37 Effect of Slaughter Practices on Contamination of Pig Carcasses with Pathogenic* Yersinia enterocolitica**
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Introduction: At time of slaughter, many pigs are asymptomatic carriers of *Yersinia enterocolitica* in their tonsils, lymph nodes and intestines. From these infected tissues, carcasses may become contaminated during different steps of slaughter.

Purpose: The objective of this study was to identify risk factors for pig carcass contamination with pathogenic *Y. enterocolitica.*

Methods: In total, 360 pig carcasses were sampled in nine slaughterhouses in Belgium. The following four areas were swabbed: pelvic duct, split surface near the sacral vertebrae, sternal region and mandibular region. Mixed-effects multivariate logistic regressions were used to examine the association between the presence of pathogenic *Y. enterocolitica* as dependent variable and different slaughter procedures, slaughterhouse properties, and the presence of the bacteria in tonsils and rectum as independent variables.

Results: Contamination at each of the four carcass sites was associated with the presence of *Y. enterocolitica* in faeces and/or tonsils. When the tonsils were incised during removal of the plug set, the odds of contamination at the mandibular region was higher than when the tonsils were intact. Significantly more carcasses were positive for *Y. enterocolitica* at the mandibular region and the split surface when the head was split together with the carcass than when the head was left intact. Cleaning of knifes before removal of the plug set was a protective factor for contamination at the sternal region. Moreover, contamination of this region was higher in large slaughterhouses than medium and small slaughterhouses.

Significance: Carcasses may become contaminated with pathogenic *Y. enterocolitica* during normal slaughter of pigs. Small adaptations during slaughter may reduce carcass contamination, such as training of the slaughterhouse personnel to respect basic hygienic rules, preventing incision of the tonsils, and avoiding splitting of the head. Moreover, interventions during primary production to reduce the number of positive animals may decrease the percentage of *Yersinia*-positive carcasses.

P1-38 Seroprevalence of Enteropathogenic* Yersinia *spp. in Batches of Pigs at Slaughter
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Introduction: Enteropathogenic *Yersinia* spp. are the third most frequent cause of foodborne bacterial infections in Europe. Slaughter pigs are the main reservoir of these pathogens and on-farm infections cause the contamination of the pig carcasses during slaughter. Information on the infection status of the pig batches at slaughter before they arrive at the slaughterhouse might be useful to allow differentiation of high risk and low risk farms.

Purpose: As it is difficult to determine the infection status of fattening pigs on farms, serology of meat juice samples from pigs during slaughter was used to determine the infection rate of farms.

Methods: Pieces of the diaphragm (± 10 g) of 7,047 pigs were collected and stored at -20°C. These pigs were originating from 100 farms. After two to three weeks, these pieces of diaphragm were thawed and 2 ml of meat juice was collected, where after an ELISA Pigtype Yopscreen (Labor Diagnostik Leipzig, Qiagen, Leipzig, Germany) was performed following the instructions of the producer. The optical density (OD) was determined with a spectrophotometer (Tecan SpectraFluor, MTX Lab Systems, Virginia, U.S.) at 450 nm. The results were positive if the absorbance exceeded the proposed cut-off value of 30%.

Results: The results of the individual pigs have a binomial-shaped distribution with modes at 0–8.3% and 58.3–66.6%. The average OD% was 51%. Sixty-six percent of the animals were positive according to the used cut-off value. The within-batch seroprevalence ranged from 0 to 100% and is also showing a binomial distribution with modes at 0–6.6% (n = 13) and 86.6–93.2% (n = 20). Twenty-one percent of the farms were negative (mean OD%<30%).

Significance: Many pigs at slaughter show there had been contact with enteropathogenic *Yersinia* spp. at the farm. In at least 79% of the farms, the pigs had ever been in contact with these pathogens.

P1-39 Indicators for Prevention of Foodborne Outbreaks in Portugal
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Introduction: To lower the incidence of foodborne outbreaks (FBO), a science/risk-based food safety system is necessary. A way to obtain data for risk analysis is to study available FBO investigation data to identify FBO major food vehicles, causative agents and contributing factors.

Purpose: The aim of this work was to study data of FBO analysed in INSA laboratories from 2009 to 2012 in order to highlight major potential critical points to be tackled to lower the incidence of FBO in Portugal.

Methods: Descriptive analysis of investigation outbreak data.

Results: Of the 65 FBO, analysed in INSA, 13 affected an unknown number of people and the remaining involved 1,112 people. The causative agent was identified (CAI) in 546 cases corresponding to 30 FBO (46.2%) and unknown (CAU) in 566 cases resulting from 35 FBO (53.8%). 135 people were hospitalized; of them 68.1% had CAI and 31.9% CAU. One death was reported with CAI. Regarding the FBO with CAI and using EFSA classifications, the major food vehicles were mixed meals 56.7%, raw ham 13.3% and bakery products 6.6%; the causative agents were *Staphylococcus* spp. 36.7%, *Clostridium botulinum* tipo B 20%, *Salmonella* spp. 13.3%, *C. perfringens* 13.3%, *Escherichia coli* 6.7% and *Yersinia enterocolitica* O:3 3.3%. The main contributing factors were storage time/temperature abuse (56.7%), cross-contamination (36.7%), infected food handler (26.6%), and unknown 16.7%. Microbiological toxins were the major pathogenic factor

(60%). *Bacillus* spp. and *Salmonella* Enteritidis caused 87% of hospitalizations; *Yersinia enterocolitica* O:3 caused a death. 53.8% of FBO with CAU corresponded to 31.9% hospitalizations.

Significance: The major findings (presence of toxins, mixed meals and storage time/temperature abuse), indicate the need to improve hygiene and food safety practices to ensure effective heat treatment and food storage time/temperature. FBO with CAU should be further investigated for representativeness of food samples and range of tested agents (virus, parasites or emergents).

P1-40 The Binding Capacity of Yeast-derived Products for Aflatoxin B1 in Buffer Solution

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Introduction: Aflatoxins are considered the most well known and widely distributed mycotoxins in food and cause negative effects to human and animal health, as they are carcinogenic, mutagenic, teratogenic, hepatotoxic and immunosuppressive. The use of yeast cells or parts of them, such as cell wall or esterified glucomannan, have been widely studied and have shown promising results for decontamination of aflatoxins.

Purpose: The objective of this study was to verify the ability of six yeast-derived products, specifically from *Saccharomyces cerevisiae*, to remove aflatoxin B1 (AFB1) from a contaminated medium.

Methods: The *in vitro* tests were performed using yeast-derived products (five commercially available and one produced from residue of beer fermentation (BFR) collected at a microbrewery, dried and milled) to binding AFB1 at 1 ppm from spiked solutions at pH 3.0 and pH 6.0.

Results: AFB1 adsorption results by all products ranged from 45.53 ± 2.81% to 69.41 ± 0.90% at pH 3.0 and from 23.99 ± 1.27% to 63.84 ± 1.19% at pH 6.0. The best results of AFB1 binding at both pHs were achieved by products containing hydrolyzed yeast cells and yeast cell wall, differing significantly from the other products containing intact yeast cells. AFB1 binding percentage by BFR was 55.02 ± 4.97% at pH 3.0 and 49.23 ± 4.53% at pH 6.0, presenting intermediate results among all products analyzed.

Significance: It was concluded that yeast cells have the ability to remove AFB1 from a contaminated medium and this binding process is dependent on wether intact or hyrolysed yeast cell walls products are used. *In vitro* results should not be used to determine their ability to protect animals without further *in vivo* testing.

P1-41 Adsorption of Zearalenone, Ochratoxin A and Deoxynivalenol by Yeast-Derived Product in Buffer Solution

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Introduction: Zearalenone (ZEA), ochratoxin A (OTA) and deoxynivalenol (DON) are mycotoxins that have been implicated alone or in combination as the causative agents in a variety of animal diseases and have been also associated with some human diseases. Various approaches have been identified to reduce or prevent the adverse effects of mycotoxins on animal health and production, the most promising is the biological detoxification of feed using yeast cells, which have been tested as a natural adsorbent.

Purpose: The objective of this study was to analyze the *in vitro* capacity of a yeast-derived product produced from beer fermentation residue (BFR) in binding ZEA, OTA and DON.

Methods: BFR were collected at a microbrewery and contain cells of *Saccharomyces cerevisiae*. The binding process were performed using ZEA, OTA and DON at a 2 ppm concentration in buffer solution at pH 3.0 and pH 6.5.

Results: The best BFR adsorption results were for ZEA, 75.10 ± 1.76% for pH 3.0 and 77.49 ± 0.38% for pH 6.5. These results differed significantly from those found for OTA (59.35 ± 1.24% and 13.18 ± 1.07%) and DON (11.62 ± 2.17% and 17.57 ± 3.53%) at pH 3.0 and pH 6.5, respectively. The binding values for OTA varied greatly depending on pH, whereas for ZEA and DON the binding values were similar for the two pHs.

Significance: It was concluded that ZEA was the only mycotoxin which BFR could adsorb efficiently at both pHs. The ZEA binding process by yeast cells, specifically *S. cerevisiae*, allows the use of this product as an organic adsorbent, avoiding much of the ZEA adsorption by the animal's gastrointestinal tract and reducing the adverse effects of ZEA on animal estrogenic activities and reproductive performance. This product needs to be tested further for *in vivo* efficacy.

P1-42 Reduction of Aflatoxin M1 Levels in Milk by Pulse-High Hydrostatic Pressure Application

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Introduction: Aflatoxin M1 (AFM1) problem in milk and milk products is evident. It is important to reduce levels of AFM1 in these products to overcome economical losses and to protect public health. In food technology, different physical and chemical approaches have been used for the reduction of aflatoxins. Our work is based on an approach using high hydrostatic pressure (HHP).

Purpose: The aim of this work was to improve the use of HHP and pulse-HHP (p-HHP) in dairy science giving outcomes to the industry by reduction of AFM1 in milk.

Methods: Different combinations of pressure (300–500 MPa) / temperature (30–50°C) / pulse (6 × 50 s and 2 × 150 s) were applied to artificially AFM1 contaminated UHT milk (0.5–100ppb). After the pressure treatment, AFM1 was regained by extraction using immunoaffinity columns. Extracts were analysed by HPLC and by LC-MS/MS (Agilent 6130 LC-MS/MS). Reduction rates were calculated as % reduction in comparison to control samples. Statistical analyses of the data were carried out using one way ANOVA, which was followed by Tukey's post hoc test (SPSS Version 16.0).

Results: For HHP treatment, the highest reduction rates were 9.13% for 0.5 ppb (at 300 MPa), 10.23% for 5 ppb (at 500 MPa), and 47.70% for 50 ppb (at 500 MPa), independently of different temperatures used. p-HHP treatments

exhibited the results as follows: 6 pulse × 50 seconds treatment for different pressures 0–9.92% for 5 ppb, 20.42–33.68% for 50 ppb and 18.90–29.02% for 100 ppb; 2 pulse × 150 seconds treatment for different pressures 0–13.63%, 24.49–34.32%, 18.95–36.55% for 5 ppb, 50 ppb and 100 ppb, respectively. The effect of 400MPa/30°C without pulse at 5ppb concentration of AFM1 was found to be statistically significant ($P < 0.05$).

Significance: The level of AFM1 in milk can be reduced with the application of pressure and temperature. The effect of the treatments was variable depending on the concentrations. This work was supported by TUBITAK-TOVAG and CNRS joint research program. The authors would like to acknowledge METU Central Laboratory and Prof. Dr. Vural GÖKMEN (Hacettepe University, Food Engineering Department) for their contributions to HPLC and LC-MS/MS analyses.

P1-43 Effect of Cultivar and Scab Disease on Patulin Production of Fresh Apple during Cold Storage

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Introduction: Patulin, a secondary toxic metabolite produced by certain species of *Aspergillus*, *Penicillium*, and *Byssochlamys*, is importantly heat-stable mycotoxin in apples and apple products and is able to cause adverse health effects in human. Consumption of patulin might result in chronic symptoms including cellular level effects such as neurotoxic, immunotoxic, and genotoxic effects. As a result, patulin is regulated by many countries throughout the world such as the U.S. and European countries.

Purpose: The objective of this study was to investigate the effect of cultivar, scab disease, and storage time on patulin production of fresh apple.

Methods: Apple cultivars of McIntosh and Cortland with 50% (surface area of infection) scab disease were used in this study and were compared to control samples (no scab disease). Physical and chemical properties including total soluble solid (°Brix), pH, % titratable acidity, ascorbic acid content, total plate count, and patulin concentration were measured every 2 weeks until the end of storage of 12 weeks at 42°F.

Results: Results showed that patulin was detected in only scabbed McIntosh apples from week 8 to the end of storage compared to other samples. No significant differences ($\alpha = 0.05$) of physical and chemical properties of mean values between control and scabbed apple in each apple cultivar. °Brix and % titratable acidity tended to decrease while pH and total plate count tended to increase during the storage time. Ascorbic content was approximately the same throughout the storage.

Significance: This study suggested that cultivar, scab disease, and storage time were significant factors on patulin production in fresh apple and this finding would be valuable information for food industry with an awareness of this mycotoxin.

Poster Session 2 – Thursday, 16 May 2013
Presenters will be at posters during coffee breaks to discuss with attendees

P2-01 Mechanisms of* Bacillus *Spore Germination and Inactivation during High Pressure Processing
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Introduction: High pressure combined with elevated temperatures allows the production of commercially sterile and shelf-stable low acid food. However, this promising sterilization technology has not yet been applied in industrial settings, which is largely due to the unknown inactivation mechanism of highly resistant bacterial spores.

Purpose: This study aimed to clarify the germination and inactivation behavior of *Bacillus subtilis* spores, within a wide pressure–temperature–time domain.

Methods: To ensure reliable process conditions, all kinetic data were derived under isothermal (20 to 80°C) and isobaric (0.1 to 1000 MPa) treatment conditions during pressure dwell time in ACES buffer solution (pH 7, 0.05 M) with a stable pH under pressure. After the treatment, the physiological state of each spore sample was analyzed using the plate count method and flow cytometry, and the amount of dipicolinic acid (DPA) released was quantified by HPLC. In order to optimize scanning electron microscopic evaluations, a focused ion beam section method was developed to investigate the internal structural changes in spores.

Results: Based on this set of methods, a mechanism was proposed in which 150 MPa at 37°C induces a physiological-like DPA release, followed by cortex and small acid soluble protein (SASP) degradation, resulting in a subsequent inactivation. When keeping the temperature at 37°C a pressure increase to 550 MPa retarded the completion of germination and disabled SASP degradation. High pressures in combination with high temperatures (≥ 60°C) caused a rapid DPA release and spore inactivation. This suggests that the most sensitive structure under these conditions is the inner spore membrane. By using a multiresponse kinetic model, isorate pressure–temperature diagrams for the release of DPA, the formation of heat-sensitive and inactivated spores were calculated. It was confirmed that the first step of inactivation under high pressure is the release of DPA. Above a threshold pressure of 600 MPa the treatment temperature dominates germination and inactivation rate. The model used enabled the comparison of kinetic data derived in two different high pressure units with different compression rates.

Significance: The kinetic data from this study and the suggested mechanisms can aid in optimizing this promising sterilization technology and will increase food safety. Regardless of the pathway, *Bacillus* endospores first release DPA, which contributes to the spore's high resistance against wet and dry heat. This is therefore the rate-limiting step in the high pressure sterilization process and could be used for screening of highly resistant spore strains under pressure.

P2-02 Identification and Characterization of* Yersinia enterocolitica *Strains Isolated from Pig Tonsils
PATRIZIA CENTORAME, **Nadia Sulli**, **Vincenz Prencipe** and **Giacomo Migliorati**, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G.Caporale," Teramo, Italy

Introduction: Yersiniosis represents the third most frequent zoonosis in EU. Human infection is usually acquired from contaminated food, particularly raw or undercooked pork meat. Pigs are considered the primary reservoir of human pathogenic types.

Purpose: This study was carried out to assess the prevalence of *Yersinia enterocolitica* in pig carcasses selected from13 farms located in different regions of Italy. Distribution of pathogenic biotypes and genetic characterization of the strains isolated were provided.

Methods: Tonsils tissue from 376 pig carcasses were tested for *Yersinia enterocolitica*. The estimate of the number of samples and technical specifications used are those proposed for the harmonised monitoring and reporting of *Yersinia enterocolitica* in slaughter pigs in the European Union according to Directive 2003/99/EC.

Results: Five farms out of the 13 analysed were positive. *Yersinia enterocolitica* was isolated from 35 out of 376 (9.3%) tonsil samples. A total of 47 strains were isolated, the prevalent bio-serotype (87.2%) was 3/O:3, followed by 4/O:3 (6.4%), 3/O:9 (4.3%) and 2/O:3 (2.1%). All strains were characterized by DNA microarray and clustered into two main groups. The first group was characterized by the presence of plasmid genes of the secretion apparatus, the role of this additional TTSS in virulence has not been addressed yet, and the second by having genes for the flagellum transport machinery, required for efficient cellular invasion.

Significance: The high prevalence among strains of *Yersinia enterocolitica* of the pathogenic biotype 3/O:3, followed by bio-serotype 4/O:3 able to infect humans and considered an emerging zoonotic pathogen, confirms the role of pigs as natural reservoir. To assess the relevance of *Yersinia enterocolitica* for public health, it should be implemented a monitoring program on food taking into account that consumption of raw or undercooked pork products is a common habit, especially in some Italian regions.

P2-03 Evaluation of a New Selective Chromogenic Agar for the Isolation of Non-O157 STEC serovars
Gregory Devulder, bioMérieux, Hazelwood, MO, United States, **PEGGY NOMADE**, bioMérieux, Marcy-l'Étoile, France, **Hari Dwivedi** and **Jennifer Bick**, bioMérieux, Hazelwood, MO, United States

Introduction: Non-O157 shigatoxin producing *Escherichia coli* (STEC) are increasingly recognized as important foodborne pathogens. Effective isolation and detection of non-O157 STEC remain challenging. Isolation of colonies of non-O157 STEC on agar media is critical for their confirmatory identification.

Purpose: To evaluate the performance of chromID EHEC media for the isolation of non-O157 STEC from artificially inoculated beef samples as compared to modified Rainbow® agar.

Methods: Selective isolation of 30 strains of O26, O45, O103, O111, O121 and O145 serovars was performed on ChromID EHEC and modified Rainbow agar for their further identification. Briefly, 375 g samples of fresh raw beef were spiked with 25-100 CFU of different strains followed by either immunomagnetic separation (IMS) or a combination of IMS and acid treatment performed according to USDA-MLG guidelines. After isolation of non-O157 STEC colonies on both media, confirmation was performed using PCR targeting *stx* and *eae* genes.

Results: When combined with IMS and acid treatment recommended by USDA-MLG 5B.03, ChromID EHEC showed 100% sensitivity as all tested strains were successfully isolated from the beef samples. Percent confirmed colonies for the tested strains ranged between 28–100% and 0–100% for the new media and Rainbow agar respectively. Some of the tested strains were not recovered on Rainbow agar. The acid treatment procedure was helpful in enhancing the specificity of the non-O157 STEC isolation procedure for both media.

Significance: With its high sensitivity and desired specificity, chromID EHEC agar could be implemented for the selective isolation of non-O157 STEC from foods. The new chromogenic media supported the growth of all non-O157 STEC strains studied, ensuring their recovery from contaminated food samples.

P2-04 Influence of Packaging on* Bacillus Amyloliquefaciens *Spore Inactivation during Pressure-assisted Thermal Processing

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Introduction: Pressure-assisted thermal processing (PATP) is an emerging technology for sterilizing low-acid foods. Different laboratory groups utilize different packaging types for evaluating microbial safety of food.

Purpose: The objective of this study was to assess the role of different packaging in the inactivation of *Bacillus amyloliquefaciens* TMW 2.479 Fad 82 spores in HEPES buffer (pH 7) during PATP treatment.

Methods: Experiments were conducted using a laboratory scale high pressure unit. Propylene glycol was used as the pressure transmitting fluid. Aliquots (2.25ml) of *B. amyloliquefaciens* spore samples (~2.09 × 10^6 spores/mL) suspended in HEPES buffer were packaged into polyethylene pouch, polyethylene tube, and semi-rigid polypropylene cryogenic vial. The spore samples with minimal headspace were pre-heated at 45°C before loading into pressure vessel. The samples were treated at 600 MPa and 105°C from 0 to 20 minutes holding time in triplicate. Processed samples were cooled immediately in ice water before counting survivors.

Results: The D-values for *B. amyloliquefaciens* spores treated in pouch, vial, and tube packages at 600 MPa and 105°C were 2.93, 2.11, and 2.96 minutes, respectively. Within the experimental conditions of the study, spores processed in semi-rigid vials had 0.48 to 0.9 log more inactivation ($P < 0.05$) than those processed in pouch and tube under 5, 10 and 20-minute treatments. The differences in spore inactivation could be attributed to uncontrolled experimental variability in different polymer properties.

Significance: In summary, in comparison to other processing variables (such as pressure, temperature and holding time), the packaging type did not have major contribution to spore reduction during PATP treatment. The findings of the study demonstrate that the packaging type may introduce some variability in PATP spore inactivation studies. A more systematic study is needed to determine the relationship between process efficacy and package material, thickness and geometry.

