



IAFP'S EUROPEAN SYMPOSIUM ON FOOD SAFETY

PROGRAMME



2021 EXHIBITORS



2021 SUPPORTERS



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New Zealand Food Safety

Ruth Petran, IAFP President Elect
Ruth Petran Consulting, LLC

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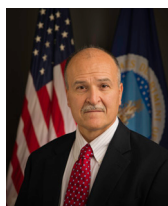
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IAFP'S EUROPEAN SYMPOSIUM ON FOOD SAFETY PROGRAMME AT-A-GLANCE

all times listed in Central European Time (CET)

recordings will be posted for access by registered attendees within 24 hours following the session

Tuesday, 27 April 2021				
Tuesday 10.00 - 10.50	Day 1 Plenary Session <i>Transformers in the Food Safety World – Food Safety Challenges to Master</i> Speaker - Marta Hugas , European Food Safety Authority			
Tuesday 11.00 - 12.30	S1 – Quantitative Microbial Risk Assessment for Food Spoilage	S2 – COVID-19: Assessing Potential Consumer Risk and Managing Value Chain Disruption	Technical Session 1 – Communication Outreach and Education; Food Law and Regulation; Food Safety Systems; General Microbiology; Packaging; Pre-harvest Food Safety; Produce; Viruses and Parasites; Water	Poster Session 1 – Antimicrobials; Beverages and Acid/Acidified Foods; Communication Outreach and Education; General Microbiology; Laboratory and Detection Methods; Meat, Poultry and Eggs; Seafood
Tuesday 12.30 - 14.00	S3 – Foodborne Zoonoses and One Health; What's New in Europe? the One Health EJP!	S4 – Validation of Control Measures for Foodborne Pathogens in Foods: Challenges and Solutions		
Tuesday 14.00 - 15.30	S5 – Biofilm Formation as an Adaptation Strategy for Food-associated Bacteria	S6 – Water Re-use in Operation – How to Clean Up Used Water Sources for Food Use and Consumer Safety in Practice		
Tuesday 15.30 - 17.00	S7 – Next Generation Sequencing (NGS): Pragmatic Considerations from Industrial Perspectives	S8 – What To Decide? Making Informed Decisions for Process Validation and Food Safety Legislation using Stochastic Risk Models		
Wednesday, 28 April 2021				
Wednesday 10.00 - 10.50	Day 2 Plenary Session <i>How AI Can Improve Food Safety</i> Speakers - Julie Pierce , UK Food Standards Agency and Cronan McNamara , Crème Global			
Wednesday 11.00 - 12.30	S9 – <i>Clostridium botulinum</i> : Re-Emerging Risk?	S10 – An Update on the Integration of “Omics” into Risk Assessment	Technical Session 2 – Antimicrobials; Food Toxicology; Laboratory and Detection Methods; Low-water Activity Foods; Meat, Poultry and Eggs; Modeling and Risk Assessment; Molecular Analytics, Genomics and Microbiome; Sanitation and Hygiene	Poster Session 2 – Communication Outreach and Education; Epidemiology; Food Processing Technologies; Food Safety Systems; Microbial Food Spoilage; Modeling and Risk Assessment; Molecular Analytics, Genomics and Microbiome; Packaging; Pre-harvest Food Safety; Produce; Retail and Food Service Safety; Viruses and Parasites; Water
Wednesday 12.30 - 14.00	S11 – Consumer Safety Aspects of Artisanal and Entrepreneurial Food Fermentations	S12 – Processing Environment Monitoring in Low-moisture Foods Production Environments. Are We Looking for the Right Thing(s)/ Microorganisms, in the Right Places?		
Wednesday 14.00 - 15.30	S13 – Distinction between <i>Bacillus Thuringiensis</i> Used in Biopesticide and Presumptive <i>Bacillus cereus</i> Strains Involved in Food Quality & Safety: A Hot Topic	RT1 – Food Safety Impacts of National and Organisational Culture		
Wednesday 15.30 - 17.00	S14 – Viruses: Looking into and Making Sense of Unforeseen Risks for Food Safety	S15 – All Food Processes Have a Residual Risk, Some are Small, Some Very Small and Some are Extremely Small: Zero Risk Does Not Exist		

Let's Make Food Safer Together



NEW DATES

October 27 – 28, 2021

Beijing

Come join 500+ food safety leaders to learn, share, discuss and discover the most recent developments in....

Anti Microbial Resistance • Risk Management in Supply Chain • Standards Harmonization
Emerging Pathogens & New Detection Methods • Food Allergen & Mycotoxin • Food Analysis
Testing & Risk Assessment of Combined Exposure to Multiple Chemicals
Future of Audits • Food Safety Culture • Risk Communications • Consumer Communications
Safety Evaluation of Substances Used for Both Food & Drug (TCM) in China
Food Formulated for Special Medical Purposes • Quality & Safety Reuse of Water in F&B
EU-China Safe Project & Collaboration • Food Integrity • Hot Topics & Industry Seminars
Enhancing Food Safety with AI, Analytics, Block Chain & Other Innovations

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PROGRAMME

27–28 April 2021



WELCOME TO THE IAFP EUROPEAN SYMPOSIUM

All times listed in Central European Time (CET).

TUESDAY, 27 APRIL

Opening Session – Transformers in the Food World – Food Safety Challenges to Master

Convenors: Helen Taylor, Anett Winkler

- 10.00 Welcome and Programme Notes
HELEN TAYLOR, ZERO2FIVE Food Industry Centre,
Cardiff Metropolitan University, Cardiff, United Kingdom
and Anett Winkler, Cargill, Krefeld, Germany
- 10.05 Introduction to IAFP
DAVID THARP, International Association for Food
Protection, Des Moines, IA, USA
- 10.10 Introduction to IAFP's European Symposium
ROGER COOK, New Zealand Food Safety, Wellington,
New Zealand
- 10.20 Food Safety Challenges Ahead to the Food Systems
Transformation
MARTA HUGAS, European Food Safety Authority,
Parma, Italy
- 10.50 Questions & Answers

S1 Quantitative Microbial Risk Assessment for Food Spoilage

Organizer: Kostas Koutsoumanis
Convenor: George-John Nychas

- 11.00 New Developments in Food Spoilage Assessment
GEORGE-JOHN NYCHAS, Laboratory of Microbiology
and Biotechnology of Foods, Department of Food
Science and Human Nutrition, Agricultural University
of Athens, Athens, Greece
- 11.20 Quantitative Microbial Risk Assessment for Food
Spoilage: Principles and Methodology
KOSTAS KOUTSOUMANIS, Laboratory of Food
Microbiology and Hygiene, Department of Food
Science and Technology, Aristotle University of
Thessaloníki, Thessaloníki, Greece
- 11.40 Quantitative Microbial Risk Assessment Applied to
Fungal Spoilage of Bakery Products and Fruit Purees
JEANNE-MARIE MEMBRÉ, Secalim, INRAE Oniris,
Nantes, France
- 12.00 Discussion and Questions and Answers

S2 COVID-19: Assessing Potential Consumer Risk and Managing Value Chain Disruption

Organizer: Leon Gorris

Convenor: Marcel Zwietering

- 11.00 COVID-19: Science Update and View on Potential Food Safety Risks
LEON GORRIS, Food Safety Expert, Food Safety Futures, Nijmegen, The Netherlands
- 11.20 Food Safety Regulatory Challenges of the COVID-19 Pandemic
WAYNE ANDERSON, Food Safety Authority of Ireland, Dublin, Ireland
- 11.40 Navigating Challenges of COVID-19 in the Food Supply Chain
JOHN DONAGHY, Nestlé S.A., Vevey, Switzerland
- 12.00 Discussion and Questions and Answers

S3 Foodborne Zoonoses and One Health; What's New in Europe? The One Health EJP!

Organizers: Christophe Cordevant, Hein Imberechts

Convenors: Anne Brisabois, Daniele Sohier

- 12.30 Foodborne Zoonoses and One Health Approach; What's New in Europe? The One Health EJP!
HEIN IMBERECHTS, Sciensano, the Belgian Institute for Health, Brussels, Belgium
- 12.50 Tox-Detect: Development and Harmonization of Innovative Methods for Comprehensive Analysis of Foodborne Toxigenic Bacteria, i.e., *Staphylococci*, *Bacillus cereus* and *Clostridium perfringens*
JACQUES-ANTOINE HENNEKINNE, Université Paris-Est, ANSES, Maisons-Alfort, France
- 1.10 Cohesive: Organizing Risk Analysis for (re-) Emerging Zoonoses: A One Health Approach
KITTY MAASSEN, RIVM, Bilthoven, The Netherlands
- 1.30 Discussion and Questions and Answers

S4 Validation of Control Measures for Foodborne Pathogens in Foods: Challenges and Solutions **Organizers and Convenors: Erdogan Ceylan, Heidy Den Besten**

- 12.30 Process and Product Parameters to Consider for Designing a Validation Study
ANETT WINKLER, Cargill, Krefeld, Germany
- 12.50 Determining Performance Criteria for a Validation Study
ELISSAVET GKOGKA, Arla Innovation Centre, Aarhus N, Denmark
- 1.10 Obtaining Scientific Evidence and Data Evaluation for Process Validation
HEIDY DEN BESTEN, Wageningen University and Research, Wageningen, The Netherlands
- 1.30 Discussion and Questions and Answers

S5 Biofilm Formation as an Adaptation Strategy for Food-associated Bacteria

Organizer: Moshe Shemesh

Convenors: Romain Briandet, Moshe Shemesh

- 2.00 Identifying the Role of Spatial Organization of Biofilms in Their Persistence
ROMAIN BRIANDET, INRAE, Paris, France
- 2.20 Affecting Biofilm Formation during Milk Processing Improves the Safety and Quality of Dairy Food
MOSHE SHEMESH, Agricultural Research Organisation, Rishon LeZion, Israel
- 2.40 Role of Biofilm Formation by *Bacilli* in Adaptation to Food Matrices
SATISH KUMAR, Agricultural Research Organization, Rishon LeZion, Israel
- 3.00 Discussion and Questions and Answers

S6 Water Re-Use in Operation – How to Clean Up Used Water Sources for Food Use and Consumer Safety in Practice

Organizer and Convenor: Leon Gorris

- 2.00 Achieving Microbiologically Safe Water Re-Use in Food Operations: Rules and Tools
PHYLLIS POSY, PosyGlobal, Jerusalem, Israel
- 2.20 What are the Options to Prevent and Control Chemical Risks Associated to Water Re-Use?
JOSEP MOLAS PAGES, Coca-Cola Company, Madrid, Spain
- 2.40 Integrating Safe Water Re-Use into the Management Systems of Food Operations
SUSANNE KNOCHEL, University of Copenhagen, Copenhagen, Denmark
- 3.00 Discussion and Questions and Answers

S7 Next Generation Sequencing (NGS): Pragmatic Considerations from Industrial Perspectives

Organizers: Francois Bourdichon, Adrienne Klijn

Convenors: Roy Betts, Francois Bourdichon

- 3.30 WGS and Implications for the Food Industry
ADRIANNE KLIJN, Société des Produits Nestlé SA, Lausanne, Switzerland
- 3.50 Food Safety and Quality Applications of NGS
MARTIN WIEDMANN, Cornell University, Ithaca, NY, USA
- 4.10 Roundtable: NGS and WGS in My Factory – Really?
FRANCOIS BOURDICHON, DiSTAS, Università Cattolica Del Sacro Cuore, Piacenza, Italy
- 4.30 Discussion and Questions and Answers

- S8 What to Decide? Making Informed Decisions for Process Validation and Food Safety Legislation Using Stochastic Risk Models**
Organizer: Heidi Den Besten
Convenor: Marcel Zwietering
- 3.30 A Definition of Probabilistic Models Including Variability of the Bacterial Response Using a Multi-level (Bayesian) Approach
 ALBERTO GARRE, Wageningen University, Wageningen, The Netherlands
- 3.50 How Can Stochastic Models Including Variability be Used in Validation of Industrial Processes?
 JEAN-CHRISTOPHE AUGUSTIN, Danone, Palaiseau, France
- 4.10 How Can Variability be Accounted for in Governmental Risk Assessments?
 WINY MESSENS, European Food Safety Authority (EFSA), Parma, Italy
- 4.30 Discussion and Questions and Answers
- T1 Technical Session 1 – Communication Outreach and Education; Food Law and Regulation; Food Safety Systems; General Microbiology; Packaging; Pre-harvest Food Safety; Produce; Viruses and Parasites; Water**
- T1-01** A Review of the Relative Proportion of Foodborne Disease Associated with Food Preparation or Handling Practices in the Home
 11.00 ANITA EVES, Elizabeth C. Redmond, Monique Raats, University of Surrey, Guildford, United Kingdom
- T1-02** The Safety of Cultured Meat
 11.15 LINSAY KETELINGS, Food Claims Centre Venlo, Maastricht University, Venlo, The Netherlands
- T1-03** Evaluation of Hygiene Parameters in Chicken Slaughterhouses in Lombardy and Emilia Romagna during 2020
 11.30 GUIDO FINAZZI, Matteo Gradassi, Irene Bertoletti, Paolo Bonilauri, Lia Bardasi, Franco Paterlini, Giuliana Cammi, Mario D’Incau, Laura Fiorentini, Food Safety Department, Istituto Zooprofilattico Sperimentale della Lombardia e dell’Emilia Romagna, Brescia, Italy
- T1-04** Evaluation of Hygiene Performance Rating for Assessment of Cattle and Sheep Slaughter Hygiene
 11.45 SIGRUN J. HAUGE, Ole-Johan Røtterud, Truls Nesbakken, Miguel Prieto, Marianne Sandberg, Gro Johannessen, Ole Alvseike, Animalia, Oslo, Norway
- T1-05*** Multi-Hurdle Approach Toward *Listeria monocytogenes* Inactivation in Fermented Meat Sausage: High Pressure Processing Assisted by Bacteriophage P100 and Bacteriocinogenic *Pediococcus acidilactici*
 12.00 CLÁUDIA MACIEL, Norton Komora, Renata A. Amaral, Joana Isidro, Sonia Castro, Jorge Saraiva, Paula Teixeira, Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
- T1-06** Assessment of Handwashing Equipment Cleanliness in Food Manufacturing Environments
 12.15 EMMA J. SAMUEL, Ellen W. Evans, Rowena E. Jenkins, Elizabeth C. Redmond, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, Wales, United Kingdom
- T1-07*** Impact of High Hydrostatic Pressure on the Stability of Bacteriophage SALMONELEX™ Towards Potential Application on *Salmonella* Inactivation
 12.30 CLÁUDIA MACIEL, Ana Campos, Norton Komora, Carlos A. Pinto, Rui Fernandes, Jorge Saraiva, Paula Teixeira, Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portugal
- T1-08** RNA-Seq Analysis Revealed Differences in the Global Transcriptome of *Clostridium perfringens* Isolates
 12.45 ABAKABIR ABDELRAHIM, Olivier Firmesse, Anses-Laboratory for Food Safety, Maisons-Alfort, France
- T1-09** GFSI Scopes II and III: Background to Their Development and Purpose
 1.00 JOHN HOLA, GFSI Hygienic Design EHEDG; and Kersia Group, Bury, United Kingdom
- T1-10** Establishment of GFSI Hygienic Design Benchmarking Requirements and Role of 3A-SSI and EHEDG
 1.15 PATRICK WOUTERS, GFSI Hygienic Design TWG; EHEDG; and Cargill, Amsterdam, The Netherlands
- T1-11** Hygienic Design as a Holistic Concept within Food Production Facilities
 1.30 DEBRA SMITH, Global Hygiene Specialist, Vikan, EHEDG UK:IE, Swindon, United Kingdom
- T1-12*** Isolation, Stability and Characteristics of High Pressure Superdormant *Bacillus* Spores
 1.45 ALESSIA I. DELBRÜCK, Yifan Zhang, Vera Hug, Clement Trunet, Alexander Mathys, ETH Zurich, Zurich, Switzerland
- T1-13*** *Listeria monocytogenes* Comes in Different Shades: Clinical- and Food-associated Strains Vary in Virulence, Stress Resistance, and Carbon Source Metabolism
 2.00 FRANCIS MUCHAAMBA, Athmanya Eshwar, Ueli von Ah, Marc J.A. Stevens, Roger Stephan, Taurai Tasara, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
- T1-14*** Behaviour of Staphylococcal *egc* Enterotoxins during Bacterial Growth and Under Food Production-like Stress Conditions
 2.15 LIVIA SCHWENDIMANN, Thomas Berger, Jacques-Antoine Hennekinne, Ivana Ivanovic, Yacine Nia, Michel-Yves Mistou, Hans-Ulrich Graber, Agroscope, Bern, Switzerland
- T1-15*** Attachment Ability and Strength of *Bacillus cereus* and *Bacillus Thuringiensis* on Spinach Leaves
 2.30 XINGCHEN ZHAO, Laurent Van den Storme, Monica Höfte, Mieke Uyttendaele, Ghent University, Ghent, Belgium

- T1-16** Hazard Prioritization of Substances Used in Printing
2.45 Inks and Adhesives Applied to Plastic Food Packaging
EDOARDO GALBIATI, Liesbeth Jacxsens, Bruno De Meulenaer, Research Group Food Chemistry and Human Nutrition (nutriFOODchem), Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
- T1-17** Differential Survivability of Two Genetically Similar
3.00 *Salmonella* Thompson Isolates on Pre-Harvest Basil (*Ocimum basilicum*) Leaves
YE HTUT ZWE, Xinyi Pang, Mei Zhen Michelle Ten, Dan Li, Department of Food Science and Technology, National University of Singapore, Singapore
- T1-18** Performance Assessment of the Canadian Food
3.15 Inspection Agency's Feed Mills Risk Assessment Model Outputs
GENEVIEVE COMEAU, Manon Racicot, Virginie Lachapelle, Alexandre Leroux, Ornella Wafo Noubissie, France Provost, Romina Zanabria, Sylvain Quessy, Canadian Food Inspection Agency, St-Hyacinthe, QC, Canada
- T1-19*** A DNA Prime/Protein Boost Flagellin-based Vaccine
3.30 Against *Campylobacter* in SPF White Leghorn Chickens: A Model to Gain Further Insights about the Role of Chicken Immune System in Response to Anti-*Campylobacter* Vaccination
NOÉMIE GLOANEC, Daniel Dory, Ségolène Quesne, Véronique Beven, Typhaine Poezevara, Alassane Keïta, Marianne Chemaly, Muriel Guyard-Nicodème, ANSES, Laboratory of Ploufragan-Plouzané-Niort, Viral Genetics and Biosafety Unit, Ploufragan, France
- T1-20** Removal of Parasite Transmission Stages from Berries
3.45 Using Washing Procedures Suitable for Consumers
TAMIRAT TEMESGEN, Norwegian University of Life Sciences, Oslo, Norway
- T1-21** Norovirus Detection in Berries – Outbreak and Surveillance Results
4.00 ANNIE LOCAS, Dominic Lambert, Helen Zhang, Rachel Bissonnette, Etsuko Yamamoto, Marina Steele, Canadian Food Inspection Agency, Ottawa, ON, Canada
- T1-22*** Evaluation of Viral Infectivity during the Frozen Storage
4.15 of Berries
ALYSSA KELLY, Brienna Anderson-Coughlin, Kalmia Kniel, University of Delaware, Newark, DE, USA
- T1-23*** Applying Sequencing Approaches to Comprehensively
4.30 Characterize the Microbiological Quality of Non-Traditional Water Sources Used for Food Crop Irrigation: A Conserve 2-Year Field Study
SUHANA CHATTOPADHYAY, Sarah Allard, Anthony Bui, Leena Malayil, Manan Sharma, Kalmia Kniel, Shirley A. Micallef, Fawzy Hashem, Salina Parveen, Eric May, Amir Sapkota, Mihai Pop, Mary Callahan, Hillary Craddock, Rianna Murray, Cheryl East, Eric Handy, Prachi Kulkarni, Shani Craigh, Maryland Institute for Applied Environmental Health, University of Maryland, School of Public Health, College Park, MD, USA
- T1-24** Occurrence of Human Enteric Virus and Coliphages
4.45 in Water Reuse Systems: From the Wastewater Treatment Plant to the Irrigation Point of Use for Leafy Greens
PILAR TRUCHADO, Maria Gil, Gloria Sánchez, Ana Allende, CEBAS-CSIC, Murcia, Spain

P1 Poster Session 1 – Antimicrobials; Beverages and Acid/Acidified Foods; Communication Outreach and Education; General Microbiology; Laboratory and Detection Methods; Meat, Poultry and Eggs; Seafood

Virtual Meeting

Antimicrobials

- P1-01 Relative Quantification of the Expression of Some Key Stress Response and Virulence Associated Genes in Stationary Phase *Listeria monocytogenes* Cells Surviving Sub-lethal Antimicrobial Exposure — Eleanna Kokkoni, Nikolaos Andritsos, Christina Sakarikou, Sofia Michailidou, Anagnostis Argiriou, EFSTATHIOS GIAOURIS, Department of Food Science and Nutrition, School of the Environment, University of the Aegean, Lemnos, Greece
- P1-02* Electrolysed Water for Fresh Produce Treatment: Effects of Organic Contaminants and Outgrowth Delays from Treated Spores — FRANZISKA WOHLGEMUTH, Rachel L. Gomes, Ian Singleton, Frankie J. Rawson, Simon V. Avery, School of Life Sciences, University of Nottingham, Nottingham, United Kingdom
- P1-03 *In Vitro* Study of Antimicrobial Activity of Essential Oils and Their Components against the Main *Clostridium difficile* PCR-Ribotypes Isolated in Belgium — CRISTINA RODRIGUEZ, Hasika MITH, Bernard Taminiou, Nicolas Korsak, Georges Daube, Antoine Clinquart, University of Liege, Faculty of Veterinary Medicine, FARAH, Food Microbiology, Liège, Belgium
- P1-04 Distribution of Antimicrobial Resistance Genes in Various Types of Food Including Meat, Produce (Vegetables and Fruits) and Dairy Products in Canada — SOHAIL NAUSHAD, Chris Grenier, Beverley Phipps-Todd, Andrea Arzate, Karen Zhao, Nur Syifa Azmil, Dele Ogunremi, Hongsheng Huang, Ottawa Laboratory - Fallowfield, Canadian Food Inspection Agency, Ottawa, ON, Canada

Beverages and Acid/Acidified Foods

- P1-05 Rapid Determination of Lactic Acid Bacteria from Fermented Green Olives Packaged in Modified Atmospheres by Means of FTIR Spectroscopy and Machine Learning — Effrosyni Karagianni, Aikaterini Tzamourani, George-John Nychas, EFSTATHIOS PANAGO, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece

Communication Outreach and Education

- P1-06 Food Safety Research Among People with Intellectual Disabilities — MARIE LANGE, Päivi Adolffson, Ingela Marklinder, Uppsala University, Uppsala, Sweden

General Microbiology

- P1-07* Effect of Heavy Water Incorporation on the Viability on *Listeria innocua* — SYLVAIN TRIGUEROS, Thomas Brauge, Sabine Debuiche, Véronique Rebuffel, Pierre Marcoux, Graziella Midelet, French Alternative Energies and Atomic Energy Commission CEA, LETI, Minatec-Campus, Grenoble, France
- P1-08 The Effect of Temperature on Development of *Salmonella enterica* ser. Enteritidis and *Pseudomonas fluorescens* Biofilms — Valentina Theodoulidou, Eirini Schoina, Efstathios Panagou, GEORGE-JOHN NYCHAS, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
- P1-09 The Effect of Seawater Salinity on Biofilm Formation of Seven Bacteria of Aquaculture Interest — George-John Nychas, EIRINI SCHOINA, Marianthi Tsimogianni, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
- P1-10* Microbiological Quality of Fresh Produce Purchased from Street Vendors and Stored in Homes in Informal Settlements of Gauteng Province — TINTSWALO BALOYI, Stacey Duvenage, Erika du Plessis, Lise Korsten, University of Pretoria, Pretoria, South Africa
- P1-11* Real-time Observation of Sub-Lethally Acid Injured *Listeria monocytogenes* and Resuscitation Capacity at Single-cell Level — MARIANNA ARVANITI, Panagiotis Tsakanikas, Vaia Ntrigiou, Artemis Giannakopoulou, Panagiotis Skandamis, Laboratory of Food Quality Control and Hygiene, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
- P1-12 Exploring Perceptions and Self-reported Food Safety Practices of Pet Owners, Providing Raw Meat-based Diets to Pets — VERONIKA BULOCHOVA, Ellen W. Evans, Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, Cardiff, United Kingdom
- P1-13* Prevalence Study of Presumptive *Bacillus cereus* from Fresh to Frozen Spinach with the Indication of Using *Bacillus Thuringiensis* Biopesticides — XINGCHEN ZHAO, Laurent Van den Storme, Pieter Spanoghe, Mieke Uyttendaele, Ghent University, Ghent, Belgium

Laboratory and Detection Methods

- P1-14* Comparison of Compact Dry and Conventional ISO Methods for Microbiological Survey of Lettuce Varieties at Retail Stage — SUSANNA AIYEDUN, Mark Swainson, Ronald Dixon, Bukola Onarinde, University of Lincoln, Holbeach, United Kingdom
- P1-15 Evaluation of the Assurance® Gds MPX Top-7, MPX-ID and Ehec-ID to Detect, Confirm and Isolate *Escherichia coli* Producing Shiga-Toxins (STEC) Serogroups in Raw Dairy Products — Marion Bouvier-Crozier, Mélissa Canizares, Cong Yu, DAVID TOMAS, Thierry Muller, Delphine Thevenot-Sergentet, MERCK Life Science, Madrid, Spain

- P1-16 Detection of Viable *Listeria monocytogenes* by Multiplex Reverse Transcriptase Real-time PCR, Using Two Target Genes — SARAH AZINHEIRO, Dipak Ghimire, Joana Carvalho, Marta Prado, Alejandro Garrido-Maestu, International Iberian Nanotechnology Laboratory, Braga, Portugal
- P1-17* Application of Recombinase Polymerase Amplification with Lateral Flow for a Naked-eye Detection of *Listeria monocytogenes* on Food Processing Surfaces — SARAH AZINHEIRO, Joana Carvalho, Marta Prado, Alejandro Garrido-Maestu, International Iberian Nanotechnology Laboratory, Braga, Portugal
- P1-18 Assessment of a Real-time PCR Method for the Detection of Shiga Toxin-Producing *Escherichia coli* — Ana-Maria Leonte, Cécile Bernez, Muriel Bernard, Christophe Quere, Maryse Rannou, DANIELE SOHIER, Evangelos J. Vadoros, Thermo Fisher Scientific, Basingstoke, United Kingdom
- P1-19 Glyphosate, Glufosinate, and Their Metabolites in Food of Animal Origin: Analytical Method Optimization and Validation — MARINE LAMBERT, Antoine Ducrocq, Fiona Lobo, Gwenaëlle Lavison-Bompard, Chanthadary Inthavong, Université Paris-Est, ANSES, Laboratory for Food Safety, Maisons-Aflort, France
- P1-20 AOAC Assessment Program of Real-time PCR Method for *Salmonella* Detection in Cocoa and Chocolate Products after Post-enrichment Pooling on Large Test Portions — Wesley Thompson, David Crabtree, Benjamin Bastin, Kateland Koch, Matthew Hahs, DANIELE SOHIER, Thermo Fisher Scientific, Basingstoke, United Kingdom
- P1-21 ISO 16140-2:2016 Validation Study of a Real-time PCR Workflow for *Salmonella* Detection in Large Test Portions of Cocoa and Chocolate Products — Wesley Thompson, David Crabtree, Benjamin Bastin, Kateland Koch, Matthew Hahs, DANIELE SOHIER, Thermo Fisher Scientific, Basingstoke, United Kingdom
- P1-22 A Multiplex Real-time PCR Kit for the Detection of Food-relevant *Listeria* Species and Identification of *Listeria monocytogenes* in a Single Reaction — BENJAMIN JUNGE, Astrid Grönwald, Cordt Grönwald, BIOTECON Diagnostics, Potsdam, Germany
- P1-23 Workflow Evaluation for a Detection Method of SARS-CoV-2 from Environmental Surfaces — Patrick Stephenson, David Crabtree, DANIELE SOHIER, Matthew Hahs, Thermo Fisher Scientific, Basingstoke, United Kingdom
- P1-24 Identification of Soya, Maize, and Rapeseed Taxon in Food and Feed GMO Samples — Hans-Henno Dörries, Ivonne Remus-Dörries, IVO MEIER-WIEDENBACH, Cordt Grönwald, Kornelia Berghof-Jäger, BIOTECON Diagnostics, Potsdam, Germany
- P1-25 Validation of the New Genedisc® Method for the Combo Detection of *Campylobacter* and *Salmonella* in Chicken Neck Skin Samples — Ezequiel Gilligan, Christelle Nahuet, Sarah Jemmal, Stéphane Bonilla, SYLVIE HALLIER-SOULIER, Pall GeneDisc Technologies, Bruz, France
- P1-26 Collaborative Study to Evaluate the Use of Next Generation Sequencing for Food Authenticity — MARIO GADANHO, Tiina Karla, Milja Tikkanen, Nicole Prentice, Amanda Manolis, Thermo Fisher Scientific, Lisbon, Portugal

Meat, Poultry and Eggs

- P1-27 Detection of Minced Beef Adulteration by Means of UV-VIS Spectrometer — LEMONIA-CHRISTINA FENGOU, Alexandra Lianou, Panagiotis Tsakanikas, Efstathios Panagou, George-John Nychas, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
- P1-28 Changes in the Diversity of Microbiota of Chicken Breast and Thigh Fillets during Shelf Life at Different Storage Temperatures Monitored Using Next Generation Sequencing — DIMITRA DOUROU, Athena Grounta, Agapi Doulgeraki, Anthoula Argyri, Nikos Chorianopoulos, Chrysoula Tassou, Hellenic Agricultural Organisation DIMITRA, Institute of Technology of Agricultural Products
- P1-29 Spoilage of Chicken Liver at Refrigerated Temperatures and Fate of Inoculated *Salmonella* Enteritidis — ATHENA GROUNTA, Dimitra Dourou, Anthoula Argyri, Nikos Chorianopoulos, Agapi Doulgeraki, Chrysoula Tassou, Hellenic Agricultural Organisation DIMITRA, Institute of Technology of Agricultural Products
- P1-30 Identification of *Salmonella* spp. and Differentiation between Enteritidis and Typhimurium in One Real-time PCR Test — Anne Rölfing, BENJAMIN JUNGE, Cordt Grönwald, Kornelia Berghof-Jäger, BIOTECON Diagnostics, Potsdam, Germany
- P1-31 Animal Species Detection in Meat and Processed Food: Identification of Pork, Beef, Horse, Donkey, and Zebra Meat in One Single Real-time PCR Reaction — Anne Rölfing, Maren Brose, IVO MEIER-WIEDENBACH, Cordt Grönwald, Kornelia Berghof-Jäger, BIOTECON Diagnostics, Potsdam, Germany

Sanitation and Hygiene

- P1-32 Temperature and Hygiene Conditions of Consumers' Refrigerators in Slovenia — ANDREJ OVCA, Tina Škufca, Mojca Jevšnik, University of Ljubljana, Ljubljana, Slovenia
- P1-33 Survey of Bacterial Pathogens at Spanish Small-scale Factories of Traditional Fermented Sausages — ARÍCIA POSSAS, Olga María Bonilla-Luque, Javier Sánchez-Martín, Antonio Valero, University of Cordoba, Department of Food Science and Technology, Cordoba, Spain

- P1-34 A Microbiological Survey of Protein Shaker Bottles and Self-reported Cleaning Practices — JAMES BLAXLAND, Simon Dawson, Ellen W. Evans, Elizabeth C. Redmond, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom
- P1-35 Use of Surrogate Bacteria for Validation and Verification of Chemical Sanitation Steps — Pauline Bouveret, Priscilla Piller, Virginie Pignard, Pierre-Olivier Beal, PIERRE-ALEXANDRE JUAN, Moussa Ndiaye, NOVOLYZE, Daix, France
- P1-36 Identifying Barriers Towards Optimal Cleaning and Sanitation Practices in a Small- and Medium-sized Enterprise (SME) Food Manufacturer — ALIN TURILA, Elizabeth C. Redmond, Cardiff Metropolitan University, Cardiff, Wales, United Kingdom

Seafood

- P1-37 Occurrence of Histamine in Canned Fish Available in Poland and Changes of Histamine Contents during Their Production — MIROSLAW MICHALSKI, Marzena Pawul-Gruba, National Veterinary Research Institute, Pulawy, Poland
- P1-38 Investigation of the Microbiological Quality of Edible Marine Algae, *Alaria esculenta*, Originated from Ireland — Anastasia Lytou, Dimitra Lymperi, Joanne Casserly, Efstathios Panagou, GEORGE-JOHN NYCHAS, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
- P1-39 Microbiological Quality Assessment of Seaweed *Alaria esculenta* Originated from Scotland and Harvested in Two Different Years — ANASTASIA LYTOU, Nick Sarris, Kati Michalek, Michele Stanley, Eirini Schoina, Efstathios Panagou, George-John Nychas, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece

WEDNESDAY, 28 APRIL

Day 2 Plenary Session – How AI Can Improve Food Safety

Convenors: Anett Winkler, Helen Taylor

- 10.00 Welcome – Day 2
HELEN TAYLOR, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom and Anett Winkler, Cargill, Krefeld, Germany
- 10.05 Welcome and Programme Notes - Day 2
RUTH PETRAN, Ruth Petran Consulting, LLC, Eagan, MN, USA
- 10.10 AI and Predictive Modelling in the Food Industry. What Can It Do for You?
CRONAN MCNAMARA, Crème Global, Dublin, Ireland
- 10.30 A Regulator's Data Journey
JULIE PIERCE, U.K. Food Standards Agency, Bristol, United Kingdom
- 10.50 Questions & Answers
- 11.00 Closing Remarks
RUTH PETRAN, Ruth Petran Consulting, LLC, Eagan, MN, USA

S9 *Clostridium botulinum*: Re-Emerging Risk?

Organizer: Stéphane André

Convenor: Louis Coroller

- 11.00 An Overview – *Clostridium botulinum* as an Emerging Risk
MICHAEL W. PECK, QIB Extra Ltd., Norwich, United Kingdom
- 11.20 Modelling the Probability of Growth of *Clostridium sporogenes* Spores as a Function of Heat Treatment and the Properties of the Recovery Medium (pH, Water Activity) in Pasteurized Olive-based Product
EMMANUELLE BOIX, Centre Technique pour la Conservation des Produits Agricoles (CTCPA), Avignon, France and Laboratoire Universitaire de Biodiversité et Ecologie Microbienne (LUBEM), Quimper, France
- 11.40 Influence of Reduced Levels of Sodium Nitrite on the Growth and Toxinogenesis of *Clostridium botulinum* Group II Type B in Cooked Meat Products
SARAH LEBRUN, University of Liège, Liège, Belgium
- 12.00 Discussion and Questions and Answers

S10 An Update on the Integration of “Omics” into Risk Assessment

Organizers and Convenors: Kalliopi Rantsiou, Heidy Den Besten

- 11.00 Can We Use (Epi-)Genetic Markers for *Listeria monocytogenes* in Hazard Characterization?
LUCAS WIJNANDS, cZ&O/RIVM, Bilthoven, The Netherlands

- 11.20 Genome-wide Association Studies to Explore *Listeria monocytogenes* Phenotypes Relevant for Exposure Assessment and Hazard Characterization
LAURENT GUILLIER, Department of Risk Assessment, French Agency for Food, Environmental and Occupational Health and Safety (ANSES), Maisons-Alfort, France

- 11.40 Enabling a Preventative Approach to Managing Microbial Risk in a Manufacturing Environment through Omics and Predictive Microbiology
BRENDAN RING, Creme Global, Dublin, Ireland

- 12.00 Discussion and Questions and Answers

S11 Consumer Safety Aspects of Artisanal and Entrepreneurial Food Fermentations Organizers and Convenors: Leon Gorris, Marcel Zwietering

- 12.30 Small Scale Food Fermentation Trends and Potential Microbiological Risks
EDDY J. SMID, Wageningen University, Wageningen, The Netherlands

- 12.50 Vegetable Fermentations as Man-made Microbial Ecosystems: Investigating Microbial Community Succession and Safety Aspects
SARAH LEBEER, University of Antwerp, Faculty of Science, Department of Bioscience Engineering, Research Unit Environmental Ecology and Applied Microbiology (ENdEMIC), Antwerp, Belgium

- 1.10 Consumer Safety Aspects of Traditional Fermented Foods
GEORGE-JOHN NYCHAS, Agricultural University of Athens, Athens, Greece

- 1.30 Discussion and Questions and Answers

S12 Processing Environment Monitoring in Low-moisture Foods Production Environments. Are We Looking for the Right Thing(s)/Microorganisms, in the Right Places?

Organizers and Convenors: Francois Bourdichon, Anett Winkler

- 12.30 Pathogen of Concerns in Low-moisture Foods – An Overview
SÉAMUS FANNING, University College Dublin, Dublin, Ireland

- 12.50 Low Moisture Food Processing Environment – What is Specific?
ANETT WINKLER, Cargill, Krefeld, Germany

- 1.10 Indicators and Their Role in PEM
ELLEN WEMMENHOVE, Arla Foods Ingredients, Nr Vium, Denmark

- 1.30 Discussion and Questions and Answers

S13 Distinction between *Bacillus Thuringiensis* Used in Biopesticide and Presumptive *Bacillus cereus* Strains Involved in Food Quality and Safety: A Hot Topic

**Organizers: Sandra Tallent, Florence Postollec
Convenor: Pamela Wilger**

- 2.00 Friend or Foe – Bt in the Spotlight of Food Microbiology
MONIKA EHLING-SCHULZ, Institute of Microbiology – Department of Pathobiology – University of Veterinary Medicine, Vienna, Austria
- 2.20 Genetic and Ecological Distinctiveness of Entomopathogenic *B. Thuringiensis* – Implications for Food Safety
BEN RAYMOND, University of Exeter, Penryn, United Kingdom
- 2.40 Update on the Implication of Presumptive *B. cereus* Associated with Food Poisoning Outbreaks in France
MATHILDE BONIS, Laboratory for Food Safety, University of Paris-Est, ANSES, Maisons-Alfort, France
- 3.00 Discussion and Questions and Answers

RT1 Food Safety Impacts of National and Organisational Culture

**Organizers: Carol Wallace, Nicola Sharman
Convenor: Carol Wallace**

- 2.00 Panelists:
SHARON BIRKETT, OSI Industries, LLC, Aurora, IL, USA
LONE JESPERSEN, Cultivate Food Safety, Hauterive, Switzerland
CAROL WALLACE, University of Central Lancashire, Preston, United Kingdom
AISHAH BIBI ZAINAB JOOMUN, University of Mauritius, Moka, Mauritius

S14 Viruses: Looking into and Making Sense of Unforeseen Risks for Food Safety

Organizer and Convenor: Efstathia (Efi) Papafragkou

- 3.30 Examples of Viral Zoonotic Infections of Relevance to Food Safety
WIM VAN DER POEL, Wageningen BioVeterinary Research, Department of Virology, Lelystad, The Netherlands
- 3.50 Lessons Learned from Testing Water for SARS-CoV-2 Transmission: Do We Need to Worry about It?
GLORIA SÁNCHEZ, Institute of Agrochemistry and Food Technology (IATA-CSIC), Valencia, Spain
- 4.10 Environmental Prospective on SARS-CoV-2 Testing: Advances and Challenges
ERIN CROWLEY, Q Laboratories, Inc., Cincinnati, OH, USA
- 4.30 Discussion and Questions and Answers

S15 All Food Processes Have a Residual Risk, Some are Small, Some Very Small and Some are Extremely Small: Zero Risk Does Not Exist

**Organizer: Marcel Zwietering
Convenor: Alberto Garre**

- 3.30 Not Detected or 12D Reduction Does Not Mean Zero Risk
MARCEL ZWIETERING, Wageningen University, Wageningen, The Netherlands
- 3.50 Residual Risk in the Era of Molecular Epidemiology and Large Scale Food Production
ROBERT BUCHANAN, University of Maryland-College Park, College Park, MD, USA
- 4.00 Different Perspectives of Residual Risk: Risk per Serving, Total Risk and Burden of Disease
MARTIN WIEDMANN, Cornell University, Ithaca, NY, USA
- 4.30 Discussion and Questions and Answers

T2 Technical Session 2 – Antimicrobials; Food Toxicology; Laboratory and Detection Methods; Low-water Activity Foods; Meat, Poultry and Eggs; Modeling and Risk Assessment; Molecular Analytics, Genomics and Microbiome; Sanitation and Hygiene

- T2-01** Metagenomic Characterization of the Microbiome and Resistome in the Milk Production Environment
11.00 SELENE RUBIOLA, Francesco Chiesa, Alessandra Dalmasso, Tiziana Civera, University of Turin, Department of Veterinary Science, Grugliasco (TO), Italy
- T2-02*** Variable Contribution of Cold Shock-Domain Family Proteins to Nisin Tolerance in *Listeria monocytogenes*
11.15 JOSEPH WAMBUI, Francis Muchaamba, Patrick Murigu Kamau Njage, Taurai Tasara, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Zurich, Switzerland
- T2-03** The ISO 16140 Series: A Never-ending but Successful Story
11.30 PAUL IN'T VELD, Netherland Food and Consumer Product Safety Authority, Utrecht, The Netherlands
- T2-04** Variation of Deoxynivalenol Levels in Corn and Its Products Available in Retail Markets of Punjab, Pakistan, and Estimation of Risk Assessment
11.45 SHAHZAD ZAFAR IQBAL, Government College University Faisalabad, Faisalabad, Pakistan
- T2-05** Quantitative Determination of *Staphylococcus aureus* Enterotoxins in Complex Food Matrices by a Multiplex Immunocapture Mass Spectrometry Method
12.00 DONATIEN LEFEBVRE, Kevin Blanco-Valle, Cécile Feraudet-Tarisse, Jacques-Antoine Hennekinne, François Fenaille, Stéphanie Simon, François Becher, Yacine Nia, Université Paris-Est, ANSES and Paris-Saclay University, CEA, INRAE, Medicines and Healthcare Technologies Department (DMTS), SPI, Gif-sur-Yvette, France

