Technical Session 1 – Wednesday, 9 June, 15.30-17.00
Food Detection News
Chair, Sarah Cahill

T1-01   Multiple Concentration and Detection of Foodborne Viruses in Produce

ROCIO MORALES-RAYAS, Julie Jean Petra, F.G Wolffs and Mansel W. Griffiths, Institute of Nutraceuticals and Functional Foods, Pavillon Paul Comtois, Universite Laval, Quebec, QC, G1V0A6, Canada

Introduction: The application of molecular methods for routine food analysis has been hampered by the lack of appropriate sample preparations that remove reaction inhibitors. The diverse and complex food matrices that have been associated with foodborne outbreaks make difficult to use a universal sample preparation. Therefore, there is still a need for sample preparation methods that concentrate/separate low amounts of pathogens from different foodstuffs without compromising the sensitivity of the detection method.

Rationale: The objective of the study was to assess the performance of two sample preparation methods for a simultaneous concentration/detection of noroviruses and hepatitis A virus in different produce. A multiplex real-time PCR assay was designed to evaluate the sample preparation methods. The multiplex assay was set up using artificial templates and validated with norovirus isolates from different outbreaks and a HM-175 HAV strain. Two RNA extraction methods were analyzed for a suitable nucleic acid extraction from the targets. Once the molecular detection method was optimized, the two sample preparation methods based on electrostatic binding were evaluated to simultaneously separate different virus concentrations from green onions, lettuce, raspberries and strawberries.

Results: Cationically charged filters yield a more consistent detection of infectious amounts of HAV and norovirus ($2-20$ viral particles/g) compared to cationically charged beads in a flow-through system. The overall recovery of the method was between $23-40\%$ from the different food matrices.

Conclusion: The developed multiplex detection protocol provides a promising alternative for a simultaneous, rapid and sensitive detection of hepatitis A virus and norovirus in produce.

T1-02   Enhanced Detection of *Listeria* spp. from Environmental Swab and Food Samples within 24 Hours Using Sample Pooling, Automated Pathatrix Re-circulating IMS Linked to Real-time PCR

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Introduction: Listeriosis is an important public health problem that produces high mortality rates. Serious infections occur mainly in pregnant women, neonates, immunocompromised and elderly individuals and result primarily from eating food contaminated with the bacterium *Listeria monocytogenes*. *L. monocytogenes* has a ubiquitous distribution and possesses properties that increases the risk of its persistence and dissemination in food processing facilities. Like other members of the genus *L. monocytogenes* is psychrophilic and can grow at refrigeration temperatures of 1°C. The potential implications of *L. monocytogenes* being present in ready-to-eat foods which support growth has led to a zero tolerance approach from both the FDA and USDA FSIS. RTE Food considered to be high risk for *L. monocytogenes* include those with pH > 4.4 and a_w > 0.92.

Rationale: Given the potentially serious implications of *Listeria monocytogenes* being present in RTE foods that support growth or in the food processing environment the aim of this study was to assess the feasibility of using an enhanced enrichment protocol, recirculating IMS and real-time PCR to develop a method which is capable of detecting the presence of *Listeria* contamination, including *L. monocytogenes*, at low level in pooled food and environmental swab samples within 24 hours.

Results: The range of *Listeria* spp. were successfully isolated from pooled food samples down to 0.004 CFU/g and environmental contact swabs (1 CFU in 100 cm^2) using recirculating IMS. Detection was achieved using real time PCR and this was confirmed by isolation of target *Listeria* on selective agar plating. Target capture employed Pathatrix IMS particles with proven inclusivity for all listeria species.

Conclusion: The Pathatrix *Listeria* pooling method described allows food production facilities to increase sample throughput during routine *Listeria* monitoring of both food and environmental contact swabs. The method has the potential to enhance HACCP and pathogen testing regimes and can be employed to validate hygiene practices and sanitizing procedures aimed at reducing the incidence and spread of listeriae in the food processing environment.

T1-03   A Comparison of VTEC O113 Isolates from Australia, New Zealand, Norway and Ireland

DECLAN BOLTON, Aine Monaghan, Brian Byrne, Anne Carroll and Seamus Banning, Teagasc-Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland

*Introduction***:** VTEC O113 is common on beef farms. In recent years intimin (*eaeA*) negative VTEC O113 has been associated with hemolytic uremic syndrome (HUS) in Australia, New Zealand, Norway and Ireland. To date there is little or no information on non-O157 HUS strains from different countries throughout the world.

Rationale and objectives: The objective of this study was to compare Irish farm, abattoir, and clinical VTEC O113 isolates with those from Australia, New Zealand and Norway using MLST, PFGE and PCR analysis of virulence factors.

Results and findings: All of the Irish isolates belonged to ST-10 with the exception of one clinical isolate, which belonged to ST-29. The Norwegian and one of the New Zealand food isolates belonged to ST-223 with ST-56 accounting for the remaining Australian and New Zealand isolates. In general, the MLST genotyping was supported by PCR analysis of the virulence gene profiles with isolates of the same ST showing similar profiles. Broadly speaking PFGE analysis grouped the 48 isolates into their respective serotypes although there were several clones within each serotype. All isolates were examined for *vt1*, *vt2*, *eaeA*, *hlyA*, *espE* (TIR), lpfAO113, *esp*P, *saa*, *sab*, *tox*B and *iha*. Farm and abattoir VTEC O113:H4 carried *vt1* and/or *vt2* and *iha* only and were not associated with human illness. The Australian and New Zealand strains carried *vt2*, *saa*, *sab* and *lpfA_{O113}* but these were absent in the Irish clinical isolates suggesting an alternative variant or adhesion factor. Furthermore, the latter carried both *vt1* and *vt2*, variants *vt1*, *vt1c*, *vt2c* and *vt2d*.

*Conclusion*s: The various analyses would suggest that at least one of the New Zealand HUS isolates is prevalent or originated in Norway and the Irish clinical isolates developed from the farm O113:H4 VTEC strains that acquired additional virulence factors including the putative adhesin Iha.

T1-04   Withdrawn

T1-05   Use of Principal Component Analysis in Order to Link Genotypic and Phenotypic Characters of Psychrotrophic *Bacillus cereus* Group Isolates to Their Cytotoxic and Spoilage Potentials in Egg-breaking Industry

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Introduction: Since liquid egg products usually receive only mild heat treatments inefficient on spores and are stored at low temperature, control of bacteria belonging to the *B. cereus* group is of high importance for the sector of egg production. Their various enzymatic activities are recognized as causing food spoilage, even at refrigerated temperatures when psychrotrophic strains are involved.

Rationale: The aim of the study was to link, using principal component analysis, genotypic and phenotypic characters of a collection of 77 psychrotrophic *B. cereus* group isolates, coming from 6 French egg breaking companies at two seasons, to their potentiality to cause food spoilage and sanitary problems in egg products.

The genetic characteristics (presence/absence of *cspA* (Francis et al., 1998), 16S rDNA-2p et 16S rDNA1-m (Von Stetten et al., 1998) signatures) and phenotypic characteristics (growth at 6 and 43°C) were studied in regard to the egg product spoilage potential (growth and enzymatic activities in liquid whole egg at refrigerated temperatures) and to the cytotoxic activity on Caco-2 cells.

Results: This study allowed distinguishing 12 profiles from genotypic and phenotypic characteristics, with a high occurrence of *B. weihenstephanensis* isolates (47%). All the 77 isolates of the collection were able to grow in whole liquid egg at refrigerated temperatures. The cytotoxic and spoilage capacities depend on the profile, the genetic signature, the ability to grow at 6°C and 43°C, the season and the company. The presence of the *cspA* psychrotrophic signature, specific of *B. weihenstephanensis* isolates, is associated with low cytotoxic activity and high spoilage potential at refrigerated temperatures.

Conclusion: To conclude, this study provides new insights on psychrotrophic *B. cereus* group strains in the sector of egg production and forms the starting point for their control in egg product industry.

T1-06   VTEC on Farms: Serotypes, Virulence Profiles and Survival

DECLAN BOLTON, Ciara Ennis, Aine Monaghan, Brian Byrne, David McDowell and Seamus Fanning, Teagasc-Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland

Introduction: Verocytotoxigenic *Escherichia coli* (VTEC) are a serious foodborne risk to consumer health. Serotype O157:H7 causes most haemolytic uraemic syndrome (HUS) cases and is responsible for the majority of fatalities. O26, O111, O103 and O145 are also deemed to be clinically significant. Indeed, approximately half of VTEC associated illness in Europe is attributed to non-O157 strains. The farm is the source of current and newly emerging VTEC.

Rationale and Objectives: The objective of this study was to examine the incidence, serotypes, virulence profiles and survival behaviour of VTEC in the farming environment using culture based, PCR and RFLP-PCR analytical techniques.

Results: Approximately 37% (1104/3000) of faecal and 27% (324/1200) of soil samples were VTEC positive. A seasonal peak was observed in May-June. O113:H4 was the most common serotype followed by O26:H11. 6/32 farms were O157:H7 positive. The prevalence of selected virulence genes were as follows: *vt1* (59%), *vt2* (77%), *eaeA* (18%), *hlyA* (26%), *tir* (14%), *katP* (0%), *etpD* (0%), *lpfA* (11%), *saa* (7%), *espA* (1%), *espB* (1%) and *espP* (24%). *Vt2d* was the most common *vt2* gene variant and the HUS associated *vt2c* and *vt2dact* variants were rarely detected. A cocktail of *E. coli* O157:H7 strains inoculated into farm water, soil, slurry and bovine faeces showed different rates of decline but were still detected by enrichment in each medium for up to 102 days.

Conclusion: The present study shows that there are a broad range of different VTEC serotypes on farms and many have the virulence factors required to cause serious clinical illness in humans.

Technical Session 2 – Thursday, 10 June, 16.00-18.00
Food Preservation and Ecology Updates
Chair, Margaret Patterson

T2-01   Fate of Aflatoxin M1 during Manufacture and Storage of Yogurt
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Introduction: Aflatoxin M1 (AFM1) may occur in milk resulting from the ingestion of aflatoxin B1 in feedstuffs by dairy cow.

Rationale: Our goal was to study what happened if contaminated milk manufactured into yogurt and stored at 4ºC ± 1 for 7, 10, 14 and 21 days.

Results: Yogurt from cow's milk artificially contaminated with aflatoxin M1 at level of 100 μg/L was fermented to reach pH 5.1 ± 0.04. Yogurt was stored at 4 degrees C for 7, 10, 14 and 21 days. Analysis of aflatoxn M1 in yogurt was carried out using solid phase extraction coupled with competitive enzyme-linked immunoabsorbent assay (ELISA) AFM1 in yogurt samples showed a significant increase ($P< 0.01$) compared with those initially added to milk. The level of AFM1 in yoghurt samples was 2.1 ± 0.07 fold. During the refrigerated storage AFM1 was rather more stable. The percentages losses of the initial amount of AFM1 in yogurt were estimated at about zero, 3.75 ± 0.05, 6.25 ± 0.16 and 13.75 ± 0.24 by the end of storage with pHs. Changes in AFM1 content of yogurt samples were found statistically insignificant ($P > 0.01$) for 3 weeks storage periods.

Conclusion: It was concluded that processing of contaminated milk with aflatoxin M1 into yogurt does not lead to appreciable degradation of the toxin content. The levels of AFM1 in manufactured yoghurt was high and seemed to pose a threat to public health. Therefore, it is important to detoxify milk before processing to be safe for human health.

Acknowledgments: I would like to acknowledge Dr. Diekmann, Professor of Food Microbiology, as well as the Institute for Microbiology, Hannover University for their valuable and kind help during my stay and work at the Institute.

T2-02   Ozone Treatment of Apple Juice at Different pH Levels; Effects on the Microbial Responses of *Escherichia coli*

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Introduction: Non-thermal processing technologies represent a preferred alternative method for fruit juice processors to produce a minimally processed food with minor quality changes. The FDA's approval of ozone as a direct additive to food in 2001 triggered interest in ozone applications development, and industry guidelines for apple juice and cider were published by the USFDA in 2004, which also highlighted gaps in the scientific knowledge (US FDA, 2004).

Rationale: Enhanced acid resistance of *Escherichia coli* O157:H7 has led to food borne outbreaks in acidic fruit juices. The objective of this research was to investigate the efficacy of gaseous ozone at a concentration of 33–40 μg/mL on the inactivation of *E. coli* ATCC 25922 and NCTC 12900 strains (10^6 CFU/mL) in apple juice over a range of pH levels; 3.0, 3.5, 4.0, 4.5 and 5.0 (adjusted by using either 10% malic acid or 1N NaOH), using an ozone bubble column for different time periods up to 18 min.

Results: The results revealed that pH showed a significant effect on the microbial inactivation kinetics during ozonation. The relationship between time required to achieve 5 log reduction (t5d) and pH for both strains was described quantitatively by an exponential expression. The ozone treatment duration required for achieving a 5-log reduction was faster (4 min) at the lowest pH than at the highest pH (18 min) studied. Both strains of *E. coli* (ATCC 25922 and NCTC 12900) showed similar trends of inactivation at different pH levels studied.

Conclusion: Ozone treatment could be a potential alternative for reducing bacteria in apple juice and the required applied treatment for producing a safe apple juice is dependant on its acidity level.

T2-03 **Comparison of Growth Limits of *Listeria monocytogenes* in Milk, Broth and Cheese**

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Introduction: Predictive modelling is frequently used to predict the behaviour of pathogens in food. Combase (www.combase.cc) is a well used example. However, the predictions of pathogen behaviour are generally based on laboratory media and then applied to food.

Rationale: The obvious differences in composition and structure between laboratory media, liquid food such as milk and solid food such as cheese raises a question about the suitability of these liquid-based models in their application to structured foods. By making predictions of the behaviour of *L. monocytogenes* in the three different matrices, the objective of this study was to determine the accuracy of models based on laboratory media when applied to solid food.

Results: In each matrix, the probability of growth (> 0.5 log increase in 8 h) of *L. monocytogenes* at an inoculation level of about 10 CFU/ml was determined in a full factorial experimental design of 4 pH values (6.5, 6.1, 5.9, 5.6) and 5 water activity values (0.99, 0.98, 0.97, 0.96 and 0.95) in 6 replicates of each combination. The non-linear regression model was applied to the data. The results showed that water activity was the growth limiting parameter. The minimum water activity where 100% growth was observed was 0.98 in cheese, 0.95 in milk and 0.97 in TSB. During cheesemaking, variable growth was observed at and below water activity values of 0.97; no growth was observed below water activity of 0.96. In milk or TSB none of the combinations used resulted in 100% no growth observations.

Conclusion: The results show that liquid-based models overestimate the growth encountered in cheesemaking conditions. For accuracy, models applied to food need to be constructed in food.

T2-04 **Differences in the Ability of Contact Materials to Support *Salmonella enterica* Biofilm Formation**

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Introduction: Salmonellosis is the second most common cause of bacterial foodborne gastroenteritis. The ability of *S. enterica* to persist as a biofilm in food processing surfaces may be associated with contamination of foods. Food contact surfaces may be a significant source in the transmission of foodborne disease and consequentially poses a serious risk to consumer health.

Rationale: Variations in the ability to establish biofilm on surfaces in food processing environments may be an important determinant of the potential for a given *S. enterica* strain to cause human foodborne infection. Glass, stainless steel, polycarbonate, glazed tile and concrete are materials that are used in different areas of food processing premises. The CDC biofilm reactor represents a convenient model system to study *Salmonella* biofilm formation

on these relevant surfaces. The objective of this project is to quantify biofilm formation by a number of different *S. enterica* strains on these surfaces.

Results: All six *Salmonella* (5 *S. Agona* and 1 *S. Typhimurium*) form a biofilm on all 5 surfaces (as assessed by scanning electron microscopy and viable counts following sonication). The highest mean viable count per standard coupon was for glazed tile for all strains tested followed by concrete and polycarbonate. Glass and stainless steel had reduced intensity of biofilm formation. Some differences in intensity of biofilm formation by different strains on a given material were observed however the reproducibility and statistical significance of these results require further assessment.

Conclusion: A convenient laboratory model for investigation of *Salmonella enterica* biofilm formation on surfaces relevant to the food processing environment has been established. This data suggests significant differences in the ability of materials to support salmonella biofilm formation. The extent of variation in biofilm formation between the strains studied is less marked than variation between materials.

T2-05   An Automated And Portable Monitoring System For HACCP Food Safety System With Sophisticated And Fast Management Responding And Supervising Feature

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Introduction: This project was developed as a combined effort of E-Control Systems, Chatsworth, California, a start up company and Gideon Zeidler, University of California-Riverside. It took eight years and several generations of the system to reach the current status which focused on foodservice. However, almost every category of the food business and agricultural production can use this system. An electronic Management Tools System which allows management a rapid and sophisticated respond to emerging problem even in a far away unit was the last feature that was added to the system.

Rationale: HACCP food safety system was originally designed as reactive and manual system and in this form it remained till the present time in most places. Performing HACCP is laborious, generating huge amount of paper work and human errors and requires constant training, especially in foodservice where employee turn around is high. As a result, it is also costly. Furthermore, management response especially in preventing HACCP failures is very limited in particular when multi-unit operations are involved.

The objectives of this project were to overcome these problems by developing an automated and remote HACCP monitoring system. It continuously transmits almost all HACCP data from the facility into a computer which processes it into reports and also initiates warnings well in advance to allow management to correct the problem before HACCP failure occurs. Eliminating most of the manual labor involved, the paper work and human errors will result in more accurate reports and by far lower operations costs.

Several additional objectives were added during the development process. 1. The connection between equipment failure and HACCP failure was recognized and all processing equipment operational parameters were collected to create an On-line Preventive Maintenance System. 2. To create an Electronic MANAGEMENT TOOLS SYSTEM to replace the manual and too slow existing system. This system allowed management to have all related data to be processed in one screen in real-time, thus greatly improving their response to complicated problems. 3. To elevate operations productivity and efficiency, which is very low especially in food service. All the objectives of this project were successfully achieved.

Results: The monitoring system is composed of three elementary components: A. The wireless Receiver/Transmitter, which collects data from the sensors and transmits it to, B. The Wireless Collector, which collect the data from all the transmitters around and sends it through the Internet into the computer, C. The computer's software processes the data, creates

reports and sends warnings when needed. It also instantly identify the problem for rapid correction before failure happen.

Two comparable systems were developed: 1. The Static System where the transmitters are connected to the terminal ports of each piece of equipment and transmit all HACCP parameters and equipment operating parameters. 2. The Portable System where the transmitters and collectors were miniaturized to the size of a credit card and can be operated by a battery. The transmitter is connected directly to the sensors and keeps monitoring even when equipment is broken or in electrical shut down. It can be attached to moving units; very little installation is needed and is extremely user friendly. The two systems can integrate any number of units regardless of distance and operate with one software only, which drastically reduces cost.

The development of this project achieved several milestones: 1. The first electronic and wireless HACCP monitoring system, 2. The first on-line preventive maintenance system which helps prevent HACCP failures and improves process operations, 3. Better utilization of labor and materials, which saved up to 50% in labor and up to 20% in materials due to better inventory and finished products control and reduction of waste and spoilage, 4. Rapid identification of problems, which can prevent HACCP failures. Checking and approving or disapproving of repairs done, 5. The recently developed software for Management Tools, which allows all relevant data to be processed in a way that can be displayed on one screen for rapid and educated management decisions. One of the features can compare different units selected operations and concentrate on elevating the performances of the worst ones first. This is the first successful system of this kind, 6. The development of the first battery operated portable system, 7. Emphasis on development of a very low cost system which allowed large number of financial strapped elementary schools to widely use the system. Better protecting school children from foodborne diseases is the ultimate achievement of them all.

Conclusion: The two versions of the HACCP monitoring system, and especially the portable one, were well accepted by foodservice and by schools, universities, hospitals and care providers. In the last few years over 5,000 schools (about 5 million students and staff) and over 750 hospitals and care providers installed the system. Convention centers, large casinos, correctional institutions, kitchen installers and turn-key commercial kitchen manufacturers are also using this system. Many restaurants, including two University of California campuses, and big names such as the U.S. Senate dining system and the new Canadian Parliament are among the users. Many cold storage facilities and several foods processors, such as meat plants, bakeries and others are among the users. The new Electronic Management Tools System is attracting mega-food companies, which are looking to replace their old, manual and inefficient systems. Some have done it successfully.

T2-06   Inactivation of *E. coli* O157:H7 in Spinach Leaves Using Gaseous Sanitization

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Introduction: Contamination of leafy vegetables with *Escherichia coli* O157:H7 has been a sporadic and recurrent problem in the fresh produce industry. Since fields are open systems, contamination via animals, birds or insects is inevitable. Thus adequate mitigation strategies should include decontamination steps within the produce chain.

Rationale: Gaseous sanitizers have been shown to be effective in penetrating crevices within produce. This work evaluated the efficacy of using ozone for decontamination at two key steps in the produce chain: vacuum cooling and transit.

Results: Baby spinach inoculated with *E. coli* O157:H7 (10^7 CFU/g) was treated in a pilot-scale system with combinations of vacuum cooling and sanitizing levels of ozone gas. This process decreased *E. coli* O157:H7 populations by up to 2.4 log CFU/g. An optimized process that inactivated 1.8 log CFU/g with no apparent damage to the quality of the spinach was determined from response surface methodology.

In a separate set of experiments, refrigerated spinach was treated with low ozone levels (8 to 16 mg/kg; 5 to 10 ppm, v/v) for up to 3 days in a system that simulated sanitization during transportation. The treatment decreased *E. coli* populations by up to 1.4 log CFU/g, and the optimum process resulted in a 1.0-log inactivation with minimal effect on product quality. In a third group of experiments, freshly harvested unprocessed spinach was inoculated with *E. coli* O157:H7 and sequentially subjected to optimized vacuum cooling and long-term sanitization processes. This double treatment inactivated 4.1 to 5.0 log CFU/g, depending on the treatment time.

Conclusion: Sanitization with gaseous ozone during vacuum cooling and subsequent transit shows potential for mitigation of spinach contamination by *E. coli* O157:H7.

T2-07 Influence of Food Processing on Detection of Hazelnut Proteins/Allergens

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Introduction: With the ongoing rise of the prevalence of food allergies the food industry is nowadays confronted with the serious task to control and validate their production processes. To date the most applied method for detection of allergens is the ELISA format, because it is cheap and easy to perform. However, few information is available on the influence of food processing on the detectability of the target proteins. During food processing proteins undergo changes such as aggregation, polymerization, degradation, random unfolding, oxidation etc. Moreover, food proteins often interact with other food components during processing, giving rise to additional protein modifications. Therefore, the antibody-protein interaction during the analytical procedure might be affected, potentially giving rise to erroneous assay results.

Rationale: The purpose of this work was to investigate the effect of different chemical modifications, which frequently occur during food processing, on the detection of hazelnut proteins/allergens by means of commercially available ELISA kits. To this end, hazelnut proteins were subjected to controlled chemical modifications in buffered model systems; Maillard reaction in the presence or absence of wheat proteins, chemical and lipid induced oxidation and pepsin hydrolysis.

Results: The results show that the detection of hazelnut proteins is affected by the different induced chemical modifications. This may lead to an altered estimation of the actual hazelnut protein concentration in the sample. The severity of the impact depends on the modification that has been induced. In addition, for the individual modifications different responses are recorded with the different ELISA kits.

Conclusion: From this work it can be concluded that food processing affects the antigenicity and thus the detectability of hazelnut proteins. The outcome of the analysis also depends on the type of assay that is used. Consequently, detection of hazelnut proteins/allergens in processed food products by means of ELISA can be drastically influenced and lead to false positive/negative results.