P2-05 Examination of Reproducibility of Liquid Egg HHP Treatment

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Introduction: In both economic and scientific circles, significant efforts have been made to weaken the undesirable effects on foodstuffs that are caused by heat. This is how non-thermal technologies have been developed. Many 'gentle' technologies have emerged, for example: ultraviolet rays, ultrasound, pulsing electric field (PEF), UV sterilization, high intensity laser and high voltage discharge. However, none is more promising than the technology of hydrostatic pressure (HHP).

Purpose: We conducted experiments on whole liquid egg (pressure treatment, determination of spore count and measurement of color changes). The results were then compared with our earlier measurements.

Methods: The current experiments occurred in a Resato FPU-100-2000 machine, where we observed the adiabatic increase in temperature and its effect, as opposed to the measurements obtained previously on a Stansted Food Lab 900. We intended to find the similarities and differences between the two sets of measurements.

Results: A close correlation was found between measurements obtained with liquid egg using varying times and equipment, but identical parameters of treatment. Therefore, it can be stated that the effect of high pressure treatment to reduce spore count is reproducible. Further, I have proved by my measurements that barely noticeable organic changes in liquid egg will occur with hydrostatic pressure less than 250 MPa. Also these conditions produce a significant magnitude 5 reduction of *Salmonella* Enteritidis live spore count.

Significance: In summary, it has been proved that the positive results achieved with HHP technology are reproducible.

P2-06 Use of EDTA and the SEA Enzyme System in the Preservation of Whole Liquid Egg

CSABA NEMETH, Capriovus Ltd., Szigetcsep, Hungary, **Marta Borosjenoi** and **Laszlo Friedrich**, Corvinus University of Budapest, Budapest, Hungary

Introduction: Generally, whole liquid egg is preserved by combined methods. In order to extend product lifetime, heat-treatment and cold storage are combined with doses of preservatives. The Codex Alimentarius Hungaricus approves for liquid egg products (with the exception of products used in pasta and noodles) the use of: citric acid, natrium benzoate,

potassium sorbate and nisin. However, the list of approved preservatives changes constantly, one example of which is the 2011 approval of nisin.

Purpose: Both foreign professional journals and Hungarian distributors of preservatives expect to see EDTA and the SEA enzyme system approved for use in liquid egg products in the near future. Therefore, we made it our goal to study their effects on microbes.

Methods: The study involved EDTA and the SEA enzyme system separately and in combination. We looked for their influence on whole liquid egg in terms of varying live spore count under heat treatment. In an additional experiment, the material was divided into doses following heat treatment. Then we determined to what degree these preservatives retard the growth of microbes in doses of 250 and 500 ppm concentrations.

Results: Based on our results, the life expectancy of whole liquid egg is definitely extended by these two preservatives, both separately and in combination. There were especially positive results in the case of the SEA enzyme system, which produced a significant effect even in rather low concentrations. The best results came from both EDTA and the SEA enzyme system in the sample containing 500 ppm concentration. Here the additives doubled the effect of the heat treatment, while in the four-week storage test, microbe growth was reduced by a magnitude of 3.5.

Significance: In summary, it can be said that with approval of EDTA and the SEA enzyme system for use in egg product factories, these preservatives will significantly and positively influence the preservation time of liquid eggs.

P2-07 Bacterial Load and Characterization of* Salmonella enterica *and* Escherichia coli *from African Giant Snail* (Achatina marginata) *Meat in a Local Market and a Modern Meat Shop in Ibadan, Nigeria

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Introduction: African Giant Snails (*Achatina marginata*) meat is a delicacy for both local and international consumers. Snails are mostly obtained from the wild or reared under primitive conditions.

Purpose: Unhygienic processing of the snail meat is a public health concern and could be a vehicle for foodborne pathogens.

Methods: This study was carried out to assess the microbial presence of bacterial contaminants in snail meat randomly obtained from a local market and a modern meat shop in Ibadan, Nigeria. Sterile swabs of the meat surface and rinsate were the specimens used for bacterial isolation. *Salmonella* spp. and *Escherichia coli* load were identified and characterized, and the load was enumerated using colony counting.

Results: The results showed that all the snail meat samples were positive for pathogens, and mean colony forming unit (bacterial load) was found to be above 10 $\log^2$ CFUg-1 recommended microbiological limits (International Commission of Microbiology Standards for Food). *Salmonella* spp. and *Escherichia coli* were isolated from all the meat samples contained which was above the regulatory limits.

Significance: The processed snail meat sold in this study area may not be fit for human consumption because of the public health implications of the microbial pathogens identified, which could result in food poisoning. Improve hygiene (Hazard Analysis and Critical Control Points, HACCP) from farm to fork and inclusion of snail meat for inspection before public consumption is recommended.

P2-08 Freezing as an Intervention to Reduce the Numbers of* Campylobacter *Isolated from Chicken Livers

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Introduction: In the UK, there is growing evidence from source attribution studies, investigations of outbreaks of campylobacteriosis and retail surveys which implicates imperfectly cooked chicken livers as a likely source of human *Campylobacter* infections. In particular, chicken livers prepared in catering volumes and consumed in the form of pâté or parfait appear to be making a disproportionately large, and increasing, contribution to gastrointestinal illness.

Purpose: Although adequate cooking kills campylobacters, over-cooked livers have unappealing colour and texture. However, there are other potential interventions which could possibly control this bacterium which do not negatively impact on the organoleptic properties of chicken livers. Freezing can reduce numbers of viable campylobacters on chicken skin and muscle, typically, reducing *Campylobacter* numbers by around one log cycle. We were unable to find information describing the effect of freezing campylobacters which contaminate chicken livers. Consequently, this study assesses the effect on numbers of *Campylobacter* if chicken livers are frozen for up to one week at temperatures chosen to mimic domestic and catering freezers. In addition, the effect of a two-freeze treatment is reported.

Methods: Fresh chicken livers were collected directly from slaughterhouses so that previously thinned final clearance birds could be selected as those most likely to be naturally contaminated with campylobacters and to provide an assurance the livers had not been previously frozen. Livers were frozen to -15^{o}C, or -25^{o}C for 24 h or 7 d. In addition, the effect of twice freezing to -25^{o}C was investigated. *Campylobacter* numbers were determined by the ISO 10272-2 protocol.

Results: Freezing chicken livers for 24 h at -25^{o}C can cause reductions to campylobacters of up to 2 logs. Two freeze treatments each for 24h duration at -25^{o}C, with a refrigerated thaw in-between, caused reductions to *Campylobacter* numbers of up to 3 logs. There were significant reductions to *Campylobacter* numbers between the first and second freeze treatments.

Significance: Freezing chicken livers can reduce, but not eliminate, *Campylobacters*. If poultry processors were to freeze livers destined for human consumption as part of routine processing, it is likely a reduction in the foodborne illness associated with the consumption of imperfectly cooked chicken livers and processed derivatives, such as pâté would occur.

P2-09 Warm Versus Cold Packing of Beef Tripe - A Comparative Microbiological Analysis

THOMAS KENNEDY, Food and the Marine, Limerick, Ireland

Introduction: Regulation (EC) 854/2004 stipulates that food business operators must ensure that offal is maintained at <3°C during processing. However, provision is made for meat to be cut at >3°C when the cutting room and slaughterhouse share the same site. Immediately post cutting and packaging, the meat must be chilled to <3°C.

Purpose: This study compares the microbiological quality of beef tripe chilled to <3°C prior to cutting / packing (cold tripe) with tripe cut at >3°C and chilled to <3°C after packing (warm tripe).

Methods: For both sets of conditions, 50 × 25 g samples of beef tripe were taken 24 hours after packaging. Total Viable Counts (TVC) and *Enterobacteriaceae* counts (TEC) were enumerated using standard methods.

Results: The $LogA_{TVC}$ [$= x + ½(\ln 10.\sigma^2)$] for cold and warm tripe were 4.88 $\log_{10}$ $CFUg^{-1}$ and 4.04 $\log_{10}$ $CFUg^{-1}$ respectively. These values were found to differ significantly ($P < 0.05$). $LogN_{TEC}$ (calculated by summing the counts in each set and obtaining the log of the sum) and $\%Neg_{TEC}$ (those samples with counts less than the detection limit) for cold tripe were $2.48g^{-1}$ and 82% respectively whilst those for warm tripe were $2.90g^{-1}$ and 70% respectively.

Significance: The results indicate the superior microbial quality of cold tripe with implications for its shelf-life and safety. Though legislation has provided for warm tripe production, chilling prior to packing must be considered best practice.

P2-10 Numerical Scoring as a Tool to Improve Animal-based Welfare Outcomes in a Beef Abattoir

THOMAS KENNEDY, Food and the Marine, Limerick, Ireland

Introduction: The pre-slaughter handling of animals is critical to food safety and meat quality. Carrier animals when stressed may succumb to disease, potentially shedding large quantities of pathogens within the abattoir environment. Stress also plays a critical role in the pathogenesis of quality issues such as dark, firm and dry beef and pale, soft and exudative pork.

Purpose: The aim of this study is to demonstrate how an animal based numerical scoring system was used to improve animal welfare standards within the abattoir.

Methods: A numerical scoring assessment as described by Grandin (2001) and available at the following link http://www.grandin.com/cattle.audit.form.html was used to assess the pre-slaughter welfare of cattle in an abattoir over a three year period. Every 3 months, 50 animals were numerically scored to determine percentage of animals that:
(a) were stunned correctly on the first attempt (target: > 95%),
(b) were sensible at bleeding rail (target: 0%),
(c) an electric goad was used to encourage the animal to move forward (acceptable performance: < 25%, excellent, < 5%),
(d) slipped or fell within the facility (target: < 3%) and
(e) vocalise during handling or stunning (target: < 3%).

Results: Results indicate that over the the study period the use of goads has decreased from an initial unacceptable level of 72% to a current level of 5%. The high goad use is indicative of problems that relate to facility design where animals are reluctant to progress forward or with operator practices whereby the goad is used due to convenience of access to it. A review of design features and work practices resulted in the following measures being taken which successfully achieved a reduction in goad use:
(a) sheeting the side rails of the race to create solid walls,
(b) supporting the floor of the stun box to remove the hollow sound when the animal walked into it,
(c) altering light patterns within the facility to prevent shadow and reflection formation,
(d) stowing the goad away from the race so it was not so readily to hand.
The remaining 4 parameters, while from the outset were within target performance limits, also improved over the study period.

Significance: Numerical scoring makes it possible to determine if practices or the condition of the animals is improving or worsening. It moves away from traditional codes of practices which lean towards engineering based parameters such as space requirements, slope gradients and stocking densities amongst others. Standards often use phrases such as 'adequate', 'sufficient' or 'appropriate' which can mean different things to different people. Numerical scoring forces facilities to manage what they measure. Continual improvements can be made in a fair, targeted and measured way.

P2-11 Investigating Factors Affecting Slaughterhouse Bovine Spongiform Encephalopathy Rapid Test Sample Quality.

THOMAS KENNEDY, Food and the Marine, Limerick, Ireland

Introduction: Obtaining the appropriate sample is critical to ensure BSE test result integrity. For BSE, this is the brain stem at the level of the obex - the area where abnormal Prion Protein (PrP^{sc}) is most consistently deposited and fortuitously first detectable. Occasionally suboptimal samples (SO) occur where the obex is absent or unidentifiable, in which case negative results are questionable.

Purpose: The relationship between SO occurrence and candidate factors such as animal age, breed category [dairy (e.g., Holstein, Friesian and Jersey), beef breeds native to the British Isles (e.g., Aberdeen Angus and Hereford) and Continental beef breeds (e.g., Belgian Blue, Limousin and Charolais)], gender, dehiding method (upward or downward) and sampler identity (n = 13) was investigated.

Methods: A stepwise logistic regression model was applied to a dataset containing records of 23,646 animals sampled at the abattoir over a 2 year period from 09 June 2009 to 30 June 2011. Details relating to SO occurence were obtained from reports submitted to the abattoir with each days rapid test results. Details relating to the animal's age and date of slaughter, gender and breed were obtained from the Animal Identification and Movement System – the national database held by the Department of Agriculture, Food and the Marine. The abattoir changed its hide removal (i.e. skinning) method on 27 October 2009.

Results: The SO incidence was 0.26%. Results indicate that samplers S_{ahlmk} (OR = 5.9; 95% CI = 1.9 - 18.4), S_{dlrkl} (OR = 3.5; 95% CI = 1.2 - 10.5), S_{emada} (OR = 5.3; 95% CI = 2.0 - 13.7), bulls (OR = 2.7; 95% CI = 1.4 - 5.3), native beef

breeds (OR = 2.3; 95% CI = 1.2 - 4.5) and continental beef breeds (OR = 2.4; 95% CI = 1.3 - 4.3) had a significant positive effect on SO occurrence. Age and hide removal method were found not to have any significant effect on SO outcome.

Significance: The results inform a basis for risk ranking animals according to breed and gender prior to sampling. The results also highlight the importance of sampler training and motivation. As animal age is not a significant contributing factor, samplers are encouraged to perfect their technique by sampling animals younger than the statutory prescribed age (currently 72 months) prior to taking official samples from older animals.
(OR = Odds Ratio).

P2-12 A Microbiological Assessment Scheme to Evaluate the Feed Safety Management System in a Category 3 Fat Melting Establishment

THOMAS KENNEDY, Food and the Marine, Limerick, Ireland

Introduction: Animal tissues used in the manufacture of pet foods pose identical risks to those used in human food production. Pet foods are often stored and prepared in the domestic kitchen, providing an opportunity for cross contamination. Pet foods must therefore be produced under a strict feed safety management systems (FeSMS).

Purpose: The performance of a FeSMS in a Category 3 fat melting establishment integrated to a beef abattoir that produces greaves for pet food production was measured using a microbiological assessment scheme (MAS).

Methods: The establishment, approved by the competent authority, is subject to official controls and maintains a FeSMS based on HACCP. Over a 10-month-period, 685 samples taken at 7 critical sampling locations (raw materials, greaves post heat treatment, post centrifugation, post packaging, the environment, personnel and water) were analysed for 8 microbial parameters (Total Viable Counts, *Enterobacteriaceae*, *Salmonella*, *Listeria* spp., faecal enterococci, *Escherichia coli*, *Clostridium perfringens* and coliforms). Findings were benchmarked against legal, industry and best practice norms.

Results: Results indicate that 100% of raw fat samples attained the same acceptable criteria as the beef carcases from which the fat was derived as determined by Regulation (EC) No. 2073/2005. *Salmonella* was not isolated from greaves samples taken within process or post packaging, however, *Enterobactericeae*, though compliant, were detected at levels of 15 CFU/g and 165 CFU/g in two final product samples. 100% of water samples were compliant. Four environmental samples had TVC greater than 10 CFU/cm^2 with *Listeria innocua* isolated from one sample. Two personnel swabs had TVC above the recommended 100 CFU/cm^2.

Significance: The findings indicate that the MAS is an effective tool to assess FeSMS performance. Results indicate that safe pet food is being produced, however, deficiencies in personnel and environmental sanitation have been identified. Post cooking contamination from these sources remains a risk. This is supported by the presence of *Enterobacteriaceae* in some samples of final product. The MAS provides the establishment with key information for prioritising resources and nvestment to improve the safety of their product.

P2-13 WITHDRAWN

P2-14 Improvement of Molecular Detection of* Salmonella *in Environmental and Poultry Samples

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Introduction: *Salmonella* is a frequent contaminant in diverse environmental farm samples, in many sectors within the meat industry and is commonly isolated in poultry meat and in various poultry environmental samples. This contamination may occur throughout the whole production chain.

Purpose: The objective of the study was to compare the efficiency of sample pre-treatment step to increase the sensitivity of the real-time PCR detection of *Salmonella* spiked in poultry sample matrices. The potential inhibitor removal and the sensitivity of the test were assessed in comparison to usual protocol including a sub-cultivation in BHI for 3 hours.

Methods: Four types of matrices, feces, fluff, dust and chick box papers, were enriched for 18 hours in BPW and spiked afterwards with separately *Salmonella* enteritidis, *Salmonella gallinarum* or *Salmonella pullorum* ranging from 10^3 to 10^5 CFU/mL. Before DNA extraction, each sample was either centrifuged to analyse the supernatant, or sub-cultured directly for 3 additional hours in 1:10 dilution in BHI. *Salmonella* detection was performed using the foodproof® *Salmonella* detection system.

Results: Results showed that *Salmonella* is detected by real-time PCR in the 4 poultry sample types without inhibition when samples are sub-cultured in BHI. This trial demonstrated also that adding a centrifugation step right after the enrichment in BPW allowed to use directly the supernatant for DNA extraction without BHI sub-cultivation and shorten the time to detection. The sensitivity of the alternate protocol showed a detection down to 1,000 CFU similar to the reference method.

Significance: The study conducted here showed that an alternate protocol including a centrifugation step enabled the detection of *Salmonella* contamination in various poultry sample types keeping the same test sensitivity. This protocol could be applied to various matrices in order to remove PCR inhibitory components and better monitored *Salmonella* contamination.

P2-15 Development of a Biochip Array for the Multi-Analyte Screening of Thirteen Coccidiostat Residues

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Introduction: Coccidiostats are antiprotozoal agents that act upon Coccidia parasites in livestock. The EU has implemented testing guidelines (outlined in group B2b of Annex 1 to Council Regulation 2377/90) to test for veterinary medicines in food. This would encompass the treatment of coccidiosis, a protozoal disease that can cause diarrhoea and dysentery in the affected animal. Control of coccidiosis is particularly important in the poultry industry, where the

prophylactic use of coccidiostats prevents the disease from developing, thus increasing the risk of drug residue carry over into meat and eggs. Maximum Residue Limits (MRLs) have been set in place for a number of coccidiostat residues.

Purpose: Biochip array technology enables the determination of multiple analytes from a single sample, and thereby increases the screening capacity in test settings. This study reports the development of a biochip array for the simultaneous determination of thirteen coccidiostat residues. This multiplex screening approach reduces time and cost spent testing samples for a residue.

Methods: Simultaneous competitive chemiluminescent immunoassays, defining discrete test sites on the biochip surface, were applied to the semi-automated analyser Evidence Investigator. The system incorporates dedicated software to process and archive the multiple data generated.

Results: Initial analytical evaluation of the simultaneous biochip assays showed sensitivity values, expressed as the half maximal inhibitory concentration (IC50), typically 2.5 ppb, 0.5 ppb, 1 ppb, 0.5 ppb, 0.5 ppb, 0.4 ppb, 1 ppb, 0.3 ppb, 2.5 ppb, 8 ppb, 15 ppb and 1 9ppb for diclazuril, halofuginone, toltrazuril, monensin, maduramicin, nicarbazin, salinomycin (also narasin is detected), imidocarb, robenidine, lasalocid, nifursol metabolite and decoquinate respectively. The intra-assay precision (n = 3) was ≤ 12% for all assays.

Significance: Data indicates applicability of this biochip array to the multiplex determination of coccidiostat residues, which is relevant for the screening of these compounds in foods to ensure compliance with regulatory MRLs.

P2-16 **Campylobacter *Transmission between Batches in a Belgian Broiler Slaughterhouse***

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Introduction: The majority of human campylobacteriosis is attributed to the consumption of contaminated poultry meat. Carcass contamination occurs mainly during slaughter of *Campylobacter*-positive batches. Additionally, slaughter practices are conducive to *Campylobacter* transfer between batches. Investigation of *Campylobacter* transmission throughout the slaughter line may contribute to defining the exact role of individual slaughter procedures in *Campylobacter* cross-contamination from one batch to another.

Purpose: The study aimed to assess the role of individual slaughter processes in the transmission of *Campylobacter* contamination between batches.

Methods: One Belgian slaughterhouse was visited 5 times. During every visit, 3 successive batches were investigated. Per batch, carcasses (n = 2) were collected at four locations on the slaughter line after one, ten and twenty minutes from the start of the slaughter. The status of every batch was defined by caeca content examination. Collected samples (breast skin, caeca content) were homogenized, serially diluted and directly plated on *Campy*Food Agar® (bioMérieux SA, France) plates for counting *Campylobacter*. Presumptive colonies were confirmed by Gram staining and PCR.

Results: *Campylobacter* was not detected (LOD=10 CFU/g) when only negative batches were slaughtered at the beginning of the sampling day. If positive birds were slaughtered afterwards, an immediate increase (> 4 log CFU/g) of the carcass contamination was seen. When all batches were positive, the concentration of *Campylobacter* started high (> 3 log CFU/g) and remained at the same high level during the whole sampling day. If a *Campylobacter* negative batch followed a positive batch, approximately 1 log CFU/g decrease of *Campylobacter* contamination was observed on the first carcasses from the negative batch compared to the contamination level on the carcasses of the previous batch. During the slaughter of the batch, the *Campylobacter* contamination of the carcasses further decreased over time.

Significance: The *Campylobacter* status of the batches has an impact on the carcass contamination: (i) if only negative batches are slaughtered, non-contaminated carcasses are produced; (ii) the slaughter of positive batches results in contaminated carcasses; (iii) *Campylobacter* is transmitted from a positive to a negative batch when they are processed successively.