- T2-06** 12.15 Characterisation of Foodborne Outbreaks Due to Emerging Staphylococcal Enterotoxins
YACINE NIA, Donatien Lefebvre, Isabelle Mutel, Florence Guillier, Fabio Zuccon, Abdelhak Fatihi, Kevin Blanco-Valle, Pascal Bouchez, François Becher, Lucia Decastelli, Jacques-Antoine Hennekinne, Cécile Feraudet-Tarisse, Stéphanie Simon, Université Paris-Est, ANSES, Maisons-Alfort, France
- T2-07*** 12.30 Immunomagnetic Separation Combined with Propidium Monoazide for the Specific Detection of Viable *Listeria monocytogenes* by Multiplex Loop-mediated Isothermal Amplification in Milk Products
FOTEINI ROUMANI, Sarah Azinheiro, Joana Carvalho, Marta Prado, Alejandro Garrido-Maestu, International Iberian Nanotechnology Laboratory, Braga, Portugal
- T2-08** 12.45 Confirmation of Foodborne Pathogens (*Salmonella*, *Cronobacter*, *Campylobacter*) with a New Platform of Mass Spectrometry Instruments
OLAF DEGEN, Markus Timke, Karl Otto Kraeuter, Thomas Maier, Bruker Daltonik GmbH, Bremen, Germany
- T2-09** 1.00 Validation of High-throughput Technologies to Speed Up the Confirmation Workflow
ERIN CROWLEY, Q Laboratories, Inc., Cincinnati, Ohio
- T2-10** 1.15 Caught Between Two Principles: How to Validate Semi-quantitative Assays
SUZANNE JORDAN, Campden BRI, Chipping Campden, United Kingdom
- T2-11** 1.30 Survival of *Listeria monocytogenes* and *Salmonella* Typhimurium on Hot-air Dried Sliced Mushrooms
MARTIN LAAGE KRAGH, Louisa Obari, Alyssa Marie Soria Caindec, Lisbeth Truelstrup Hansen, DTU Food, Kongens Lyngby, Denmark
- T2-12** 1.45 Surveillance of *Salmonella* in French Poultry Production Highlights Potential Cross-Contaminations between Poultry and Cattle Farms
AMANDINE THEPAULT, Louise Baugé, Marianne Chemaly, Laetitia Bonifait, ANSES, Laboratory of Ploufragan-Plouzané-Niort, Ploufragan, France
- T2-13*** 2.00 Behaviour of *Listeria monocytogenes* in High Pressure Processed Dry-cured Ham during Storage Under Refrigeration
CRISTINA SERRA-CASTELLÓ, Noemie Desriac, Anna Jofré, Louis Coroller, Sara Bover-Cid, IRTA. Food Safety and Functionality Programme, Monells, Spain
- T2-14** 2.15 Food Safety Risk-based Categorization of Manufactured Foods for Inclusion in the Canadian Food Inspection Agency's Establishment-based Risk Assessment Model
Romina Zanabria, Nassim Haghighi, ELISABETH MANTIL, Tamazight Cherifi, Manon Racicot, Suzanne Savoie, Anna Mackay, Sylvain Quessy, Canadian Food Inspection Agency, Ottawa, ON, Canada
- T2-15** 2.30 The Canadian Food Inspection Agency Work Tasking Logic Model Pilot Project
HAORAN SHI, Raphael Plante, Genevieve Comeau, Suzanne Savoie, Sylvain Quessy, Ornella Noubissie Wafo, Anna Mackay, Manon Racicot, Canadian Food Inspection Agency, Ottawa, ON, Canada
- T2-16** 2.45 A Deep Learning Approach to Predict *E. coli* Growth Using Micro-Isothermal Calorimetry (MIC) Data
IMRAN AHMAD, Toni-Ann Benjamin, Florida International University, North Miami, FL, US
- T2-17** 3.00 Ever-changing STEC Evolution: Illustration with O26
SABINE DELANNOY, Patricia Mariani-Kurkdjian, Sandra Jaudou, Mai-Lan Tran, Hattie E. Webb, Stephane Bonacorsi, Patrick Fach, ANSES, Maisons-Alfort, France
- T2-18** 3.15 Genomic Characterization of *Listeria monocytogenes* Isolated from *Agaricus bisporus* Mushroom Production Facilities
FRANK LAKE, Leo van Overbeek, Jeroen Koomen, Johan Baars, Tjakko Abee, Heidi Den Besten, Wageningen University and Research, Wageningen, The Netherlands
- T2-19*** 3.30 Variability in the Survival of *Salmonella enterica* in Response to Heat and Desiccation
HANNAH PYE, Muhammad Yasir, Keith Turner, Mark Kirkwood, Matt Bawn, Gaetan Thilliez, Robert Kingsley, Quadram Institute Bioscience and University of East Anglia, Norwich, United Kingdom
- T2-20** 3.45 Heterogenous Contamination of Food Products, A Paradigm Shift?
Lucas Wijnands, Angela van Hoek, Ellen Delfgou - van Asch, El Bouw, Heidi Den Besten, Wilma Hazeleger, Jiansheng Wang, Susanne Pinto, Elisa Beninca, INDRA BERGVAL, Rozemarijn van der Plaats, cZ&O/RIVM, Bilthoven, The Netherlands
- T2-21** 4.00 Significance of Viable but Non-culturable Bacteria in the Fresh-cut Produce Industry
PILAR TRUCHADO, Maria Gil, Ana Allende, CEBAS-CSIC, Murcia, Spain
- T2-22** 4:15 Variation of Deoxynivalenol levels in Corn and Its Products Available in Retail Markets of Punjab, Pakistan and Estimation of Risk Assessment
SHAHZAD ZAFAR IQBAL, Government College University Faisalabad, Faisalabad, Pakistan
- T2-23*** 4.30 The Locus of Heat Resistance Confers Resistance to Chlorine and Oxidative Stress in *Escherichia coli*
ZHIYING WANG, David Simpson, Lynn McMullen, Michael Gänzle, University of Alberta, Edmonton, AB, Canada

P2 Poster Session 2 – Communication Outreach and Education; Epidemiology; Food Processing Technologies; Food Safety Systems; Microbial Food Spoilage; Modeling and Risk Assessment; Molecular Analytics, Genomics and Microbiome; Packaging; Pre-harvest Food Safety; Produce; Retail and Food Service Safety; Viruses and Parasites; Water

Virtual Meeting

Communication Outreach and Education

- P2-01 Piloting a Support Program to Enable Small- and Medium-sized Food Manufacturing Businesses in Wales to Obtain Food Safety Certification — HELEN TAYLOR, UK, Cardiff, United Kingdom
- P2-02 Attitudes Related to Food Safety Behavior Among Students in Sweden — INGELA MARKLINDER, Gustav Eskhult, Roger Ahlgren, Anna Blücher, Stina-Mina Ehn Börjesson, Madeleine Moazzami, Jenny Schelin, Marie-Louise Danielsson-Tham, Uppsala University, Uppsala, Sweden
- P2-03 Most Food Control Officers Have Suspected Food Fraud at Inspections — JASMIN JOENPERÄ, Janne Lundén, Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland
- P2-04 Everyday Risks Every Time We Eat – Global Poll Findings of Perceived and Experienced Risks from Unsafe Foods — SARAH CUMBERS, Lloyd's Register Foundation, London, United Kingdom
- P2-05 A Review of Consumer Food Safety Advice from International Government Agencies — SIMON DAWSON, Ellen W. Evans, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, South Wales, United Kingdom
- P2-06 Determination of Food Safety and Technical Skills Shortages Challenging the Food and Drink Manufacturing Processing Industry in Wales, UK — LEANNE ELLIS, Sharon Mayho, Elizabeth C. Redmond, Cardiff Metropolitan University, Cardiff, South Wales, United Kingdom
- P2-07 The Features of Cabin-crew Food Safety Training: A Content Analysis — ELIZABETH C. REDMOND, Ayman Safi Abdelhakim, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom

Epidemiology

- P2-08 Seven-year Foodborne Botulism Outbreaks in Northern Italy: Associated Foods and Detected Toxins — Guido Finazzi, ELENA DALZINI, Roberto Benevenia, Sara Arnaboldi, Luigi Bornati, Barbara Bertasi, Marina-Nadia Losio, IZSLER, National Reference Centre for Emerging Risks in Food Safety, Brescia, Italy
- P2-09 Listeriosis Infection Leading to Uncover Widespread Persistent Contamination in Poultry Meat — Virginia Filipello, Maria Gori, Erika Scaltriti, Luca Bolzoni, Alessandro Massone, Camillo Gandolfi, Mariagrazia Zanoni, Elisabetta Tanzi, Stefano Pongolini, Marina Nadia Losio, GUIDO FINAZZI, Food Safety Department, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

Food Processing Technologies

- P2-10 UV-C Technology Optimization to Inactivate *Salmonella* Enteritidis in Soymilk Flavoured with Cocoa and Vanilla — ARÍCIA POSSAS, Antonio Valero, Rosa Maria Garcia-Gimeno, Poliana Mendes De Souza, University of Cordoba, Department of Food Science and Technology, Cordoba, Spain

Food Safety Systems

- P2-11 Occurrence of Virulent and Antibiotic-Resistant *Staphylococcus* spp. in Ready-to-Eat *Rhynchophorus phoenicis* and *Archachatina marginata* Vended Along the Port Harcourt-Bayelsa Route — ONORIODE C. ERUTEYA, Chioma Ngoka, Abiye A. Ibiene, University of Port Harcourt, Nigeria, Port Harcourt, Nigeria
- P2-12 Assessment of *Listeria* spp. and *Escherichia coli* Contamination on Surfaces in a Broiler Abattoir — LEILA BOUAYAD, Radia Bouhamed, Sara Lezzoum, Siham Azzi, Taha Mossadak Hamdi, Laboratory of Food Hygiene and Quality Insurance System (HASAQ) National Veterinary School, Algiers, Algeria
- P2-13 Developing and Maintaining Food Safety Culture through Implementation of GFSI Benchmarked Standards: A Success Story — MUHAMMAD SHAHBAZ, Abdul Moiz, Muhammad Bilal, Shugufta Mohammad Zubair, Akhlaq Ahmad, Mawarid Food Company - KSA (Pizza Hut, Taco Bell), Riyadh, Saudi Arabia
- P2-14 Determination of Food Safety Culture in a Low-risk Food Production Site: Identification of Key Strengths and Weaknesses — LAURA HEWITT, Paul Hewlett, Elizabeth C. Redmond, Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, Northallerton, United Kingdom
- P2-15 Microbiological Quality Evaluation of Meal-kits (Non-heated Food and Food after Heating Treatment) Compared by Their Storage Times — SEO-JIN KIM, Jae-hee Park, Hye-Kyung Moon, Changwon National University, Changwon, South Korea
- P2-16 Academic and Food Industry Management Perspectives of Food Safety Culture — Sharon Birkett, Ellen W. Evans, ELIZABETH C. REDMOND, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom
- P2-17 Strategic Continued Growth Platforms in Wales, UK: The Development of Food Sector Small to Medium Enterprises (SMEs) — SHARON MAYHO, Darren Mumford, David Lloyd, Elizabeth C. Redmond, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom
- P2-18 Next Generation Sequencing Workflow for Detection of *Salmonella* Serotypes: From Food Sample to Serotype Identification — MARIO GADANHO, Tiina Karla, Hanna Lehmusto, Heikki Salavirta, Milja Tikkanen, Thermo Fisher Scientific, Lisbon, Portugal

- P2-19 Impact of Neutralizing Buffers of Disinfectants on the Viability of *Listeria monocytogenes* Cells from Monospecies Biofilm — THOMAS BRAUGE, Guylaine Leleu, Anthony Colas, Lena Barre, Graziella Midelet, ANSES, Laboratory for Food Safety, Bacteriology and parasitology of Fishery and Aquaculture Products Unit, Boulogne-sur-mer, France
- P2-20 Exploring Management Attitudes Towards Leadership Roles in Food Manufacturing — EMMA J. SAMUEL, Ellen W. Evans, Elizabeth C. Redmond, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, Wales, United Kingdom
- P2-21 Factors Influencing Food Safety Compliance in Home Food Production Businesses — ELIZABETH C. REDMOND, Schani Naude, ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom

Microbial Food Spoilage

- P2-22 Interaction between *Saccharomyces cerevisiae* and *Aspergillus carbonarius* in *Vitro* and in *Situ* on Table Grapes — Danai Katsarou, PASCHALITSA TRYFINOPOULOU, George-John Nychas, Efstathios Panagou, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
- P2-23 Rapid Evaluation of Sea Bream Fillets Quality Using FTIR Spectroscopy, Microbiological and Sensory Analysis — MARIA GOVARI, Paschalitsa Tryfinopoulou, Efstathios Panagou, George-John Nychas, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
- P2-24 Spoilage Assessment on Chicken Thighs Surface Via Non-Invasive Multispectral Image Analysis (MSI) — EVGENIA SPYRELLI, Christina Papachristou, Anastasia Lytou, Efstathios Panagou, George-John Nychas, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece
- P2-25 Multivariate Data Analysis for the Development of Classification Models Assessing Spoilage of Two Different Types of Poultry Meat — EVGENIA SPYRELLI, Christina Papachristou, Valeriu Culcinschi, Anastasia Lytou, Efstathios Panagou, George-John Nychas, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece

Modeling and Risk Assessment

- P2-26 Thermal Inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in Curd Stretching Used for Mozzarella Cheese — ELENA DALZINI, Simone Russo, Elena Cosciani-Cunico, Paola Monastero, Daniela Merigo, Marina-Nadia Losio, Filippo Barsi, Matteo Ricchi, IZSLER, National Reference Centre for Emerging Risks in Food Safety, Brescia, Italy

- P2-27 Log Reduction of *Listeria monocytogenes* during the Domestic Heating of Meat Products: Different Approaches — ELENA COSCIANI-CUNICO, Elena Dalzini, Paola Monastero, Daniela Merigo, Giorgio Varisco, Marina-Nadia Losio, IZSLER, National Reference Centre for Emerging Risks in Food Safety, Brescia, Italy

- P2-28 Risk Estimation for Aflatoxin M1 Due to Milk Consumption in Chilean Children — CLAUDIA FOERSTER, Andrea Rivera, Gisela Rios-Gajardo, Universidad de O'Higgins, Rancagua, Chile

- P2-29 The Canadian Food Inspection Agency Work Tasking Logic Model Parameter Selection and Prioritization — Manon Racicot, Raphael Plante, Genevieve Comeau, SUZANNE SAVOIE, Haoran Shi, Sylvain Quessy, Canadian Food Inspection Agency, Moncton, NB, Canada

- P2-30* Probabilistic Modelling of Exposure to Coliform Bacteria in Raw Milk — RODNEY FELICIANO, Géraldine Boué, Fahad Mohssin, Muhammad Mustafa, Jeanne-Marie Membré, Secalim, INRAE / Oniris, Nantes, France

Molecular Analytics, Genomics and Microbiome

- P2-31 Microbial Diversity of Fermented Greek Table Olives of Halkidiki and Konservolia Variety from Different Regions as Revealed by Metagenomic Analysis — Konstantina Argyri, Athena Grounta, Agapi Doulgeraki, Anthoula Argyri, CHRYSOULA TASSOU, Hellenic Agricultural Organisation DIMITRA, Institute of Technology of Agricultural Products, Sof. Venizelou 1, Lycovrissi, Attica, Greece

- P2-32 A Metagenetic Analysis of Bacterial Community in Inoculated Fermentations of Conservolea Variety Black Olives with Multifunctional Starter Cultures in Reduced Salt Brines — EVANTHIA MANTHOU, Angeliki Kochila, Aikaterini Tzamourani, Stephane Chaillou, George-John Nychas, Efstathios Panagou, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece

- P2-33* Whole Genome Sequencing of Extended-Spectrum- and AmpC β -Lactamase-producing *Enterobacteriaceae* Isolated from Spinach Supply Chains in Gauteng Province, South Africa — LOANDI RICHTER, Erika du Plessis, Stacey Duvenage, Lise Korsten, University of Pretoria, Pretoria, South Africa

Packaging

- P2-34 Lactic Acid Bacteria and Yeast Species Diversity of Non-thermally Preserved Green Spanish-style Olives during Modified Atmosphere Packaging — Aikaterini Tzamourani, Aikaterini Kasimati, George-John Nychas, EFSTATHIOS PANAGO, Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece

Pre-harvest Food Safety

P2-35 The Canadian Food Inspection Agency Establishment-based Risk Assessment Model for Feed Mills: Algorithm Principles — Alexandre Leroux, Manon Racicot, Virginie Lachapelle, GENEVIEVE COMEAU, Ornella Wafo Noubissie, Romina Zanabria, Marie-Lou Gaucher, Marcio Costa, Younes Chorfi, Richard Holley, John Smillie, My-Lien Bosch, Andre Dumas, Egan Brockhoff, Stephanie Collins, Philip Snelgrove, Sylvain Quessy, Canadian Food Inspection Agency, St-Hyacinthe, QC, Canada

Retail and Food Service Safety

P2-36 Great Challenges Ahead for Global Food Safety Community: Practices in a Developing Country after Legislation — MUHAMMAD SHAHBAZ, Abdul Moiz, Muhammad Bilal, Mawarid Food Company – KSA (Pizza Hut, Taco Bell), Riyadh, Saudi Arabia

Viruses and Parasites

P2-37 Sarcocystis Species Detection by Multiplex-PCR in Cattle Affected by Eosinophilic Myositis — Selene Rubiola, Davide Vercellino, Daniela Tripolini, Massimo Sinelli, Annalisa Guida, Ingrid Castellani, FRANCESCO CHIESA, University of Turin, Department of Veterinary Science, Grugliasco (TO), Italy

Water

P2-38 Assessment of Water Quality Index (WQI) of Commercially Available Drinking Bottled Water — PAMELA IHEOZOR-EJIOFOR, Bukola Onarinde, University of Lincoln, National Centre for Food Manufacturing, Holbeach. Lincolnshire, Holbeach, United Kingdom



SYMPOSIUM ABSTRACTS

27–28 April 2021

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Opening Session – Transformers in the Food World – Food Safety Challenges to Master

Food Safety Challenges Ahead to the Food Systems Transformation

Marta Hugas

European Food Safety Authority, Parma, Italy

The world needs food systems that can feed a growing population without increasing land use while reducing emissions. The EU Green Deal, in-line with the UN SDGs, sets targets for a sustainable food system, which will ensure environmental, social and economic sustainability.

Transitioning towards sustainability needs to be supported by fit-for-purpose scientific advice from risk assessors. This is relevant to EFSA, which supports risk managers with advice to protect European consumers from food-related risks, helping maintain trust in the food chain.

Regulatory agencies face challenges ranging from current priorities, such as tackling AMR, to the process for identifying/appraising emerging risks, where methodologies or data may be lacking, such as microplastics in the food chain, to new areas with potential relevance to risk assessment, such as the role of human and environmental microbiomes in human health and the environment. At the same time, policy and market developments, such as the circular economy and product innovation, e.g., novel food, require risk assessors being able to identify potential associated risks and advise risk managers accordingly.

Investing in overcoming such challenges, via individual and collaborative effort, is essential for improving the sustainability of our food systems.

Day 2 Plenary Session – How AI Can Improve Food Safety

AI and Predictive Modelling in the Food Industry. What Can It Do for You?

Cronan McNamara

Creme Global, Dublin, Ireland

Companies are discovering how to capitalise on the vast array of data they have access to. This is driving industry-wide improvements in everything from food safety to new product development.

The application of Artificial Intelligence (AI) in the food industry is undergoing a remarkable change in recent years. This has only accelerated during the pandemic.

Learn how companies that are leading this AI revolution are gathering and interrogating all relevant data to identify trends and discover anomalies, providing business-critical foresight. The data is often freely and easily accessible, such as QA data, factory environmental data, ingredient specifications, processing parameters, shelf-life studies. Visualising these data sets and trends can bring huge value. Use AI to predict, prevent and control events before they happen, and to reduce time to market for new products in rapidly changing global markets.

When new and rich datasets such as genomics, IIOT, social media, or other public data are integrated correctly, you dramatically enhance the commercial benefit and predictive power of your AI solutions.

This talk will provide an overview and case studies on what AI can do for you.

A Regulator's Data Journey

Julie Pierce,

U.K. Food Standards Agency, Bristol, United Kingdom

The Food Standard Agency routinely uses data science to identify emerging risks before they threaten health by utilising a variety of (mostly open) data sources and increasingly sophisticated analytics techniques. Through this capability, the FSA is developing a picture of the food system, its risks (safety / authenticity / assurance) and vulnerabilities, so that FSA, and others within the system, can better manage risk.

Using machine learning and artificial intelligence, the Agency has developed various data science solutions, including tools that identify risky imported food and feed products coming into the country through various ports, tools that scan food risk incidents being reported across the globe, and tools to predict the risk of hazards associated with certain commodities from certain countries.

We also apply the data tools and techniques to address risks closer to home. We have developed methods to identify unregistered food businesses trading online and our capability to predict Food Hygiene Rating Scheme (FHRS) ratings, which would help Local Authorities to prioritise inspections.

The Agency works in the open as far as is possible and closely with industry through focussed collaborations. One technique we are now exploring is a data trust for sharing data across many actors within the food system, with the common objective of reducing global food risks.

S1 Quantitative Microbial Risk Assessment for Food Spoilage

Food safety management at the international level has been moved towards a more risk-based approach with regulators around the world adopting the risk analysis framework and the quantitative microbial risk assessment (QMRA) approach as the basis for their decision-making. Despite the extensive use of QMRA within the context of food safety its application to food spoilage management is only limited. Food spoilage may have considerably negative impact to the food industry especially when it occurs before the end of expiration date. In addition, microbiological spoilage contributes greatly to the amount of food which ends up wasted and to the associated financial losses.

Probabilistic QMRA models for food spoilage can provide a transparent scientific basis to support food quality management decisions by food business operators (FBOs) while replacing empirical-based decisions. Probabilistic QMRA models for food spoilage can simulate what-if scenarios with different combinations of settings regarding product formulation, process and storage conditions, expiration dating etc. and assess their impact on the risk of spoilage. This can support the FBOs in selecting an effective expiration date (use-by or best before date), which leads to the maximum exploitation of the "true" product's shelf life, while minimizing the risk of spoilage to an acceptable (by the managers) level, in accordance to the Appropriate Level of Protection (ALOP) for food safety. Furthermore, QMRA can provide the elements for a cost-benefit analogy in relation to the identified mitigation strategies for reducing the risk of spoilage and/or extending the shelf life of foods.

This symposium aims at describing the potential use of QMRA for assessing the risk of spoilage, providing information regarding the working principles, methodology and overall, a risk-based food quality management system. Moving towards a risk-based decision-making perspective for both safety and quality will support the food industry to meet the increased consumer demands.

New Developments in Food Spoilage Assessment

George-John Nychas

*Laboratory of Microbiology and Biotechnology of Foods,
Department of Food Science and Human Nutrition,
Agricultural University of Athens, Athens, Greece*

The issue of assessing quality, safety and authenticity of food commodities is vital in recent years and although it is constantly reviewed in the light of new scientific evidence, its implementation is not always efficient across food chain. The current assessment of food spoilage is based on conventional microbiology (e.g., colony counts) and chemical analytical techniques. These are invasive, laborious and usually provide retrospective information limiting their applicability in- or on-line. The potential of using non-destructive methods (e.g., vibrational spectroscopy and surface chemistry sensors) to (i) overcome the limitations of conventional food microbiology and (ii) automatic monitoring of food spoilage in all stages of the food chain, gains more and more attention. The main restriction of the above-mentioned approaches is related to (big) data derived from these analytical spectra and expression profiles that can be considered as unique fingerprints. So far, the data analytics of the measurements acquired from sensors have been only limited applied in food sector. In order to proceed and make this discipline approachable to food scientists, data analytics should be considered as an essential step, for providing solid and valid information to stakeholders.

Quantitative Microbial Risk Assessment for Food Spoilage: Principles and Methodology

Kostas Koutsoumanis

*Laboratory of Food Microbiology and Hygiene, Department
of Food Science and Technology, Aristotle University of
Thessaloniki, Thessaloniki, Greece*

Management of food safety at the international level has been moved towards a risk-based approach with regulators and food business operators (FBOs) around the world adopting the risk analysis framework. Despite the extensive use of Quantitative Microbiological Risk Assessment (QMRA) within the context of food safety, the application of such a risk-based approach for food spoilage is limited. The present study provides a detailed description of QMRA structure applied for assessing the risk of spoilage in foods by presenting the main principles, the methodological standards and the application scheme for a risk-based food quality management. The output of QMRA for a quality hazard such as food spoilage can provide a transparent scientific basis to support food quality management decisions by food business operators (FBOs) while replacing empirical-based decisions. Probabilistic QMRA models for food spoilage can simulate what-if scenarios with different combinations of settings regarding product formulation, process and storage conditions, expiration dating etc. and assess their impact on the risk of spoilage. This can support the FBOs in selecting an effective expiration date (use-by or best before date), which leads to the maximum exploitation of the “true” product’s shelf life, while minimizing the risk of spoilage to an acceptable (by the managers) level, in accordance to the Appropriate Level of Protection (ALOP) for food safety. Furthermore, QMRA can provide the elements for a cost-benefit analogy in relation to the identified mitigation strategies for reducing the risk of spoilage and/or extending the shelf life of foods.

Quantitative Microbial Risk Assessment Applied to Fungal Spoilage of Bakery Products and Fruit Purees

Jeanne-Marie Membré

Secalim, INRAE, Oniris, Nantes, France

Food safety management has evolved towards a risk-based approach, in which modelling approaches to microbial risk assessment assist regulators and food operators in their decision-making. Probabilistic models are used to assess the risk but also to evaluate the impact of various risk mitigation strategies by scenario analysis.

Some food companies are applying these modelling techniques to the management of microbial spoilage, but there are still few. In this presentation, two examples of quantitative microbial risk assessment applied to fungal spoilage are presented. For manufactured products, food spoilage by fungi results often from an in-factory fungal contamination followed by mould growth on food products up to a visible mycelium.

The food products chosen here are pasteurised strawberry purees (potentially contaminated with *Aspergillus fischeri*) and bakery products (potentially contaminated with *Aspergillus niger*, *Eurotium repens* and *Penicillium corylophilum*). These are two products on which moulds are able to grow while bacteria are not.

These two examples will serve to illustrate in a practical manner how the microbial models were set and run to assess the effect of different combinations of product formulations, process, storage conditions and expiration dates on the risk of spoilage.

S2 COVID-19: Assessing Potential Consumer Risk and Managing Value Chain Disruption

The globalization of food sourcing and trade, the extensive movement of people and intensive food production practices are challenging the resilience and sustainability of food systems and value chains around the world.

The COVID-19 public health pandemic caused by SARS-CoV-2 illustrates the challenges for governments, industries and scientists to assess consumer risks potentially associated to new, rapidly emerging zoonotic agents, and to manage potential disruptions of food supply chains within and across borders.

The International Commission on Microbiological Specification of Foods has been closely following the developments in the food safety related risk of SARS-CoV-2. Whilst COVID-2 is not a foodborne illness, the perception in many parts of the world may have been very different from the onset of the pandemic. Moreover, the public health and occupational safety impacts of COVID-19 had significant impact on the operations of food industries, the operability of food supply chains and the movement of food in international trade.

The learnings from managing this disruption, even when not foodborne, will be valuable for making food safety management more resilient towards possible future, truly foodborne zoonoses.

This session will discuss the current scientific insights on the relationship of COVID-19 to food safety and will illustrate the experiences of government and industry in overseeing and managing the ongoing assurance of food safety in the face of the COVID-19 challenges.

Food Safety Regulatory Challenges of the COVID-19 Pandemic

Wayne A. Anderson

Food Safety Authority of Ireland, Dublin, Ireland

Consumers have a right to safe food that is accurately labelled and marketed. This right does not diminish in a pandemic and the normal food safety hazards do not go away. Government bodies with responsibility for the oversight of food safety rules have had to adapt and respond to many challenges that have affected the food chain throughout the COVID-19 pandemic. It has been a struggle for Authorities to maintain the standard of official controls carried out in food businesses whilst at the same time managing the health and safety of its workers, resource restriction due to front line deployment and remote working issues that impacted on day to day life. At the same time supply chain disruption for food businesses, their closure and re-opening cycles in response to lockdowns and their altered business models presented new threats to food safety. Then there was and is, the ongoing debate about whether SARS-CoV-2 is a food safety risk and what are proportionate controls.

This presentation will provide an overview of how the Food Safety Authority of Ireland and its Official Agencies responded to the crisis. What worked and what was difficult. It will show what were the real and perceived food safety issues and will provide some key learnings for the future when the next pandemic strikes.

COVID-19: Science Update and View on Potential Food Safety Risks

Leon Gorris

Food Safety Expert, Food Safety Futures, Nijmegen, The Netherlands

The globalization of food sourcing and trade, the extensive movement of people and intensive food production practices are challenging the resilience and sustainability of food systems and value chains around the world.

The ongoing COVID-19 public health pandemic caused by SARS-CoV-2 illustrates the challenges for governments, food industries and scientists to bring together sound science- and risk-based information in a timely way and to confidently assess consumer risks potentially associated to new, rapidly emerging zoonotic agents, and to manage potential disruptions of food supply chains within and across borders.

Whilst COVID-19 is not a foodborne illness, the perception in many parts of the world may have been very different at the onset of the pandemic. This paper discusses the evolution and current status of scientific insights regarding the relationship between COVID-19/SARS-CoV-2 and food/ food safety as well as whether good hygienic practices and food safety management systems can help food operations to help manage the occupational and general health risks associated to SARS-CoV-2.

Navigating Challenges of COVID-19 in the Food Supply Chain

John Donaghy

Nestlé S.A., Vevey, Switzerland

While not a food safety issue, the COVID-19 pandemic has put unprecedented pressure on the food industry to protect their workers from any occupational health risks, while fulfilling their operational roles. Furthermore, food processors and manufacturers have had to demonstrate resilience, to ensure business continuity, under the constraints of supply challenges, resource challenges, abnormal upturn in demand for key food products, shifts in supply channels, while at the same time dealing with an infodemic of diverse opinions and strategies to deal with the virus.

This presentation will describe practical consequences for a food manufacture in supply chain management, manufacturing continuity and supply channel re-orientation. The talk will address preventative measures put in place to ensure safety of workers, enhanced personal hygiene and facility sanitation regimes put in place, the contingencies put in place to on-board new suppliers (raw and packaging materials), while assuring food safety and quality. The opportunities presented for the use of remote technologies, in the sphere of auditing and factory remote technical assistance will be described. Among the challenges which will be described is the 'license to operate' afforded by different global jurisdictions during the pandemic and the implications of these for a global manufacturer.

19 member states in Europe. Their objective is to enable significant advances in three major fields of high importance in Public Health: Foodborne zoonoses, Antimicrobial resistance and Emerging Threats. Coordinated by Anses and Sciensano, the OH EJP aims to acquire new knowledge in the fields of foodborne zoonoses, antimicrobial resistance and emerging threats. The first talk will set up the scene for brief overview of this EJP and its impact on Public Health in Europe. There are currently 29 projects running. The basis for these projects is a defined via a strategic research agenda that is built on programs supported by the national authorities and by ECDC and EFSA. Among the on-going projects, TOX-Detect, dealing with the development and harmonization of innovative methods for comprehensive analysis of foodborne toxigenic bacteria, i.e., Staphylococci, *Bacillus cereus* and *Clostridium perfringens*, will be presented. Finally, there is an increasing attention for sharing (surveillance) information within a country as well as between countries. The COHESIVE project is focusing on how to organize the signaling, risk assessment, risk management and communication of (re)-emerging zoonoses and AMR in a joint multidisciplinary fashion within countries.

Foodborne Zoonoses and One Health Approach; What's New in Europe? The One Health EJP!

Hein Imberechts

Sciensano, the Belgian Institute for Health, Brussels, Belgium

In Europe, while efficient national systems for surveillance and risk management of foodborne zoonoses and AMR exist, there is still room for improvement in cross-sector and cross-border collaboration between animal health, public health and food safety organisations. For example, surveillance programmes can be more efficiently aligned, procedures for detection and characterisation can be further harmonised, data sharing and data access improved and intervention methodologies should be harmonized.

Building on the Med-Vet-Net Network of Excellence funded by the European 6th Framework Programme and further on the MedVetNet Association, the One Health European Joint Programme (One Health EJP, is funded by the Horizon2020 Framework Programme. It started in January 2018, runs for 5 years and has a budget of €90M, with 50% co-funding by the European Commission. At the beginning of 2021, it is comprised of 44 partners from 22 member states including the MedVetNet Association.

The One Health EJP is successfully creating a European framework through the integration and alignment of public health, veterinary and food safety institutes that perform reference laboratory functions. The organisations work together through joint prioritisation and undertaking research, integrative activities, and training and education exercises in the domains of foodborne zoonoses, antimicrobial resistance and emerging threats. As such, the consortium addresses the needs of its main stakeholders, i.e., European (EEA, EMA, ECDC, EFSA, WHO-Europe), global (FAO and OIE) and national authorities, risk managers and policy makers. Applying a One Health approach, the consortium strengthens its preparedness in line with the 'prevent-detect-response' concept. All One Health EJP partners have the mandate of their national or regional authorities, i.e., mainly Ministries competent for foodborne zoonoses, antimicrobial resistance and emerging threats. As such, the One Health EJP is optimally positioned to provide the evidence-based risk evaluation for a science-to-policy dialogue with its stakeholders. Both the overarching actions of the One Health EJP and its scientific activities (research projects, integrative projects and PhD projects) add to the building of a sustainable network addressing existing and emerging threats of zoonoses and antimicrobial resistance at the interface of humans, animals, food, and environment.

After three years, the One Health EJP has become an established, unique network of cross-sector collaboration and alignment in Europe, with structures for efficient science-to-policy translation of its outcomes. Further challenges have

S3 Foodborne Zoonoses and One Health; What's New in Europe? The One Health EJP!

The international concept of "One Health" recognizes that human health is tightly connected to the health of animals and the environment, and that contaminants that affect human health, animal health and the environment through food in particular are closely linked. In this context, a European Joint Programme (EJP) on "One Health" was launched in 2018. The One Health (OH) EJP is the third EJP funded by the European Commission with a total financial support of € 90 Millions, 50% of which is co-financed by the EU. This EJP is an example of the 'One Health' concept, and boasts a landmark partnership between 37-acclaimed food, veterinary and medical laboratories and institutes across

been identified and are currently dealt with by promoting the outreach of the One Health EJP also beyond the EU and further encouraging the attention toward human health and environmental aspects.

Tox-Detect: Development and Harmonization of Innovative Methods for Comprehensive Analysis of Foodborne Toxicogenic Bacteria, i.e. *Staphylococci*, *Bacillus cereus* and *Clostridium perfringens*

Jacques-Antoine Hennekinne
Université Paris-Est, ANSES, Maisons-Alfort, France

Bacterial toxins produced by *Staphylococcus* spp., *Bacillus* spp. and *Clostridium* spp. are responsible for a large number of food-poisoning outbreaks (FPOs) in the European Union. Moreover, the true incidence of FPOs caused by these toxicogenic bacteria is underestimated due to a lack of relevant detection tools and to a common symptomatology that makes outbreak investigation challenging.

The OH EJP ToxDetect project uses three different non-NGS approaches for a better detection of bacteria and quantification of some bacterial toxins, including emerging threats that remain currently undetectable.

In this project, we focus on both bacteria identification and toxin characterization.

MALDI-ToF has been developed for an accurate and rapid identification and characterization of toxicogenic bacteria whereas proteomics approaches based on LC-MS/MS experiments have been selected as a relevant choice for their high specificity and possible quantitation.

In parallel, High Content Analysis approaches have been used for the characterization of the toxicity and the identification of the molecular mechanisms involved in the toxicity of different strains.

Moreover, for an easier implementation in end-user laboratories, we also develop some immuno-enzymatic methods for selected targets.

Finally, all the developed methods have been shared among partners and will be submitted to ring trials for performance characteristic evaluation purposes.

Cohesive: Organizing Risk Analysis for (re-) Emerging Zoonoses: A One Health Approach

Kitty Maassen
RIVM, Bilthoven, The Netherlands

Emerging zoonoses can pose serious threats to both human and animal health. It is clear that working towards a world with less zoonotic disease burden requires collaboration at all levels and between the veterinary, human, food and environmental sectors. In Europe already a lot is arranged in legislations, regulations and for many (especially foodborne) zoonoses surveillance systems are in place, providing early warning and timely response and control. However, it is not always realized that also new and emerging zoonoses which are not monitored can become human and/or animal health threats. Every country can face such threats unexpectedly, look at COVID-19. The UK and The Netherlands have implemented a risk analysis structure covering signalling, risk assessment, risk management and risk communication in a One Health approach after going through a crisis.

However, there is no blue-print for such One Health risk analysis structures (OH-RAS) due to different organisation of food production systems, organization of ministries, geographic factors, differences in cultures between countries. In the One Health EJP project COHESIVE implementation guidelines are drafted to support countries to organise risk analysis activities at the national level. These implementation guidelines also address barriers for collaboration, such as lack of political will.

S4 Validation of Control Measures for Food-borne Pathogens in Foods: Challenges and Solutions

Food facilities are required to implement a written food safety management plan and document that their control measures are effective against the identified hazards. The effectiveness of control measures to meet a pre-defined process control objective must be validated prior to implementation of the food safety management plan. A validation study provides evidence that control measures are capable of effectively controlling the identified hazards and therefore meet the process control objective. Scientific data and technical evidence may also come from a variety of other sources including safe harbors (i.e., well documented and accepted processes), published references, guidance documents, and mathematical modelling. Determining key requirements that must be included in a validation of control measures involves significant challenges such as identifying the hazard(s) to be controlled, setting critical control parameters, understanding the interactions between the hazard and the food and collecting adequate scientific, technical and/or experimental evidence. Recently, an international, multidisciplinary expert group, convened under the aegis of the European Branch of the International Life Science Institute (ILSI Europe), wrote a guidance document on validation studies. In this symposium, expert group members from industry and academia will give examples and guidance on how to successfully plan and perform a validation study.

Process and Product Parameters to Consider for Designing a Validation Study

Anett Winkler
Cargill, Krefeld, Germany

In order for a validation to be successful certain steps and considerations have to be performed before the actual study can take place. This presentation will focus on necessary considerations for the process(es) and product(s) to be validated. The success and reliability of a validation study depends on understanding the process mechanisms and product characteristics that influence pathogen inactivation. Attention will be given to determine the relevant process step(s) best suitable to control the identified hazard(s), providing insights into their principles of action. Furthermore, product characteristics and their influence on growth and survival of foodborne pathogens will be presented.

Determining Performance Criteria for a Validation Study

Elissavet Gkogka
Arla Innovation Centre, Aarhus N, Denmark

A validation study aims to demonstrate that control measures effectively control identified hazards by reducing their frequency and/or concentration to a specified target level. This presentation focuses on the identification/selection of target hazards for control and the determination of performance criteria for validation studies of key food commodities such as dairy, meat, fish and egg products. Furthermore, it provides an overview of existing performance criteria that through consensus or regulation are recognized as "safe harbors" and discusses the use of surrogate microorganisms in situations where it is not possible to use pathogenic microorganisms to provide evidence of the efficacy of different control measures.

Obtaining Scientific Evidence and Data Evaluation for Process Validation

Heidy Den Besten
Wageningen University and Research, Wageningen, The Netherlands

A validation study provides evidence that control measures are capable of effectively controlling the identified hazard. This presentation will discuss how collection and evaluation of already available microbiological data from various sources on the target pathogen in the product type of interest can be used as an initial confirmation of the efficacy of an inactivation intervention and can serve as part of the

weight of evidence to support a validation study. In addition, guidance will be given which decisions have to be made when designing a challenge study and how to evaluate experimental data, taking into account variability in batches, replicates and samples.

S5 Biofilm Formation as an Adaptation Strategy for Food-associated Bacteria

Despite advances in food preservation techniques, bacterial spoilage remains a leading cause of global food loss. Nearly one-third of all food produced worldwide is estimated to be lost post-harvest, and much of this loss can be attributed to microbial spoilage. A major source of the contamination of many food products is often associated with biofilms formed on the surfaces of the processing equipment. Biofilms are highly structured multicellular communities, which allow bacteria to successfully adapt and survive in hostile environments. Biofilms are not only a potential source of contamination, but can also increase corrosion rate of metal pipes and equipment used in the food industry, reduce heat transfer and increase fluid frictional resistance. Therefore, contamination of food products due to the presence of bacterial biofilms is a major concern to modern food manufacturers, especially with current trends towards longer production runs, the use of complex equipment, the automation of plants and increasingly stringent microbiological requirements.

Currently, many strategies are been employed to remove, prevent and/or delay the formation of biofilms in the food industry, but with limited success. This is, in part, because little is known about the biofilm structure and behavior at conditions relevant to food-associated environments. The proposed session will be dedicated to food-associated biofilm formation by mostly problematic species, with special attention to the role of biofilm in the adaptation and survival in food processing environment. In addition, a novel anti-biofilm means will be discussed in the context of food industry.

Identifying the Role of Spatial Organization of Biofilms in Their Persistence

Romain Briandet
INRAE, Paris, France

The traditional perception of microbes as unicellular life forms has deeply changed over the last decades with the collection of scientific evidences showing that microorganisms predominantly live in dense and complex communities known as biofilms. Biofilms are dynamic habitats which constantly evolve in response to environmental fluctuations and thereby constitute remarkable survival strategies for microorganisms. The modulation of biofilm functional properties is largely governed by the active remodeling of their three-dimensional structure and involves an arsenal of microbial self-produced components and interconnected mechanisms. The production of matrix components, the spatial reorganization of ecological interactions, the generation of physiological heterogeneity, the regulation of motility, the production of active enzymes are for instance some of the processes enabling such spatial organization plasticity. The possibility to harness such characteristics to sculpt biofilm structure as an attractive approach to control their functional properties, whether beneficial or deleterious, is also explored.

Affecting Biofilm Formation during Milk Processing Improves the Safety and Quality of Dairy Food

Moshe Shemesh
Agricultural Research Organisation, Rishon LeZion, Israel

Biofilm is the predominant form of bacterial living in natural and industrial settings. Thus, hygienic problems caused by biofilm-forming bacteria during dairy food processing are a fundamental threat to safety and quality of milk products. Biofilm formation depends on the synthesis of an extracellular matrix that holds constituent cells together.

In the model bacterium *Bacillus subtilis*, expression of the matrix genes is induced by environmental cues via a signal transduction pathway. These signals interact with components of the cytoplasmic membrane, which presumably play a key role in inducing biofilm formation by *B. subtilis*. Moreover, we find that mitigating the biofilm formation causes high sensitivity of bacterial cells, to heat pasteurization undertaken during food processing. The downregulation in the expression of genes responsible for the production of the biofilm matrix could account for the increased sensitivity of bacterial cells to heat treatments. Besides, we find an improvement in the technological properties of food products such as soft cheeses following mitigating biofilm formation. Thus, we believe that affecting biofilm formation during dairy food processing provides an opportunity for developing safer and qualitative food products.

Role of Biofilm Formation by *Bacilli* in Adaptation to Food Matrices

Satish Kumar
Agricultural Research Organization, Rishon LeZion, Israel

Biofilms formed by probiotic bacteria can be beneficial and thought to enhance the quality of the food. Besides, beneficial biofilm may confer effective adaptation to food matrices that help bacteria to survive unfavourable conditions. Thus, those matrices for instance dietary fibers of various food products might serve as the scaffold for bacteria to colonize and form biofilm. The fibres might essentially protect the bacteria against various environmental and physical stresses that usually might kill the free-living planktonic cells. We report about the tight interaction of gram-positive probiotic bacterium *Bacillus subtilis* with the dietary fibres in legumes through floating biofilm formation. Our findings show that the interaction and pellicle formation is mainly dependent on the matrix genes (*tasA* and *epsH*) and is directly controlled by the Spo0A/SinI signal transduction pathway. It is believed that biofilm formation on fibres is an adaptation response of bacteria for maintaining different physiological processes such as cellular metabolism and growth.

S6 Water Re-Use in Operation – How to Clean Up Used Water Sources for Food Use and Consumer Safety in Practice

The availability of potable water for food production along the farm-to-fork food chain is swiftly diminishing. While suitable water sources are drying up, regulations continue to require potable water quality for every aspect of food production, without regard to what quality actually is needed for a particular process or purpose.

The default regulatory standard water quality for food use around the world is potable water. This is equivalent to drinking water quality water, in terms of the global WHO, FAO and Codex guidelines that WTO will concur to. This regulatory viewpoint hampers efforts of the industry to address water security challenges by developing fit-for-purpose water supplies and manage them in day-to-day operation such that consumer food safety is not compromised.

A fit-for-purpose and risk-based approach has been advocated by Codex Alimentarius, but this only focuses on key principles for assessing and managing risks and does not go into operational matters. Yet, it is the operational ability for large as well as small food businesses to deliver suitable quality water supplies for safe food production and processing ongoingly where the ultimate challenge of is.

The technical capability to understand safe water reuse operationally and to ensure chemical, microbiological and physical suitability of reuse water sources is not commonly available to many food businesses as yet, but more and more experience is being gained by some industry sectors and other organizations.

In this symposium, experts will share and discuss their experiences and views on the needs and solutions to clean-up used water for food reuse, and how to manage the re-use water supplies as an integral part of the management system of a food business operation.

Achieving Microbiologically Safe Water Re-Use in Food Operations: Rules and Tools

Phyllis B. Posy

PosyGlobal, Jerusalem, Israel

Rather than policy or regulatory generalizations, this presentation will focus on rules of thumb to apply practical, available approaches and technologies for safe water re-use. Three key questions will help you determine if you have a viable candidate for water re-use that will be microbiologically safe over the long term. Step-by-step routes for each, including what data you need and how to get it, will show how to develop answers and a working plan. Major non-chemical and chemical tools for assuring safety will be discussed as well as effective implementation strategies for integrating them into your production system.

What are the Options to Prevent and Control Chemical Risks Associated to Water Re-Use?

Josep Molas Pages

Coca-Cola Company, Madrid, Spain

Food and beverage producers use water in their facilities for several purposes, like disinfection, cleaning and rinsing of processing equipment, toilet and handwashing facilities, watering, etc. However, the most critical use is in cases where the water may come in indirect contact with a food stuff or be used as an ingredient in the final product.

In the European Union the water used in food facilities shall meet the quality criteria in the Drinking Water Directive 2020/2184, although some exemptions can be granted in case the authority is satisfied with the quality of the water, which cannot affect the wholesomeness of the finished foodstuff.

Water reuse in food and beverage industry can be done applying HACCP principles and taking into consideration the intended use of the reused water. A critical aspect in food and beverage processes is the microbiology, but also traces of chemicals and by-products that may be present due to different processing and origin of the water.

Reuse of water implies decision trees to ensure that final product and health of the workers and consumers is not affected. A fit-for-purpose reuse needs to be established. Water origins need to be carefully assessed, and reconditioning evaluated accordingly to ensure compliance with applicable regulations and food law.

In a world where fresh water resources are stressed, the reuse of water shall be considered and practiced where feasible.