T2-08 The Effect of Heat Shrink Treatment and Storage Temperature on the Time to Onset of 'Blown Pack' Spoilage

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Introduction: *Clostridium estertheticum*, *Clostridium gasigenes* and a newly discovered species, *Clostridium ruminantium* cause blown pack spoilage (BPS) in vacuum packaged (VP) meat. BPS is characterized by the production of sufficient amounts of carbon dioxide and hydrogen to cause pack distension, within four to six weeks of chilled storage. BPS costs the meat industry in excess of several million euro per annum. Science based control activities that could be incorporated into the meat processors HACCP prerequisite programme are urgently required.

Rationale and Objectives: Vacuum pack heat shrinkage (85–90°C for 2–3 s) may activate BPS clostridial spores naturally present on the meat thus producing metabolically active cells and promoting rapid spoilage. The objective of this study was to investigate the effects of VP heat shrinkage and subsequent chilled storage temperatures on the time to BPS.

Results: Beef and lamb steaks were inoculated with 1,000 CFU per square cm of spore suspensions of five gas producing clostridia, vacuum packed and treated as follows: no heat, 50ºC/15 s, 70ºC/10 s or 90ºC/3 s and stored at -1.5, 1 and 4ºC. These were examined daily to determine 'time to onset' (TTO) of BPS. For each strain, pack treatment and storage temperature had significant ($P < 0.05$ and $P < 0.001$, respectively) effects on TTO of BPS. The general trends were as follows: 90ºC/3 s < 70ºC/10 s < 50ºC/15 s ≤ 'no heat' and 4ºC < 1ºC < -1.5ºC.

Conclusion: BPS can be prevented in commercial meat plants by avoiding high temperature heat shrinkage and/or reducing chilled storage temperatures.

Technical Session 3 – Friday, 11 June, 09.00-10.00
Food Safety Management and Quantitative Aspects
Chair, Vickie Lewandowski

T3-01 A Comparative Observational and Microbiological Analysis of Mechanical Hide Pulling Techniques in an Irish Cattle Abattoir

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Introduction: The animal hide is the primary source of beef carcass contamination. Preventing carcass contamination during hide removal is difficult due to the nature of the process. Superior skinning techniques are therefore critical. In Irish abattoirs two skinning methods are employed – Upward and Downward Hide Pulling (UHP and DHP).

Rationale and Objectives: As part of this study, a survey of Irish abattoirs shows a definite and continuing shift from UHP usage in favour of the DHP type. Abattoirs cite a reduction in contamination of the carcass forequarter as the basis for this trend. To establish a scientific basis for this hypothesis, an abattoir that was changing its hide pulling method from the UHP to the DHP type was selected. Animals with a Clean Livestock Policy hide score of 3 (Dept. of Agriculture and Food, Ireland: 2005) were chosen for this study. Eight carcass sites (ham, rump, bung, flank, brisket, shin, chuck and neck) were sampled, using the wet-dry double swab technique, on 72 carcasses divided into two groups of 36 carcasses skinned by each technique. Total viable counts and *Enterobactericeae* counts for each site were determined.

Results and Findings: No significant difference was seen in overall carcass contamination levels between the methods. Significant differences ($P < 0.05$) in microbial contamination were observed at flank, shin, brisket and chuck. Critical observation of each hide removal method attributed the site-specific differences to factors that were not attributable to the hide-puller per se. Deficiencies in the abattoir's HACCP Prerequisite Programmes (HPRPs) were identified during observation.

*Conclusion***s:** Results indicate that implementing sound and robust HPRPs is more critical in the hide pulling process than the hide puller type. This is the first comparative study of its kind, it facilitates abattoirs who may be considering changing their skinning method in making a more informed decision.

T3-02 Prevalence Data Concerning Foodborne Viruses in Europe

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Introduction: Human enteric viruses are the main cause of viral gastroenteritis. During the last winter 2009–2010, several European alerts have been reported implicating norovirus and hepatitis A virus in different types of food (berries, salads, tomatoes, shellfish).

Rationale: This underlines the need for simple, sensitive, fast, standardized and reliable methods for the identification of viral contamination to offer analytical solutions to the food industries. The developed methods must make it possible to the food industries to anticipate the future guidelines of the Codex Alimentarius concerning the problem of enteric viruses.

Results: Methods have been developed on the bases of the future standardized method under validation by the European Comittee of Normalization. Elution and concentration methods have been optimized for different types of food matrices. An extraction control, the mengo virus vMC0 was used to validate the method, check for inhibition and estimate an extraction efficiency. RNA extraction has been performed using the NucliSens reagents and the miniMag automate (bioMérieux). The detection of different virus, was made using ceeramTools™ detection kits. The developed methods were validated by different reference laboratories with identical performance criteria.

Since 2007, 700 food samples without relation to foodborne disease were submitted for analysis by several food industries from Europe. These samples gather shellfish, fruits and vegetables (fresh, frozen, dried, lyophilised, mashed, smoothies...), ready to eat food, delicatessen. Prevalence levels of 9.5% for NoV GI, 17.7% for NoV GII and 10% for HAV were found.

Conclusion: Using the developed methods, the integration of the viral risk in HACCP plan of food company can lead to a better managment of microbial food security and consummer protection. These data underline the need for a regulation concerning the viral risk in food.

T3-03   **The Relevance of Within-batch Sampling Distributions to Microbiological Criteria in Foodstuffs**

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Introduction: Traditionally, the sampling distribution of microorganisms within batches has been identified as lognormal indistinctively for bacteria present in high numbers as for those present in low numbers. Despite its alternative theoretical interpretations (whether a process is true contagious or simply heterogeneous), the negative binomial distribution has been recently shown to efficiently represent microbial data consisting of a large proportion of zero counts.

Rationale: This work investigates the relevance of the sampling distribution assumption and its effect on microbiological criteria with an application of *Escherichia coli* on pre-chill beef carcasses. The unknown heterogeneous concentration of *Escherichia coli* among carcasses (λ) was separately modelled as a lognormal and a gamma distribution, by fitting the plate counts data (n = 1354 consisting of ~40% zero counts) to the Poisson-lognormal and Poisson-gamma distributions, respectively.

Results: In all sampling batches and overall, the gamma distribution (mean 0.21; 95% CI: 0–1.55 CFU/cm^2; DIC = 4795) modelled the observed data significantly better than the fitted lognormal (mean 0.50; 95% CI: 0–3.04 CFU/cm^2; DIC = 4967), as the latter consistently predicted a lower proportion of zeros, with a higher skewness that overestimated the values of λ. Consequently, setting microbiological criteria with basis on the common lognormal assumption would result in a lower level of safety than expected. A hypothetical attributes sampling plan of n = 5, c = 0 operating under the lognormal assumption, would apparently distinguish batches of mean concentrations higher than 0.027 CFU/cm^2 at 5% risk, while in fact under the negative binomial assumption the level of safety is 0.098 CFU/cm^2.

Conclusion: Furthermore, it is expected that for lower microbial counts (higher proportion of zeros), the lognormal assumption would become more misleading, which is relevant to pathogens and certain hygiene indicators as contemplated by EC No. 2073/2005. Finally, this work discusses further on the need and implications of embracing more realistic assumptions such as bacterial clustering or aggregation and its relation to mean and sample size.

T3-04   Statistical Aspects of *Cronobacter sakazakii* in Powder Infant Formula

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Introduction: The microbiological criteria established in the EC 2073/2005 for *C. sakazakii* in PIF, are based on two-class attribute sampling plans, where the sample results are qualitative (sample indicates presence or absence) and the lot is rejected if any samples are positive.

Rationale: The performance of a sampling plan is revealed by its OC curve which plots the probability of acceptance against possible values of proportion defective. The objective of this study is to generate several OC curves assuming different distributions of *C. sakazakii* in PIF, in order to determine the probabilities of rejecting/accepting the lot and the respective level of contamination.

Results: The OC curves presented graphically are obtained by montecarlo simulation and results are compared. Assuming a lognormal distribution of the bacteria within the lot (standard deviation 0.8) we are 95% confident of a rejecting a lot with mean 0.048 CFU/g (1 CFU in 20 g). Instead, assuming a Poisson lognormal distribution, such that the bacteria is lognormally distributed within the lot, while the distribution in the sample follows a Poisson process, the mean decreases at 0.0161 CFU/g (1 CFU in 62 g). Extending the assumption of the lognormal distribution also between the lots, and simulating for several values of standard deviation for the between and within lot variability, confidence levels of rejecting the contaminated lot diminish drastically. Graphically are also compared several OC curves based on statistical distributions describing the clustering of the bacteria in PIF.

Conclusion: The statistical distribution of *C. sakazakii* in PIF and the sampling plan implemented play a crucial rule in determining the confidence level of rejecting a contaminated lot. Currently, work is being performed in order to determine the distribution of the bacteria in PIF and consequently estimate the correct level of contamination in the final product.

Poster Session 1 – Wednesday, 9 June

P1-01   Comparison of SimPlate *Campylobacter* ⁻ Color Indicator and Campy CEFEX Agar Methods for the Enumeration of *Campylobacter jejuni* and *Campylobacter coli* from Poultry Rinse Samples

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Introduction: *Campylobacter* continues to emerge as a food-borne pathogen of concern for poultry processors. Standard reference culture methods for the detection of *Campylobacter* require the use of multiple plates, lack specificity and require up to 5 days to obtain results.

Rationale: To compare the specificity of SimPlate *Campylobacter* Color Indicator (C-CI), Campy CEFEX Agar, Abeyta-Hunt-Bark (AHB) agar and Line Agar. To compare the recovery and enumeration of *Campylobacter* from poultry carcass rinses using SimPlate C-CI and Campy CEFEX Agar.

Results: There was good correlation for the enumeration of 37 different strains of *Campylobacter jejuni* and *Campylobacter coli* between the SimPlate C-CI and three selective agar media. Additionally, SimPlate C-CI did not detect any of the 27 non-*Campylobacter* organisms tested.

A total of 168 carcass rinse samples were analyzed via both the Campy CEFEX and SimPlate C-CI methods during a field trial at 3 poultry processing plants. Regression analysis comparing the results produced a slope and correlation coefficient of 0.91 and 0.96, respectively.

Conclusion: SimPlate C-CI was found to be comparable to the Campy CEFEX culture method for the detection and enumeration of *Campylobacter* from poultry rinse samples. SimPlate C-CI offers an advantage to food processors by providing results in 48 hours.

P1-02   Development of a Novel Method for the Detection of the Top 6 Non-O157 Seropathogenic Shiga Toxigenic *E. coli* Using Immunomagnetic Separation and DNA Amplification

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Introduction: Shiga toxigenic *E. coli* (STEC) are a risk to public health, as exemplified by *E. coli* O157:H7, which is well documented and routinely tested for in the food supply. However, there is increasing recognition that STEC of serotypes other than O157 may also represent a significant health threat. Currently, foods are not widely tested for non-O157 STEC and available testing protocols are very cumbersome. Our goal is to develop an accurate, rapid, and convenient testing method for the six most clinically significant non- O157:H7 STEC seropathotypes ("Top STEC") in food samples. This group includes pathogenic isolates of serotypes O145, O26, O103, O45, O111, and O121 which define seropathotype group B (Karmali, et al., 2003, J. Clinical Microbiology, 41:4930-4940).

Rationale: Our goal is to develop an accurate, rapid, and convenient testing method for the six most clinically significant non- O157:H7 STEC seropathotypes ("Top STEC") in food samples. This group includes pathogenic isolates of serotypes O145, O26, O103, O45, O111, and O121 which define seropathotype group B (Karmali, et al., 2003, J. Clinical Microbiology, 41:4930-4940).

Results: Assurance GDS correctly detected all 31 members of the top 6 target STEC serogroups and did not detect any potential cross reacting organisms tested. Assurance GDS correctly detected

59 of 60 positive 375 g beef trim samples inoculated at a level of 1.8 CFU per sample, demonstrating a 98.3% sensitivity level.

Conclusion: The Assurance GDS method can provide food processors with a fast, accurate and actionable results for the detection of non-O157 pathogenic STEC in food samples.

P1-03 Persistence and Thermal Adaptation of Vancomycin Resistant *Enterococci* during Composting

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Introduction: Enterococci are well-known for either their intrinsic ruggedness or having acquired resistance with relative ease to many antibiotics which are essential for maintaining public health. Vancomycin resistant enterococci (VRE) have played a major part in causing nosocomial illnesses.

Rationale: This study determined if VRE can survive the composting process in a field setting by acquiring the thermal resistance.

Results: In this study, two dairy compost heaps and one vegetable scrap compost heap were constructed on two research farms in Clemson, SC. Samples were taken from each heap from different locations and analyzed for enterococci and VRE counts by spread plating on Bile Esculin agar (BEA) and BEA containing 6 µg/ml of vancomycin, respectively. The initial populations of enterococci and VRE in compost were in the range of 6.46–7.43 and 5.36–6.70 logs CFU/g, respectively. After 30 days of active composting, the average VRE populations declined ca. 4.6, 4.07, 3.97 and 1.6 logs at the top, center, bottom and surface locations of the heaps, respectively, whereas the enterococci populations declined for 4.36, 3.54, 3.10, and 2.28 logs, respectively. During the compost trials, the temperatures at the top, center, and bottom locations were in excess of 60°C for 10–14, 8, and 4 days, respectively, whereas temperatures of the surface samples never exceeded 32°C. A total of 88 VRE isolates was picked and identified to species level. All VRE isolates were confirmed to contain the *vanA* gene for vancomycin resistance. The D-values of selected VRE isolates (n = 8) from Trial 1 compost heaps were in the range of 9.7-17.73 min at 60°C, 4.73–12.57 min at 65°C, and 1.59–4.44 min at 70°C. The z-values for those VRE isolates ranged from 11.92–7.87°C. The results revealed that the isolates from day 30 had the highest D-values while isolates from day 60 had the highest z-value, as compared with isolates from day 0 which had the lowest D- and z-values. Box-PCR analysis of VRE isolates (n = 12) from Trial 1 revealed that 42% were identical and appeared on compost days 7, 14, and 60, suggesting that this specific strain was able to adapt to the elevated temperatures in compost by developing heat resistance.

Conclusion: Our studies demonstrated that VRE can be inactivated during composting process but some strains may develop heat resistance during the thermophilic phase of composting. This is important because it suggests that improperly composted manures may serve as a means for the spread of VRE on food products intended for human consumption.

P1-04 Pre-limit of Detection Pooling Strategy for the Detection of *E. coli* O157:H7 in Beef Trim

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Introduction: *E. coli* O157:H7 is a foodborne pathogen sometimes found in raw beef and produce that can cause serious, sometimes fatal, illness at a very low infectious dose (as few as 10

organisms). Since culture-based methods can be difficult and time-consuming, and since the organism is difficult to isolate when in the presence of an excess of competing flora, well validated rapid methods for the detection of this pathogen are needed. Pooling strategies for this organism are needed to increase the volume of material tested without significantly increasing testing costs. These strategies must not decrease sensitivity for the target pathogen.

Rationale: In order to ensure that the target pathogen is enriched to a detectable level, the following strategy was employed:
The principal elements of the method are:
1) Pooling before the target organism reliably reaches a detectable level.
2) Addition of extra nutrition with shaking to ensure the targets exceed the level that will allow for detection by PCR.
3) Continuing to incubate the un-pooled enrichments so that if the target is present in the pooled sample, each individual lot's enrichment can be screened to determine where the target originated.

Results: A commercial pcr test kit was used to demonstrate effectiveness of the method, followed by culture confirmation of all presumptive negative samples. Spiked 375 g beef trim samples were enriched in 1500 ml of pre-warmed BAX® MP test media. Testing using this method on beef trim that was artificially inoculated with a low level of *E. coli* O157:H7 (~1.5 CFU/375 g sample) demonstrated that the pooled method following 7 hours of enrichment un-pooled plus 4 hours of pooled enrichment with shaking and added nutrients gave identical performance to the corresponding results where the samples were enriched without pooling for 10 hours. Additionally all negative controls tested negative, and all spiked pcr negative enrichments tested negative using the USDA-FSIS culture method.

Conclusion: This pooling method demonstrates equivalent sensitivity to an unpooled method of detection. Further, the method demonstrated sensitivity at or near a single cell limit of detection for the target pathogen on 375 g and 5 × 375 g (1875 g) sample sizes.

P1-05 ***Listeria monocytogenes* in the Irish Beef Chain – A Quantitative and Epidemioloical Study**

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Introduction: Although numerous studies have investigated the prevalence of *Listeria* spp. in food, few have quantitatively tracked clinically significant serogroups of *L. monocytogenes* through the beef chain.

Rationale: In order to determine the prevalence of clinically significant *L. monocytogenes* serotypes in the Irish beef chain, a quantitative survey was carried out. Bovine hides (n = 400), carcasses (n = 400), ground beef (n = 100) and ready-to-eat beef products (n = 200) were examined and epidemiological tools were used to identify potential routes of transmission. In addition, virulence and antimicrobial resistance profiles were generated to establish the potential public health threat of the recovered isolates.

Results: *L. monocytogenes* was isolated from 26%, 14% and 19% of bovine hides, carcasses and ground beef at levels of 0.25 –150 CFU/cm^2 and 0.5 – 200 CFU/g^{-1} respectively. Ready-to-eat products were negative for *L. monocytogenes* populations. The 1/2a, 3a serogroup was the most commonly encountered serogroup in both animal and meat samples. The gene imo2821, associated exclusively with virulent strains of *L. monocytogenes* was detected in 65% and 70% of meat and animal isolates respectively. PFGE profiling demonstrated that direct contamination during the dehiding process did not play a major role in carcass contamination and transmission was more likely to occur between animals sequentially on the slaughter line. Based on a relatedness criterion of 80%, a high level of genetic diversity was observed between isolates of meat and animal origin. Antibiotic profiling identified several isolates with multi-resistance to 3 or

more antibiotics, however 55% of isolates tested were sensitive to all clinically relevant drugs tested.

Conclusion: Despite a high prevalence in beef cattle, quantitative analysis showed that < 1% of Irish beef contained *L. monocytogenes* above the critical limit of 100 CFU g^{-1}. However, the presence of virulent, multi-antibiotic-resistant strains highlight the need for vigilant retail and domestic hygiene and thorough cooking of ground beef products.

P1-06   Withdrawn

P1-07   BIOTRACER: Outputs from an EU-funded 6th Framework Integrated Project

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Introduction: BIOTRACER is an Integrated Project funded by the EU 6th Framework Program. The work is planned for 4 years and is organized in 5 Research Areas. The practical work is organised into 5 food-chain areas, covering tracing and tracking of contamination in feed, meat and dairy chains, in addition to accidental and deliberate contamination of bottled water. The BIOTRACER Consortium consists of 47 partners, including Europe's largest food/feed industries, several SMEs, and relevant INCO countries. The Consortium includes experts in predictive microbiology, database developers, software companies, risk assessors, risk managers, system biologists, food and molecular microbiologists, legislative officers, standardization and validation members and food retailers.

Rationale: Using a total food chain approach, BIOTRACER aims to develop recommendations to control food safety risk through integration of novel genomic and metabolomic data resulting in a better understanding of the physiology of the microorganisms, combining these with advances in predictive food-based microbiological models.

Results: The outputs to date contribute to the novel concept of biotracing, which can be defined as the ability to use downstream information to point to materials, processes or actions within a particular food chain that can be identified as the source of undesireable agents. These outputs include: development in detection technologies for *Salmonella*, *Bacillus*, *Clostridium*, *Norovirus* and *Campylobacter*; improved extraction technology for *S. aureus*; statistical based sampling strategies and innovative sampling technology for *Campylobacter*, *B. cereus* and *B. anthracis*; mathematical modelling approaches and models developed, such as modular process risk modelling for *Salmonella* and domain models for *S. aureus* in milk and *Salmonella* in pork; incorporation of field sampling data into mathematical models; improved understanding of pathogen physiology and of immobilised cells.

Conclusion: The results of the project will ensure a more reliable and rapid response to a contamination event.

P1-08   The Effectiveness of Food Industry Biocides against *Salmonella* and *Cronobacter*

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Introduction: Biocides play an essential role in limiting the spread of food-borne disease. However, concern is growing over the risk of emerging biocide resistant bacteria and the potential for selecting bacteria displaying a cross resistance to antibiotics. In this study we investigated the susceptibility of 150 *Salmonella* and 90 *Cronobacter* strains to eight biocides currently used in the food industry.

Rationale: This study investigated the relative susceptibility of *Salmonella* and *Cronobacter* isolates to biocides currently used in the food industry and the effectiveness of these biocides against planktonic, biofilm and surface dried cells. Antibiotic susceptibility profiles against 15 antibiotics were determined for all strains to investigate whether there was a correlation between biocide resistance and antibiotic resistance. The bacteriocidal or bacteriostatic nature and stability of the biocides was also assessed.

Results: A total of 7/8 biocides were highly effective in killing all bacterial strains in a planktonic state. All eight biocides showed significantly decreased activity against surface dried and biofilm cells; three were ineffective against biofilms and two against surface dried cells. The biocide susceptibility varied considerably amongst the *Salmonella* and between the *Salmonella* and *Cronobacter*. There was little variation amongst the *Cronobacter* strains. There was no correlation between biocide and antibiotic resistance. All biocides were bacteriocidal in action and retained this bacteriocidal activity over a period of 8 days without the presence of organic matter. All but one of the biocides retained their activity in the presence of organic matter.

Conclusion: All but one of the biocides were effective at killing planktonic *Salmonella* and *Cronobacter* strains. However, activity was reduced, or ineffective, against biofilm and surface dried cells. This has implications for the effectiveness of biocides in factory settings, for the reliability of some biocide assays, and for the potential mutation of pathogens exposed to sub-lethal concentrations of biocides.

P1-09   **Ozone Inactivation of Acid-stressed *Listeria monocytogenes* and *Listeria innocua* in Orange Juice**

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Introduction: *Listeria monocytogenes* is more resistant than many food borne pathogens to organic acids and can be difficult to control in food processing facilities. It has been isolated from unpasteurized juice blends although no outbreaks involving this pathogen in fruit juices have been reported.

Rationale: Thermal pasteurization of orange juice can cause degradation of the product's quality. Consumers tend to prefer recently extracted fresh juices with fresh taste and minimal flavor or vitamin losses. The FDA's approval of ozone as a direct additive to food in 2001 triggered interest in ozone applications development.

The objectives of this study were to investigate quantitatively (i) the efficacy of gaseous ozone treatment at a concentration of 72–75 μg/mL for reduction of *L. monocytogenes* (ATCC 7644 and NCTC 11994) and *L. innocua* NCTC 11288 (10^6 CFU/ml) at ambient temperature in orange juice, using an ozone bubble column (ii) ozone treatment efficacy in orange juice inoculated with the acid stressed *Listeria* population using acid stress conditions namely; mild acid stressed, mild acid stress-habituated and acid stressed cells habituated in orange juice.

Results: Ozone treatment of mild acid stressed and mild acid stress-habituated (pH 5.5) cells of *L. monocytogenes* resulted in higher inactivation times compared to control non-acid stressed cells. Additionally acid stressed cells habituated in orange juice (ATCC 7644 & NCTC 11288), showed higher inactivation times during ozonation by comparison with the control as well as the mild-acid stressed cells.

Conclusion: The efficacy of ozone treatment was found to be a function of strain and duration of acid stress-habituation conditions. Direct ozone diffusion treatment could be used as a potential alternative to traditional thermal pasteurisation for control of *Listeria* populations in fruit juices or other liquid foods.

P1-10   Presence of *Listeria* along the Gorgonzola PDO Production Chain

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Introduction: *L. monocytogenes* represents a concern in the production of Gorgonzola PDO cheese. The ubiquitous nature of the pathogen and its adaptability pose a risk for this production.