P2-17 ***Mold Pollution on Fresh Red Deer (Cervus Elaphus) Meat in Latvia***

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Introduction: Meat contamination consists not only of microorganisms, but also of microscopic mold fungi. Our task was to determine deer (Cervus elaphus), fresh meat possible contamination degree and intensity of the microscopic fungi.

Purpose: Define a degree of contamination by microscopic fungi and its intensity in the fresh venison.

Methods: Microscopic moulds fungi were defined by generally accepted methods for the determination.

Results: The conducted microbiological studies show that game meat is significantly contaminated by microorganisms during its obtaining process. The further examinations were also associated with studying the possible presence of opportunistic pathogenic microscopic fungi, because they also impair the quality of products and incur losses. In our study, the microscopic fungi *Ascomycota* and *Zygomycota* are mainly classified and presented. Dominating species were *Scedosporium* 21%; *Aspergillus* and *Rhizomucor* 16%; *Cladosporium* and *Penicillium* 14%; *Mucor* 9%; *Rhizopus* 5% uncommonly (3%) genera *Acremonium, Apophysomyces* are isolated. The highest contamination degree in the red deer meat was by fungi of the genera *Mucor* (47.9%) and *Scedosporium* (29.9%) that may be connected with infecting the meat during processing, not following the temperature regimen and doing pre-processing of the game meat defectively. Conversely, among the microscopic fungi the most intensively growing fungi was of genus *Mucor*, and the most slowly growing – fungi of the genus *Penicillium*. The mean intensity of contamination by microscopic fungi in red deer meat was (CFU number log is 4.59).

Significance: The mean log vales of the total contamination differed significantly between genera of the microscopic fungi ($P > 0.05$ or P – value 0.594). The highest contamination degree in the red deer meat was by fungi of the genera *Mucor* and *Scedosporium*. The study data showed microscopic fungi contamination of the obtained venison.

P2-18 Feasibility Study for Processing a Reference Material for Staphylococcus aureus Enterotoxin A in Cheese

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Introduction: Reference Materials (RMs) are crucial laboratory quality assurance tools for method validation (e.g., trueness estimation) and method performance qualification. ANSES in its function as the European Union Reference Laboratory (EU-RL) for Coagulase Positive Staphylococci has highlighted the demand to have available suitable RMs for a number of staphylococcal enterotoxins in food matrices. ANSES and IRMM agreed to collaborate for the development of a RM for SEA in cheese, currently seen as a priority analyte/matrix combination.

Purpose: The aim of the study was to establish a suitable processing procedure to produce homogeneous and longlife cheese powders with sub nano-gram per gram target levels for *Staphylococcus* enterotoxin A.

Methods: Processing steps and their order were investigated in view of homogeneity, defined particle size, dilution of blank with highly contaminated powder to achieve desired target level, logistical aspects and possibilities for upscaling. This included the following processing steps: decrusting, cutting, pre-grinding, freeze-drying of cubes and pre-ground cheese and cheese slurry (spiked or unspiked), cryogenic milling, turbula mixing and filling of materials. A confirmatory double-sandwich ELISA with a preceding extraction and dialysis concentration step was used to determine the SEA levels in the materials.

Results: Cryogenic milling allowed to reproducibly manufacture materials (particle size), which can be readily reconstituted. The optimised processing procedure comprises both the freeze-drying of cheese cubes milled to a powder (blank) as well as freeze-drying of spiked cheese slurry with subsequent grinding and a step-wise dilution of the two intermediate materials to obtain the final material with SEA around the target level of 0.25 ng/g.

Significance: A suitable approach to produce candidate RMs (cheese powders containing sub ng/g levels of SEA) was developed. The RM once processed, value-assigned and released will be a valuable quality assurance tool for laboratories and will contribute to establish and maintain reliable measurement results for effective consumer protection.

P2-19 Probabilistic Modeling of* Bacillus sp. *Spore Lag Time Applied to the Sterility Testing of UHT Milk

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Introduction: In association with Monte Carlo simulations, predictive microbiology models allow to simulate and quantify rare events that may not be evidenced through laboratory testing. Taking into account the lag time when predicting the growth of spore-formers is often crucial because the lag time may represent a significant part of the time to growth to an unacceptable level, or the time to detection. The lag time varies as a function of the strain, the conditions of sporulation, the stresses the population of spores was subjected to, and the environmental conditions of outgrowth. On top of that, the germination process seems to display a stochastic pattern, leading to between-cell variability.

Purpose: The purpose of our study was to model the outgrowth of a heat-stressed spore of a *Bacillus* sp. strain in milk. This model was intended to estimate the time needed for the pre-incubation of UHT milk in the context of sterility testing, assuming that a unit may be contaminated by as little as 1 spore.

Methods: A strain of *Bacillus* sp. was selected and, for the purpose of the study, spores cultures were subjected to a heat stress of 101°C for 30 seconds. The optimal growth rate in milk was estimated by challenge-testing. Individual lag times were measured in BHI by Bioscreen.

Results: The optimal growth rate in UHT milk was estimated at 2.3 h-1. Individual lag times in BHI at 35°C (146 values) ranged from 0.5 h to 32 h with a median value of 2.8 h. Results were combined with other data from the literature to develop a stochastic model intended to predict the outgrowth of a heat-stressed spore of *Bacillus* sp. in milk.

Significance: This predictive model will help quality managers to establish the time needed for the pre-incubation of UHT milk in the context of sterility testing.

P2-20 Label-friendly Reduction of Yeast Spoilage in Yogurt

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Introduction: Yeasts play a major role in spoilage of different types of dairy products and thus can lead to high economic losses. Chemical preservatives like organic acids and their salts can be used to preserve dairy products and protect the shelf life. Drawbacks in using chemical preservatives are the labelling requirements (often as E numbers) and potential adverse effects on the sensory properties of the foodstuff. Label friendly alternatives are bio-protective cultures limiting the outgrowth of spoilage fungi in dairy products.

Purpose: The objective of the study was to evaluate the inhibitory activity of two antifungal cultures each consisting of a *Lactobacillus* and a *Propionibacterium* strain against yeast spoilage in yogurt.

Methods: Yogurt samples were prepared using whole milk with 3.5% fat. The milk was inoculated with a commercial available thermophilic yogurt starter culture. Test samples were additional inoculated with the antifungal culture blends. The fermentation was done for about 6 – 7 hours at 43°C until the pH reached 4.60 in the yogurt samples. After cooling, the yogurt samples were inoculated with a pool of two yeasts added at a final level of 8 CFU per 10 g of yogurt. The yogurt samples were stored at 5°C and yeasts were enumerated on YGC agar throughout a period of 33 days.

Results: The application of antifungal cultures limited the outgrowth of yeasts compared to the sample without protective culture added. The yeast counts increased to 6.7 $\log_{10}$ CFU/g in the samples without antifungal culture after 19 days of storage. The yeast counts in the samples prepared with the two antifungal cultures increased only to 4 – 5 $\log_{10}$ CFU/g throughout the storage period of 33 days.

Significance: The results demonstrate that antifungal cultures can limit the outgrowth of spoilage yeasts in yogurt and thus maintain the shelf life and reduce food waste.

P2-21 Improving Honey Heat Process for a Better Quality and Safety Product

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Introduction: Honey is a concentrated solution of sugars, containing many organic acids, pigments and other compounds. Heating processes are usually applied to melt the honey sugar crystals, resulting in a more homogeneous and acceptable product and, sometimes, to pasteurize the product respecting either marketing recommendations or commercial agreements. This melting process is quite onerous and high temperatures for extended times may change many honey properties, like decreasing diastase activity and increasing hydroxymetylfurfural (HMF) contents, important thermal abuse indicators. On the other hand, when pasteurization is needed, the heat resistance of bacteria in hypertonic sugar solutions is affected and results can be misunderstood.

Purpose: The aims of this work were to obtain the reaction kinetics parameters for HMF formation, diastase degradation and to compare these data to those of microorganisms' death kinetic in order to improve honey heating treatment.

Methods: Two kinds of eucalyptus honey were used. The physicochemical parameters evaluated were pH, acidity, formaldehyde level and color. Diastase activity and HMF contents at 50 up to 90°C were also obtained. All the analysis carried out was based on AOAC official methods.

Results: Results demonstrated that production of HMF and degradation of diastase could be described by a first order kinetic model with thermal index z (°C) 31.0 for HMF production, and 19.2 for diastase destruction. Considering a 6 log reduction of a hypothetical heat resistant fungi showing a z value of 6.7°C and a D65.6 of 15 minutes, it was possible to find a binomial time x temperature region to achieve pasteurization effect, to keep the HMF increment below 1 log and the diastase drop below 1/10 of its original value.

Significance: Heating treatments for liquefying crystallized honey, pasteurizing the product and keeping quality and safety characteristics at acceptable levels can be employed by the honey processor, providing the kinetic parameters are estimated. These data can be used to design new equipment with higher heat transfer coefficients in all steps of the process.

P2-22 Cassava and Yam Production in Developing Countries: Safety Assessment

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Introduction: Cassava and yam are crops widely used in several countries, constituting a main food source for about 700 million people. They can either be directly consumed as crops or employed to make other food products, such as flour, snacks, etc. In developing countries, often these processes are performed in SMEs or in household level facilities, which may not always implement appropriate safety protocols. This may result in health hazard and/or poor quality products.

Purpose: The aim of this work is to establish critical analysis of the food safety procedures used by SMEs and households in four different developing countries – Nigeria, Ghana, Thailand and Vietnam in the processing of yam and cassava.

Methods: Several companies located in the countries mentioned above, which produce yam or cassava and/or derivate products, were visited. The visits were organised in cooperation with local universities and/or research institutions. The various phases of the production process(es) and steps were analysed; in this way, safety level adopted was established. Moreover, possible critical points, which could lead to contamination and/or health hazard, were also evidenced.

Results: Noticeable differences in food safety standard were observed between the different companies visited. Many SMEs showed acceptable safety procedures; household facilities, on the other hand, were often lacking in adequate safety protocols and good hygiene practices. The potentials of extracting valuable compounds from by-products and wastes were also evaluated, especially for the SMEs (as the amount of waste produced is greater) and safety baseline was also established.

Significance: Improving the safety conditions in cassava and yam processing is an issue which has remarkable implications for the consumer health but also in the productivity of these producers. Moreover, the upgrading of by-products and wastes can affect positively both the environment and the economies of these countries.

P2-23 Optimization in the Use of Plum Concentrates as an Alternative Ingredient in Marinated Catfish Fillets

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Introduction: Polyphosphates have been utilized to reverse freezer burn and lipid oxidation, to increase weight pickup, and to extend shelf life in refrigerated and frozen catfish. Plum extracts are considered GRAs and have been approved for use in beef and poultry.

Purpose: This research investigated the functionality of two different concentrations of plum concentrates applied to hybrid catfish (*Ictalurus furcatus* X I. punctatus) fillets.

Methods: For each of the duplicate treatment, approximately 2 pounds of fillets were marinated with a brine solution formulated for a pick-up over weight of 15%. The fillets were vacuum-tumbled (4^{o}C, 20 mmHg, 20 minutes, 18 rpm). The finished marinated product contained approximately 0.45% or 1% of plum concentrates; the concentration of salt was 0.5% salt. The marinated fillets were placed in polyethylene plastic bags and stored at 4^{o}C for 24 hours. The following characteristics were evaluated: marinade % pick-up, pH, and drip after 24 h.

Results: Results from duplicates showed a higher marinate % pick-up for fillets treated at 1% plum concentrates when compared with those treated at 0.45% (14.12% versus 11.81%). Drips after 24 h were higher for fillets at 1% plum concentrates (10.05 versus 8.25%). The pH of treated fillets was 6.73 for fillets treated at 1% and 6.82 for fillets treated at 0.45%.

Significance: These results are comparable to those of various phosphates where catfish fillets were treated with a marinated solution for a 15% pick-up containing 0.45% phosphates and where pick-up were 8.6–9.1% and pH 6.52-6.68 (Kin et al., 2009). These preliminary data justify further study in the use of plum concentrates in treatment of catfish fillets.

P2-24 Challenges Encountered by Welsh Food Sector SMEs When Implementing Issue 6 of the British Retailer Consortium (BRC) Global Standard

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Introduction: Achieving the BRC 3rd party accreditation standard is required by food companies to enable standardisation of quality, safety, operational criteria as well as fulfilment of legal obligations. This standard was most recently revised from issue 5 to issue 6 in January, 2012. For food-sector SMEs to meet technical demands required for business sustainability there is a need for attainment/maintenance of 3rd party accredited standards (such as BRC) needed for customer base expansion/retention.

Purpose: Assessment of food safety/quality management challenges encountered by SMEs when implementing BRC (6) and evaluation of how a knowledge-transfer-exchange mechanism has been used to overcome such challenges in different food sector SMEs.

Methods: In-depth case studies have been conducted with SMEs in raw meat, dairy and processed food (ready-to-eat/ready-to-cook) sector companies (n = 8) who have achieved BRC(6). Analysis included SME assessment, operational and procedural gap analyses (including HACCP-based audits), BRC-audit non-compliance reviews, qualitative in-depth interviews (n = 20) and focus groups (n = 12 affiliates/food-technologists) with knowledge-transfer partners.

Results: Overall, implementation of a knowledge-exchange-transfer mechanism in Welsh food-sector SMEs has resulted in attainment of 22 BRC accreditations in the past 4 years (many graded 'A'). This includes mentoring 8 SMEs from conformance with BRC (5) to achieving BRC (6). Key changes to BRC (6) that have challenged these SMEs and required increased technical mentoring and implementation of controls include (a) segregation/'zoning' of high-care/high-risk controls; (b) allergen management; (c) supplier approvals and, (d) raw material risk assessment. A significant barrier experienced by all SMEs included capital outlay to enable BRC6 compliance, for example, purchase/embedding of new metal detection equipment (with documented systems) in processing operations. Other challenges reported by SME managers and knowledge-transfer food technologists included labelling, on-site fabrication issues and a widespread lack of knowledge needed to detail raw food product risks particularly related to allergens.

Significance: Implementation of the knowledge-exchange-transfer mechanism has been identified as a critical variable required by Welsh SMEs, from a broad range of food sectors, to facilitate embedding of food safety/technical controls needed for attaining BRC 6 compliance and enable consequent business retention and sustainability.

P2-25 Impact Assessment on Food Safety in Korea Due to Climate Change Using Simulated Scenario Analysis

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Introduction: Climate change may have both direct and indirect impact on food safety at various stages of the food chain as well as the host environmental factors that affect food safety, such as global trade, socio-economic and technological development in Korea. Therefore, to evaluate the overall impact on food safety caused by climate change, food chain as well as the surrounding factors will be considered.

Purpose: To identify the overall impact of climate change on food safety in Korea, integrated impact assessment including its host environmental factors was conducted by using simulation-based scenarios developed in this study.

Methods: We analyzed 5 steps of simulation-based scenarios, 1) definition of scope, 2) standardization of critical factors, 3) integration, 4) simulation, 5) development of scenarios, developed in this study. Firstl, environmental analysis was conducted. The critical factors were extracted from host environmental analysis, and we defined proxy index for critrical factors by the method of expert AHP analysis. Datasets of defined proxy index were national statistical data in Korea. For simulation, mathematical models of proxy index were developed with Statistica Ver. 10 (StatSoft). For the future trends, the climate change scenario produced in KMA (Korean Meterological Administration) based on the RCP (RCP 4.5 and 8.5) scenarios were used.

Results: The results of host environment analysis (AHP-based weighted analysis) shown that food industry (weight value: 0.238) obtained the highest scores, followed by consumer behaviour (0.173), science & technology (0.168), government & policies (0.154), information (0.138), and social & economy (0.128). Based on our analysis (of the Expert AHP questionnaires), 18 critical factors and 56 proxy index were determinated. For simulation-based scenario analysis, 2 axes of uncertainty, RCP4.5+FBDOs (Foodborne disease outbreaks) and RCP8.5+FBDOs, were used. The simulation results suggested food industry (14.6-15.2%), consumer behaviour (16.3-18.2%), and social & economy (10.6-12.5%) will be reduced in 2030, and negative impact may be affected food safety in Korea. But science & technology (9.7-10.3%), government & politics (8.3-9.6%), and information (5.9-6.3%) will be build up adaptation capacity on food safety. In conclusion, the overall level of food safety in Korea will be down from 8.8% to 14.4% in 2030.

Significance: The study concluded that impact of climate change on food safety in Korea would be negative, but additional studies using measurable food safety index or economic assessment are necessarily for developing adaptation polices. The simulation-base scenario models play an essential tool for developing food safety programs and climate change adaptation in Korea.

P2-26 WITHDRAWN

P2-27 RIDASCREEN®FAST Soya (R7102) Sandwich ELISA to Detect Traces of Soya in Native as Well as in Processed Food

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Introduction: Soya belongs to the so called "Big 8" allergens, and following the food labeling directive soya has to be labeled as ingredient if used in food. Parallel to this worldwide, more and more soya allergies appear.

Purpose: The new sandwich ELISA RIDASCREEN®FAST Soya (R7102) is made to detect the typical allergenic soya proteins like beta-Conglycinin (Gly m5) and Glycinin (Gly m6), not only in untreated food, but also in heated samples (e.g., sausages, bakery goods, soups, sauces, margarine, ice cream and beverages).

Methods: The ELISA antibody is specific for denaturized soya proteins from raw soybean, flour, protein concentrates and other soya products. The testing of soya protein isolates and hydrolyzates is recommended with restriction.

Results: The detection limit (LOD) of this fast assay is 0.31 mg/kg (ppm) soya protein and the limit of quantification (LOQ) is 2.5 mg/kg (ppm) soya protein. The ELISA shows low cross reactivity to beans (0.0017%) and common tare (0.0003%) but not to peanut, lentil, pea or lupine.

Significance: These facts and the laws make a measurement of soya traces in food essential. Soya contaminations have to be prevented during food production and food samples must be checked regularly.

P2-28 Performances Assessment of the TEMPO AC Method According to the ISO 16140 Standard for Aerobic Microflora Count in Foods, Pet Foods and Environmental Samples

Justine Baguet, **Muriel Bernard**, **Cecile Bernez**, **Claudie Le Doeuff**, **Sarah Péron**, **Maryse Rannou** and **DANIELE SOHIER**, ADRIA, Quimper, France

Introduction: The TEMPO® system is an automated method associating an innovative card with a medium adapted to ensure a rapid enumeration of several quality indicators. It replaces serial dilutions and tedious plate reading with a simple 1/10 dilution and an automated enumeration based on a miniaturized card using 16 tubes MPN (Most Probable Number) method. The AC method allows Aerobic microflora Count in foods, pet foods and environmental samples.

Purpose: An independent study was conducted at ADRIA, to validate AC method in comparison to the ISO 4833 reference method, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: Meat, dairy, fruits/vegetables, seafood and catering food categories were covered in the scope of the validation study, as well as pet foods and environmental samples. The ISO 16140 method comparison gathered a linearity study done on 7 (matrix/strain) pairs, a relative accuracy study with 82 samples tested in duplicate.

Results: The alternative AC method showed satisfying linearity performances, with linear correlation coefficients superior to 0.98. The intercepts close to 0 and the slopes close to 1 were validated for all the tested 7 categories in the accuracy study. Biases between both methods were characterized by low values, varying from -0.00 to + 0.175 Log CFU/g.

Significance: The AC method is a reliable alternative method for Aerobic Total Count in foods and pet foods, and offers important economic savings by reducing time to result, handling time and the required incubation space. The TEMPO® AC card incubation time varies from 24 h and 28 h or 40 h and 48 h for raw meats and raw poultry meats, fruits and vegetables, catering foods, pet foods, environmental samples, while it is comprised between 40 h and 48 h for cooked delicatessens, dairy products, raw and processed seafood stuffs.

P2-29 A New Method to Detect **Salmonella** *spp. in Primary Production Samples: Certification According to the ISO16140 Standard*

Justine Baguet, **Muriel Bernard**, **Cecile Bernez**, **Claudie Le Doeuff**, **Sarah Peron**, **Maryse Rannou** and **DANIELE SOHIER**, ADRIA, Quimper, France

Introduction: *Salmonella* infections in primary production are major food safety concerns, since these are important transmission routes from the food chain to humans. The ISO and CEN Committees have, thus, decided a few years ago to describe standardized protocols for the detection of such zoonotic agents, which are often found in low numbers in primary production samples. The Annex D of the CEN/ISO 6579 was published in 2007, and is mainly based on *Salmonella* mobility detection in MSRV followed by streaking on selective agars and appropriate confirmation tests. An AFNOR standard (NF U47-100 method) is also available that includes a second selective step in MKTTn broth.

Purpose: An independent study was conducted at ADRIA, to validate the VIDAS® UP *Salmonella* (VIDAS SPT) method in comparison with the ISO 6579-Annex D and the NF U47-100 reference methods, as part of the NF Validation approval process and according to the ISO 16140 standard.

Methods: The VIDAS SPT method is an automated phage-ligand assay including a selective enrichment in SX2 broth for 6 h – 24 h at 41.5°C ± 1°C after an overnight culture in a selective BPW. The confirmation tests are done by streaking the SX2 broth on selective agar(s) before running API tests on typical colonies.