Integrating Safe Water Re-Use into the Management Systems of Food Operations

Susanne Knochel

University of Copenhagen, Copenhagen, Denmark

While the obvious place to start water savings is to avoid spillage and unnecessary use, more technically challenging steps such as in-house treatment of reclaimed water and process water with subsequent reuse is attracting increasing interest. Using other qualities than the default "potable" water represents new challenges within microbiological quality and safety management not only for the responsible processors but also for regulators and inspection services. The intended use will define the microbiological quality required. From a risk perspective, water as an ingredient would normally be potable "first-use" water, while reuse water (reclaimed, recirculated or recycled) can replace potable water for a range of other purposes. If used for non-food contact surfaces, food safety is not an issue provided unintentional contact can be avoided. If used for contact with food or food contact surfaces, the whole process needs to

be integrated in the safety management. Specific knowledge is needed on the exact source of the water and potential hazards, the capture, treatment technologies applied and their performance, distribution and storage conditions, monitoring possibilities and requirements for the intended use and potential impact on the final food product all the way to the consumer. Due to the specificity, it is difficult to make a "one-size-fits-all" but guidelines are being and have been developed at different levels. Guidelines should ideally also cover non-safety issues of importance e.g., prevention of quality problems, biofouling, work environment etc. Collaborative efforts between industry, academia and regulators as well as showcases and training are helpful in bringing the field fast forward.

S7 Next Generation Sequencing (NGS): Pragmatic Considerations from Industrial Perspectives

2012 was a date to remember for genomic sequencing technique. 8 years later, numerous sequencing solutions with faster throughput and longer reading have been made available, together with a deluge of communications! They are all referred as NGS – Next Generation Sequencing. Among them, the most notorious being the capacity to sequence the whole genome of a bacterial isolate, WGS – Whole Genome Sequencing.

Early adopted by food safety authorities for source tracking in foodborne outbreaks, these techniques have not been yet widely considered for their potential by food producers. A workshop was organized within a network of 17 global food companies having experienced WGS with the objective to better define the benefits and barriers of the technology implementation for source tracking. According to the outcomes, it is unlikely that WGS will replace all other typing tools in the near future...

The first talk will provide the industry perspectives on WGS: the added values, but as well the pain points and. The second talk will clarify the difference between WGS and NGS to put the troops into order; it will as well draw industrial expectations regarding these technologies while tones of sequences are available for the development of complete solutions. The last 35 min will be dedicated to a round-table gathering various stakeholders such food producers, method developers, food technology centre, university and consultant operating in hygiene control, in order to get a return of experiences, practical examples, and expectations.

WGS and Implications for the Food Industry

Adrienne Klijn

Soci t  des Produits Nestl  SA, Lausanne, Switzerland

Much has been written about Whole Genome Sequencing (WGS) as a tool for outbreak detection and investigation. The point of view from authorities and academia is well documented, but a broad perspective on its use by the food industry has been missing so far. A workshop was held in September 2019 in Lausanne, Switzerland, bringing together several companies from the food industry to define a more general point of view, share experiences around implementation and explore synergies from working together. Prior to the workshop, a survey was sent out to collect information. The survey and discussions provided unique insights into how, when and for what purposes WGS is used by these companies.

Food Safety and Quality Applications of NGS

Martin Wiedmann

Cornell University, Ithaca, New York, USA

Next generation sequencing (NGS) is a technology platform that can be used for different applications from whole genome sequencing to metagenomics and targeted amplicon sequencing. This presentation will provide an overview of different next generation sequencing-based applications in food safety and quality. Specific case studies on the use of WGS for outbreak detection, trace-back investigations, and

characterization of different foodborne pathogens will also be provided to illustrate the challenges and opportunities associated with application of NGS in the food industry. Finally, this presentation will also discuss challenges and roadblocks that stand in the way of increased uses of NGS technologies as well as possible approaches to overcome these challenges in order to achieve a future that provide for truly risk-based precision approaches to addressing microbial food safety and quality issues.

S8 **What to Decide? Making Informed Decisions for Process Validation and Food Safety Legislation Using Stochastic Risk Models**

Food safety management usually involves dichotomous decisions: What is an acceptable microbial concentration in food? What is the target reduction of a critical control step? However, food safety is not black and white because microbial behaviour is variable. Also, critical parameters like temperature and time vary along the food supply chain and significantly amplify the variability. Because variability is part of the system, it should be included in risk assessments that are used to come to informed decisions. In this symposium, we will show how this can be done using stochastic models built following a Bayesian approach. We will illustrate the application of these models to aid in food safety management: we will give examples of how industries use it to validate processes, and how government includes it to optimize quantitative microbial risk assessment.

A Definition of Probabilistic Models Including Variability of the Bacterial Response Using a Multi-level (Bayesian) Approach

Alberto Garre

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Variability is an inherent to microorganisms and can be very relevant for food safety. In this context, variability refers to differences in the thermal resistance of growth fitness of different cells of the microbial population. However, its quantification and separation from uncertainty is a challenge from the point of view of experimental design and statistical analysis.

In this talk we describe a modelling methodology based on multi-level models and Bayesian statistics to analyse the variability of the microbial response. This approach enables the definition of stochastic hypotheses for the contribution of variability and uncertainty to the microbial response. Once the model has been fitted to the data, it can be used for making predictions in risk assessment including different sources of variation (uncertainty and/or variability). The application of the modelling approach is illustrated using a case study related to the inactivation of *Listeria monocytogenes*.

How Can Stochastic Models Including Variability be Used in Validation of Industrial Processes?

Jean-Christophe Augustin

Danone, Palaiseau, France

The example developed during this presentation is related to the validation of the shelf life after opening for a multi-servings new product. In case of microbial contamination after opening by the consumer, this product supports the growth of *Listeria monocytogenes* and the residual shelf-life duration will act as a critical control measure to maintain the contamination at an acceptable level.

The estimation of the number of bacterial cells ingested by the consumers requires information about the initial contamination, the growth of *L. monocytogenes* in the considered product and about storage conditions. For this kind of food products which are relatively favorable to the growth of *L. monocytogenes*, it is of primary importance to consider the actual variability of influential factors to adopt a risk-based management option.

The variability of consumers practices according to the expected shelf life was estimated and combined with biological variability of *Listeria* to estimate the risk of

listeriosis. The duration of the shelf life after opening was adopted considering the available risk estimates for *L. monocytogenes* in other ready-to-eat foods. This kind of information, published by official bodies, is really useful for business operators to benchmark their own risk estimates and take the decision about the acceptable risk.

How Can Variability be Accounted for in Governmental Risk Assessments?

Winy Messens

European Food Safety Authority (EFSA), Parma, Italy

EFSA's Panel on Biological Hazards (BIOHAZ Panel) deals with questions on biological hazards relating to food safety and foodborne diseases, including foodborne zoonoses, food hygiene, microbiology, transmissible spongiform Encephalopathies (TSE). The scientific assessments are diverse and very often a new approach needs to be established to deal with a mandate. Variability (and also uncertainty) need to be considered and add to the complexity. Amongst the many factors, time and temperature along the supply chain are the most relevant for assessing the biological hazards. Predictive modelling of pathogens is a valuable tool for this. Two recent BIOHAZ scientific opinions dealing with the impact of on-land transport/storage of fresh fishery products (FFP) on the survival and growth of biological hazards will be used to illustrate the complexity and numerous factors to be considered. One opinion compares the use of "tubs" with freshwater and ice with the currently authorised practice (fish boxes with ice). The other one deals with superchilling of FFP, i.e., lowering the fish temperature to between the initial freezing point of the fish and about 1–2°C lower, and compares superchilled FFP in boxes without ice with the currently authorised practice in boxes with ice.

S9 ***Clostridium botulinum*: Re-emerging Risk?**

There is an increasingly large demand for natural food products. Consumers reject some ingredients leading to the development of "clean-labels" by industry. This rejection is justified by increasing awareness of potential negative health impacts of some food ingredients, the rejection of the production method resulting from intensive agriculture, concerns over the long-distance transport of food ingredients, the search for more natural products, and fears over the lack of knowledge of the food chain and production methods.

To meet this demand, manufacturers must limit the use of preservatives, reduce salt or sugar content, constantly improve the organoleptic quality of the processed, preserved products. Changes in consumer practice could also impact the risks due to foodborne pathogens. Recently, only a few botulism outbreaks have involved commercial products (a majority of botulism cases have been due to home-prepared foods). There is concern that this may now change, and lead to the re-emergence of cases of *Clostridium botulinum* for commercial manufactured products.

The purpose of this symposium is to present the risk of re-emergence of botulism outbreaks due to *Clostridium botulinum* species, both for psychrophilic and mesophilic strains, following the reduction of the various hurdles used for decades to stabilize food. In this context, an overview of the present situation, and then two scenarios will be presented: i) a refrigerated product, where limiting the use of nitrites could become problematic, or ii) a heat-treated shelf-stable product, for which the combination of a minimal heat treatment or none, associated with combination of low level of physicochemical parameters of formulation is used for product stability.

An Overview – *Clostridium botulinum* as an Emerging Risk

Michael W. Peck

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Clostridium botulinum is a heterogeneous species comprising at least four distinct Groups of Gram-positive endospore-forming anaerobic bacteria. Strains of *C. botulinum* Group I and Group II are responsible for most cases of foodborne

botulism, a severe and deadly intoxication associated with consumption of the highly potent botulinum neurotoxin that has been formed in food. *C. botulinum* Group I (proteolytic *C. botulinum*) is a mesophilic bacterium, while *C. botulinum* Group II (non-proteolytic *C. botulinum*) is a psychrotrophic bacterium.

There is an increasing consumer demand for natural food products and “clean food labels.” This is driven by consumer concerns over factors such as intensive agriculture and long-distance transport of ingredients, potential negative health impact of some ingredients, and the desire for more natural mildly processed food products. To meet this demand manufacturers are reducing the concentrations of preservatives and salt in processed food products, addressing the sourcing of ingredients and improving organoleptic quality

Foodborne botulism is associated with home-prepared foods, and more rarely with commercially-prepared foods. But, there is a concern that in addressing these consumer demands, without strong vigilance, there may be an opportunity for the re-emergence of foodborne botulism associated with commercially-prepared foods. This presentation will provide an overview of the present situation with respect to *C. botulinum* and foodborne botulism, and consider the risk of re-emergence of foodborne botulism.

Modelling the Probability of Growth of *Clostridium sporogenes* Spores as a Function of Heat Treatment and the Properties of the Recovery Medium (pH, Water Activity) in Pasteurized Olive-based Product

Emmanuelle Boix

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Clostridium sporogenes has been widely used as a surrogate for proteolytic *C. botulinum* for validating thermal processes in low-acid cans. In fact, *C. botulinum* is the most heat-resistant pathogen spore-forming bacteria and its elimination requires strong heat treatments that can harm foods. To limit the intensity of heat treatments, industrials must use other ways of control as an association of acidic and saline environment after a low heat treatment like found in the pasteurized olive product.

First, the growth limit of pH (5.1-4.4), salt concentration (0-11% m/v) and heat resistance (2-10 min at 102°C and 110°C) were studied on 40 strains of *C. sporogenes* isolated from spoiled cans. Some strains were able to grow below pH 4.6 and above 10% of salt concentration, which are growth limit commonly assumed for *C. botulinum*. The thermoresistance of *C. sporogenes* spores was heterogeneous with strains resistant at 10 min at 110°C (< 2 log of destruction) and others very sensitive with complete destruction after 2 min at 102°C.

Secondly, *C. sporogenes* PA 3679 spores were inoculated in PYGm broth with NaCl (0-11% w/v) or pH (4.4-7). The absence of germination and recovery of spores of *C. sporogenes* PA 3679 was observed under conditions allowing vegetative cell growth (pH 4.4 - 7; NaCl 0-10% (w/v)).

Thirdly, we studied the interactions between salt (0-11% w/v), pH (7-4.4) and heat treatment (80°C, 10 min or 100°C, 1.5 min) on the growth probability of *C. sporogenes* PA 3679 spores. Results shown than the low-heat treatment decreases the growth probability in association with non-acid pH and low salt concentration.

In conclusion, the strains of *C. sporogenes* selected according to the physiological study (growth limit and thermoresistance) and strains of *C. botulinum* will be added to validate the growth probability obtained with *C. sporogenes* PA 3679.

Influence of Reduced Levels of Sodium Nitrite on the Growth and Toxinogenesis of *Clostridium botulinum* Group II Type B in Cooked Meat Products

Sarah Lebrun

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There is a high demand to reduce nitrite (NaNO₂) incorporation rates in meat products in order to limit the risk associated with nitrosamines formation. Taking into account its preventive effect against *Clostridium botulinum*, the objective of this study was to evaluate the risk associated with psychrotrophic *C. botulinum* Group II (non-proteolytic) type B in cooked ham and Frankfurter type sausages in function of various NaNO₂ and salt incorporation rates (0, 30, 60 and 80 mg NaNO₂/kg; 12 to 19 g NaCl/kg). Raw materials were mixed with appropriate ingredients, then inoculated or not with a cocktail of spores of three *C. botulinum* Group II (non-proteolytic) type B strains at 3.5 log CFU/g and vacuum packed in portions of 50g. The products were cooked and cooled in conditions simulating industrial processes and stored 2 weeks at 4°C/1h at 20°C/4 to 11 weeks at 8°C. Growth and toxinogenesis were observed during the shelf life. In conditions applied during the present experiment, reduction of NaNO₂ incorporation at 30 mg/kg or higher allowed to prevent *C. botulinum* growth and the associated toxinogenesis during around 6 weeks. In contrast, total removal of nitrite didn't prevent growth and toxin production in the tested meat models.

S10 An Update on the Integration of “Omics” into Risk Assessment

Omics approaches can serve to reduce the uncertainty in the different steps of risk assessment – hazard identification, exposure assessment, hazard characterization, and risk characterization. Understanding the molecular mechanisms of growth, survival, adaptation throughout the food chain and how such phenotypes vary within pathogenic species, is a necessary step in the process of fine tuning exposure assessment. Similarly, studying the virulence mechanism of foodborne pathogens, how they interact with the human host to cause disease, and identifying key molecular events that determine such virulence and their distribution among members of the same species, is fundamental for an upgraded hazard characterization. This symposium has the goal of providing an update regarding the integration of “omics” into risk assessment as a following-up of the IAFP meeting in 2017 and speakers from industry, academia and government will share recent advances in this field.

Can We Use (Epi-)Genetic Markers for *Listeria monocytogenes* in Hazard Characterization?

Lucas Wijnands

cZ&O/RIVM, Bilthoven, The Netherlands

Hazard characterization is an important part of microbial risk assessment, as it should provide data on the hazard posed by a foodborne microorganism, at best in combination with a food product. In its best form, hazard characterizations are based upon a species, but differentiation within a species would be a big improvement.

The species *Listeria monocytogenes* is subdivided in 13 serotypes. Some of these serotypes are mainly linked to human disease. Thus, *L. monocytogenes* occurring in food and/or patients can be more precisely specified, and more precise hazard characterizations and with that more specific risk assessments can be set up.

In a simulation model of the gastrointestinal passage we investigated some of the human disease related serotypes for their capacity to invade epithelial cells. Marked differences were shown.

The results of the comparison of invasion capacity show that the invasive capacity of serotypes is different. More specific description of *L. monocytogenes*, as shown here in serotypes, can be used to improve hazard characterization and thus lead to improved risk assessment. Other (molecular) characterizations, such as MLST, clonal complexes and/or WGS/NGS, are also available and can therefore also improve hazard characterizations. Moreover, hazard characterization and the concurring risk assessments could

even be further improved by investigating the influence of matrix and host on the invasive potential of *L. monocytogenes*. Such improvements do not only count for *Listeria monocytogenes*, but for all foodborne pathogens.

Genome-wide Association Studies to Explore *Listeria monocytogenes* Phenotypes Relevant for Exposure Assessment and Hazard Characterization

Laurent Guillier

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The application of whole-genome sequencing (WGS) to the risk assessment of foodborne pathogens is a key challenge. WGS offers the highest level of strain discrimination for more precise hazard identification, hazard characterisation and exposure assessment.

Genome-wide association studies (GWAS) represent today powerful tools for the identification of associations between genomic elements and microbial phenotypic properties. Other cutting-edge tools include supervised classification technique (such as machine learning) or statistical methods to characterize phenotype distribution on a phylogenetic tree. A panorama of the available techniques will be presented in this talk with a focus on the interpretation of significant results and their inclusion in risk assessment.

Listeria monocytogenes is a major pathogen of concern for risk assessment agencies. In recent years, several studies applied GWAS or machine learning methods for improving hazard identification, hazard characterisation and exposure assessment of this pathogen. A review of relevant studies will be presented. It will present the studies that identified markers related to higher persistence in the food chain, better growth in foods and higher virulence.

Enabling a Preventative Approach to Managing Microbial Risk in a Manufacturing Environment through Omics and Predictive Microbiology

Brendan Ring

Crema Global, Dublin, Ireland

Food safety and consumer health are front of mind for many in the food industry. There are numerous procedures and controls in place to minimise food contamination. However, foodborne illness still occurs. New approaches are required to enable companies to take a more preventative approach to food safety. Genomics may be the tool that industry has been waiting for.

Driving this next-generation approach to managing microbial risk in the manufacturing environment is genomics. This rich dataset is a valuable source of information detailing what is happening right throughout the production environment. Companies that are learning how to use this data are discovering insights that enable them to identify what is happening in their factory, predict and rank future risks so that you can implement early preventative action. This is a step-change in improvement from traditional culture techniques.

Further value can be achieved by feeding genomic data into food safety predictive algorithms. These algorithms use many data sources, including incoming material specification, temperature and humidity in the factory and historic data. Combining these data sources greatly enhances the output and value to the users.

Learn how these new techniques are enabling a truly preventative approach to food safety.

S11 Consumer Safety Aspects of Artisanal and Entrepreneurial Food Fermentations

The spontaneous nature of food fermentation has been exploited for thousands of years in all regions and civilizations around the globe for preservation and improvement of quality and taste of plant and animal raw materials. Different fermentation approaches have developed around the globe, for instance varying with the food raw

materials and ingredients being fermented, the fermentation conditions, starters used and process control exerted. They all have in common that they promote growth of beneficial microorganisms that mostly occur in particular succession for an optimal fermented end product. Typically, detrimental and/or pathogenic microbes may be inhibited in successfully designed fermentation approaches.

The succession of different microbes working in cooperation, symbiosis, or competition is a complex process, especially where the indigenous microbiota of the raw material is relied on. At the industrial scale, starter cultures are commonly used for faster growth and better control of quality and safety of the fermentation end products. Consumer risks associated to non-well controlled fermentation have been voiced, but safety incidents so far seem limited.

Food fermentation is a booming trend at smaller “entrepreneurial” scales, such as in restaurants, small food service businesses and in the consumer home. In most of these settings the focus may mainly be on the health and other benefits of food fermentation, and there may be rather little attention on potential food safety risks to consumers and how to control hazards consistently. Importantly, the advanced or even the basic skills and knowledge of the producers necessary to ensure safe, fermented foods is lacking in many of the countries where this trend is apparent.

This short symposium will consider the theory and practice of the consumer safety of small-scale artisanal or entrepreneurial food fermentation. Key questions: what are significant hazards and is there already epidemiological evidence of consumer risks?

Small Scale Food Fermentation Trends and Potential Microbiological Risks

Eddy J. Smid

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The public interest for fermented foods and beverages is rising, particularly in western markets. This trend is boosted by the public perception of fermentation as a natural preservation method, delivering foods and drinks with health benefits. Public interest in fermentation is evidenced by the growing numbers of (i) small scale producers (ii) chefs in quality restaurants offering various fermented ingredients and (iii) more and more people producing fermented foods at home. Also large industrial food producers have regained interest in fermentation as a technique delivering options to diversify their product portfolio with characteristic food products.

This lecture will address the microbiology as well as the food safety risks of traditional fermented food products. Most of these products are made without starter cultures and rely on either spontaneous fermentation or backslopping. To keep the fermenting microbes alive for their anticipated health benefit, the end-products of fermentation usually are not pasteurised. In this context, I will discuss (i) the natural barriers for food pathogenic bacteria to propagate in fermented foods, (ii) fermentation related risk factors for the generation of toxins and (iii) measures to be taken by small scale producers to mitigate food safety risks of traditional fermented foods.

Vegetable Fermentations as Man-made Microbial Ecosystems: Investigating Microbial Community Succession and Safety Aspects

Sarah Lebeer

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Recently, artisanal plant-based fermentations have regained interests by the broad public and haute cuisine chefs, because it also increases the organoleptic properties of food. Fermented carrot juice is an example of such an artisanal vegetable fermentation, implemented by collaborating chefs and home-fermenting enthusiasts. It is a robust man-made

ecosystem which is dominated by lactic acid bacteria. Using an RNA-based amplicon sequencing approach allowed a more precise monitoring of the active community with less sequencing depth. This study also reemphasized the need for attention to the initial peak of *Enterobacteriaceae* at the start of the fermentation, because it could harbor a food risk. In addition, we explored the food safety of the spontaneous carrot juice by doing a challenge test with the three common food pathogens *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* Typhimurium and *Escherichia coli* O157:H7. These pathogens could survive and persist within the fermentation up to 8 days of fermentation. Therefore, dedicated starter culture could be used to guide and speed up the fermentation and reduce potential pathogen load as early as possible.

Here, starter cultures from the three important genera of the carrot juice fermentation were evaluated: (1) *Leuconostoc*, which is the first genus to dominate the fermentation, (2) *Lactiplantibacillus*, which dominates the final phase of the fermentation and (3) *Lactocaseibacillus*, a genus which occurs within the carrot juice fermentation but seldomly dominates it. *Lactocaseibacillus* also harbors very well characterized probiotic such as *L. rhamnosus* GG and *L. rhamnosus* GR-1. *Leuconostoc* starter cultures had the largest impact on the acidification of the fermentation in the first days and their fermentation resulted in a pH lower than 4.6, an important food safety threshold, after one day of fermentation. In general, the sympatric starter cultures belonging to the *Lactocaseibacillus* and *Lactiplantibacillus* were able to better guide the fermentation towards a uniform end community than their allopatric counterparts, with some exceptions. The successful isolates studied were shown to harbor a range of tools such as a metabolic repertoire that allows fast/efficient conversion of the available carrot carbohydrates in lactic acid and presence of putative bacteriocin genes that could help them combat other competing bacteria.

To conclude, carrot juice fermentation as a model for vegetable fermentations is generally safe to consume when fermented for at least 8 days. The fermentation could be further improved by using dedicated starter culture, e.g. by the use of combination of a *Leuconostoc* strain with an additional starter culture from the *Lactiplantibacillus* genus. This could result in a faster acidification and more uniform end community, but will require careful monitoring of the microbial competition. Also addition of probiotic strains as starter culture is feasible when additional functionalities or validated probiotic properties are desired for specific applications or markets.

Consumer Safety Aspects of Traditional Fermented Foods

George-John Nychas

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Traditional fermented foods have been evolved over centuries according to local culture and artisanal practices and constitute a major part in modern diet. Consumer concern for food safety and high demand for traditional food products is a challenge for producers. Meat fermentation is a two-stage process of fermentation and ripening, resulting in the development of an inhibitory environment for most pathogens. This holds true for fermented dry sausages where the hygienic status of the processing environment and equipment plays a pivotal role in their microbiological safety. Transmission of pathogenic bacteria through the production of table olives at artisanal level has not been documented so far, provided that the olives have been subjected to proper fermentation in terms of salt and acid control. It seems that the brine environment is hostile for the survival of most pathogenic bacteria. Eastern Asia is also famous for a variety of traditional fermented foods, such as fermented soybean and fermented vegetable products. *Bacillus cereus*, aflatoxins and biogenic amines could be considered as major safety hazards due to inconsistent quality of raw materials. However, significant efforts have been undertaken to standardize the processing of these foods, especially at small-scale production to improve sanitation and safety.

S12 Processing Environment Monitoring in Low-moisture Foods Production Environments. Are We Looking for the Right Thing(s)/Microorganisms, in the Right Places?

Over recent years, both industry and regulators have become aware of the role that microbiological monitoring of the processing environment makes to improved food safety. Such monitoring is often referred to as processing environment monitoring (PEM). Its importance in the manufacture of low-moisture foods (LMF) is key to pathogen control. Many documents, certification schemes and regulations underline its importance, provide general guidance and even require PEM as part of Food Safety management systems. PEM is required in EU Regulation 2073 since 2005.

Recent foodborne outbreaks involving low moisture foods in Europe highlights that a well-designed PEM is increasingly important in the context of food safety management systems. However, PEM can only form an integral part of food safety systems when it is designed and implemented adequately assure that the processing environment will not contribute to contamination of products.

From a practical point of view, there are still many questions on how to set up and evaluate a useful PEM to provide not only early warnings of potential product contamination, but also ongoing verification of environmental control in dry production environments. Within ILSI Europe, an expert group has evaluated the current existing scientific data on microorganisms of importance and/or concern in dry production environments. This includes pathogens as well as indicators and their role, i.e., what do they indicate and how could indicators be used to improve food safety. The final report will help industry and regulators set up and evaluate targeted PEM Programs.

Pathogen of Concerns in Low-moisture Foods – An Overview

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Controlling the food processing environment is an important first step in reducing food safety risk to the consumer, whilst maintaining the protection of a reputable food brand. The relevance of low-moisture foods (LMF) and their production environment is highlighted following reports of several recent outbreaks. This development in turn focuses attention on the foodborne pathogens of concern that are found to colonise these ecological niches and, importantly, how these biological hazards might be controlled. In particular, the mechanisms that aid their survival within a low-moisture environment remain to be fully elucidated. At the outset, a better understanding of the nature of these bacteria of importance to food safety is required and, in particular, the development of strategies designed to break the link between contamination and their persistence under low-moisture niche selection.

Several biological hazards are recognised, some of which, based on current epidemiological information are of greater relevance when compared with others. Among these, *Salmonella* is the bacterial pathogen of most concern in LMF products. This genus possesses an innate ability to persist for extended time periods in a dried state within raw material, finished product and the factory environment. *Cronobacter* species is also recognised as being of increased risk for special populations (such as neonates and older individuals) and has been documented in outbreaks associated with powder infant formula. This bacterium is recognised in particular for its long-term tolerance to desiccation. Pathotypes of *Escherichia coli*, though less commonly associated with LMF have been reported. Although *Bacillus cereus* is a known bacterial pathogen of concern in LMF, and is normally found at very low levels, such foods, if inappropriately reconstituted, may pose a public health risk after vegetative growth and toxin production.

This short presentation will provide an overview of the current knowledge of selected pathogens of concern. By expanding our knowledge of these LMF bacteria of concern and, in particular, understand how they persist in these niches, we may be in a better position to exploit them to improve food safety and protect public health and brand reputation.

Low Moisture Food Processing Environment – What is Specific?

Anett Winkler

Cargill, Krefeld, Germany

The presentation will start with providing the definition and some specific facts about low moisture foods and dry production environments. Then it will point to the analyses of outbreaks being helpful to determine necessary steps and changes to existing processing environment monitoring programs (PEM). Furthermore, more detailed information will be given to the elements of a PEM program. That includes search for meaningful sampling points and looking at them in the context of root-cause analyses. Different sampling strategies depending on the circumstances will be presented, and the elements of corrective and preventive actions highlighted.

Indicators and Their Role in PEM

Ellen Wemmenhove

Arla Foods Ingredients, Nr Vium, Denmark

Microbiological examination of the food manufacturing environment is key for an effective food safety and quality system. Pathogen detection may be complicated in dry processing environments. In these environments, pathogens may be present at low concentrations while being spread heterogeneously. Under these conditions, a sampling bias may occur in the dry processing environment. Microbial indicators are widely used for monitoring of pathogens in dry processing environments. Analysis of microbial indicators may not be very targeted, however it is sometimes preferred over more specific types of analyses. The presentation focuses on the difference between microbial indicators and index organisms, the history of use of microbial indicators, and their purposes of use. A detailed overview with examples of microbial indicators in the dry processing environment will be presented.

Friend or Foe – Bt in the Spotlight of Food Microbiology

Monika Ehling-Schulz

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Bacillus cereus is the name giving species of a group of genetically closely related Gram-positive endospore-forming bacteria, which is also often referred to as *B. cereus sensu lato* (s.l.). During recent years, toxigenic *Bacillus cereus* s.l. has gained prominence as an important food borne pathogen as well as the causative agent of systemic and local infections. While the relevance of the name giving species of the group, *B. cereus sensu stricto* (s.s.), as a major cause of foodborne infections and intoxications is undisputed, the role of the closely related *Bacillus Thuringiensis* in food microbiology is currently under debate. *B. Thuringiensis* is a widely used biopesticide, which is gaining increasing importance in frame of the ecological transformation process of food production and vector control. However, there is also growing evidence suggesting that certain *B. Thuringiensis* strains can represent a food safety risk, underpinning the importance of assessing the hazardous potential of each strain used as biopesticide. Thus, this lecture will focus on *B. Thuringiensis* in the spotlight of food microbiology and will also discuss the potential of novel diagnostic tools to move from the currently taxonomic driven to a more risk orientated diagnostics.

Genetic and Ecological Distinctiveness of Entomopathogenic *B. Thuringiensis* – Implications for Food Safety

Ben Raymond

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The *Bacillus cereus* group contains vertebrate pathogens such as *B. anthracis* and *B. cereus* as well as the invertebrate pathogen *B. Thuringiensis* (Bt). DNA sequencing studies along with ecological and experimental work has shown that the *B. cereus* group is heterogeneous and formed of distinct groups with substantial differences in biology, ecology and host association which should be re-classified into distinct species. The group posing the greatest risk (the *anthracis* clade) is distantly related to the group containing all biopesticides. Recent papers have suggested that Bt may be a causative agent of food-poisoning. However, a critical examination of available data provides no solid evidence that Bt causes diarrhoea. Bt and *B. cereus* spores are common at low doses in the environment, in foods and in salad crops. Moreover, MLST genotyping of >2000 isolates show that biopesticide genotypes have never been isolated from any clinical infection. In general, Bt is very rarely identified from stool samples, and is not infectious in oral toxicity tests of vertebrates. No recent data provide evidence to suggest that these oral toxicity tests are invalid, nor is there any new data that confirm a causal link between ingestion of Bt spores and food poisoning.

Update on the Implication of Presumptive *B. cereus* Associated with Food Poisoning Outbreaks in France

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Bacillus cereus sensu lato (Bc) is a group of bacteria well known for their involvement in human illnesses, in particular foodborne outbreaks (FBOs). Bc is considered the second most common causative agent of FBOs in France after *Staphylococcus aureus*, with more than 300 suspected and confirmed outbreaks and more than 3000 human cases in 2019 (SPF, 2019). *Bacillus Thuringiensis* (Bt) is one of the eight species that compose the Bc group. It distinguishes itself by its capacity to produce insecticidal crystals, making of it the most widely used microbial pesticide on the world market. However, the search for these crystals is not routinely performed in food safety, and the actual involvement of Bt in the occurrence of FBOs is still not known. In a retrospective study of 250 Bc-associated FBOs

S13 Distinction between *Bacillus Thuringiensis* Used in Biopesticide and Presumptive *Bacillus cereus* Strains Involved in Food Quality and Safety: A Hot Topic

Bacillus Thuringiensis (Bt) is a widespread spore-forming bacteria commonly found in soil. Due to its ability to produce parasporal crystalline inclusions that show insecticidal properties, it has become the main microorganism used for pest control in organic farming since the '50s. Bt-based products containing crystal proteins and spores are applied to foliage, soil, water environments or even food storage facilities. While the use of commercial phytosanitary product based on Bt strains is an efficient, easy-to-use and low-cost treatment for crop protection, the persistence of spores on treated vegetables is overpassing microbiological criteria applied in the food industry.

Indeed, at a taxonomic level, Bt belongs to presumptive *Bacillus cereus* which also contains strains that may be involved in food quality and safety. In response of EFSA scientific opinion published in 2016, there are now available tools to distinguish and trace strains authorized and used in commercial biopesticide from presumptive *B. cereus* strains. This session will provide an update and overview of available knowledge and tools regarding this field to fork issue.

declared in France between 2007 and 2017, we revealed that Bt was isolated in the context of 49 toxic episodes (i.e., 20%). Most of Bt isolates were found undistinguishable from some Bt pesticides, based on genomic characterization. This calls for further investigations on the pathogenic potential of Bt pesticides and for the development of monitoring tools for their traceability in food.

S14 Viruses: Looking into and Making Sense of Unforeseen Risks for Food Safety

This symposium will bring together expertise and perspectives from academia and industry to provide scientific information related to emerging or re-emerging viral pathogens of concern to public health that could impact food safety.

Although there is no evidence of transmission in food, the rapid emergence of SARS-CoV-2 and the subsequent scientific response can offer insights on creating awareness, preparedness, and evaluation of approaches that could be used for an emerging viral food pathogen.

In line with the One Health concept, the session will cover topics such as zoonotic transmission of viruses in the past and what lessons those epidemiological linkages have taught us for identifying and assessing potential risk for foodborne transmission. Being able to adopt and develop the appropriate tools to detect such emerging pathogens can assist in controlling their dissemination in order to protect public health and the food industry. Scientific advances in methods to improve traceability will be discussed, as will their implications and limitations. Specifically, addressing the relative risk associated with interpretation of molecular diagnostic tests and how they correlate to measurable risk of transmission and actual health risk will help further our understanding of how to better manage such viral pathogens.

Examples of Viral Zoonotic Infections of Relevance to Food Safety

Wim van der Poel

Wageningen BioVeterinary Research, Department of Virology, Lelystad, The Netherlands

Foodborne viral infections are increasingly recognized as causes of illness in humans. A lot of different viruses can be transmitted by foods, but just a few are common causes of illness in industrialised countries and have a zoonotic origin. Foodborne virus infections primarily are considered person-to-person transmissions in which food is a vehicle. Therefore, any food item that has been handled by an infected person and is not treated before consumption is potentially a risk food.

Consequences differ greatly, depending on where the contamination occurs, and foodborne virus outbreaks range in size from small (when the contamination occurs at the end of the food chain) to massive, with a shellfish-borne hepatitis A outbreak affecting 300.000 persons in China as an example of the effects of source contamination.

Examples of zoonotic viruses of relevance to food safety include hepatitis E virus (HEV) and SARS-CoV-2. Most foodborne viruses replicate in the intestinal tract. By far the most important clinical feature is gastroenteritis. However, foodborne viruses of zoonotic origin like HEV may cause hepatitis and neurologically based symptoms, and severe respiratory symptoms in the case of SARS-CoV-2.

HEV is widely distributed in pigs and to prevent transmission from pork, meat and sausages should be appropriately cooked. Elimination of the virus from the entire pork production through biosecurity management would be preferred, however additional intervention methods as vaccination strategies may be needed to achieve that.

At several occasions workers in the food industry have been tested positive for COVID-19, which may have been acquired from SARS-CoV-2 contaminated frozen food, packaged locally or imported from a COVID-19 affected country.

Lessons Learned from Testing Water for SARS-CoV-2 Transmission: Do We Need to Worry about It?

Gloria Sánchez

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The excretion of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in stools and urine has been used to globally implement wastewater based epidemiology (WBE) as a tool for monitoring the spread of COVID-19 pandemic. Detection of SARS-CoV-2 RNA by molecular techniques in wastewater, sewers, and sludge has showed its potential as an early warning tool, and different countries are currently implementing sewage surveillance into their national or regional COVID-19 monitoring programs. WBE can determine the presence of both asymptomatic and symptomatic infected individuals in a community and can be used as an epidemiological indicator, as well as an indicator of emerging variants of concern. Although the presence of SARS-CoV-2 RNA in sewage and effluents waters, these molecular techniques alone cannot discriminate between potentially infectious viral particles and inactivated particles. For this reason, an easily applicable in situ evaluation tool for viral infectivity would complement the current routine molecular analysis, thus providing information on the spread of the virus, on the quantitative estimation of the risks of transfer and exposure, and in turn, facilitating the public health response. From this experience we build on our learning on how to better prepare and respond to other emerging viral pathogens that could impact food safety.

Environmental Prospective on SARS-CoV-2 Testing: Advances and Challenges

Erin Crowley

Q Laboratories, Inc., Cincinnati, OH, USA

It is certainly no secret that we have all been faced with a multitude of impacts of the COVID-19 crisis in both our professional and domestic environments. Over the past year, SARS-CoV-2, the causative agent of COVID-19 illness has become a public health issue of epic proportions and direct transmission of the virus from person-to-person is the main route of contamination. However, some food industries have faced contamination issues among co-workers, stability of SARS-CoV-2 on various surfaces have been demonstrated, and transmission of the virus from contaminated surfaces might be possible.

In light of these findings, emergency responses were set up to support the food industry. Several methods have been developed to detect SARS-CoV-2 in the food production environment to evaluate the efficiency of control measures designed to eliminate the virus from surfaces. The AOAC International Emergency Response Validation program allows for evaluation and review of multiple test kit models simultaneously, to accelerate the availability and variety of certified test kits.

For food manufacturers, contract laboratories, and others in the food supply chain, these certifications provide independently validated tests they can use to ensure their sanitation protocols are effective and to provide a culture of safety and security for their essential employees.

A discussion of method validation study design, end-user impact and future implications for virus surveillance will be presented.

S15 All Food Processes Have a Residual Risk, Some are Small, Some Very Small and Some are Extremely Small: Zero Risk Does Not Exist

As risk-based approaches are increasingly recognized and used to manage food safety hazards, their implementation requires a recognition and appreciation of residual risk. We define residual risk as the one that remains even after a fully compliant food safety system has been implemented. As true "zero risk" is essentially unattainable, understanding and assessing the residual risks for different products is essential for the different actors involved in the food production system. Understanding residual risk is particularly

critical as improved surveillance systems (e.g., facilitated by whole genome sequencing) can detect small outbreaks and potentially link cases to a product, even when they are consequences of residual risk rather than a noncompliant food safety system. Future work on assessing residual risk for different pathogen-food combinations are essential at both the company and governmental level to further fine tune food safety systems with the definition of an acceptable residual risk. In this symposium the perspective will be reflected on from the risk for the consumer, the industry and the government.

Not Detected or 12D Reduction Does Not Mean Zero Risk

Marcel Zwietering

Wageningen University, Wageningen, The Netherlands

Consumers, food industries and governments typically desire foods that are 'free of any risk.' In practise this is not achievable. The MRA methodology is nowadays one of the tools that can be used by food industries and regulatory bodies to estimate the risk associated with different food products. Inactivation treatments in food processing are usually designed targeting a number of log-reductions of the microorganisms of interest, but inactivation is never absolute. Even after a severe treatment some residual risk remains. Also, if food products are sampled and no contaminants are detected, this clearly is no proof that a full batch is free of the organism. And even if during a longer time period positives are never found, this again is no proof at all for absolute 'safe' product. For both cases illustrative examples will be shown.

Residual Risk in the Era of Molecular Epidemiology and Large Scale Food Production

Robert L. Buchanan

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The development of molecular biology-based tools has led to the ability to 'fingerprint' microorganisms associated with foodborne disease outbreaks. In large scale production lots with extremely low levels of a foodborne pathogenic bacterium coupled with the modern molecular epidemiology systems could lead to detection of an outbreak caused by

pathogen levels well below that which a food manufacturer can verify by traditional testing. This leads to the potential policy gaps when regulatory agencies or food distributors/retailers provide realistic and practical testing guidelines and specifications for foods that are substantially less stringent than the ability to detect a low-level outbreak after literally millions of servings have been consumed by the public. Therefore residual risks of microbial hazards can be a topic of substantial debate and legal challenges in the coming decades. Importantly however, identification of a residual risk that could not be detected by end product testing and classical isolate identification techniques can now be detected, what can be further used to prevent future cases.

Different Perspectives of Residual Risk: Risk per Serving, Total Risk and Burden of Disease

Martin Wiedmann

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For a consumer, the risk associated with the consumption of one serving may be of high relevance, whereas for a government the total number of cases linked to a specific product (e.g., produce, deli meats) may be more important. The relevance of the residual risk will also be influenced by severity of the consequences. This presentation will describe how the different risk metrics available can be used to understand the risk associated to a given food product: risk per serving, total risk in a population and burden of disease. A risk per serving might be more valuable for an individual consumer when deciding between food products, while the number of illness cases or the number of disability adjusted life years (DALYs) lost per year will be more relevant for risk managers. While it therefore is best to report values at both scales for better interpretation, industry may require additional metrics to determine the enterprise risk associated with residual risks to help assess the need to reduce the residual risk that is associated with food safety systems that focus solely on regulatory compliance. Importantly, comparison of expected residual risks to actual risks (which include risks due to failures to apply appropriate and mandated interventions and control strategies) will also be important to determine whether preventative efforts should focus on more stringent regulations or improved enforcement of existing regulations.



TECHNICAL ABSTRACTS

27-28 April 2021

TECHNICAL ABSTRACTS

* Student Award Competitor

Abstracts with an underlined title were previous session presentations and may be formatted differently

TUESDAY, 27 APRIL 2021

T1 Technical Session 1 – Communication Outreach and Education; Food Law and Regulation; Food Safety Systems; General Microbiology; Packaging; Pre-harvest Food Safety; Produce; Viruses and Parasites; Water

Communication Outreach and Education

T1-01 A Review of the Relative Proportion of Foodborne Disease Associated with Food Preparation or Handling Practices in the Home

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Introduction: The home is recognised as a point in the food chain where risks of consumers contracting foodborne disease can be minimised through application of good hygiene practices. However, these practices, for complex reasons are frequently not implemented. The extent of foodborne disease arising in the home is unclear and an updated understanding of associated literature may help inform development of targeted, and potentially more effectual, consumer food safety interventions.

Purpose: The study aimed to estimate the relative proportion of UK foodborne disease caused by faults in food preparation/handling within the home and to identify associated risk factors linked to illness.

Methods: The systematic review was guided by a PICOS (population-intervention-comparator-outcomes) approach to structure eligibility criteria. Key academic databases were interrogated to source academic literature (from 1990, English language and from countries with similar dietary practices to UK); grey literature was identified through papers, reports, reviews and food safety networks/organisations.

Results: Of 278 academic articles evaluated (84 incidence studies, 192 behavioural studies and 2 covering both), 71 were included in the review, supplemented with 21 items from grey literature. Results showed a complicated picture for attribution of incidence to setting, with variable results and limitations associated with data consolidation. Key risk-factors linked to illness were derived from case-reports, case-control studies, refrigerators and microbiological studies, and observation studies. Widespread pathogen contamination in the domestic kitchen was determined, with hands/chopping boards most frequently linked to cross-contamination, particularly of surfaces such as knobs and handles; domestic refrigerators were commonly found to operate at >5°C. Observation studies highlighted many hygiene contraventions, largely focussing around inadequate hand washing, inadequate sanitation of boards/knives and poor temperature control (storage/cooking).

Significance: Findings from the systematic review have identified the potential links between food activities in the domestic kitchen to the point-of-consumption. Data have informed development of generic and pathogen-specific theoretical frameworks.

Food Law and Regulation

T1-02 The Safety of Cultured Meat

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Introduction: Cultured meat is proposed as an innovative solution to the adverse effects of the meat industry. Only limited research has touched upon safety aspects of cultured meat or related cellular agricultural products. Scientific evidence that can demonstrate a food does not pose safety risks to human health is however essential in bringing new products to the market, as regulated by the Novel Foods Regulation in Europe.

Purpose: This study aims to create more depth in what safety of cultured meat entails and what this safety means to different stakeholders active in this field.

Methods: In a qualitative explorative study, we have investigated possible safety concerns of cultured meat that could play a role in the authorisation procedure. Potential food safety and regulatory challenges were explored by interviewing different stakeholders via semi-structured, in-depth interviews.

Results: Provided that safe production requirements are met, cultured meat is not believed to be putting consumers at risk according to the interviewed experts. It is also pointed out that legislators, risk assessors and food business operators should intensively collaborate to ensure a safe product enters the market, partially due to the complexity of this product and its production techniques. Scientific testing and risk management requirements will aid in creating a cultured meat product for which the safety is guaranteed for future consumers.

Significance: The results of this research highlight that to ensure food safety and therefore public health, scientific testing and adequate risk management are essential in introducing a safe food to the market. This not only applies to cultured meat, but also to every novel food that that will be introduced onto the European market in the future.

Food Safety Systems

T1-03 Evaluation of Hygiene Parameters in Chicken Slaughterhouses in Lombardy and Emilia Romagna during 2020

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Introduction: *Campylobacter* is considered the first food poisoning agent in the European Union. Despite this, in EU legislation, *Campylobacter* spp. is indicated as a process hygiene criterion to be evaluated at the end of the slaughter chain for chicken carcasses, in conjunction with the verification of the presence of *Salmonella* spp.

Purpose: The aim of this study was to evaluate the results of Official Sampling Plan for the verification of slaughterhouse hygiene conducted in Lombardy and Emilia Romagna during 2020.

Methods: The sampling plan involved two of the most important Italian Regions for livestock production. Samples were collected in nine provinces and 18 different poultry slaughterhouses. A total of 466 samples, consisting of three chicken neck skins each, were collected and analyzed conforming to the indications of Regulation 2073/05CE for *Salmonella* detection according to ISO6579-1:2017, and for *Campylobacter* count, according to ISO10272-2:2017. All *Salmonella* and *Campylobacter* isolates were also typed.