Rationale: The aim of this study was to investigate the presence of genus *Listeria* in the Gorgonzola production chain, in order to identify dissemination pathways. For this reason one producer and all the conferring farms (N = 20) were selected.

A total of 200 samples were collected in four visits (May 2008 –October 2009), divided into: milk from each farm (N = 20), milk from receiving tanks (N = 3), and sponges from equipment, environments and surfaces (N = 27).

Samples were processed using ISO 11290-1 method. Suspected *L. monocytogenes* colonies were identified through specific PCR, whereas the others were challenged with 16S DNA PCR and then sequenced.

Results: *Listeria innocua* was identified in 3 samples of farm milk. *Listeria* spp. and *L. monocytogenes* were retrieved from 20 sponges out of 108 (19%). *L. monocytogenes* alone was identified in 7 samples, *L. innocua* in 12 and both were identified in one sample. Contaminated sponges were retrieved from stewing, ripening, perforating and salting equipments, carts, sink and cutting blade. In 5 surfaces/equipments contamination was recurrent.

Conclusion: Noteworthy, low levels of contamination were detected in milk samples prior to pasteurisation. Contrarily, surfaces and equipment in contact with ripening cheese were frequently contaminated. The finding of contaminated salting and perforing equipments, as well as moving carts may contribute to dissemination. The detection of *Listeria* in the same site on different visits should be looked at carefully, as it may indicate the presence of persistent strains adapted to specific niches.

Nonetheless, considering that rinds are not edible according to EU legislation, the presence of the pathogen on cheese rinds cannot be considered a risk for the consumer.

P1-11   Distribution and Virulence Profile of Farm Verocytotoxigenic *Escherichia coli* (VTEC) Isolates

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Introduction: Over 380 different VTEC strains causing human illness have been identified. The majority of human disease is associated with O157, O111, O26, O145 and O103. Hemolytic colitis and hemolytic uremic syndrome are generally associated with VTEC that produce attaching and effacing lesions. However there are at least 39 other virulence factors associated with clinical illness in humans. Ruminants are the primary source of clinically significant VTEC.

Rationale: There is a dearth of information on the range of serotypes on farms and their virulence profiles. The objective of this study was to identify sources, serotypes, dissemination patterns and virulence profiles of VTEC on farms in the same water catchment area using culture based, PFGE and PCR analytical techniques.

Results: All of the 12 farms tested were VTEC positive and many had multiple serotypes. O157:H7 was the predominant serotype and strains were undistinguishable by PFGE. O157:H7, O157:H16, O26:H11, O76:H34 and O3:H12 had vt1 and or vt2 and the *eaeA* gene, while O-:H11, O-:H8 and O113:H36 had a verocytotoxin encoding gene and an alterative adhesin factor (saa).

Conclusion: O157:H7 is common on the farms in this study and has been disseminated over long distances between closed farms that have no obvious contact. O76:H34, O3:H12, O-:H11, O-:H18 and O113:H36 are potentially clinically significant and may emerge in future clinical cases. Farm workers and visitors should be warned of the presence of the pathogen and necessary hygiene practices/facilities put in place.

P1-12   **Prevalence of *Campylobacter* spp. on Chicken Packaging and Retail Display Cabinets**

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Introduction: Campylobacteriosis is the most common bacterial cause of gastroenteritis in the Republic of Ireland and internationally chicken meat is recognised as a major source of campylobacteriosis.

Rationale: The purpose of this national survey was to determine: 1) the prevalence of *Campylobacter* spp. on (a) the external surface of chicken packaging and (b) the surface of retail display cabinet shelves; and 2) to establish whether handling and cooking instructions deviate from accepted best practice.

Results: Seven hundred eighty-five paired samples were taken by environmental health officers from retail establishments in the Republic of Ireland between September and December 2008 and were analysed in the food microbiology laboratories of the Health Service Executive (HSE).

Campylobacter spp. were detected on 13.2% (104/785) of the external surface of packaging and 10.9% (86/785) of the surface of display cabinets.

The study included a questionnaire which captured information on the sample (e.g. source, packaging type, etc.) and had a response rate of 75% (590/785). The type of packaging was shown to be important, whereby contamination was more prevalent on the exterior of conventional packaging (18.9%; 68/361) than on leak-proof packaging (2.1% 4/189). Conventional packaging is where the plastic is wrapped around the tray and sealed underneath, while in leak-proof packaging the plastic is sealed directly onto the tray. The contamination detected on the display cabinet which was in contact with the packaging and the evidence of leakage reported to be visible on that display cabinet further supported this finding.

This study also found that handling and cooking instructions (through location and content) on some of the packaging had the potential to encourage risky food-handling practices among consumers.

Conclusion: Irish retailers should change to sourcing chicken in leak-proof packaging and ensure that handling instructions do not encourage risky consumer practices.

P1-13   The Potential for Biocide Resistance in Verocytotoxigenic *E. coli*

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Introduction: Biocides are deployed at all stages of the farm to fork chain to eliminate pathogenic microrganisms. The use of biocides to control pathogens has increased significantly, yet there is less understanding of biocide resistance. An increase in biocide resistance would be of concern as it could contribute to increased persistence of foodborne pathogens and subsequent human exposure to such agents.

Rationale: The purpose of this study is to establish the level of resistance exhibited by verocytotoxigenic *E. coli* to commercial biocides and biocidal components and to establish the ability of VTEC to acquire resistance to these agents. A micro-dilution method was used to determine biocide MIC in a micro-titre plate assay. Subculturing in stepwise increases in biocide concentrations was used for the isolation of potential biocide resistant mutants.

Results: MICs expressed as a percentage of the manufacturer's recommended working concentration were found to range from 10 to 50% for 2 of the 8 commercial biocides, while the MIC for the other 6 was 0.39% to 6.25%. The biocidal component MICs ranged from 0.01 mg/L to 12.5 mg/L dependent on the component. 6 strains were isolated with increased MICs for 3 commercial biocides but this increase wasn't stable. In the case of triclosan mutants were more readily isolated with growth at 1000 mg/L observed.

Conclusion: In the majority of cases biocide MICs were found to be less than the manufacturer's recommended in use concentration and isolates with a stable resistance to the agents were difficult to isolate. Nonetheless, VTEC showed significant tolerance to triclosan, a commonly used agent in consumer products, indicating the potential for resistant bacterial populations to develop where such an agent is relied upon

P1-14   Uncertainty of *Listeria monocytogenes* Plate Count through Statistical Process Control (SPC) Charting

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Introduction: *Listeria monocytogenes* is a foodborne pathogen often associated with ready-to-eat (RTE) foods that can support its growth. EU Reg. 2073/2005 states that RTE foods containing <100 CFU/g and that do not support the growth of this organism are considered low risk. Nevertheless, microbiological results of *L. monocytogenes* plate count must be appropriately interpreted considering the uncertainty associated with them.

Rationale: Statistical Process Control (SPC) charting was used to compare enumeration methods for *L. monocytogenes* using quantified lyophilized pellets to inoculate 10 ml of ultra high temperature (UHT) milk. The inoculated samples were allowed to resuscitate for 1 h in 90 ml of buffered peptone water and enumerated using the ISO 11290-2: 2005 method and the Rapid L.

mono (RLM) BioRad protocol as outlined in the manufacturer's instructions. Colony forming units per gram were determined after 24 to 48 h of incubation at 37°C. Trials were conducted at seven European laboratories using three different lots of reference material.

Results: Mean values of log (CFU/g) recovered for the three lots and each lot were determined for both methods. The ISO method showed a consistently higher recovery level of *L. monocytogenes* after 24 h of incubation: 0.29 log (CFU/g) across the three lots, and 0.29, 0.23 and 0.49 log (CFU/g), respectively, for lots 1, 2 and 3. Method-associated variation was also determined for each lot as defined by the difference between the upper and lower control limits. Variation between the two methods was overall smaller in the ISO method, 0.09 log (CFU/g) across lots; 0.04 log (CFU/g) for lot 1 and 0.23 log (CFU/g) for lot 3; while RLM showed a smaller variation in lot 2 (0.12 log (CFU/g)).

Conclusion: Differences in overall recovery and variation of *L. monocytogenes* enumeration methods are not well defined. Nonetheless, SPC charting has already provided preliminary information that can be used to determine method uncertainty to better interpret results given the low target level of <100 CFU/g of *L. monocytogenes* in certain RTE foods.

P1-15 Effect of the Concentration of Nitrites and Nitrates on the Microbiological Quality of Dry Fermented Sausages Inocculated with *Listeria*

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Introduction: Nitrites and nitrates are typical additives for the production of cured meats. They contribute to the colour and flavour of these products and exert antioxidant and antimicrobial effects, related to the inhibition of spoilage and pathogenic bacteria such as *Clostridium botulinum*. Despite of these beneficial activities, there is great concern regarding the role of these additives in the formation of nitrosamines, which carcinogenic, mutagenic and teratogenic effects are well documented.

Rationale: This is a study on the effect of different concentrations of nitrites and nitrates on the typical microbiota and the inactivation of *Listeria* in dry fermented sausages. For this purpose, Spanish "salchichón" type sausages were manufactured with combinations of sodium nitrite and sodium nitrate of 150 (at present the maximum amount permitted by the European Union for this kind of products with the exception of certain traditional varieties), 112.5 and 75 ppm each. Control sausages without nitrites and nitrates were also prepared. "Salchichón" was inoculated (10^5 CFU/g) with *Listeria innocua*, as surrogate for *Listeria monocytogenes*, and ripened for 30 days.

Results: In comparison with the sausages added with the maximum levels of nitrites and nitrates, the final numbers of Gram positive catalase positive cocci were 1 and 2 log CFU/g higher in the products manufactured with 75 ppm, and in the control sausages respectively. *Enterobacteriaceae* and *L. innocua* counts were also higher, approximately 1 log CFU/g in the sausages with 75 and 112.5 ppm, and 2 log CFU/g in the control ones.

Conclusion: The concentration of nitrites and nitrates affects the microbiological quality of dry fermented sausages. This should be taken into account in view of a future reduction of the amounts of these curing additives authorised by the European regulations.

P1-16   Efficacy of Pulsed Light for the Inactivation of *Escherichia coli* 157:H7 on Beef Carpaccio

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Introduction: The demand of minimally processed foods has greatly increased over the last few years. This category includes, among other foods, meat products that are eaten raw, such as beef *carpaccio*. The consumption of raw meat products may pose a microbiological safety risk, since processing operations may allow pathogenic microorganisms to colonize the surface and reach infective doses along shelf life. Pulsed light (PL), a non thermal technology for surface decontamination, could be a useful approach for ensuring the safety of these products. It consists in the application of short (10^{-3}–10^2 milliseconds) pulses of an intense broad-spectrum light (200-1000 nm), being the UV-C component the main responsible for microbial inactivation.

Rationale: The aim of this work was to study the capability of PL to inactivate *Escherichia coli* O157:H7 on beef *carpaccio*. For this purpose, meat slices were superficially contaminated up to 10^3 CFU/cm^2, vacuum packaged and PL treated with increasing fluences from 0 to 8.4 J/cm^2.

Results: The level of inactivation achieved by PL treatment ranged from 0.6 to 1.2 log CFU/cm^2. All the fluences assayed significantly ($P < 0.05$) modified the colour parameters of meat at a different extent. Samples treated at 4.2 J/cm^2, in which an inactivation of 1 log cfu/cm^2 was obtained, only showed differences in redness when compared to control slices.

Conclusion: PL treatment gave a reasonable level of inactivation of *E. coli* O157:H7 on the surface of meat, although to increase the safety of raw meat products, PL should be combined with other technologies, such as the addition of biopreservatives.

P1-17   Evaluation of a Real-time PCR Method to Detect *Salmonella* in the Poultry Meat, Down and Faeces Matrices

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Introduction: Traditional *Salmonella* culture methods are labor intensive and require days before to get results. In the context of the Dutch *Salmonella* and *Campylobacter* control program in the poultry sector, the performances of the iQ-Check *Salmonella* II alternative method were compared to those of the MSRV/XLD branch method recommended by the Dutch Production Boards for Livestock, Meat and Eggs (PVE).

Rationale: The objective of this study was to determine if this real-time PCR method can be used as a reliable alternative to the PVE - branch method for *Salmonella* detection in the following poultry matrices: meat, down and faeces.

Two independent labs performed the comparison study on a total of three hundred seventy-four samples, with at least 120 samples (including 40 positive samples) per matrix.

Results: Relative specificity, sensitivity and accuracy, were found to be over 95%.

Conclusion: This study demonstrated that the iQ-Check *Salmonella* II method can be used as an alternative to the classical MSRV reference culture method, in the poultry primary production sector.

P1-18   ***Listeria monocytogenes* in Ready-to-Eat Foods for Sale in the Republic of Ireland**

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Introduction: The presence of *Listeria monocytogenes* (LM) ready-to-eat (RTE) foods raises concern as it can grow at refrigeration temperatures. For most RTE foods, Commission Regulation 2073/2005 on microbiological criteria for foodstuffs requires LM at levels.

Rationale: Since 2001, the Food Safety Authority of Ireland, in conjunction with the official agencies (Health Service Executive and Dept. of Agriculture, Fisheries and Food) conducted 17 surveys to investigate the safety of RTE foods on sale in the Republic of Ireland with respect to LM.

Results: Qualitative tests showed that LM was present in 2.6% (147/5,686) of RTE food samples tested between 2001 and 2009. LM was detected in prepacked sandwiches (11%; 52/475), precut fresh fruit and vegetables (4.1%; 21/513), sprouted seeds (3.8%; 1/26), prepacked mixed salads (2.7%; 19/714), fermented meat (2.6%; 20/757), mushrooms (1.1%; 8/719) and prepacked cooked sliced ham (1/618), but was not detected in any of 67 unpasteurised fruit or vegetable juice samples tested qualitatively. Although LM was not detected in any of the 410 samples of cheese made from raw, thermized or pasteurized milk sampled at processing level; at retail level, it was detected in 1.1% (10/880) of cheese made with pasteurized milk and 3% (15/507) of cheese made with raw or thermized milk.

Overall quantitative testing was performed on 8,484 RTE food samples. Ten (0.1%) were judged unsatisfactory when assessed against the food safety criteria in Commission Regulation 2073/2005.

Conclusion: 99.9% of RTE food samples were satisfactory. When an unsatisfactory result was detected, follow-up action was taken ranging, as appropriate, from inspecting premises, analyzing follow-up samples or initiating a product recall.

P1-19   **Chemical Decontamination of Poultry Carcasses**

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Introduction: *Campylobacter* is the most common cause of gastroenteritis in developed countries. Several studies have demonstrated the effectiveness of chemical decontamination of broiler carcasses to reduce *Campylobacter*. Although applied in the USA these are currently outlawed by the European Commission. However approval for use in Europe is likely in short to medium term.

Rationale: The objective of this study was to investigate the effect of different chemical decontaminants on the microbiological status of broiler carcases immediately and over a 2-week period in chilled storage using culture based analytical techniques.

Results: At concentrations of 10%, 1%, 1%, 100 ppm and 500 ppm, tri-sodium phosphate (TSP), lactic acid, citric acid, peroxyacids and acidified sodium chlorite, achieved 1.45, 0.89, 0.87, 0.86 and 1.07 $\log_{10}$ per square cm reductions in *Campylobacter* were obtained, respectively. Over two-weeks storage *Campylobacter* levels decreased from approximately 6 $\log_{10}$ per square cm to 2.5 $\log_{10}$ per square cm regardless of treatment. While a small immediate reduction in total viable counts (TVC) (mesophiles), TVC (psychrophiles), total *Enterobacteriaceae* counts (TEC), lactic

acid bacteria (LAB), *Pseudomonas*, yeasts ands molds was observed, these increased consistently during storage.

Conclusion: At the concentrations tested TSP was the most effective broiler carcass decontaminant and its application would reduce the risk of campylobacteriosis to consumers. Interestingly none of the decontaminants improved shelf life.

P1-20   Flow Cytometric Comparison of Gentle Treatment Techniques

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Introduction: Decontamination of fresh produce requires gentle treatment techniques that reduces microbial load and maintain product quality. Chemical disinfection such as peracetic acid and ozone treatment is applied to fresh produce with varying success. Non-thermal plasma treatment is of grown interest in food microbiology but its potential regarding mild food surface decontamination is not fully investigated yet.

Rationale: The objective of this study was to compare the inactivation effects of peracetic acid, ozone and non-thermal plasma using flow cytometric methods and to derive relevant process parameters for surface decontamination of fresh produce.

Results: The inactivation of *E. coli* was measured by conventional plate count method with a detection limit of 101 CFU ml-1 for peracetic acid (PAA) and ozone (O3) and 102 CFU ml-1 for plasma. Membrane integrity, esterase activity, pump activity, and membrane potential of the bacteria cells was measured by flow cytometry. *E. coli* cells were completely depolarized after treatment (15 s) with 0.25% PAA at 10°C, and after treatment (10 s) with 2.8 mg l-1 O3 at ~ 10° C. The membrane potential of plasma treated cells remained almost constant at 20 W over a time period of 3 min, and subsequently decreased within 30 s of further treatment. Complete membrane permeabilization was observed after 10 s O3 treatment, but treatment with PAA and plasma did not completely permeabilize the cells within 2 min and 4 min, respectively. Similar results were obtained for esterase activity. O3 inactivates cellular esterase but esterase activity was detected after 4 min plasma treatment and 2 min PAA treatment.

Conclusion: Flow cytometry enables to monitor and to differentiate inactivation effects of gentle treatment techniques. When compared to PAA and ozone treatment, plasma application allows better and controlled handling.

P1-21   Molecular and Phenotypic Characterization of *Salmonella* from Infant Foods

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Introduction: As powdered infant food is a non-sterile product, it is susceptible to contamination at many stages of production. There have been a number of outbreaks of salmonellosis in infants over the past several years that have been linked to contaminated powdered infant formula. In 1985 *Salmonella ealing* caused a major outbreak in the United Kingdom which was linked back to one brand of infant formula. Also in the years 1996–1997 there was an outbreak due to *Salmonella anatum* in both the United Kingdom and France.

Rationale: Various isolates from the *Salmonella ealing* and *Salmonella anatum* outbreaks were subjected to molecular subtyping by pulsed-field gel electrophoresis in comparison with unrelated

strains. Physiological and virulence characteristics of these *Salmonella* isolates were examined to determine factors that may have contributed to increased risk along the food chain.

Results: Molecular subtyping by pulsed-field gel electrophoresis in comparison with unrelated strains and indicated clonality within the two outbreaks. Growth rates in IFM, D60-values, and survival at low pH indicate ability to proliferate in reconstituted formula and survive ingestion. Other virulence characteristics include the detection of SPI-1 in the isolates; attachment and invasion in CaCo-2 cells and persistence in J774 macrophages.

Conclusion: This study identified a number physiological and virulence factors that could have aided these particular *Salmonella* isolates in causing serious infection in infants during major outbreaks of salmonellosis from milk powder.

P1-22 **Investigation of Potential Improvements to the Proposed ISO Standard for Detection of *Cronobacter***

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Introduction: *Cronobacter* belong to the *Enterobacteriaceae* family. These bacteria are found widely in the environment however they can also contaminate powdered infant formula (PIF). *Cronobacter* may only be present in low numbers in PIF (< 1 CFU/ml), but can cause meningitis, necrotizing *enterocolitis* and *bacteraemia* in neonates.

Rationale: The International Organization for Standardization (ISO) horizontal method for detection of *Cronobacter* in foods, such as powdered infant formula, is currently being developed. This study examined possible improvements to different stages of the proposed method including pre-enrichment, chromogenic agar. Biochemical and molecular identification techniques were assessed.

Results: Buffered peptone water (BPW) is commonly used as a pre-enrichment to recover stressed Gram-negative cells however background flora can affect the growth of *Cronobacter*. For 4/10 strains recovery of stressed cells was 80–100% the same with supplements added to BPW; for 4/10 recovery was greater using growth supplements; and for 2/10 recovery was

Conclusion: During pre-enrichment *Cronobacter* can be outgrown by Gram positive species which may make detection difficult. Addition of supplements to BPW could improve *Cronobacter* isolation by inhibiting the growth of Gram positive organisms, leading to a more sensitive detection method. Also the newly proposed species-specific PCR may provide a quick, useful method in identifying any isolated *Cronobacter*.

P1-23 **Examination of the Internalization of *Escherichia coli* O157:H7 in Mung Bean (*Vigna radiate*), Following Seed Contamination**

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Introduction: *E. coli* O157:H7 has been associated with numerous outbreaks involving fresh produce in recent years, which has resulted in significant research efforts aimed at methods to prevent outbreaks from occurring, yet the problem still persists. Previous studies have shown that bacteria can be internalized into plant tissue and that this may act as a source of protection from

sanitizers and antimicrobial chemicals, as well as various environmental conditions. The internal plant tissue may also serve as a carbon source for bacterial growth.

Rationale: The techniques used to examine internalization have not addressed the types of tissue and cellular locations the bacteria occupy in the plant. In this study, immunocytochemical techniques were used to localize the bacteria following contamination. Mung bean seeds were soaked with *E. coli* O157:H7 expressing GFP. Stem tissue was sampled following germination and it was fixed, embedded in paraffin, and serially sectioned. Immunocytochemistry was then performed and the tissue examined using epi-fluorescence microscopy to determine the location of each internalized bacterium.

Results: An average of 13 bacteria/mm^3 were localized in the sampled stem tissue following contamination of intact seeds. The bacteria were found to be associated with every major tissue and corresponding cell type (cortex, phloem, xylem, epidermis, pith), with the cortical cells located to the outside of the vascular bundles containing the majority of bacteria (61%). The bacteria were primarily located in the apoplast between the plant cells, and not within the cells themselves. Growth experiments also demonstrated mung bean plants could support high numbers of bacteria (10^7 CFU/plant) over a 12 day period.

Conclusion: Together these results show that the bacteria are able to be internalized in many different tissue types following seed contamination and that the bacteria are able to persist within the plant.

P1-24   Withdrawn

P1-25   Efficacy of High Intensity Pulsed Light for the Microbiological Decontamination of Packaging Materials and Contact Surfaces Associated with Chicken

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Introduction: The external and internal packaging surfaces of chicken have frequently been shown to be contaminated with pathogens including *Campylobacter, Salmonella* and *Escherichia coli*. These bacteria are recognised as the most frequent causes of bacterial foodborne gastroenteritis worldwide. Contamination of external packaging with these organisms pose a potential opportunity for cross-contamination of surfaces and other foods in retail premises and consumers homes.

Rationale: The aim of this study was to investigate the effectiveness of high intensity light pulses (HILP) for the decontamination of packaging materials and contact surfaces. HILP is an emerging technology that has been shown to be highly effective against a wide range of pathogenic microorganisms. This technology was applied at various doses ranging from $0.9 - 6.0$ J/cm^2 to a number of packaging materials and contact surfaces commonly encountered along the chicken processing line. Packaging materials examined included polypropylene, polystyrene and aluminum trays, polyethylene-polypropylene, polyvinyl chloride and polyolefin films. Contact surfaces examined included stainless steel and a polyethylene cutting board.

Results: Reductions of 3.56, 4.69 and 4.60 $\log_{10}$ CFU/cm^2 were obtained following HILP treatment of *C. jejuni, E. coli* and *S. Enteritidis* inoculated onto packaging materials and contact surfaces respectively.

Conclusion: Preliminary findings indicate that HILP could be applied as a rapid surface decontamination technology for the control of pathogenic organisms associated with packaging materials and contact surfaces.