Results: 94 primary production samples (bootstocks, faeces rectal, dust samples, hygiene swabs, drinking waters, litters, hatchery samples, etc….) were analysed for the relative accuracy, sensitivity and specificity. These results clearly show that the VIDAS SPT method exhibits equivalent performance to the reference methods. The relative detection limit was found between 0.6 and 1.5 CFU/25 g for the reference methods and between 0.5 to 1.5 CFU/25g for the SPT method. The selectivity and specificity of the alternative method was confirmed by testing 50 target strains and 30 non-target strains.

Significance: The VIDAS SPT is a reliable alternative method for *Salmonella* spp. detection in primary production samples, and offers important economic savings by reducing time to result and handling time.

P2-30 IBISA Method for the **Salmonella** *Detection in Foods: A Dissolving Tablet to Optimize Laboratory Workflow*

JEROME THEPAUT, **Corinne Delanoe** and **Laura Goiset**, AES Chemunex/BioMérieux, Combourg, France

Introduction: IBISA method for *Salmonella* detection requires the addition of a selective liquid supplement (ISS) prior to enrichment step. This step can be improved to be quicker and optimized in an ergonomic workflow for microbiological laboratories.

Purpose: The aim was to evaluate the practicability of a new dispensing system for ISS : a tablet form and its distributors - and to compare its performances to the current liquid presentation within the IBISA method.

Methods: A practicability study was performed for the supplementation step by comparing ISS tablet presentation and the liquid one. Criteria such as time, aseptic condition and consumables needs were taken into account. Due to the presence of excipients in ISS tablets, it was necessary to study the impact of these components on *Salmonella* recovery by evaluating *Salmonella* growth in Buffered Peptone Water (BPW) supplemented with placebo tablets (only excipients). An evaluation between liquid and tablet form of ISS was performed by comparing recovery level and kinetic growth using IBISA method parameters (Diluent : BWP - incubation : 41.5°C for 16–20 hours). Both ISS presentations were then compared by studying IBISA method to detect *Salmonella* in 20 artificially contaminated food samples in comparison to the reference method ISO 6579.

Results: The study proved that the excipients in ISS tablets have no effect on *Salmonella* growth. Moreover, no significant difference was observed between tablets and liquid form of ISS on the recovery and kinetic growth on *Salmonella* pure cultures. On 20 food samples, 19 were found positive and 1 negative by all 3 tested methods: ISO 6579, IBISA with Liquid ISS and IBISA with tablet ISS.

Significance: With same performances, the new presentation of the IBISA Selective Supplement - ISS in tablets associated with its specific distributor will help the end-user by improving his laboratory workflow for Salmonella detection.

P2-31 ALOA COUNT, the First ISO16140 Validated Method for Listeria Species Enumeration

JEROME THEPAUT, AES CHEMUNEX/bioMérieux, Combourg, France and **Gaillard Nicolas**, Laboratoire de Touraine, Tours, France

Introduction: Due to the needs of industrial laboratories to optimize the quality of their products and process, it has been asked to propose an alternative method for the enumeration of *Listeria* species.

Purpose: The aim of this study was to evaluate the performances of ALOA COUNT according to the ISO16140 Standard for the enumeration of *Listeria* species versus the ISO 11290-2 reference method. The new protocol allows to avoid any resuscitation step and proposes also to perform the enumeration directly from the Half Fraser with selective supplements by a surface or pour plate method.

Methods: According to ISO 16140 validation rules a relative accuracy study has been performed on a minimum of 10 samples by food categories (meat, seafood, vegetables and egg products, milk products and environmental samples) with more than 25% naturally contaminated samples. All samples were analysed with ALOA COUNT method (by surface and pour plate technique from Half Fraser without resuscitation step) and compared with the ISO11290-2 protocol. An additional linearity and relative sensitivity study has been completed by contaminating each category samples with a range of Listeria from 0 to 10000 CFU/g.

Results: By analysing the bidimensional graphics, the comparison study showed a good correlation between ALOA COUNT and the reference method for the enumeration of *Listeria* species. Whatever the inoculation protocol the repeatability limits are very similar between both methods, the angle between ALOA Count and ISO11290-2 method is low (-0.015 or -0.051 Log) and the correlation coefficient is around 0.99.

Significance: ALOA COUNT is a reliable method to perform an accurate enumeration of *Listeria* species in food and environmental samples. The possibilities to avoid the resuscitation step and to work directly from the Half-Fraser help the labs to optimize their analytical workflow.

P2-32 Evaluation of Agglutination Assays for the Confirmation of the Top 5 Shiga Toxin-producing* Escherichia coli *(STEC) Serogroups as Described in the ISO/TS 13136 Reference Method

Marion Crozier and **Delphine Sergentet**, vetagro, Marcy letoile, France and **DAMIEN CÔTE**, bioMérieux, Marcy l'etoile, France

Introduction: Although Shiga toxin producing *Escherichia coli* (STEC) may belong to a large number of serogroups, those that have been firmly associated with the most severe form of deseases, in particular haemolytic uremic syndrome, belong to O157, O26, O103, O111 and O145. Due to the lack of a specific agar medium, the confirmation of STEC is an issue and there is a need for new assays to facilitate this critical step.

Purpose: HyGluTex assays are novel agglutination assays based on the use of specific recombinant phage proteins for each STEC serogroup. This study was designed to evaluate these new assays for confirmation of the top 5 STEC serogroups from a selective agar.

Methods: The latex test was performed by mixing a colony taken directly from a selective agar with a drop of the blue latex suspension. A positive agglutination is obtained within 1 minute of mixing. For each serogroups, the inclusivity study was performed on 20 specific strains and the exclusivity on 33 non target strains from other *E. coli* serogroups or other gram negative or gram positive bacteria.

Results: The inclusivity study showed a high specificity: all target organisms produced positive results in less than one minute. The exclusivity study showed that none of the latex assays cross reacted with the other *E. coli* serogroups or bacterial species tested.

Significance: These rapid, sensitive and reliable agglutination assays can be used for the identification of presumptive isolated STEC colonies from selective media. They can be an economic, rapid and sensitive alternative to PCR for identification of the positive colonies to be further studied for the presence of *eae* and *stx* virulence genes, as described in the ISO TS 13136 reference method.

P2-33 Detection of* Listeria monocytogenes *from Selective Enrichment Broth Using MALDI-TOF MS

SNEHAL JADHAV, **Danielle Sevior**, bioMérieux Australia Pty Ltd, Baulkham Hills, Australia, **Mrinal Bhave** and **Enzo Palombo**, Swinburne University of Technology, Hawthorn, Australia

Introduction: *Listeria monocytogenes* is an important foodborne pathogen responsible for the disease listeriosis. This disease has severe manifestations in pregnant women, neonates, immunocompromised patients and aged populations. Stringent laws to control the presence of *Listeria* in food processing environments and expensive food recalls associated with

contaminated foods highlight the demand for rapid and cost effective methods for detecting *Listeria* from food samples and processing environments.

Purpose: The current research focussed on investigating the ability of a MALDI-TOF MS-based proteomic approach to detect *L. monocytogenes* directly from selective enrichment broth containing spiked UHT milk, without the requirement of an extra culturing step on solid media.

Methods: A standard *L. monocytogenes* strain was used in pilot experiments performed in non-selective BHI broth to test the ability of MALDI-TOF MS to detect *Listeria* from broth culture. Subsequent experiments in selective enrichment broth were performed using the standard culture. Spiked milk samples were incubated in a selective enrichment broth (Oxoid Novel Enrichment (ONE) broth-*Listeria*) for 24 h, followed by an additional 6h 'recovery' period in non-selective BHI broth. The procedure was repeated using a dairy *L. monocytogenes* isolate. Bacterial cells were collected from broths by centrifugation and were treated with formic acid and acetonitrile. They were then spotted onto MALDI-TOF target plates and overlaid with CHCA matrix. MALD-TOF MS analysis was performed using a Shimadzu Axima Performance. Peaks obtained in the mass range of 2 to 20kDa were exported to SARAMIS (Version 1) for identification. Each spectrum was generated by accumulating 100 profiles for each sample. Identification was carried out based on the pattern-matching algorithm using the manufacturer's default settings.

Results: UHT milk spiked with as low as 1 colony-forming unit of *L. monocytogenes* per ml of the selective broth culture was detected. Identification to the species level by MALDI-TOF MS was possible within 30 h of enrichment.

Significance: The current study provides evidence that MALDI-TOF MS can be used to detect *L. monocytogenes* directly from selective enrichment broth using a commercially available database. This technique is more rapid and cost-effective compared to the conventional methods used for detection, thereby reducing overall time for reliable detection of this important food pathogen.

P2-34 A Toolbox for Pathogen Live-Dead Viability PCR

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Introduction: Real-time PCR (rt-PCR) provides a fast and powerful tool to analyze samples for the presence of potentially harmful microbes. However, there is a risk of false positives by the detection of DNA from harmless dead cells. Viability rt-PCR utilizes the DNA-masking compound propidium monoazide (PMA) which is able to enter dead and membrane-compromised pathogen cells and to intercalate into DNA, rendering the DNA from dead cells inaccessible to and thus not detectable by rt-PCR.

Purpose: The aim of this study was to develop a universally applicable toolbox centering on PMA. We have pre-developed viability PCR as a complete standardized system to be used with a new illumination device designed to catalyze the PMA reaction.

Methods: To develop the toolbox, mixtures of live/dead pathogens (*Salmonella* spp., *Listeria* spp., *Legionella* spp.) were prepared, and treated with PMA. The compound will enter dead and membrane-compromised cells, allowing it to irreversibly bind to DNA following activation by illumination with a specific wavelength. The reagent efficiently suppresses amplification signals from dead cell DNA allowing a direct differentiation between live or dead pathogens.

Results: Titrations of different live/dead pathogen ratios allow the determination of the sensitivity of the method. Workflow data was developed with pathogen relevant matrices to show PMA efficiency. This workflow data shows results of life and killed pathogens treated and not-treated with PMA to demonstrate the extent of the masking effect of PMA on dead cells, including all specifically designed workflow controls.

Significance: Live/dead differentiation can play an important role in procedures such as hygiene testing (success of decontamination processes), water testing (distinguishing between live and dead legionella for regulatory compliance) and human diagnostics (monitoring medication efficiency in pathogen killing).

P2-35 Development of a Food Safety Verification Risk Model

Louise Manning, Royal Agricultural College, Cirencester, United Kingdom and **JAN MEI SOON**, Universiti Malaysia Kelantan, Jeli, Malaysia

Introduction: Audit risk in the food safety context is the risk that an auditor may arrive at the wrong conclusions and opinions as a result of verification activities. The audit risk model is defined by: Audit Risk (AR) = Inherent Risk (IR) × Control Risk (CR) × Detection Risk (DR).

Purpose: To develop a verification risk (VR) model to identify the components of VR that prevent weaknesses or actual non-conformances within the food safety management system (FSMS) being identified and addressed.

Methods: This paper is built upon the audit risk (AR) model to develop a verification risk model (VR). VR is defined as follows: Verification Risk (VR) = Validation Risk (VaR) x Detection Risk (DR).

Results: An organisation must determine the degree of VR associated with their activities and how they can reduce the level of risk. VR will be minimised by developing a suitable FSMS based on appropriate scientific and technical content and by reducing VaR through appropriate initial validation and revalidation activities. VaR is therefore a combination of both IR and CR. DR will be minimised by determining and implementing appropriate monitoring and verification activities.

Significance: Product and process validation is the key to designing systems and processes that are capable of consistently producing safe food, by reducing VaR. The development of appropriate real-time monitoring and associated verification activities which reduces DR and provides sufficient objective evidence that the systems are functioning and effective.

P2-36 The FACET Software: Databases and Models to Assess Dietary Exposure to Food Packaging Migrants

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Introduction: Estimating the dietary dose to contaminants from food packaging in a population of consumers requires a number of inputs. The concentration of migrant in food must be known, together with the level of consumption of the food. In order to assess total aggregate dose to the packaging migrant, this must be known for all combinations of food and food packaging, together with the variability in the dietary intake of the foods in the population of interest.

Purpose: To develop databases, models and software to estimate consumer exposure to food packaging migrants.

Methods: 15 dietary surveys from 8 member states were recoded into a harmonised food categorisation system for food packaging. Market study data on the distribution of pack types in the EU was recoded into the same harmonised system, which was in turn linked to food packaging data provided by industry. The composition of the packaging raw materials as well as their arrangement in packaging structures was also included. A probabilistic diffusion model and related physicochemical parameters was developed to assess the concentration of migrants in foods to generate a database of migrant concentrations in foods. This is turn can be linked to a probabilistic dietary exposure model based on food consumption.

Results: The developed databases and models were integrated into a desktop software system. Options in an exposure assessment include assessing specific food types, specific packaging types, and specific consumer demographics. A distribution of exposure per unit body weight is generated. Exposure in population can be broken down by food category for a specific packaging type or conversely broken by packaging type for a specific food category or total diet.

Significance: The software tool presents an advance towards a realistic methodology for assessing dietary exposure to food packaging migrants. This work was funded by the EU Seventh Framework Programme (FP7).

P2-37 The Establishment of ALOP (Appropriate Level of Protection), FSO (Food Safety Objective), and PO (Performance Objective) Using MC (Microbiological Criteria) as Sampling Plans Based on QMRA Simulation Modeling

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Introduction: Quantitative microbial risk assessment (QMRA) can be used to provide scientific advice to risk managers who will use the information to decide upon the risk management option(s) will be implemented to achieve the appropriate level of (consumer) protection (ALOP) from microbial hazards. This public health goal must be converted into parameters that can be monitored by the responsible public health agency; one approach is to use scientifically-derived food safety objectives (FSOs) for the product at the time of consumption. Food processors are able to achieve the FSOs if they use performance objectives (POs) at different stages of production.

Purpose: The objective of this study was to provide a method for establishment of ALOP, FSO, and PO using the microbiological criteria (MC) as sampling plans based on QMRA modeling as an example of *Clostridium perfringens* in an animal product.

Methods: A QMRA simulation model was constructed in an Excel spreadsheet. Exposure assessment consisted of three process steps (produced products, retail, and consumption as a post process) including a predictive growth model, a novel dose-response model, serving size, etc included for hazard characterization and risk characterization. In addition, the Normal distribution was used for the set up of MC as two, three-class attribute plans. The developed model was simulated with @RISK. Totally 12 scenarios by sample plans were simulated with temperature at distribution and storage time etc.

Results: According to simulation results, in Korea the suggested reasonable PO and FSO were the level of *C. perfringens* which, in animal products must not exceed 50 and 50,000 CFU/g, respectively, with risk level (probability of illness per person per day) was $10^{\wedge -7}$ (mean) as ALOP, and the MC also proposed n = 5, c = 1, m = 10 CFU/g, M = 100 CFU/g as three-class attributes plans at the point of production.

Significance: This study can be used as scientific information and showed the practical possibility using QMRA model for the establishment of an ALOP, FSO, PO and MC as sampling plans in a food.

P2-38 Consumer Consumption of Raw Milk in the United States: Development of the Mental Model Approach

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Introduction: Milk sold at retail in the USA is generally pasteurized; but, milk producers can freely consume milk from their own cows. Dairy herd ownership is a loophole that non-producers use to obtain raw milk in states where legal sale is prohibited, a practice called herd sharing. A dairy farmer sells ownership shares in their herd in return for a share of raw milk the herd produces. No food safety standards are associated with the herd share contract because no food is sold under the contract.

Purpose: Our objective is to query raw and pasteurized milk consumers to identify distinguishing beliefs and perceptions that under lies the behavioral decision to consume raw milk, and then to develop a graphical representation (i.e., influence diagram) of the mental model that will be useful for subsequent research and data analysis.

Methods: The preliminary mental model is based on the Risk Information Seeking and Processing (RISP) model and Theory of Planned Behavior. The first model addresses underlying factors and beliefs that result in information seeking and the use of that information to form a belief structure about a risky behavior. The second model predicts consumer behavior that begins with the belief structure identified in the RISP model and how the consumer translates information and beliefs into behavioral action. A preliminary study to test the model was completed using structured survey (n = 81) and focus group (n = 15) techniques.

Results: The preliminary mental model has been tested with 36 raw milk consumers and 45 pasteurized milk consumers with divergent factors separating the preliminary model into working mental models of raw or pasteurized milk consumption. A national survey and expert review will follow to refine and validate the two working models.

Significance: Although, consumption of raw milk is associated with high health risk, it is one that is freely accepted by the raw milk consumer. Risk managers wish to reduce the consumption of unpasteurized milk; however, raw

milk drinkers have strongly held beliefs about the health benefits they perceive to be gained from raw milk consumption making risk communication attempts challenging.

P2-39 Combining Information from Outbreaks and Expert Elicitation to Attribute Foodborne Illness to Food Commodities

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Introduction: Attribution of foodborne disease to food sources is critical for resource allocation and for developing and evaluating food safety interventions. Numerous attribution approaches have been developed, each with distinct strengths and weaknesses, but few attempts have been made to combine information from multiple data sources into integrated estimates.

Purpose: This study evaluates foodborne outbreak data, expert elicitation, and case-control studies as information sources for food attribution, and creates an integrated set of attribution estimates for 14 major pathogens.

Methods: This study expands upon prior work developing measures to evaluate reliability of outbreak attribution on a pathogen-by-pathogen basis, including outbreak density, the ratio of estimated disease incidence to reported outbreak cases, the difference between outbreak and expert-based estimates, expert variance, and comparison with case-control results. We develop new methods for using expert elicitation results to weigh available evidence to create combined estimates of attribution that incorporate multiple primary data sources. We present a range of previously unpublished analyses on the influence of modeling assumptions on attribution estimates.

Results: We find that outbreak data for four foodborne pathogens – *Campylobacter*, *Toxoplasma gondii*, *Cryptosporidium parvum* and *Yersinia enterocolitica* – do not provide reliable attribution estimates. Outbreak attribution is found most reliable for *E. coli* O157:H7, non-cholera *Vibrio* spp., and *Cyclospora*.

Significance: Expert elicitation is shown to be uniquely powerful as a tool for evaluating strength of available attribution information from different data sources, particularly when the instrument has been designed for direct comparisons. Outbreak data is shown to have variable reliability as a source of attribution information. Our findings suggest that integrated estimates of attribution of illnesses to foods are possible and may be more reliable for public health policy than estimates based on a single data source.

P2-40 Microbiological Contamination on the Hands of Food Handlers as an Indicator of Handwashing Efficacy in the Convenience Food Industry in South Africa

IZANNE HUMAN, Cape Peninsula University of Technology, Cape Town, South Africa, **Andre Lambrechts**, Swift Laboratories, Cape Town, South Africa and **Ryk Lues**, Central University of the Free State, Bloemfontein, South Africa

Introduction: The hands of ready-to-eat food employees have been shown to be vectors in the spread of foodborne disease, mainly because of poor personal hygiene. It is of utmost importance that high standards of sanitation, cleanliness and good housekeeping be maintained at all times, as any laxness in this regard may result in a serious epidemic or infection. Statistical evidence indicates that food poisoning caused by the catering industry is 70% higher than that caused by any other sector. From this brief description, it should be evident that people involved with every stage of food production, from farm to fork, must take responsibility to prevent infections and destroy pathogens. According to Government Regulation 962 of 2012, promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, No. 54 of 1972, it is a requirement for food handlers to wash their hands with soap and hot and/or cold water before handling any food product or container or working in a food facility. This regulation further stipulates that a maximum of 100 viable organisms are allowed per cm^2 after cleaning and sanitation of food contact surfaces has occurred. The same standard will be applied to workers' hands, as they come into direct contact with the ready-to-eat food produced.

Purpose: The purpose of this study is to evaluate the efficacy of hand washing practices and sanitation amongst food handlers before they commence working in convenience food plants, as well as to add to the existing body of knowledge on hand washing and sanitation in the ready-to-eat food industry.

Methods: A total of 230 samples were collected, involving 100% of the food handlers in eight selected convenience food outlets, with their main focus on preparing ready-to-eat foods. The workers cleaned and disinfected dominant hands, which are normally in direct contact with the food, were sampled after staff passed through the hand washing area and before they commenced work, according to SABS method 762 regarding the swab technique (1975). One manufacturing plant per day was sampled and samples were transported to the laboratory on ice and analysed on the same day. A total of 88 samples were collected for Total Plate Count analysis, 77 samples for the presence of *Escherichia coli* and 65 samples to test for *Staphylococcus aureus*. In order to ensure the consistency of workers' normal practices in washing and disinfection, they had no prior knowledge of the planned sampling runs. The samples were collected on working days and adequate time was allowed for workers to clean and sanitize their hands. Results are the means of duplicate analyses.

Results: The highest bacterial count from the hand samples was 7.4×10^3 CFU cm^{-2} and the lowest showed no detectable growth. Although hands with a count of 0 CFU cm^{-2} were found in all of the plants, the results indicated that all of the premises sampled exceeded the legal limit of < 100 CFU cm^{-2} when the average bacterial counts on hands were compared. Sixty percent of the Total Plate Counts analysed exceeded the legal limit for food surfaces or hands of < 100 CFU cm^{-2} and only 18% of the food handlers had no bacteria detectable on their hands. One sample tested positive for *Escherichia coli*, which is generally viewed as an indication of faecal contamination. *Staphylococcus aureus* could not be detected on the hands of any of the food handlers.