Results: Regulation 2073/05CE and s.m.i. indicate specific process hygiene criteria for chilled broiler carcasses sampled at slaughterhouse. Positive samples for *Salmonella* greater than 14% and a percentage of samples with *Campylobacter* above 10^3 CFU/g beyond 30% are considered unsatisfactory. Our data show *Salmonella*'s prevalence of 19.31% and 35.68% of samples with a *Campylobacter* count $>10^3$ CFU/g. Nine slaughterhouses, out of 18 plants, weren't compliant for *Salmonella* and 11 weren't for *Campylobacter*'s criterion. Considering both parameters, in 14 slaughterhouses it's necessary to improve the hygiene of production process. *C. jejuni* was the 65.7% of isolates and *S. infantis* was the 70%.

Significance: The poultry production chain represents one of the main reservoirs for *Salmonella* and *Campylobacter* food infections. Therefore, monitoring and control activities are of fundamental importance to keep the levels of contamination within acceptable limits, otherwise improvement of hygiene process it's mandatory, starting from the slaughterhouses but also in the farms.

T1-04 Evaluation of Hygiene Performance Rating for Assessment of Cattle and Sheep Slaughter Hygiene

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Introduction: Regulatory microbiological testing of carcasses cannot be used to guarantee 100% food safety in meat, and thus, the strategy is to ensure that abattoir premises and procedures are sufficiently hygienic for the risk to be at an acceptably low level. This is done by Good Hygiene Practice and HACCP approach conceptualized auditing and microbiological testing. The auditing scheme of Hygiene Performance Rating (HPR) has been used for 10 years in Norwegian abattoirs.

Purpose: The objective was to evaluate HPR for assessment of slaughter hygiene in ovine and bovine abattoirs in Europe, assessed by microbiological testing.

Methods: Ten cattle slaughter lines were visited in Norway, Denmark, Germany, and Spain and 10 sheep slaughter lines were visited in Norway, UK, and Spain. The HPR focus on the operators' hygienic behavior and risk handling of the carcasses. Scores obtained for each operation were summed up into a total score. The standardized study sampling of 25 warm carcasses was performed by swabbing of 800 cm² of cattle carcasses and 600 cm² of sheep carcasses. The abattoirs' own routine microbiological testing used different types of equipment, methods, and analyses.

Results: A close relationship was found between the HPR score and the *Enterobacteriaceae* and *E. coli* results obtained by the standardized study testing. For cattle,

R^2 values were 0.69 and 0.62 for *Enterobacteriaceae* and *E. coli*, respectively, and for sheep the values were 0.62 and 0.60. The correlations between the HPR results and the results from the abattoirs' mandatory sampling were low, as 90% of the samples were below the limit of detection.

Significance: The correlation between the HPR results and the standardized study testing of the carcasses suggests that HPR could be a useful proxy measure for improving slaughter hygiene and risk management.

T1-05* Multi-hurdle Approach Toward *Listeria monocytogenes* Inactivation in Fermented Meat Sausage: High Pressure Processing Assisted by Bacteriophage P100 and Bacteriocinogenic *Pediococcus acidilactici*

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Introduction: The consumers' quest for healthier, natural, locally produced, greener and sustainable foods, renders the demand for these products increasingly prominent. Concerning traditional foods, this challenge may represent an extensive effort since consumers' perceptions regarding these specialties are entirely associated to sensorial and organoleptic features.

Purpose: The purpose of the present work was to evaluate the impact of a non-thermal multi-hurdle approach, which associated mild high pressure processing (HPP), bacteriophage Listex™ P100 and pediocin PA-1 producing *Pediococcus acidilactici*, as a novel minimal processing towards *Listeria monocytogenes* eradication in *Alheira* (a Portuguese fermented meat sausage).

Methods: Two batches of *Alheira* artificially contaminated with 10^5 CFU g⁻¹ of *L. monocytogenes* were inoculated with P100 (10^8 PFU g⁻¹) and *P. acidilactici* HA-6111-2 (10^7 CFU g⁻¹). One set of samples was submitted to HPP (300 MPa, 5 min, 10°C) and stored under refrigeration (4°C) for 60 days, concomitantly with a non-pressure treated control. Physicochemical parameters (pH, colour, lipid peroxidation), texture and microbial diversity (pyrosequencing of V3-V4 region of 16S rRNA) were evaluated throughout storage.

Results: With respect to the multi-hurdle treated samples, a USDA-FSIS 5 logarithmic reduction requirement on *L. monocytogenes* numbers was achieved immediately following pressure processing, with no regrowth being observed. Moreover, no significant differences ($P > 0.05$) on the quality attributes were observed and the *in situ* biosynthesis and semi-quantification of pediocin PA-1 was documented for the first time in *Alheira*. Regarding the synergistic system impact on the microbial communities of *Alheira*, slight differences were observed in the bacterial diversity between treated samples and its counterparts, albeit the main trends in microbial groups during shelf-life was followed by both sets.

Significance: The HPP-bacteriophage-lactic acid bacteria system may represent a novel approach of minimal processing concerning *Listeria* decontamination of cured and fermented meat products, while preserving the authenticity of the organoleptic features of traditional foods.

T1-06 Assessment of Handwashing Equipment Cleanliness in Food Manufacturing Environments

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Introduction: Hand hygiene is linked to food safety at every food production, preparation and service stage. Adequate handwashing with soap and effective drying is necessary to

* Student Award Competitor

prevent cross-contamination of pathogens to food and food-contact surfaces. For food manufacturing, where product volumes are high, accessible hand-hygiene equipment is paramount for food safety programmes to succeed. However, such equipment - if unclean - may inadvertently contaminate hands during the very process undertaken to ensure they are cleaned.

Purpose: To assess hand-hygiene equipment cleanliness in food manufacturing facilities ($n = 3$) as a potential source of hand contamination.

Methods: Hand sinks ($n = 14$), soap dispensers ($n = 13$), towel dispensers ($n = 10$) and sanitiser dispensers ($n = 13$) were sampled post-cleaning ($n = 360$) and post-production ($n = 354$) on repeated occasions using dipslides to assess total viable count (TVC) ($n = 238$) and presumptive *Enterobacteriaceae* ($n = 238$), and adenosine triphosphate (ATP) bioluminescence ($n = 238$).

Results: Hand-hygiene equipment cleanliness, whether post-production or post-cleaning, returned similar findings. Overall, ATP swabs indicated a cautionary (≥ 150 -299 RLU) or unacceptable (≥ 300 RLU) measurement on 72% of occasions while TVC, with counts ranging from 2.5 CFU/cm² (very slight growth) to 100 CFU/cm² (heavy growth), were detected in 64% of samples. Surfaces sampled for *Enterobacteriaceae* were negative (zero growth) on 74% of occasions. While towel dispensers indicated unacceptable ATP at ≥ 300 RLU in 65% of samples post-cleaning, hand sinks more frequently indicated unacceptable ATP measures ≥ 300 RLU (42%) in combination with positive TVC results (71%) and positive *Enterobacteriaceae* (46%). Little variation was seen in sanitiser dispenser cleanliness with 66% of TVC and 14% *Enterobacteriaceae* samples positive (2.5 CFU/cm² or above) post-production in comparison to 54% TVC and 15% *Enterobacteriaceae* (2.5 CFU/cm² or above) post-cleaning.

Significance: Environmental monitoring and appropriate cleaning can ensure that potential reservoirs of contamination are detected and maintained to support hand decontamination post-washing. Data from this study not only highlighted common reservoirs but also areas for intervention focus.

T1-07* Impact of High Hydrostatic Pressure on the Stability of Bacteriophage SALMONELEX™ Towards Potential Application on *Salmonella* Inactivation

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Introduction: The quest for alternative biological approaches to guarantee the safety of food products has been addressed, namely the exploitation of multi-hurdle technologies based on the association of bacteriophages with high hydrostatic pressure (HHP), which is claimed to be a more environmental-friendly, minimally processing option comparatively to conventional thermal processes.

Purpose: The present work consisted in the first preliminary study in which the potential to exploit the bacteriophage cocktail SALMONELEX™ in combination with HHP towards *Salmonella* inactivation in egg was evaluated.

Methods: In order to investigate the effect of different pressure treatments on the stability of the bacteriophage, aliquots with a 10^{11} PFU mL⁻¹ titre were exposed to pressure magnitudes in the range of 200-600 MPa (5 min, 10°C) and the infectivity was determined. Moreover, the HHP impact on the morphology and structural integrity of SALMONELEX™ was evaluated through transmission electron microscopy (TEM). The influence of pH (5-8) and egg components (lysozyme, albumin and alkaline pH) on the bacteriophage barotolerance (300 MPa) was also analyzed.

Results: The assessment of HHP impact on the bacteriophage viability pointed out a stability in the range of 200 to 400 MPa and from 400 MPa onwards, the inactivation was potentiated by an increase in the pressure magnitude, demonstrating a prominent baroresistance of SALMONELEX™ up to 500 MPa. Concerning morphological features, TEM unveiled that processing at 500 and 600 MPa elicited a detrimental impact on the bacteriophage integrity. Moreover, it was noteworthy the barotolerance of SALMONELEX™ previously exposed to different pH conditions, which proved not to undermine its infectivity. Regarding the influence of egg components on the bacteriophage inactivation induced by HHP, a protective effect on the bactericidal activity of SALMONELEX™ was observed.

Significance: The promising results highlighted the notable potential of SALMONELEX™-HHP system concerning *Salmonella* (a resilient pathogen of prominent relevance in the food industry) inactivation in egg.

T1-08 RNA-Seq Analysis Revealed Differences in the Global Transcriptome of *Clostridium perfringens* Isolates

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Introduction: *Clostridium perfringens* is estimated to be the fourth most common bacterial cause of food poisoning outbreaks (FPO) in France causing hundred FPO and more than one thousand cases each year. Based upon the production of six major toxins *C. perfringens* is classified into seven types (A-G). It has been demonstrated that the enterotoxin CPE encoded by *cpe* gene is essential to the development of gastroenteritis. Through its complex regulatory system, *C. perfringens* orchestrates the expression of a collection of toxins and extracellular enzymes that are crucial for the development of the disease.

Purpose: The objective of this study was to analyse differential expression of CPE-positive *C. perfringens* isolates collected from FPO (16SBCL940, 17SBCL79) and human (168-2017, 197-2019) under different conditions.

Methods: RNA-Seq-based global transcriptome analysis was performed to compare the transcriptome under vegetative and sporulation conditions.

Results: Many genes involved in amino acid metabolism and carbohydrate degradation were similarly upregulated in all four isolates confirming that sporulation on *C. perfringens* can be triggered by upregulation of these genes. The genes encoding RNA polymerase σ factors involved in different stages of *C. perfringens* sporulation were uniformly upregulated in the investigated isolates. The most prominent difference was observed for *cpe* gene expression. However, isolate 16SBCL940 showed increased transcription of *cpe* gene encoding CPE which was further identified as powerful CPE producer compared to the other four isolates included in this study. Similarly, genes encoding putative virulence factors were downregulated. These downregulated genes are predicted to be expressed during vegetative growth of *C. perfringens* isolates.

Significance: These findings demonstrate that there are growth phase-specific differences in the global transcriptomes of CPE-positive *C. perfringens* isolates, and highlight the utility of comparative transcriptomics for identifying additional factors that are directly or indirectly involved in *C. perfringens* foodborne diseases.

T1-09 GFSI Scopes JI and JII: Background to Their Development and Purpose

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It is widely recognized that poor hygienic design of food processing equipment and buildings can lead to major food poisoning incidents in both raw materials pre-decontamination treatment¹ or ready-to-eat food products post-decontamination treatment².

To update and strengthen their benchmark requirements on the hygienic design of new and existing food buildings and processing equipment, from farm to fork, the Global Food Safety Initiative (GFSI) organization established a Technical Working Group, *Hygienic design of facilities and equipment*, in 2018. The output of this TWG led to the publication of two new scopes in the GFSI Benchmarking Requirements Version 2020³: Scope JI for building constructors and equipment manufacturers, and Scope JII for building and equipment users.

The basis of JI and JII is a new hygienic design process on the lifecycle of hygienic design and considers the design and construction of a food building or item of equipment and its subsequent use throughout its working life. Specifically, the process considers:

- The intended use of the food building or processing equipment
- The risks of any hazards that might be associated with the building/equipment that could affect food safety within its intended use
- The mitigation of such risks by appropriate hygienic design
- The safe construction of buildings and equipment, so that no additional hazards arise that could affect food safety
- The safe commissioning of buildings and installation of equipment, so that no additional hazards arise that could affect food safety
- The mitigation of any remaining hazard risks in use, via building/equipment cleaning and maintenance programmes
- The periodic review of existing (legacy) buildings and equipment to ensure they are still hygienically fit for their intended use

The presentation will describe the philosophy behind the hygienic design process and the implications this may have to the farm to fork food chain.

T1-10 [Establishment of GFSI Hygienic Design Benchmarking Requirements and Role of 3A-SSI and EHEDG](#)

Patrick Wouters

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In 2018 the Global Food Safety Initiative (GFSI) organization established a team of industry hygiene thought leaders to develop and deliver a high-level set of hygienic design benchmarking requirements with the objective of enhancing food safety from farm-to-fork. With the 2020 publication of two new hygienic design benchmarking requirements, the Global Food Safety Initiative (GFSI) paves the way to incorporate hygienic design in food safety management programs. The new GFSI benchmarking requirements for the *Hygienic Design of Food Buildings and Processing Equipment* are published in 2 scopes: JI for building constructors and equipment manufacturers, and scope JII for building and equipment users¹. Compliance with these standards will benefit supply chain stakeholders and consumers with consistent food safe, quality products across the global food supply chain.

Since the GFSI benchmarking process is widely adopted by the worldwide food industry, these new benchmarking requirements are bound to change how hygienic design is integrated in food safety management systems. Consequently, hygienic design will assume a central role in food equipment development processes. It is also expected that this will have a harmonizing effect on certification systems, since these new hygienic design benchmarking requirements set a clear and unified baseline for all standards. Two organization in hygienic engineering and design, 3-A SSI and EHEDG (European Hygienic Engineering & Design Group) have a long history of developing criteria for the hygienic design of equipment and facilities in the form of standards and guidelines which are essential for equipment manufacturers, building constructors and food manufacturers to meet food safety and quality objectives and for government agencies and regulatory authorities to achieve public health objectives.

This lecture will explain the roadmap, achievements and what especially EHEDG stand ready for, and what practical implications can be expected for food processing companies and their equipment suppliers.

1GFSI Benchmarking Requirements Version 2020 (February 2020) https://mygfsi.com/news-and-resources/?type=publications&fwp_news_topics=benchmarking

T1-11 [Hygienic Design as a Holistic Concept within Food Production Facilities](#)

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In the food industry the use of equipment that is easy to clean and maintain, i.e., hygienically designed, is fundamental to ensuring food safety and quality. Hygienic design also extends to the safety of the materials used in contact with the food, there being a legal requirement for these to be food contact compliant. Surprising then that awareness of hygienic design and food safe material compliance is often unknown, or overlooked, by those that manufacture, purchase, and use food industry equipment. The European Hygienic Engineering & Design Group (EHEDG) and 3-A SSI (USA) both have long histories in the promotion of hygienic design, through provision of training, guidance, certification, and expert advice. Previously this information has focused on food processing equipment but now there is a realisation that the hygienic design of the buildings the food is processed in; the facilities that service those buildings; and the supplementary equipment used in factory environments, is also key to maintaining food safety and quality.

As a consequence, EHEDG now publish guidance on 'Hygienic Design Principles for Food Factories', (Guideline 44), which includes information on site location; construction and placement of lighting; doors; windows; walls; floors; ceilings; and drainage systems; and guidance on 'Air Quality Control for Building Ventilation', (Guideline 47).

Food industry cleaning tools and utensils have long been identified as a major source and vector of contamination^{1,2,3}, and the importance of their hygienic design is reflected in GFSI food safety standard requirements. EHEDG Guidelines regarding basic principles of cleaning and disinfection; and control of foreign bodies are also in preparation.

This presentation summarises the recent and developing EHEDG guidance, and GFSI food safety standard requirements, related to these supplementary sources of contamination within food production areas, and investigates the future of hygienic design as a holistic concept within food production facilities.

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General Microbiology

T1-12* [Isolation, Stability and Characteristics of High Pressure Superdormant *Bacillus* Spores](#)

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Introduction: High pressure (HP) of 150 MPa can trigger spore germination, leading to resistance loss and increased susceptibility to inactivation strategies. Successful implementation of such a mild germination-inactivation

* Student Award Competitor

strategy is hampered by germination heterogeneity within a population. Of special concern are HP superdormant (HPSD) spores, i.e., spores that remain dormant after exposure to the germination trigger.

Purpose: The purpose of this study was to develop a suitable isolation protocol for HPSD spores allowing their subsequent characterization as a mean to better understand superdormancy.

Methods: *Bacillus subtilis* spores were HP treated at 150 MPa/37°C and isolated by means of buoyant density centrifugation and fluorescence-activated cell sorting. Important characteristics like their prevalence, stability, and germination capacity were investigated with flow cytometry. The microscopic structure was analyzed with transmission electron microscopy.

Results: HPSD spores prevalence strongly depended on the HP dwell time, with increasing treatment times reducing their prevalence. Spore mutants lacking major germinant receptors further showed a highly increased prevalence of HPSD spores ($93.1 \pm 0.6\%$, $n = 3$) even after prolonged dwell times of 40 min, emphasizing the importance of germinant receptors in 150 MPa germination. Microscopic analysis did not reveal any visible structural differences between HPSD and initial dormant spores. Quantitative analysis also showed no significant difference in cortex thickness ($M = 105 \pm 21$ nm and $M = 107 \pm 20$ nm, t -test $P = 0.231$, $n = 50$). HPSD spores are likely not genetically different from the rest of the population as re-sporulated HPSD spores showed similar germination capacity compared to initial dormant spores. Moreover, the majority of HPSD spores germinated when exposed a second time to the same treatment, indicating that superdormancy is likely cumulative or transient ($15.4 \pm 0.5\%$ of HPSD spores isolated after 6 min HP treatment remained dormant in a second treatment, $n = 3$).

Significance: These results shed light on properties of HPSD spores and potential causes of superdormancy, thereby contributing to the development of mild HP-based spore control strategies.

T1-13* *Listeria monocytogenes* Comes in Different Shades: Clinical- and Food-associated Strains Vary in Virulence, Stress Resistance, and Carbon Source Metabolism

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Introduction: *Listeria monocytogenes* is a public health and food safety challenge due in part to its natural stress resistance and virulence traits.

Purpose: Evaluation of phenotypic and genotypic diversity underlying variable *L. monocytogenes* distribution in foods and clinical cases.

Methods: Sixty-two *L. monocytogenes* isolates of food and clinical origin were characterized based on growth in Brain heart infusion (BHI) with and without 8% NaCl (osmotic) and benzalkonium chloride (BC) [MIC in BHI] stress tolerance, virulence (zebrafish embryo microinjection and hemolysis), phenotype microarrays (carbon-source utilization, osmotic and pH stress), and whole-genome analysis. The significance of differences in virulence and growth kinetics under stress between the strains was determined using ANOVA.

Results: Despite high genome conservation (76.0% core genome conservation) the strains differed significantly ($P < 0.05$) in osmotic and BC stress tolerance, and zebrafish pathogenicity. Clinical lineage I serotype 4b, CC1, CC2, CC4, and CC6 ($n = 28$) strains displayed significantly higher zebrafish pathogenicity [100% mortality ($n = 30$ embryos per strain) at 24 h post-infection (hpi)], whilst food associated lineage II, CC8 ($n = 5$) and CC9 ($n = 11$) strains were less virulent [$\leq 30\%$ mortality at 24 hpi]. Lineage I, CC2 and CC4 strains were significantly ($P < 0.05$) more tolerant whereas lineage II, CC9 strains were more sensitive [2.1 vs 4.13-fold increase in lag phase] to osmotic stress. Phenotypic

microarrays revealed significant variation ($P < 0.05$) in C-source utilization and confirmed osmotic and pH stress resistance variation. A lineage II, serotype 1/2a outbreak strain utilized more (51 vs 34-39) C-sources than other strains. Strain-dependent alkaline stress inhibition patterns upon inclusion of β -phenylethylamine (2/8 strains inhibited) were observed indicating a potential for its exploitation in *L. monocytogenes* control.

Significance: We provide evidence of both virulence and stress resistance stratification with *L. monocytogenes* genetic backgrounds. Phenotypic microarray data generated provides a potential basis for improved *L. monocytogenes* detection media design and novel listeriosis control strategies.

T1-14* Behaviour of Staphylococcal *egc* Enterotoxins during Bacterial Growth and Under Food Production-like Stress Conditions

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Introduction: According to the European Food Safety Authority (2019), 77 out of 114 outbreaks caused by staphylococcal enterotoxin (SFPO) are weak evidence outbreaks. However, only five out of 27 enterotoxins can be analyzed using commercially available kits. Especially the presence of the so called “new enterotoxins”, that have been described being involved in SFPOs, cannot be determined. A group of these new enterotoxin genes (*seg*, *sei*, *sem*, *seo*, and *seu*) is located on the same enterotoxin gene cluster (*egc*).

Purpose: The aim of the present study is to improve the understanding of the parameters and conditions in which *egc* enterotoxins are produced to better control their expression during food production and storage.

Methods: A selection of eight strains from different origin were chosen according to their genetic diversity (genetic structure) and origin. For them the enterotoxin expression (mRNA) of *seg*, *sei*, *sem*, *sen* and *seo* was measured using RT-qPCR at three different points during the bacterial growing phase (start, mid-log and end-log). Based on these results three strains were selected to study the enterotoxin gene expression under stress conditions: salt concentrations up to 100 g/L, higher temperature (45°C). In addition, each sample was tested on Staphylococcal enterotoxins G and I expression using an in-house sandwich ELISA method.

Results: *Egc* enterotoxins are mostly expressed in the mid-log phase of bacterial growth and seem to switch off at the end of the log phase. SEG and SEI are already produced at an early stage of the growing phase. Interestingly, common salt and temperature stress, mimicking conditions found in food production, seem not to affect the expression of *egc* enterotoxins, even though differences between strains were observed.

Significance: The study gives insights on the production of *egc* enterotoxins under stress conditions. This information will enhance the availability of methods controlling *egc* enterotoxins in food production and storage.

T1-15* Attachment Ability and Strength of *Bacillus cereus* and *Bacillus Thuringiensis* on Spinach Leaves

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Introduction: *Bacillus cereus* as an opportunistic foodborne pathogen and *B. Thuringiensis* (Bt) as a biological control agent are both frequently isolated from leafy greens. The attachment and persistence of *B. cereus* including Bt on these leafy greens with high levels ($>10^5$ CFU/g) might pose a risk to humans.

Purpose: This study aims to compare the attachment ability and strength of *B. cereus* and Bt including biopesticide strains with the form of vegetative cells and spores on spinach leaves.

Methods: The 1.3-cm diameter spinach discs were inoculated in 12-well plates containing 10^6 CFU/mL vegetative cell or spore suspension from 3 Bt and 4 *B. cereus* strains at 12°C. At specific time intervals, loosely attached cells were removed from discs by vortex, then strongly attached cells were removed by homogenization from the same discs. A series of dilutions of these solutions were spread on MYP agar plates, and the results were counted from these plates after incubation at 30°C for 24 h.

Results: Attachment index (AI) and attachment strength (S_R , %) values were calculated to determine the attachment ability and attachment strength of vegetative cell or spore on spinach leaves. Both spores and vegetative cells of *B. cereus* strains have higher AI values (but not S_R values) than Bt strains. The highest S_R values in all tested strains were observed by commercial Bt spores Dipel and Dipel vegetative cells at 24 h and 48 h, respectively.

Significance: In general, *B. cereus* strains have higher attachment ability on spinach leaves than Bt strains with both spores and vegetative cells. However, strain-specific attachment strength of tested strains on spinach leaves was observed. The highest S_R values of Dipel might indicate its persistence and thus residual presence of high numbers presenting a potential risk for foodborne intoxication when used as a biopesticide on leafy greens.

Significance: This study has shown that more than 1,500 substances used in inks and adhesives have intrinsic hazardous properties. By applying the hazard prioritization strategy, 696 substances were classified as very high priority and should be considered the most urgent candidates to be further evaluated.

Packaging

T1-16 Hazard Prioritization of Substances Used in Printing Inks and Adhesives Applied to Plastic Food Packaging

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Introduction: More than 6,000 intentionally added substances are currently used in printing inks and adhesives. However, most of them have not been sufficiently assessed for their risk towards human health. The lack of prioritization hampers the work of control authorities, since it is not clear which substances should be monitored in first priority. Consequently, the food safety is compromised.

Purpose: The purpose of this study is to set priorities for the evaluation of substances used in inks and adhesives applied to plastic food packaging, based on their hazard.

Methods: An inventory of substances was filtered according to several exclusion criteria aimed to retain a workable amount of substances with known chemical identity. For instance, duplicate values, substances without CAS number or without definite molecular formula were removed. Based on the concept of the Threshold of Toxicological Concern, the retained substances were investigated for their hazardous properties and compared with three 'Substances of Concern' lists and RASFF notifications. Afterwards, the substances were classified as high, medium and low priority. Additionally, a panel of five experts was asked to evaluate which high or medium priority substances should be considered relevant or not for further investigation. Upon the information collected, a group of very high priority substances was established.

Results: Of the 7,413 substances collected in the inventory, 2,300 were evaluated and prioritized from 1 (highest) to 3 (lowest) as follows: 1. High priority: 636 substances; 2. Medium priority: 1,024 substances; 3. Low priority: 640 substances. Following the experts' evaluation, 696 substances were identified as very high priority.

Pre-harvest Food Safety

T1-17 Differential Survivability of Two Genetically Similar *Salmonella* Thompson Isolates on Pre-harvest Basil (*Ocimum basilicum*) Leaves

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Introduction: Produce is increasingly being implicated as a vehicle for transmission of *Salmonella* spp. which is traditionally considered to be a zoonotic pathogen. Hence, knowledge of plant-pathogen interactions is increasingly becoming vital to food safety.

Purpose: This study seeks to decipher the underlying reasons for the differential survivability of two genetically similar *Salmonella* Thompson strains ST 889B (isolated from fresh basil imported from Israel to Belgium in 2014) and ST 688C (a clonal subculture of the *S. Thompson* strain RM1984 responsible for the California coriander outbreak in 1999).

Methods: *S. Thompson* survival was enumerated 0-, 1-, and 6-days post-inoculation on basil leaves and in basil juice. Biofilm forming ability, motility, Type III secretion system (T3SS) activity and their corresponding gene expressions were compared. The flagellar gene *fliC* was knocked out in ST 889B to investigate its role in forming "hypersensitive response (HR)-like lesions".

Results: Upon inoculation, a "dry spot" or a macroscopic "hypersensitive response (HR)-like lesion" formed. In "dry spots", ST 889B survived significantly better than ST 688C (2.83 v. 2.18 log CFU/sample at 6 days post-inoculation) likely owing to the former's significantly higher biofilm forming ability. In "HR-like lesions", no significant difference in survivability was observed. However, ST 889B caused the formation of "HR-like lesions" at a significantly higher rate (70% v. 40%) than ST 688C. Both strains survived better in these lesions than on the "dry spots" (ST 688C: 4.39 v. 2.18 and ST 889B: 4.78 v. 2.83 log CFU/sample). ST 889B exhibited 8.6-fold higher expression of T3SS gene *prgH* while the *fliC* mutation did not have a significant change in "HR-like lesion" formation.

Significance: Findings from this study shed additional light on the *Salmonella*-basil interaction and may contribute to the knowledge of food safety in produce.

T1-18 Performance Assessment of the Canadian Food Inspection Agency's Feed Mills Risk Assessment Model Outputs

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Introduction: The Canadian Food Inspection Agency (CFIA) has developed an Establishment-based Risk Assessment model for feed mills (ERA-Feed Mill model) to allocate inspection resources according to the feed safety risk, from both animal and human health perspectives, associated with each feed establishment. To do so, previous studies identified 34 feed safety-related risk factors and weighted 203 assessment criteria to be included in this model.

Purpose: The objectives of the current study were to validate the model data collection tool and assess the model performance to confirm its applicability on commercial feed mills.

* Student Award Competitor

Methods: A pilot project was first completed with 31 randomly selected Canadian commercial feed mills. Using data collected during this step, risk results were calculated using the ERA-Feed Mill model and grouped in 5 risk categories. Risk factors' information used as input for the ERA-Feed Mill model were summarized and 22 senior CFIA inspectors categorized 10 establishments, including controls, based on their feed safety risk, using a 5-categories scale. The outputs of the ERA-Feed Mill model were assessed by correlating them with the results obtained from the assessment done by inspectors on the same mills. Statistical analyses were performed using Excel 2016 and R version 4.0.3.

Results: Results showed a Spearman correlation coefficient of 0.78 ($P < 0.01$) between the model outputs and the risk assessment performed by CFIA's senior inspectors. The data collection tool was also improved afterward considering results of the analyses and feedback from feed mills and inspectors. No adjustment was made to the preliminary model algorithm as no specific discrepancies were identified between both assessments.

Significance: By assessing the feed safety risk represented by each feed mill under CFIA's jurisdiction, this tool will help determine the level of oversight required for risk management, contributing to enhance human and animal health protection.

T1-19* A DNA Prime/Protein Boost Flagellin-based Vaccine Against *Campylobacter* in SPF White Leghorn Chickens: A Model to Gain Further Insights about the Role of Chicken Immune System in Response to Anti-*Campylobacter* Vaccination

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Introduction: Campylobacteriosis is the most commonly reported zoonosis in Europe and poultry is the main reservoir of *Campylobacter*. According to EFSA, reduction of *Campylobacter* in broiler caecal concentrations would reduce the risk of human campylobacteriosis attributable to broiler meat. However, no effective control measure is available yet, and vaccination is a strategy to explore. A previous study determined that flagellin-based vaccine induced a clearance of *Campylobacter jejuni* in Specific Pathogen Free (SPF) Leghorn chickens after 42 days of rearing.

Purpose: The aim of this work was to deepen our understanding about immune pathways stimulated in response to this flagellin-based vaccine and *Campylobacter* challenge.

Methods: Two groups of SPF Leghorn chickens were used: a vaccinated group receiving a flagellin-based vaccine consisting in two immunizations (DNA prime-protein boost regimen) at day 5 and 12 and a placebo group. Chickens were challenged by *C. jejuni* at day 19. *Campylobacter* loads were assessed in caeca by the decimal dilution method; IgY in serum and IgA in bile levels were analyzed by ELISA tests at days 22, 28, 35 and 40. The statistical analysis was performed using Mann-Whitney tests to compare the two groups.

Results: Clearance of *C. jejuni* was not observed as previously, but a significant reduction of *Campylobacter* loads (1.26 log, $P < 0.05$) was observed in the vaccinated group at day 40 compared to the placebo group. In the vaccinated group, IgY and IgA levels increased significantly after *Campylobacter* challenge from day 22 to day 40 compared to the placebo group ($P < 0.05$).

Significance: These encouraging results highlight a possible implication of systemic and mucosal immune responses in *Campylobacter* reduction using this vaccination model.

However, further investigations are in progress to explore the role of cytokine expression implicated in the T-cell pathway. These new data could allow the improvement of future anti-*Campylobacter* vaccination protocols.

Produce

T1-20 Removal of Parasite Transmission Stages from Berries Using Washing Procedures Suitable for Consumers

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Introduction: Due to the delicate nature of berries and their reduced shelf life once washed, producers usually do not wash berries prior to sale. Therefore, consumers are recommended to wash berries prior to consumption, and this might be an effective way of infection prevention. However, the efficacy of how people wash berries before consumption in removing parasite contaminants from the berries' surfaces has not been investigated.

Purpose: The aim of the present study was to compare the efficacy of three different washing techniques in removing parasite transmission stages from the surfaces of berries.

Methods: Three alternatives to washing berries before consumption were compared using berries artificially contaminated with oo/cysts of *Cyclospora cayetanensis*, *Cryptosporidium parvum*, and *Giardia duodenalis*. Five independent replicates of spiked berries were analyzed per washing technique, including the control group, which was not subject to any of the three washing alternatives but directly processed for DNA extraction and qPCR analyses.

Results: The results show that simple washing of berries under cold tap water for 1 min could remove on average at least 80% of the parasites, except for *C. cayetanensis* oocysts which seem to be stickier than both *G. duodenalis* cysts and *C. parvum* oocysts. The percent removal was slightly lower for raspberries than blueberries. Although the differences are expected, a relevant result of the study is that washing contaminated berries prior to consumption by the consumer removes a considerable proportion of parasite transmission stages and thereby lowers the risk of infection.

Significance: Several studies have been conducted to assess the parasite contamination of berries and have indicated that berries have the potential to act as vehicles for transmission of foodborne parasites (FBP). Therefore, approaches to reduce or prevent the transmission of FBP are highly needed.

Viruses and Parasites

T1-21 Norovirus Detection in Berries – Outbreak and Surveillance Results

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Introduction: Berries have been implicated in several outbreaks linked to norovirus (NoV) and hepatitis A virus (HAV).

Purpose: (1) Present results from surveillance and outbreaks testing, compare occurrence, and assess importance of testing. (2) CFIA conducted surveillance of various types of frozen berries for the presence of NoV and HAV. (3) CFIA provided support during 5 outbreak investigations related to frozen berries between 2016 and 2020.

Methods: The samples were tested for NoV (GI and GII) and/or HAV using a silica beads-based and RT-qPCR methods and/or ISO-15216 method-based protocols. Surveillance samples: 3,014 frozen berry samples were collected at retail locations across Canada. Investigation samples: Samples were tested for NoV ($n = 24$) and HAV ($n = 42$). Samples including berries and berry-containing products were collected at various locations based on the investigations.

Results: Surveillance: NoV were detected in 11/3,014 (0.36%; 95% CI 0.20,0.65) of the frozen berries samples. HAV was not detected in any samples. Investigations: During 4/5 investigations, NoV GI (12/24), GII (6/24) or GI and II (2/24) were detected in frozen raspberries, and HAV (5/42) was detected in frozen mixed berries samples and strawberries.

Significance: The results confirm that frozen berries could be a vector for viral contaminants. The differences in the data sets do not allow for a direct comparison of the occurrence; however, a somewhat higher occurrence was observed in the outbreak samples versus the surveillance samples, which was as expected. The results show the value of testing consumer and investigation samples to identify the causative agent. Surveillance of the food supply could allow for rapid detection of potential issues. In the absence of guidelines linked to the presence of viruses in foods in Canada, both activities helped the CFIA to implement timely risk management actions such as specific control strategies. These risk management measures help minimize the public health impact and increase the control of potentially contaminated products into Canada.

T1-22* Evaluation of Viral Infectivity during the Frozen Storage of Berries

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Introduction: Raspberries and strawberries are vulnerable to contamination by enteric viruses, which are generally unaffected by frozen storage. Data gaps remain regarding long-term storage of frozen berries. Large outbreaks associated with viruses have been epidemiologically linked to contaminated frozen berries.

Purpose: This study assessed the impact of frozen storage on infectivity of Hepatitis A Virus (HAV) and norovirus-surrogate Tulane virus (TV) on strawberries and raspberries.

Methods: Composite samples were prepared in duplicate (average weight 15 g, 1 strawberry or 3 raspberries) and inoculated with 6-log TCID₅₀/mL TV or HAV using a 0.1 mL volume. Samples were stored in a frost-free freezer with an average temperature of -10.9°C and processed for viral infectivity on days 1, 10, 20 and every 30 days up through 690 days. Berry pH and color were processed in triplicate on days 1, 20 and every 30 days through day 180 followed by every 60 days until day 660. Viruses were recovered by rinsing two combined samples with 10 mL PBS, rinsing 30x total, and infectivity determined using TCID₅₀ with LLC-MK2 cells for TV and FRhK-4 cells for HAV. Data were analyzed using a one-way ANOVA with the significance $P < 0.05$.

Results: After 660 days, there was a significant change in pH detected for raspberries ($P < 0.0001$) and strawberries ($P < 0.0001$). Changes in color (ΔE^*_{ab}) were first noted at day 540 for strawberries (19.84) and at day 30 for raspberries (20.32). Statistically significant changes in infectivity were detected for both HAV and TV after 690 days for raspberries ($P < 0.0001$) and strawberries ($P < 0.0001$). HAV appears to be more stable than TV under freezing conditions. A statistically significant decline in TV infectivity was observed at day 20 for raspberries ($P = 0.004$) and strawberries ($P = 0.007$). TV infectivity was relatively constant until day 690.

Significance: These findings reinforce the importance of good agricultural practices, hygiene practices, and manufacturing practices to reduce risk of contamination on berries before freezing.

Water

T1-23* Applying Sequencing Approaches to Comprehensively Characterize the Microbiological Quality of Non-Traditional Water Sources Used for Food Crop Irrigation: A Conserve 2-Year Field Study

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Introduction: Climate change, population growth and escalating freshwater shortages call for an urgent need to explore non-traditional irrigation water sources for food crop production. However, there is a dearth of comprehensive data regarding the overall microbiological quality of these sources.

Purpose: To address this knowledge gap, our team within the CONSERVE Center of Excellence applied a 16S rRNA gene and metagenomic sequencing approach to comprehensively evaluate the microbiological quality of diverse water sources (e.g., recycled water, non-tidal freshwater, tidal brackish water, untreated pond water) from 12 sites in the Mid-Atlantic United States.

Methods: We collected 338 water samples (1L) bi-weekly over a two-year period (2016–2018). Samples were filtered, DNA-extracted, and sequenced on the Illumina HiSeq 2500, targeting the 16S rRNA gene. Additionally, metagenomic sequencing was performed on a subset of samples to characterize antimicrobial resistance and virulence genes.

Results: Alpha and beta diversity within and between samples were significantly ($P < 0.001$) different across sites and water types. Over 65% of the bacterial community variation was due to sites, while 60% of the variation was explained by water type and ~10% by season. The most common antimicrobial resistance genes identified coded for resistance against aminoglycosides, beta-lactams and macrolides, and the most common virulence genes identified were *Pseudomonas aeruginosa* gene int1, and *Enterobacter aerogenes* gene tniC. We also detected pathogenic bacterial species, such as *Propionibacterium acnes*, *Pseudomonas alcaligenes*, *Clostridium butyricum*, *Enterobacter cloacae*, *Campylobacter curvus*, and *Prevotella melaninogenica*.

Significance: Our results suggest that the tested water sources may require treatment to remove diverse bacterial communities (that could pose potential risks to food safety) before these water sources are used to irrigate food crops. Overall, our data can be applied in the development of mitigation strategies to ensure the use of safe irrigation water, bolstering resilient agricultural communities and sustainable food production in the face of ongoing climate change.

T1-24 Occurrence of Human Enteric Virus and Coliphages in Water Reuse Systems: From the Wastewater Treatment Plant to the Irrigation Point of Use for Leafy Greens

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Introduction: The reuse of treated wastewater as agricultural water for irrigation is a priority to alleviate water scarcity. However, any potential health and environmental risk issues need to be avoided, mostly food safety risks associated with the irrigated crops. Special precautions must be achieved on fresh produce where irrigation water gets in contact with the edible part of the crop, such as leafy greens. The European Commission has recently approved the new regulation on minimum quality requirements for reused water (2020/741), which includes new and more strict microbial thresholds.

Purpose: The present study focuses on the evaluation of two water reuse systems where water is used for irrigation of leafy greens, including romaine lettuce and baby spinach.

Methods: The efficacy of water treatments implemented in two different wastewater treatment plants (WWTPs) was evaluated as well as the impact of operational steps of the water reuse system (distribution, storage, and irrigation systems) on the levels of noroviruses genogroups I and II (GI and GII) and coliphages. The levels of enteric viruses in leafy greens irrigated with reclaimed water were also evaluated during the growing cycle.

Results: The results showed that reclamation treatments applied in the WWTPs significantly reduced the prevalence and the levels of Noroviruses GI, GII, and coliphages. However, the coliphages reductions (c.a. 5 log) obtained at the WWTP outlet were below the minimum microbiological requirements established in the new legislation (≥ 6.0 log). Noroviruses were not detected in the growers' water reservoirs near the growing field, probably due to the solar radiation. On the other hand, the prevalence of noroviruses and coliphages found in leafy greens were very low (5/95). Results obtained indicate that current WWTP effectively reduces enteric viruses.

Significance: More efforts are necessary for the establishment of advanced disinfection treatments and the maintenance of the distribution system.

WEDNESDAY, 28 APRIL

T2 Technical Session 2 – Antimicrobials; Food Toxicology; Laboratory and Detection Methods; Low-water Activity Foods; Meat, Poultry and Eggs; Modeling and Risk Assessment; Molecular Analytics, Genomics and Microbiome; Sanitation and Hygiene

Antimicrobials

T2-01 Metagenomic Characterization of the Microbiome and Resistome in the Milk Production Environment

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Introduction: Antibiotics are used extensively in the dairy industry to treat mastitis and other bacterial diseases, leading to the presence of antimicrobial resistance genes (ARGs) in milk and dairy products. Among Next Generation Sequencing (NGS) techniques, shotgun metagenomic sequencing allows the generation of a vast amount of data which can be interrogated to generate the desired evidence, including resistome. However, host DNA poses a major challenge to metagenome analysis.

Purpose: The goal of this study was to characterize the microbiome and the resistome of different dairy farms through a shotgun metagenomic sequencing approach.

Methods: A pilot study was performed on a sample of bulk milk and the in-line milk filter of a dairy farm; each sample was divided into 4 aliquots which were sequenced to different sequencing depth and through different host DNA depletion treatments. Taking advantage of the results reported, bulk tank milk filters ($n = 10$) were collected aseptically in ten different dairy farms; both shotgun metagenomic sequencing (sequencing depth: 50 M reads 2×150 bp) and 16S rRNA amplicon sequencing were applied.

Results: Milk filters proved to be the most suitable matrices to evaluate the presence of ARGs. The investigation of the microbiome of different dairy farms revealed a high proportion of sequences assigned to Gram-negative bacteria; the resistome profiling showed the presence of 59 groups of genes conveying multi-drug resistance ($n = 26$) and resistance to tetracyclines ($n = 9$), aminoglycosides ($n = 9$), β -lactams ($n = 5$), sulphonamides ($n = 1$), MLS (macrolides, lincosamides and streptogramins, $n = 6$), rifampin ($n = 1$), Fosfomycin ($n = 1$) and phenicols ($n = 1$).

Significance: To our knowledge, this is the first study to unveil the resistome of dairy farms using a shotgun metagenomic sequencing approach applied to milk filters, thus confirming the circulation of ARGs in the milk production environment and highlighting the importance of tracking microbes from farm to fork.

T2-02* Variable Contribution of Cold Shock-Domain Family Proteins to Nisin Tolerance in *Listeria monocytogenes*

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Introduction: Cold shock-domain family proteins (Csp) are global gene expression regulators involved in innate stress tolerance in *L. monocytogenes*. The bacterium possesses numerous mechanisms that confer innate tolerance to nisin, a bacteriocin produced by *Lactococcus lactis*, but the role of Csp to the innate tolerance has not been fully characterized.

Purpose: To examine the functional contribution of Csp to *L. monocytogenes*' tolerance to nisin stress.

Methods: Growth kinetics of *L. monocytogenes* EGDe wild type (WT), single, double and triple csp deletion mutant strains were determined in 5 ppm nisin through spectrophotometry for 24 hours at 37°C. Net cell surface charge and response of the WT and triple csp deletion mutant to cell envelope targeting antimicrobials, benzalkonium chloride (BC), ampicillin (AMP) and polymyxin b (PB) were compared through cytochrome c binding assay and survival assays or minimum inhibitory concentration, respectively. Expression of csp genes and genes involved in nisin response, virR, dltA and mprF was also determined.

Results: Compared to the WT, the triple deletion mutant lacking all three csps showed increased sensitivity to nisin ($P < 0.05$). Mutants lacking one csp ($\Delta cspA$ and $\Delta cspB$) showed higher tolerance while $\Delta cspD$ was more sensitive to nisin ($P < 0.05$). Conversely, $\Delta cspBD$ and $\Delta cspAB$ mutants lacking two csps were more sensitive and tolerant to nisin than WT, respectively, ($P < 0.05$). The three csps therefore had hierarchical role in nisin tolerance whereby $cspD > cspB > cspA$. mRNA analysis also showed similar hierarchical expression of csp transcripts under nisin stress ($P < 0.05$). Further analysis revealed loss of all three csps increased sensitivity to BC, AMP and PB, reduced transcripts of dltA and mprF and increased cytochrome c ($P < 0.05$) suggesting increased loss of cell membrane integrity and negative charges in the former.

Significance: These data show Csp contribute to innate nisin tolerance in *L. monocytogenes* and can be targeted to enhance effectiveness of nisin.