P1-26   **The Effect of Nutrient Starvation on the Resistance of *E. coli* O157:H7 to Chemical Sanitisers and Refrigeration Hurdles**

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Introduction: *Escherichia coli* O157:H7 is a zoonotic pathogen that is transmitted to humans from ruminant animals via direct animal contact, untreated water or foods, notably salad vegetables and ground meat. Ground meat is usually manufactured from beef or veal. The prevalence of *E. coli* O157:H7 on beef carcasses in New Zealand is extremely rare, however low numbers have been detected on very-young (<10 day old) veal carcasses. This necessitates the use of antimicrobial interventions to decontaminate veal carcasses.

Rationale: The most likely source of carcass contamination is either directly or indirectly from the hide, with the primary source being faeces. Bacteria located on animal hide for any length of time are likely to be in a stressed state as this is likely to be a low-nutrient, temperature-variable, water-limited environment. In recent trials using bacteria inoculated onto veal carcasses and sprayed with acidified sodium chlorite (ASC), we have shown that there is a significant reduction in the effectiveness of ASC on nutrient-starved cells compared to metabolically active cells. To determine how this finding affects the survival of the bacteria on veal under different manufacturing conditions, nutrient-starved *E. coli* O157:H7 were inoculated onto veal carcasses and subjected to three distinct processes.

Results: When the veal was hot-boned, numbers increased marginally following exposure to a freezing curve simulating meat at the centre of a 27 kg box after three weeks storage. If the carcasses were treated with ASC prior to boning and freezing, a reduction of approximately 1 log10 CFU/cm² was observed. Reductions in excess of 3 log10 CFU/cm² were observed when the carcasses were first treated with ASC sprays, and then chilled overnight to 2°C, boned out, and finally the meat was frozen.

Conclusion: There is a significant reduction in the effectiveness of ASC on nutrient-starved cells compared to metabolically active cells. Significant reductions in pathogen load can however still be achieved, if ASC treatment is combined with freezing or chilling and freezing.

P1-27   **Increased Resistance to Biocides of *Salmonella* Typhimurium Grown in Presence of Plant-derived Antimicrobials**

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Introduction: Addition of preservatives is one of the most frequent approaches to enhance microbiological safety of food products. In recent years, consumer-driven demands have arisen for the provision of natural foods with minimal processing while maintaining the control of food safety. In that context, aromatic plants can provide essential oils and terpenes compounds which can be used as natural antimicrobials. Nevertheless, more information is needed about the ability of pathogenic bacteria to adapt to these antimicrobials before using them in food systems. Actually, it is well known that non-optimal environmental factors can promote bacterial adaptation leading to membrane lipid modifications and induced resistance to various inactivation processes.

Rationale: The aim of this study is thus to characterize the adaptation of *Salmonella* Typhimurium to sub-lethal concentrations of four terpenes extracted from aromatic plants: thymol, carvacrol, citral, eugenol, and their combinations. *Salmonella* Typhimurium has been taken as bacterial model as *salmonellosis* is an important public health problem throughout the word. Hence, bactericidal activity of two commonly used biocides (peracetic acid and didecyl dimethyl ammonium bromide) was tested against adapted and non-adapted *Salmonella* cells.

Results: Growth in presence of any terpenes or their combinations induced increased resistance of *Salmonella* to both biocides. To explain the differences, membrane fatty acid composition was analysed as its modification is the main bacterial adaptation mechanism to environmental disturbances. As a matter of fact, bacterial membrane of adapted cells had higher proportions of saturated fatty acids than non-adapted ones. The observed saturation of cytoplasmic membrane can certainly lead to harder penetration of antimicrobials into the membrane which induces increased resistance of cells.

Conclusion: Plant-derived terpenes can be a good alternative to chemical preservatives in food, but concentrations have to be optimized in order to avoid bacterial adaptation and induction of tolerance to bactericidal activity of usual biocides.

P1-28   *Staphylococcus aureus* **and** *Pseudomonas aeruginosa* **Biofilm Resistance to Disinfectants**

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Introduction: Currently, in industrial or medical environments, treatment for cleaning and disinfection are undertaken to ensure hygiene of surfaces. According to regulatory standards, the antimicrobial activity of disinfecting agents is tested on cells in suspension or deposited and dried. However, microorganisms are usually found as biofilms in which environmental conditions are very different from planktonic state. Biofilms mostly generates increased resistance of microbial cells to the activity of disinfectants and thus increases the persistence of potentially pathogenic bacteria in the food chain. While the precise mechanisms underlying this resistance are still poorly understood, it appears as a multifactorial process primarily related to the biofilm characteristics. In this context, we sought to better understand the biofilm architecture and to identify the matrix composition that may be involved in the resistance to biocides of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms, two species which are known to be involved in a large number of human infections.

Rationale: In this context, we sought to better understand the biofilm architecture and to identify the matrix composition that may be involved in the resistance to biocides of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms, two species which are known to be involved in a large number of human infections. Using the Calgary biofilm device, the susceptibility of planktonic and 24 h-biofilm cells were evaluated for three disinfectants having different modes of action (peracetic acid, benzalkonium chloride and O-phthalaldehyde).

Results: Results underlined the greater resistance of some biofilms to disinfectants as for *P. aeruginosa* to benzalkonium chloride. The structural study of biofilms by confocal laser scanning microscopy (CLSM) helped to characterize the architecture heterogeneity of these multicellular structures. a characterization of the matrix composition (exopolysaccharides, proteins) by PM-IRAS FTIR spectroscopy and biochemical assays was also explored leading a deeper understanding of the resistance mechanisms in the different biofilms.

Conclusion: This study contributes to a better understanding of the relation structure / function of multicellular bacterial communities. In order to guarantee the effectiveness of cleaning and disinfection treatments, the biofilm lifestyle should be considered in the establishment of new regulatory standards for assessing bactericidal activity of disinfectants.

P1-29   Combined DVC-FISH Method for in Situ Detection of Viable *V. parahaemolyticus* and *V. vulnificus* Cells

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Introduction: *Vibrio parahaemolyticus* and *Vibrio vulnificus* are Gram-negative, halophilic bacteria that occurs naturally in estuarine and marine environments worldwide. Bacterial illness is associated with seafood consumption and to exposure to contaminated waters. The virulence and pathogenicity capabilities of *V. parahaemolyticus* are somewhat less pronounced and serious than *V. vulnificus*, and fatalities associated with this bacterium are rare.

Vibrio is able to enter into viable but non-culturable (VBNC) state. This bacterial state supposes a problem to detect bacteria by cultural methods and therefore a potential human risk. On the other hand, a method able to differentiate the presence of viable or dead *Vibrio* cells is prioritary to determine the real infection risk of a food sample.

Rationale: The aim of this study was developed a method combined DVC procedure with FISH technique in order to discriminate and enumerated rapid and easily viable and non viable cells of viable *Vibrio parahaemolyticus* and *Vibrio vulnificus* for a future application on seafood and raw food detection.

For the DVC procedure, bacteria must be incubated in a broth media with an optimal concentration of antibiotics which inhibit cell replication but allows other synthetic pathways to continue. Therefore, in these conditions viable bacteria continue to metabolize nutrients and become elongated but not replicated.

Several antibiotics, different concentrations of each antibiotic and incubation times were tested to establish the optimal conditions to elongate the cells.

Results: For DVC assay, ciprofloxacin was the most effective gyrase inhibitor of both *V. parahaemolitycus* and *V. vulnificus*. A period 5 hours of incubation was optimal for *V. parahaemolitycus* elongation while for *V. vulnificus* elongation time was increased to 8 h. LIVE/DEAD BacLight bacterial viability kit of Molecular Probes (Molecular Probes, Inc., Eugene, Oregon) was used to evaluate cellular dead after antibiotic treatment.
The number of viable cells was obtained by detection of specific VIB3 probe hybridizated cells which were elongated at least 2 times their original size.

Conclusion: Results show that this DVC-FISH procedure with the use of Ciprofloxacin is a rapid and culture-independent useful method to detect specifically viable Vibrio cells in different seafood samples. Besides fluorescent intensity signal of the hybridized cells following DVC treatment was stronger than before the treatment allowed a easier detection in spite of small number of cells and the background of the sample.

P1-30   Occurrence of *Campylobacter* ssp. in Broiler Carcasses in a Slaughterhouse of Latium Region

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Introduction: *Campylobacter* spp. is the main responsible for foodborne disease in Europe (EFSA, 2009).

This study gives the results of a single slaughterhouse survey of Latium Region on the prevalence of *Campylobacter* spp. in 300 samples of broiler carcasses. The survey was based on the sampling scheme described in DEC 516/2007/UE, with the aim to collect data, currently scarce, on the contamination level of broiler carcasses in our region.

Rationale: Estimate the prevalence and the number of *Campylobacter* spp. in broiler carcasses in a slaughterhouse of Latium region. Compare the results obtained by ISO 10272-1and 2:2006, and biomolecular test (PCR).

Results: The prevalence of *Campylobacter* spp. by ISO 10272-1:2006 was 98%. Almost a quarter was classified as *C. jejuni* and a quarter as *C. coli*. The ISO 10272-2:2006 method pointed out a high number of *Campylobacter* spp. (96%) with a variable distribution amongst the different classes of values examined. The higher count of *Campylobacter* spp. ranged between 100–999 cfu/g with a frequency of 60% of total samples. Data obtained with ISO 10272:1–2 and PCR had an accordance, respectively, of 38.4% and 59.8%.

Conclusion: The high prevalence of *Campylobacter* spp. detected in this study shows that poultry meat could represent a major mean of infection for consumers. Infact, it has been shown that once contaminated poultry meat is introduced into the kitchen, it can serve as a source of cross-contamination to other foodstuffs and surfaces during meal preparation (FAO/WHO, 2009). The comparison between different methods gave comparable results. The study points out the importance of PCR real time as a confirmatory method of the results given by the microbiological technique and for the discrimination of *Campylobacter* pathogen to humans (*C. coli*, *C. jejuni*, *C. lari*) only.

P1-31 **A Novel Colorimetric Screening Assay for *E. coli* O157:H7 Utilizing Simultaneous Capture and In Situ Labeling during Automated Re-circulating IMS**

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Introduction: *E. coli* O157:H7 is notorious because of its low infective dose and the severity of the disease in vulnerable individuals. Raw ground beef and produce has been implicated as the source of *E. coli* O157:H7 in a significant number of foodborne disease outbreaks and food safety recalls during the past two decades. Detecting the presence of this STEC at low levels presents significant challenges to both the food industry and regulatory agencies.

Rationale: This study describes the development and validation of a robust screening assay for *E. coli* O157:H7 in raw fresh beef samples based on in situ labeling of the target STEC captured during re-circulating IMS. The method is applicable to analyzing ground beef and trim samples, produce and dairy and RTE foods for the presence of *E. coli* O157:H7 where initial pathogen levels are in the 1–5 CFU per sample range.

Results: Reliable detection of initial low level *E. coli* O157:H7 in a range of food samples was achieved using the labelling of target STEC cells in conjunction with the automated RIMS capture and washing procedure. No natural contamination of uninoculated samples with *E. coli* O157:H7 was encountered. Based on comparing the screening assay result with target isolation on selective agar plates and real time PCR methodologies no false positive or false negative results were obtained. Recovery of *E. coli* O157:H7 colonies on selective agar plates confirmed the colorimetric screening assay result in all cases.

Conclusion: The *E. coli* O157:H7 screening method described in this study offers a flexible and cost effective approach to identifying the presence of this STEC in a wide variety of food samples including raw beef, dairy and produce and RTE foods. It offers next day results and reliability. User handling is significantly minimized as target capture, labelling and washing steps are an integral part of the automated RIMS procedure.

P1-32   **Relative Risk of Escherichia coli Contamination in Lettuce (*Lactuca sativa*) as Influenced by Different Irrigation Systems**

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Introduction: Much of the leafy vegetables produced in the southwestern United States are irrigated with surface water diverted from rivers. The influence of irrigation systems on the contamination risk of *Escherichia coli* in commercial lettuce (*Lactuca sativa* L.) field conditions was investigated. These studies were prompted by the need for more information of survival of pathogenic *E. coli* under field conditions.

Rationale: Non-pathogenic *E. coli* K-12 strains, LMM1010 and ATCC 25253, were used for the simulation in replicated field trials. Bacteria stock was injected into the water stream used to irrigate iceberg and romaine lettuce plants through drip, furrow and overhead sprinkler systems. Samples of lettuce tissue and soil were collected to determine any presence of *E. coli*.

Results: The results show that product samples were positive for *E. coli* during 7 days following overhead sprinkler irrigation, whereas no product samples was found positive for *E. coli* when using furrow or drip irrigation methods. Soil samples were initially positive in all three irrigation systems; however survival of the bacteria indicator was longest in the furrow irrigated areas. We further investigated survival in soil through the year under desert conditions, and found survival is dependent on both temperature and moisture. Based on our results we estimate survival of *E. coli* in the surface layers of soil can range from 17 days in winter months to 5 days in the summer.

Conclusions: The results of this study confirm the enhanced risk of *Escherichia coli* contamination when using overhead sprinkle irrigation. These studies further reveal the importance of an early irrigation termination for both sprinkler and furrow irrigation methods.

Poster Session 2 – Thursday, 10 June

P2-01   **An Innovative e-Learning Training Program in Food Safety Legislation - From Farm to Fork European Food Safety Legislation (F4ESL) Training Program**

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Introduction: EU authorities address the "farm-to-fork" approach by giving priority to consumer demand and the right to access to safe and high quality food. To achieve this, an efficient and effective food control system along the food chain should be established and implemented. At this point, Food Safety Legislation becomes very important to obtain food safety.

Rationale: Food Safety Legislation has many positive effects for food sector such as; protecting public health, providing consumers with relevant and accurate information so that they can make an informed choice, promoting fair trade by ensuring a consistent standard among related sectors, ensuring that all stakeholders of the food chain fulfill their role (including suppliers and customers), providing guidance to all stakeholders on food safety and also increasing the confidence of consumers in the food supply.

F4ESL - From Farm to Fork European Food Safety Legislation Training Program is an innovative and integrated online training program on EU Food Safety Legislation ensuring vocational education for stakeholders of food chain via newest learning methods, and is going to be piloted across all European countries in two sessions, in 2011.

Five European member countries and a candidate country participated in this project to share their knowledge and experiences on food safety legislation and e-learning technology. To reward their endeavor the EU decided to fund the project through the Lifelong Learning Program (LLP) "Leonardo da Vinci" (2009-1- TR1-LEO05-08647).

Results: F4ESL is developed to fill the legislation gaps for each profession working in food sector with a common "from farm-to-fork" food safety approach covering all related EU regulations. Since it is laborious to read and comprehend all the related legislations; simple and comprehensible language will be used in the F4ESL training as well as giving some case studies about regulations in the lectures to make them easier to understand. The national food safety legislation of Turkey and EU food safety legislation will be compared within the context of this project.

The training program will be developed by a Pan European committee of top professionals as to bring about a state of the art curriculum which consists of five modules: Introduction, Legislation on product, Legislation on process, Labeling, and Public Powers.

Conclusion: About 500 trainees are planned to be trained by F4ESL free of charge. The effects of the program will be measured by surveys. In addition, trainees will have opportunity to share the feedbacks about F4ESL project in seminars organized in all partner countries and in valorization conference in Turkey.

Acknowledgments: Thanks to the great contributions of the experts and instructors of F4ESL for developing the curriculum, as well as the extensive financial contribution of the LLP Leonardo da Vinci Programme of EC which has made this project possible.

P2-02   Assessment of the Potential for Psychrotrophic *Clostridium botulinum* to Grow in Pressure-treated Cooked Poultry Meat

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Introduction: High pressure processing can be used to improve the microbiological safety and shelf-life of many foods, including cooked meats. It can be regarded as a "cold pasteurisation" process as it can give significantly reduce numbers of vegetative pathogens. However, *Clostridia* spores are known to be pressure-resistant and will survive treatments normally applied to foods.

Rationale: The purpose of this study was to assess the potential of psychrotrophic spores of *Clostridium botulinum* type B to survive and grow in pressure-treated cooked poultry meat. Raw meat was inoculated with a cocktail of *C. botulinum* spores, to give an inoculum level of 9.1×10^5/g. Samples were cooked to an internal temperature of $80 \pm 0.2°C$ for 1 minute, cooled on ice and stored at 4°C for 24 ± 2hr before pressure treatment (600 MPa/2 min), or left untreated as controls. Samples were stored for up to 95 days at 8°C and numbers of surviving clostridia (total count and spore count) were assessed weekly for 35 days and again after a further 30 and 60 days.

Results: The cooking process did not significantly reduce the numbers of spores. However, the subsequent pressure treatment did cause numbers to be reduced by almost 1 log, to 1.1×10^5/g. It is proposed that the cooking induced heat shock and caused some of the spores to germinate. Pressure treatment the following day then inactivated these germinated cells.

Spore numbers continued to decrease during storage of the pressure treated meat and reached 3.2×10^4/g after 35 days, before increasing slowly over time to 1.4×10^5/g after 95 days. Numbers in the control samples increased slightly, to $\sim 3 \times 10^6$/g after 95 days at 8°C.

Conclusion: These preliminary findings suggest that the risk of psychrotrophic *C. botulinum* growing in pressure-treated cooked meats during extended refrigerated storage is likely to be low.

P2-03   Comparative Genomic Indexing of Clinical and Bovine Isolates of *E. coli* O157

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Introduction: *Escherichia coli* O157 is a foodborne pathogen of significant public health concern due to the severity of the illness which it can cause in humans. Characteristics which contribute to its significance as a foodborne pathogen include possession of key virulence factors such as verocytotoxin genes and the locus of attachment and effacement. The genomic content of a strain has a direct influence on a strain's pathogenicity potential. Microarray based comparative genomic indexing (CGI) allows discrimination of bacterial isolates based upon comparisons of the gene content of each strain to that of a reference strain or strains.

Rationale: *E. coli* O157 strains show extensive genomic diversity, with some strains being considered of less clinical significance than others. The purpose of this study was to examine Irish and US bovine and clinical *E. coli* O157 isolates using CGI in order to determine the differences between strains from different sources.

Results: Irish strains were compared to data available for American *E. coli* O157 isolates and phylogenetic analysis indicated that the strains formed separate subclusters based on geographical origin. A major reason for this was an apparent multigene deletion in the Vt2 encoding bacteriophage in the strains of Irish origin, which was observed in strains from bovine and clinical sources. It was also found that the Irish clinical isolates clustered together.

Conclusion: CGI has been shown in this and other studies as a powerful discriminatory tool. As well as its discriminatory power it can be used to examine, for example, virulence gene profiles, potential for antibiotic resistance or strain evolution. It also allows for the possibility to identify more precise markers for strain identification and association with human infection.

P2-04   Dry Heat Resistance of *Cronobacter* and *Salmonella* in a Simulated Powdered Infant Formula Processing Environment

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Introduction: *Cronobacter* (*Enterobacter sakazakii*) is an opportunistic pathogen associated with bacteremia, necrotizing enterocolitis and meningitis in neonates after ingestion of contaminated powdered infant formula (PIF). Neonatal *salmonellosis* cases have also been associated with PIF. Environmental studies of milk powder factories indicates that contamination often occurs in and around the spray drier.

Rationale: Dried milk products are pasteurized prior to drying, therefore contamination of PIF is attributed to post-process contamination events. The presence of water in processing environments is kept to a minimum. Therefore the combination of low a_w and high temperatures are environmental stresses likely to be encountered by potential contaminants. Determination of D and z values is frequently undertaken in liquid suspension. In this study comparison was made between D and z values in dry as well as liquid cultures. *Cronobacter* and *Salmonella* in infant formula were dried to stainless-steel coupons. These were then exposed to temperatures from 60–200°C in a dry oven for 0–120 min. The surviving cells were enumerated using impedance technology. This simulated the factory environment where bacteria are likely to exist as dry films on stainless-steel surfaces.

Results: *Cronobacter* strains included a factory persistent clonal strain as well as clinical isolates. *Salmonella* strains consisted of outbreak strains, which were isolated from PIF and infants. The *Cronobacter* factory isolate exhibited a higher D60 value in comparison to the other isolates, indicating that this strain has built up a higher thermotolerance to temperatures that it is regularly exposed to in the factory.

Conclusion: The thermal death characteristics of *Cronobacter* and *Salmonella* in dry environments vary considerably from those observed in liquid suspensions. Isolates exposed to elevated temperatures in combination with low water availability may develop greater thermotolerance leading to an increased risk of persistence in the processing environment and potential product contamination.

P2-05   *Giardiasis* and *Cryptosporidiosis* in Calves from Farms Selling Pastoral Food in Cefalù area (Sicily, South Italy)

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Introduction: The protozoa *Giardia duodenalis* and *Cryptosporidium parvum* have emerged as important parasites of cattle because of their pathogenicity and of the potential public health significance of zoonotic transmission.

Rationale: The aim of this study was to obtain data about the prevalence of these two parasites in dairy calves from seven farms in Cefalù area, where are selling retail cheeses and unpasteurized

milk; calves are frequently infected with *Cryptosporidium* and milk can be contaminated through mechanisms such as poor udder hygiene.

Results: Faecal samples were collected from 47 dairy calves, between 7 and 180 days of age, and examined for *Cryptosporidium* and *Giardia* (oo)cysts after concentration and immunofluorescent staining (IFA) (Merifluor Cryptosporidium/Giardia, Meridian Bioscence). Nested-PCR protocols were used to amplifly fragments of the TPI gene of *Giardia*; genotypes were determined by means of DNA sequencing of amplicons and subsequent sequence alignment.

Of the 47 investigated samples, 23 (48,9%) resulted positive for *G. duodenalis* by IFA and 16 by PCR; six positive *Giardia* samples were coinfected with *C. parvum*. Infection peaked in calves of 7-30 days old for both parasites. The zoonotic genotype, *G. duodenalis* Assemblage A, was isolated from 1 calf while Assemblage E (non zoonotic) was found in 6 dairy calves; the remaining nine PCR products are in progress.

Conclusion: The health status of animals that are destined to enter the human food supply chain may be an important factor in predicting the risk of human foodborne infections. Consequently, interventions that reduce the incidence of giardiasis and cryptosporidiosis in cattle might also help to avoid possible contamination on pastoral food as milk. The presence of both zoonotic parasites (*C. parvum* and *G. duodenalis* assemblage A) suggests that further studies are necessary to evaluate the occurrence of *Giardia* and *Cryptosporidium* on unpasteurised milk sold in the analyzed farms.

Acknowledgments: The authors would like to thank Dr. Carmelo Calcò who provided us with access to farms of Cefalù. We also grateful to all the farmers who contributed to the realization of this study.

P2-06   **Survey on Monitoring of Antimicrobial Usage in Food Animals and Antimicrobial Resistance in Bacteria from Food Animals in WHO European Region**

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Introduction: An important component in the prevention and control of antimicrobial resistance is the implementation of both a national monitoring program on the non-human usage of antimicrobial agents and a national monitoring program on antimicrobial resistance in bacteria from food animals.

Rationale: The objective of this survey was to obtain an overview of the existence of antimicrobial usage and antimicrobial resistance monitoring programs associated with food safety in the WHO European region. In 2007 a questionnaire was developed and sent to the food safety counterparts in the 53 member countries in the WHO European Region.

Results: The overall response rate was 72%. The major classes of antimicrobial agents used by the countries in food producing animals included "critically important" antimicrobials such as quinolones, macrolides, penicillins, aminoglicosydes and 3rd and 4th generation cephalosporines. Five responding countries reported that they do not require veterinary prescriptions for use of antimicrobials in food animals. Only 39% and 47% of the responding countries reported to have in place a monitoring program on antimicrobial usage in food animals and in humans, respectively. Only 50% and 45% of the responding countries replied to have in place monitoring programs on antimicrobial resistance in bacteria from food animals and in humans, respectively.