Significance: The microbiological quality of food can be improved, especially with regard to contamination from bacteria on food handlers' hands. The study revealed that hand hygiene is unsatisfactory and it underlines the need to improve food handlers' hygiene knowledge by focusing on the importance of further training to improve food handlers' knowledge of good hand washing practices.

P2-41 WITHDRAWN

Poster Session 3 – Friday, 17 May

Presenters will be at posters during coffee breaks to discuss posters with attendees

P3-01 From Farm-to-Fork: Merck Singlepath® Direct* Campy *Poultry Rapid Test Kit For Farm-based Direct Detection of* Campylobacter *spp. in Faecal and Caecal Samples from Live Chicken

Lisa John, **Joerg Slaghuis** and **Charlotte Lindhardt**, Merck Millipore, Darmstadt, Germany, **Maria Wadl**, Robert Koch-Institute, Berlin, Germany, **Gerhard Schallegger** and **Martina Glatzl**, Tierarzt GmbH, Vienna, Austria, **Beatrix Stessl**, **Martin Wagner** and **Thomas Poelzler**, University of Veterinary Medicine, Vienna, Austria, **Tomasz Seliwiorstow** and **Lieven DeZutter**, University of Ghent, Ghent, Belgium and **HEIKE WULFF**, Merck Millipore, Darmstadt, Germany

Introduction: The 2012 EFSA Scientific Opinion on meat inspection (EFSA Journal 2012;10(6):2741) proposed testing the *Campylobacter* status of live broiler flocks ≤ 3 days prior to slaughter, to identify the 'high shedding' flocks and allow segregation from low-shedding at slaughter, thereby avoiding cross-contamination of carcasses and reducing human consumption of *Campylobacter* spp. Such a strategy requires on-farm testing and a method which requires no specialized equipment or laboratory-trained personnel. Lateral Flow technology fulfils this requirement and offers a reliable, fast, user-friendly, alternative detection method to the laboratory-based cultural reference methods.

Purpose: To develop and evaluate a qualitative immunochromatographic assay for direct (non-enrichment) detection of high shedding (>7.0 $\log_{10}$ CFU/g of faecal/caecal sample) *C. jejuni* and *C. coli* broiler chicken flocks, within 2 hours of sampling, as a rapid and farm-based alternative to standard cultural reference methods to monitor *Campylobacter* status of flocks and assist slaughter scheduling.

Methods: A sandwich Lateral Flow assay was developed, using gold labelled specific antibodies for *Campylobacter* spp. A non-enrichment sample preparation protocol was developed to enable a time-to-result of within 1 hour of sampling. Evaluation was by field studies conducted both on-farm (faceal/caecal droppings) and at slaughterhouse (caecal contents) using a cross-seasonal representative set of broiler chicken faecal/caecal samples. Reference method comparison was with ISO 10272 method and quantitative real-time PCR.

Results: In a field trial of faecal droppings collected on-farm, Singlepath® Direct Campy Poultry achieved a sensitivity of 88.9% (% correctly classified positive) and a specificity of 91.0% (% correctly classified negative) based on a Limit of Detection of >6.0 $\log_{10}$ CFU/g of faeces. Overall agreement with q-PCR was 90.6%. In a field trial of caecal contents collected at slaughter, Singlepath® Direct *Campy* Poultry achieved a sensitivity of 92.4% (% correctly classified positive) and a specificity of 95.2% (% correctly classified negative) based on a Limit of Detection of >6.0 $\log_{10}$ CFU/g of faeces. Overall agreement with cultural plate count (ISO 10272) was 93.1%.

Significance: Merck Singlepath® Direct Campy Poultry Rapid Test Kit provides an alternative, fast and simple method for detection of high shedding (>7.0 $\log_{10}$ CFU/g of faecal/caecal sample) *C. jejuni* and *C. coli* broiler chicken flocks, on-farm or at slaughter, and can assist in monitoring *Campylobacter* spp. status of flocks and in slaughter scheduling.

P3-02 Trichinellosis in Pigs and Wild Boars in Poland

EWA BILSKA-ZAJAC, **Miroslaw Rozycki**, **Ewa Chmurzynska**, **Tomasz Cenek** and **Jacek Karamon**, National Veterinary Research Institute in Pulawy, Pulawy, Poland

Introduction: Trichinellosis in humans is still a current problem in Poland. Despite the obligation of meat examination, new cases of human trichinellosis are noted each year. 932 cases were reported in the years 2000 – 2010.

Purpose: The aim of the study was to determine the percentage of pigs and wild boars infected by *Trichinella* spp. and identification of species of the occurring parasite.

Methods: Samples were collected from all over the country (Poland) in the years 2009 – 2011. The first step of investigation was performed by the Veterinary Inspection Service (VIS). Samples (muscle tissue) were examined by artificial digestion according to the EU Regulation 2075/2005, Annex I, Chapter I. Samples derived from carcasses assessed as positive in VIS laboratories were sent to the National Reference Laboratory (NRL) for genus identification. For species identification, isolated larvae were examined by Multiplex-PCR.

Results: Over 57,993,199 pig carcasses and 262,810 wild boars were examined. Results obtained by VIS by artificial digestion show that the percentage of *Trichinella* positive pigs was 0.000059% (i.e., 34 positives), while wild boars was 0.58% (i.e., 1,524 positives). In our laboratory 15 positive samples from pigs and 381 from wild boars were used for genus identification. During species identification *T. spiralis* was found in all samples of larvae isolated from pigs. However, in samples from wild boars different species were recognized, namely: 75% of larvae was identified as *T. spiralis*, 24% as *T. britovi* and in 1% coinfection of *T. spiralis*-*T. britovi* was found.

Significance: Occurrence of *Trichinella* positive wild boars in 0.58% caused high risk of infection in humans, which is confirmed by a relatively high number of human trichinellosis cases. Presence of *T. spiralis* in pigs and wild boar populations is dangerous because of high pathogenicity of that species for humans. Identification of *T. britovi* in 24% of infected wild boars shows that this could be also a danger for human health, because of resistance of this species to low temperature treatment.

P3-03 Rapid Detection of Foodborne Pathogens Using Isothermal Nucleic Acid Amplification

ROB LANGLEY, Neogen Europe Ltd, Ayr, United Kingdom, **Paul Norton**, **Edan Hosking**, **Michael Wendorf**, **Mark Mozola** and **Jennifer Rice**, Neogen Corporation, Lansing, MI, United States

Introduction: A new family of tests, ANSRTM, has been developed for rapid detection of pathogenic bacteria in foods and environmental samples. The tests are amplified, isothermal nucleic acid assays based on the nicking enzyme amplification reaction (NEARTM). It allows users to process sample serially or in parallel form as well as eliminating many of the limitations of antibody-based technologies. The tests have been granted AOAC approval for a range of food and environmental matrices.

Purpose: ANSR provides the food industry both the DNA-definitive test results they need, and the much easier and quicker methodology that they require. Recent food recalls have only emphasized the point that the food industry needs easier and quicker precise pathogen tests to lessen the chance that contaminated food products ever reach the consumer.

Methods: Using molecular beacon probes, the tests generate fluorescent signal which is measured in real-time using a simple incubator/fluorescence reader. Following sample enrichment, assays are completed in approximtely 30 minutes including sample preparation.

Results: The *Salmonella* assay has a limit of detection of 1,000-10,000 CFU per mL and high specificity for serovars of both *S. enterica* and *S. bongori*. Single-step enrichment ranges from 10 to 24 hours in duration depending on the sample type. The method has been validated for use with a variety of sample types including sponge or swab samples from environmental surfaces, poultry and red meats, and pasteurised egg products. The *Listeria* assay targets high copy number ribosomal RNA sequences specific to *Listeria* spp. and has a limit of detection of less than 100 CFU per mL. Method validation is focused on environmental samples, processed meats and seafoods, and dairy products. Tests for *Salmonella* spp. and *Listeria* spp. have been developed, and a test for Shiga toxin-producing *Escherichia coli* will follow.

Significance: Considering their accuracy, speed, and simplicity, the ANSR tests represent a significant advancement in diagnostic methodology for food safety management. High assay sensitivity allows the use of abbreviated enrichment protocols, and assays are completed within minutes and require only simple, low-cost instrumentation.
Acknowledgement: NEAR™ Technology - This product utilizes the patent pending NEAR isothermal technology and is sold under li¬cense from Ionian Technologies, San Diego, CA, and may be used under Ionian Technologies patent rights only for tests on food, beverage, and water safety. ANSR is a trademark of Neogen Corporation

P3-04 A High Throughput Method for the Detection of STEC Top7 in Meat Samples

SYLVIE HALLIER-SOULIER, **Sirine Assaf**, **Valerie van Wilder**, **Sarah Jemmal** and **Sebastien Bouton**, Pall GeneDisc Technologies, Bruz, France

Introduction: *Escherichia coli* O157:H7 and the top six (O26, O45, O103, O111, O121, and O145) non-O157 Shiga toxin-producing *Escherichia coli* (STEC) have emerged as important public health threats. Here, we propose a complete new work-flow including a one step multiplex PCR based method using GeneDisc® technology to screen STEC O157 and non-O157 in food products. The combination of the Top7 targets and genes associated to virulence factors, allows a higher level of discrimination, and an enhanced focus on the highest "at risk" STEC strains.

Purpose: The purpose of this validation was to evaluate the specificity of the new method (inclusivity and exclusivity), the limit of detection of the PCR assays for the various gene targets, and the comparison of performances to the USDA/FSIS 5B.01 for raw ground beef (375 g) and raw beef trim (375 g).

Methods: After enrichment of 375 g raw beef samples (ground beef, beef trim) in 1.5 L Buffered Peptone Water for 10 h at 41.5°C, bacterial DNA was extracted by high throughput sample prep, then analyzed with a multiplex PCR assay targeting the virulence genes and the specific genes of the Top7 serogroups. When the virulence genes and at least 1 specific gene of the Top7 serogroups were co-detected, the GeneDisc Cycler software automatically displayed an alert message indicating presumptive presence of the STEC Top7.

Results: The specificity of each PCR assay was demonstrated by analysis of DNA extracts from 122 *E. coli* strains. The limit of detection of each PCR assay was determined at 25 GU/PCR well, which theoretically corresponds to 3E + 04 CFU/ml after enrichment. Validation study of the entire method with ground beef and beef trim samples artificially contaminated by O26, O103 or O157 showed 100% presence for all target genes. Evaluation of the method was also carried out with 400 ground beef and 150 beef trim processed in 4 beef facilities across the U.S. and compared to the USDA-FSIS MLG 5B.01 method. Presumptive positive samples were confirmed according to the reference methods. The multiplex approach and the reference method gave 3 and 7 presumptive positive samples, respectively. Cultural confirmation yielded 3 Top7 STEC isolates whose 2 were given as presumptive positive sample by the GeneDisc method. None of the presumptive positive samples obtained by the reference method MLG 5B.01 was confirmed.

Significance: This work demonstrates that this new method is rapid, specific, sensitive and reliable. Hence, it can be used for routine screening of beef meat samples for STEC Top7 serogroups with a time to result inferior to 12 hours.

P3-05 Comparative Validation Study to Demonstrate the Detection of **Salmonella** *in Products Containing Probiotics with Assurance GDS*® **Salmonella** *TQ*

Philip Feldsine, **Andrew Lienau**, **Markus Jucker** and **DAVID KERR**, BioControl Systems Inc., Bellevue, WA, United States

Introduction: One of the new trends in the food industry is the addition of probiotics in select foods to aid consumer health. Unfortunately, *Salmonella* can be difficult to detect in foods containing probiotics. This study proposed a novel detection method, Assurance GDS, utilizing Immunomagnetic Separation (IMS) to isolate *Salmonella* from the food samples containing probiotic inhibitors.

Purpose: To demonstrate the equivalence of Assurance GDS for *Salmonella* Tq to the reference culture methods for the detection of *Salmonella* in selected probiotic containing foods.

Methods: Three food matrices (infant formula, oatmeal cereal and rice cereal) which have been implicated in foodborne *Salmonella* illness were included in the study. 20 samples were inoculated with low levels of *Salmonella* (0.0018 – 0.0261 CFU/g) for each sample type. 375 g samples were enriched with a 1:5 sample to media ratio in Buffered Peptone Water (BPW) plus supplements for 18 – 24 h at 36 °C. All samples were analyzed using Assurance GDS for *Salmonella* Tq according to the directions for use and reference culture methods. For all samples, the reference method used was ISO 6579:2002.

Results: After analysis the data showed 43 out of 60 samples tested positive for *Salmonella* using Assurance GDS. This indicated that the Assurance GDS method was 100% in agreement with the reference culture method for all three probiotic food matrices. The overall sensitivity and specificity were both 100%.

Significance: This new detection method for probiotic containing foods gives customers a faster option for testing *Salmonella* than culture methods, while retaining the necessary accuracy for this difficult food matrix.

P3-06 Comparative Validation Study to Demonstrate the Detection of* Salmonella *in Cocoa and Chocolate Containing Products with Assurance GDS® Salmonella TQ

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Introduction: Traditionally, cocoa and chocolate products are considered difficult food matrices due to cocoa's dark color and natural inhibitory compounds. This study proposed a novel detection method, Assurance GDS, utilizing Immunomagnetic Separation (IMS) to isolate *Salmonella* from cocoa-based food samples containing these inhibitory compounds.

Purpose: To demonstrate the equivalence of Assurance GDS for *Salmonella* Tq to the reference culture methods for the detection of *Salmonella* in selected cocoa and chocolate containing foods.

Methods: Three food matrices (cocoa powder, milk chocolate and chocolate cake) which have been implicated in foodborne *Salmonella* illness were included in the study. 20 cocoa powder samples were inoculated with low levels of *Salmonella*, 40 samples were inoculated with high levels of *Salmonella* and 10 uninoculated samples were included as controls. 20 milk chocolate and chocolate cake samples were inoculated with low levels of *Salmonella*, 20 samples were inoculated with high levels of *Salmonella* and 5 uninoculated samples were included as controls. 25 g samples of cocoa powder and milk chocolate were enriched with a 1:10 sample to media ratio in UHT milk + Brilliant Green media for 18–24 hr at 36°C. 25 g samples of chocolate cake were enriched with the same ratio, temperature and time but in Buffered Peptone Water (BPW). All samples were analyzed using Assurance GDS for *Salmonella* Tq according to the directions for use and reference culture methods. For all samples, the reference method used was ISO 6579:2002.

Results: After analysis the data showed that for all three cocoa containing food matrices, McNemar's Chi Square showed the performance of Assurance GDS *Salmonella* was equivalent to the reference culture method.

Significance: This new detection method for cocoa containing foods gives customers a faster option for testing *Salmonella* than culture methods, while retaining the necessary accuracy for this difficult food matrix.

P3-07 Comparative Validation Study to Demonstrate the Equivalence of an 8 Hour Dual* Salmonella *and* Escherichia coli *O157:H7 Enrichment for Assurance GDS® to Culture Methods for the Detection of* Salmonella *in Selected Foods

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Introduction: Many foods are commonly tested for both *Salmonella* and *Escherichia coli* O157:H7. Typically, these foods are enriched separately, increasing time and lab costs. This study proposed harmonizing these enrichments so food samples could be enriched in a single modified EHEC (mEHEC®) broth validated for use with Assurance GDS for *E. coli* O157:H7 (OMA 2005.04).

Purpose: To demonstrate the equivalence of an 8 hr enrichment time in mEHEC media to reference culture methods for the detection of *Salmonella* in selected foods.

Methods: Eight food matrices which have been implicated in foodborne illness were included in the study. 20 samples were inoculated with low levels of *Salmonella* for each sample type, 5 samples were inoculated with high levels of *Salmonella* for each sample type, and 5 uninoculated samples were included for each sample type as controls. 25 g samples were enriched with a 1:10 sample to media ratio in mEHEC for 8 – 18 h at 42°C. All samples were analyzed using Assurance GDS for *Salmonella* according to the directions for use and reference culture methods. For the following matrices: cooked poultry, raw beef trim, raw ground beef, leaf lettuce, spinach, mixed greens, strawberries and almonds the reference method used was ISO 6579:2002. Inclusivity and exclusivity of Assurance GDS was determined by analyzing 105 strains of *Salmonella* and 30 strains of potentially cross-reacting organisms.

Results: After analysis with Assurance GDS *Salmonella*, the data showed a sensitivity rate of 98.3% and a specificity rate of 100%. Additionally, both the inclusivity and exclusivity rates were 100%.

Significance: The newly harmonized mEHEC enrichment for both *Salmonella* and *E. coli* O157:H7 saves time and lowers lab testing costs for customers.

P3-08 The Influence of Different Freezing and Frozen Storage Temperatures on* Clostridium perfringens *Type A in Chicken Wings

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Introduction: *Clostridium perfringens* is an important anaerobic pathogen causing food poisoning in humans. *C. perfringens* type A food poisoning currently ranks as the third most common foodborne illness. Freezing and frozen storage are used to control bacterial growth in foods.

Purpose: The aims of the present study were to investigate the survival ability of *cpe* positive *C. perfringens* type A at different freezing and frozen storage temperatures.

Methods: Chicken wings were surface inoculated with vegetative *cpe* positive *C. perfringens* cells using a sterile drigalski spatula into the inoculum. The inoculated samples were divided into three groups as follow: (I) frozen and stored at -12 ± 1°C, (II) frozen and stored at -18 ± 1°C, and (III) frozen at -40 ± 1°C and then stored at -18 ± 1°C. The samples were analysed on 0, 1, 7, 14, 28, 56, 98 and 112 days of storage.

Results: In group I, the numbers of the pathogen decreased significantly ($P < 0.05$) compared to group II and III, during 112 day storage. No significant difference between group II and III was detected during the storage ($P > 0.05$). At the end of the storage (112th day), *C. perfringens* level dropped below the detectable level (<1.0 $\log_{10}$ CFU/cm^2) in group I, and decreased to 4.23 and 4.30 $\log_{10}$ CFU/cm^2 in group II and III, respectively.

Significance: It can be speculated that frozen chicken meat stored at -18°C and below, may still pose public health risk since the treatment did not show the expected reduction on the survival of vegetative *C. perfringens* cells.

P3-09 Detection and Typing of* Clostridium perfringens *from Retail Chicken Meat Parts

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Introduction: *Clostridium perfringens*, an important anaerobic bacterial pathogen, causes enteric diseases in animals and food poisoning in humans. Common reasons for *Clostridium perfringens* food poisoning include high-protein foods of animal origin such as meat, meat products and meat dishes.

Purpose: The objectives of the present study were to investigate the presence of *Clostridium perfringens* in chicken meat parts (breast, wing, drumstick and leg quarter) by culture methods and to detect the *cpa*, *cpb*, *etx*, *iA*, *cpe* and *cpb2* toxin genes by multiplex PCR.

Methods: A total of 200 samples including breasts (n = 50), wings (n = 50), drumsticks (n = 50) and leg quarters (n = 50) were collected from various retail stores around Elazig between May and August 2011. The chickens were of various known brands each representing different national broiler companies. Presumptive isolates obtained from these samples were identified as *Clostridium perfringens* based on the Gram staining and biochemical tests. DNA extracted from the isolated bacteria for molecular typing was performed by the multiplex PCR with specific primers.

Results: The results demonstrated that 94% (47/50) of wings, 80% (40/50) of leg quarters, 66% (34/50) of drumsticks, and 66% (33/50) of breasts were found to be contaminated with *Clostridium perfringens.* Also, 558 positive isolates obtained from these samples were identified as *C. perfringens*. Of these, 545 of them (97.6%) contained only *cpa* toxin gene (type A), 12 (2.1%) of them both *cpa* and *cpb2* toxin gene (type A-*cpb2*), and only 1 (0.1%) of them included both *cpa* and *cpe* toxin genes (type A-*cpe*), according to the multiplex PCR results, targeted *cpa*, *cpb*, *etx*, *iA*, *cpe* and *cpb2* genes.

Significance: This study is the first to report the presence of *cpe* and *cpb2* toxin genes in *Clostridium perfringens* isolated from chicken meats in Turkey.

P3-10 Decontamination of* Salmonella *on Shell Eggs by Summer Savory

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Introduction: Salmonellosis associated with shell eggs is a serious public health concern.

Purpose: In this study, we purpose a natural antimicrobial summer savory (*Satureja hortensis*) hydrosol for the decontamination of *Salmonella enterica* subsp. enterica serovar Typhimurium ATCC 14028 on shell eggs.

Methods: Fresh and unfertilized egg samples (63 ± 3g) were purchased from a chain supermarket in Turkey and stored at 4°C. After washing and brushing shell eggs with tap water, the eggs were surface sanitized with ethanol (75% v/v) for 30 mins, rinsed with distilled water, and dried in a biosafety cabinet for 30 min. Then, shell eggs were dip inoculated with *S. enterica* subsp. enterica serovar Typhimurium for 10 s and kept in a biosafety cabinet for 1h. Decontamination of shell eggs was carried out by immersing the eggs in sterile beakers containing summer savory hydrosol for 10 20, 30, 40, 50 and 60 min (7 eggs per 500 mL of the hydrosol).