Food Toxicology

T2-03 The ISO 16140 Series: A Never-ending but Successful Story

Paul In't Veld

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ISO TC34 SC9 (microbiology of the food chain) has several Working Groups (WG) on specific topics. WG 3 of SC9 is involved with all aspects of method validation. WG3 is responsible for drafting the ISO 16140 series and ISO 17468. They started their work in 2005. The ISO 16140 series comprises currently 6 different parts. The main standard of this series is ISO 16140-2 (validation of alternative (proprietary) methods against a reference method). This standard serves as the basis for the other standards. The most recently (January 2021) published part is ISO 16140-3 which describes the protocol for verification of a method in a laboratory. This standard is relevant for all laboratories that want to introduce a reference or alternative method in their laboratory. In this presentation an overview of the standards and their relation will be presented. WG 3 is also working on new topics and on the revision/amendment of existing standards. New developments are, for example:

- development of ISO 16140-7 on the validation of identification methods;
 - review of ISO 16140-2 for use in combination with non-culturable organisms such as viruses or parasites;
 - use of larger test portion sizes for qualitative methods.
- A short explanation on these topics will be presented as well. After 15 years many challenging topics still exist for the working group experts.

T2-04 Variation of Deoxynivalenol Levels in Corn and Its Products Available in Retail Markets of Punjab, Pakistan, and Estimation of Risk Assessment

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Introduction: The contamination of food and food products with mycotoxins is a serious food safety concern. Deoxynivalenol (DON) in cereal products is major concern in developing countries.

Purpose: There are very few reports on the assessment of DON in cereal products. In current research, corn samples

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from different corn producing locations were analyzed for the presence of DON. Furthermore, the levels were compared with the permissible limits implemented by European Union.

Methods: A total of 1,220 samples of corn and products were analyzed for the detection of DON. The samples were analyzed using HPLC with UV detector.

Results: The limit of detection (LOD) and limit of quantification were 25 and 50 µg/kg, respectively. Furthermore, 539 (44.2%) samples were found to be positive with DON ($n \geq$ LOD). Furthermore, 92 (7.5%) samples of corn & products have levels of DON, higher than the proposed limits of EU. The lowest and highest exposure and hazard quotient (HQ) of 0.92 and 9.68 µg/kg bw/d were documented in corn flour samples.

Significance: The data are significantly different from normal distribution for DON in corn and products samples and from different locations ($P < 0.05$) for Shapiro-Wilk and Kolmogorov-Smirnov values. However, significant difference of levels of DON were found between corn and corn products types ($P \leq 0.05$).

Laboratory and Detection Methods

T2-05 Quantitative Determination of *Staphylococcus aureus* Enterotoxins in Complex Food Matrices by a Multiplex Immunocapture Mass Spectrometry Method

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Introduction: Staphylococcal food poisoning outbreaks (SFPO) are caused by the ingestion of food contaminated with staphylococcal enterotoxins (SEs) produced by strains of coagulase positive *Staphylococci*. To date, 27 SEs are described in the literature but only 5 classical toxins (SEA to SEE) can be routinely detectable via commercially available immunoassays (EN ISO 19020). Liquid chromatography coupled to mass spectrometry (LC-MS) approach is highly specific and allows the analysis of a wide range of toxins comparing with immunoassays.

Purpose: We propose to develop a Multiplex method by immunocapture-LC-MS for the detection and quantification of SEs in food matrices.

Methods: This method is based on selective capture by antibodies and targeted high resolution LC-MS. Briefly, samples were incubated with magnetic beads coated with toxin-specific antibodies. After toxin extraction, on-bead trypsin digestion was performed and recovered peptides were analyzed by LC-MS. This multiplex method was optimized for 8 staphylococcal enterotoxins (SEA to SEE and SEG, SEH and SEI).

Results: Limit of detection was estimated at 0.1-0.5 ng/mL in milk, which was in perfect agreement with toxic dose (20-100 ng/person) to be ingested to provoke symptoms. This method was optimized and tested (i) on artificially contaminated foods, (ii) *S. aureus* strains carrying targeted enterotoxins genes and (iii) on naturally contaminated samples issued from SFPO and official controls (especially cheeses, meat). Results demonstrated that this method was sensitive, specific and able to detect SEs in naturally contaminated matrices. For example, SEA was detected at 0.7 ng/g in ham responsible of SFPO in south of France in August 2019.

Significance: In absence of a real confirmation test for the official method (EN ISO 19020), LC-MS approach could be used as confirmatory method.

T2-06 Characterisation of Foodborne Outbreaks Due to Emerging Staphylococcal Enterotoxins

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Introduction: Among the 27 SEs reported in literature, only five can be detected with commercially available immunoassays: SEA, SEB, SEC, SED, and SEE. Due to the lack of suitable antibodies, no validated method is available for the detection/quantification of enterotoxin types other than SEA-SEE, which are yet known to be a risk for consumers. At the level of European Reference network for Coagulase Positive *Staphylococci* (CPS), several foodborne outbreaks in Europe have shown a typical SE symptomatology but no classical enterotoxins could be detected. However, genes encoding other enterotoxins were detected by typing methods (PCR and WGS) indicating, probably, the presence of toxins recently described.

Purpose: Investigation of two foodborne outbreaks (FBO) occurred in two European member states in 2019, using newly developed ELISA and Liquid Chromatography coupled Mass Spectrometry methods (LC-MS) targeting emerging staphylococcal enterotoxins.

Methods: In addition to epidemiological investigation, newly developed ELISA and LC-MS based methods were used to detect emerging staphylococcal enterotoxins (SEG, SEH and SEI). Typing methods (PCR, WGS) and official methods (EN ISO 6888 and EN ISO 19020) were implemented for CPS enumeration, se genes and classical enterotoxins detection.

Results: Production of classical enterotoxins SEA and emerging enterotoxins such SEH at ng/g level was confirmed by newly developed methods (ELISA and LCMS). Presence of CPS and se genes was confirmed only for the first FBO (cheese matrices), but no CPS and se genes were detected in the second FBO due to heat treatment of the roasted beef.

Significance: Methods targeting emerging staphylococcal enterotoxins should be implemented in official laboratories. The European official method EN ISO 19020 should be expanded to cover emerging enterotoxins.

T2-07* Immunomagnetic Separation Combined with Propidium Monoazide for the Specific Detection of Viable *Listeria monocytogenes* by Multiplex Loop-mediated Isothermal Amplification in Milk Products

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Introduction: Foodborne diseases are a growing public health problem. Particularly *L. monocytogenes* infections report mortality rates as high as 20-30%. Traditional detection methods based on culturing require up to 6 days. Due to the need for faster methods, isothermal amplification techniques, such as LAMP, have emerged. These techniques allow for simpler assays and reduction of costs when compared to the gold standard PCR/qPCR. A major limitation of these methods is the inability to differentiate among viable and non-viable microorganisms. Consequently, false negative results can lead to unnecessary product recalls and economic losses.

Purpose: In this study, a novel multiplex LAMP method was developed targeting the *hly* gene of *L. monocytogenes*, along with a competitive IAC for higher confidence. By combining immunomagnetic separation (IMS), to concentrate the

bacteria and purify the sample, and propidium monoazide (PMA) to block the amplification of DNA from non-viable microorganisms, specific detection of viable *Listeria monocytogenes* present in milk products was achieved.

Methods: A four-step sample treatment including (1) cleaning, (2) PMA treatment, (3) IMS treatment, and (4) DNA extraction was evaluated. A multiplex fluorescence-LAMP assay was optimized and the two targets, namely the *hly* and IAC, were differentiated based on a melt curve analysis. The methodology evaluation was performed in a total of 59 samples including UHT milk and powdered infant formula.

Results: Overall, the methodology provided results higher than 95% in terms of sensitivity, specificity and accuracy, as well as a Cohen's k of 0.97, reaching a LOD_{95} of 2.7 CFU/25 g. The method was capable of effectively eliminating undesired amplification, in samples inoculated with up to 10^6 - 10^7 CFU of dead microorganisms/25 g.

Significance: Next-day detection of viable *L. monocytogenes* was achieved by combining LAMP with an IMS-PMA treatment. This study can be of advantage for the food industry by reducing economical losses due to faster analysis.

T2-08 Confirmation of Foodborne Pathogens (*Salmonella*, *Cronobacter*, *Campylobacter*) with a New Platform of Mass Spectrometry Instruments

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Introduction: Mass spectrometry is a useful Rapid Microbiological Method. The MALDI Biotyper (MBT) is a benchtop instrument, and can be used for microorganism identification and confirmation of foodborne pathogens.

Purpose: Our study should show the performance of the new MBT sirius instrument platform with Gram negative *Salmonella*, *Campylobacter* and *Cronobacter* species. The positive ion mode were tested for confirmation of food pathogens from non-selective and selective media.

Methods: MALDI target plates ("target") were used for applying of samples, or Bacterial Test Standard at a precise target position and separated from each other. One part of the MBT sirius, and MBT sirius one System is a microflex mass spectrometer equipped with smartbeam laser technology. The mass spectrometer has the two purposes: Soft ionization of biological compounds and mass spectrometric analysis of the respective ions (acquiring mass spectra). The prepared MALDI target was introduced into the mass spectrometer, measured and identified by using the dedicated reference libraries.

Results: We tested 15 different strains of *Salmonella*, *Campylobacter* and *Cronobacter* with the new MALDI Biotyper sirius platform. Different media were used for cultivation of test organisms, and results were compared for: Columbia blood agar with 5% sheep blood, CCI Agar (*Cronobacter*), mCCDA Agar (*Campylobacter*), XLD Agar (*Salmonella*). Different sample preparation procedures were applied in parallel and compared with a focus on the user-friendly direct transfer (DT) method. In addition, we calculated 225 different mass spectra with the new MBT Compass HT software, and compared all data with existing standard software.

Significance: Equivalence of MBT sirius Systems to other mass spectrometry instruments was demonstrated for confirmation of *Salmonella*, *Campylobacter* and *Cronobacter* tested. Reliable and fast identification of Gram negative pathogens is possible from colony material of non-selective and selective media with a significant lower workload than the workload of traditional confirmation methods.

T2-09 Validation of High-throughput Technologies to Speed Up the Confirmation Workflow

Erin Crowley

Q Laboratories, Inc., Cincinnati, OH

Identification and characterization of microbial isolates provide crucial information to decision makers, from the tracking of foodborne pathogens to the monitoring of spoilage indicators or technological strains. For the past three decades, molecular phylogeny has significantly changed systematics and microorganisms classification, while conventional identification methods are still widely used despite some limitations have been clearly demonstrated. Nonetheless, it is clear evidence that genomic and proteomic technologies are now perceived as performing alternatives to characterize, identify or routinely confirm microbial isolates with significant savings in time. We see them and their power to produce critical data supporting recalls in foodborne illness outbreaks thus allowing regulators to more accurately trace the epidemiology of an implicated strain. These technologies require harmonization and standardization in order to strengthen the use of bioinformatics platforms, to be implemented in food testing laboratories, to share and compare data sets. The establishment of ISO 16140-6 has provided us with the long-awaited guidance needed to properly validate confirmation and identification methods against traditional characterization procedures. This standard has been recognized as by certification bodies such as MicroVal and AOAC and has set a precedent in the journey to harmonization of methods and certification. This presentation will present the study design and outcomes of validating alternative methods against this milestone standard as labs continue to pursue higher throughput solutions to testing volumes all over the world. The need of quality assurance and method robustness to run reliable comparison and enable information exchanges will also be presented.

T2-10 Caught Between Two Principles: How to Validate Semi-quantitative Assays

Suzanne Jordan

Campden BRI, Chipping Campden, United Kingdom

Introduction: Advances in microbiological analysis have enabled the development of rapid detection techniques for foodborne pathogens and indicator organisms including semi quantitative methods. Semi quantitative methods detect microorganisms at set thresholds and can be used either to monitor compliance with regulatory criteria or product screening against specifications.

Purpose: Qualitative methods typically detect the absence of organisms in defined sample sizes, and validations standards have been designed around these requirements. Semi quantitative methods however require defined contamination levels e.g., colony forming units per g. The presentation will use the validation of Neogen Soleris® S2-EBAC9 vials as an example of the challenges for experimental study design and data analysis.

Methods: The performance of Soleris® *Enterobacteriaceae* S2-EBAC9 vials was compared to a direct plating reference method ISO 21528-2:2017 following the validation procedure ISO 16140-2 (2016). A single plate of the reference agar was used with the presence of one or more colonies being equivalent to the detection of *Enterobacteriaceae* at >10 CFU/mL. This approach enabled the plate count to be used as a qualitative rather than a quantitative result.

Results: Neogen Soleris® S2-EBAC9 vials were successfully validated for the detection of *Enterobacteriaceae*, with equivalent performance to ISO 21528-2:2017. The results also showed that Soleris® S2-EBAC9 vials were more sensitive than ISO 21528-2:2017 with no significant differences seen between results obtained by reference method and Soleris® S2-EBAC9 vials in the interlaboratory trial.

Significance: Neogen Soleris® *Enterobacteriaceae* S2-EBAC9 vials was the first MicroVal certification of a semi quantitative method as a qualitative method using the independent method validation protocol ISO 16140-2 (2016). Semi quantitative methods offer many advantages

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to the food microbiologist in terms of speed, throughput and standardisation of protocol and interpretation of results. The development of a relevant study plan is essential for validation of these methods to ensure that the alternative method performance is appropriately assessed.

Low-water Activity Foods

T2-11 Survival of *Listeria monocytogenes* and *Salmonella* Typhimurium on Hot-air Dried Sliced Mushrooms

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Introduction: The view on low-moisture foods (LMFs) as safe due to the lack of microbial growth in these foods is challenged by reports of outbreaks and recalls caused by contaminated LMFs.

Purpose: The purpose of this study was to determine the survival of *S. Typhimurium*, and *L. monocytogenes* on sliced Portobello mushrooms during hot-air drying and subsequent storage at ambient temperature.

Methods: Fresh Portobello mushrooms were sliced before being spiked with *S. Typhimurium* or *L. monocytogenes* and dried with hot-air for 8 hours in a food dehydrator. The dried mushrooms were vacuum-packed in plastic bags and stored 2 months at room temperature to simulate storage of the final consumer product. Samples were taken before, during and after hot-air drying and during storage to analyze weight, water activity and number of surviving bacteria. The ability of surviving cells to regrow was tested to simulate the process where consumers soak the mushrooms before use.

Results: Hot-air drying reduced the water activity (a_w) of the mushrooms to 0.17, which is well below microbial growth limits. *S. Typhimurium* and *L. monocytogenes* displayed total reductions of 2.5 and 2.6 log CFU/g, respectively, with two months storage of the vacuum-packed dried mushrooms further reducing *L. monocytogenes* by 2 log CFU/g. *S. Typhimurium* were not further reduced during storage and the higher stability of *S. Typhimurium* are reflected in the number of reports in the European Rapid Alert System for Food and Feed system of the presence of this organism in dried mushrooms. Both pathogens regrew to high concentrations when dried mushrooms were soaked in peptone saline, simulating a scenario where mushrooms are improperly rehydrated for 24 h at room temperature.

Significance: The present study concludes that hot-air drying and subsequent storage at low a_w cannot be relied on alone to reduce the microbial load on Portobello mushrooms and that additional inactivation methods should be applied to produce a safe food product.

Meat, Poultry and Eggs

T2-12 Surveillance of *Salmonella* in French Poultry Production Highlights Potential Cross-Contaminations between Poultry and Cattle Farms

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Introduction: *Salmonella* is responsible for the second major bacterial foodborne zoonoses in Europe, and poultry products are described as the main source of human contamination. The French National Reference Laboratory (NRL) for *Salmonella* collects *Salmonella* spp. isolates from French official controls performed in poultry farms, allowing the monitoring of *Salmonella* infections in these productions. In specific cases, the NRL can be mandated to investigate contamination sources and establish relationships between isolates through genotyping.

Purpose: A French region is regularly subject to *Salmonella* infections in poultry farms. In order to understand this recurrence and identify potential contamination sources, we investigated several poultry farms contaminated by *Salmonella* Enteritidis and located in this region.

Methods: This investigation consisted of the genome sequencing (Illumina) of 21 *Salmonella* Enteritidis isolates, previously genotyped by PFGE, and genome comparison using the *Salmonella* cgMLST scheme available on Enterobase Platform. Among these isolates, 8 were collected from four poultry houses, 2 from two surrounding cattle farms, 4 isolates were extracted from the NRL database for their potential epidemiological link with isolates from the case, and 7 additional were collected from poultry the same year in different French regions.

Results: Comparison of the sequenced genomes revealed 21 distinct STs. Using the hierarchical clustering at the level 5 (HC5), which allows a maximum of 5 allelic differences for cluster definition, 14 clusters including from 1 to 5 isolates were observed. Within the cluster including 5 isolates, 4 were collected from two different poultry houses, and 1 was from a surrounding cattle farm. Another clustering of poultry and isolates from the second cattle farm was also observed at a higher level of hierarchical clustering (HC20).

Significance: These clusterings of cattle and poultry isolates highlight epidemiological links between farms, suggesting the existence of cross-contaminations between both animal productions. This work constitutes a basis for broader investigations on the relationships between *Salmonella* circulating in poultry and cattle sectors.

T2-13* Behaviour of *Listeria monocytogenes* in High Pressure Processed Dry-cured Ham during Storage Under Refrigeration

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Introduction: High pressure processing (HPP) is applied to inactivate *Listeria monocytogenes* to increase the safety of ready-to-eat meat products. The efficacy of this process has to be validated and monitored as a control measure.

Purpose: The aim of this study is to quantify the inactivation of *L. monocytogenes* by the HPP treatment and the fate of survivors during refrigerated storage of dry-cured ham (DCH), taking into account HPP intensity and product a_w .

Methods: *L. monocytogenes* CTC1034 was inoculated in DCH with different a_w (0.87-0.98) at 6-7 log CFU/g, vacuum packaged, pressurized at 300-750 MPa for 5 min and stored at 7°C for up to 2 months. *L. monocytogenes* was periodically enumerated on chromogenic agar. The gamma concept was used to quantify the effects of the a_w and storage temperature on the *L. monocytogenes* fate.

Results: The a_w was the main factor affecting the growth/no growth interface of *L. monocytogenes* during the refrigerated storage of the DCH. *L. monocytogenes* was able to grow in DCH at a_w over 0.96 independently of the HPP intensity applied. Moreover, the intensity of HPP significantly influenced the maximum density reached by *L. monocytogenes* population during the storage of the pressurized DCH. The application of high HPP intensities (750 MPa) enhanced a log increase of ca. 5 and 9 logs in DCH at a_w of 0.96 and 0.98, respectively, compared to the one-log increase of the pathogen reached in DCH pressurized at the lowest pressure (300 MPa).

Significance: The threshold for a_w value determining the growth/no growth boundary of *L. monocytogenes* in post-HPP DCH during refrigerated storage is 0.96. In conditions allowing growth, the log increase is enhanced by increasing HPP intensity, raising a concern in relation to the risk associated with these products.

Modeling and Risk Assessment

T2-14 Food Safety Risk-based Categorization of Manufactured Foods for Inclusion in the Canadian Food Inspection Agency's Establishment-based Risk Assessment Model

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Introduction: The Canadian Food Inspection Agency has developed the Establishment-based Risk Assessment (ERA) model to support inspection resources' allocation for food establishments. To assess the food safety risk, the model considers the hazards associated with a specific food commodity (e.g., dairy) and product (e.g., cheese), the mitigation strategies implemented by industry, and the establishment compliance history. Having the categorization and health burden allocation at the commodity and product level is then critical; however, this is limited for manufactured foods which includes diverse food items.

Purpose: To categorize the most significant groups of manufactured foods (beverages, grain derived products, confectionery and multiple foods) based on their food safety risk.

Methods: A literature review of scientific and technical papers (including Canadian foodborne outbreak/recall data) published in the last 20 years was completed. Considerations included the products' intrinsic properties, ingredients, processing applied (e.g., fermentation), storage conditions, number of incidents, and industry practices impacting the food safety risk. Categories were created when products had unique characteristics, did not fit into others, and/or posed a level of risk warranting its assessment at the sub-product level. They should also be mutually exclusive, jointly exhaustive and only contain items with similar risk levels.

Results: Pathogens of interest (e.g., *Listeria monocytogenes*, *Salmonella* spp.) and factors impacting the product food safety risk were identified. This resulted in the expansion of the current ERA algorithm product classification system. New categories included 8 beverages (e.g., alcoholic/carbonated, non-shelf stable), 7 grain derived products (e.g., filled pastries, dried grains), 3 confectioneries (e.g., chocolate/cocoa-based products, candies), and 6 multiple foods (e.g., frozen, ready-to-eat). Next, an expert elicitation to estimate the source attribution for the most significant pathogen-product combinations will be conducted.

Significance: Results will be integrated in the ERA algorithm to enhance risk-informed oversight for work planning, to proportionally allocate inspection resources based on food safety risk.

T2-15 The Canadian Food Inspection Agency Work Tasking Logic Model Pilot Project

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Introduction: The Canadian Food Inspection Agency (CFIA) is developing a risk management algorithm, the Work Tasking Logic Model (WTLM), to digitally allocate inspection resources based on risk. The WTLM facilitates the delivery of Preventive Control Plan (PCP) inspections based on the Establishment-based Risk Assessment model risk intelligence and inspection parameters, and the unbiased delivery of monitoring sampling plans.

Purpose: A pilot project was conducted to examine the delivery and practicality of the WTLM.

Methods: The pilot, conducted from February to March 2020, targeted all federally regulated dairy establishments in Ontario ($n = 100$) and involved inspection staff ($n = 33$) who oversaw these establishments. The inspection staff were asked to follow a weekly schedule recommended by the WTLM, which identified establishments for PCP inspections, including inspection scope, and establishments for sample collection. A post-pilot survey was conducted, using SimpleSurvey, to assess the participants' appreciation of the WTLM. The descriptive results were done in Microsoft Excel 2016.

Results: Seventeen (38%) of the 45 inspections recommended by the WTLM were delivered. Unexpected events such as the Covid-19 pandemic accounted for 74% of the uncompleted inspections. The inspection staff conducted the inspection scope recommended by the algorithm in eight (47%) inspections. Over the 8-week pilot, 76 (60%) of the 126 recommended samples were collected. Nineteen (58%) of the 33 inspection staff completed the appreciation survey. On a scale from 1 to 10 (10 = completely agree), the respondents agreed (median = 7) that the WTLM was relevant, and moderately agreed (median = 6) that it helped risk-based inspection task delivery. They also agreed (median = 8) that the parameters used in the algorithm were appropriate. The pilot also identified areas of improvement to further refine the model and improve data quality.

Significance: The WTLM allows the CFIA to target inspection activities in higher risk establishments and to adapt in a timely manner to changing risks and redirect capacity as needed.

T2-16 A Deep Learning Approach to Predict *E. coli* Growth Using Micro-Isothermal Calorimetry (MIC) Data

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Introduction: Prediction of microbial growth in a food sample is important for shelf-stable food products. Improper storage and inadequate packaging leads to enhanced risk of growth of foodborne pathogens. The micro-isothermal calorimetry (MIC) captures very small amounts of heat produced due to metabolic activity of microorganisms and it has been related to microbial growth. MOs are reported to produce heat to an average of 1-3 μW per cell, which can be monitored in real time using modern isothermal calorimeters. Bacterial heat production is associated with CFU/g, which can be recorded and calibrated to be proportional to the heat flow (μW). However, MIC technique is organism specific and hence leads to insufficient analysis.

Purpose: When MIC data is coupled with the morphological information of a microorganism, advanced modelling techniques such as deep learning could successfully predict food spoilage. We explored the application of deep learning as an alternative method to interpret bacterial growth by developing ANN based optimization technique.

Methods: We used Hyperparameter Optimization (HPO) – a mechanism for automatically exploring a search space of potential Hyperparameters. The procedure allows optimization of ANN architecture, building a series of models and comparing them in terms of error. A training dataset ($n = 30$) of *E. coli* (MG1655) colonies were prepared by inoculating the strain 20 mL of TSB broth and incubated in the calorimeter at 37°C for 48 – 96 h. Salient data features (from the growth curve) were recorded and modelled.

Results: The HPO-ANN model yielded the training parameters (hidden layers, nodes, weight change momentum and learning rate). The MSE for the training and validation set was 0.003 and 0.022, respectively. Whereas an independent cross-validation procedure ($N = 10$) yielded high R^2 values (>0.9) with low avg. RMSE (0.0202).

Significance: The MIC data has the great potential to predict likelihood of microbial growth if advanced modelling techniques are used.

T2-17* [Ever-changing STEC Evolution: Illustration with O26](#)

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) of serotype O26:H11/H- constitute a diverse group of strains and several clones with distinct genetic characteristics have been identified and characterized. Especially, a clone possessing the *stx2* gene has emerged in the last decade.

Purpose: The objective of our study was to gain a better insight into the phylogenetic relationships of the various O26:H11 strains circulating in France.

Methods: Whole genome sequencing was performed using Illumina, Oxford Nanopore MinION and PacBio technologies on 150 O26:H11 strains circulating in France (of clinical and animal/food origin), including some *stx2*-positive O26:H11 strains. A combination of bioinformatics methodologies was used to perform a comparative and phylogenetic analysis of these strains with other O26:H11 genomes obtained from publicly available databases.

Results: Comparative analyses of the whole genome of the O26:H11 strains indicate that several clones of STEC O26:H11 are co-circulating in France. Phylogenetic analysis of the strains indicates that they are separated in two distinct lineages, one of which comprises the “new French clone” that appears genetically closely related to *stx*-negative attaching and effacing *E. coli* strains. Numerous MGEs were identified in each strain, including a large number of prophages and up to four large plasmids, representing overall 8.7 to 19.8% of the total genome size. Analysis of the prophage pool of the *stx2*-positive strains shows a considerable diversity with a complex history of recombination. Each clonal complex (SNP-CC) is characterized by a unique set of plasmids and phages, including *stx*-prophages, suggesting evolution through separate acquisition events.

Significance: These data provide insight on the phylogenetic relationships of the various O26:H11 strains circulating in France. Overall, the MGEs appear to play a major role in O26:H11 intra-serotype clonal diversification.

Molecular Analytics, Genomics and Microbiome

T2-18 [Genomic Characterization of *Listeria monocytogenes* Isolated from *Agaricus bisporus* Mushroom Production Facilities](#)

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Introduction: *Listeria monocytogenes* is a foodborne pathogen ubiquitously found in nature and can be isolated from food processing environments including the production and processing environments of frozen sliced mushrooms (*Agaricus bisporus*). Hygienic measures are therefore taken to control *L. monocytogenes*. The genetic diversity of *L. monocytogenes* strains isolated from mushroom environments is not well understood and therefore genomic insight could support identification of genomic traits that may affect factory ecology, persistence and virulence of *L. monocytogenes*.

Purpose: The objective of the study is to determine the genomic diversity using whole genome sequencing of *L. monocytogenes* strains isolated during the production and processing chain of frozen sliced mushrooms.

Methods: Samples of fresh and frozen sliced mushrooms and swabs of product contact surfaces before and after cleaning and disinfection were enriched following the ISO 11290-1:2017 procedure. In total, 153 *L. monocytogenes* strains were serotyped and 44 selected strains isolated from processing environments and final products were whole genome sequenced. The selected *L. monocytogenes* strains were also tested for antibiotic and benzalkonium chloride (BC) susceptibility.

Results: The *L. monocytogenes* strains were grouped in three serogroups, namely, 1/2b-3b-7, 1/2a-3a and 4b-4d-4e highlighting high strain diversity along the whole chain. Comparative WGS analysis of 44 selected strains revealed the presence of 11 Clonal Complexes (CCs), with *Listeria* Pathogenicity Island-1 (LIPI-1) and internalins *inlA* and *InlB* present in all strains, LIPI-3 present in all CC1, CC4, CC6 and CC224 strains and LIPI-4 present in all CC4 and CC87 strains. Yet, all tested 44 strains were sensitive towards a wide range of antibiotics and only one CC5 isolate carrying the *bcrABC* genes showed resistance towards BC.

Significance: This study highlights the diversity of *L. monocytogenes* strains in the frozen sliced mushroom production and shows the importance of controlling *L. monocytogenes* along the whole mushroom production chain.

T2-19* [Variability in the Survival of *Salmonella enterica* in Response to Heat and Desiccation](#)

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Introduction: Non-typhoidal *Salmonella* is an important foodborne pathogen causing over 400,000 deaths worldwide annually. Historically, *Salmonella* has been associated with products of animal origin; however, more recently salmonellosis cases have been associated with fruits and vegetables. Despite control measures in place, *Salmonella* contamination is still common during food production. The popularity of plant-based food products has been increasing, however there is a gap in scientific knowledge about *Salmonella* survival in vegetarian food products under various stresses.

Purpose: This study aims to assess the variability of *Salmonella* survival to stresses relevant to food production, such as heat and desiccation.

Methods: Single knock out mutants, high throughput mutagenesis, and genomic analysis are used to identify genetic factors responsible for survival in stress conditions. Various *Salmonella* serovars were subjected to heating at 60°C for 30 seconds in a vegetarian food product and surviving colonies enumerated.

Results: *S. Schwarzengrund* was the serovar most sensitive to heat during this study. Serovars were also exposed to desiccation for 24 hours and *S. Typhimurium* strain U288 1960-05 showed the lowest survival rate in this stress condition. Phylogenetic and genome analysis revealed an accumulation of pseudogenes in the U288 lineage which could explain the reduced survival after desiccation and hence, single mutations were constructed in a *S. Typhimurium* laboratory strain, SL1344, to assess the role of the genes in survival to desiccation. No significant difference was observed between single mutants and the wild type, suggesting that none of the candidate genes had a significant impact on their own.

Significance: A strong understanding of the variability in *Salmonella* stress response will help increase food safety, especially in plant-based food products.

T2-20 Heterogenous Contamination of Food Products, a Paradigm Shift?

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Introduction: Most routine methods currently used for the surveillance of food-associated pathogens are focused on identification of a single bacterial contaminant and are thus not equipped to detect potential heterogenous contamination with multiple genotypes/pathogenic species in the same matrix. We determined the microbial composition on consumer products in order to investigate the co-occurrence of multiple bacterial contaminants.

Purpose: Investigate the co-occurrence of multiple pathogens/genotypes of *Listeria* spp. *Salmonella* spp. or *Campylobacter* spp. in/on various food products.

Methods: A collection of ~70 different food products was investigated by RT-PCR and shotgun metagenome analysis, without prior enrichment by culturing. Each of the products was at least culture-positive for either *Listeria monocytogenes*, *Salmonella* spp. or *Campylobacter* spp. In addition, another selection of consumer products was analysed by 16S sequencing, directly upon purchase and again after 1 week storage at 4°C in our lab. The products were either purchased in the supermarket or at the local butcher's.

Results: In the majority of samples the bacteria observed with microbiological methods were also observed by metagenomic analysis, however with the metagenomic approach we could further classify these bacteria into subspecies/genotypes. By both methods, multiple pathogens/genotypes were observed in >20% of products investigated. No (known) foodborne pathogens were found in the collection analyzed by 16S sequencing, but the bacterial profiles were predictive of storage period and place of purchase (butcher or supermarket).

Significance: Our findings indicate that heterogenous contamination of food products may be more common than previously thought. This may have major implications on surveillance, but also on source attribution, cluster analysis and risk assessment models. Finally, microbiome profiles obtained from food products may be predictive of origin, type and perhaps relative risk. These data suggest that a focus on bacterial populations rather than single bacterial strains, is more appropriate in food microbiology and associated disciplines.

T2-21 Significance of Viable but Non-culturable Bacteria in the Fresh-cut Produce Industry

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Introduction: Sanitizers are used to maintain the microbiological quality of wash water, avoiding bacterial cross-contamination of fresh produce during washing. However, sanitizers can induce pathogenic bacteria to change to a viable but non-culturable (VBNC) stage and not be detected by traditional culture media. The occurrence of VBNC cells of bacterial pathogens in food has been identified as a public risk.

Purpose: This study focuses on optimizing a suitable methodology to differentiate dead and VBNC cells of *L. monocytogenes* in process water from the fresh-cut industry.

Methods: The methodology includes a quantitative polymerase chain reaction (qPCR), and the combination of different DNA-dyes such as an improved version of the propidium monoazide (PMAxx) dye plus ethidium monoazide (EMA).

Results: The results after sanitation treatment showed that the use of 10 µM of EMA and 75 µM incubated a 40°C for 40 min followed by 15 min-light exposure gave the best resolution to reduce the overestimation of viable cells with intact membrane. The selected methodology was validated in different types of process water including that from washing shredded lettuce and cabbage, diced onions, and baby spinach inoculated with *L. monocytogenes* and treated with chlorine (20-25 mg/L) or chlorine dioxide (ClO₂) (2-3 mg/L) for 1 min, mimicking the industrial conditions of high organic matter. Chlorine inactivated the presence of *L. monocytogenes* in the different process water, while ClO₂ reduced the levels of culturable pathogenic bacteria but induced the VBNC state of the remaining cells.

Significance: Chlorine's operational limits were satisfactory to inactivate foodborne pathogens present in process water, preventing cross-contamination. However, the antimicrobial activity of ClO₂ to maintain the microbiological quality of the process water could have been overestimated when establishing the operational limits because of the use of conventional plate count methods.

Sanitation and Hygiene

T2-22 Variation of Deoxynivalenol levels in Corn and Its Products Available in Retail Markets of Punjab, Pakistan and Estimation of Risk Assessment

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Introduction: The contamination of food and food products with mycotoxins is a serious food safety concern. Deoxynivalenol (DON) in cereal products is major concern in developing countries.

Purpose: There are very few reports on the assessment of DON in cereal products. In current research corn samples from different corn producing locations were analyzed for the presence of DON. Furthermore, the levels were compared with the permissible limits implemented by European Union.

Methods: Total 1220 samples of corn and products were analyzed for the detection of DON. The samples were analyzed using HPLC with UV detector.

Results: The limit of detection (LOD) and limit of quantification were 25 and 50 µg/kg, respectively. Furthermore, 539 (44.2%) samples were found to be positive with DON (n ≥ LOD). Furthermore, 92 (7.5%) samples of corn & products have levels of DON, higher than the proposed limits of EU. The lowest and highest exposure and hazard quotient (HQ) of 0.92 and 9.68 µg/kg bw/d were documented in corn flour samples.

Significance: The data is significantly different from normal distribution for DON in corn and products samples and from different locations (*P* < 0.05) for Shapiro-Wilk and Kolmogorov-Smirnov values. However, significant difference of levels of DON were found between corn and corn products types (*P* ≤ 0.05).

T2-23* The Locus of Heat Resistance Confers Resistance to Chlorine and Oxidative Stress in *Escherichia coli*

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Introduction: Chlorine-resistant *E. coli* isolates frequently harbor the Locus of Heat Resistance (LHR), a genomic island conferring heat resistance. The activities of the LHR-encoded proteins indicate a role in resistance to chlorine; however, this role has not been confirmed experimentally. Moreover, the relationship between the LHR and virulence factors is unexplored.

* Student Award Competitor

Purpose: This study aimed to determine whether the LHR confers resistance to chlorine and alters induction of Shiga toxin prophages by oxidative stress in *E. coli*.

Methods: Survival of LHR-positive and -negative strains of *E. coli* was assessed after treatment with NaClO, H₂O₂, and peroxyacetic acid. Isogenic strains of *E. coli* were generated by cloning of pLHR or the control plasmid pRK767. Fluorescent probes quantified the protective effect of the LHR against chlorine and oxidative stress on oxidation of cytoplasmic proteins and membrane lipids. Quantification of GFP fluorescence in the reporter strain *E. coli* O104:H4Δstx2::gfp::amp^r is an indication of Shiga toxin expression.

Results: Cloning of the LHR reduced the lethality of treatment with NaClO, H₂O₂ and peroxyacetic acid by 2.07 ± 0.56, 2.49 ± 0.33 and 1.75 ± 0.13 log (CFU/mL),

respectively ($P < 0.05$). The protein homeostasis module of LHR, which encodes heat shock proteins, prevented oxidation of cytoplasmic proteins by 0.12 ± 0.05 nm/nm ($P < 0.05$) while the oxidative stress module encoding KefB, prevented oxidation of membrane lipids by 15.03 ± 5.23 % ($P < 0.05$). Cloning of the LHR in *E. coli* O104:H4 Δstx2::gfp::amp^r reduced the population of induced cells by 21.10 ± 2.46 % ($P < 0.05$). Taken together, the LHR contributes to both chlorine and oxidative stress resistance by protecting multiple cellular targets.

Significance: Chlorine is used in water sanitation and for disinfection by food processing plants; the resistance of *E. coli* to chlorine is thus of concern to public health and food safety. The LHR protects *E. coli* against chlorine and other oxidizing chemicals, adding to our knowledge of the tools used to resist stress.



POSTER ABSTRACTS

27-28 April 2021

POSTER ABSTRACTS

* Student Award Competitor

Poster Session 1

Antimicrobials; Beverages and Acid/Acidified Foods; General Microbiology; Laboratory and Detection Methods; Meat, Poultry and Eggs; Seafood

Antimicrobials

P1-01 **Relative Quantification of the Expression of Some Key Stress Response and Virulence Associated Genes in Stationary Phase *Listeria monocytogenes* Cells Surviving Sub-Lethal Antimicrobial Exposure**

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Introduction: *Listeria monocytogenes* is an important foodborne pathogenic bacterium provoking listeriosis, a life-threatening disease mainly for susceptible individuals. Alarmingly, it can progressively adapt to stress, not only displaying stress-hardening, but also in some cases increasing its virulence.

Purpose: To quantify the expression of some key stress response and virulence associated genes in *L. monocytogenes* following their sub-lethal antimicrobial exposure.

Methods: The expression of ten genes (*groEL*, *hly*, *iap*, *inlA*, *inlB*, *lisK*, *mdrD*, *mdrL*, *prfA*, and *sigB*) was determined through qPCR, in two *L. monocytogenes* strains belonging to the most frequent listeriosis-associated serovars (1/2a and 4b) following sub-lethal exposure to each one of three common antimicrobials: a general-purpose synthetic biocide (benzalkonium chloride), a natural plant terpenoid (thymol), and a broad-spectrum beta-lactam antibiotic (ampicillin). To this end, each antimicrobial was applied for 2 hours at 37°C against stationary phase cells of each serovar, at a concentration that was higher than that needed to inhibit the bacterial growth. Specifically, benzalkonium chloride, thymol, and ampicillin were tested at concentrations of 4, 312.5, and 0.5 ppm, respectively.

Results: In general, and rather fortunately, the expression of most of the studied genes remained either stable or was downregulated following the antimicrobial exposure, with some strain specific differences yet to be recorded. THY was the compound that provoked downregulation of most of the studied genes, significantly limiting the expression of 6/10 genes in one strain (ser. 1/2a), and 4/10 genes in the other strain (ser. 6b), including those coding for the master regulators of stress response and virulence (SigB and PrfA, respectively), in both strains. Nevertheless, at the same time, the two genes coding for the invasion internalin proteins, with crucial role in the onset of *L. monocytogenes* pathogenesis, were importantly up regulated in ser. 6b strain.

Significance: Results obtained increase knowledge on stress physiology of *L. monocytogenes* under certain sub-lethal antimicrobial conditions that could be encountered within the food industry and in clinical settings.

P1-02* **Electrolysed Water for Fresh Produce Treatment: Effects of Organic Contaminants and Outgrowth Delays from Treated Spores**

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Introduction: The oxidising sanitiser electrolysed water (EW) has diverse proposed applications including the surface-sanitisation of fresh foods. There is a need to understand limiting factors associated with chemical matrices presented by the relevant applications, as well as to understand how spoilage organisms respond to the sanitiser.

Purpose: This study investigates the impact of different, incidental organic-material sources on EW efficacy which also leads to new insights into the mechanism of action of EW in cells. It further assesses the spore-to-spore variation and growth delay arising from EW treatment of the food spoilage mould *Aspergillus niger*.

Methods: Fungi were treated with EW in suspension (5 min exposure), with or without the addition of incidental organic-material sources, and the survival was assessed with standard microbiological methods on agar plates or in suspension. Experiments were conducted in biological triplicates.

Results: The EW (360–400 mg mL⁻¹ free available chlorine) retained partial fungicidal activity against *A. niger* at high levels of added soils (30–750 mg mL⁻¹), commonly associated with harvested produce. Addition of pure proteins or amino acids (≤ 1 mg mL⁻¹) fully suppressed EW activity, and among the amino acids, cellular methionine was found to protect against EW also in a yeast model. Low EW concentrations (2–5 mg mL⁻¹ free available chlorine), as might occur in the presence of EW-suppressing organics, significantly delayed the subsequent development of fungal colonies from EW treated spores ($P = 0.0002$, 3 biological replicates) and affected the colony-colony variation in outgrowth ($P = 0.0002$), independent of the spore survival rate.

Significance: The data help to understand, monitor and predict the antimicrobial efficacy of chlorine-based sanitisers such as EW against spoilage or pathogenic microorganisms in industry and domestic settings.

P1-03 **In Vitro Study of Antimicrobial Activity of Essential Oils and Their Components against the Main *Clostridium difficile* PCR-Ribotypes Isolated in Belgium**

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Introduction: Essential oils are suggested as potential agents to treat intestinal dysbiosis and as natural preservatives against spoilage in food products. *In vitro* antimicrobial effects against foodborne pathogenic bacteria have been demonstrated. To the present, there are few available data in the literature describing the capacity of some essential oils to inhibit the growth of the intestinal pathogen *Clostridium difficile*.

Purpose: The aim of this study was to evaluate the antibacterial activity of ten commercial essential oils and some of their main components against *C. difficile* strains isolated from hospital patients and foods in Belgium.

Methods: The antibacterial spectrum of ten essential oils (*Cinnamomum cassia*, *Cymbopogon nardus*, *Coriandrum sativum*, *Cinnamomum verum*, *Eugenia caryophyllus*, *Origanum compactum*, *Origanum heracleoticum*, *Origanum majorana*, *Salvia sclarea*, *Thymus vulgaris thymoliferum*) and individual constituents (including carvacrol, *trans*-cinnamaldehyde, eugenol, linalool and thymol) was determined by paper disk diffusion assay, in comparison with six antibiotics (chloramphenicol, clindamycin, erythromycin, gentamicin, metronidazole and vancomycin). Each data of the experiment was presented as mean value \pm standard deviation of triplicates. The means were analyzed by one-way analysis of variance, followed by Tukey test to determine the significant difference between essential oils at *P*-values \leq 0.05.

Results: Strains of *C. difficile* isolated from both patients and food samples were significantly inhibited by all tested essential oils and their main components. Oils of cinnamon (*Cinnamomum cassia* and *C. verum*) and cinnamaldehyde showed the highest activity, followed by thyme and thymol, and oregano oil and carvacrol (*P* \leq 0.05). The antibacterial spectra of cinnamon oils and cinnamaldehyde were comparable and even higher than tested antibiotics.

Significance: This study is one of the few to report on susceptibilities of human and food *C. difficile* strains to the essential oils and their components. These findings can be exploited in preventing *C. difficile* infection by natural alternative.

P1-04 Distribution of Antimicrobial Resistance Genes in Various Types of Food Including Meat, Produce (Vegetables and Fruits) and Dairy Products in Canada

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Introduction: Distribution of microorganisms with anti-microbial resistance (AMR) genes in food including meat, produce (vegetables and fruits) and dairy products has been recognized as a growing public health concern worldwide. Food contaminated with microorganisms are responsible for numerous foodborne illnesses, including outbreaks caused by AMR gene-containing bacteria.

Purpose: This study is aimed at determining the distribution of AMR genes in various commodities including meat, produce and dairy products for a better understanding of the associated hazards and facilitating quantitative risk assessment.

Methods: Food samples (*n* = 59), including meat (*n* = 18), produce (*n* = 30, vegetables = 21, fruits = 9) and dairy (*n* = 15), were randomly purchased at different times from local retailers. Microbes in food were enriched (24 h at 37°C) using modified Schaedler media, followed by genomic DNA extraction and detection of 87 AMR genes relevant to human, animal and agriculture using a commercial AMR gene qPCR array kit (Qiagen).

Results: Thirty-three AMR genes had at least one occurrence in all three groups with potential resistance against aminoglycoside, fluoroquinolone, tetracycline, macrolide and Class C beta-lactam. Eight AMR genes including erythromycin-resistance genes were not detected in any of the food samples. The meat contained the highest number of AMR genes/sample (mean = 14, ranged from 2 to 28), followed by produce (mean = 10, 1 to 34) and dairy products (mean = 7, 1 to 23). Four AMR genes including *vanB* (vancomycin resistance) were only detected in meat samples, 12 and 4 other AMR genes were only detected in produce and dairy products, respectively. The *mecA* gene, encoding a methicillin-resistant *Staphylococcus aureus*-specific penicillin-binding protein, was also detected in produce and dairy products but not in meat.