Conclusion: Data show that efforts have to be undertaken by the national public health and food safety authorities and by the international organizations in order ensure the implementation of proper risk management actions for antimicrobial resistance, which include monitoring programs of antimicrobial resistance in bacteria from both humans and food animals, the monitoring of

usage of antimicrobial agents in humans and food animals, intersectoral collaboration, enforcement of prudent use policy including prescription requirements, and proper and timely risk communication.

P2-07   The Role of Exopolysaccharide in Environmental Stress Resistance of *Cronobacter* (*Enterobacter sakazakii*)

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Introduction: *Cronobacter sakazakii* is a neonatal pathogen associated with bacteraemia, meningitis and NEC linked to ingestion of contaminated infant formula. In comparison to other *Enterobacteriaceae*, *Cronobacter* are known to be relatively resistant to desiccation, to grow at elevated NaCl concentrations and elevated temperatures. Many strains of *C. sakazakii* produce an exopolysacharide capsule (EPS) in the presence of high C:N media such as infant formula. It is speculated that EPS assists in protecting the cells from physiological stress.

Rationale: Over 100 *Cronobacter* strains from clinical cases, infant formula and food processing environments were screened for production of EPS. Selected strains were assayed to determine environmental factors that induce EPS. Induced and non-induced strains, along with mutant strains constitutive for +/- EPS expression, were assayed to determine the protective effect of EPS in a variety of physiological environments (heat, acid, and desiccation). Relative resistance to antibiotics and industrial biocides, as well as attachment/biofilm formation, were also determined. Additionally, phenotype microarray (PM) analysis of mutant EPS +/- strains investigated metabolic differences in the presence of nearly 2000 environments including carbon, nitrogen, phosphorus and sulphur sources, osmolytes and pH, as well as antimicrobial agents.

Results: MboII restriction analysis of the colanic acid operon indicated taxonomic differences between *Cronobacter* species. Restriction fingerprints could not be associated with levels of EPS expression and it is likely that morphological differences relate to variation in regulatory rather than biosynthesis genes. No differences were found in the metabolism of EPS +/- strains under any of the conditions in the PM array. Elevated carbohydrate was the only factor that induced EPS. There was no correlation between EPS production and antimicrobial resitance, however there may be a relationship between acid resistance and EPS production.

Conclusion: While EPS production in *E. coli* has been shown to increase tolerance to heat, salt, acid and desiccation, it is likely that *C. sakazakii* has developed additional strategies for survival under harsh environmental conditions to compliment the effects of EPS.

P2-08   Fish Species Differentiation in Seafood by PCR-RFLP Analysis

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Introduction: Food authenticity is presently in the centre of interest of food authorities, because incorrect labelling of foods represents a comercial fraud. The misleading labelling is very important concerning the presence of allergic food.

Rationale: Fish and seafood substitution has become an important concern in marketplaces, in part due to increased international trade, per capita seafood consuption, and processed food. Most DNA-based methods for species identification in food consist on the higly specific amplification of DNA fragments by means of polymerase chain reaction (PCR).

Results: PCR-RFLP analysis was used to identify fish species in 60 fish samples belonging to the families Gadidae, Scombridae, Gemphylidae, Salmonidae and Anguillidae) obtained from the local markets in the Czech Republic. At 47 samples (78.3%), the results were in agreement with declarations of the producers and 10 samples (16.7%) contained other fish species.

Conclusion: Even if in three cases the analysis was unsuccessful the method is useful for control of the adulteration of food with fish tissue content and in this way it contributes to better consumer awareness.

P2-09   Diversity of Serine-ß-lactamase Genes among *Aeromonas* Isolates from Pigs Slaughtered for Consumption

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Introduction: Antimicrobial resistance in foodborne pathogens has become a public health issue. The intense and indiscriminate use of antibiotics is undoubtedly the major force associated with the high bacteria resistance worldwide. Some *Aeromonas* species cause both gastrointestinal and extraintestinal infectious diseases in humans. Many studies suggest members of *Aeromonas* developed multiple antibiotic resistances which constitutes a limitation to the treatment of infections associated to these bacteria.

Rationale: The aim of this work was to evaluate the susceptibility patterns of the isolates obtained from pigs slaughtered for consumption and to assess the occurrence and diversity of ß-lactamase genes.

Results: A total of 106 *Aeromonas* strains were isolated from 50 (73%) of the 69 samples of pig carcasses and diaphragm muscle analysed. The strains, identified by gyrB gene sequencing, belong to eight different species (*A. hydrophila*, *A. bestiarum*, *A. salmonicida*, *A.caviae*, *A. media*, *A. veronii*, *A. allosaccharophila* and *A. aquariorum*) and were tested against 8 families of antibiotics (27 antimicrobial agents). The ß-Lactamases encoding genes blaTEM, blaSHV, blaCTX-M, blaOXA, blaFOX and blaMOX were detected by PCR. The susceptibilities profiles revealed a high level of resistance to several groups of antibiotics. The genes blaOXA-B (1) and blaOXA-C (1) were detected in strains of *A. hydrophila* and the gene blaCTX-M (1) was presented in A. *media*. In *A. aquariorum* and in one *A. caviae* the gene blaMOX was presented. The genes blaFOX and blaOXA-aer were detected in 71% and 49% respectively of the strains tested. There was no evidence for the presence of blaTEM and blaSHV genes.

Conclusion: In the present work we verified multiresistance mainly to the cephalotin (ß-lactam) and erythromycin (macrolide) in different *Aeromonas* strains. In several strains, the presence of more than one ß-Lactamase gene, from different classes, was detected. The genes blaFOX and blaOXA-aer showed a high dispersal in some species of the *Aeromonas* genus.

P2-10   Detection of Metallo-ß-lactamases in *Aeromonas* Isolated from Alheira: A Traditional Portuguese Meat Product

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Introduction: Members of the genus *Aeromonas* are facultative anaerobic gram-negative rods, oxidase-positive that are widely distributed in aquatic environments and have also been isolated from different kinds of food. Gastroenteritis is considered the most common Aeromonas clinical presentation. Susceptibility patterns of these species revealed an increase in resistance to ß-lactam antibiotics, which is attributed to the presence of ß-lactamases. Zinc-dependent class B metallo-ß-lactamases (MBLs), chromosomally mediated and those encoded by transferable genes, are of special importance because display an extremely wide spectrum of hydrolysis that includes also carbapenems.

Rationale: The aim of this study was to determine the susceptibility patterns, to ß-lactam antibiotics, of *Aeromonas* strains isolated from alheira, a traditional Portuguese meat sausage and the occurrence of CphA, a chromosomally mediated MBL, and VIM, an acquired MBL.

Results: Thirty-two samples purchased in different local markets were analysed. A total of 84 presumptive *Aeromonas* spp. were obtained and subjected to genotyping ERIC-PCR analysis. The 20 strains showing differences in the ERIC-pattern were identified by gyrB gene sequencing and tested against 15 ß-lactam antibiotics. Metallo-beta-lactamases encoding genes were detected by PCR and positive results were confirmed by sequencing. All the strains studied belonged to the species *A. hydrophila* (5), *A. caviae* (6), *A. media* (6), *A. salmonicida* (2) and *A. allosaccharophila* (1). Tested antibiotic susceptibilities in these strains indicated a noticeable level of multiresistance. blaCphA gene was detected in 8 strains (40%). There was no evidence for the presence of blaVIM genes in any isolate.

Conclusion: In this study a high percentage of resistance to cephalotin and ticarcillin and low percentage to other ß-lactam antibiotics have been detected in *Aeromonas* spp. from alheira. Despite blaCphA gene was detected in all strains of the species *A. hydrophila*, *A. salmonicida* and *A. allosaccharophila*, only one strain of *A. salmonicida* showed resistence to imipenem.

P2-11 **Food Safety Awareness and Risk Perception of Turkish Consumers**

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Introduction: Food safety is a growing global concern for consumers and professionals in the food and foodservice sectors. As a consequence of the food crisis in EU in the late 1990s, EFSA has foreseen the inclusion of food safety and risk perception as a key point to be surveyed in consumer surveys regularly conducted in the EU member states for gathering and analyzing information about the EU consumers, as part of the EU public opinion research series with EUROBAROMETER's methods. Turkey, still being a candidate country, is naturally not included in these surveys. However, in 2008, The Turkish Food Safety Association has attempted to run a similar study in Turkey by using the same questionnaires employed in the EUROBAROMETER surveys, to obtain the current status of consumer perceptions related with food in Turkey.

Rationale: The general purpose of the survey was to determine consumer's risk perception and awareness level on food safety in Turkey. The survey's field work, conducted during April 20 – May 7, 2008, covered a total of 661 interviews, among consumers selected as being representative of the Turkish demographic population, both geographically and socio-economically, according to NUTS definitions. CATI (computer assisted telephone interview), a quantitative research technique, has been utilized in the study. Respondents have been chosen from among real people who are responsible for purchasing food items for their households. 74% of consumers surveyed were women, 26% were male, the same ratio being a common feature in Turkish homes for people who are doing the family food shopping

Results: Areas of inquiry included food safety perceptions, awareness of foodborne risks, sources of food safety information, confidence in food safety authorities, food handling and safety practices at homes. There were some striking differences as well as some similarities in opinions of the EU and Turkish consumers on food-related issues and risks. One striking difference was on consumer confidence in public authorities on food safety issues: While almost half of the European consumers think that their public authorities will take satisfactory action with regard to food safety risks, about one-third of the consumers found these actions insufficient. In Turkey, 77% of Turkish consumers thought that the actions of Turkey's public authorities were insufficient, as opposed to the 21% who found it sufficient. When Turkish consumers were asked for their first impression of the word "food", 39% of Turkish consumers answered "nutrition", whereas EU citizens had answered as "taste" when the same question was asked. When they were asked for their major health concerns related with foods , 38% stated "food poisoning" and 27% stated "cancer". Turkish consumers also generally believe that food safety management is on the downgrade in the last 10 years.

Conclusion: There were many informative points and some gaps identified in food safety knowledge of Turkish consumers during this study. The poster will cover a brief compendium and graphical analysis of all the survey results. Since education of consumers is an effective strategy for reducing foodborne illness and economic losses associated with foodborne diseases, there seems to be a need for effective public education programs in Turkey, targeting risks associated with foods and safe practices in good hygiene at homes.

P2-12 Formation of Hydroperoxides from Outer Membrane Lipids and Cytoplasmic Accumulation of L-lactic Acid of *Escherichia coli* O157:H7 Following Exposure to Hot Water

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Introduction: Application of interventions consisting of hot water and lactic acid has been shown to be effective in reducing levels of *Escherichia coli* O157:H7 on beef carcasses. However, the mechanisms by which these interventions inactivate the pathogen remain unknown.

Rationale: The objectives of this study were to determine the potential for degradation of outer membrane lipids of *E. coli* O157:H7 following hot water exposure and to determine the impact of hot water-induced bacterial membrane damage on L-lactic acid penetration into the bacterial cell.

A cocktail containing four strains of rifampicin resistant *E. coli* O157:H7 was dispensed into sterile disposable tubes and exposed to hot water at 25, 65, 75, or 85°C for 0, 5, 15, 30, and 60 s, and to L-lactic acid (0 and 5 % (w/v). Following treatment, membrane lipids were extracted using methyl-tert-butyl ether (MTBE). Formation of lipid hydroperoxides were determined spectrophotometrically using the ferric oxide (FOX) method, absorbance at 560 nm. Cell accumulation of organic acid was measured via L-lactic acid-derived nicotinamide adenine dinucleotide (NADH), absorbance at 340 nm.

Results: The maximum log reduction achieved was at 85°C for 60 s (6.9 ± 0.8 log CFU/ml). Log reductions for 60 s at 25, 65, and 75°C were 0.01 ± 0.01, 5.6 ± 0.8, and 6.5 ± 0.6 log CFU/ml, respectively. The peroxide milliequivalents/kg at 25 and 85°C (60 s) were 0.02 ± 0.01 and 0.05 ± 0.01, respectively. L-lactic acid accumulation at 25°C and 85°C (60 s) were 0.01 ± 0.001, and 0.04 ± 0.004 Nmol/cuv, respectively.

Conclusion: This study indicates hot water exposure may increase *E. coli* O157:H7 inactivation by resulting in damage to outer membrane components but does not necessarily result in significant increase in accumulation of L-lactic acid in the bacterial cell. These data serve as first steps to

understand the modification in pathogen sensitivity to antimicrobial as a result of prior hot water exposure.

P2-13   Growth Monitoring of *Salmonella* spp. According to the Packaging Technique and to the Inoculums Levels on Pork Minced Meat

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Introduction: *Salmonella* was in 2008, the second most often reported zoonotic disease in humans, and 131,468 confirmed cases of human salmonellosis were reported in Europe. Microorganisms counting techniques are a subject of major concern in the field of food hygiene. The European Community Regulation No 2073/2005 defined a quantitative limit for 100 CFU.g-1 which was is applicable for certain types of food during their shelf life. However an adequate counting method for low contaminating levels is still lacking.

Rationale: The aim of the present work was first to develop a new method to enumerate *Salmonella* spp. at low concentration on meat products. This method is based on membrane filtration method without using treatment to solubilize food components. Further more, this study wanted to compare *Salmonella* spp. growth according to the packaging and to the inoculums levels. The final aim of the present work was to develop and to validate a quicker and more sensitive genetic method for quantification of *Salmonella* spp. in meat products by the quantitative real-time PCR.

Results: Experiments were carried out on pork's irradiated and not irradiated minced meat. These meats were packaged either in expanded polystyrene trays wrapped with permeable stretch film or under modified atmosphere (70% O2/30% CO_2) in sealed trays. Food matrixes have been artificially contaminated with 4–12 CFU.g-1 or 100 CFU.g-1 of *Salmonella* spp. For enumeration, 25 g of samples were used for solubilisation with water peptone buffer (dilution 1/10) using a Pulsifier or a Stomacher. For low levels of inoculation, food suspension was filtered on a single use membrane of 47 mm diameter and 0.45 μm pore size. The filters and filtrates (1 ml) were laid on specific plated media according to EN ISO 11290-2 standard. After preliminary tests for primers and probes choice, the genetic method was validated with classical microbial methods (ISO 11290-2:1998, ISO 6579) using challenge tests. A mixture of 3 strains was realized: 1 referee strain (*S. Typhimurium* ATCC 14028) and 2 lab isolates. The initial inoculum was homogenized in minced meat before packaging in trays. They were incubated for 14 days at +8, +10°C and +12°C. In the 2 tested meats, growth speeds as well as the final populations increased according to the temperature. In the not irradiated minced meat, growth speeds of the pathogenic flora as well as the associated final populations were lower than those observed in the irradiated matrices. As soon as the total flora reached its stable growth phase, growths of the various pathogenic stagnated.

Conclusion: As expected, level of inoculums has an impact on the latency period according to the incubation temperature. The growth of the original flora inhibited partially the growth of pathogens inoculated. Generally, growth of the total flora is partially inhibited by modified atmosphere packaging. The analytical methods of molecular biology allow faster and less heavy analyses, in terms of hand of work and execution, than the methods of classic microbiology.

P2-14   Influence of the CsgA Protein on Autoaggregation, Hydrophobicity and Attachment of *Escherichia coli* O157:H7

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Introduction: The attachment of *Escherichia coli* O157:H7 to abiotic surfaces in the food processing industry may be mediated by surface structures such as curli (*CsgA*) and thus contribute to the contamination of food products.

Rationale: In order to assess the role of the *CsgA* protein in attachment, this study had the following aims: 1) create *csgA* knockout mutants in two strains of *E. coli* O157:H7; determine the differences between parent and mutant strains with respect to 2) autoaggregation; 3) hydrophobicity using Bacterial Adherence to Hydrocarbons (BATH) and Contact Angle Measurements (CAM); 4) attachment to Teflon, glass and stainless steel (SS) using epifluorescence microscopy and 5) determine the effect of growth media by conducting all studies following growth in Nutrient Broth (NB), Nutrient Agar (NA), Luria Bertani broth (LB) and LB Agar.

Results: The *csgA* gene was successfully replaced with a chloramphenicol antibiotic resistance cassette in both strains. Autoaggregation was not always significantly different ($P > 0.05$) suggesting that properties other than curli production may contribute to autoaggregation in these strains. CAM results suggest that *csgA* mutant strains were more hydrophilic than parent strains under most growth conditions ($P < 0.05$), however, hydrophobicity determined by BATH suggested the mutants were more hydrophobic when cultured on agar ($P < 0.05$). Mutant strains attached in significantly fewer numbers to Teflon and glass under most growth conditions ($P < 0.05$). Attachment of mutants to SS was significantly higher when cultured in NB and NA ($P < 0.05$).

Conclusion: The results of this study highlight the complexity of *E. coli* O157 attachment to abiotic surfaces. Multiple factors are likely to play a role in attachment as deletion of the *csgA* gene did not consistently affect autoaggregation, hydrophobicity or attachment in these strains.

P2-15   Withdrawn

P2-16   Microbial Characterization of Pitina, a Sausage-like Produced in Friuli Region (Italy)

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Introduction: Pitina is a typical smoked and fermented meat product of North-East Italy. Its origin is dated back to at least the first half of the XIX century. Nowadays it is still produced following a local recipe by some artisan plants and families, using ground sheep meat, mixed with variable proportions of pork lard and flavored with salt, pepper and spicy herbs. Their smoking comes from the burning of local non-resinous firewood.
The main characteristic of Pitina is the absence of casing. In fact this sausage-like product is prepared by hand coated with corn flour. The repeated application of flour during processing allows the formation of a mould cover on the surface of the product, beginning with a white thin layer after a fortnight, and as an almost entire coat, after a month.

Traditionally cooked before consumption, nowadays it is also consumed in an uncooked and sliced form.

Rationale: Little is known about its fermentation and its safety for consumption. In this paper, we describe the microbial ecology of Pitina during the entire fermentation period. Moreover, strains of Coagulase Negative Catalase Positive Cocci (CNCPC) were isolated, subsequently identified by molecular methods, and characterized by the use of Repetitive Extragenic Palindromic sequence analysis.

Results: The results underlined a good hygienic quality of Pitina ready-to-eat: *Staphylococcus aureus* and *Escherichia coli* dropped below the detection limit (< 10 colony forming unit (CFU)/g and < 1 CFU/g, respectively). *Salmonella* and *Listeria* spp. were always absent/25 g of product. Lactic acid bacteria (LAB) and CNCPC increased during the first day of fermentation to reach value of 10^7–10^8 CFU/g and 10^5–10^6 CFU/g, respectively. The 53% of CNCPC strains isolated were identified as *Staphylococcus xylosus*.

Conclusion: The product resulted safe for consumption even not cooked. It is essential to underline once more the importance of *S. xylosus* specie in meat fermentations.

P2-17   Withdrawn

P2-18   Multilocus Variable Number Tandem Repeat Analysis (MLVA) of *Salmonella enterica serovar* Typhimurium DT104/b

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Introduction: *Salmonella enterica* subspecies *serovar* Typhimurium is known as an important and pathogenic clonal group which continues to cause sporadic cases and outbreaks of foodborne illness in humans. This study describes a multiple locus variable number tandem repeats analysis (MLVA) method for the sub-typing of *Salmonella* Typhimurium.

Rationale: Emphasis was given to the most predominant phage types DT104 and DT104b. The method comprises of a multiplex PCR specifically amplifying repeated sequences targeting seven different loci in the *S.* Typhimurium genome followed by fragment size analysis using microchip based capilliary electrophoresis on the Agilent 2100 bioanalyser. A total of 144 animal, environmental and retail isolates for the two phage types were used for the analysis.

Results: Results showed that three loci STTR5, STTR-10 and STTR-6 provided most variation in tandem repeat numbers between the 144 *S.* Typhimurium isolates with 16, 15, and 11 alleles detected for each locus respectively. The remaining loci provided less allelic discrimination. Some isolates did not produce amplified product with PCR for various loci, providing an additional level for discrimination. The different alleles at each locus were assigned allele numbers, which were used for strain comparison.

Conclusion: The MLVA is a highly discriminative method for *S.* Typhimurium strains even within a single phage type. It is easy to use and cost effective compared to other molecular methods and therefore is a useful tool for surveillance for *S.* Typhimurium.

P2-19   Probiotic Microorganisms and Their Application in Food Safety Enhancement

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Introduction: Probiotics are defined like beneficial bacteria, which positively affect the host by regulating the microbial balance, restoring the normal intestinal permeability and gut microecology. They are applied in prevention of different infectious diseases, which can compromise the food safety including salmonellosis.

Rational: The goal of the study was *in vivo* evaluation of preventive effect of *Enterococcus faecium* EF55 against salmonellae in chickens.

Results: *Salmonella* spp. was monitored in caecum, liver and spleen after preventive per oral application of *E. faecium* EF 55 (Institute of Animal Physiology, SAS) and consecutive infection with *S*. Enteritidis at age of 8 days. Detection of pathogen was performed by PCR with standardized set of primers targeting invA gene. In birds treated with *E. faecium* EF 55 (group E) was recorded decreased number of *Salmonella* spp. positive individuals from 28.5% 2 days post infection (pi) to 10% 14 days pi when the difference between group E and group S was significant ($P < 0.01$). On the contrary in non-treated birds (group S) the percentage of *Salmonella* spp. positive animals increased from 12.5% 2 days pi to 83.3% 14 days pi. The highest differences recorded in distribution of salmonellae between E and S during experiment was in ceacum 2.5 times, in liver 2.5 times and in spleen 3 times in favour of group S. According to preliminary RT-PCR results, application of *E. faecium* EF55 stimulated expression of mRNA for the IFN-gamma in group E more than twice when compared to group S.

Conclusion: Preventive application of *E. faecium* EF55 significantly reduced colonization of chicken's caecum with salmonellae and minimized their translocation into the liver and spleen. Simultaneously, it stimulated the immunoregulatory mechanism against intracellular bacterial infection.

P2-20 Rapid Genus and Species Specific Identification of *Cronobacter* spp. by MALDI-TOF Mass Spectrometry

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Introduction: *Cronobacter* spp. are Gram-negative opportunistic foodborne pathogens and known as rare but important causes of live-threatening neonatal infections. Rapid and reliable identification of *Cronobacter* species and their differentiation from phenotypically similar, apathogenic *Enterobacter turicensis*, *Enterobacter helveticus* and *Enterobacter pulveris* has become increasingly important.

Rationale: We evaluated here the application of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for rapid genus and species identification of the 6 *Cronobacter* species recognized so far. To this end, we developed a reference MS database library including 54 *Cronobacter* target as well as 17 non-target strains.

Results: The strains provided reproducible and unique mass spectra profiles covering a wide molecular mass range (2,000 to 30,000 Da). Genus and species-specific biomarker protein mass patterns were determined. The defined biomarker mass patterns (SARAMIS SuperSpectrum™) were validated using 36 strains from various *Cronobacter* species as well as eight non-target strains. For all strains the mass spectrometry-based identification scheme yielded identical results compared to a PCR based identification system. All strains were correctly identified and no non-target strain was misidentified as *Cronobacter*.

Conclusion: Our study demonstrates that MALDI-TOF-MS is a reliable and powerful tool for the rapid identification of *Cronobacter* strains to the genus and species level.

P2-21 **Studies on the Growth of *Listeria monocytogenes* and *Lactococcus lactis* in Mixed Cultures**

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Introduction: There is an increasing interest in the food industry for new biological preservation methods. The preservative effect of the different metabolites of lactic acid bacteria (LAB) has been known for a long time. Numerous researchers studied the preventive effect of LAB metabolites to increase the safety of foods by suppressing the growth of foodborne pathogenic bacteria. As lactobacilli inhibit certain pathogens and growth of *L. monocytogenes* can occur during cheese production, the aim of the present work was to study the inhibitory effect of the inoculum level of *Lactococcus lactis* and incubation temperature on the growth of *Listeria monocytogenes* in milk.