Results: Savory hydrosol treatment reduced *S. enterica* subsp. enterica serovar Typhimurium population by 1.7, 1.8, 2.0, 2.2, 3.6, >4 log CFU/g after 10, 20, 30, 40, 50 and 60 min treatments, respectively, compared to the no hydrosol (control) treatment.

Significance: *Salmonella* was effectively inactivated on shell eggs by a natural antimicrobial summer savory hydrosol and this treatment could be an alternative way of decontaminating shell eggs.

P3-11 Screening the Inhibitory Effect of* Listeria innocua *Strains on* Listeria monocytogenes *Strains

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Introduction: *Listeria monocytogenes* is a well known foodborne human pathogenic bacterium that has a significant public health risk. Scientific literatures discussed that *L. innocua* might have inhibitory effect on *L. monocytogenes*, which can lead to detection problems. *L. innocua* is able to overgrow *L. monocytogenes* in different enrichment broths and environmental circumtances. Studies showed that the potential inhibitory effect was due to an inhibitory substance produced by *L. innocua*.

Purpose: The aim of our work was to study the growth ability of several *L. monocytogenes* in presence of inhibitory substance produced by some *L. innocua* strains.

Methods: 57 *L. monocytogenes* strains with different origins were tested in presence of one *L. innocua* cell free supernatant in Brain Heart (BH) broth incubated at 37°C. The growth was monitored in Multiskan microplate reader. Based on the results, two *L. monocytogenes* strains were selected and tested against 8 different *L. innocua* cell free supernatant with the above-mentioned method. The data were analyzed by PAST statistical program.

Results: Based on the results, irrespective of the origin of *L. monocytogenes* strains the range of inhibition was very wide, from 7 to 85%. In some cases *L. innocua* stimulated the growth of certain *L. monocytogenes* strains.

Significance: The overgrowth of *L. monocytogenes* by *L. innocua* may lead to false negative results during detection methods. This work was supported by the TÁMOP-4.2.1/B-09/1 and TÁMOP-4.2.2/B-10 project.

P3-12 The Effect of Acid Adaptation on the Chlorine Tolerance to* Listeria monocytogenes *in Phosphate Buffer Saline

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Introduction: Acid adaptation of *Listeria monocytogenes* not only cause the resistance to lethal acid but also induce the cross protection to heat resistance.

Purpose: The purpose of this study is to evaluate the chlorine tolerance of acid adapted cells of *Listeria monocytogenes.*

Methods: *Listeria monocytogenes* (101, 108, 310, Scott A, and V7) were grown in trypticase soy broth supplemented with 0.6% yeast (TSBYE) extract for 18 h and then each strains was mixed and induced to be an acid adapted cells by culturing in TSBYE pH 5.5 for 1 h and to be a non acid adapted cell by culturing in TSBYE pH 7.2 for 1 h. Both cells type were harvested by centrifuge and the pellets were suspended in phosphate buffer saline pH 7.4. After that 0.1 ml of acid and non acid adapted cells of *Listeria monocytogenes* (8.0 and 7.8 log CFU/ml, respectively) were treated with 0.9 ml of sodium hypochlorite at a different concentration (0.7, 0.8, or 0.9 ppm) for 0, 0.25, 0.5, 1, 5, 10, 15, and 30 min. The survival of both cells were determined by plating on trypticase soy agar supplemented with 0.6% yeast (TSAYE).

Results: The results showed that the survival of both cells were dramatically decreased with in 1 min and were not changed until 30 min. Non-acid adapted cells significantly resisted to chlorine better than that acid adapted cells. After being treated with 0.7, 0.8 and 0.9 ppm chlorine for 5 min, the acid adapted cells were reduced 0.65, 1.53, and 4.48 log CFU/ml, while non acid adapted cells were reduced 0.18, 0.59, and 1.58 log CFU/ml, respectively.

Significance: The data from this study may be useful to industrials to improve the food safety.

P3-13 Study of* Bacillus cereus *Adhesion on Mucin Surfaces as Influenced by Environmental Factors

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Introduction: Adhesion is an essential bacterial mechanism associated with the ability of microorganisms to persist in the gastrointestinal tract. The potency of *Bacillus cereus*, an important foodborne pathogen, to adhere to the gut mucosa is a determinant of virulence. Although adhesion of *B. cereus* on the intestinal epithelium has been documented, the attachment to the mucus layer that overlies the epithelial cells of a healthy host can be considered an important prerequisite to the virulence process.

Purpose: We evaluated the capability of *B. cereus* to adhere to mucus covered surfaces that are encountered in the human gut.

Methods: Using plates coated with mucin, we monitored the initial colonization of pathogenic *B. cereus* on mucin agar in vitro. The role of several environmental factors such as pH, oxygen and nutrient conditions in modulating the mucus adhesion of *B. cereus* was evaluated.

Results: Our tests showed that not all pathogenic *B. cereus* strains tested could adhere well on mucin surfaces, thus adhesion is strain specific and may not be directly linked to virulence. Adhesion was not affected by the reduced oxygen availability (1.7%) prevailing in the small intestine (air with ~ 21% oxygen was used as control). Additionally, the neutral pH of the small intestine slightly improves bacterial attachment compared to that of slightly acidic conditions (pH 5.9). In terms of the nutrient conditions affecting the adhesion of *B. cereus* on mucin, no differences were observed between a 10% solution of peas, a carbohydrate and protein rich feed and the supernatant of a simulated small intestinal suspension. Bacterial concentration in the liquid phase above the mucin layer did not vary among the media, but in the absence of a mucin layer, *Bacillus* growth was differentially supported by these media. We observed that *B. cereus* could grow on an unidentified component present in the mucin agar, which explains why adhesion was not reduced when low nutrient media, such as diluted intestinal suspension, were used.

Significance: It appears that *B. cereus* can adhere well on mucin surface because under all conditions tested, components present in the mucin agar resulted in growth in the liquid phase in contact with the mucus (lumen). As long as the experimental parameters do not influence the growth in the lumen, the adhesion potential of a given *B. cereus* strain remains unaffected.

P3-14 Invasion Efficiency of* Listeria monocytogenes *after Various Incubation Treatments

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Introduction: *Listeria monocytogenes* (*Lm*) is a Gram-positive bacterium, capable of causing foodborne disease. Pregnant women and elderly people are especially at risk. Although the incidence of *Lm* foodborne disease is not high (72 confirmed cases in The Netherlands in 2010, ECDC), the disease burden per disease case is one of the highest among foodborne pathogens. To cause disease, *Lm* has to invade epithelial cells of the small intestine. The mechanism behind the onset of disease is well understood. The influence of stress conditions in food products on the invasion capacity of *Lm*, however, is understudied.

Purpose: Three incubation conditions were compared for their influence on the invasion efficiency into epithelial cells. In addition, three methods of assessing the invasion efficiency were compared.

Methods: *Lm* was incubated in three conditions: brain heart infusion broth (BHI) at pH 7 (control), BHI at pH 5, and sausage-slurry at pH 5. Thereafter, the bacteria were exposed in three procedures to decreasing number of steps in a simulated gastrointestinal system including the interaction with differentiated Caco-2 cells was determined. To assess strain variability, three human and three non-human strains were used. Invasion efficiency was expressed as the percentage of cells that invaded the Caco-2 cells.

Results: The invasion efficiency decreased with increasing number of gastrointestinal steps and the invasion capacity of *Lm* pre-incubated in BHI pH 7 was in general the highest. The invasion efficiency of *Lm* incubated in sausage slurry was higher than after incubation in BHI pH 5, even though the pH of the sausage slurry was identical. The variability in invasion efficiency was highest in the non-human strains.

Significance: Incubation of *Lm* in sausage generally reduced the invasion capacity compared to incubation in BHI pH 7. However, sausage should still be considered a risk product with respect to *Lm.*

P3-15 Preliminary Results on the Prevalence of Nematodes, Cestodes and Ectoparasites in Fish Organs and Fillets

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Introduction: Protozoan and metazoan parasites frequently infest fish worldwide. The increasing consumption of seafood in France as well as the trendy consumption of raw fish products are two main reasons to identify hazards caused by parasites for the consumers and to define efficient prevention strategies to improve the safety of fish and fishery products.

Purpose: The aim of the study is to identify parasites infesting organs and fillets of 15 fish species. These fish species were selected by risk ranking on the basis of their consumption in France, the consumer exposure (sold fresh or frozen, consumed raw or cooked) as well as their level of parasite infestation reported in the literature. In fine, the prevalence of each species of parasites will be established per fish species, geographical localization, fish organ or fillet, etc.

Methods: So far, 1,475 individual fishes or fish fillets have been sampled at sea (English Channel, North Sea, Mediterranean sea, Bay of Biscay and Atlantic ocean), in freshwater lakes or from inland stockholders. External visual inspection of the fish, careful organ dissection as well as pepsic digestion of the fillets allowed the isolation of macro-parasites (mainly nematodes) in 57.6% of the sampled fish or fillets. Anisakid parasites were identified by sequencing a Cox2 gene fragment.

Results: The preliminary results showed that the main parasite genus isolated from the digestive tract is Hysterothylacium, present in 63.9% of the studied organ samples. Anisakis nematodes were isolated from the corporal cavity (52.6%), the liver (65.3%) and the fillets (59.1%).

Significance: The refined assessment of parasite prevalence in the fish species consumed most frequently in France is necessary to adapt efficient prevention measures for the consumer and to prevent economic loss for the fish industries. In parallel, an automated vision-assisted candling system is currently developed to allow a more efficient detection of the anisakid larvae in fish fillets for use in the fish industries. This work is supported by the French National Agency of Research (ANR) in the framework of the action 'Fish-Parasites' (ANR-10-ALIA-004).

P3-16 Comparative Evaluation of ISO 6579:2002 and Mericon* Salmonella *spp. Real-Time PCR Workflow for the Detection of* Salmonella *spp. in All Foods, Feedstuffs and Environmental Samples

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Introduction: During the past years, the food industry has been presented with increasing food safety concerns and a resulting demand for rapid, accurate, and easy-to-use pathogen detection systems. Increasingly, real-time PCR is relied upon t meet these demands. The mericon™ *Salmonella* spp. method combines one of two straightforward sample preparation methods with real-time PCR detection: the manual mericon™ DNA Bacteria Kit or the automated QIAsymphony mericon™ Bacteria Kit. For either procedure, the resultant purified DNA is combined with PCR Master Mix and real-time PCR is conducted on the Rotor-Gene Q platform.

Purpose: The purpose of the preliminary and collaborative evaluations was to conduct a comparison of the new method to the ISO 6579:2002 reference method for the detection of *Salmonella* as part of the AFNOR Certification validation process.

Methods: The preliminary method comparison analyzed all foods, animal feed and environmental samples. There were at least 30 positive and 30 negative samples in each group for a total of 397 samples analyzed. A relative detection level study was performed on one sample type from each group (meat products, dairy products, seafood products and vegetables, egg products, feed products and environmental samples). Each matrix was inoculated with a different serotype of *Salmonella* at four to five levels (from 0.2 to 5.4 CFU/25 g) and an uninoculated control. For each sample, DNA was extracted by both the manual DNA and automated DNA extraction kits, analyzed by the RotorGene real-time PCR system and compared to the ISO 6579:2002 reference method. Test kits were evaluated for inclusivity and exclusivity. A collaborative study was performed. Ground beef (8 samples of each level) was inoculated at 4 and 32 CFU/25 g, or uninoculated controls. Samples were sent to 13 laboratories for enrichment culture, DNA extraction with the manual method and *Salmonella* real-time PCR assay and comparised to the ISO 6579:2002 method.

Results: The method comparison demonstrated no significant differences in the number of positive samples detected between the mericon method and the ISO method for all samples studied. All 50 inclusivity strains tested were positively detected. All 30 non-target exclusivity strains tested were not detected. The collaborative study indicated that there were no differences between the new real-time PCR method and the ISO 6579:2002 reference method.

Significance: This new method is an efficient and reliable alternative to the traditional reference methods of detecting *Salmonella* in a variety of foods, feed and environmental samples.

P3-17 Validation of a Commercial Real-time PCR Assay for Screening* Salmonella *in Foods

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Introduction: *Salmonella* is found in many food and environmental sources and can cause serious illness. Since its isolation is long and difficult when in the presence of competing flora, non-culture, rapid detection methods are needed for this organism. To improve assay performance, a real-time version of the BAX® System *Salmonella* assay was designed, which reduces instrument processing time to approximately one hour.

Purpose: This study evaluated the effectiveness of the test kit for screening *Salmonella* from ground beef, lettuce, chicken, cream cheese, dry pet food and stainless steel environmental surfaces.

Methods: Artificially contaminated foods and environmental surfaces were tested and results compared with the appropriate Health Canada, USDA or FDA reference culture method(s). Samples were inoculated with Salmonella at levels expected to yield fractional positive results based on preparatory studies. All sample types were enriched in the appropriate reference method primary enrichment (LB or BPW). Corresponding replicates were also enriched in an alternative media (BAX® System MP media or TSB with novobiocin) where appropriate to improve method performance. For ground beef testing, the reference method was tested on 25 g analytical portions while the alternative method was tested on 375 g portions (25 g spiked sample combined with 350 g of unspiked material) to reflect industry testing norms. Secondary enrichment and culture confirmation from all enrichments was conducted using the appropriate reference method(s).

Results: Testing included 240 spiked and 60 unspiked samples. For ground beef, lettuce, chicken and cream cheese, 29/100 spiked reference method enrichments were culture positive, while 28/100 spiked test method samples were positive by PCR from the alternative enrichments. For pet food and environmental testing, LB and BPW enrichments were found to be equivalent when testing by culture and by the BAX® System method, with 5/20 spiked pet food samples and 13/20 spiked environmental samples being PCR positive for each enrichment method. All PCR positive samples culture confirmed and all PCR negative samples were negative by culture. Statistical analysis revealed no significant difference in the alternative PCR and reference culture methods.

Significance: This study indicates that PCR detection of *Salmonella* using the BAX® System real-time assay is rapid and sensitive. Test kit results demonstrate no significant difference when compared with the reference culture methods.

P3-18 **Campylobacter *spp. and* Listeria monocytogenes *Prevalence Study in 2012***

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Introduction: Human listeriosis is a relatively rare but serious zoonotic disease which can be life threatening. High prevalence and numbers of *Listeria monocytogenes* are often linked to ready-to-eat (RTE) fish and meat products. *Campylobacters* are the most common registered bacterial causes of human intestinal infections in EU-countries and *Campylobacter enteritis* is mostly associated with consumption of poultry meat.

Purpose: Estimate the prevalence and counts of *L. monocytogenes* in RTE fish and meat products of Estonian origin, and to determine the prevalence and counts of *Campylobacter* spp. in fresh broiler chicken meat products sold in Estonian retail outlets.

Methods: The isolation and enumeration of *L. monocytogenes* and *Campylobacter* spp. was carried out in accordance of EVS-EN ISO methods.

Results: According to our results the prevalence of *Campylobacter* spp. in fresh broiler chicken meat was 35% from the total of 220 samples obtained from the biggest retail outlets of the Estonia. The percentage of *Campylobacter* positive samples among Estonian, Lithuanian and Latvian fresh broiler chicken meat products available in Estonian retail markets was 18.6%, 48.8 and 45%, respectively. The average *Campylobacter* counts in Estonian, Lithuanian and Latvian *Campylobacter* positive products were 3.3 × 102 CFU/g, 1.4 × 103 CFU/g and 2.8 × 103 CFU/g, respectively. *Campylobacter* prevalence study at Estonian broiler chicken farm level in 2012 showed that the contamination appeared from July to September and among 380 cecal/fecal samples the *Campylobacter* positive percentage was 39.2% in total. *Listeria monocytogenes* prevalence among 101 Estonian RTE meat products and among 89 RTE fish products in 2012 was 6.9% and 18%, respectively. Among positive Estonian RTE meat and fish products 78.3% had *L. monocytogenes* counts less than 1.0 × 101 CFU/g.

Significance: The findings of present study showed that the prevalence of *L. monocytogenes* in RTE meat and fish products was generally low in Estonia, and only in one RTE food product the legal safety limit for *L. monocytogenes* counts was exceeded. Prevalence of *Campylobacter* spp. in fresh broiler chicken meat was low in Estonian products and it was significantly ($P < 0.001$) higher in Latvian and Lithuanian products. There was seasonal variation in proportions of *Campylobacter* positive samples with seasonal peak on summer months in present study.

P3-19 ***Invasion of* Kudoa septempunctata *Increases Permeability of Human Intestinal Epithelial Monolayer***

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Introduction: Outbreaks of an unidentified foodborne disease associated with the consumption of raw *Paralichthys olivaceus* (olive flounder) have increased in Japan and average more than 100 cases per year. The lag phase is only 1 to 20 h, and symptoms include transient but severe diarrhea and emesis. The causative agent of this disease has been long debated, but in our previous study, we demonstrated that *Kudoa septempunctata*, a recently described myxosporean species, was the causative agent of this novel foodborne disease. The oral administration of purified *K. septempunctata* spores induces diarrhea and emesis in suckling mice and house musk shrews, respectively. However, the mechanisms of this disease are poorly understood.

Purpose: In order to examine possible mechanisms by which *K. septempunctata* may cause diarrhea, we studied the toxicity and behavior of *K. septempunctata* in cultured human intestinal cells.

Methods: *K. septempunctata* spores were inoculated in Caco-2 human intestinal cells. After incubation, the transepithelial electrical resistance (TER) across the cell monolayer was measured. The behavior of *K. septempunctata* on intestinal cells was investigated by electron microscopy.

Results: When *K. septempunctata* spores were inoculated in Caco-2 cells, *K. septempunctata* sporoplasms were released from spores, and they invaded the cells. Electron microscopic observations revealed that the sporoplasm invasion severely damaged the Caco-2 cells. The inoculation of *K. septempunctata* spores eliminated TER. Inhibiting the invasion of the sporoplasms prevented the observed loss in cell layer integrity as illustrated by the rapid elimination of the TER.

Significance: Our results suggested that the invasion of intestinal epithelial cells by *K. septempunctata* sporoplasms is the one of contributing factor to the diarrhea associated with this pathogen.

P3-20 A Simplified Growth/No Growth Modeling Approach for the Quantification of Preservative Factors Affecting* Listeria monocytogenes *Growth in Ready-to-Eat Products

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Introduction: The severity of infection and high mortality increase the importance of *Listeria monocytogenes* as a gastro-intestinal pathogen and underline the necessity to control not only the frequency but also the level of contamination of this pathogen in foods. This requires a great understanding of the effect of combined inhibitory parameters on the growth/no-growth (G/NG) conditions of the pathogen.

Purpose: The purpose of this study was to illustrate a simplified G/NG interface model (Polese et al., 2011) for the quantification of factors affecting stability of minimally processed ready-to-eat (RTE) meat and fish products with respect to *L. monocytogenes*.

Methods: Through a normalization constant, the simplified G/NG model, which is based on the Gamma concept (Zwietering et al., 1992), allows the estimation of the growth probability of the pathogen as a function of distance from its cardinal limits of growth. The fractional contribution of each inhibitory factor to growth probability was evaluated as a function of the difference between the actual level of the factor and the inhibiting value, adjusted for the sub-optimal interval of the factor. Product characteristics, such as pH, aw, concentration of the inhibitory substances at the end of the production were analyzed or collected from published papers. Microbiological data were generated by assessing the growth of a mixture of four *L. monocytogenes* strains inoculated in the RTE products under storage at 4°C.

Results: The tested RTE meat products were found unable to support growth of the pathogen mainly for the contribution of temperature and pH. Although the RTE fish products were smoked, most of them showed a limited stability mainly due to the effect of pH.

Significance: The contribution of each preservative factor to growth probability could provide food operators with information exploitable in possible intervention strategies. This work was supported by the MIERI project financed by MiPAAF (Italian Ministry of Agricultural Food and Forestry Policies).

P3-21 Comparative Proteomic Analysis of* Salmonella enterica *serovar* Enteritidis *PT4 Planktonic and Sessile Cells on Stainless Steel Surface Provides New Insights in Protein Determinants Involved in the Maintenance of a Biofilm community

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Introduction: Numerous studies with various microorganisms have demonstrated that biofilm formation triggers the expression of specific sets of proteins, compared to planktonic cells. However, nothing is yet known about the proteomic profile inside a *Salmonella* biofilm formed on stainless steel (SS), an abiotic substratum commonly used in food processing equipment.

Purpose: In order to better understand the cellular mechanisms sustaining a surface-associated lifestyle of *S.* Enteritidis in food related environments, the differential protein patterns of this bacterium cultivated as biofilm on SS versus planktonic mode were comparatively studied in the present work.

Methods: By using 2-D PAGE in combination with MALDI-TOF MS analysis, 30 proteins were identified as differentially expressed between the two growth modes on an “on-off” basis, that is, proteins that were detected in one case but not in the other.

Results: In particular, 20 proteins were identified solely expressed in biofilm cells, of which half (10 out of 20) have also been found to be implicated in biofilm formation and / or other related events in other bacteria (ArcA, Dps, TrxA, Crr, DppA, GpmA, RibB, SseA, Ssb and MipA). Biofilm related proteins were mainly related to global regulation and stress response, nutrient transport, degradation and energy metabolism.