Significance: The results in this study provide useful baseline data on AMR gene presence in food in Canada and indicate that different types of food may acquire microorganisms containing certain AMR genes from different sources.

Beverages and Acid/Acidified Foods

P1-05 Rapid Determination of Lactic Acid Bacteria from Fermented Green Olives Packaged in Modified Atmospheres by Means of FTIR Spectroscopy and Machine Learning

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Introduction: The application of Fourier transform infrared (FTIR) spectroscopy has increased in food studies over the last years and has become a powerful tool in the determination of quality in a variety of food products.

Purpose: To investigate the efficacy of FTIR in the rapid determination of the population of lactic acid bacteria (LAB) in fermented green olives during modified atmosphere packaging.

Methods: Fermented green olives of Halkidiki and Conservolea varieties were packed in multi-laminated pouches under modified atmospheres (100% N₂) and stored at room temperature for 12 months. Every month, FTIR spectra were acquired from the olives together with microbiological analyses for the determination of the population of LAB, yeasts and enterobacteria. PLS-regression (PLS-R) was employed to provide quantitative estimations of microbial counts during storage. The spectra were pre-processed by multi-scatter correction (MSC) followed by 1st derivative (Savitzky-Golay algorithm, 2nd order polynomial and 15-point moving window). The spectra were divided into calibration set (80%) and prediction set (20%) according to the Kennard-Stone method.

Results: No enterobacteria could be detected throughout storage in both varieties, whereas yeasts could be enumerated only at the beginning of fermentation in populations ranging from 5.2-5.5 log CFU/g. LAB were enumerated systematically during storage of olives and thus FTIR spectra were associated with LAB counts to provide quantitative estimations. The PLS-R model developed with spectral data in the region 900-2,000 cm⁻¹ was able to provide satisfactory predictions of LAB counts with root mean squared error of calibration (RMSEC), cross-validation (RMSECV) and prediction (RMSEP) of 0.281, 0.408, and 0.479 log CFU/g, respectively.

Significance: Spectroscopic data in tandem with appropriate algorithms exhibit promising potential for the rapid detection of LAB in packaged green olives.

Acknowledgment: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-04110).

General Microbiology

P1-06 Food Safety Research Among People with Intellectual Disabilities

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Introduction: In many countries people with Intellectual Disabilities (ID) were previously housed in different institutions but have now become individual consumers with private households. They live in the society as other people and taking care of cooking by themselves or with support of staff. Their everyday life in relation to food safety is hardly studied and their knowledge of food safety is relatively unknown. Good hygiene practices can inhibit foodborne diseases and diminish the waste of food and instead increases the sustainability and give economic effort.

* Student Award Competitor

Purpose: To develop a method for investigating food safety knowledge and practices in relation to everyday life among people with ID who live by themselves in Sweden.

Methods: A special interview guide with pictures was developed to be used in focus group interviews, due the intellectual limitations of the study group. The pictures were related to the Four C (Cooking, Cleaning, Chilling and to avoid Cross-contamination). The pictures are to be shown on a computer and discussed, one at a time. With support of recognizable pictures illustrating the Four C might make it easier for individuals with ID to reflect on everyday actions. Focus groups interviews are planned for 2-3 participants per group in a familiar context. To discuss everyday life in a focus group with other people in similar living conditions is supposed to give an extra inspiration to the discussion.

Results: This method can provide new important knowledge to food safety research and lead to future food safety education and teaching material tailored for this group.

Significance: To strengthen their position as consumers, people with ID need adapted methods to be included in food safety research. This is an unexplored group of importance to include into food safety prevention work to improve public health as well as of high interest internationally.

P1-07* Effect of Heavy Water Incorporation on the Viability on *Listeria innocua*

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Introduction: *Listeria innocua* is a Gram-positive ubiquitous bacterium and is the most frequently encountered non-pathogenic *Listeria* species, although excessively rare cases of *L. innocua* septicemia and meningitis infections have been reported in humans and ruminants. *L. innocua* is a close relative of *Listeria monocytogenes* specie, an important foodborne pathogen and the etiological agent of human listeriosis, a rare but frequently fatal disease. Bacteria in the viable but nonculturable (VBNC) state have very low metabolic activity and do not divide. Consequently, VBNC cells do not grow on standard microbiological media but retain the ability to recover and become culturable under favourable conditions (i.e., resuscitation). Different environmental stresses can induce the VBNC state during food processing, such as starvation, oxidative stress, temperature changes and disinfection. An innovative approach is to use mass spectrometry, Raman spectroscopy coupled with heavy water labelling of metabolism appears to be an innovative technique for the study of bacterial metabolism and spectral changes.

Purpose: We evaluated the impact of heavy water incorporation on the viability state of *L. innocua* cells (Viable Cultivable (VC) or VBNC) by Raman spectroscopy.

Methods: We exposed the *L. innocua* bacterial suspension to different heavy water concentrations (0%, 25%, 50% and 75%) during 0h30, 1h00, 1h30, 2h, 4h, 6h, 12h, 24h, 48h, 72h and 96h. For each condition, total, viable (VC and VBNC) and VC populations were quantified by qPCR, PMA-qPCR and plate count agar, respectively. In parallel, we analyzed heavy water absorption by Raman spectroscopy.

Results: The results of the quantification showed that exposure to heavy deuterium does not affect the viability of *L. innocua* cells.

Significance: This study shows for the first time that heavy water incorporation has no impact on *L. innocua* cell viability. This heavy water incorporation is essential for the development of an innovative approach for detecting the viable non-cultivable state of the bacteria by Raman spectroscopy.

P1-08 The Effect of Temperature on Development of *Salmonella enterica* ser. Enteritidis and *Pseudomonas fluorescens* Biofilms

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Introduction: Biofilms are considered to be the predominant form of bacterial life and can be a major source of contamination in food industries thus increasing the probability of public health's risk. Temperature and the co-existence of multi-bacterial species may considerably affect the biofilm formation of individual strains.

Purpose: To investigate the effect of temperature on development of *Salmonella enterica* ser. Enteritidis and *Pseudomonas fluorescens* in mono-culture and co-culture biofilms.

Methods: Biofilm formation on stainless steel coupons in Tryptic Soy Broth (TSB) of *S. Enteritidis* and *P. fluorescens* at 5°C, 15°C, 30°C was tested, in mono- and co-culture for 144 hours. At 3, 48 and 144 h of incubation, sessile cells were retrieved using the bead vortexing method and enumerated on Tryptic Soy Agar (TSA) in the case of monocultures and in the case of co-culture XLD Agar for *S. Enteritidis*, CFC Agar for *P. fluorescens* and TSA for both bacterial species.

Results: The population of *P. fluorescens* in monoculture biofilms was higher in both 15°C and 30°C than those of *Salmonella* although the differences in terms of population did not exceed 1 log CFU*cm⁻² in most cases. *S. Enteritidis* was affected by temperature in a larger extent. Indeed, differences greater than 2.5 log CFU*cm⁻² were observed between 5°C and 15°C. Between 15°C and 30°C differences exceeded the 4 log CFU*cm⁻². The results observed in the case of co-culture were similar to those obtained by both monocultures. At 5°C and 15°C, *P. fluorescens* was the dominant bacterium of the biofilm. At 30°C, *S. Enteritidis* approached *P. fluorescens*' population without surpassing it.

Significance: Temperature appeared to play an important role in biofilm development of the pathogenic *S. Enteritidis* but not of *P. fluorescens*.

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P1-09 The Effect of Seawater Salinity on Biofilm Formation of Seven Bacteria of Aquaculture Interest

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Introduction: The effect of sodium chloride on biofilms has been studied in many bacteria. NaCl has been previously reported as a factor that influences biofilm's three-dimensional structure. However, to our knowledge, the effect of salinity of seawater on biofilm formation has not been well studied.

Purpose: To evaluate the effect of seawater's salinity on the biofilm formation and development of bacteria of aquaculture interest.

Methods: Bacterial strains found in aquaculture installations were used: *Pseudomonas fluorescens*, *Vibrio harveyi*, *Vibrio atypicus*, *Tenacibaculum discolor*, *Pseudoalteromonas* sp¹, *Pseudoalteromonas* sp², *Algibacter* sp. Biofilm formation was assessed on stainless steel coupons. Attachment was performed in five different solutions (4 seawater solutions of 10, 20, 30 and 40 PSU and ¼ strength Ringer solution). Then, coupons were transferred in Marine Broth and incubated at 15°C for 48 hours. Sessile cells were retrieved using the bead vortexing method and enumerated on Marine Agar plates. Simultaneously, coupons were retrieved before bead vortexing, fixed with methanol, stained with Acridine Orange dye and observed under epi-fluorescent microscope.

Results: Seawater salinity influenced the adhesion of microbial cells on stainless steel. When exposed at lower salinities, *P. fluorescens* and *V. harveyi* exhibited higher attachment. *Pseudoalteromonas* sp.1, *Pseudoalteromonas* sp.2 and *Algibacter* sp., *V. atypicus* and *T. discolor* exhibited none to minimum adhesion at Ringer's solution and increased adhesion at seawater solutions, despite the salinity level. Mature biofilms' populations were not affected by initial salinity condition. Epi-fluorescent microscopy showed no differences in the structure of just adhered cells exposed at different salinities. However, the 3-D structure of the mature biofilms showed major differences among the bacterial species, regardless of the initial exposure at different salinity conditions.

Significance: Seawater salinity affected the adherence of planktonic cells, but did not have further impact on the mature biofilm, whose 3-D structure was distinct for each bacterial species.

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P1-10* Microbiological Quality of Fresh Produce Purchased from Street Vendors and Stored in Homes in Informal Settlements of Gauteng Province

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Introduction: Improper handling of fresh produce can lead to cross-contamination with foodborne pathogens, which could result in human illnesses. Proper storage is one of the critical steps in the supply chain needed to maintain good microbiological quality of fresh produce. However, implementation of good handling practices in informal settlements can only be achieved as critical challenges are addressed such as infrastructure, regulation and awareness.

Purpose: The study aims to assess the microbiological quality of fresh produce from the point of purchase from street vendors and post storage in homes in informal settlements of Gauteng Province.

Methods: A total of 360 fresh produce samples (180 spinach and 180 tomato) were purchased from ten street vendors and given to 50 families living in informal settlements to simulate final stage of food handling "at home". Analysis of the fresh produce included the detection and isolation of foodborne pathogens (*Escherichia coli*, *Salmonella* spp. *Listeria* spp. and extended spectrum β -lactamase *Enterobacteriaceae*). Isolates were further characterized for antimicrobial resistance, virulence genes and relatedness determined by phylogenetic analysis and repetitive PCR.

Results: *Listeria monocytogenes* was detected from one sample, *Salmonella* spp. from five samples, and ESBL *Enterobacteriaceae* was detected from 20 samples. *Escherichia coli* was detected from 9.17% ($n = 33$) of the 360 samples. None of the selected *E. coli* isolates ($n = 62$) contained diarrheagenic virulence genes and 40.32% were phylogenetically characterised as category generalist (B1). However, a large number of isolates (75.81%), were found to be multi-drug resistant.

Significance: Fresh produce sold in informal settlements contain multidrug-resistant *E. coli* which can be considered a human health risk in communities with high levels of immunocompromised people. The study highlights the need for effective food safety training of street vendors and consumers to improve personal hygiene and prevent food cross-contamination in poorly resourced areas lacking adequate cold storage, potable water and sewage removal systems.

P1-11* Real-time Observation of Sub-lethally Acid Injured *Listeria monocytogenes* and Resuscitation Capacity at Single-cell Level

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Introduction: Exposure of *Listeria monocytogenes* to sub-lethal stresses related with food processing may induce sub-lethal injury that is stochastically expressed at single-cell level, with varying resuscitation capacity.

Purpose: (i) Examine, at single-cell level in conjunction with population level, sub-lethal injury in *L. monocytogenes*, (ii) outline the distribution of culturable, injured and dead cells during exposure to stress, and (iii) monitor real-time resuscitation with direct time-lapse cell imaging.

Methods: Acidic conditions (acetic and hydrochloric acid adjusted to pH 3, 2.7, 2.5 at 4 and 20°C for 5 hours) were used to evaluate injury of *L. monocytogenes* Scott-A. To differentiate the resistant sub-population from the total, Tryptic Soy Agar with 0.6% Yeast Extract (TSAYE) supplemented or not with 5% NaCl were comparatively used. Sub-lethally injured cells were determined by comparing plate counts with fluorescent microscopy, coupled with CFDA (metabolic active) and Propidium-Iodide (dead). Resuscitation on TSAYE was monitored with time-lapse microscopy at 37°C. Fluorescent and time-lapse images were analyzed by a customized software for cell quantification and tracking along time.

Results: Acetic acid treated cells showed detectable logarithmic reduction of total population and an induction of injury and death, at single-cell level. *L.monocytogenes* retained its culturability towards hydrochloric acid exposure, while cells remained metabolically active, exhibiting green fluorescence. In terms of single cell resuscitation, growth properties of control samples were quantified and compared to stressed samples. In all cases of stresses apart from pH 2.5, there were cells able to form colonies and "recover" even with larger lag phases than the control group.

Significance: Identification of potential risks underestimating a product's microbial status and understanding the kinetics governing revitalization of the sub-lethally injured cells upon resuscitation.

Acknowledgment: This project has received funding from the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT), under grant agreement No [1788].

P1-12 Exploring Perceptions and Self-reported Food Safety Practices of Pet Owners, Providing Raw Meat-based Diets to Pets

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Introduction: As the trend of raw meat-based diets for domestic pets is becoming more popular, there is a growing concern, regarding the potential food safety implications of such practice to pet owners. In order to reduce the risk of foodborne illness, when practicing raw meat-based diets, pet owners must be adequately informed about the potential hazards and implement appropriate food safety practices.

Purpose: To explore pet owners' knowledge and perceptions about safety of raw meat-based feeding and determine self-reported food safety practices during raw meat-based pet food preparation.

* Student Award Competitor

Methods: An online questionnaire, comprised of 23 questions, was distributed via social media platforms and completed by pet owners, practicing raw meat-based feeding ($n = 174$). The data was analysed using descriptive statistical analysis.

Results: Almost every participant (95%) reported confidence that their pets' raw meat-based food is safe. Although respondents (67%) reported to have researched food safety information about raw meat-based diets, only 8% asked a veterinarian for food safety advice. Despite reporting awareness that harmful pathogens may be present in raw meat-based diets, respondents did not report a consistent implementation of food safety practices. Unsafe practices of rinsing meat (27%) and the absence of segregation of utensils and kitchen surfaces (52%) were also reported. Whilst the participants aged 65 years and over did not report any serious malpractices, they did not employ sufficient practices, in order to safeguard themselves from foodborne illness. Majority of respondents (89%) perceived the risk of foodborne illness, associated with raw feeding practice to be 'low', suggesting 'optimistic bias'.

Significance: There is a need for raising pet owners' awareness of the potential risks of raw meat-based feeding and informing them about appropriate food safety practices that they should employ in order to safeguard their health. Pet owners, aged 65 years and over, should be encouraged to implement correct food safety practices consistently.

P1-13* Prevalence Study of Presumptive *Bacillus cereus* from Fresh to Frozen Spinach with the Indication of Using *Bacillus Thuringiensis* Biopesticides

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Introduction: The *Bacillus cereus* group is ubiquitous in the environment and also likely to be detected in various food products, even in fresh-frozen vegetables. *B. Thuringiensis* (Bt) used as a biopesticide can also persist on crops and residual numbers to be found on the final produce through food supply-chain.

Purpose: This study aims to evaluate whether there are any differences in the occurrence and residual numbers of presumptive *B. cereus* and Bt from fresh spinach to frozen spinach with or without prior treatment of Bt biopesticides.

Methods: Fresh spinach from field, fresh washed and frozen spinach samples were prepared by 3 companies and delivered to the laboratory. Typical presumptive *B. cereus* colonies were counted from MYP agar plates after incubation at 30°C for 24 h. Isolates from positive samples were checked by the production of parasporal crystals under phase-contrast microscope for the identification of Bt.

Results: Presumptive *B. cereus* and Bt were detected in all fresh ($n = 4$) and frozen spinach ($n = 8$) samples treated with Bt biopesticides with the range of $1.95 \times 10^4 - 1.75 \times 10^5$ CFU/g and $250 - 1.15 \times 10^3$ CFU/g, respectively. A total of 17 of 17 and 47 of 49 isolates from Bt-treated fresh and frozen spinach samples were identified as Bt. For samples without prior Bt biopesticide treatment, 7 (50%) fresh spinach from field, 6 (67%) fresh washed spinach and 4 (33%) frozen spinach were detected by presumptive *B. cereus* with the range of 100-850 CFU/g, and in which 0 (0%), 6 (100%) and 3 (75%) samples were positive for Bt. A total of 20 of 23 and 3 of 4 isolates from untreated fresh washed and frozen spinach samples were identified as Bt.

Significance: Higher occurrence and counts of presumptive *B. cereus* with more Bt populations were found in fresh and frozen spinach samples prior sprayed with Bt biopesticides than samples without using of Bt biopesticides.

Laboratory and Detection Methods

P1-14* Comparison of Compact Dry and Conventional ISO Methods for Microbiological Survey of Lettuce Varieties at Retail Stage

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Introduction: Prevalence of foodborne outbreaks linked with fresh produce consumption calls for exploring rapid methods to investigate diverse microbial communities associated with fresh produce.

Purpose: This study evaluated the performance of compact dry and conventional methods in determining the microbial ecology of lettuce varieties at retail stage.

Methods: Microbiological analysis was carried out using compact dry and conventional methods for 7 lettuce varieties (including cos, curly leaf, iceberg, little gem, romaine hearts, round and sweet gem). Samples were analysed for aerobic bacteria, yeasts and moulds, coliforms and *Enterobacteriaceae* while pathogen levels were determined for *Salmonella* spp., *Staphylococcus aureus* and *Listeria monocytogenes*.

Results: Using conventional methods, mean microbial counts ranged from 6.1×10^3 CFU/g to 1.4×10^6 CFU/g (aerobic bacteria); 3.2×10^3 CFU/g to 1.7×10^5 CFU/g (yeasts and moulds); 1.2×10^2 CFU/g to 1.3×10^5 CFU/g (*Enterobacteriaceae*); 1.0×10^2 CFU/g to 1.1×10^5 CFU/g (coliforms); 0.0 CFU/g to 3.0×10^3 CFU/g (*Salmonella* spp.); 0.0 CFU/g to 1.3×10^4 CFU/g (*Staphylococcus aureus*) and 6.0×10^2 CFU/g CFU/g to 1.0×10^5 CFU/g (*Listeria monocytogenes*). However, mean microbial counts using compact dry methods ranged from 6.0×10^3 CFU/g to 1.5×10^6 CFU/g (aerobic bacteria); 2.8×10^3 CFU/g to 1.7×10^5 CFU/g (yeasts and moulds); 1.2×10^2 CFU/g to 1.1×10^5 CFU/g (*Enterobacteriaceae*); 1.2×10^2 CFU/g to 1.0×10^5 CFU/g (coliforms); 0.0 CFU/g to 2.0×10^3 CFU/g (*Salmonella* spp.); 0.0 CFU/g to 1.2×10^4 CFU/g (*Staphylococcus aureus*) and 3.0×10^2 CFU/g to 1.0×10^5 CFU/g (*Listeria monocytogenes*). Furthermore, mean microbial counts of both methods revealed a positive correlation ($r = 0.9593$).

Significance: Compact dry method is a reliable alternative for timely investigation of microbial communities associated with fresh produce like lettuce.

P1-15 Evaluation of the Assurance® Gds MPX Top-7, MPX-ID and Ehec-ID to Detect, Confirm and Isolate *Escherichia coli* Producing Shiga-toxin (STEC) Serogroups in Raw Dairy Products

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens implicated into human infection that can be acquired through the ingestion of critical foods such as raw dairy products. In order to limit the risk of STEC outbreaks it is important to develop rapid and accurate STEC detection methods.

Purpose: Evaluate the alternative method Assurance® GDS MPX-TOP7, MPX-ID and EHEC-ID combining immunomagnetic concentration (IMS) and real-time PCR (PCR) with a reference method adapted from ISO/TS-13136:2012 for STEC analysis in raw dairy products.

Methods: A total of 100 samples of raw milk (cow, sheep and goat) and raw milk cheeses, including 10 uninoculated and 90 samples spiked with stressed cells (<10 CFU/25 g-ml) of O26, O103, O111, O145 and O157 STEC, were analyzed using both methods. The alternative method includes enrichment in proprietary broth at 41.5°C for 18 h followed by primary screening of TOP-7 STEC (IMS+PCR) containing *eae*, *stx*₁, *stx*₂ genes and O157:H7 markers, secondary screening for serogroup identification using MPX-ID/EHEC-ID (IMS+PCR), and finally IMS+cultural confirmation on two selective agars. For reference method, enrichment was performed in BPW at 41.5°C for 18 h.

Results: Relative trueness (agreement between both methods) was 95%. Five negative deviations (positive by ISO and negative by alternative) were detected in some replicates from Roquefort, Cantal and Comté cheeses spiked with *E. coli* O157 and O111 at low level (4, 2 and 5 CFU/25 g, respectively). One positive deviation (negative by ISO and positive by alternative) was detected for raw cow milk spiked with *E. coli* O157. All PCR positive results were confirmed by IMS+cultural isolation for both methods.

Significance: This study demonstrated accuracy and sensitivity of the alternative method for STEC analysis in a complex matrix like raw dairy products, enabling fast screening and identification of Top 5 STECs.

P1-16 Detection of Viable *Listeria monocytogenes* by Multiplex Reverse Transcriptase Real-time PCR, Using Two Target Genes

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Introduction: Foodborne infections are a major public health issue, and those associated with *L. monocytogenes* cause the highest number of deaths worldwide. Due to the need to detect this pathogen before the food product reaches the consumer, faster methodologies have been developed, being those based on PCR/qPCR the most popular. However, traditionally target DNA techniques can originate false negative result due to the detection of dead bacteria DNA, leading to unnecessary product recalls and economic losses.

Purpose: Different approaches for the viable bacteria detection have been studied, among them mRNA detection, represents a good option due to its short half-life. In this study, we developed a multiplex RT-qPCR for the specific detection of viable *L. monocytogenes* targeting two species-specific genes, *hly* and *actA*, to improve the analysis sensitivity and specificity.

Methods: The methodology was optimized to determine a suitable enrichment broth (higher levels of target genes expression), and to select the best RNA extraction kit and protocol (higher RNA concentration with lower DNA contamination). The methodology evaluation was performed in 24 smoked salmon samples, spiked with different concentrations of live and dead cells, and the results were compared against the ISO 11290-1.

Results: A general medium (mTA10) was selected, due to low expression levels of *hly* using Half-Fraser broth. After RNA extraction optimization, ΔCq between RT-qPCR and qPCR was 26.0 ± 2.2 and 15.8 ± 1.0 for *hly* and *actA*, respectively, showing very low DNA contamination (ΔCq of 5, indicates 3% gDNA contamination), and lack of DNA interference from dead cells in viable bacteria detection. The methodology allows a proper discrimination between live and dead cells with a LOD_{50%} of 1.2 CFU/25 g.

Significance: Next-day detection of viable *L. monocytogenes* by mRNA analysis, was possible. This approach shows a real advantage for the food industry, allowing to reduce losses, and increasing analysis speed.

P1-17* Application of Recombinase Polymerase Amplification with Lateral Flow for a Naked-eye Detection of *Listeria monocytogenes* on Food Processing Surfaces

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Introduction: The incidence of *L. monocytogenes* has not significantly decreased in the last few years, which highlights the need for more control measurements in the food industry. The verification of food processing plants is the key to avoid cross-contamination and assure the safety of the food products. However, the analysis of the food contact surface needs improvement.

Purpose: The use of isothermal DNA amplification techniques to overcome the drawback of classical analysis showed promising results, reducing time and the need of complex equipment. In this study a new methodology based on Recombinase Polymerase Reaction (RPA) combined with a lateral flow strips (RPA-LF) was developed. The method targeted *L. monocytogenes* and was tested on stainless steel coupons, which mimic food processing surfaces

Methods: Different approaches for the recovery of the bacteria from the surface, the enrichment step and downstream analysis by RPA-LF were tested and optimized. A total of 32 samples, 100 cm² each, were spiked with different *L. monocytogenes* concentrations, and were analysed to evaluate the methodology. Finally, the results were compared against ISO 11290-1 and qPCR.

Results: Sponges were more efficient than cotton swabs for the recovery of the bacteria, and a 24-h enrichment in ONE broth was needed for the most sensitive detection. By RPA-LF it was possible to clearly detect down to 1.1 pg/ μ L. The limit of detection of the methodology was determined to be LoD₅₀ of 4.2 CFU/cm². The results obtained by RPA-LF were comparable to those of the reference methodology, which also demonstrated more sensitivity than qPCR.

Significance: The developed approach will allow for a faster and easier analysis compared with the classical methodologies applied in the food industry. Additionally, it will also allow to reduce the cost of the analysis compared with other DNA-based techniques.

P1-18 Assessment of a Real-time PCR Method for the Detection of Shiga Toxin-producing *Escherichia coli*

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are important enteric pathogens worldwide, causing diarrhea with or without blood visibly present and hemolytic uremic syndrome. Cattle and other ruminants are the natural reservoir of STEC. The sources of these infections are most commonly foods that are intended to be eaten raw or part-cooked (including meat, vegetables & fruits).

Purpose: The purpose of this study was to evaluate the performance of the SureTect™ *Escherichia coli* O157:H7 and STEC PCR Assays for screening and identification of STEC from two different food categories with large sample sizes (375 g) to improve sampling plans for (i) meat (excluding poultry) and (ii) vegetables & fruits.

Methods: An unpaired study was conducted versus the ISO/TS 13136:2012 reference method according to the technical rules of the ISO 16140-2 standard. A minimum of 90 samples were tested for each category, to meet the ISO 16140-2 requirements for the sensitivity and relative level of detection (RLOD) studies. All samples were enriched using a short protocol (8 hours for meat samples, 10 hours for vegetables

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& fruits samples) and a long protocol (24 hours) then tested with the SureTect PCR workflow. The samples were also tested after a 72-hour cold storage hold post-enrichment. Positive PCR results were confirmed using a combination of culture and molecular techniques. As part of the inclusivity and exclusivity studies, 50 strains of STEC (covering O26, O103, O111, O145 and O157 serogroups) and 30 non-target strains were tested.

Results: The number of positive deviations was significantly higher than the negative deviations. This indicates a skew in performance in favour of the alternative method for the meat and vegetables categories. For inclusivity and exclusivity studies, all strains provided expected results.

Significance: The data indicates that the SureTect method is a suitable alternative to the ISO/TS 13136:2012 method for 375 g meat (excluding poultry) and vegetables & fruits.

P1-19 Glyphosate, Glufosinate, and Their Metabolites in Food of Animal Origin: Analytical Method Optimization and Validation

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Introduction: Among pesticides which cannot be analyzed through multi-residue methods, polar pesticides represent a group of pesticides particularly difficult to analyze. Glyphosate, the main representative of this group, is a non-selective herbicide extensively used worldwide to fight against weeds in crops since the 1970s and is classified as "probably carcinogenic to humans". Due to its use on several crops that might be fed to livestock, monitoring of glyphosate levels in food of animal origin had been introduced in the European Multiannual Control Program (MACP) in 2019. To the same extent, glufosinate and its metabolites have been introduced in the MACP in 2021 for food of animal origin.

Purpose: As French National Reference Laboratory for Single Residue Methods (SRM), mandated by the French Ministry for food, agriculture and fisheries, we optimized and validated an analytical method for glyphosate, glufosinate, and their metabolites in milk, liver and eggs, to analyze the French official samples of the MACP.

Methods: The EU Reference Laboratory for SRM has recently published a method (Quick Polar Pesticides) for analyzing these residues in foods of animal origin. On this basis, the influence of different parameters on method performances were tested: (i) water addition during extraction, (ii) purification sorbent phases, (iii) use of ultra-filtration as an additional purification step, and (iv) dilution of the extracts.

Results: The resulting method consists of an extraction of the residues by acidified methanol, followed by SPE on HLB Prime short cartridges and an ultra-filtration of the extracts before LC-MS/MS analysis (HILIC mode). Validation according to guideline SANTE/12682/2019 has been performed, from 0.025 to 0.4 mg/kg for each individual residue.

Significance: This method has been applied to around 60 samples of milk and bovine liver from the MACP in 2019 and 2020. This method will be applied on chicken eggs samples of MACP.

P1-20 AOAC Assessment Program of Real-time PCR Method for *Salmonella* Detection in Cocoa and Chocolate Products after Post-enrichment Pooling on Large Test Portions

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Introduction: With a water activity below 0.85, low-moisture foods are often considered as low-risk products regarding microbial contamination. However, *Salmonella* is very well-known for being able to survive in dry conditions in cocoa and chocolate products. Proper monitoring should

be ensured while current practices on low-moisture food increases the sample size combined with an appropriate sampling plan and pooling.

Purpose: A workflow combining PATHATRIX[®] 10-pooling *Salmonella* spp. kit with MicroSEQ[™] *Salmonella* spp. Real Time PCR assay (candidate workflow) was evaluated through an AOAC-PTM matrix study. The FDA/BAM Chapter 5 was used as Reference Method.

Methods: Four challenging matrices were tested: cocoa powder, cocoa butter, cocoa liquor and > 70% dark chocolate. Two contaminations were run with bulk inoculation: one low level to achieve fractional recovery for 20 test portions, and one higher inoculation level for 5 test positions. Non-inoculated test portions were included as well. Large sample sizes of 375 g were tested. Two enrichment procedures were used for the candidate method: pre-warmed non-fat dried milk and pre-warmed BPW. Post-enrichment pooling was run combining one inoculated test portion from the inoculated bulk with four non-inoculated test portions using the PATHATRIX Auto Instrument prior to RT-PCR analyses. The FDA/BAM Chapter 5 method was performed on single samples of 25 g.

Results: According to the probability of detection (POD) calculations, there were no statistically significant differences between the number of positive samples detected by the candidate workflow and the reference methods for all four matrices.

Significance: PATHATRIX combined with MicroSEQ Real Time PCR enables sensitive detection of *Salmonella* after post-enrichment pooling of 5 test portions of 375 g cocoa and chocolate products within one day. Positive PCR data can be easily confirmed by direct streaking onto Thermo Scientific[™] *Brilliance*[™] *Salmonella* or XLD Agars.

P1-21 ISO 16140-2:2016 Validation Study of a Real-time PCR Workflow for *Salmonella* Detection in Large Test Portions of Cocoa and Chocolate Products

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Introduction: Although pathogens can't grow in low-moisture foods, they can survive in this environment for months, if not years! The survival ability of several *Salmonella* in cocoa and chocolate products is very well known. Proper sampling plans should be developed when utilizing dry pooling methodology in large composite test portions.

Purpose: The purpose of this study was to evaluate the Thermo Scientific[™] SureTect[™] *Salmonella* PCR Assay (candidate method) for detection of *Salmonella* from cocoa and chocolate products according to the ISO 16140-2:2016 technical rules.

Methods: Large sample sizes of 375 g were tested for the candidate method, with two enrichment procedures: the usual ISO 6887-4:2017 protocol in non-fat dry milk or UHT milk, and pre-warmed BPW. The candidate method was compared to the ISO 6579:2017 reference method within a paired and an unpaired data study. A sensitivity study was run on 81 samples of raw materials (e.g., cocoa beans, cocoa butter, cocoa liquor), cocoa powders and chocolate samples from various origins collected in the US and in Europe. The Relative Limit of Detection (RLOD) was evaluated on cocoa powder.

Results: The sensitivity and the RLOD studies both demonstrated that there were no statistically significant differences between the candidate method and the reference method. The data interpretations met the Acceptability Limits for all the tested conditions.

Significance: The Thermo Scientific SureTect *Salmonella* PCR Assay enabled sensitive detection of *Salmonella* from 375-g test portions of cocoa and chocolate products within one day. Flexibility to end users is offered with two possible enrichment procedures. All the positive PCR data was easily confirmed by directly streaking onto Thermo Scientific[™] *Brilliance*[™] *Salmonella* or XLD agars.

P1-22 A Multiplex Real-time PCR Kit for the Detection of Food-relevant *Listeria* Species and Identification of *Listeria monocytogenes* in a Single Reaction

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Introduction: *Listeria monocytogenes* is considered to be one of the most important foodborne pathogens. It can lead to severe illness, including meningoencephalitis, and abortion, with mortality rates up to 33%. Infections have been traced to the consumption of contaminated foods that have relatively short shelf lives, emphasizing the need for rapid detection methods.

Purpose: *L. monocytogenes* is often found in samples that contain other *Listeria* spp. Therefore, the detection of *Listeria* species is used as an indicator for the presence of *L. monocytogenes*. The purpose was to develop a method that detects both in one test, *Listeria sensu stricto* species and *Listeria monocytogenes*.

Methods: As such, BIOTECON Diagnostics has developed the foodproof *Listeria* plus *L. monocytogenes* Detection LyoKit – a rapid, accurate and sensitive qPCR method for the simultaneous detection of food-relevant *Listeria* species and the specific identification of pathogenic *L. monocytogenes* in a single reaction.

Results: To shorten the enrichment time, we have internally validated different rapid enrichment broths for *Listeria*. These broths enable the safe detection of *Listeria monocytogenes* and the food-relevant *Listeria* species in less than 24 h. Depending on the throughput, the method can be used with different foodproof DNA extraction procedures for both manual and fully automated DNA extraction. All kits, including the rapid enrichment broths and the ISO reference broth, half-Fraser, are currently in the process of being validated according to ISO 16140 by NordVal. This validation is being carried out with a broad variety of food categories including dairy, meats, vegetables, fish, frozen as well as ready-to-eat foods and environmental samples.

Significance: This is the first lyophilized qPCR kit on the market that is able to detect *Listeria monocytogenes* and all the other food-relevant *Listeria* species: *L. innocua*, *L. welshimeri*, *L. ivanovii*, *L. seeligeri*, and *L. marthii* in a single reaction.

P1-23 Workflow Evaluation for a Detection Method of SARS-CoV-2 from Environmental Surfaces

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Introduction: Food processing plants have been confirmed as the source for a number of SARS-CoV-2 outbreaks in 2020 and the virus has been shown to remain viable on surfaces for extended periods of time. This supports the theory that surface-to-human transmission may be occurring in the factory setting.

Purpose: Emergency validation according to the AOAC PTM program was conducted to evaluate performance of the Thermo Scientific™ Real-time PCR Detection of SARS-CoV-2 on Food Packaging and Environmental Surfaces Assay (candidate method) against the U.S. Centers for Disease Control and Prevention 2019-Novel Coronavirus (2019-nCoV) RT-PCR Diagnostic Panel (reference method).

Methods: Inclusivity and exclusivity studies were performed via *in silico* analysis using the GISAID and GenBank Viral NCBI databases by comparison to 15,764 SARS-CoV-2 sequences and 65 exclusivity organisms. An unpaired study comparing the probability of detection (POD) of the candidate and reference methods was conducted by contaminating 2x2 inch areas of stainless steel with SARS-CoV-2. A total of five areas were contaminated with a high level (POD = 1), twenty with a low level (POD = 0.5) and five were uncontaminated (POD = 0) per method. Samples were transported from the site of sample preparation to the site of analysis and testing completed within 24 hours, replicating true sample handling conditions. The candidate method workflow is compatible with two different extraction instruments and PCR thermal cyclers; all combinations were evaluated in this study.

Results: The candidate method achieved 99% inclusivity and 100% exclusivity following *in silico* analysis with the GISAID and GenBank Viral NCBI databases. All combinations of instrumentation for the candidate method showed comparable detection to the reference method during the POD analysis.

Significance: The candidate method demonstrated a specific and sensitive option for SAR-CoV-2 detection from environmental surfaces. This enables end-users to employ appropriate hygiene intervention strategies minimize contamination risks and factory shutdowns.

P1-24 Identification of Soya, Maize, and Rapeseed Taxon in Food and Feed GMO Samples

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Introduction: Screening for transgenic regulatory elements in food and feed samples is the standard when testing for the presence or absence of genetically modified (GM) plants. Knowing which plant taxon is present drastically speeds up the subsequent identification/quantification of the specific GMO event in the sample. Moreover, it removes any risk of confusion associated with botanical impurities.

Purpose: To develop multiplex real-time PCR plant taxon screening and identification assay, that covers the most common GMO-containing plants (soya, maize and rapeseed), to reduce time, effort and cost of GMO analysis. To design an automated solution at a low cost to replace the time-consuming column-based approach.

Methods: As an automation solution, the foodproof[®] Magnetic Preparation Kit III was used to extract DNA. The foodproof[®] Plant Taxon Screening LyoKit, able to detect soya, maize and rapeseed in one single PCR reaction, was tested for inclusivity, exclusivity and limit of detection. Additionally, new multiplex GMO Soya and Maize identification assays complement the GMO screening and identification workflow. The assays comply with ISO 21570 and JRC methods. An internal amplification control is included.

Results: Specificity was verified against different modified and non-modified plants. Twenty-seven different matrices were tested successfully, including vegetable burgers, flour products and cream cheese. The LOD for the assays is 0.2 target copies/μL. Even in competing matrices, the target could be detected, e.g., 0.1% soya flour in 99.9% corn flour.

Significance: The flexible GMO LyoKit screening and identification assays offer an easy and cost-effective approach for analyzing genetically modified foods.

P1-25 Validation of the New Genedisc[®] Method for the Combo Detection of *Campylobacter* and *Salmonella* in Chicken Neck Skin Samples

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Introduction: *Campylobacter* and *Salmonella*, the two leading sources of foodborne illness, are associated with the consumption of poultry products. Hazard analysis critical control point (HACCP) programs for food processing have been initiated in poultry processing plants to reduce the risk of foodborne illness. In that context, Pall GeneDisc Technologies proposes a new real-time PCR-based method enabling to simultaneously monitor both pathogens, with presumptive results available in less than 24 h.

Purpose: This new method was validated on a range of chicken neck skin samples.

Methods: Seventy-seven chicken neck skin samples issued from poultry industry were analyzed by both the GeneDisc method and the ISO reference methods (ISO 10272-2:2017 & ISO 6579-1:2017). For the GeneDisc method, the samples were enriched for 20-24 h, then, a one-step DNA extraction was carried out before PCR analysis with the GeneDisc plate *Campylobacter* & *Salmonella*. All samples were confirmed by plating on chromogenic media.

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Results: Results highlighted that the GeneDisc method gave equivalent results to the culture methods for both targets, i.e., *Campylobacter* and *Salmonella*. Moreover, no impact on the GeneDisc method performance was observed according to the sample type (fresh or frozen sample), demonstrating the robustness of the method.

Significance: The GeneDisc *Campylobacter* & *Salmonella* offers greater efficiency and flexibility when compared to the reference methods, with a 20-24 h single-step enrichment combined with common sample preparation and common PCR analysis, for monitoring both key pathogens in poultry plants.

P1-26 Collaborative Study to Evaluate the Use of Next Generation Sequencing for Food Authenticity

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Introduction: The introduction of NGS into the food sector revolutionizes food authenticity testing. NGS enables accurate detection and differentiation of thousands of different species in each sample using DNA sequencing that is recognized as the most reliable method for species identification. However, NGS is new to the food sector and there is a need for validation and recognition of the method.

Purpose: A collaborative study with several European laboratories was organized to evaluate the complete Thermo Fisher food authenticity workflow in a broad range of food samples. The selected species groups were meat, fish, and plants.

Methods: The food samples include both real samples and control samples prepared with mixtures of DNAs from several meat, fish, and plant species. The real food samples were selected based on the type of processing treatment used for their production. This included canned, dry, raw, liquid, and frozen food samples. A total of 72 samples were analyzed by each participant. The complete workflow tested include DNA extraction, library preparation with the SGS Allspecies ID kits, NGS run using the ION CHEF and S5 GeneStudio instruments and sequence data analysis with the SGS AllspeciesID software providing species identification.

Results: The results obtained by each participant are compared with the expected result and conclusions are taken based on the number of species identified for each meat, fish and plant sample. Additionally, the processing treatment of each food sample and its impact on the species identification result is also evaluated. The databases used for species identification were also evaluated to ensure a reliable identification result.

Significance: This is the first automated NGS workflow tested for food authenticity in a collaborative study. Like metagenomics analysis for microbe profiling, NGS can be used to generate a species profile of the meat, fish or plant content of a food product.

Meat, Poultry and Eggs

P1-27 Detection of Minced Beef Adulteration by Means of UV-VIS Spectrometer

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Introduction: Food fraud has been an emerging food protection issue, with minced meat being a rather vulnerable to adulteration food commodity. Specifically, intentional substitution of minced meat with different (than the one claimed) animal species/tissues can be practiced for economic gain. Hence, the rapid detection of minced meat adulteration is a major priority in terms of food protection.

Purpose: This study was conducted in order to assess the potential of spectroscopic methods coupled with multivariate data analysis to detect minced beef adulteration with beef offal.

Methods: Beef and offal (bovine hearts), purchased from four different butcher shops, were minced and appropriate portions of the two tissue types were mixed so as different adulteration levels (25, 50 and 75%) to be attained, whereas pure meat types (100% beef and 100% offal) also were studied. Six different samples were prepared for each one of the abovementioned levels. In total, 120 samples (5 levels × 6 samples × 4 batches) were prepared and spectral data were acquired. The used UV-VIS sensor (spectrometer Hamamatsu C12880MA) was employed for visible range spectroscopy (VIS) and for fluorescence signal (FLUO) detection. The data corresponding to three batches were used for model training and the data of the fourth batch for testing (external validation). Samples were classified as (i) pure beef, (ii) pure offal or (iii) adulterated using Partial Least Square Discriminant Analysis (PLSDA) and Support Vector Machines (SVM).

Results: The overall correct classification (OCC) of the test set samples using SVM for the VIS and FLUO data was 80 and 70%, respectively. Application of PLSDA yielded lower OCC, namely 77% for VIS and 53% for FLUO.

Significance: Spectroscopic data (VIS, FLUO) coupled with appropriate algorithms exhibit promising potential for the rapid detection of adulterated minced beef samples.

This work has been partially funded from the HORIZON 861915 EU project with the acronym DiTECT.

P1-28 Changes in the Diversity of Microbiota of Chicken Breast and Thigh Fillets during Shelf Life at Different Storage Temperatures Monitored Using Next Generation Sequencing

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Introduction: Microbial communities associated with chicken meat are important for safety and quality. Next generation sequencing (NGS) analysis may provide insight into the chicken-associated microbiome during shelf life.

Purpose: To monitor changes of microbial communities on chicken breast and thigh fillets stored aerobically at 0, 5 and 10°C for 5 days using NGS.

Methods: Microbiome sequencing of bacterial DNA extracted from samples was performed using Ion Torrent PGM targeting the V2-4-8 and V3-7-9 hypervariable regions of 16S rRNA gene.

Results: *Proteobacteria* and *Firmicutes* were the dominant phyla across samples. Fresh chicken breast and thigh fillets were characterized by a complex bacterial structure that composed of 96 and 65 families, respectively. Among them, *Comamonadaceae*, unclassified Burkholderiales, *Moraxellaceae*, *Pseudomonadaceae*, *Bradyrhizobiaceae* and *Flavobacteriaceae* accounted for ca. 63% of the entire families on breast, while *Moraxellaceae* and *Pseudomonadaceae* for ca. 69% on thigh. The taxonomical structures of bacterial families were less diverse after 5 days of storage at all temperatures and on chicken thigh compared to breast. The core microbiota of chicken breast was dominated by *Vibrionaceae*, *Listeriaceae* and *Pseudomonadaceae* (abundances up to 79%, 27% and 7%, respectively, depending on temperature). Conversely, chicken thigh samples core microbiota composed mostly of *Moraxellaceae*, *Listeriaceae*, *Carnobacteriaceae*, *Shewanellaceae* and *Vibrionaceae* (up to 35%, 24%, 5%, 17% and 46%, respectively). At genus level, *Pseudomonas* and *Brochothrix*, the two specific spoilage organisms of meat, were present across most samples at abundances of up to 19% and 27%, respectively. Surprisingly, *Photobacterium* predominated towards the end of shelf life (8%-48%), despite its minor (<1%) initial presence.

Significance: Results provide in-depth information on the biodiversity of chicken-associated bacterial communities that may be responsible for spoilage or safety.

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P1-29 Spoilage of Chicken Liver at Refrigerated Temperatures and Fate of Inoculated *Salmonella* Enteritidis

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Introduction: Spoilage of chicken liver has received little attention in comparison with other types of poultry meat despite the fact that it has been recognized as a vehicle of foodborne diseases including salmonellosis.

Purpose: To monitor the spoilage of chicken liver and examine the survival/growth of inoculated *Salmonella* Enteritidis at aerobic storage at chill (0, 4°C) and abuse (8°C) temperatures.