Rationale: The effect of nisin production of *Lactococcus lactis* on the growth characteristics of *Listeria monocytogenes* was examined in milk. The effect of temperature (7, 20°C) and the co-ratios with Lc. lactis at different cell count ratios (10:1, 1000:1) on the growth of two different *L. monocytogenes* strains were studied. The Baranyi model was fitted to the obtained growth curves.

Results: There was a difference between the nisin resistance of the two examined *L. monocytogenes* strains in broth but not in milk. A decreasing correlation was observed between the logarithmic maximum population of *L. monocytogenes* and the initial log counts of lactococci. The outcome of the experiments was dependent on the temperature, especially at 20°C. There was no difference between the growth of the two *L. monocytogenes* strains regardless of their nisin resistance.

Conclusion: The Baranyi equation provided good fit for the lag and exponential phase of *L. monocytogenes*. According to our observations, pH decrease does not seem to be the main factor of early stationary phase induction of *L. monocytogenes* in milk.

P2-22 **The European Project BASELINE "Selection and Improving of Fit-for-purpose Sampling Procedures for Specific Foods and Risks"**

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Introduction: Food Safety Objectives (FSOs) and Performance Objectives (POs) are new criteria complementing the existing concepts of microbiological criteria. To achieve these objectives it is critically important a harmonisation of food safety control procedures. The EU project "Selection and improving of fit for purpose sampling procedures for specific foods and risks"-BASELINE, funded under the FP7 program, intends to obtain the following objectives:

Rationale: 1) to review the sampling schemes currently available for food authorities and food producers to collect data for quantitative risk assessment at EU level; 2) to assess the relevance and suitable limit values of POs and FSOs for biological risks, including *Listeria monocytogenes*; 3) to evaluate the need for new or adapted methods for sampling and testing the identified biological risks; 4) to develop predictive mathematical models for the target biological risks; 5) to validate and harmonise the sampling schemes and the alternative detection methods developed in the project; 6) to share and disseminate the scientific knowledge coming out from the project to stakeholders.

Results: The project results will be translated in clear recommendation to the European Commission as well as end users and they will have a significant impact on protection of human health.

Conclusion: A poster detailing the project objectives, strategies and expected impact will be presented at the conference.

P2-23 **The Use of Carvacrol for the Inhibition of *Escherichia coli* O157:H7 on Bovine Hide and Carcass Pieces**

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Introduction: *Escherichia coli* O157:H7 is a major food safety concern worldwide. Contamination of the pathogen from the hide and/or faeces onto the carcass can occur during slaughtering and processing. Antimicrobial intervention strategies can potentially reduce the risk of contamination and transfer of *E. coli* O157:H7 from animals to food and reduce the risk of infection. Essential oil components such as carvacrol (from thyme/oregano oil) have been identified to have strong antimicrobial properties.

Rationale: The aim of this study was to evaluate the antimicrobial activity of carvacrol against *E. coli* O157:H7 on bovine hide and carcass pieces. Different concentrations of carvacrol (0, 1, 2, 3 % v/v) were sprayed onto hide and carcass pieces inoculated with *E. coli* O157:H7. The numbers of *E. coli* O157:H7 following treatment was determined using a swabbing and excision method.

Results: The abilities of the swabbing and excision methods to enumerate *E. coli* O157:H7 from the samples did not significantly ($P > 0.05$) differ. However, analysis of individual data sets for each method found that the numbers of *E. coli* O157:H7 recovered from carcass pieces treated with 3% carvacrol were significantly ($P > 0.05$) differ to the control. For the hide samples, the numbers of *E. coli* O157:H7 following treatment with 3% carvacrol was significantly less (1.3 log CFU/cm^2 reduction) compared to the control for both swab and excision methods.

Conclusion: Results from the study suggest that carvacrol can inhibit *E. coli* O157:H7 on hide and carcass pieces. Further work is required to optimise the use of carvacrol as an antimicrobial intervention strategy as well as investigating the sensory and toxicity issues that may arise with its use in the beef chain.

P2-24 **Thermal Destruction of *Listeria monocytogenes* in Liquid Egg**

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Introduction: Nowadays food manufacturing plants prefer ready-to-use liquid egg products to eggs. However, the shelf life of pasteurized liquid egg products is relatively short, there is a need to elaborate new technologies – like the long term (6–24 hours) heat treatment of liquid egg products at 53–55°C.

Rationale: The aim of our work was to develop a model for a new long term heat treatment under laboratory conditions to reduce viable count of *Listeria monocytogenes* (NCAIM B1371) by this procedure. Liquid egg samples (white, yolk, whole) were inoculated with *Listeria monocytogenes* to reach log 6 CFU/ml, and incubated at 53 and 55°C. Viable cell count was examined in every 30 minutes during 6 hours, and after 24 hours of heat treatment.

Results: Surviving cell count of *Listeria monocytogenes* during long term incubation at 53°C and 55°C was significantly different ($P < 0.05$) in the various liquid egg products. Reduction of viable cells was the fastest in the egg white and slowest in the yolk in each case.

Our measurements have shown that treatment at low temperatures for long time reduced the cell count of *Listeria monocytogenes* below the detection level in all samples irrespectively of the treatment temperature, the previous life of bacteria

Conclusion: The long term heat treatment at 53–55 °C can be used to reduce *Listeria monocytogenes* counts to ensure the microbiological safety of liquid egg products.

P2-25   **Towards the Management of Food Hazards through Risk Analysis in Peru**

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Introduction: In Peru, food safety assurance systems have been incorporated over 15 years ago, and have evolved and progressed in time. While the essential functions of monitoring, inspection, hazard containment and outbreak management have been effectively addressed by regulatory agencies, research activities towards improving food safety have been somewhat disregarded.

Rationale: In today's globalized world, Peruvian authorities have recognized the need to move towards the management of food hazards through the use of risk analysis. The Peruvian Food Safety Law (2008) establishes that food safety management measures should, when possible, be supported by objective, transparent and independent risk analysis studies. Given the economic, social and cultural contrast found in Peru, the realization of risk analysis becomes a great challenge, and given the current policies promoting our agro-export capability, this challenge cannot be delayed. Thus, an initial assessment of the requirements to perform risk analysis in Peru has been conducted.

Results: Although substantial amounts of internal information and data are available, they may neither be in a format suitable for risk assessment nor be of sufficient quality. Regarding activities of monitoring and surveillance, while they are conducted, they should be more comprehensive, frequent, and with a preventive sense. Additionally, a reliable mechanism to collect, aggregate and store sampling/surveillance data is needed. In relation to technical expertise, there is a need for capacity building. Expertise on quantitative risk assessment exists only in a few countries, but transfer of knowledge and experience can be achieved by the establishment of collaborative studies between international organizations (such as FAO, ILSI), academia and Peruvian regulatory agencies.

Conclusion: In this context, the non-profit Peruvian organization "Food is Health" aims to be the link between food safety research and regulatory agencies, with the capacity to scientifically support their national-level food safety management decisions. As such, "Food is Health" welcomes the participation and technical cooperation from expert organizations.

P2-26   Validation of a Model for the Thermal Inactivation of *Bacillus pumilus*, *B. licheniformis*, *B. megaterium* and *B. subtilis* in Soups

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Introduction: Thinning of soups is an important spoilage problem in the food industry. This is believed to be caused by the growth of *Bacillus* spp. that have survived the heating process. Whereas extensive work has been conducted to determine the thermal resistance of *Bacillus* spp. in buffer solutions, to our knowledge no work has been done to validate their resistance in soups.

Rationale: The purpose of this work was to validate a polynomial model which was used to describe the thermal resistance of bacilli and was examined in different soups, tomato ($pH = 4.16$), potato and leek ($pH = 4.94$) and chicken ($pH = 5.90$) at 95, 100 and 105°C. Bias and accuracy factors were used to determine the performance of the model.

Results: Overall bias factors of 0.56, 1.02, 0.45 and 0.46 with accuracy factors of 1.78, 1.25, 2.21 and 2.28 were observed for *B. pumilus*, *B. licheniformis*, *B. megaterium* and *B. subtilis* when heated at different temperatures for the 3 types of soup tested. The predicted model for *B. licheniformis* showed to be fail-safe, whereas for the other 3 bacilli were fail-dangerous. For *B. licheniformis*, the bias factor changed with temperature, with values of 1.37, 1.03 and 0.75 at 95, 100 and 105°C respectively, showing that the model was considered fail-dangerous as temperatures were increased.

Conclusion: The D-values observed in soups for 3 out of the 4 *bacilli* tested were higher than predicted when using data obtained in buffer solutions. This may be attributed to the viscosity of the soup offering protection to the test organisms during the heat treatment, alternatively, other ingredients in the soup recipes may cause this effect. This work will aid the design of thermal processes for the elimination of *Bacillus* spp. in soups.

P2-27   Effect of Irradiation (e-beam) and Heat Treatments on the Microbial Lag Phase

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Introduction: Heating and irradiation reduce or eradicate microorganisms, pathogens or not, in foods. Both may be used in minimally processed foods, or in foods to be consumed raw. However, the minimal processing increases the microbial growth risk.

Rationale: Variability of individual cell lag phase after heat and other preserving processes has been studied. However, no data are available on the effect of irradiation on the survivor micro-organism lag phase variability. Variability is not usually considered in predictive growth/inactivation models and microbial risk assessment, although it may be quite large, especially at low cell concentration levels.

Our objective is to analyze the lag phase of survivors to electron beam irradiation and heat treatments and to compare them in a model system.

Results: Variability of the lag phase was significantly lower (Bartlett test) in controls than in treated samples. Variance of the lag phase was significantly different between the different treatments (0 to 3 decimal reductions), resulting that the more severe treatment, the greater variability in the lag phase was observed. Furthermore, the distribution of the lag phase of the survivors also depended on the type of treatment applied; being the survivor lag phase after irradiation longer than that of heat shocked organisms, while the standard deviation of irradiated samples was lower for the same logarithmic reduction.

Conclusion: Lag phase of survivor cells to heat treatment is more variable than that of irradiated cells, although lag phase of irradiated organism is longer. Furthermore, the more intense the microbicidal treatment, the more variable the outcomes were. We conclude that food manufacturers and microbiological behaviour modellers should take into account the variability among individual cells in a bacterial population in order to improve the accuracy of risk estimation.

P2-28   Variability Analysis of *Pseudomonas fluorescens* Inactivation by Acidification

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Introduction: Twenty-first century consumers like minimally processed foods because of their good sensorial properties, high nutritional value and, usually, convenience of cooking. Food industry must offer this kind of products, although it means the assumption of higher microbiological risks than those of conventional processing. Several preservative techniques may be combined to minimize such risks. Acidification may be one of them. Microbial responses to any preservative technique are variable, even in the case that all conditions are under strict control.

Rationale: In the present study, the *Pseudomonas fluorescens* CECT378 inactivation variability by acidification in three substrates (citrate buffer, peptone / acetic acid and chicken soup/acetic acid) has been analyzed. The final aim of this project is the improvement of the inactivation predictions through different preservative processes.

Results: Standard deviations of control batches (10 samples each) always were lower than those of acidified batches (75 samples each). Furthermore, the more intense the acidification treatments, the bigger the standard deviations (variability) were, although, obviously, the number of survivors was lower. The same findings have been reported with other preserving treatments (heating and irradiation).

Conclusion: This fact, the noticeable variation in the survivor number after intense preserving treatments warns to the need to consider such variation in predictive inactivation models in order to apply a determined food safety objective, mainly in the special case of absence/presence of a microorganism.

P2-29   Preliminary Risk Assessment *Salmonella* in Formulated Dry Foods

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Introduction: Recent U.S. *Salmonella* outbreaks and recalls, including the Peanut Butter Corporation of America outbreak, the Plainview non-fat dry milk recall, and the Basic Food Flavors hydrolyzed vegetable protein recall have highlighted the importance of controlling *Salmonella* in Formulated Dry Foods.

Rationale: This risk assessment was undertaken to assist food companies in managing the risks associated with formulated dry food products that do not support the growth of *Salmonella*.

Results: Specific model components include: serving size, weight of contaminated ingredient per serving, *Salmonella* cells per gram, the effect of negative test results on *Salmonella* prevalence, the effect of thermal processing on *Salmonella* in the dry state and the effect on storage time on *Salmonella* survival. A component of the model was also created to use the effect of environmental sampling test results to predict finished product risk. Estimated number of illnesses resulting from contaminated servings was calculated using the FAO/WHO beta-Poisson dose-response model for *Salmonella*. The risk model was developed using the Microsoft Excel add-in, @Risk (Palisade Corporation, Ithaca, NY).

Conclusion: Results show that even when foods are contaminated with very low levels of *Salmonella*, when millions of servings are simulated, hundreds or thousands of illnesses are predicted to result. Product manufactured with significantly (~1 year) older ingredients represent a measureable lower risk due to *Salmonella* die-off during storage. When hundreds of negative test results are obtained, the predicted risk is lower. Finally, when low water-activity foods are processed, *Salmonella* survival may present a significant risk unless very high temperatures and long times are used.

Poster Session 3 – Friday, 11 June

P3-01   Hazard Analysis Critical Control Points (HACCP) on Food Safety Management System

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Introduction: The HACCP system and guidelines for its application were defined by the Codex Alimentarius Commission. This Commission implements the Joint Food and Agriculture Organisation (FAO) of the United Nations and World Health Organisation (WHO) Food Standards Programme.

Rationale: Hazard Analysis Critical Control Point (HACCP) is a systematic preventive approach to food safety and pharmaceutical safety that addresses physical, chemical, and biological hazards as a means of prevention rather than finished product inspection. HACCP is used in the food industry to identify potential food safety hazards, so that key actions, known as Critical Control Points (CCPs) can be taken to reduce or eliminate the risk of the hazards being realized.

Results: The system is used at all stages of food production and preparation processes including packaging, distribution, etc. The Food and Drug Administration (FDA) and the USA Dept. of Agriculture (USDA) say that their mandatory HACCP programs for juice and meat are an effective approach to food safety and protecting public health.Meat HACCP systems are regulated by the USDA, while seafood and juice are regulated by the FDA. The use of HACCP is currently voluntary in other food industries.

Conclusion: FSIS is continuing the HACCP-based Models Project because the Agency believes that the project has been shown to improve food safety and other consumer protections and expects to publish a proposed rule. The new models capitalize on the food safety and other consumer protection gains garnered by the HIMP project thus far, while still meeting the demands of the inspection laws. Under the Models Project, FSIS is requiring improvements in the protections that are currently achieved under the traditional inspection. Data collected from this project show significant improvements in both food safety and other consumer protections.

P3-02   Antibacterial Effects of Three Iranian Plant Extracts on the Growth of *Listeria monocytogenes* and *Enterococcus faecalis* in Nutrient Broth Medium

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Introduction: Foodborne illness resulting from consumption of food contaminated with pathogenic bacteria has been of vital concern to public health. Consumers are also concerned about the safety of foods containing synthetic preservatives. To reduce health hazards and economic losses due to food borne microorganisms, the use of natural products as antibacterial compounds seem to be an interesting way to control the presence of pathogenic bacteria and to extend the shelf life of processed food. Among these compounds extracts from spices, medicinal plants and herbs have been shown possess antimicrobial activities and could serve as a source of antimicrobial agents against food pathogens.

Rationale: The aim of this work was to evaluate the antimicrobial effects of Wild Garlic (*Allium hirtifolium Boiss.*), Mint (*Mentha piperita L.*) and, Willd Mint (*Mentha Longifolia L. Hudson*) on *Listeria monocytogenes* and *Enterococcus faecalis* using disk diffusion method as a preliminary step and Bioscreen C which is basd on optical density measurements.

Results: Results showed that *Allium hirtifolium* Boiss. extract showed MIC of 1.56% (v/v) for *Listeria monocytogenes* and 0.78% (v/v) for *Enterococcus fecalis*. Using *Mentha piperita* l. MIC

of 0.39 % (v/v) for *Enterococcus faecalis* was obtained but *Listeria monocytogenes* did not grow on exposure to it. And *Mentha Longifolia L. Hudson* showed MIC of 0.78% (v/v) and, 3.125% (v/v) for *Listeria monocytogenes* and *Enterococcus faecalis*, respectively.

Conclusion: The named essential oils exhibit antibacterial activity against the mentioned bacteria in definite amounts, so they may be used as antimicrobial agents in foods but they require more study before commercial applications.

P3-03   Withdrawn

P3-04   Withdrawn

P3-05   Survival to Simulated Gastrointestinal Transit of Lactic Acid Bacteria with Antimicrobial Activity

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Introduction: Some Lactic Acid Bacteria (LAB)are potential as probiotic since they benefit health of the host. Anticrobial activity against pathogens as *H. pylori* and *Salmonella* of this probiotic bacteria has been demonstrated previously and therefore its combination with antibiotic therapy has been accepted. In fact, the reporter from the Maastricht 2000 consensus conference on *H. pylori* include probiotics as "possible" tools for management of the infection. Otherwise, it is require that LAB survival to gastrointestinal conditions (gastric juices as pepsin and pancreatin) and must be able to colonise the intestine and exert its beneficial effect.

Rationale: Determine survival to simulated gastrointestinal conditions of sheep milk LAB isolates with tested antimicrobial activity against *H. pylori* and *Salmonella* strains in order to determine its usefulness as coadjuvant of antibiotic therapy.

Results: Each BAL broth culture (*L. acidophilus* (9O3), *L. paracasei paracasei* (1O10), *L. pentosus* (2O7), *L. plantarum* (Q2,Q3) and *Lc. Lactis lactis* (1O3)) was adjusted to 10^8 CFU/g. Viability to pepsin (pH 2) was evaluated after 0, 5, 40, 180 min of exposition and resistance to pancreatin (pH 8) after 0, 240, 360 min. Total counts (viable and dead) were made by using LIVE/DEAD Bac light™ kit and count platting in MRS agar.

After 3 hours of pepsin exposition, strains 2O7, 1O10, 9O3 presented survival rates of 0.23, 1.21, 1.89%, respectively. Strain 1O3 showed a viability percentage of 11% until 40 min exposition and Q2, Q3 strains yielded viability percentages of 16–6% after 5 min. Plate counting exhibit counts varied between 6.02 and 7.18 log cel/ml. All strains assayed survived for 6 hours in pancreatin juice showed viability percentages between 16–51%.

Conclusion: This study indicate that survival of LAB to gastrointestinal juices resulted to be strain dependant. Then, gastrointestinal tolerance of potential probiotic LAB has to be investigated previously.

Viable cell counts were higher using LIVE/DEAD Bac light™ kit than by culture method. Thus LIVE/DEAD Bac light™ kit method resulted a more effective and rapid tool to determine the viability of LAB strains after exposition to gastrointestinal in vitro conditions.

P3-06   Antibiotic Resistance in *Bifidobacteria* Isolated from the Meat Production Chain

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Introduction: The genus *Bifidobacterium* forms an important part of the intestinal flora in man and animal. Furthermore, representatives of this genus play a role as probiotic component in human and animal nutrition. Today, there are not only concerns about the transfer of antibiotic-resistant pathogens but also about the risk of transfer of antibiotic resistance genes from animal commensals to human pathogens. Additionally, the absence of acquired resistance traits has been recommended as safety criterion in the field of probiotics. Unfortunately, earlier research was mainly focused on antimicrobial susceptibility testing of pathogens and there has been only little discussion regarding the antimicrobial susceptibility of bifidobacteria.

Rationale: Among the recent efforts undertaken to develop a standardized method for antimicrobial susceptibility testing of bifidobacteria, a test medium and standard operation procedures were established. In the scope of these developments, the present study aimed to investigate the antimicrobial susceptibility of bifidobacteria from the meat production chain. Strains exhibiting atypical resistance were screened for resistance genes using molecular tools.

Results: All strains were susceptible to ampicillin and vancomycin. On the contrary, all tested strains were resistant to the aminoglycosides gentamicin and streptomycin. This intrinsic resistance can be traced back to the lack of an electron transport system in anaerobes. In contrast, acquired resistance could be assessed for tetracycline, erythromycin and clindamycin. Hence, underlying resistance determinants were found for the displayed resistant phenotypes.

Conclusion: As shown here, bifidobacteria may act as a potential reservoir for antibiotic resistance genes. Furthermore the results strenghten the requirement to carefully test the antimicrobial susceptibility of bifidobacterial strains intended for use as probiotics in foods.

P3-07   Aspects of Systems Theory in the Analysis of Molecular-biological Based Detection Methods

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Introduction: The implementation of molecular-biological based food pathogen detection is a frequently and intensively discussed topic. Molecular-biological methods for food analysis comprise a detection chain consisting of sample preparation, target purification and a detection assay. Given this systemic character systems theory provides basis for discussion of principles and application of testing methods derived from various scientific areas for specification and validation of pathogen detection.

Rationale: This work describes the structure and strategy of a possible alternative approach for validation and specification of molecular-biological methods to accelerate their broad range implementation into food pathogen detection.

The hypothesis is established that systems theory provides the basis for implementation of test systems, derived from other scientific or technical areas, to specification and validation of molecular-biological food pathogen detection methods, or alternatively as supplemental to existing international standards. The categorisation of black box and white box systems demonstrates a possible classification for pathogen detection methods. Furthermore, the transformation of models from systems theory to the analytical chain of food detection provides new insights for opportunities and restraints for such methods, especially when compared with conventional microbiological methods.

Results: The resulting applicability of Physical-Modelling-Synthesis and System-Identification as used in systems analysis provides two strong instruments for validation of the completely molecular-biological detection process. Equivalence-Class-Formation and Limit-Analysis, which

is the underlying test principle for approvable application of System-Identification by means of Poisson-Analysis, both support specification of the enzymatic assay building the core of a molecular-biological detection chain. This alternative approach is based on validation of the method per se and supports conventional comparative validation according to ISO 16410.

Conclusion: The application of systems theory to problems of food detection by molecular-biological methods provides a strong tool and an alternative approach for the validation of new methods, specification of the enzymatic core method and evaluation of related and unanswered questions in this context.

P3-08 **Cloning and Characterization of a Δ-*prfA Listeria monocytogenes* Strain Containing a Single Copy Genomic Artificial Internal Amplification Control (IAC) for Use as Internal Sample Process Control**

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Introduction: Real-time PCR for food pathogen detection is mostly used with internal amplification controls to monitor the enzymatic reaction and for determination of the efficiency of the reaction. Preliminary methodical steps such as sample preparation and DNA isolation and purification are not included in this kind of control and if at all, checked by external controls. This does not allow for control of single samples and thus negative results imply the possibility of false verification of the pathogen status of the investigated food samples. For this purpose there is need for an internal process control covering the whole detection process.

Rationale: Development and characterization of an internal process control based on a model organism as close related to the actual target pathogen as possible which does not influence the quantitative results of the underlying method for detection of the aimed pathogen.

A Δ-*prfA L. monocytogenes* EGDe strain was cloned with a phage insertion vector to result in a single copy inserted artificial real-time PCR target (IAC) amplified with the primers binding the prfA locus of *L. monocytogenes* resulting in a fluorescence signal not interfering with the respective signal of the *L. monocytogenes* wild type strain.

Results: The Δ-*prfA L. monocytogenes* EGDe strain was characterized as *L. monocytogenes* EGDe, the single copy status of the DNA insertion was demonstrated and the strain was used in context with matrix lysis sample preparation both with artificially and naturally contaminated food samples to demonstrate the use of the control. The resulting corrected values as obtained by the whole molecular detection protocol corresponded to the respective values of contamination as determined according to ISO 11920-2.