Significance: Present results clearly show that under surface-associated growth *Salmonella* over-produces proteins mainly related to stress management, supporting the well established view that biofilms are examples of multicellular behavior which enhance the capacity of microorganisms to survive multiple stresses Unambiguously, the ability to recognize “how and why” *Salmonella* attach to food-contact surfaces and form biofilms on them is an important area of focus, since a better understanding of this ability may provide valuable ways towards the elimination of this pathogenic bacterium from food processing environments and eventually lead to reduced *Salmonella*-associated human illness. The action THALIS: “Biological Investigation Of the Forces that Influence the Life of pathogens having as Mission to Survive in various Lifestyles; BIOFILMS”, has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: THALES. Investing in knowledge society through the European Social Fund.

P3-22 The Analysis of Microbial Contamination of Soils and Farm Products Near Animal Carcass Burial Sites

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Introduction: Animal carcass burial sites can spread pathogenic bacteria to nearby farmland consequent on contamination.

Purpose: This study investigated microbial contamination in soils and farm products nearby 253 animal carcass burial sites (FMD:241, AI:12) which were made between 2010 and 2011 in Gyeonggido, Korea.

Methods: During 2012, a total of 253 soil samples were collected from agricultural lands (paddy:72 , upland:181) who were cultivating crops around burial sites. They were analyzed the presence of *Clostridium perfringens* as indicator of

biological safety in soil of burial sites. The crops, which were corn (n = 5), green onion (n = 1), job'tear (n = 2), pepper (n = 13), rice (n = 14) and squash (n = 2) from *C. perfringens* contaminating soil were examined to see if they contained *Bacillus cereus*, *C. perfringens*, *Escherichia coli*, *E. coli* O157:H7, *Salmonella* and *Staphylococcus aureus*.

Results: 46 soil samples (18.1%) were positive for *C. perfringens*. The detection frequencies of *C. perfringens* were higher near the burial sites of AI (33.3%) compared to those of FMD (17.3%). Peddy soils (16.5%) had similar frequencies of *C. perfringens* compared with upland soils (15.5%). The microbiological counts of cereals (rice, corn and job'tear) were very low. Four pepper samples (28.9%) were contaminated by *E. coli* and its levels ranged from 2.1 to 3.1 log CFU/g. *B. cereus* was detected in 11 (29.7%) samples: pepper (63.6%), corn (18.2%), rice (9.1%), squash (9.1%) and its contamination levels were from 1.0 to 2.1 log CFU/g. *C. perfringens* was isolated for four pepper samples (28.9%) at 0.96 log CFU/g (from 0.7 to 1.2 CFU/g). *E. coli* O157:H7, *Salmonella* and *S. aureus* were not detected on any of samples. As microbial contamination could occur on near animal carcass burial sites, the hygienic control is more required.

Significance: This study provides basic database about microbiological quality of soils and farm produces around animal carcass burial sites.

P3-23 The Analysis of Microbial Contamination of Fresh Vegetables Directly Transferred from Farms and Preprocessed Vegetables from Retail Markets

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Introduction: Fresh vegetables are under risk of pathogenic bacteria contamination in every production step including, harvesting, processing, packing, distribution and retail.

Purpose: This study investigated microbiological safety of fresh vegetables on pre-harvest and preprocessed vegetables in retail with regard to the farm-to-fork continuum.

Methods: From April to November in 2012, a total of 107 samples (22 kinds) were directly collected from 53 farms (n:53) and from 11 supermarkets (n: 54) in Gyeonggi-do, Korea. Aerobic Plate Count (APC), Total Coliforms (TC), *Escherichia coli*, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli* O157:H7, *Salmonella* and *Staphylococcus aureus* were tested.

Results: : The majority of APC, TC, *B. cereus*, *C. perfringens* and *E. coli* was 5–7 log , 4–6 log , 1–2 log , 1-2 log and 1–3 log CFU/g on 107 samples, respectively. *B. cereus* was isolated in 29.0% (n:31), *C. perfringens* 14.0% (n:15) and *E. coli* 12.1% (n:13). The overlapped bacterial contamination were found in Lettuce (n:4), crown dais (n:1), green pepper (n:3) from farms and washed celery (n:1) from market. *E. coli* O157:H7, *Salmonella*, *S. aureus* were not detected in any of the samples. The highest contamination level of APC and TC was observed on washed root vegetables (bonnet bellflower, balloon flower and ginger). Leafy green vegetables from farms were highly contaminated with *B. cereus* 75.0% (n:15), *C. perfringens* 25.0% (n:5) and *E. coli* 30.0% (n:6). All *C. perfringens* isolation (n:15) revealed CPA by analyzing real-time PCR. By comparing preharvest with preprocessed samples, APC, TC and *E. coli* were not different ($P > 0.05$), while the prevalence of *B. cereus* and *C. perfringens* were lower in preprocessed samples ($P < 0.05$). Even in washed products may contain pathogenic microorganisms and represent potential microbiological hazard. Consumers should remove residual contaminants on vegetables prior to consumption.

Significance: This study provides basic database about microbiological quality of vegetables during growing and marketing in Korea.

P3-24 Avoid of Chemical Contaminants through Usage of TPE as Legally Food-safe Material

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Introduction: The EU-Directive 10/2011 PIM concerning FCM specifies that apart from global migration, many specific migration values must also be adhered to. This also applies for sealing compounds of metal closures. These have been produced on the basis of PVC to date. In order to make this naturally rigid material pliable and elastic, plasticizers are required. The disadvantage: Plasticizers are fat-soluble which means that they can migrate into fatty foods upon contact.

Purpose: With TPE as a recyclable and legally food-safe material, it was possible to dispense with the use of PVC and the plasticizers contained therein, thereby avoiding a potential impairment to smell, taste and health.

Methods: Already in use since the early 1980s as a sealing compound for crown caps and subsequently for aluminum and plastic seals, complex development over several years led to the solution of PROVALIN<sup® as sealing material free of PVC and plasticizers for vacuum twist caps - for the protection of the consumer and in line with the demands of the food industry. This compound is an innovative thermoplastic elastomer, which is inherently elastic and easily malleable with the result that it does not require any plasticizers. Nor can plasticizers migrate into the jar contents.

Results: This compound thereby complies with all EU guidelines while guaranteeing maximum food safety. This has been tested within long-term studies of independent test laboratories.

Significance: The development of a sealing compound may appear to be a small step, but it can give rise to a revolutionary change when combined with the associated technology. Today it is available in different variants and for lots of applications including hot or cold filling or subsequent heat treatment such as pasteurization and sterilization and for different seal sizes.

P3-25 Use of Irradiation to Ensure the Safety of Fresh Pre-cut Vegetables

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Introduction: In past decades, a number of large foodborne outbreaks attributed to consumption of fresh, pre-cut and minimally processed produce were reported in several countries. Therefore, the consumption of fresh fruits and

vegetables is not allowed in low microbial diets. Ionizing radiation is increasingly recognized as an effective method to ensure the microbiological safety of these products.

Purpose: The aim of this project was to investigate the effect of low dose irradiation on the survival of inoculated *Listeria* strains and the natural microbiota of tomato and carrot irradiated with doses of 0–2 kGy gamma irradiation and their survival/growth during chilled storage. Furthermore, chemical parameters and sensory properties were investigated.

Methods: Microbiological analysis was carried out by traditional culturing methods. Chemical parameters (vitamin C, tocopherol and carotene) were determined by HPLC. Unirradiated control and irradiated batches were analysed sensorically by a sensory panel directly after the treatment on the basis of hedonic scores on color, odor, taste and texture.

Results: Treatment with 2 kGy irradiation dose reduced considerably the microbiological contamination of carrot and tomato on the day of exposure. The microorganisms surviving the irradiation on tomato were able to grow, while the number of microorganisms on carrots did not grow significantly during refrigerated storage. The effect of irradiation on *Listeria* strains was dose-related on tomatoes, while on carrot doses above 0.5 kGy decreased its number below detection limit. Radiation doses of 0–2 kGy had no significant effect on sensory properties. Chemical parameters did not seem to be dose dependent.

Significance: The results of these studies suggest that radiation processing can ensure safety of minimally processed foods of pre-cut selected vegetables. Assistance of AGROSTER Co. Ltd Budapest in irradiation of samples is highly acknowledged. This work was supported by IAEA Nr.16243 and the TÁMOP-4.2.1/B-09/1, TÁMOP-4.2.2/B-10 projects.

P3-26 Incidence of* Salmonella *in Organic Fruits and Alternative Control of this Pathogen in Post-harvest Mangoes

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Introduction: Data on the microbiological safety of organic fruits are still scarce. Tomatoes and strawberries are among the most consumed organic produce. An outbreak of salmonellosis in the United States associated with consumption of Brazilian mangoes has been documented. A hot water immersion treatment to kill fly larvae is thought to be responsible for contamination. Alternative treatments such as a mixture of warm water and ethanol have not been evaluated for effectiveness in killing *Salmonella* on mangoes.

Purpose: To study the incidence of *Salmonella* in organic fruits (strawberry, mangoes and tomatoes) and to evaluate the combined effects of warm water and ethanol for controlling this pathogen on post-harvest mangoes.

Methods: Organic fruits (mangoes, tomatoes and strawberries) were collected from markets and analysed for *Salmonella* presence according to the BAM method. An alternative treatment was also studied for *Salmonella* control in mangoes. Mangoes were spot-inoculated with *Salmonella*, dried, and immersed in water containing ethanol (0, 1, 3, 5 and 7) at 46^{o}C for 70 min, then cooled in water at 21^{o}C for 30 min. Populations of *Salmonella* on mangoes were evaluated before and after treatments. Physical-chemical analysis of treated and control mangoes stored for at 25^{o}C/ 75% RH and at were 10^{o}C/90% RH were also performed.

Results: *Salmonella* was not detected in the three organic fruits analyzed. Mangoes spot-inoculated with *Salmonella*, dried, immersed in warm water (46^{o}C/70 min) containing ethanol (0, 3, 5 and 7%) and cooled in water at 21^{o}C for 30 minutes still showed the pathogen presence. Quality of treated mangoes was affected during subsequent storage at 10^{o}C/90% RH but not at 25^{o}C/75% RH.

Significance: Organic tomatoes, mangoes and strawberries grown in Brazil were not found to be contaminated with *Salmonella*. Other treatment methods should be tested to achieve elimination of *Salmonella* without compromising fruit quality during storage at refrigerated temperature.

P3-27 Development and Evaluation of an Immunochromatographic Rapid Assay for the Detection of Pathogenic* Vibrio parahaemolyticus *in Marine Food

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Introduction: *Vibrio parahaemolyticus* is an important foodborne pathogen throughout the world, particularly in Asian countries where it is a major cause of foodborne illnesses. Infections are mostly associated with the consumption of contaminated raw or undercooked seafood and manifest in gastroenteritis. The thermostable direct hemolysin (TDH) is known as the major virulence factor. Standard detection methods are mainly cultural based, labour-intensive and time-consuming (3 to 7 days). Rapid deterioration of raw seafood in particular, requires faster detection methods. Lateral Flow technology offers a reliable, fast and user-friendly, alternative detection method.

Purpose: To develop and evaluate an immunochromatographic assay for detection of pathogenic *Vibrio parahaemolyticus* from marine food, as a rapid alternative to standard reference methods.

Methods: A sandwich Lateral Flow assay was developed, using gold labelled specific antibodies for detection of TDH of *Vibrio parahaemolyticus*. Evaluation included determination of detection limit of TDH and of pure cultures, inclusivity testing of 23 *tdh*-positive *V. parahaemolyticus* strains and exclusivity testing of 69 *tdh*-negative *V. parahaemolyticus* strains, other vibrios and non-vibrios. Furthermore, sensitivity and specificity were tested using artificially contaminated seafood samples compared to reference method ISO/TS 21872-1:2007.

Results: Detection limit of TDH was 125 pg/ml and for pure cultures of TDH-positive *V. parahaemolyticus* 3.3 × 10^6 to 1.9 × 10^7 CFU/ml. The test produced an inclusivity of 81% and exclusivity of 100%. *V. parahaemolyticus* was detected in fresh food samples artificially contaminated with 10^1 to 10^2 CFU/g and in frozen samples at 10^3 to 10^4 CFU/g. After 24 h sample enrichment and implementation of a pre-treatment step (centrifugation), a sensitivity and specificity of

100% was attained. Performance was equivalent to the culture based ISO reference method. The LFA reduced time-to-result to a 24 h enrichment plus 1 h sample pre-treatment and assay performance.

Significance: The developed Lateral Flow Assay provides a fast detection of pathogenic *V. parahaemolyticus* in food with easy handling.

P3-28 24 Hours Enrichment and Real Time-PCR Detection of* Vibiro *spp. and* Vibrio cholerae *in Raw Oysters

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Introduction: Currently 12 species of the genus *Vibrio* have been reported as potential human pathogens. 8 of these species are directly associated with food. Since *vibrios* are abundant in estuarine waters, seafood is the major source of infections. *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* are responsible for the majority of seafood-borne infections, with *V. cholerae* and *V. vulnificus* being responsible for most fatal infections. Furthermore, cholera, caused by *V. cholerae*, is a disease with epidemic potential. A higher risk of infection with pathogenic vibrio species is achieved if seafood such as oysters, are consumed raw. Standard cultural detection methods take at least 3 to 7 days, and so are unsuitable for easily perishable raw seafood, such as oysters, which must be consumed within several days of fishing. The combination of a 24 hour enrichment step with subsequent Real Time-PCR enables the rapid and sensitive testing of oysters for absence of *Vibrio* spp. and *Vibrio cholerae* within 2 days.

Purpose: Combination of 24 hours enrichment with Real-time PCR for detection of *Vibrio* spp. and *Vibrio cholerae* from oysters to provide a fast alternative to standard reference methods.

Methods: Samples of fresh oysters were artificially contaminated with 10^1 to 10^2 CFU/10 g with strains of *V. alginolyticus*, *V. cholerae*, *V. mimicus*, *V. hollisae*, *V. parahaemolyticus* and *V. vulnificus*. After 24 hours enrichment in ASPW, DNA was extracted using the MMB Bacteria Prep Kit. Real-Time PCR detection was performed using the *Vibrio* screening (V) Kit and MMB *Vibrio cholerae* (V) Kit.

Results: All artificially contaminated samples were tested positive after 24 hours enrichment using the *Vibrio* screening Kit. In addition, all samples contaminated with *V. cholera* were tested positive using the MMB *Vibrio cholerae* (V) Kit. Analysis of fractional positive samples for *V. cholerae* (inoculated with 0.7 – 1.1 CFU/10g) using Real-Time PCR detection showed equivalent performance to the culture based ISO reference method.

Significance: The described method allows the Real-Time PCR based detection of *Vibrio* spp. and *V. cholerae* in oysters within 2 days which is a faster alternative to standard cultural detection methods.

***P3-29 Identification through Transposon Mutagenesis of Genes Involved in Osmotic Stress in* Cronobacter sakazakii**

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Introduction: *Cronobacter sakazakii* is a Gram-negative, rod-shaped bacterium that can cause necrotizing enterocolitis, meningitis and bacteraemia in infants, with mortality rates of up to 40-80%. *C. sakazakii* has been isolated from a wide variety of food sources, with powdered infant formula (PIF) being the most common vehicle involved in newborn infections. The microorganism has been shown to be highly resistant to osmotic stress and, interestingly, it has been shown to persist for long periods in dried PIF samples, with some capsulated strains surviving up to 2.5 years. However, little is known to date on the physiological response of *C. sakazakii* to osmotic stress.

Purpose: This study characterizes the growth in hyperosmotic media and the resistance to desiccation of a collection of fifteen *C. sakazakii* strains. In addition, a transposon mutagenesis approach was used to identify genetic systems involved in the response of *C. sakazakii* DPC 6529 to hyperosmotic conditions.

Methods: Growth in hyperosmotic media was determined in LB broth containing various concentrations (up to 10% w/v) of NaCl or KCl by determining the optical density at 600. Sensitivity to desiccation was monitored after air drying and incubation of samples at room temperature for up to 12 days. A transposon mutagenesis library was constructed for the strain *C. sakazakii* DPC 6529 by using the EZ-Tn5 <KAN-2>Tnp Transposome kit. Transposon mutants were screened for a defect in growth in hyperosmotic media. Transposon insertion sites were identified by modified single-primer PCR.

Results: *C. sakazakii* strains showed similar abilities to growth/persist under osmotic stress conditions to strains from other related *Enterobacteriaceae*. Nevertheless, some degree of heterogeneity among *C. sakazakii* strains could be observed, and in general, strains isolated from clinical sources showed the greatest robustness, which supports an association between the capacity to cope with drying and the likelihood of disease. We obtained evidence that de novo protein synthesis, repair of damage in macromolecules and maintenance of the structure and integrity of the cellular envelope are essential processes for the cell under osmotic stress. Moreover, some metabolic activities were also important, including the synthesis of glutamine as a compatible solute and the regulation of nucleotide and nucleoside pools. The Cpx system, known as an envelope stress response regulator, and the sigma factors RpoN and RpoS seem to be the main signals regulating the bacterial response to hyperosmotic conditions. Among the identified salt-sensitive mutants, only those disrupted in dnaK and dnaJ, encoding two molecular chaperones, were important for *C. sakazakii* survival under desiccation.

Significance: The identification of molecular mechanisms underlying the osmotic tolerance of *C. sakazakii* may ultimately be useful in the development of control strategies in PIF factories.

P3-30 A Microbiological Survey of Pre-packed Ready-to-Eat Sliced Meats at Retail in UK Small to Medium-sized Enterprises for* Listeria *spp.

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Introduction: In the UK, the incidence of listeriosis in older members of the population has risen recently, and such people are more likely to purchase foodstuffs in small to medium sized (SME) enterprises.

Purpose: The objective of this survey was to carry out a UK-wide study to determine the prevalence and levels of microbiological contamination of cooked sliced meats offered for sale to UK consumers by SME shops.

Methods: Numbers of *Listeria* spp., *L. monocytogenes*, *Enterobacteriaceae* and *Escherichia coli* were determined, and also pH, water activity and salt content. Sample temperature was measured prior to purchase and collections reflected population density across the UK.

Results: Overall, 1,049 samples were analysed and the mean sample temperature was 6.80°C ± 3.01, with 72.4% of samples exceeding the Chilled Foods Association guideline of 5°C. Almost a third of samples (32.7%) exceeded 8°C, the limit in the UK for such foodstuffs. *Listeria monocytogenes* was detected in 3.8% of packs, but only enumerated in four (0.4%) and in no case did it exceed 100 CFU/g. *Enterobacteriaceae* exceeded the UK guidelines of 10^4 CFU/g in 9.2% of samples. There was no correlation between numbers of *Enterobacteriaceae* and pack temperature.

Significance: A previous UK survey of RTE meats was undertaken in 2007 and mainly sampled in larger retail outlets. It reported a mean temperature of 4.54°C, and only 3.6% of samples exceeded 8°C (n = 1,680). This survey therefore indicates poor temperature control of RTE sliced meats in SME retailers, although no samples exceeded guidelines for the presence of *Listeria monocytogenes*.

P3-31 **Listeria monocytogenes *in Certain Ready-to-Eat Foodstuffs at Retail: Results of the European Union Coordinated Monitoring Program in Italy***

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Introduction: *Listeria monocytogenes* is a Gram-positive, intra-cellular facultative bacteria that causes severe foodborne disease. YOPI (Young, Older, Pregnant and Immunocompromised) are most frequently infected and may result in mortality rate up to 20%. More than 1600 cases of human listeriosis are yearly confirmed in the EU.

Purpose: The absence of harmonized data on prevalence and levels of contamination at retail determined the necessity of a baseline study aimed to the most risky RTE products, precisely soft and semi-soft cheese, cooked meat products and smoked fish.

Methods: Decision 2010/678/EU assigned to Italy 400 samples for each category. Sampling program was planned to reflect the actual market distribution of products throughout Italian territory, according to available commercial data and to the size of local population. A specific information system was made available for data collection.

Results: Prevalence ranged from 1% in soft and semi soft cheese to 2% in cooked meat products and 20.2% in smoked fish. The 100 CFU/g microbiological criterion was not respected in 0.5% of meat samples and in 3.3% of fish samples. The highest level of contamination was found in smoked salmon (1.3×10^6 CFU/g). Significant difference was found between fishes tested at different moments of their shelf-life ($\chi 2 = 4.992$, $P < 0.05$). The presence of statistical significant difference between fishes from different countries was found. Smoked fish from some establishments was far more likely to carry Listeria monocytogenes contamination than products from others ($\chi 2 = 193.22$; $P < 0.05$), prevalence for different premises ranging from 0 to76.9%.

Significance: Even if this program was planned to be significant at the EU level, some remarkable results were obtained also at the Country level. An interesting picture of Listeria monocytogenes contaminations in RTE foods at retail in Italy was yielded, being the starting point for more national surveys to evaluate the actual risk posed to Italian consumers.

P3-32 ***Effect of Climate Change on Foodborne Pathogens***

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Introduction: Increasing temperatures and high water levels are reputed to lead to increases in the prevalence of waterborne and foodborne pathogens. Food handling mistakes during extreme weather events can have detrimental outcome.