Methods: Liver samples (50 ± 2 g) of two batches were inoculated with ca. 3 log CFU/g of 4-strain inoculum of *Salmonella* Enteritidis and stored aerobically under isothermal (0, 4 and 8°C) and dynamic temperature conditions (0, 4, 8°C/8 h). Non-inoculated (control) samples were also included. The dynamics of total viable counts (TVC), pseudomonads, *Brochothrix thermosphacta*, lactic acid bacteria (LAB), *Enterobacteriaceae*, yeasts and *Salmonella* Enteritidis were determined by microbiological analyses ($n = 4$) and the pH values were also measured.

Results: Chill isothermal temperatures (0, 4°C) did not favor the growth of *Salmonella*, which managed to increase substantially only at 4°C after 96 h, reaching 4.5 ± 0.2 log CFU/g at the end of storage. At 8°C, the pathogen increased after 44 h reaching a final population of 5.0 ± 0.2 log CFU/g. At dynamic conditions *Salmonella* levels remained practically unaffected during aerobic storage for 186 h. The dynamics of microbial spoilage showed that, *Pseudomonas* spp. was the dominant group followed by *Brochothrix thermosphacta*, LAB, *Enterobacteriaceae* and yeasts at both isothermal and dynamic conditions. The initial pH of inoculated samples was 6.48 ± 0.08 log CFU/g and exhibited minor decreases ranging from 0.09 to 0.27 depending on the temperature.

Significance: The results highlight the significance of cold chain and good hygiene maintenance in poultry plants since even low pathogen populations may survive during cold storage.

Acknowledgment: The project QAPP: T1EDK-04344 is co-financed by the EU and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, RESEARCH - CREATE - INNOVATE.

P1-30 Identification of *Salmonella* spp. and Differentiation between Enteritidis and Typhimurium in One Real-time PCR Test

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Introduction: Food products contaminated with *S. Enteritidis* (SE) and *S. Typhimurium* (STM) in 2018 are still the largest source of *Salmonella* outbreaks in the EU. A fast and easy diagnostic tool for detecting the *Salmonella* genus, in particular SE and STM, in food as well as in primary production places is essential to minimize the damage to humans and the food or veterinary markets. Being able to react quickly to salmonellosis outbreaks is difficult with ISO 6579, where the identification process takes up to five days.

Purpose: To validate a rapid tetraplex PCR *foodproof*[®] *Salmonella* Genus plus SE & STM Detection LyoKit with regard to specificity, sensitivity and matrix compatibility.

Methods: The *foodproof*[®] *Salmonella* Genus plus SE & STM Detection LyoKit was analyzed for specificity with 380 *Salmonella* spp. strains including *Salmonella bongori* and *enterica* including all five subspecies, 63 Enteritidis strains, 59 mono/biphasic Typhimurium and 30 non-target strains. Sensitivity was determined with purified DNA and in combination with different *foodproof*[®] DNA isolation kits. 10^3 - 10^5 CFU/mL SE and STM, blended with an excess of background flora, was analyzed. Matrix compatibility was tested with different food and primary production samples. One hundred naturally contaminated samples were examined after 16 h of BPW enrichment in comparison to ISO 6579.

Results: Specificity results showed 100% success for inclusivity and exclusivity. The kit detected 1 GE and 10^2 CFU/mL of *Salmonella* genus, SE and STM. With strain blending, 10^3 CFU/mL could be correctly analyzed. All tested matrices are compatible with the *foodproof*[®] *Salmonella* Genus plus SE & STM Detection LyoKit. Naturally contaminated samples were tested with comparable accuracy to ISO 6579.

Significance: The new *foodproof*[®] *Salmonella* Genus plus SE & STM Detection LyoKit measured up successfully with ISO 6579, achieving comparable specificity, sensitivity and matrix compatibility.

P1-31 Animal Species Detection in Meat and Processed Food: Identification of Pork, Beef, Horse, Donkey, and Zebra Meat in One Single Real-time PCR Reaction

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Introduction: Animal species testing is crucial for product quality control, whether due to food adulteration or because of religious requirements, like halal or kosher food. The detection, identification and quantification of potential contaminations in processed and ready-to-eat food, milk powder, vegetarian food and pharmaceutical products has become increasingly important.

Purpose: The development of a multiplex qPCR method, which identifies pork (*Sus scrofa*), beef (*Bos taurus*, *Bos indicus*) and *Equidae* (horse, donkey, zebra) in one PCR test. A rapid extraction workflow for DNA isolation carried out in a single tube for minimizing handling steps and contamination risks. This single tube DNA extraction is more convenient, faster and cheaper than regular column-based methods. Together with a reference material an estimation of the content can be achieved.

Methods: For DNA extraction, the rapid extraction buffer *foodproof*[®] StarPrep Five was compared with silica-based DNA extraction methods. The *foodproof*[®] Animal Detection 1 LyoKit was designed to detect porcine, bovine and equine DNA in specific channels and in one multiplex PCR reaction. A differentiation of equine was done by melting curve analysis, to distinguish between horse, donkey and zebra. For sensitivity testing, a reference material (extracted DNA of 1.0%, 0.1%, 0.01% pork, beef and horse in chicken meat) was used.

Results: Inclusivity and exclusivity was tested to be 100%. The detection limit of amplification and melting curve for spiked matrices is at least 0.001% for different matrices like raw and processed meat. Milk powder, gelatin or pharmaceutical products can also be analyzed with a high sensitivity. The detection limit with *foodproof*[®] StarPrep Five Kit is comparable to column-based DNA isolation methods.

Significance: This method is not only convenient, precise and additionally saves time, it also cuts monitoring costs because of its multiplex aspect and single tube DNA isolation.

Sanitation and Hygiene

P1-32 Temperature and Hygiene Conditions of Consumers' Refrigerators in Slovenia

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Introduction: In today's modern society, cold storage is one of the most widely used methods of preserving perishable foods, both from the point of view of food safety and food quality.

Purpose: The purpose of this study is to gain insight into refrigeration temperatures in relation to refrigerator and household characteristics that could potentially affect refrigeration temperature.

Methods: Households were recruited through "snowball" sampling method. During a 24-h period, the test product's internal temperature, refrigerator air temperature, and ambient air temperature were measured at 15-min intervals using a data logger. The internal temperature of the test product was measured using the prepared "Karlsruhe Test Material." Refrigerator and household characteristics were recorded using a predefined observation sheet and a short structured questionnaire.

Results: A total of 50 households and their refrigerators were included. The overall arithmetic mean of the internal temperature of the test product was 5.95°C (SD = 2.24). Temperature displays were present in 16%, while control thermometers were not observed at all; 20% of refrigerators allowed a 24-h average internal temperature of less than 4°C, 30% between 4 and 6°C, and 50% above 6°C. Refrigerator age, type, and load had noticeable but no significant effect, suggesting that thermostat setting is a key factor influencing refrigerator temperatures. The distribution of food in the refrigerators was related to the loading of the refrigerator, with a significant risk of cross-contamination in overcrowded refrigerators.

Significance: High temperatures combined with unsystematic distribution of food in the refrigerator, expired expiration dates, and unsystematic cleaning strategies create favourable conditions for foodborne infections at the end of the food supply chain.

P1-33 Survey of Bacterial Pathogens at Spanish Small-scale Factories of Traditional Fermented Sausages

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Introduction: Fermented sausages are ready-to-eat products that generally do not allow the growth of pathogenic bacteria, due to their physicochemical characteristics and the presence of curing agents. However, once introduced into processing environments through raw materials, pathogens can persist and form biofilms on food-contact surfaces (FCS) or/and overcome the production processes of these products.

Purpose: The aim of this study was to investigate the prevalence of *Listeria monocytogenes* and *Staphylococcus aureus* at small-scale Spanish factories producing traditional fermented sausages.

Methods: Two different factories, namely A and B, participated in the survey, which was performed in 3 different days. Samples of raw meat ($n = 5$) and Iberian raw-cured sausage 'Salchichón' ($n = 5$) were microbiologically analysed by using ISO methods. A total of 12 FCS at the factories were also analysed. Presumptive colonies of both pathogens were further submitted to identification by MALDI-TOF.

Results: *L. monocytogenes* was detected in 13% of *Salchichón* samples produced by factory A. In factory B, the pathogen was detected in 3 FCS, namely in cutting

and stuffing machines and on drying hooks and shelves. Moreover, 26% of raw meat samples and at least 13% of *Salchichón* samples from factory B were positive for *L. monocytogenes*. Regarding the presence of *S. aureus*, 33% of raw meat samples from factory A and at least 20% of *Salchichón* samples from factory B were found to be positive. *S. aureus* was not detected in FCS from both factories A and B.

Significance: The results highlight differences in the hygienic-sanitary conditions between the factories evaluated, being factory B a major concern regarding the presence of *L. monocytogenes*. Overall, the presence of both pathogens surveyed in raw materials and final products and the presence of *L. monocytogenes* in FCS indicate that control measures must be taken by the participating factories to avoid the exposure of consumers to bacterial pathogens.

P1-34 A Microbiological Survey of Protein Shaker Bottles and Self-reported Cleaning Practices

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Introduction: The "sport-related" protein-product sector is booming and the popularity of protein shakes has increased. The preparation of protein shakes requires the reconstitution of protein powder in a specifically designed 'shaker bottle.' Indeed, the protein shaker bottle has become the ultimate fashion accessory for many professional and recreational athletes. Despite previous studies exploring microbial contamination of bottles used for reconstituting powdered infant feeding formula, no studies have explored the potential food safety risks associated with popular shaker bottles.

Purpose: Determine microbiological contamination of shaker bottles and self-reported cleaning practices.

Methods: An intercept survey was conducted with recreational athletes leaving a gym carrying a shaker bottle ($n = 18$). The survey captured self-reported usage/cleaning practices and conducted microbiological analysis of 'in-use' shaker bottles.

Results: The reported usage of shaker bottles ranged <1 month to >36 months. Reported replacement varied from "every few months" to "two to four years," this was normally as a result of damage or loss. All participants reported that shaker bottles were cleaned before use; however, details regarding the adequacy of cleaning practices were lacking. The majority of participants reported protein powder was reconstituted after training and drank immediately; however, 28% reported advanced preparation (<12 hours). Microbiological analysis determined 94% of bottles were contaminated with microbiological colonies (average 5.0×10^7 recovered CFU/mL). *Enterobacteriaceae* were not isolated in any shaker bottles. Methicillin-resistant *Staphylococcus aureus* was detected in a 'clean, in-use' shaker bottle that had reportedly been "left in a car for a couple of days."

Significance: Self-reported practices may indicate inadequate cleaning, preparation and storage of protein drink shaker bottles, which may have contributed to high level of microbiological contamination. Observed behavioral data regarding the cleaning of shaker bottles is required to complement these self-reported data. Furthermore, there is a need to explore the perceived risks associated with shaker bottles use among professional and recreational athletes.

P1-35 Use of Surrogate Bacteria for Validation and Verification of Chemical Sanitation Steps

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Introduction: Use of surrogate bacteria for process validation and verification is a well-established methodology to estimate the lethality of industrial processes against

pathogenic microorganisms. To answer food safety issues, Novolyze has developed a range of bacterial surrogates and continuously performs intensive R&D trials to confirm the compatibility of its surrogates for new applications. Among these, use of chemical treatments for sanitation of vegetables and surfaces is an emerging application to ensure safety of fresh products as well as of environmental surfaces.

Purpose: The objective of the study was to evaluate the appropriateness of surrogate microorganisms to validate chemical treatments in oakleaf lettuce leaves and stainless steel (SST) surfaces.

Methods: Oakleaf lettuce leaves and SST pieces were independently inoculated with *Listeria monocytogenes* or a dry ready-to-use surrogate preparation derived from *Enterococcus faecium*. Inoculated lettuce leaves were immersed for 15 minutes in a 0.1% chlorine bath, while SST pieces were immersed for 15 minutes in either a 0.1% chlorine bath or a 0.01% peracetic acid (PAA) bath. After treatment, lettuce leaves and SST pieces were immersed in a neutralization solution to inactivate the chemical agents. Samples were then collected and enumerated using nonselective medium to estimate the microbial lethality of the process.

Results: In the conditions of the test, surrogate preparation seemed to be more resistant than *Listeria monocytogenes* after 15 minutes of chlorine treatment in oakleaf lettuce. Regarding results obtained on SST pieces, surrogate preparation showed also a higher resistance than *Listeria monocytogenes* within the 15 minutes of treatment, for both treatments tested.

Significance: These data show promising results for using surrogate methodology to validate and verify lethality of chemical sanitation steps against pathogenic microorganisms, either on vegetables or environmental surfaces. Further tests should be conducted on a large range of matrices/surfaces to confirm these findings.

P1-36 Identifying Barriers Toward Optimal Cleaning and Sanitation Practices in a Small- and Medium-sized Enterprise (SME) Food Manufacturer

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Introduction: Cleaning/sanitation practices in the food industry have been traditionally improved through technological advancements focusing on better hygienic design. This can be expensive and not affordable to SMEs. Incorporating elements of cleaning and sanitation optimization into the culture of the company may be a more cost-effective, feasible and effectual alternative for manufacturers.

Purpose: To explore and identify barriers associated with management and food-handlers behaviors related to cleaning and sanitation practices in a high-risk food manufacturing company.

Methods: Semi-structured, in-depth interviews ($n = 13$) captured qualitative data related to SME food-handler and management knowledge, attitudes, beliefs, and self-reported practices in relation to the cleaning and sanitation practices. The sample included staff directly or indirectly involved in company cleaning activities. Interviews were transcribed and coded using NVivo (QSR-International, 2018) and analysed using theoretical thematic analysis.

Results: Overall, respondents had a positive attitude about the current cleaning and sanitation practices and indicated the belief that the cleaning in the company is "good." Respondents indicated awareness of microbiological/allergen risks associated with improper cleaning and sanitation. The most common potential barriers that emerged related to negative attitudes expressed towards improvement of current practices "what we are doing now works." A common belief among food-handlers that "common sense should prevail" over the written cleaning instruction cards

was indicated and not consulting written cleaning procedures deemed acceptable as "we have the procedures burned on our retinas." Some of the respondents reported they work as "a big family" in the business, and that there is a "sense of pride" in doing a good job. Further barriers related to communication between departments have been identified in the context of management and food-handler perception of risk, control and responsibilities.

Significance: Findings from this study will inform the development of bespoke, targeted interventions to correct behaviors associated with the identified barriers.

Seafood

P1-37 Occurrence of Histamine in Canned Fish Available in Poland and Changes of Histamine Contents during Their Production

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Introduction: Histamine is a heterocyclic biogenic amine naturally occurring in the human body which is derived from decarboxylation of the amino acid histidine. Formation of histamine in fish is related to free histidine content in fish muscle and the presence of bacterial histidine decarboxylases produced by microorganisms in certain environmental conditions. The criteria for histamine are laid down in Commission Regulation No. 2073/2005 only for fishery products from fish species associated with a high amount of histidine.

Purpose: The aim of this study was to determine the level of histamine contamination in all species of canned fish available on the Polish market.

Methods: Histamine was determined by high performance liquid chromatography with diode array detection (HPLC-DAD), accordance with the accredited procedure PIWet-PIB, ZHZ/PB-16 "Determination of histamine in fish and fish products by HPLC". Histamine was extracted from fish samples by 0.2 M trichloroacetic acid. Extract was purified on SPE-column (Strata X-AW Polymeric Weak Anion). Separation was performed on reversed phase C18 column with the mobile phase comprising 0.1 M phosphate buffer with 1.6 mM sodium octane sulfonate + methanol (82:18; v/v). LOQ 3.3 mg/kg, LOD 1.3 mg/kg.

Results: In our research a total of 59 samples of different canned fish were investigated. In 16 (27.12%) samples of fish cans the concentration of histamine ranged 3.38 – 34.77 mg/kg. Maximum level of histamine was in "Sprats in oil." In canned herrings (11 samples) were free of histamine. For 16 series of production process of canned fish in 4 samples of raw material (fish) histamine concentration ranged from 4.96 to 177.93 mg/kg. Higher level of histamine was found in canned cod liver. Seven samples of final product (preserve) contain histamine in range 4.63 – 34.77 mg/kg.

Significance: This study showed that canned fish available at Polish markets are safe for consumers. No correlation between raw material and final product.

P1-38 Investigation of the Microbiological Quality of Edible Marine Algae, *Alaria esculenta*, Originated from Ireland

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Introduction: Seaweeds consumption has gained wide consumer interest in recent years. They are usually marketed fresh or dried and consumed without any further cooking or processing apart from a rehydration process. Despite their increasing popularity, there is still a lack of information regarding the microbiological quality of these products.

* Student Award Competitor

Purpose: The investigation of culturable microbial populations present in fresh, dried and rehydrated *Alaria esculenta* obtained from Ireland, throughout storage at different temperature conditions.

Methods: Fresh, dried and rehydrated samples were separated into 50-g portions and stored at 5, and 15°C for 10 days, 15 and 25°C for 30 days and 4 and 12°C for 6 days, respectively. Microbiological and nutritional (proteins, fat, carbohydrates, ash, moisture content) analyses were performed at the day of arrival at the lab and at certain days of storage for the estimation of Total Viable Counts (TVC), *Pseudomonas* spp., Lactic acid bacteria, *Enterobacteriaceae*, *Bacillus* spp., *Vibrio* spp., *Aeromonas* spp., Yeast and Molds, *E. coli*, *Salmonella* and *Staphylococcus* spp.

Results: The initial microbial load in fresh, dried and rehydrated products was 6.0, 7.0 and 4.0 log CFU/g, respectively. Regarding dried samples, a 3.0-log unit decrease was observed after storage at 25°C for 30 days, while in fresh samples, the population reached the level of 8.0 log CFU/g at day 8. The microbial population in rehydrated samples reached the level of 7.0 log CFU/g after 6 days of storage. The initial high population of *Enterobacteriaceae* in dried products decreased below the enumeration limit after 30 days of storage.

Significance: Information about the microbiological quality of such products could be useful for the aquaculture industry to optimize the processing and produce safe and high quality edible marine algae.

Acknowledgment: This work has been supported by project "IMPAQT" (EU H2020 research and innovation programme under Grant Agreement No 774109).

P1-39 Microbiological Quality Assessment of Seaweed *Alaria esculenta* Originated from Scotland and Harvested in Two Different Years

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Introduction: Seaweed consumption has attracted growing interest both in Europe and North America. The microbiological quality assessment of such products is crucial for the optimization of the processing and development of this food sector.

Purpose: To investigate the microbiological quality of fresh, dried and rehydrated *Alaria esculenta* obtained from Scotland throughout storage at different temperature conditions.

Methods: Fresh, dried and rehydrated samples were separated into 50-g portions and stored at 5 and 15°C for 10 days, 25°C for 6 months and 5 and 10°C for 6 days, respectively. Samples were microbiologically analyzed on the day of arrival at the lab and at certain days of storage for the estimation of Total Viable Counts (TVC), *Pseudomonas* spp., Lactic acid bacteria, *Enterobacteriaceae*, *Bacillus* spp., *Vibrio* spp., *Aeromonas* spp., Yeast and Molds, *E. coli*, *Salmonella* and *Staphylococcus* spp.

Results: The initial microbial load in fresh, dried and rehydrated products was 4.0, 7.0 and 5.5 log CFU/g, respectively. In dried samples, a 3.5-log unit decrease was observed after storage at 25°C for 6 months, while in fresh and rehydrated samples, the population increased during storage and reached the level of 8.0 log CFU/g at day 5 and 3, respectively. Regarding the specific spoilage microorganisms, none of the tested ones exceeded the initial level of 3.0 log CFU/g and the level of 6.0 log CFU/g at the end of storage.

Significance: These findings provide useful information since there is limited knowledge on the microbiological quality of edible seaweed.

Acknowledgment: This work has been supported by project "IMPAQT" (EU H2020 research and innovation programme under Grant Agreement No 774109).

Poster Session 2 – Communication Outreach and Education; Epidemiology; Food Processing Technologies; Food Safety Systems; Microbial Food Spoilage; Modeling and Risk Assessment; Molecular Analytics, Genomics and Microbiome; Packaging; Pre-harvest Food Safety; Produce; Retail and Food Service Safety; Viruses and Parasites; Water

Communication Outreach and Education

P2-01 Piloting a Support Program to Enable Small- and Medium-sized Food Manufacturing Businesses in Wales to Obtain Food Safety Certification

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Introduction: To enable growth of the food-sector in Wales, the Welsh Government has recognized there is a need to support small food-manufacturing businesses to obtain food-safety certification. SALSA (Safe and Local Supplier Audit) is a robust and effective food-safety certification scheme appropriate for smaller food-manufacturers, which is seen as a precursor to obtaining more complex, international-certification such as BRC (British Retail Consortium). Previous research has identified the barriers for Welsh businesses to obtain food-safety certification as "knowledge and skills;" "time, cost and resources;" and "access to information." Consequently, the Welsh Government wants to determine the most appropriate way to support food-manufacturers/processors in Wales to overcome barriers and obtain food-safety certification.

Purpose: To design, develop and pilot a bespoke support-program for small food-manufacturing businesses in Wales to obtain food-safety certification.

Methods: A support-program was designed to overcome identified barriers by addressing three areas: "knowledge and skills development," "accessing financial support," and "improving information and communication." The program consisted of six support-mechanisms: (i) self-assessment tool, (ii) internal systems review, (iii) "audit-ready" workshop, (iv) pre-audit factory inspection, (v) post-audit support, and (vi) audit-fee contribution. Welsh food-manufacturers ($n = 62$) expressed an interest in joining the support-program; eligible businesses ($n = 9$) joined the program.

Results: Participation in the support-program took businesses 4 to 10 months to complete. Two-thirds of the businesses completed the program ($n = 6$). Knowledge of the SALSA scheme and attitudes towards food-safety certification increased significantly ($P < 0.05$) following each support-mechanism. Seven companies underwent the official SALSA audit; however, only those that had completed the support-package program ($n = 6$) obtained SALSA certification.

Significance: This pilot study has successfully designed, developed, delivered and evaluated a support-program that has resulted in 100% of small food-manufacturing businesses that completed the support-program obtaining food-safety certification. Launch of the support-program will assist to accelerate food-industry sector growth in-line with Welsh Government aspirations.

P2-02 Attitudes Related to Food Safety Behavior Among Students in Sweden

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Introduction: It has earlier been shown through an online questionnaire performed on 606 students from 24 different Swedish universities that the foremost sources of food safety knowledge were family and friends. However, more than a third of the students had experienced food safety education which was shown to provide knowledge and promoted more optimal food safety behavior.

Purpose: Self-reported food safety attitudes, knowledge and behavior among university students in Sweden were investigated through multivariate path analysis in order to identify factors' influence on behavior.

Methods: A nationwide web-based questionnaire targeting university students in Sweden was distributed through social media, email and various university contacts. A structural equation model was applied on statistics from the questionnaire. Four factors: Background, Knowledge, Attitude and Behavior were derived from the data. The factors, built up from variables with sufficient factor loadings, were set up in a predetermined structure. The structure was based on whether background affects knowledge and whether knowledge affects behavior and attitude, and whether attitude affects behavior. The structure has been confirmed valid in previous studies done.

Results: The factor loadings were ranging from -1 to 1 where the closer to 1 indicates a stronger loading. Background affected Knowledge (0.841). Attitude has a stronger influence on the Behavior (0.457) than Knowledge (0.278). However, Knowledge has directly a strong effect on Attitude (0.606). Out of 606 respondents, 408 answers were deemed usable for the analysis. More than half of the variables have sufficient loadings to their respective factors to be included. The goodness-of-fit indices, indicated that the model had a good fit to the data, and this including hypothesis testing with a significance of < 0.005.

Significance: It can be confirmed that background such as attending a food safety education strongly influenced knowledge. Knowledge in turns strongly affects attitudes but it does not directly affect behavior. Thus, attitudes seemed to have a mediating role between food safety knowledge and behavior.

P2-03 Most Food Control Officers Have Suspected Food Fraud at Inspections

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Introduction: Food fraud is an increasingly recognized challenge in official food control. However, little is known about the occurrence of food fraud and the knowledge food control officers (FCO) have regarding food fraud detection.

Purpose: We conducted this study in order to investigate the experiences and views of Finnish FCOs on food fraud detection at inspections.

Methods: We sent an online questionnaire to Finnish FCOs in all local food control units ($n = 62$) in May 2019. The questionnaire included multiple-choice and open-ended questions on food fraud detection at inspections. A total of 93 FCOs responded to the questionnaire. To ensure anonymity and allow respondents to comment freely, the name of the food control unit was not required. Yet, we received responses from the regions of all six Regional State Administrative Agencies.

Results: In total, 35.3% (30/85) of the respondents had detected one or more food fraud cases within the past five years; however, only 63.3% (19/30) of them had reported the cases to the police. In addition to the detected cases, 65.5% (55/84) of the respondents had suspected food frauds. Only 50% (43/86) of the respondents evaluated that they have realistic chances to detect possible fraudulent activities during inspections and 53.5% (46/86) thought that current inspection guidelines neglect some aspects important to food fraud detection. Furthermore, 25.6% (22/86) reported that if they suspected fraudulent activities in a food establishment, they would not be sure how to proceed.

Significance: Most FCOs had suspected or detected food fraud cases highlighting the scale of the problem. Still, there is a need for more training in order to improve recognition of food frauds at inspections and to respond accordingly.

P2-04 Everyday Risks Every Time We Eat – Global Poll Findings of Perceived and Experienced Risks from Unsafe Foods

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Introduction: To provide the first ever global picture of the differences between people's perception of risk from unsafe food and their experiences of those risks.

Purpose: (1) To provide the first globally comparable, publicly available dataset on public understanding of risk from unsafe food; (2) To provide data where little or no official data exists on perceptions and experience of food-related risk; and (3) To provide insight that aids the development of evidence-based interventions that empower people to take action, that saves lives and helps people feel safer.

Methods: The poll was conducted by Gallup and was based on in-depth, one-to-one qualitative and quantitative interviews, conducted face to face or via telephone, with over 150,000 respondents in 142 countries.

Results: Seventeen percent of poll respondents – equivalent to one billion people worldwide – experienced serious harm, or know someone who experienced serious harm, caused by the food they ate in the two years prior to polling. The greatest levels of harm from food occur in East Africa (29% experienced harm) and the Middle East (27% experienced harm). Over half of the world's population, 60% of people worldwide, say they are worried about the food they eat. However, perception of risk from food is comparatively lower in the countries that experience most harm – highlighting an opportunity for action.

Significance: This is the first globally comparable, publicly available dataset on public understanding of risk from unsafe food. It provides insight that aids the development of evidence-based interventions that empower people to take action, that saves lives and helps people feel safer.

P2-05 A Review of Consumer Food Safety Advice from International Government Agencies

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Introduction: Domestic food handling/preparation by consumers has been associated with sporadic incidence of foodborne illness internationally. Subsequently, governments across the world have developed sources of domestic food safety advice specifically for consumers. To date, there has not been a review of international government-based consumer food safety advice, therefore, very little is known about how these sources relate and compare to each other.

Purpose: To identify commonalities and differences between food safety advice from international government agencies and assess the suitability and adequacy of advice.

Methods: An inclusion/exclusion criterion was utilized to identify online-sources of domestic food safety consumer advice from international government agencies ($n = 14$). A content analysis-approach was utilized to assess and compare sources.

* Student Award Competitor

Results: Food safety practices ($n = 494$) were included in reviewed sources ($n = 14$), in which five key food safety themes (cook, chill, clean, cross-contamination and check) were identified utilizing the content analysis approach. A total of 47 common food safety practices were present in three or more Government sources. The most frequently recommended food safety advice related to the refrigeration of perishable/cooked foods, which was included in the majority of sources. Despite widespread inclusion of practices relating to refrigeration, many failed to include recommended refrigeration temperature to ensure food safety ($5^{\circ}\text{C}/41^{\circ}\text{F}$). The inclusion of guidance detailing cooking was lacking. Terminology changed frequently between Governments with 'use-by' replaced with 'sell-by' and even 'best before' date for food safety. One source suggested consumers should "*always taste and smell products... if the flavour is normal, they are safe to eat*" such guidance may encourage food safety malpractices and increase the risk of foodborne illness to consumers.

Significance: Reviewed consumer food safety advice from international government agency websites had many commonalities regarding food safety advice, however, details to inform consumers 'how' these actions are adequately carried out, or 'why' they are needed differed greatly.

P2-06 Determination of Food Safety and Technical Skills Shortages Challenging the Food and Drink Manufacturing Processing Industry in Wales, UK

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Introduction: In Wales, the Food and Drink Manufacturing/Processing (FDMP) sector contributed £15.5 billion/annum into the economy, employing 22,400 workers. Facilitating and maintaining a technically skilled workforce is required to accelerate business growth and sustainability. Technical-skills gaps and workforce shortages are reported by 40% of businesses and >7900 new food sector workers are required in Wales by 2025. Currently a composite analysis of food sector skills shortage data is limited and such data is required to inform targeted future FDMP employment.

Purpose: The purpose of this study is to determine the food-safety/technical-skills shortages and identify Food-Safety-Management challenges in the FDMP-sector in Wales, UK.

Methods: Quantitative data from three projects were collated via electronic questionnaires ($n = 22$; $n = 34$; $n = 130$) and qualitative data from electronic interviews ($n = 10$), have been assembled to show the true skills-need situation.

Results: Overall, data from this study indicate that the FDMP-sector experiences reported skills gaps and workforce shortages in technical (61%), production (35%), and management (58%) roles ($n = 34$). Skills gaps are evident in food safety third-party accreditation (e.g., supplier approval and raw material handling), traceability, allergens, VACCP and sensory analysis.

Significance: The food sector in Wales is a significant employer, however, a large proportion are low-skilled and associated with low-pay. Data from this composite study draws stakeholder reports to illustrate the critical need for the sector to attract more technical employees and upskill the existing workforce to support and progress sector innovation. Further research is required into the specific needs of FDMP businesses to determine how these challenges can be overcome.

P2-07 The Features of Cabin Crew Food Safety Training: A Content Analysis

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Introduction: To be effective, the features of food safety training should be based on a training needs analysis, job analysis and trainees' characteristics. Food safety training features, including content, type/nature, methods, delivery,

context, certification and recurrence of training may influence potential effectiveness. Substantial research has been conducted on food handler's food safety training in different sectors of catering industry, limited data has been obtained for cabin crew.

Purpose: This study aims to explore and identify the different features of cabin crew food safety training among international airlines.

Methods: In-depth, semi-structured interviews with airline cabin crew managers and trainers ($n = 18$) were conducted; qualitative findings were analysed using a content analysis. A document analysis was undertaken using cabin crew training manuals and training materials; collated data was classified, coded and categorized using NVivo12.

Results: Findings indicated variable approaches to food safety training among different airlines, in some cases with "*no [food safety] training for different cabin crew roles.*" For airlines that did provide cabin crew food safety training, different training methods were reported, such as "*...on-the-job training, classroom with an instructor, individually based computer based.*" Training materials reportedly included "*PowerPoint/handouts, videos, bulletins/newsletters and e-modules.*" Additionally, managers/trainers indicated that, for most of the airlines included in this study, food safety instruction/training of cabin crew occurred during induction training and specifically "*...the training time... was undertaken during the initial training of the service module.*" This was reflected on the source of training as most (83%) airlines depended on "*in-house training.*" Furthermore, such variation is apparent in the content of cabin crew training.

Significance: Different timings, approaches, content and materials used for cabin-crew training may influence potential effectiveness of instruction and awareness of food safety behaviours. Absence of food safety training among cabin crew may result in failure to control on-board food safety hazards required to reduce the risk of foodborne disease.

Epidemiology

P2-08 Seven Year Foodborne Botulism Outbreaks in Northern Italy: Associated Foods and Detected Toxins

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Introduction: *Clostridium botulinum* (*Cb*) is an anaerobic, gram positive, spore-forming bacterium, which can produce botulinum neurotoxins. Foodborne botulism is a potentially fatal illness caused by consumption of food contaminated with neurotoxins produced by *Cb*. Current understanding of the epidemiology of a botulism outbreak relies on limited data from very few reports.

Purpose: The aim of this study was to evaluate the spread of *Cb* in foods in Northern Italy in comparison to the reported cases of possible human botulinum intoxication.

Methods: From 2012 to 2019, a survey study was carried out on 3,964 food samples collected in Northern Italy, using a Real-time PCR method for the detection of *Cb* neurotoxin BoNT(s) genes fragments. In the same years, the Food Control Division of IZSLER also provided support to Northern Italy Hospitals in detecting preformed toxin and *Cb* strains in food or human biological samples. Toxin detection was carried out with the Mouse Test, that requires equipped structures authorized for the use of laboratory animals.

Results: During the survey study, a total of 25/3,964 food samples showed the presence of genes BoNT(s) (6 Meat products, 4 Dairy products, 4 RTE products, 2 Flavorings, 4 Vegetable preserves, 2 Cereal products, 1 Fruit, 1 Spice, 1

Other). Among these, 4 foods were “homemade” products. From 43 events of suspected human botulism: 12 were confirmed and involved 15 persons. The suspected involved foods were the homemade vegetable preserves (in 5 cases), smoked salmon (2 cases), chamomile sachets (1 case), pasta sauces (5 cases) and tuna (1 case). The involved toxins were Type A (in 3 patients), Type B (in 8 patients) and Type E (1 patient). In 2 cases the strains were not typed.

Significance: Type B toxin accounted for the majority of outbreaks, which were due to point exposure following the ingestion of homemade fruit and vegetable preserves.

P2-09 Listeriosis Infection Leading to Uncover Widespread Persistent Contamination in Poultry Meat

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Introduction: On February 7, 2020, a woman of 68 years was admitted to the hospital of Esine (Camonica Valley, Lombardy, Italy) with a stroke diagnosis. A concurring listeriosis infection was diagnosed after isolation of *L. monocytogenes* from bloodstream. The following investigation starting from the patient's home led to track a *L. monocytogenes* contamination not correlated to the clinical case but widespread in several butchereries of the Valley.

Purpose: *L. monocytogenes* presence at the household and in food processing plants was tracked to identify the source of infection and to prevent further cases.

Methods: Sampling was carried out by local health authorities. *L. monocytogenes* was detected by PCR and resulting isolates were typed by MLVA for screening and by cgMLST to confirm possible genomic correlations.

Results: The strain isolated from the patient was identified as ST1. From the household, four different strains were isolated from five different foods, namely: ST1 from soft cheese, ST37 from chicken meat and pork loin, and ST9 from beef and pork chops with two different MLVA profiles. cgMLST identified the ST1 isolates as uncorrelated. The trace-back of ST37 led to detect a widespread contamination on poultry meat in three different poultry butchereries of the valley, each of which also had a concurring persistent environmental contamination by *L. monocytogenes* ST9, ST121, and ST155, respectively. This situation suggests a probable reservoir role of broiler farms for ST37, and specific contaminations in the different processing environments.

Significance: ST37, ST121, and ST155 are responsible for a number of listeriosis cases in the regional territory, and therefore of concern from a public health standpoint. The investigation, although failing to identify the source of infection, led to uncover widespread and persistent *L. monocytogenes* contamination linked to poultry meat. The actions taken subsequently in the processing plants allowed for an improvement in the safety of meat products.

Food Processing Technologies

P2-10 UV-C Technology Optimization to Inactivate *Salmonella* Enteritidis in Soymilk Flavoured with Cocoa and Vanilla

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Introduction: The growing consumption of soymilk and soymilk-based beverages is encouraged by soy's high-quality proteins, essential fatty acids, bioactive compounds and its lack of cholesterol, gluten or lactose. Heat treatment is currently applied for pasteurization of soymilk but it may negatively affect its nutritional and organoleptic quality. To preserve the overall quality of soymilk, the Ultraviolet technology (UV-C) is proposed as a non-thermal pasteurization technology.

Purpose: The aim of this study was to evaluate and optimize the application of UV-C technology to inactivate *Salmonella* Enteritidis in soymilk flavoured with cocoa or vanilla at different processing temperatures.

Methods: UV-C inactivation studies were carried out by placing petri dishes containing 30 mL of inoculated samples (nearly 6 log CFU/mL of *Salmonella*) and magnetic spin bars on top of a magnetic stirrer, located inside of a stainless-steel UV-C chamber equipped with a 9 W lamp that predominantly emits 253.7 nm. Samples containing cocoa (0.5% w/v) or vanilla (0.01% w/v) and control samples were exposed to different doses of radiation (0-10 mJ/cm²) at different temperatures (4-30°C). *Salmonella* counts were determined at different exposure times by count in plate methodology.

Results: *Salmonella* inactivation levels increased with the increase in temperature from 4 to 18°C. The increase in temperature from 18 to 30°C did not influence inactivation levels ($P > 0.05$). Maximum reductions achieved varied between 1.10 and 5.70 log CFU/mL from 4 to 30°C at 10 mJ/cm². The addition of vanilla flavour increased the reductions of *Salmonella* from 4-12°C, while at the other temperatures no additional antimicrobial effect was marked due to the addition of the flavouring agents.

Significance: The data presented in this study may help to optimize the application of UV-C technology to inactivate *Salmonella* in flavoured soymilk by setting appropriate processing conditions.

Food Safety Systems

P2-11 Occurrence of Virulent and Antibiotic-resistant *Staphylococcus* spp. in Ready-to-Eat *Rhynchophorus phoenicis* and *Archachatina marginata* Vended Along the Port Harcourt – Bayelsa Route

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Introduction: In Nigeria, African palm tree weevil larva (*Rhynchophorus phoenicis*) and African land snail meat (mainly *Archachatina marginata*) are regular edible insects and mollusks respectively that are vended by migratory food vendors on the busy roads of Niger Delta states.

Purpose: This study was aimed at determining the occurrence of *Staphylococcus* species in ready-to-eat (RTE) *Rhynchophorus phoenicis* (edible larvae) and *Archachatina marginata* (land snail) vended along Port Harcourt-Bayelsa route.

Methods: Eighty samples from four locations were analysed employing standard techniques for proximate and microbiological analyses; virulence determination and antibiotic susceptibility. Data were statistically analyzed using ANOVA and *t*-test.

* Student Award Competitor

Results: The mean proximate results revealed presence of protein (26.01/13.6%), lipid (18.9/3.88%), fibre (5.12/2.01%), ash (3.40/1.11%), moisture (13.47/59.5%) and carbohydrate (32.43/20.07%) for edible larvae/snail, respectively. Of the 80 samples examined, 33 (41.25%) and 52 (65.00%) had total viable bacterial counts and total staphylococcal counts above acceptable microbiological limits, respectively for RTE foods. Seven of the 81 characterized *Staphylococcus* produced the expected band of 950bp with SEA virulent genes while 3 produced expected bands of 550bp with SEB virulent genes. Three *S. aureus* strains from edible larvae harboured both virulent genes. The virulent genes bearing *Staphylococcus* were 100% resistant to augmentin, ceftazidime and cloxacillin but showed varying resistance against ceftriaxone (57.14%), cefuroxime (28.57%), vancomycin (42.86%), oxacillin (42.86%) and cefoxitin (42.86%).

Significance: The study showed that these RTE foods are potential sources of staphylococcal food poisoning in commuters; hence food vendors need to conform to standard practice.

P2-12 Assessment of *Listeria* spp. and *Escherichia coli* Contamination on Surfaces in a Broiler Abattoir

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Introduction: *Listeria* and *Escherichia coli* continue to contaminate poultry, despite all the risk management systems implemented to ensure food safety. The exploration of contamination routes via surfaces in abattoirs allows us to identify ecological niches that continue to spread germs and make any cleaning and disinfection operation inefficient.

Purpose: The objective of this study is to evaluate the contamination of surfaces in a poultry abattoir by these two germs before and after cleaning and disinfection.

Methods: A total of 48 surface swabs were carried as recommended by ISO 18593 (2004) out on 7 types of surfaces in contact or not with poultry products at four stages of the slaughter process. Twenty-four swabs were taken prior to cleaning and disinfection operations and the other 24 swabs were taken afterwards. Standards NFV08-055 and NF V08-060/2009 are used for the detection of *Listeria* and *E. coli*, respectively.

Results: A total of 71% and 42% of the surfaces tested were contaminated with *Listeria* before and after cleaning and disinfection. Five of seven (5/7) of the surface types showed 100% *Listeria* contamination. Cleaning and disinfection failed to reduce the occurrence of contamination. Twenty-two percent of surfaces were contaminated with *E. coli*. Simultaneous contamination of *Listeria* and *E. coli* was observed in 17% of the surfaces.

Significance: These results suggest that *Listeria* is persistent in this abattoir and that cleaning and disinfection operations remain ineffective. Surfaces that are doubly contaminated by these two bacteria are a significant source of direct or indirect contamination of poultry intended for human consumption.

P2-13 Developing and Maintaining Food Safety Culture through Implementation of GFSI Benchmarked Standards: A Success Story

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Introduction: Access to safe and quality food is of paramount importance and an essential requirement for consumers to maintain their health and wellbeing. The meticulous efforts of food producers to demonstrate their commitments to food safety and fulfil customers preferences and expectations can gain more attention if organizations demonstrate well established quality and food safety cultures. Top management commitment and involvement is mandatory to imbed positive food safety culture at all levels in the organizations

Purpose: The purpose of this study was to depict adoption of innovative ideas and reflection of collective attitude, beliefs and behaviours of organizations' top management, managers, supervisors and food handlers towards resolving food safety and hygiene issues and setting contemporary trends which leads towards transforming existing food safety practices into more sophisticated and regimented food safety culture.

Methods: In the present study, a survey of food manufacturing units and distribution centers on quarterly basis of Mawarid Food Company was conducted in the kingdom of Saudi Arabia.

Results: Results of this study showed that appropriate trainings, empowering the employees to share their ideas, motivations, strong top management commitments are ways which lead towards transforming existing food safety practices into more sophisticated and regimented food safety culture.

Significance: This study is quite helpful for food producers and retailers to guide how they can turn their dreams into reality when they successfully attain certification of their food facilities against FSSC 22000 benchmarked standards by a prestigious GFSI-approved certification body.

P2-14 Determination of Food Safety Culture in a Low-risk Food Production Site: Identification of Key Strengths and Weakness

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Introduction: Food-safety-culture (FSC) is an emerging concept and positive culture can provide a strong foundation to a robust food-safety (FS) management system. Owing in-part, to the Global-Food-Safety-Initiative's incorporation of FSC into benchmarked standards, FSC measurement is now a requirement within the food-and-drink-manufacturing-production (FDMP) industry. FDMP companies are now required to monitor FSC and design/implement improvement plans.

Purpose: To determine attitudes towards FS and associated FSC dimensions in a low-risk FDMP company. Cumulative and categorised data will be used to determine a targeted approach, intervention development and FSC improvement in the company.

Methods: Using in-depth company-management/food-operative interview data, a bespoke quantitative questionnaire was designed to evaluate FS and FSC attitudes. Questionnaires were distributed to all operational staff (n = 210) electronically; descriptive and inferential statistics were used for analysis.

Results: Cumulatively, positive attitudes towards FSC categories were determined (82.9% of company-managers; 75.9% production-operatives indicated concern for FS) however significant differences ($P < 0.001$) were identified between company managers and production-operatives attitudes toward key FSC parameters including FSC awareness, people, process, purpose and proactivity components. Conversely, factory-based staff perceived FS risks to be higher than office-based staff ($t = 2.06$, $df = 27$, $P = 0.049$). Company managers agreed that "health and safety is more important than FS in the business" more

strongly than production operatives ($U = 142, P < 0.001$). Similarly, production operatives agreed with “FS issues due to operative errors are not frequent in my company” more strongly than company managers ($U = 281, P < 0.001$). Despite attitudinal differences, there was a positive attitude toward empowerment with 80.0% of company managers indicating they would be comfortable stopping a production line if there were a FS risk, 62.1% of production operatives agreed. Likewise, 88.6% company managers felt trusted, 62.1% production operatives felt similarly.

Significance: Whilst overall attitudes toward FSC parameters in the company were positive, implementation of the bespoke survey identified attitudinal differences between management and operative groups. This has informed targeted intervention development for FSC improvement in the business.

P2-15 Microbiological Quality Evaluation of Meal Kits (Non-heated Food and Food after Heating Treatment) Compared by Their Storage Times

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Introduction: As meal kits can have microbiological quality changes depending on the storage times after vacuum-packaging, such meal kits should be produced safely and proper storage times should be established.

Purpose: The purpose of this study is to assess safety of meal kits by analyzing the microbiological quality of foods (non-heated food and food after heating treatment) for different storage times.

Methods: Chicken breast and four kinds of washed and sterilized raw vegetables (radish, scallion, lettuce and cherry tomato) were those food materials included in the radish salad (non-heated food) and chicken salad (food after heating treatment). Twenty-five g each of food materials (vacuum-packaged, respectively, stored at 3° for 0-3 days) and cooked foods were diluted ($10^{-1} \times 10^{-7}$) and quantitatively analyzed through pour plating method in duplicate. *Salmonella* spp., *Listeria monocytogenes*, *Cronobacter* spp., and Shiga toxin-producing *Escherichia coli* (STEC) were qualitatively analyzed using 3M MDS.