Conclusion: The internal sample process control based on IAC+, Δ-*prfA L. monocytogenes* EGDe enables in-sample control for real-time PCR detection of *L. monocytogenes* in food samples. The application of internal sample process controls is essential to cover all steps and integrated methods which are included in molecular biological pathogen detection to provide reliable results.

P3-09   Performance Testing of Selective Enrichment Media for *L. monocytogenes* Using Single Bacterial Cell Manipulation

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Introduction: In the present study, a recently developed method for single bacterial cell manipulation (SBCM) was applied to validation of enrichment media.

Rationale: The purpose of this study was performance testing of selective and unselective enrichment media in the range of < 10 cells avoiding stochastic effects caused by dilution.

The performance of Oxoid One-broth-Listeria and Half-Fraser broth was compared to tryptone soy broth with 6% yeast extract (TSB-Y). The growth of both stressed and unstressed cells was investigated. *L. monocytogenes* cells were manipulated by SBCM as previously published. Inocula of 1, 2, 3 and >3 cells were added to the respective media and the samples were analyzed after 24 and 48 hours of incubation by determination of the optical density of the offspring cultures. Additionally enrichment broths/samples were streaked on selective and unselective solid media by the semi-quantitative three-loop technique and real-time PCR targeting the prfA gene were performed for determination of cell counts of the offspring cultures.

Results: A significant difference in the performance of selective media compared to unselective TSB-Y was identified for unstressed *L. monocytogenes* cells. Chilling stress resulted in no offspring cultures of *L. monocytogenes* in Half-Fraser broth as well as in Oxoid One-broth-Listeria when 1 cell was inoculated compared to 70% offspring in TSB-Y. A coherence of increasing cell numbers in the inoculums to the number of positive offspring cultures was determined.

Conclusion: The use of SBCM generating single cell inocula independent from stochastic effects caused by dilution is a novel and successful tool in performance testing of enrichment media supporting conventional validation procedures. This approach avoids the influence of Poisson distribution and enables the direct evaluation of bacterial cell growth in the range of < 10 initial cells. This provides additional information for more precise determination of the performance of selective and unselective media.

P3-10   Withdrawn

P3-11   Prevalence and Genetic Characterisation of *Campylobacter* spp. in the Irish Beef Chain

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Introduction: *Campylobacter* is the leading cause of foodborne illness in Ireland. Few studies have examined the role of beef in the transmission of *Campylobacter* to humans and even fewer have quantitatively tracked this organism through the beef chain.

Rationale: The aim of this study was to establish the prevalence and diversity of clinically significant *Campylobacter* spp. in the Irish beef chain. To this end, bovine hides (n = 400), corresponding pre-chill, pre-eviscerated carcasses (n = 400), post-chill (24–48 h) carcasses (n = 50) and retail ground beef samples (n = 100) were examined. The virulence potential of all recovered isolates was determined by testing for the presence of genes associated with cytolethal distending toxin activity (cdt). Epidemiological tools were used to establish the relatedness of isolates.

Results: The prevalence of *Campylobacter* spp. on bovine hides and carcasses was 51% and 16% respectively. The level of contamination was between 0.25 and 250 CFU/cm^2. The incidence in post-chill carcasses and ground beef was lower at 8% and 1% respectively. The most predominant

species isolated was *C. jejuni*, followed by *C. coli*, *C. lari*, *C. fetus* and *C. curvus*. Virulence profiling showed that 51 % of *C. jejuni* and 79% of *C. lari* tested positive for the cdt A, B and C subunits, suggesting a high potential for invasion of human cells. PFGE profiles demonstrated both high and low levels of genetic diversity between *C. jejuni* isolates and *C. lari* isolates respectively.

Conclusion: Despite a high incidence of potentially virulent strains of *Campylobacter* on bovine hides and carcasses, the prevalence on retail meats was low, indicating that *Campylobacter* does not survive the stresses of processing. However, the extremely high prevalence and diversity of species encountered within the slaughter environment, combined with the reportedly low infectious dose of the genus, suggest that front-line abattoir personnel are the population most at risk to intra- and extra-intestinal *Campylobacter* infections.

P3-12   Methods for Enumeration of Aerobic and Anaerobic Spore-forming Microorganisms

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Introduction: Thermoresistant spore formers play a special role in food industry. Due to the heat resistance of spores, they survive heat treatment steps and can cause spoilage or intoxication of the finished product. Especially *Bacillus* and *Clostridium* species determine the shelf-life of a variety of heat-treated milk products. In order to minimize problems caused by bacterial spores in foods and food production processes a chain management approach, from raw materials, ingredients to environmental sources, is most effective. Therefore reliable methods for the detection of thermoresistant spore formers are crucial to ensure best microbiological quality.

Rationale: We performed studies to figure out the best detection methods for thermoresistant aerobic and anaerobic spore formers. For the analyses spore solutions of different bacilli and clostridia (*Bacillus cereus*, *Bacillus subtilis*, *Bacillus sporothermodurans*, *Clostridium pasteurianum*, *Clostridium sporogenes*, *Clostridium butyricum*) were prepared. To simulate best a naturally product γ-sterilized infant milk powder was spiked with different spore solutions and used for the analyses. Pasteurization step was initially performed at 80°C to ensure that all spores are detected. General nutrient media, dependent on growth temperature of the microorganisms, were chosen to culture the broadest range of spore formers.

Results: Repeatability and reproducibility of the elaborated methods for enumeration of aerobic and anaerobic mesophilic thermoresistant spore formers (MTS) were shown. MTS aerobic and anaerobic method provides reproducible results within a variation range of approximately 25%. The limit of detection of plate count method is theoretically 1 CFU. However, due to the calculated variation range 1 CFU/g might not always be detectable. For MTS aerobic the limit of detection is experimentally determined as 3 CFU/g, for MTS anaerobic as 4 CFU/g.

Conclusion: We here demonstrated the successful implementation of an enumeration method for spores building a basis for a range of methods for testing improvements in the chain management.

P3-13   Rapid High Throughput Micortitre Plate-based Analysis of Bacterial Load (TVCs)

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Introduction: A rapid high-throughput method for the assessment of microbial metabolism is presented and applied to the determination of Total Viable Counts in raw meat. The method uses a

water-soluble oxygen-sensitive phosphorescent probe (Redlight™) to monitor the oxygen consumption of microbial samples.

Rationale: A proof of concept study was carried out to identify the appropriate threshold (probe signal) at which a marked increase in sample oxygen consumption is observed. The higher the initial microbial load of the sample, the earlier this threshold level is reached; expressed as a characteristic onset time t_O. The objective of this work was to determine if this metric could be used to assess microbial contamination in food samples and to validate the method against the industry standard.

Results: This method was validated against the industry standard 'aerobic plate count' method (ISO:4833:2003), and a strong correlation was observed,($r^2 >0.90$). The developed method is shown to be an alternative to conventional culture methods allowing rapid, high throughput determination of TVC (30°C) in meat samples. Contamination levels as low as low as 1×10^3 cfu/g can be measured within 12 hours instead of the 48 hours required by the conventional method, while samples at $\sim 1 \times 10^8$ CFU/g are identified within an hour. Speed to a CFU/g result is therefore vastly improved. The assay is also less labour and materials intensive, with a single 96-well microtitre plate being the equivalent to 1000 Agar plates. Assay Ruggedness was also assessed and shown to be satisfactory.

Conclusion: The presented rapid TVC test provides a simple, fast, convenient and high throughput alternative to conventional TVC testing. Also, as Redlight allows for the specific detection of microbial oxygen consumption, such measurements can also provide insight into the metabolic effect of a various manipulations.

P3-14 **The 2008 Irish Pork Dioxin Contamination – Lessons for European and Global Risk Managers**

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Introduction: This poster will provide an overview of work undertaken by legal researchers from the UCD Institute of Food and Health on the contamination of Irish pork with dioxins in 2008. This incident was one of the most significant food scares and food recalls in the EU over the past decade. The research has made a number of findings about the effectiveness of food regulation in Europe, with wider implications for risk management generally.

Rationale: The research aims to detail the weaknesses and successes in the Irish management of the 2008 Irish dioxin contamination of pork products. The research objective is threefold: i) to assess the effectiveness of network governance across EU Member States in dealing with the contamination incidents at as early a stage as possible, ii) analyse the adequacy of the Irish approach to food crisis management through comparative analysis with a similar dioxin incident in Belgium in 1999, and iii) investigate the interaction between product liability rules and co-regulatory arrangements for HACCP planning in feed business operations.

Results: Question i): In terms of network governance, weaknesses were identified in the levels of communication and openness between different food safety regulators in different EU Member States in sharing food risk information; this has come about because of a formalization of information exchange networks at the European level and could have negative consequences in another food crisis scenario.

Question ii): Irish crisis management was found to be effective, partly due to the establishment of clear establishment legal limits for dioxins in food and feed and clear sampling and analysis criteria. In addition there was direct learning from previous regulatory failures in Belgium in 1999.

Question iii): Weaknesses in the incentives for public regulators to contribute more substantially to the validation of HACCP plans in feed business operations was also identified. This is partly attributable to European product liability rules which insufficiently account for the role of the regulator when imposing a primary legal responsibility on feed manufacturers to ensure safe production systems.

Conclusion: The conclusions points to a number of aspects of European food regulation which could be enhanced in order to create greater incentives for active protection of public health against food risks. These lessons are not specific to the EU and can benefit food risk managers globally.

P3-15 Monitoring of Ochratoxin A Summer Exposure Through Urinary Biomarker – Portuguese North Residents

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Introduction: The mycotoxin ochratoxin A (OTA), known to be an enzyme inhibitor, immunosupressant, teratogen, nephrotoxin, and a carcinogen, and whose producing fungi find optimal substrates in a variety of cereals and their derived products, allowing the toxin to find its way into the human bloodstream from which it is eliminated through urine. The half-life for this process is rather long, however, which means that OTA can be found in both those fluids. Though levels are generally higher in the bloodstream, advances in methodology and technology have made it possible to achieve an accurate measurement of OTA's concentration level in urine, levels which, studies have shown, possess a better correlation with those of ingestion. Collection of urine is also a less invasive, and therefore preferable, procedure.

Rationale: The OTA in that urine can then be extracted by a solution of $NaHCO_3$ (5%) and the use of immunoaffinity columns, and quantified with recourse to liquid chromatography coupled with fluorescence detection. This method was applied to urine samples collected in three cities in northern Portugal, Bragança, (11 men, 19 women), Coimbra (7 men, 6 women) and Porto (11 men, 10 women) during the summer of 2008.

Results: The method found an OTA incidence of 73.3, 100, and 57.9%, and average values of 0.015, 0.021, and 0.017ng/mL, respectively. These results stand in stark contradiction with those found for the same populations in the winter of 2007, in which Coimbra presented much lower values for both incidence and mean values, while Porto and Bragança, a much higher values for the same parameters.

Conclusion: The results suggest the need for ensuing studies to ascertain if the observed seasonal differences are maintained in a constant pattern in each region, or a one-time situation because of particular environmental conditions.

P3-16 Monitoring of Ochratoxin A Summer Exposure through Urinary Biomarker – Portuguese South Residents

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Introduction: Exposure to Ochratoxin A (OTA) has been reported as widely occurring on the Portuguese mainland given the previous published results from urinary surveys conducted in the season of the winter of 2007. However, surveys on OTA foodstuffs contamination show an increase during the summer periods, which suggests a higher risk of exposure to the consumers, possibly further influenced by anthropometrical parameters and regional dwelling.

Rationale: To contribute to the knowledge on the pattern of OTA exposure as measured by urinary biomarkers was the stirring objective of this work. In the targeted south Portuguese regions, urine was collected from 99 healthy inhabitants (40-Lisbon; 30-Alentejo; 29-Algarve) after informed consent. The samples were then analyzed for OTA using immunoaffinity columns clean-up and HPLC-FD determination (LOQ = 0.008 ng/ml).

Results: Overall results showed a broad incidence (93.9%) at an averagely low level (0.021 ± 0.011 ng/ml). Urine from Lisbonian inhabitants was the least contaminated, both in frequency and mean level (90%; 0.015 ± 0.008 ng/ml), and the ones from Alentejo were the most contaminated (100%; 0.024 ± 0.013 ng/ml). Algarve provided samples presented in-between values, with an incidence of 93.1% and a mean level of 0.022 ± 0.009ng/ml. The same regional pattern of incidence of contamination (Alentejo>Algarve>Lisbon) was observed in the previous studied season, winter 2007, although with no correspondence with the mean levels. In winter season, overall frequency of contamination was inferior to the summer, although with high mean levels.

In a gender analysis, no considerable differences were found between female and male-provided samples, neither in incidence (94% vs. 93.9%) nor in mean level (0.019 ± 0.009 ng/ml vs. 0.023 ± 0.012 ng/ml) in accordance with earlier national and foreign studies.

Conclusion: The results of this study show a south-wide extensive exposure to OTA, more pronounced in the interior region, than in the coast regions, although with no relation with gender.

P3-17 **Melamine Contamination of Milk Powder and Infant Formula on the African Market – A Significant Global Issue with Public Health and International Trade Implications**

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Introduction: Chemical food safety has emerged as a significant global issue with public health and international trade implications. The most recent example was the epidemic of melamine poisoning in China. Locally sold contaminated milk powder and infant formula originating from China have been identified in several countries worldwide, with, until now, the exception of Africa.

Rationale: The present study aimed to determine the incidence of melamine in milk powder and infant formula imported to the (East) African market and the distribution of the contaminated products either through formal/legal or informal/illegal channels. The study took place in Dar-es-Salaam, Tanzania, East Africa. Dar-es-Salaam is one of the fastest growing African megacities, the largest seaport and the centre of international trade in East Africa. For this reason Dar-es-Salaam is a suitable representative location for sampling milk powder products imported to the East African market.
Melamine determination was carried out using the commercially available AgraQuant[®] Melamine Sensitive Assay (Romer Labs[®], Singapore-Pte-Ltd., Jalan Bukit Merah, Singapore). Two categories of samples were collected: (i) market brands of all international companies supplying the East-African market and (ii) illegally imported unlabelled products from the informal channels.

Results: Despite the national import prohibition of Chinese milk products and unlabelled milk powder in Tanzania, 11% (22/200) of the inspected micro-retailers sold milk powder on the local black market. Manufacturers could be identified for only 55% (27) of the 49 investigated batches. Six percent (3/49) of all samples and 11% (3/27) of all international branded products tested, revealed melamine concentrations up to 5.5 mg/kg milk powder. This amount represents about twice the tolerable daily intake (TDI) as suggested by the U.S. Food and Drug Administration (FDA). All melamine-contaminated batches revealed production dates from September 2007 to May 2008.

Conclusion: Our study demonstrates for the first time that, despite official regulations, melamine contamination of milk powder is a significant problem in Africa and clearly indicates that melamine-contaminated milk powder and infant formula had been processed and exported to Africa long before the melamine scandal became a real topic of international attention.

P3-18   Withdrawn

P3-19   Ochratoxin A Exposure Assessment of Porto Bread Consumers during the Summer of 2008

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Introduction: Cereals in general and bread in particular are considered main contributors to ochratoxin A (OTA) exposure. The exposure to this mycotoxin is related to toxic effects, namely nephrotoxicity and carcinogenicity (IARC 2B).

Rationale: The aim of this study was to conduct a detailed exposure assessment of the inhabitants of Porto, by means of measuring OTA content in several types of bread. For that, seventy bread samples (forty-eight white breads, fourteen conventional broa, and eight typical Avintes broa) obtained from assorted retail local stores during the summer of 2008 were analyzed for OTA contamination. The analytical methodology included PBS:CH_3OH extraction, immunoaffinity clean-up and HPLC-FD determination (LOQ=0.1 ng/g).

Results: Almost 96% of the samples were contaminated, up to the maximum level of 1.423ng/g. Contrarily to maize bread, all of the analyzed white bread, which included not only wheat bread, but also whole-grain and seeds-enriched bread, were OTA-positive. However, its average value (0.206 ± 0.095 ng/g) is lower than that for conventional maize (0.328 ± 0.181 ng/g) and Avintes maize bread (0.869 ± 0.508). If these figures are compared to the ones reported from the previous season (winter 2007) in Porto, is evident a general increase in the average values and incidences in bread commercialized during the summer.

If the bread contamination data is further used for estimate dietary intake (EDI), is possible to see that because more consumed the white bread contributes to more than half (58.8 %; 0.371 ng/kg.bw/day) of the OTA EDI through bread consumption (100%; 0.631 ng/kg.bw/day). A value higher than the reported for the previous winter season (0.514 ng/kg.bw/day), reflecting the increase of the mean values, without however surpassing the maximum established levels.

Conclusion: This survey shows that Porto consumers are exposed to bread frequently contaminated with OTA, with visible differences amongst seasons and type of grain-based bread.

P3-20   Ochratoxin A Exposure Assessment through Bread Consumption in Alentejo during the Summer of 2008

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Introduction: With cereals and their derivates being one of the most fundamental food groups in human diet, it's no surprise that more and more importance is being given to its potential contaminants. One such category of contaminants is composed of both fungi that can grow on them and any toxins they might produce. Two of those fungi, *Aspergillus ochraceus* and *Penicillium verrucosum*, are both widespread and mycotoxigenic – specifically, they produce a group of compounds known as ochratoxins, of which at least one, ochratoxin A (OTA), is known to have a variety of potentially deadly toxic effects.

Rationale: It is therefore of great importance to maintain a close monitoring of the amount of that toxin present in the food group. To that effect, 35 units of wheat bread were purchased in the Portuguese region of Alentejo during the summer of 2008 and their OTA content extracted through immunoaffinity columns and then quantified by liquid chromatography coupled with fluorescence detection, a method with a quantification limit of 0.1ng/g.

Results: With exception of one sample (96.97%) all were found to be positive, with nearly one-third (30.30%) containing an OTA amount in excess of the quantification limit. Their values ranged from 0.114 to 0.204 ng/g, with an average of 0.158 ± 0.028 ng/g. Though these results remain well below the EC-established limit of 3 ng/g, there is a noted change over the values registered during the previous winter, when only 60% of the samples were positive and none of which were above the LOQ.

Conclusion: This radical increase instigates the need for a continuous monitoring of this mycotoxin's occurrence values, so as to ensure that the population is not put at risk through the consumption of this fundamental food group.

P3-21   Harmonization of the Pepsin Digestion Method for Anisakis Inspection in Fish Products

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Introduction: The lack of a reference framework for materials and methods has historically hampered the standardization of diagnosis of parasites in seafood. Fish inspection for anisakids is a good example, since up to now no international standards have been developed to inspect this biological hazard in fish products. Available detection methods, like simple visual inspection, candling, pepsin digestion, UV illumination, RT-PCR or immunodiagnoses have been largely used by fishery operators and laboratories as an integrated strategy in official and self-control tests, although nor of them have been standardized.

Rationale: Herein we assess a food safety analytical protocol on the basis of a modification of the artificial digestion of fish flesh by comparing this with that of largely-recognized for frozen and smoked fish, CODEX STAN 244-2004 and CX/FFP 08/29/7 from FAO. To this end we evaluated the current industry chemicals and conditions of the digestion assay in fresh and frozen samples, both in fat and non-fatty fish species, with special attention to the different commercial pepsins.

Results: The results showed that our improved digestion test considerably reduces the assay time. By the other hand, the new method was less expensive that the largely used one for frozen and smoked fish, CODEX STAN 244-2004 and CX/FFP 08/29/7 from FAO.

Conclusion: We can conclude that the new digestion method it is a low-cost, sensitive and reproducible off-site tool that can be useful in the implementation of regional screening programs for the prevention of human anisakidosis (and associated gastroallergic disorders) due to the consumption of raw or undercooked seafood products.

P3-22   The Accuracy of Visual Inspection for Preventing Risk of Anisakis Infection

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Introduction: Anisakids are marine cosmopolitan parasites highly prevalent in wild fish stocks of commercial interest. They are found in great numbers in the third larval stage on the gut cavity and belly flaps during fish inspections. They are recognized as a human health hazard responsible for emergent zoonoses called anisakiasis causing gastro-allergic disorders in consumers, and occupational-asthma in fish-farming workers. EU legislation (EC 853/2004; EC 2074/2005) pointed out that visual inspection of the whole fish abdominal cavity (including liver, gonad and egg mass) should be done by fish operators to control the risk of visible parasites. The accuracy of a visual inspection method in fish industry largely depends on a well-tested statistical significance between the number of observable parasites free or encysted in the abdominal cavity and the number of parasites in the edible part of the fish. This is especially true when expending untreated fresh fish products, because no prophylactic processes have been carried out to kill Anisakis larvae or inactivate their somatic and metabolic antigens during harvest and distribution, making the final consumer manages the hazard.

Rationale: This work deals with the statistical significance of relationships between gut and muscular parasites in commercial lots of 322 fresh individuals of blue whiting *Micromesistius poutassou* and 230 horse-mackerel *Scomber scombrus* caught in Galician waters.

Results: Results revealed no relationships between the number of parasites in gut and muscular of the blue whiting. However, the number of anisakids in gut of Atlantic mackerel was significantly related to the number of anisakids in hypaxial flesh.

Conclusion: This study suggests the low efficiency of visual inspection as a robust statistical predictable value to infer muscular anisakids based on the evidence of gut parasites. Our results can be considered very useful to updated epidemiological and medical findings related to anisakiasis, and to assess the public health risk associated.

P3-23   Toxic Metals (Hg, Pb and Cd) in *Thunnus thynnus* from Thyrrenian Sea (Mediterranean Sea)

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Introduction: Fish is a excellent source of proteins, vitamins and essential minerals, in addition to omega-3 fatty acids, known to support good health. Several studies have documented the long term cardio-protective benefits for human, as well as the reproductive profits of eating fish. However benefits may be off set by the presence of contaminants, particularly toxic metals such as mercury, cadmium and lead. Tunas, as top predators, are able to concentrate large amounts of these metals in their bodies representing, thus, one of the primary dietary sources of these elements to human.

Rationale: Following this premise, the objective of this work is to acquire information on the metal content (Hg, Pb, Cd) in the muscle tissue of *Thunnus thynnus* from the Thyrrenian Sea (Mediterranean Sea) in order to ensure whether their consumption is safe for human. The estimated weekly intake (EWI) of these metals are also evaluated for possible human health risks.

Results: Our results show that mercury (average: 0.61 μg g^{-1} wet weight) concentrations are significantly higher than lead (average: 0.07 μg g^{-1} wet weight) and cadmium (average: 0.01 μg g-1 wet weight) ones ($P < 0.0001$). Concerning mercury, levels exceeding the limit established by the European Union regulation is encountered in 20.0% of the samples analysed, while lead and cadmium concentrations are below the permitted levels in all the samples analysed. The intake of lead and cadmium remains within the established safety margins by the WHO, while for mercury the consumption of larger specimens gives high levels in comparison to PTWI.

Conclusion: These results confirm that mercury intake through the consumption of this type of fishery product is a cause for concern.

P3-24   Comparison of Two PCR Methods Targeting Two Different Gene Sequences for the Detection of *Bacillus cereus* Group Bacteria in Egg Products

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Introduction: *Bacillus cereus* group bacteria are able to produce toxins and their various enzymatic activities are also recognized as causing food spoilage, even at refrigerated temperatures, when psychrotolerant strains are involved. The lack of a rapid and accurate detection method, suitable for a simple routine analysis, is currently the main hurdle for the control of these populations in the sector of egg product manufacturing.