Purpose: Using the general principles of systematic review and scoping study, a scoping study was conducted to identify available evidence of the climate change effects on 30 important human pathogens.

Methods: The comprehensive literature search, repeatable two-level relevance screening (abstract and article levels), and characterization of primary articles identified and catalogued the number of publications, characteristics, and knowledge gaps in available literature on the subject.

Results: A total of 6,245 citations were screened. Of the 247 identified relevant articles that often reported on one or more research topics, one half were literature reviews (n = 123). The number of publications grew exponentially over the last several decades. The primary research was published in 85, diverse-topic scientific journals, 92% after year 2004. The effects of temperature changes and precipitation on *Vibrio cholerae* and other *Vibrio* spp., *Escherichia coli*, *Crypstosporidium* spp. and *Leptospira* spp. were studied in over 70% of articles. The most commonly measured outcomes variables were prevalence of human disease (n = 62) and prevalence of pathogens in the environment (n = 34). Fifty-six percent of research was conducted in the USA, Canada or Europe, while a substantial number of studies (24.6%) came from Asia. A limited number of publications described environmental ingression and dissemination patterns of common and emerging human pathogens. For example, there was a paucity of studies investigating the effect of climate change on the most common foodborne pathogens such as norovirus (n = 4) and *Listeria* spp. (n = 1).

Significance: We highlight research with data feasible for pooled analysis and areas warranting additional investigation. These finding can be used to inform future risk assessments and for development of evidence-based public health policies.

P3-33 **Arcobacter*: Prevalence, Virulence and Resistance in Meat***

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Introduction: The ε-Proteobacteria *Arcobacter* spp. have received attention as emerging human pathogens and are widely distributed in water, animals and foods of animal origin. *Arcobacter* spp. are taxonomic relatives of the pathogen *Campylobacter*. Predominantly, *A. butzleri* have been associated with cases of abortions in animals, but human illness occurs

with clinical symptoms resembling campylobacteriosis. Also a number of putative virulence determinants have been identified in *A. butzleri*; some of these are homologs of *Campylobacter* virulence genes.

Purpose: We wish to investigate the prevalence of *Arcobacter* spp. in Danish retail meat and establish the virulence potential in terms of presence of putative virulence genes. Further, the antimicrobial resistance profiles of the meat isolates are determined.

Methods: Danish meat obtained from supermarkets was subjected to selective cultural enrichment. Presumptive *Arcobacters* were confirmed and species identified by PCR. Virulence genes were detected by PCR. Antibiotic resistance was evaluated by the agar disc diffusion method.

Results: We isolated *Arcobacter* spp. from Danish retail meat in 22% of pork meat (n =121) and 100% of chicken meat (n = 10) in 2008/2009 and in 89% of chicken meat (n = 38) in 2012. Twenty-seven PCR-validated *A. butzleri* meat isolates were screened for nine putative virulence genes. Results showed that 78-93% of these contained homologs of six *Campylobacter*-associated genes; *cadF*, *ciaB*, *cj1349*, *mviN*, *pldA* and *tlyA*. We determined the antibiotic resistance of *A. butzleri* meat isolates (n = 40) from 2008/09 and found a high frequency of resistance to e.g., ampicillin (80%), amoxicillin-clavulanic acid (37.5%) and the 3rd generation cephalosporin cefotaxime (90%). Within the *A. butzleri* isolated in 2012, 97% of isolates (n = 29) were resistant to ampicillin.

Significance: Danish pork and chicken meat are naturally contaminated with *Arcobacter* spp. and the presence of virulence genes in these isolates indicates that *A. butzleri* has potential as a human pathogen. The resistance to antimicrobials is generally remarkably high and could represent a risk factor.

P3-34 **Bacillus cereus *and* Bacillus thuringiensis *Spores in Selected U.S. Retail Food***

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Introduction: Spores of *Bacillus* spp. are typically found in soil and marine sediments and toxigenic species have the potential to contaminate raw foods associated with these sources.

Purpose: Historically levels of spores of *Bacillus* species in raw foods have been reported without regard to their enterotoxingenic potential. Here the levels and toxigenicity of *Bacillus cereus* and *Bacillus thuringiensis* in U.S. retail seafood, rice and spices were determined.

Methods: Spore levels were detemined after heat selection following by MPN and confirmation by FDA protocols or by the use of a chromogenic agar. *B. cereus* isolates were distinguished from *B. thuringiensis* by the presence of an intracellular inclusion during sporulation. The presence of the genes for HBL and NHE enterotoxins, and the emetic toxin were determined by the PCR.

Results: A total of 760 samples of retail seafood, rice and spices were examined for the presence of spores of toxigenic *Bacillus cereus* and *Bacillus thuringiensis*. Levels of each species ranged from 3.6 to >1100/gm and 3.6 to 240/gm respectively. Approximately 30% (226 isolates) of total samples contained enterotoxigenic *B. cereus* able to produce NHE enterotoxin (90% of isolates) and HBL enterotoxin (50% of isolates). Twenty-one enterotoxigenic *B. thuringiensis* isolates were obtained but only two of the *B. cereus* isolates were of the emetic toxin type.

Significance: Enterotoxigenic *B. cereus* spores are widely distributed in non-outbreak, retail foods associated with soil and sediments, i.e, seafood, rice, and spices. By contrast the emetic toxin types are relatively uncommon in the commodities examined.

P3-35 ***Distribution of Clonal Complexes among* Campylobacter jejuni *Isolates from Human Clinical Samples and Broiler Products in Lithuania***

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Introduction: *Campylobacter jejuni* is the leading cause of human gastroenteritis in the EU. Also, in Lithuania, campylobacteriosis over the last few years increased and the number of registered cases has increased from 18.74 to 32.89 per 100,000 population from 2006 to 2010.

Purpose: The aim of this study was to evaluate distribution and population diversity of *C. jejuni* genotypes isolated from humans and raw broiler meat.

Methods: From October (2011) till May (2012) 245 samples were examined for presence of *C. jejuni*. Overall 108 *C. jejuni* isolates from humans stool samples (56), broiler wings (12), drumsticks (11), livers (15), minced meat (3) and marinated products (11) were genotyped by multilocus sequence typing (MLST).

Results: Characterisation of 108 *C. jejuni* isolates by MLST revealed 27 sequence types (STs). Twenty-four STs, representing 75 (69.4%) isolates, were assigned to 13 previously described clonal complexes (CCs). Four isolates were assigned to 3 STs which could not be refered to any of the known CC. The remaining 29 isolates were identified as a new STs, which most often represented by isolates from raw broiler livers (53.3%) and marinated products (63.6%). Human *C. jejuni* isolates were grouped in two dominant clonal complexes (CCs): ST-21 (26.8%) and ST-353 (30.4%). Isolates from raw broiler meat (wings, drumsticks, livers, minced meat and marinated products) were grouped in three dominant CCs (ST-21 (7.7%), ST-353 (13.5%) and ST-464 (21.2%)). Thirty-four humans and 24 broiler *C. jejuni* isolates were assigned to the same four CCs (ST-464, ST-607, ST-353 and ST-21). Interestingly, that clonal complex ST-464 was the dominant among isolates from broiler products, however only sporadically identified among *C. jejuni* isolates from humans.

Significance: Despite that 60.7% of *C. jejuni* isolates from human clinical cases showed the same MLST genotypes identified among isolates from raw and marinated broiler meat products, different dominant CCs were found in both sources (ST-21, ST-353 and ST-464, respectively). This research was funded by a grant (No.SVE05/2011) from the Research Council of Lithuania.

P3-36 ***Within-batch Prevalence and Quantification of Human Enteropathogenic* Yersinia *spp. in Pigs at Slaughter***

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Introduction: Yersiniosis is the third most common bacterial zoonose in Europe. The consumption of pork is the main source of human infection and healthy pigs are known to be the primary reservoir of human pathogenic *Yersinia enterocolitica*. However, little information is available about the prevalence of this pathogen within pig batches.

Purpose: The aim of this study was to obtain data about the within-batch prevalence of enteropathogenic *Yersinia spp.* in Belgian pig batches at time of slaughter.

Methods: The tonsils of 7,047 fattening pigs, originating from 100 farms, were aseptically collected immediately after evisceration in two Belgian slaughterhouses. Fattening pigs from both farrow-to-finish and specialized slaughter pig production were included. The batch size varied between 70 and 930 pigs. The number of pigs to be sampled per batch was calculated based on an expected batch prevalence of 50%, a confidence level of 95% and an accepted error of 10%. On average, 70 pigs were sampled per batch. The tonsils were analyzed using direct plating on cefsulodin-irgasan-novobiocin (CIN) agar plates and the number of suspect *Yersinia* colonies was counted. The results were verified using a multiplex Polymerase Chain Reaction.

Results: Pathogenic *Y. enterocolitica* serotype O:3 were found in tonsils of 2,009 pigs (28.5%), originating from 85 different farms. The within-batch prevalence in positive farms ranged from 5.1 to 64.4%. The number of yersinias in positive pigs varied between 2.01 and 5.98 $\log_{10}$ CFU g^{-1} tonsil, with an average of 4.00 $\log_{10}$ CFU g^{-1} tonsil. The mean *Yersinia* count of positive batches varied between 2.91 and 4.67 $\log_{10}$ CFU g^{-1} tonsillar tissue.
Y. pseudotuberculosis was found in seven farms, for which the within-batch prevalence varied from 2 to10%. In five of these farms, both *Y. enterocolitica* and *Y. pseudotuberculosis* were found.

Significance: Enteropathogenic *Yersinia spp.* are widespread in slaughter pig batches in Belgium, as 87% of the tested batches are infected with this pathogen at time of slaughter. A high proportion of pig batches represent a potential risk for public health.

P3-37 Intra- and Extracellular Survival of Human Pathogenic Yersinia enterocolitica *in Coculture with the Bacterivorous Protozoon* Acanthamoeba castellanii

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Introduction: Free-living protozoa are unicellular, eukaryotic microorganisms ubiquitous in natural aquatic and terrestrial environments and present in diverse anthropogenic and food related habitats. Free-living protozoa are bacterial predators, but some bacteria are able to evade protozoan uptake and/or digestion, turning the protozoan into a reservoir, shelter, vector or virulence training ground. *Yersinia enterocolitica* is the third most reported foodborne pathogen in Europe and is associated with the consumption of raw or insufficiently heated pork.

Purpose: *In vitro* co-cultivation assays were set up to test if *Y. enterocolitica* is resistant to predation by *Acanthamoeba castellanii*. Furthermore, we assessed if environmental factors and bacteria specific characteristics influence this interaction.

Methods: Four *Y. enterocolitica* strains with different virulence properties (absence or presence of the *Yersinia* virulence plasmid pYV) and different bioserotypes (4/O:3 and 2/O:9) were cocultivated with *A. castellanii*.

Results: The four *Y. enterocolitica* strains resisted predation by *A. castellanii* for at least 14 days, irrespective of medium (nutrient rich/poor) and temperature (7, 25 and 37°C) used. Factors excreted by *Y. enterocolitica* showed a permeabilizing effect on the protozoa, which was temperature and strain dependent. Long-term intraprotozoan survival of *Y. enterocolitica* was dependent on nutrient availability and temperature, with up to 2.8 log CFU/ml bacteria surviving intracellular at 7°C for at least four days in nutrient rich medium. Transmission electron microscopy revealed that intra-amoebal yersiniae were located in the amoebal cytosol.

Significance: As *Yersinia* and *Acanthamoeba* share similar ecological niches, this interaction suggests a role of free-living protozoa in the ecology and epidemiology of *Y. enterocolitica*.

P3-38 Toxoplasma gondii *in Turkeys and Persistence in Meat*

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Introduction: As one of the most common zoonotic parasites worldwide, *Toxoplasma gondii* is able to infect many avian species. From the point of view of food hygiene and foodborne transmission, chickens have been the preferred bird species to focus on. Besides chickens, turkeys are the second most consumed poultry worldwide. Only a few studies about toxoplasmosis in turkeys have been published. In Germany, the per capita consumption of turkey meat is about two thirds higher than in the rest of the EU-27 member states.

Purpose: For that reason, we focused on the question, if there is a potential risk of humans being infected with *T. gondii* by turkey meat or turkey meat products.

Methods: Serological examinations were done with a newly established kinetic ELISA based on recombinant GRA7 and GRA8 proteins. *T. gondii* DNA was detected by nested PCR based on the B1 gene. Tenacity studies were performed using mouse bioassay and real-time PCR based on the 529 bp fragment.

Results: To gather information about the serological state of conventionally raised turkeys in Germany we screened 1,913 sera. Out of these, 18.4 % were seropositive, giving the first evidence that German turkeys, which are intended for human consumption, do have contact with the parasite. Experimental infections were performed with different doses, *T. gondii* strains and routes of infection. The infected animals were examined serologically and for the presence of *T. gondii* DNA in 14 different tissues. There were no differences regarding infection dose or *T. gondii* strains, however, animals infected intravenously showed significantly more often presence of *T. gondii* DNA in breast muscle and livers and less often in brains compared to orally infected animals. Altogether, infection of turkeys can lead to the presence of the

parasite in edible tissues. Tenacity of the parasite against pH and salt were tested. *T. gondii* tissue cysts are tolerant of pH as they survived a pH of 5.0 for up to 26 days. But the cysts are very sensitive to NaCl and curing salt, especially at concentrations above 2.0 %, where they retained infectivity for at most 1 day. When short fermented sausages containing tissue cysts were produced with 2.0 % NaCl or curing salt infectivity of the parasite was retained for 12 and 24 hours, respectively.

Significance: Results indicate that there might be a risk for consumers to be infected with *T. gondii* by undercooked turkey meat and some special low salted, short fermented sausages.

P3-39 Qualitative Analysis of the Biofilms from* Listeria monocytogenes *Strains of Different Phylogenic Lineages

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Introduction: *Listeria monocytogenes* is an opportunist pathogen that causes listeriosis. Few data on the matrix composition of *Listeria monocytogenes* biofilms are available. However, it is well-known that the extracellular matrix plays an important role in biofilm development, resistance to chemicals and to detachment.

Purpose: The aim of the study was to characterize the ability of *Listeria monocytogenes* strains to form biofilm (strain lineage, growth medium and incubation temperature), using complementary methods. For the first time, a quantitative analysis of total carbohydrate, protein and eDNA content in the biofilm matrix was carried out.

Methods: Total biomass of 27 strains of *Listeria monocytogenes* belonging to lineages I or II was evaluated in different conditions (temperature and medium) by using crystal violet assay. Carbohydrate concentrations were determined by phenol-sulfuric acid assay. The protein content was measured using the Bio-Rad Protein Assay. The eDNA concentration was determined by OD 260 nm.

Results: Lineage II strains produced significantly more biofilm than lineage I strains. Biofilm quantities were greater in MCDB 202 vs. TSBYE medium (confirmed by Scanning Electron Microscopy (SEM) analysis) and at 37°C vs. 22°C. Conversely, cultivable bacteria were enumerated in greater quantities in TSBYE than in MCDB 202 medium. These opposite results would suggest the presence of uncultivable and/or dead bacteria in the biofilm. The SEM investigation established that *Listeria monocytogenes* biofilms produce extracellular matrix in both media at 37°C. The amount of exopolymers in the extracellular matrix and the pH values were significantly higher in TSBYE than in MCDB 202 medium. The exception was the ScottA strain which presented similar pH values and exopolymers content in both media. Proteins were the most abundant exopolymer component, followed by DNA and polysaccharides the least.

Significance: The characterization of *Listeria monocytogenes* biofilm composition may help to develop new strategies to prevent the formation and promote the detachment of biofilms.

***P3-40 Potential Cross-contamination of Food from Food Contact Surfaces Contaminated with* Listeria monocytogenes**

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Introduction: *Listeria monocytogenes* is commonly found to be associated with equipment surfaces in food industry environments. The transfer of bacteria depends on environmental and intrinsic factors. But no works study the residual bacteria on the surface (conveyor belts) after the contact between food and adherent bacteria, and the physicochemical properties which are involved in these phenomena.

Purpose: This study was designed to evaluate the ability of adherent *Listeria monocytogenes* to cross-contaminate foods in contact, and to identify which bacterial properties would affect this transfer to food.

Methods: Stainless steel surfaces were contaminated in static condition and successive blotting of the contaminated surfaces were performed with slices of agar. The initial removal rate and the percentage of residual bacteria were calculated. Both parameters were then confronted to data on bacterial surface properties.

Results: The strains of *Listeria monocytogenes* were electronegative and hydrophilic with the variation in the hydrophilic character in function of the strains and the incubation temperature. Significant differences were observed between the strains and between the temperature of bacterial adhesion for the initial slope ($P = 0.0000$). We had analysed the correlation between different variables (physico-chemical parameters, adhesion, initial slope) and the similarity between the strains which would make it possible to classify them in distinct groups. We had data processing by PCA. Two components were extracted and counted for 46.1% of the variability in the original data. The first component represented 24.23% of the total variation of the data.

Significance: The risk of foodborne infection associated with cross-contamination depends on two factors: the level of contamination on the surfaces and the probability of its transfer of the foods being consumed.

P3-41 Evaluation of the Efficacy of a Process of Pulsed-UV Light against* Cryptosporidium parvum *Oocyst* in Vivo *Infectivity in Suckling Mice

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Introduction: *Cryptosporidium* spp. is recognized as a main pathogenic protozoa problematic for public health.

Purpose: This study was designed to investigate the effects of an established procedure of pulsed light irradiation on the infectivity of *Cryptosporidium parvum* oocysts (bovine isolate) artificially contaminating a food plant matrix, basil (*Ocimum basilicum*) leaves, which were chosen as at risk from hydric contamination.

Methods: After oocyst spreading on both sides, contaminated leaves were exposed to 2 pulsed-UV light two flashes of 1 J/cm^2 (i.e. 2 J/cm^2 total fluence). Irradiated and non-irradiated parasites were extracted from leaf matrices as follows: 1) oocyst elution with glycine buffer, 2) oocyst oncentration by slow centrifugation, 3) oocyst purification using immuno-magnetic beads, 4) microscopic oocyst counting. For the evaluation of oocyst infectivity, 5 day-old mice were gavaged by doses of irradiated or non-irradiated oocysts. On the seventh day post-infection, the mice were sacrificed and the presence of oocysts in the entire small intestine (mucosa and intra-intestinal content) was quantified using 1) immunofluorescence, and 2) quantitative PCR assays.

Results: The validity of the model was confirmed (absence of spontaneous contamination of animals and the basil leaves). After a dose of 10 and 100 no irradiated oocysts, the ratios of infected animals were 1/11 and 10/12, respectively. After a dose of 1000 to 10000 irradiated oocysts, the ratios of infected animals were 0/10 and 2/8, respectively, on day 7 post-infection. Data suggest that pulsed UV irradiation at the present dose results in a decrease of 2 to 3 $\log_{10}$ in the oocyst infectivity. At irradiation doses as above, no alteration of morphologic/organoleptic characteristics of leaves was observed.

Significance: In view of expected *C. parvum* contaminations in natural environments, results prompt further assays in industrial contexts, and further investigations on other plants of economic interest such as raspberries.

P3-42 Campylobacter jejuni: Survival Strategies That Maintain Virulence

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Introduction: In spite of its fastidious nature, *Campylobacter jejuni* remains the leading cause of bacterial gastroenteritis in the developed world. *C. jejuni*, a zoonotic pathogen requiring a microaerobic environment and temperature range of 30–45°C for growth, may survive outside these conditions by forming biofilms or becoming viable but non-culturable (VBNC).

Purpose: To evaluate propidium monoazide qPCR (PMAqPCR) as a method to quantify viable *C. jejuni* cells and to compare biofilm and planktonic *C. jejuni* entry into a VBNC state in response to starvation at refrigeration temperatures in order to assess the associated food safety risk.

Methods: Three strains of *C. jejuni* biofilm and planktonic cells were stored in phosphate buffer saline at 4°C in air. Cells were considered non-culturable once they were unable to culture on supplemented agar after 24 hours of enrichment. Viability was assessed using PMAqPCR and *Bac*Light™ biovolume analysis at 10 day intervals.

Results: PMAqPCR provided results consistent with both fluorescence-based viability staining (*Bac*Light™) and standard plate counting methods for both biofilm and planktonic cells ($P < 0.05$). Biofilm cells became VBNC after 10 days and planktonic cells after 60 days in buffer at 4°C. Viable cell counts did not decline significantly over the 60 days of treatment for 3 of the 6 samples ($P < 0.05$). *C. jejuni* NCTC11168 strain V1 biofilm cells had the largest significant reduction of 1.7 $\log_{10}$ cells/ml, still leaving 5.59 $\log_{10}$ cells/ml, which although non-culturable were considered viable and potentially infectious.

Significance: *C. jejuni* biofilm cells pose an even greater risk to food safety than previously recognized. Biofilms provide cells with protection from stresses and are difficult to remove from surfaces. This is the first study to show that they are capable of becoming VBNC very rapidly when exposed to refrigeration conditions showing little to no significant reduction in viable counts for 50 days. In light of the fact that current food safety detection methods are based on culturing, this new insight into the survival strategies used by *C. jejuni* highlights the need for culture-independent detection methods.