Rationale: The aim of this study was to evaluate the performances of two PCR methods for the detection of *B. cereus* group bacteria. Whole liquid egg products (174 samples) provided by different companies were enriched in 5 g/L lithium chloride in peptone water. After DNA extraction, the detection of *B. cereus* group bacteria was assayed by a classical PCR method targeting a cspF gene sequence, as described by Francis et al. (1998) and by a real-time PCR method targeting a sspE gene sequence, as described by Kim et al. (2005).

Results: Our results show good correlation between both methods for 86% of the analyzed samples. Ten percent of positive samples assayed by real-time PCR, targeting the sspE gene sequence, were found negative under classical PCR detection, targeting the cspF gene sequence. Only 4% of the analyzed samples were negative under real-time PCR detection and positive under classical PCR.

Conclusion: Our results show good correlation between both the PCR methods described in the literature for the detection of *B. cereus* group bacteria in liquid whole egg and probably in other food, by targeting two different specific gene sequences. The method using real-time PCR seems to be more sensible and presents the advantage to be faster than the classical PCR method. Depending on the availability of their laboratory material, one of these methods could be proposed to manufacturers in order to detect this food spoilage and putative pathogenic microbiota.

P3-25   Impact of the Refrigeration of Enrichment Broth on the Performance of Methods of Detection of *Salmonella* and *Listeria monocytogenes*

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Introduction: *Salmonella* or *Listeria monocytogenes* presence is of major concern in food products which, due to their nature, require immediate testing upon reception at the laboratory. Food samples arriving on Friday necessitate the continuation of testing operations throughout the weekend, which can be sometimes problematic. ISO 7218:2007 quotes that "unless otherwise stated, the incubated enrichment broths may only be refrigerated after evaluation of the impact of refrigeration on the results and only if clearly stipulated in the test report." The present study illustrates the evaluation of weekend post-incubation enrichment broth refrigeration impact on the detection of *Salmonella* and *Listeria monocytogenes*.

Rationale: The aim of this study is to measure the impact of refrigerated storage (24 hours at 4°C) of enrichment broths after incubation, and prior to proceeding with the next step in the methods. Two methods were included in the study, the MSRV method (internal validation according to the reference method EN ISO 16140) for the research of *Salmonella* and the OAA method (AFNOR validation BIO 12/14-04/05) for the research of *Listeria monocytogenes*. The evaluation of the performance was made by calculation of the relative detection level, according to the Spearman-Kärber method (LOD50). For each of the two methods, the study was carried out on the two different enrichments (buffered peptone water for the MSRV method and half Fraser for the OAA method, respectively) and with six families of products. Four levels of contamination (between 0 and 9 CFU/25 g) were tested for each of paired analysis (matrix/strain). Six replicates of each condition were conducted.

Results: The level of detection obtained for the method with traditional enrichment was between 0.4 and 5.0 cells per 25 g for *Salmonella* and between 0.1 and 3.8 cells per 25 g for *Listeria monocytogenes*. The results obtained with refrigerated enrichments were essentially the same. The level of detections from broths stored for 24 hours at 4°C after enrichment were 0.4 to 5.0 cells per 25 g for *Salmonella* and 0.1 to 2.3 cells per 25 g for *Listeria monocytogenes*.

Conclusion: Theses results indicate that refrigeration for up to 24 hrs does not affect the sensitivity of the two test methods.

P3-26   ISO 16140 Validation of a New "Next Day" Method for Detection of *Listeria monocytogenes*

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Introduction: Rapid screening of *Listeria monocytogenes* is important for food safety because of the potential for serious disease, and a zero tolerance policy of the European and the US food regulating bodies.

Rationale: This study was designed to provide preliminary and interlaboratory data for the VIDAS *Listeria monocytogenes* (LMX) method, as part of the AFNOR Certification approval process.

Results: The test is based on an automated Enzyme Linked Fluorescent Assay (ELFA) targeting specific *Listeria monocytogenes* (LMO) antigens. Samples were culturally enriched for a total of 26h in LMX broth, before testing in the VIDAS® instrument. Positive results were then confirmed by streaking enrichment broths onto a chromogenic selective agar. A specific enrichment protocol

was proposed for raw milk cheeses. The new method was compared to the ISO 11290-1 reference method, according ISO 16140 standard.

Inclusivity and exclusivity, determined following the whole enrichment process, were both 100% using 60 LMO and 31 non LMO strains The 50% detection limit was found to be between 0.2 and 1.8 CFU/25 g for the new method and between 0.2 and 1.3 CFU/25 g for the reference method.

The comparative study included 384 products, 62 meat, 125 dairy, 66 seafood, 71 vegetable products and 60 environmental samples. 190 samples were confirmed positive by one of the methods, 19 by the immunoassay only, 18 by the cultural method and 153 by both methods. Mac Nemar's analysis at the 5% level showed no significant difference between the VIDAS LMX and the Reference method.

Conclusion: The VIDAS LMX method was certified as an alternative method to the reference ISO 11290-1/A4 method for the detection of *Listeria monocytogenes* in human food products and environmental samples. It provides a rapid, sensitive and convenient method allowing a presumptive result within 27 hours of sample set up.

P3-27   ISO 16140 MicroVal Evaluation of a Chromogenic Medium for the Enumeration of Coagulase-positive Staphylococci in Foods

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*Introduction***:** Oxoid *Brilliance*™ Staph 24 Agar is a new chromogenic medium for the enumeration of coagulase-positive staphylococci (CPS) in foods within 24 hours. Traditionally, Baird-Parker Agar supplemented with Egg Yolk and Tellurite (BPA) has been used for enumerating CPS, but presumptive positive isolates are often difficult to interpret because of the presence of typical and atypical colonies of staphylococci, which both require confirmation.

Methods: This new, alternative medium was evaluated against BPA for the enumeration of CPS from five identified food categories, detailed in ISO 16140:2003. Testing was performed according to ISO 6888-1:1999. Subsequent to the comparative laboratory study, an interlaboratory study was conducted across 11 laboratories, using eight blind samples of pasteurized milk in accordance with ISO 16140:2003.

*Results***:** The alternative medium showed good equivalence to the reference method with dairy, meat, seafood, bakery products and composite/ready-to-eat food samples. Results for inclusivity, limit of detection and quantification limit (LOD=2, LOQ=4) were equivalent for the reference and alternative method. Exclusivity testing of the new medium showed it was more specific than BPA, with no false positive results (0/48) compared to BPA, where 13/48 non-CPS isolates produced typical/atypical colonies. Statistical analysis demonstrated that the alternative medium showed excellent linearity and accuracy. The relative accuracy of the reference and alternative methods were shown to be equivalent (R = 0.999) for all food categories analysed. During the collaborative study, the performance of both media was comparable, in terms of both repeatability (no significant F_r- value for all inoculum levels) and reproducibility (no significant F_R- value for all inoculum levels).

Conclusions: Brilliance Staph 24 Agar proved to be a suitable alternative to Baird-Parker Agar with Egg Yolk Tellurite in both the comparative and collaborative laboratory studies of this ISO 16140 validation. The new medium showed greater specificity than Baird-Parker Agar and enabled accurate results to be gained within 24 hrs.

P3-27   ISO 16140 Validation Study of the *Listeria* Precis Method for *Listeria monocytogenes* Detection in Foodstuffs and Environmental Samples: Inter-laboratory Study

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*Introduction***:** The Oxoid Listeria Precis™ method combines the benefits of:
- ONE Broth-Listeria, a specific medium improving *Listeria* recovery and background microflora inhibition.
- *Brilliance*™ Listeria Agar, a chromogenic and selective medium.

Characteristic colonies are easily and rapidly confirmed with a simple O.B.I.S. ™ Mono test or the tests outlined in the ISO 11290 standards.

*Rationale***:** Comparison of this alternative method with the ISO 11290-1 standard was performed according to the ISO 16140:2003 standard and the AFNOR technical rules. This study has clearly shown that the accuracy, selectivity, specificity and detection limits of the Listeria Precis method are comparable with the standard method, as are the inclusivity and exclusivity results.

*Results***:** In order to assess the variability of the results, a ring trial involving 12 laboratories was organized, using pasteurised milk samples. Eight non-contaminated samples and 16 artificially contaminated samples were analysed by both the Listeria Precis and ISO 11290-1 reference methods by each laboratory. Two hundred eighty-eight data points were generated, gathering 191 positive agreements, 96 negative agreements, 0 negative deviations and one positive deviation, which was confirmed using the ISO 11290-1 tests. The calculated accordance, concordance and odds ratio of the Listeria Precis and ISO 11290-1 reference methods are in agreement. According to the ISO 16140 standard, the relative accuracy, sensitivity and specificity were 100%, 99,7% and 100% respectively, confirming the results of the comparative study.

*Conclusion***:** The interlaboratory study clearly shows that precision in the Listeria Precis method is equivalent to the ISO 11290-1 standard and represents a valuable alternative and user-friendly method for *Listeria monocytogenes* detection in foodstuffs and environmental samples. The Listeria Precis method offers important economic savings to laboratories by minimizing the time taken to obtain results and reducing the number of experimental steps compared to other methods.

P3-29   NordVal Validation pf the DuPont Qualicon BAX® Real-time PCR Assay for Qualitative Analysis of *Campylobacter jejuni, C. coli a*nd *C. lari* in Chicken Cloacae Swab Samples

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Introduction: Currently, *Campylobacter* is the most prevalent microorganism causing foodborne illness in Denmark and many other countries. In many cases, illness is caused by ingestion of only a few bacteria. Therefore, stringent controls during food production are of great importance. Today, many methods of *Campylobacter* testing are available, and the need for faster and more accurate analysis continues. The DuPont Qualicon BAX® Campylobacter Real-Time assay is one of these new methods. It is an automated qualitative method for the direct analysis of *Campylobacter* from poultry cloacae samples in less than three hours.

Rationale: This study details the evaluation of the BAX® Real-Time PCR assay for *Campylobacter jejuni/coli/lari* for directly analysing chicken cloacae swab samples, according to NordVal validation criteria (a Nordic system for validation of alternative microbiological methods) against ISO 10272-1.

Results: In the comparison study, equivalence between the BAX® Real-Time Campylobacter assay and the reference method was demonstrated. To determine test performance (relative accuracy,

relative sensitivity and relative specificity) 60 samples were tested to give approximately 30 positive and 30 negative results by both the reference and alternative methods. All parameters of the test performance were shown to be 100% and therefore very satisfactory. Furthermore, five portions of negative cloacae sample material were spiked with *Campylobacter jejuni* to different levels (0–10 CFU/ml, 1–10 CFU/ml, 10–100 CFU/ml, 100–1000 CFU/ml and 1000–10,000 CFU/ml) and the relative detection level between the two methods demonstrated: 1 CFU/ml for ISO 10272-1 and 100 CFU/ml for the BAX® Real-Time Campylobacter assay. In the collaborative study, the variability of the results obtained by the alternative method in different laboratories and the expert laboratory using identical samples was determined. The specificity (SP) of all methods was shown to be 100%. The accuracy (AC) and the sensitivity (SE) were found to be different, depending on the spiking level and reference method used (ISO 10272-1 or direct plating). AC and SE of 100% were obtained for all methods and for both the seven collaborative laboratories and the expert laboratory when analysing samples containing 100-1000 CFU/ml and above. The level of *Campylobacter* in 30 naturally positive chicken cloacae swab samples analysed in the comparative study must therefore have been at least 100 CFU/ml, as this was demonstrated to be the detection level of the BAX® Real-Time Campylobacter assay. Although having a higher level of detection (100 CFU/ml) the sample volume for the BAX assay is far lower (50 µl) than for ISO 10272-1 (1 ml).

Conclusion: The difference in detection levels of the two assays was not shown to be of concern as *Campylobacter* are found at very high levels (>300 CFU/g) in poultry faecal material and would therefore be detected by the BAX® Real-Time Campylobacter assay. The selectivity of an alternative microbiological method must also be tested according to NordVal validation requirements. This has been previously evaluated during the AOAC-RI study and accepted by NordVal.

P3-30 **Performance Assessment of the Aovel BAX® Real-time *E. coli* O157:H7 Method for *E. coli* O157:H7 Detection in Beef Meat and Raw Vegetables: ISO 16140 Expert Laboratory Study**

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Introduction: The DuPont Qualicon BAX® Real-Time *E. coli* O157:H7 assay is based on new PCR chemistry enabling a shortened enrichment time and PCR steps for *Escherichia coli* O157:H7 detection. The performance of the BAX® Real-Time *E. coli* O157:H7 assay with enrichment times of 7 to 24 hours for raw beef meat products and 8 to 24 hours for raw vegetables was assessed according to ISO 16140:2003 and the AFNOR technical rules, using the ISO 16654:2001 standard as the reference method during a method comparison study according to were validated in the expert laboratory study of this ISO 16140 validation. A 30 minute lysis step, with only a few minutes "hands-on" time, precedes the PCR amplification and detection, which is completed in less than 1 hour. This means that same day results are possible, depending on a laboratory's working day.

Rationale: The BAX® PCR positive results were confirmed by streaking 50 µl of BAX® MP enrichment broth onto CT-SMAC (Sorbitol MacConkey Agar with cefixime and tellurite). Characteristic colonies were confirmed using the Wellcolex™ <*E. coli* O157:H7 latex test (Thermo Fisher Scientific). A specific Qualicon protocol was used to confirm the PCR results of two positive samples which were negative with the reference method. This protocol combines an immuno-magnetic concentration step and plating onto CT-SMAC as a final confirmation step.

Results: The shortest and longest incubation times applicable to each food matrix were evaluated. 148 individual samples of raw beef and raw vegetables, from a range of origins were analysed to determine the relative accuracy, selectivity and specificity: 83 samples gave positive results by one of the methods tested. 63 samples gave a negative result with both methods. 26 positive deviations and 12 negative deviations were observed with the shortest enrichment step, 34 positive deviations and five negative deviations were observed with the longest enrichment step. The

detection limits were defined by analysing two (matrix/strain) pairs, with four contamination levels and six replicates per contamination level in ground beef and frozen spinach. Detection levels varied from 0.1 to 0.9 CFU/25 g for the reference method and from 0.1 to 1.0 CFU/25 g for the alternative method, regardless of incubation time. The inclusivity and exclusivity of the BAX® Real-Time *E. coli* O157:H7 assay were studied, using 50 target strains and 30 non-target strains. All target strains were detected and no cross reactions were observed with the non-target strains.

Conclusion: The results of this study clearly show the satisfactory performance of the BAX® Real-Time *E. coli* O157:H7 method, which represents a valuable and user friendly alternative method for *E. coli* O157:H7 detection in raw beef meat and raw vegetable samples with a very fast time to result possible.

P3-31 Mixture of Chitosan and Cinnamon Oil as Antimicrobial Preservative in Storage of Rainbow Trout

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Introduction: Food quality and safety are major concerns in the food industry as consumers prefer fresher products. In particular, bacterial contamination of products is of concern to human health. Antimicrobial activity of essential oils (EO) were recognized long ago, but their application as natural antimicrobials has recently received increased attention in the food industry. Antibacterial sprays or dips have been done to overcome those contaminations [1]. However, direct surface application of antibacterial substances has some limitations because the active substances could be neutralized, evaporated or diffused inadequately into the bulk of food [2].Currently, studies dealing with edible films or coatings with antimicrobial properties are on the increase. These coatings could prolong the shelf life and safety of foods by preventing growth of pathogenic and spoilage microorganisms as a result of their lag-phase extension and/or their growth rate reduction [3].

Rationale: This study was carried out to evaluate the microbiological quality of fresh rainbow trout filet coated by dipping in 2% (w/v) aqueous solution of chitosan (Ch) and chitosan incorporate with cinnamon (Ch+C), and stored at 4±1°C.

Results: Variations in the value of total viable counts (TVC) during the refrigerated storage are presented in Table 1. The initial TVC ($\log_{10}$ CFU/g) in trout fillet ranged from 3.51 in Ch+ C - coated samples to 3.86 in controls. The increase of TVC in fish flesh during storage has been demonstrated. By the day 8 of storage, however, TVC in trout fillet for all of the different treatments was still below 6 $\log_{10}$ CFU/g, while that of controls attained a count of 7.88 at 12 day, which is higher than the maximal recommended limit of 7 $\log_{10}$ CFU/g for TVC in raw fish [4], indicating a microbiological shelf life of about 9–10 days for the control samples. the initial PTC (day 0) of trout fillet ranged from 2.88 $\log_{10}$ CFU/g, in Ch + C - coated samples, to 3.85 $\log_{10}$ CFU/g in controls (Table 2). Additionally, the growth pattern of PTC showed the same behavior as that of TVC, with control also being the highest at day 16 (8.43 $\log_{10}$ CFU/g), followed by samples coated with Ch (6.79 $\log_{10}$ CFU/g), and a lowest count (6.68 $\log_{10}$ CFU/g) was detected in samples coated with Ch + C.

The count of lactic acid bacteria (LAB) was lower than the other bacterial counts (except for the *Enterobacteriaceae*) determined in this study at the time of spoilage. The initial count ($\log_{10}$ CFU/g) of LAB ranged from 0.33 in Ch + C in coated samples to 0.92 in the controls (Table 3). A final count of 4.19, however, was reached in the control samples at the end of the storage period (day 16), whereas samples coated with Ch did not attain any significant reduction in the LAB count ($P > 0.05$) although they contained 1.6 -logs, lower than the controls. On the contrary, a significant ($P < 0.05$) reduction in LAB count was realized in Ch + C - coated samples when compared with the controls (2.26 versus 4.19).

The growth of *Enterobacteriaceae* was slower than that of the other microbial groups (except for the lactic acid bacteria), starting by less than 0.53 $\log_{10}$ CFU/g and never exceeding 4.69 $\log_{10}$ CFU/g in the control samples. By the end of the storage (day 16), however, much lower ($P < 0.05$) counts of 3.2 and 1.12 $\log_{10}$ CFU/g were achieved in samples coated with Ch and Ch + C, respectively, when compared with controls.

The antimicrobial properties of chitosan coating have been reported in the literature [5–7]. Jeon et al. (2002) described how bacterial growth reached the stationary phase in all chitosan-coated cod and herring samples after 6 days, and also how there was a reduction of up to 3 log cycles between coated samples and controls after 12 days of chilled storage [5]. Also Tsai et al. (2002) found that pretreatment of fish fillets (*Oncorhynchus nerka*) for 3 h with 1% chitosan solution retarded the increase in the counts for mesophiles, psychrotrophs, coliforms, *Aeromonas* spp. and *Vibrio* spp. [6]. L´opez-Caballero et al. (2005) reported that a coating consisting of a blend of chitosan dissolved in acetic acid and gelatin exerted an inhibitory effect on the gram-negative flora of fish patties [7]. Various factors affect the antimicrobial action of chitosan and its mechanism of action appears to be related to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membrane [8] as well as to its function as a barrier against oxygen transfer [5]. In the present study, the coating with CH + C was observed to exert a higher inhibitory effect on the native flora in comparison with Ch coating.

Conclusion: The results generated from this study showed that edible coatings can be developed by incorporating natural compounds with antimicrobial properties against spoilage bacteria.

P3-32   Sample Preparation for Real-time Detection of Foodborne Pathogens

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Introduction: Real-time PCR uses DNA markers to determine the presence or absence of a pathogenic organism, and can be designed to detect a single serotype (e.g. *E. coli* O157:H7), a single species (e.g. *Listeria monocytogenes*), a genus (e.g. *Listeria* species), or higher phylogenetic levels. Real-Time PCR offers advantages of fast time-to-results and multiplex detection of several targets. After enrichment samples must be treated to lyse bacteria and to remove potential PCR inhibitors. Sample preparation can vary in complexity depending on the organism, the sample matrix, and the concentration of the organism.

Rationale: A number of different sample preparation methods were developed and evaluated for improving Real-time PCR detection of pathogenic organisms when present in difficult matrices or for simplifying workflows when less complex matrices are tested.

Results: Food samples were prepared and enriched according to standard procedures using 25 g food in 225 mL enrichment broth. Enriched fruit juices showed no evidence for inhibition of Real-time PCR when added directly to a PCR reaction mix based on amplification of a control plasmid, whereas more complex matrices such as cheese and ground beef showed inhibition. Simple protocols were developed and tested for detection of *E. coli* O157:H7 and *Salmonella* in cheese, ground beef, and vegetable samples after overnight enrichment (16 h) with positive results. For detection of *E. coli* O157:H7 in under 8 h more complex sample preparation was required to process larger volumes of enriched sample and included concentration and clean-up procedures.

Conclusion: Our results demonstrate that sample preparation for Real-Time PCR detection of pathogens in food and environmental samples can be adjusted between the need for fast time-to-result and ease-of-use (and cost savings) depending on enrichment time allowed for bacterial growth.

P3-33 **TEMPO TVC Express: Evaluation of the New Next Day TEMPO Application for Enumeration of Total Aerobic Mesophilic Flora in Raw Meat and Poultry Products**

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Introduction: The rapid quality control of fresh products with a short shelf life is important in the food industry. Part of this quality control process is the enumeration of Total Aerobic Mesophilic Flora. Rapid results for this testing category would represent a significant advantage for meat and poultry producers. Today, the current reference method provides results in three days with most alternative methods providing results in two days. In this study, a new TEMPO application, TEMPO TVC Express, was evaluated. The TEMPO TVC Express provides Total Aerobic Mesophilic Flora enumeration results in 24 hours for raw meat and poultry products. The TEMPO method was compared to the official method ISO 4833: Aerobic Plate Count (APC) incubated for 72 hours at 30°C.

Rationale: Currently, the ISO validated TEMPO TVC (Total Viable Count) provides a result in two days for all food product categories. This new TEMPO application is able to give a next day result on raw meat and poultry products. TVC Express was compared to the ISO reference method for this study. A wide variety of raw meat samples were tested (beef, veal, pork, chicken, turkey, sheep, lamb...) representing more than 70 naturally contaminated samples. A combination of regression analyses, difference in log_{10} distributions and T-tests at the 5% level were used to analyse the data and compare performances.

Results: The performance of the TEMPO TVC Express application compared to the ISO reference method was similar with a 97.1% agreement on the 102 results collected. Regression analysis demonstrated the absence of bias (0.06 [-0.03; 0.15]) between both methods.

Conclusion: The new rapid method, TEMPO TVC Express, is equivalent to the ISO method for enumerating total aerobic mesophilic flora and allows a time to result "next day" compared to 3 days for the reference method. This rapid solution is an additional benefit related to the automated TEMPO system.

P3-34 **Economical Impact of a Dioxin Crisis in the Dutch Dairy Chain**

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Introduction: Food-dioxin crises are a threat to animal and human health as well as the public economy and agri-food chains involved. Control measures that are applied after an incident can influence the magnitude of this threat.

Rationale: The aim of this study is to quantify the financial consequences of a milk-dioxin crisis on the stages of the involved dairy chain in The Netherlands.

Results: Assuming the dioxin contamination started at one feed supplier processing plant, and it was detected after two weeks of the initial contamination, 714 dairy farms, 26 milk processors and 2,664 retailers were involved. The consequent economical impact totaled €151.9 million. The stages of the chain that contributed most to this total are the milk processor (71.9%) and the dairy farm (19.1%). The contribution of the retailer and the feed supplier was 7.9% and 1.1%, respectively. Contamination detected earlier, such as at the third day after its start, could reduce the number of food business involved minimizing the economical impact as low as €11.9 million. The most influential inputs of the model are: 1) the sale price of consumption milk at the processing stage and the daily amount of milk processed per processing plant and; 2) the farm-blocking period and the daily amount of milk produced per farm. However, their effect on the economical impact value is less than 10%.

Conclusion: The results of this study show that the economical impact of a dioxin incident can be high and that early detection of the contamination is very important to limit this impact. These results can be used for underpinning the application of control measures such as to ensure food safety and limit the economical and public health impact of the crisis.