Results: In the microbiological analysis of cooked foods using the meal kits, APC and coliforms were confirmed at a 100% acceptable ratio regarding the microbiological recommendation standard for foods. In the results between main food materials and cooked foods, chicken breasts were $APC 3.77 \pm 0.51 \log CFU/g$, coliforms $2.00 \pm 0.48 \log CFU/g$, and *Salmonella* spp. was detected. However, after cooking, APC and coliforms were significantly decreased ($P < 0.05$, using a *t*-test by SPSS 25.0) to $1.24 \log CFU/g$ and $0.60 \pm 0.56 \log CFU/g$, respectively, and foodborne diseases were not detected. There was no significant difference between the food material of radish and the radish salad.

Significance: Food materials pretreated hygienically, stored at refrigerator temperature for 0-3 days showed no problems in cooked foods, and it was confirmed that sufficient heating was important in the cooking process after heating. Therefore, in order to prevent foodborne diseases, food materials should be pretreated in a hygienic method in producing meal-kits, and foodservices should cook them in a certified method such as HACCP.

P2-16 Academic and Food Industry Management Perspectives of Food Safety Culture

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Introduction: Food safety culture (FSC) determinants are desirable attitudes and behaviors that enable and encourage food safety throughout an organisation. Demonstrating FSC and meaningful, positive improvement is challenging and understanding FSC perceptions and related improvement approaches may be achieved from determination of industry and academic perspectives.

Purpose: To explore FSC perspectives from professional influencers in the food industry and academia to ascertain effective improvement approaches and key actions used in industry to facilitate culture change.

Methods: Semi-structured, in-depth interviews ($n = 22$) were conducted with professional and academic influencers to obtain insights into perceptions of FSC, including desirable FSC key actions, characteristics, behaviors and attitudes associated with facilitation culture change. A thematic analysis of transcribed interviews was undertaken using NVivo.

Results: An in-depth understanding of FSC improvement complexities was obtained and variances between academic and industry professional perceptions of FSC identified. When describing an ideal, strong, positive FSC, industry professionals visualized and verbalized what it would look like, for example “everything is clean...and well ordered...and on the boards...[with] complete alignment and engagement from the very top of your organization [through to the bottom]”. Furthermore, FSC was described more about ensuring employee understanding of roles/responsibilities, awareness and tools to execute jobs consistently and easily and enabling self-correction when food safety challenges occur. From an academic perspective, FSC was defined in a more formal, complex and less practical way, where it was agreed that FSC is prevailing, constant, with learned beliefs, values or attitudes that are socialized contributing to hygienic behaviours related to food safety. Consultants/academia bring to life the ideal positive strong food safety culture as “high-performing teams” and “high-level of compliance.”

Significance: Industry professional and academic differences in perceptions of FSC have the potential to make application of FSC improvement approaches in the food industry challenging. Exploring and understanding such differences may help overcome such challenges.

P2-17 Strategic Continued Growth Platforms in Wales, UK: The Development of Food Sector Small to Medium Enterprises (SMEs)

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Introduction: Between 2008 and 2020, the Welsh Government (WG), in response to the need for knowledge transfer of necessary technical and food safety/science skills to the food-drink-manufacturing-and-processing (FDMP) sector, engaged in four SME and University tripartite growth programmes. The KITE project, Food Innovation Rapid Response Programme (FIRR), Barriers to Accreditation (BTA) and Project HELIX facilitated food-business innovation, growth and sustainability, via technical competence, compliance with food safety regulations and engagement in 3rd party accreditations.

Purpose: This study aimed to identify the impact of strategic and tactical development growth platforms' continued technical and food safety knowledge transfer support, on the Welsh FMDP sector.

Methods: A mixed-method qualitative and quantitative analysis was performed to evaluate WG, KITE, FIRR, BTA and HELIX project documentation, reports and media articles ($n \geq 260$). Projects' process and output data, based on 60 FDMP SMEs participation in 89 knowledge transfer delivery interventions, were assessed.

Results: The cumulative data identified 513 delivery project interventions facilitating knowledge transfer in in 252 SME FDMPs with the most frequent activities implemented including systems development ($n = 89$), provision of technical information ($n = 85$), training/mentoring/skills ($n = 42$) and validation of systems ($n = 42$). The ten types of delivery programmes of KITE, BTA, FIRR and HELIX designed for SME needs resulted in FDMP outputs including £207.5m increased sales, 906 new jobs created and 129 new markets accessed. KPIs detailed 1767 new products created, 2317 jobs in the food sector safeguarded and 64 new business start-ups supported.

* Student Award Competitor

Significance: The strategical objectives of the Welsh Government to have achieved a 30% increase in Gross Value Added for the sector was contributed to by the longer strategic KITE and HELIX programmes and the FIRRIP and BTA tactical tripartite growth platforms that implemented improved food-safety/technical compliance and competence.

P2-18 Next Generation Sequencing Workflow for Detection of *Salmonella* Serotypes: From Food Sample to Serotype Identification

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Introduction: The identification of *Salmonella* serotypes in food samples proposes a challenge to food producers as culturing methods can be inaccurate and open to interpretation errors. Whole-genome sequencing methods that have been used for strain typing analysis are expensive and involve time consuming sample handling.

Purpose: The NGS workflow proposed in this study enables the use of both lysates from enrichment samples and plate cultures as a sample material. Allowing the use of direct lysates from enrichment samples allows the user to utilize this method as a next-step after a PCR positive result without additional culturing or DNA extraction steps from the original enrichment sample. The proposed method applies targeted sequencing on Ion Torrent platform, enabling fast and accurate detection of *Salmonella* serotypes.

Methods: The method was applied to food enrichment samples from minced beef and chicken spiked with 20 *Salmonella* serotypes. Enrichment protocol of validated *Salmonella* species detection method was followed (SureTect *Salmonella* spp.) and the presence of *Salmonella* was confirmed with PCR. NGS libraries from the lysates were prepared and analysed on S5 Genestudio Food Protection instrument. Resulting sequences were mapped against *Salmonella* database for serotype identification. The NGS library construction included amplification of several selected target DNA regions identified as unique for each serotype.

Results: The results show that *Salmonella* serotyping by targeted NGS method can be applied directly to lysates from food enrichment samples as well as to pure cultures. A total of 80 serotypes were tested both in silico and in the wet lab for reliable discrimination and identification.

Significance: A complete workflow for *Salmonella* serotyping based on NGS is proposed. This will enable a quick and reliable serotyping identification that include NGS data analysis based on comparison with a custom-made database.

P2-19 Impact of Neutralizing Buffers of Disinfectants on the Viability of *Listeria monocytogenes* Cells from Monospecies Biofilm

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Introduction: Ready-to-eat products can be contaminated during processing by pathogenic or spoilage bacteria, which persist in the industrial environment. Some bacterial species are able to form biofilms which protect them from environmental conditions. To check the bacterial contamination of the surfaces in the food industries, the professionals must regularly use surface sampling methods (sponges, swabs, gauze pads...) to detect pathogens such as *Listeria monocytogenes*. Due to the presence of disinfectant residues on surfaces and sometimes within the biofilm, many sampling methods are contained in a nutrient broth combined with a neutralizing buffer to inactivate

disinfectant residues that can cause excess bacterial mortality during sample transport. This could be a source of false negatives.

Purpose: However, questions arise about the impact of this neutralizing buffer on the viability of the bacteria after sampling and more specifically on the viability of *L. monocytogenes*.

Methods: To answer this question, biofilms of *L. monocytogenes* were cultured on stainless steel for 24 hours at 8°C or 20°C. The biofilms were treated with disinfectants or with water (control) and brought into contact with 6 commercially available neutralizing buffers at different compositions. The bacterial populations were then detached by swab and analyzed directly after sampling and after 24 hours of incubation at 8°C to simulate the storage of surface samples before analysis (recommendation ISO 18593 standard, 2018). The analyses included agar enumeration to quantify the viable cultivable population as well as qPCR and PMA-qPCR quantification to assess the proportions of dead and viable populations of *L. monocytogenes*.

Results: These results showed that neutralizing buffers have variable impact on *L. monocytogenes* cells.

Significance: This is the first study on the impact neutralizing buffers of disinfectants on the viability of *L. monocytogenes* cells contained in surface samples.

P2-20 Exploring Management Attitudes Toward Leadership Roles in Food Manufacturing

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Introduction: As a driving force, leaders play a pivotal role in setting positive examples. For food businesses, 'walking the talk' is a vital management trait to ensure food safety is never compromised. Being a credible and accessible leader is paramount to support an open, trustworthy, thriving food safety culture (FSC).

Purpose: To explore how managers perceive their roles as leaders across hierarchical levels to identify opportunities to enhance communication and working relationships to support FSC progress.

Methods: In-depth interviews were conducted with senior, middle and low-level managers ($n = 16$) from food manufacturing sites processing a range of raw and high-risk products ($n = 3$). Discussions related to aspects underpinning the Global Food Safety Initiative (GFSI) FSC dimensional framework including leadership behaviour, beliefs and mindset.

Results: Between sites, senior and middle manager leadership attitudes ($n = 4$) had both detrimental and beneficial impacts on morale and culture. At the manufacturing site producing high-risk food products, getting "bogged down" with work often prevented the site manager from visiting production which was perceived by low-level managers as a detachment or disinterest in production operations. As such, production-based managers felt "whiny" whenever they sought additional food safety support from senior managers. Conversely, leaders ($n = 2$) who reported visiting production departments at the raw meat site more frequently (if only for a brief amount of time) considered the regularity important as it helped "build trust in the guys downstairs." From mid and low-level manager perspectives, the involvement was welcome as managers were regarded as accessible and communicative, ensuring the food production team developed "every day."

Significance: Poor communication can ultimately have consequences for food safety if low-level and middle managers feel reluctant or unable to approach senior managers for additional support. Interventions enhancing two-way communication and sharing best 'leadership' practices between sites would be advantageous to support FSC progress.

P2-21 Factors Influencing Food Safety Compliance in Home Food Production Businesses

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Introduction: In recent years, market growth of home food production businesses (HFPBs) has been reported. Prior to food production, HFPBs require Environmental Health (EH) approval/registration, including demonstrated understanding of food hygiene principles and a HACCP plan. To date, limited research has explored factors that may influence compliance relating to HACCP implementation and FSM in HFPBs.

Purpose: This study explored EH regulatory requirements, processes and information provision in HFPBs in conjunction with reported food safety compliance/non-compliances.

Methods: In-depth, semi-structured telephone/face-to-face interviews were carried out with UK-EH-practitioners ($n = 13$). The interview schedule investigated EH regulatory processes, reported HFPB contact, perceptions of HFPB FSM awareness, EH experience indicating observed food safety in HFPBs and associated information provision. Interview transcriptions were analysed using a thematic, content-analysis using NVivo-software.

Results: As expected, EHs reported a consistent regulatory approach to HFPB approval/registration; however, challenges relating to this were identified including a lack of defined continuity, so “when a hobby becomes a business could be a grey area.” EH-practitioners reported that most HFPBs lacked awareness about registration/ approval processes as well as “lack of awareness of food safety problems [by HFMBs].” Indeed, “over-familiarity with the setting led to non-compliances” and other key factors influencing non-compliance included inadequate knowledge of HACCP, inadequate paperwork and training and poor temperature control. Positioning/availability of domestic facilities reportedly made “domestic segregation challenging” with one EH-practitioner indicating “the use of a domestic kitchen for commercial purposes isn’t always ideal.” EH information provision was perceived to be “sufficient” and usually provided prior to company registration. Variable EH experiences were provided to further understand HFPB food safety compliance.

Significance: This study has enabled increased understanding of HFPB regulatory processes and identified potential barriers for food safety regulatory compliance. Data will contribute to the development of targeted information materials to potentially improve HFPB FSM awareness, EH support and food safety compliance.

Microbial Food Spoilage

P2-22 Interaction between *Saccharomyces cerevisiae* and *Aspergillus carbonarius* in Vitro and in Situ on Table Grapes

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Introduction: Biological control agents could be employed as alternative and advantageous approaches to improve food quality and safety. *Saccharomyces cerevisiae* is one of the most promising yeast species against ochratoxin producing *A. carbonarius*.

Purpose: To investigate the efficacy of biological control of *A. carbonarius* by *S. cerevisiae* through competition experiments *in vitro* and *in situ* on Fraoula variety table grapes.

Methods: The growth kinetic parameters and conidia production of *A. carbonarius* were determined in the presence and absence of *S. cerevisiae* on Czapek Yeast

Extract Agar. The plates were incubated at 25°C for 10 days. The interaction of *A. carbonarius* and *S. cerevisiae* was also investigated on table grapes under the following treatments: inoculation with (a) *S. cerevisiae*, (b) *A. carbonarius*, (c) *S. cerevisiae* and *A. carbonarius*, and (d) uninoculated berries. All samples were stored at 25°C for 22 days. For the expression of the results both microbiological and molecular techniques, namely conventional PCR (for *A. carbonarius* detection) and Real Time PCR (for *A. carbonarius* quantification) were employed.

Results: According to the results, the presence of *S. cerevisiae* inhibited the growth of *A. carbonarius* both *in vitro* and *in situ* on grape samples. Nevertheless, *A. carbonarius* continued to produce conidia when co-cultured *in vitro* with *S. cerevisiae*. The range of the haploid genome for the Real Time PCR standard curve was from 24 to 24 x10³ haploid genomes/g of grape and for the grape samples it was from 28.25 to 19.3 x10³ haploid genomes/g of grape.

Significance: The inhibition of *A. carbonarius* by *S. cerevisiae* is a very important fact for food safety, because yeasts, such as *S. cerevisiae* can be used instead of chemical agents to prevent the growth of mycotoxigenic fungi.

P2-23 Rapid Evaluation of Sea Bream Fillets Quality Using FTIR Spectroscopy, Microbiological and Sensory Analysis

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Introduction: Microbial activity is the most significant cause of fish spoilage. The innovative technique of Fourier Transform Infrared Spectroscopy (FTIR) for the prediction of microbial growth and fish spoilage presents significant research interest.

Purpose: The aim of the present study was to examine the microbial spoilage of gilthead sea bream (*Sparus aurata*) fillets by using FTIR spectroscopy, microbiological and sensory analysis.

Methods: Aqua cultured gilthead sea bream fillets were stored aerobically at 0, 4, 8 and 12°C. Duplicate samples of the fish fillets were subjected to microbiological analysis for the enumeration of Total Viable Counts (TVC), *Pseudomonas* spp., H₂S-producing bacteria, *Brochothrix thermosphacta*, *Enterobacteriaceae*, Lactic Acid Bacteria (LAB) and yeasts. Sensory analysis of odor and skin color of fish fillets was also performed. In parallel to microbiological and sensory analysis, FTIR spectral data of fish fillets were acquired and subjected to analysis by means of Partial Least Squares Regression (PLS-R).

Results: According to sensory results, fish samples were assessed as spoiled after 6, 3, 2 and 1 days, at 0, 4, 8 and 12°C, respectively. *Pseudomonas* spp. and H₂S-producing bacteria were the dominant spoilage microorganisms at all temperatures, while *Enterobacteriaceae*, *B. thermosphacta*, yeasts and LAB presented lower population levels. The developed PLS-R models based on FTIR spectra of fish fillets exhibited satisfactory performance in the estimation of TVC, as the coefficient of determination (R²) was 0.89, whereas the value of the root mean square error (RMSECV) was approximately 0.5 log CFU/g and the slope was 0.94.

Significance: FTIR spectroscopy seems to be promising for the rapid and non-invasive assessment of the microbiological quality of sea bream fillets.

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P2-24 Spoilage Assessment on Chicken Thighs Surface Via Non-invasive Multispectral Image Analysis (MSI)

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Introduction: Consumer demand for quality meat, combined with poultry meat's vulnerability to spoilage, led to the implementation of rapid spectroscopic methods for the assessment of spoilage of meat products. Multispectral image analysis is a promising and non-invasive method which has been applied on meat products resulting in the development of spoilage predictive models.

Purpose: The aim of this research was the correlation of microbiological to multispectral imaging data acquired during spoilage experiments of chicken thigh fillets. The microbiological quality on the surface of chicken was predicted by Partial Least Squares-Regression (PLS-R) models.

Methods: Chicken thigh fillets ($n = 198$, two independent experiments) were stored aerobically at four isothermal conditions (0, 5, 10, and 15°C) for up to 456 h. At regular intervals, samples were subjected to microbiological analysis for the determination of total viable counts (TVC) and *Pseudomonas* spp. in parallel to multispectral image acquisition (wavelengths: 405-970 nm). Kinetic parameters of growth for TVC and *Pseudomonas* spp. were determined based on the primary model of Baranyi and Roberts. PLS-R analysis was undertaken for the assessment of TVC and *Pseudomonas* spp. counts.

Results: *Pseudomonas* spp. was the dominant spoilage microorganism on samples while low storage temperatures prolonged the lag phase of this microorganism. Specifically, the lag phase and μ_{max} of *Pseudomonas* spp. on samples stored at 0°C were 63.9 h and 0.059 h⁻¹, respectively. PLS-R models predicted efficiently TVC with RMSE_p and r_p (correlation coefficient) values of 0.858 and 0.818, respectively. Moreover, RMSE_p and r_p values for *Pseudomonas* spp. PLS-R model were 0.972 and 0.814, respectively.

Significance: These findings could assist poultry industries to evaluate rapidly the level of spoilage in their products and hence improve their quality.

Acknowledgment: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-04344).

P2-25 Multivariate Data Analysis for the Development of Classification Models Assessing Spoilage of Two Different Types of Poultry Meat

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Introduction: Multispectral imaging (MSI) and Fourier Transform Infrared Spectroscopy (FTIR) are two rapid and non-destructive methods commonly employed in spoilage assessment of a variety of fresh or processed meats.

Purpose: The purpose of this study was to evaluate the performance of these two spectroscopic methods and develop classification models assessing spoilage of two chicken products.

Methods: Chicken breast ($n = 427$) and chicken thigh ($n = 402$) samples were subjected to spoilage experiments (isothermal conditions: 0, 5, 10, 15, 20, 25, 30, and 35°C; dynamic conditions: winter transportation scenario, summer transportation scenario). Samples were analyzed microbiologically (Total Viable Counts, TVC) while in parallel

MSI and FTIR measurements were performed. Sensory analysis was also conducted by 14 individuals for the evaluation of fresh and spoiled samples. Linear Discriminant Analysis (LDA) and Quadratic Support Vector Machines (QSVM) models were developed for the assessment of quality in samples.

Results: Sensory outcomes established the threshold of spoilage at 6.22 and 6.68 log CFU/cm² for chicken breast and thigh, respectively. LDA and QSVM models performed satisfactorily for chicken thigh models. More specifically, for chicken thigh, MSI combined with QSVM analysis exhibited overall accuracy, sensitivity and specificity of 91.7, 88.9 and 94.4%, respectively. In contrast, for chicken breast, FTIR and QSVM model identified the two classes with overall accuracy being at 61.2% while model could not classify satisfactorily spoiled samples (specificity at 30%).

Significance: The implementation of the developed models coupled to rapid sensors could be efficient for the assessment of quality in poultry products. Apart from the increase of quality and safety, these findings could result in the reduction of food waste.

Acknowledgments: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T1EDK-04344).

Modeling and Risk Assessment

P2-26 Thermal Inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in Curd Stretching Used for Mozzarella Cheese

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Introduction: A role of *Mycobacterium avium* subsp. *paratuberculosis* (MAP), the causal agent of Johne's disease in cattle and other ruminants, in human diseases is still debated, but raw milk and unpasteurized products are considered the main source of human exposure. Mozzarella is a soft cheese made by stretched curd and despite the use of pasteurized milk is generally indicated for these productions, the use of fresh raw milk is also allowed.

Purpose: The aim of this study was to investigate the kinetics inactivation of MAP in curd used to produce Mozzarella cheese.

Methods: Twenty-seven samples of mature curd (pH of 5.2) (weight 100 g), made by pasteurized milk spiked with MAP ATCC 19698 (5 log CFU/mL), were under-vacuum packed and treated in hot water at 65, 70 and 75°C (isothermal profiles). Two replicate experiments were performed in this study. MAP inactivation was estimated by plate count method and Geeraerd-tail model in the add-in GlnaFit (Geeraerd et al., 2000; 2005) was used to estimate the kinetics survival parameters as the K_{max} (specific inactivation rate). The D_{value} of MAP was also calculated for each temperature.

Results: During the isothermal experiments, the K_{max} (min⁻¹) values were 0.95, 3.64 and 16.71 min⁻¹ at 65°C, 70°C and 75°C, respectively, showing wide changes of the inactivation rates for MAP in curd as a function of the temperatures. The D_{value} were 2.42, 0.63 and 0.14 min at 65°C, 70°C and 75°C, respectively.

Significance: The most significant criteria to define the pathogen heat resistance include its K_{max} and D_{value} data which enable the effective design and theoretical evaluation of the thermal processes. Knowing the effect of the thermal treatments on fresh curd in hot water is a critical control point of MAP survival and might be pivotal in the safety improving of raw cheeses.

P2-27 Log Reduction of *Listeria monocytogenes* during the Domestic Heating of Meat Products: Different Approaches

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Introduction: Heat treatment is one of the most efficient methods to improve the microbiological safety of foods. Among non-spore forming bacteria, *L. monocytogenes* is ubiquitous and one of the most heat-resistant food pathogens. Food Business Operators (FBOs) have to indicate the heating time for the domestic preparation of their products.

Purpose: This study considered two approaches to follow the fate of *L. monocytogenes* in meat products during the domestic heating: A) experimental inoculation, B) temperature profiles registration and mathematical models application.

Methods: A) A three-strain cocktail, fresh or lyophilized, of registered strain (ATCC 19115) and wild strains (previously isolated from meat) was used to contaminate (1% v/w) fishburgers, sausages and cooked poultry meat products with an inoculum size of ca. 8 log CFU/g. The pathogen log reductions were estimated by plate count method after the heating of 9 replicates for each meat product following the label advices. B) Nine Data Loggers were positioned in the coldest point of as many roasted shanks, already cooked *cotechino* and *zampone* and then heated following the label advice. Temperature profiles for each product were registered and the worst case scenario was used to calculate the theoretical log reduction by on-line software using literature parameters.

Results: A) In the experimental contaminated products the log reductions were 5.83 ± 0.7 , 1.83 ± 0.92 , 6.37 ± 1.04 log CFU/g for fishburgers, sausages and poultry meat. B) No theoretical log reduction was determinate in the shank heated by oven, while by boiling 8.5-, 17.3- and 122-log reductions were calculated for shank, *cotechino* and *zampone*, respectively.

Significance: The study may encourage the FBOs of meat products and competent authority to pose more attention to the domestic heating advice to increase the safety of consumers.

P2-28 Risk Estimation for Aflatoxin M1 Due to Milk Consumption in Chilean Children

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Introduction: Aflatoxin M1 (AFM1) is the hydroxylated metabolite of AFB1 found in milk obtained from livestock that has ingested contaminated feed. AFM1 is a carcinogen to humans being in group 1 according to IARC.

Purpose: The aim of this work was to estimate AFM1 exposure by milk consumption in children under 10 years old in Chile.

Methods: Consumption was estimated based on an online survey $n = 214$ (question: How much milk consume your son/daughter?). In terms of the responses, we assumed a triangular distribution with min 150 mL, max 700 mL, and most frequent 400 mL. AFM1 concentration in milk was assessed according to the Chilean Surveillance Program of Mycotoxins during the 2017–2019 period, adjusted by the lowest Akaike information criterion into a Pareto distribution, assuming in <LOD to LOD/2. Weights of the children were taken from the Health Ministry guide of ideal

weights, modeling a triangular distribution. Probabilistic models of each variable were sampled by the hypercubic Latin sampling method and variables were associated with a Montecarlo simulation. MoE was calculated as the ratio between reference BMDL at the 10% effect level (BMDL10) according to Udovicki et al. 2019 (570 ng/kg bw/day), and the estimated EDI; a MoE <10,000 was considered of high health concern (EFSA 2020).

Results: Occurrence of AFM1 in milk was 10.5% (17/162), with a mean level of 0.0034 ng/g and a max of 0.029 ng/g. The mean EDI was of 0.059 (0.023) ng/kg-bw/day, with a worst-case scenario (P95) of 0.101 ng/kg-bw/day. MoE was estimated in 9,583 in mean consumption and 5,627 in high consumers.

Significance: Based on these estimations, dairy must be considered of public health concern in children of 2 to 10 years old in Chile. Current Chilean regulation do not consider vulnerable groups, so it seems urgent to generate risk assessment based on local information, so food safety managers in Chile can take more accurate and informed decisions.

P2-29 The Canadian Food Inspection Agency Work Tasking Logic Model Parameter Selection and Prioritization

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Introduction: The Canadian Food Inspection Agency (CFIA) is developing a risk management algorithm, the Work Tasking Logic Model (WTLM), designed to digitally integrate Establishment-based Risk Assessment (ERA) model risk intelligence and inspection parameters into tactical work planning to allocate inspection resources to food facilities based on the risk they represent to consumers.

Purpose: Select the model parameters and determine their relative importance for prioritizing the inspection scheduling and determining the inspection scope.

Methods: Nineteen CFIA experts with significant experience in work planning were prompted to identify the parameters that influence the inspection planning and assess their relative importance by completing two surveys (Microsoft Word and SimpleSurvey, respectively). Parameter scoring was established by ranking each parameter according to their level of importance, from one to five (1 = most, 5 = less). Descriptive results and the average ranks assigned by experts were calculated in Microsoft Excel 2010.

Results: Overall, 16/19 identified experts participated in the first survey, and 15/16 completed the second one. For scheduling establishment visits, the following parameters were selected and are presented by importance: risk results and categories generated by the ERA model, establishment's food safety control, proportion of inspection tasks completed according to the risk category, days since the last non-compliance, days since the last inspection, and days since the last visit. For defining the inspection scope, experts prioritized: task involved in repetitive non-compliance, tasks having the greatest impact on risk results, task involved in non-compliance at the last visit, task unassessed for the longest period of time, and task involved in non-compliance during the penultimate visit.

Significance: By digitally integrating the ERA model intelligence into tactical planning, the Agency will be able to more efficiently allocate resources based on risk, adapt to changing risks and redirect capacity as needed, compared to manual yearly planning.

P2-30* Probabilistic Modelling of Exposure to Coliform Bacteria in Raw Milk

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Introduction: Probabilistic modelling tools are increasingly being developed to take into account the different sources of variability and uncertainty along the food safety continuum in exposure or risk assessment models. These models are also popular to reflect real-life data and generate what-if scenarios to inform decision makers.

Purpose: Probabilistic modelling tools were utilized in developing a coliform bacteria exposure assessment model in raw milk in Kingdom of Saudi Arabia (KSA). This country was taken as proxy of what will happen in Europe in the near future due to climate change.

Methods: The initial coliform concentration in raw milk was derived from industrial dairy farm data (around 1695 data) while microbial growth was determined across various scenarios of time and temperature storage, using existing databases. The exposure to coliform was interpreted considering KSA and EU standards. The probabilistic model, with uncertainty and variability separated, was implemented in R using the mc2d while to fit the data, the nls function and the packages nlstools and fitdistrplus were used.

Results: The level of exposure to coliform concentration was compared to KSA and EU standards as function of various time and temperature conditions. The impact of raw milk, storage time, chilled temperature conditions on the compliance regarding these standards was analysed in details. The upcoming climate change may affect the storage temperature but also the milk quality due to potential cow heat stress.

Significance: The application of probabilistic modelling tools to assess current exposure can be expanded to other food systems. The ways on how the variability and uncertainty from data inputs and storage scenarios were addressed, using second order Monte Carlo procedure, provide an added-value to develop realistic exposure assessment models and suggest mitigation options.

Molecular Analytics, Genomics and Microbiome

P2-31 Microbial Diversity of Fermented Greek Table Olives of Halkidiki and Konservolia Variety from Different Regions as Revealed by Metagenomic Analysis

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Introduction: Current knowledge from conventional microbiological methods does not provide sufficient information on the microbial diversity of different table olive varieties. The use of next generation sequencing (NGS) technologies will enable the comprehensive analysis of their microbial community, providing microbial identity of table olive varieties and their designation of origin.

Purpose: To evaluate the bacterial and yeast diversity of fermented olives of different varieties from different regions – green olives, cv. Halkidiki, from Kavala and Halkidiki and black olives, cv. Konservolia, from Magnesia and Fthiotida – by conventional microbiological methods and NGS.

Methods: Total Viable Counts, Lactic acid bacteria (LAB), yeast and molds, and *Enterobacteriaceae* of the samples were enumerated. In addition, microbial genomic DNA was directly extracted from olive's surface and subjected to NGS for the identification of bacteria and yeasts.

Results: Microbial counts revealed a similar growth pattern of the main microbial groups in all samples, characterized by the dominance of LAB. NGS analysis showed no difference in LAB diversity since *Lactobacillus* was the most abundant genus in all samples. In relation to yeast diversity among cultivars and regions it was noted that *Wickerhamomyces* was the most abundant yeast genus in Konservolia olives from Magnesia region, while *Pichia* sp. and *Pichia membranifaciens* dominated in Konservolia olives from Fthiotida and *Pichia manshurica* in Halkidiki olives from both regions.

Significance: This study is an attempt to use NGS to investigate the microbial ecology of Greek fermented table olives. The results will contribute to the microbial identity of table olive varieties in relation to their origin, thus assisting in fraud detection and quality assessment.

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P2-32 A Metagenetic Analysis of Bacterial Community in Inoculated Fermentations of Conservolea Variety Black Olives with Multifunctional Starter Cultures in Reduced Salt Brines

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Introduction: During fermentation of table olives, lactic acid bacteria (LAB) not only determine the quality and sensory characteristics of the final product, but also provide functional properties. In addition, salt reduction is a great challenge for establishing additional health-promoting features in olive products.

Purpose: The aim of this study was to characterize the bacterial community of Conservolea variety natural black olives and determine the changes on the bacterial diversity during fermentation with different starter cultures and reduced salt using a metagenetic approach.

Methods: Conservolea natural black olives were fermented in 6% NaCl brines, while 50% substitution of NaCl by KCl was attempted for the preparation of reduced salt olives. Two starter cultures, namely *Lactobacillus plantarum* and *Lactobacillus pentosus* were tested. In total 18 olive samples were collected from 0, 75, and 146 days of the fermentation process. The bacterial communities were characterized at species-level based on *gyrB* amplicon sequencing.

Results: *Lactobacillus collinoides* prevailed during fermentation in both 6% NaCl and reduced salt olives inoculated with *L. plantarum*. In the case of inoculated fermentation with *L. pentosus*, the starter dominated in reduced salt olives at 75 days, while *L. collinoides* reached similar abundance at 146 days. In 6% NaCl olives, the latter species was the most abundant at 75 days, but *L. pentosus* finally dominated at 146 days.

Significance: The metagenetic approach, revealed the bacterial composition to the level of species and introduced the potential of new species in olive processing.

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P2-33* Whole Genome Sequencing of Extended-spectrum and AmpC β -Lactamase-producing *Enterobacteriaceae* Isolated from Spinach Supply Chains in Gauteng Province, South Africa

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Introduction: Contaminated irrigation water has been recognised as a source of potential pathogenic antimicrobial resistant bacteria in fresh produce production systems. The increasing occurrence of multidrug-resistant (MDR) extended-spectrum β -lactamase- (ESBL) and/or AmpC β -lactamase-producing *Enterobacteriaceae* in fresh produce represent risks related to environmental integrity and food safety. In South Africa, information about the prevalence of ESBL/AmpC-producing *Enterobacteriaceae* from non-clinical sources is limited, particularly in the water-plant-food interface.

Purpose: The purpose of this study was to characterise selected MDR ESBL/AmpC-producing *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia fonticola* and *Salmonella enterica* isolates from spinach- and associated irrigation water-samples from two commercial spinach production systems.

Methods: The 19 selected isolates were subjected to whole-genome-sequencing (Illumina MiSeq). Antibiotic resistance genes were identified with ABRicate. Plasmid typing, detection of mobile genetic elements, virulence factors (*E. coli*), serotyping (*E. coli*) and prediction of pathogenicity of the strains was conducted (Centre for Genomic Epidemiology platform). The virulence factors and K-antigen typing of *K. pneumoniae* were determined using the Institut Pasteur's *Klebsiella* database and Kaptive Web, respectively. SeqSero and SPIFinder were used for prediction of the *Salmonella* strains' serotype and *Salmonella* Pathogenicity Islands, respectively.

Results: Antibiotic resistance genes from eight different classes were present, with *bla*_{CTX-M-15} the dominant ESBL and *bla*_{ACT} the dominant AmpC detected. The *bla*_{CTX-M-15} harboured by *K. pneumoniae* was the only β -lactamase resistance gene associated with plasmids. Integron In191 was observed in six strains. All strains showed relevant similarity to human pathogens.

Significance: This study adds to the global knowledge base regarding the prevalence and characteristics of ESBL/AmpC-producing *Enterobacteriaceae*. This is the first study to characterise MDR pathogenic strains in fresh produce production systems from the farm, through processing and up to retail in South Africa, highlighting the need for expanded surveillance, required for future risk analysis.

Packaging

P2-34 Lactic Acid Bacteria and Yeast Species Diversity of Non-thermally Preserved Green Spanish-style Olives during Modified Atmosphere Packaging

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Introduction: Spanish-style green olives are one of the main trade preparations in the international market. Nowadays, there is an increasing trend to use plastic packaging due to reduced weight, lower costs, flexibility and convenience.

Purpose: To investigate the diversity of the technological microbiota of Spanish-style fermented green olives (lactic acid bacteria and yeasts) during modified atmosphere packaging in multilaminated pouches, using culture dependent molecular techniques.

Methods: Green pitted olives of cvs. Conservolea and Halkidiki were packaged in high barrier, multilaminated pouches under modified atmospheres (100% N₂) and stored at room temperature for 12 months. Microbial consortia (lactic acid bacteria – LAB, yeasts, enterobacteria) were monitored during storage. LAB and yeast species diversity was evaluated at the initial (0 days), middle (180 days) and final (360 days) period of storage by RAPD-PCR genomic fingerprinting with the oligo-nucleotide primer M13. The identity of the isolates was obtained by partial sequencing analysis 26S rDNA and 16S rRNA for yeasts and LAB, respectively.

Results: The microbiota consisted of LAB (5.2-5.5 log CFU/g) and yeasts (4.6-4.8 log CFU/g). No enterobacteria could be detected in both olive varieties. Yeasts could not be detected after the beginning of storage on both varieties. In the end, LAB dominated in populations exceeding 5.2 \pm 0.29 and 4.6 \pm 0.10 log CFU/g for cvs. Halkidiki and Conservolea, respectively. Molecular analysis revealed that the dominant LAB species at the beginning, 6 and 12 months of storage were *Pediococcus ethanolidurans*, *Lactobacillus pentosus*, *Lactobacillus rafi*, *Lactobacillus buchneri* and *Lactobacillus paraffraginis*. As for the yeasts' isolates, *Pichia manshurica* prevailed in both cultivars.

Significance: The survival of LAB combined with the probiotic potential of this microbial group creates new possibilities for the use of non-thermally preserved olives as a functional food.

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Pre-harvest Food Safety

P2-35 The Canadian Food Inspection Agency Establishment-Based Risk Assessment Model for Feed Mills: Algorithm Principles

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Introduction: The Canadian Food Inspection Agency (CFIA) has developed a quantitative risk assessment model to estimate the feed safety risk (i.e., risks for food safety and animal health) associated with Canadian commercial and on-farm feed mills.

Purpose: To help allocate inspection resources to facilities based on risk in a scientific, evidence-based, standard, consistent and transparent approach.

Methods: The identification and selection of risk factors ($n = 34$) and their associated assessment criteria ($n = 203$) were completed through a literature review and experts advice. During a face-to-face Delphi elicitation, experts ($n = 28$) were asked to estimate the relative risk of each assessment criterion to the feed safety risk of a feed mill. Based on these findings, a questionnaire was developed to gather data on the applicable criteria for 432 feed mills. The model algorithm was designed in Lumina Analytica (Lumina Decision Systems, USA).

* Student Award Competitor

Results: The algorithm is a multiplicative model. The feed safety risk assessment result of a feed mill is expressed as the proportion of the volume of outgoing feed distributed in Canada and exported to foreign countries by a feed mill to the total Canadian production volume of feed. This relative distribution volume value is multiplied by the relative risk of applicable feed safety criteria and then adjusted by the maximum increase or decrease in risk attributed to the three clusters of risk factors previously defined: inherent, mitigation and compliance. Therefore, three risk assessment results are calculated for each feed mill: inherent, mitigated and final risk results. Finally, the final risk results are grouped in risk categories to define inspection parameters and allocate resources.

Significance: By assessing the feed safety risk represented by each feed mill under the CFIA's jurisdiction, this new risk assessment model will help the Agency to appropriately allocate inspection resources and will further improve the protection of both public and animal health in Canada.

Retail and Food Service Safety

P2-36 Great Challenges Ahead for Global Food Safety Community: Practices in a Developing Country after Legislation

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Introduction: The food safety system in Pakistan is facing new challenges due to rapid population growth, globalization of food trade, poor sanitation, unhygienic practices and lack of consumer awareness. Increasing use of pesticides and insecticides in agriculture and toxic chemicals in food processing industries has raised many concerns to public health.

Purpose: The purpose of this study was to assess and evaluate the current food safety practices adopted by various food establishments and food safety knowledge and awareness among food handlers.

Methods: In the present study, a cross-sectional survey of 500 food establishments (fast food chains, dairy and milk shops, restaurants and hotels, sweets and bakers, bottled water and beverages, marriage halls and caterings, cafes and canteens and manufacturing industries) of 9 towns of Lahore capital which are under jurisdiction of Punjab Food Authority was carried out in collaboration of Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences Lahore Pakistan with the Punjab Food Authority.

Results: Results of this study showed that food establishments have not properly adopted food safety practices. On the part of management and personnel, the situation at food establishments is highly pathetic: 36% of food premises need immediate improvement, 75% need major improvement, only 2% of food establishments have showed good sign. The situation of personnel hygiene is also very pathetic: 17% of food establishments need immediate improvement and 26% require major improvement and none of the establishments meet the "very good" criteria. Almost 80% of food establishments require immediate improvement because their maintenance and infrastructure. Sixty percent of food establishments require urgent improvement in response to control of operation.

Significance: This study is quite helpful for food establishments to improve and adopt food safety practices. It has highlighted that appointment of competent and certified people in charge and food safety training are means of improving food safety culture.

Viruses and Parasites

P2-37 Sarcocystis Species Detection by Multiplex-PCR in Cattle Affected by Eosinophilic Myositis

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Introduction: Sarcosporidiosis in cattle may lead to Bovine Eosinophilic Myositis (BEM), pathological lesions due to inflammatory reactions to sarcocysts. Gross lesions, characteristically green, focal stripes or diffused patches that fade to off-white when exposed to air, can be observed upon meat inspection or during meat cutting. The condemnation of the affected carcasses leads to consistent economic losses. Research to date has not yet determined the contribution of the different *Sarcocystis* species to BEM pathogenesis.

Purpose: The specific objective of this study was to detect the *Sarcocystis* species present in BEM lesions from condemned cattle carcasses in Italy.

Methods: Lesional and non-lesional tissue was sampled from heart and skeletal muscle of 20 cattle carcasses condemned for BEM at the largest Italian slaughterhouse, for a total of 89 samples (37 non-lesional and 52 lesional tissue samples). Gross lesions were categorized in 4 types: green focal lesions (GFL), diffused green patches (GDP), white focal stripes (WFS) and "others." A multiplex PCR targeting 18S rRNA and COI genes was performed after DNA extraction.

Results: Out of 20 carcasses, 19 showed the presence of at least one *Sarcocystis* species. *S. cruzi* was more frequent in heart samples (49%), while *S. hominis* and *S. bovifelis* prevailed in the skeletal muscle (41 and 24%, respectively). GFLs represented 52% of the gross lesions observed; DNA from at least one sarcosporidian species was present in all these lesions. Either *S. hominis* or *S. bovifelis* were present in 90% of these samples, while *S. cruzi* and *S. hirsuta* counted for only 22% and 3%, respectively; notably, a putative new *Sarcocystis* sp. was found in 2 samples.

Significance: The study contributes to our understanding of the importance of different *Sarcocystis* species in the BEM pathogenesis. The results indicate *S. hominis* and *S. bovifelis* as the major sarcosporidian species involved.

Water

P2-38 Assessment of Water Quality Index (WQI) of Commercially Available Drinking Bottled Water

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Introduction: Bottled drinking water is becoming increasingly popular due to its convenience and perceived benefits. By 2017, consumption of bottled water was estimated to have reached 391 billion litres. WQI is considered as the most effective method used for overall description of the quality of water bodies used for different purposes. Water quality is characterized based on physical, chemical, and microbiological parameters and human health is at risk if values exceed acceptable limits.

Purpose: The aim of this study was to determine the WQI of different bottled water from different sources sold commercially in different packaging.

Methods: Commercially available bottled water from four different water sources (spring, underground aquifer, reservoir, and tap) and in different packages (PET, paper carton and glass bottles) were evaluated for electrical conductivity ($\mu\text{S}/\text{cm}$), pH, resistivity ($\text{k}\Omega/\text{cm}$), total dissolved solids (TDS in ppm), oxygen reduction potential (ORP, mV), nitrates (mg/L) and heterotrophic count (HPC). Calculated WQI was used to confirm the quality of these bottled water samples using the Weighted Arithmetic index method.

Results: Electrical conductivity, resistivity, salinity, total dissolved solids, Oxygen reduction potential, nitrates, and heterotrophic counts were significantly different among the samples. The values of the parameters recorded ranged as follows: electrical conductivity (208.3 – 607.3 $\mu\text{S}/\text{cm}$) pH (7.20 – 8.22), resistivity (1.62 – 5.02 $\text{k}\Omega/\text{cm}$), TDS (107.4 – 307.3 ppm), ORP (142.6 – 224.6 mV), nitrate (2.04 – 12.09 mg/L), and HPC (50 – 110 CFU/mL). The WQI values ranged from 8.14 – 40.19 (excellent – good). This study showed that

all parameters were within acceptable limits. Furthermore, for the analysed bottled waters, irrespective of the source and packaging type, the water quality index ranged from excellent to good.

Significance: The analysis of these commercially bottled waters is relevant for qualitative examinations since they are bottled and sold to the public from various sources such as groundwater, spring, distilled and tap.

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- Piller, Priscilla**, *NOVOLYZE* (P1-35)
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Wohlgemuth, Franziska, *School of Life Sciences, University of Nottingham* (P1-02*)

Wouters, Patrick, *GFSI Hygienic Design TWG; EHEDG; & Cargill* (T1-10*)

Yamamoto, Etsuko, *Canadian Food Inspection Agency* (T1-21)

Yasir, Muhammad, *Quadram Institute Bioscience* (T2-19)

Yu, Cong, *MilliporeSigma* (P1-15)

Zanabria, Romina, *Canadian Food Inspection Agency* (P2-35, T2-14, T1-18)

Zanoni, Mariagrazia, *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna* (P2-09)

Zhang, Helen, *Canadian Food Inspection Agency* (T1-21)

Zhang, Yifan, *ETH Zurich* (T1-12)

Zhao, Karen, *Ottawa Laboratory - Fallowfield, Canadian Food Inspection Agency* (P1-04)

Zhao, Xingchen, *Ghent University* (P1-13*, T1-15*)

Zubair, Shuguftha Mohammad, *Dubai Municipality* (P2-13)

Zuccon, Fabio, *Laboratorio Controllo Alimenti – IZS PLV, Via Bologna, 148* (T2-06)

Zwe, Ye Htut, *National University of Singapore* (T1-17*)

Zwietering, Marcel, *Wageningen University* (S15*)

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PAST EUROPEAN STUDENT TRAVEL SCHOLARSHIP RECIPIENTS

2014 – Erika Georget
2015 – Emily Jackson
2016 – Amanda Demeter
2017 – Christian Hertwig

2018 – Katrien Begyn and Giannis Koukkidis
2019 – Maria Gkerekou and Yifan Zhang
2020 – Alessia Delbrück and Hannah Pye

STUDENT AWARD COMPETITION RECIPIENTS

2009 **Overall:** Peter Rossmanith
Posters: Antje Frohling and Mary Pia Cuervo
2010 **Technical:** Rocio Morales-Rayas
Poster: Orla Condell and Shane Cooney
2011 **Technical:** Srianant Wanassen
Poster: Era Taludhar
2012 **Technical:** Srianant Wanassen
Poster: Srianant Wanassen
2013 **Technical:** Kai Reineke
Poster: Brenda Magajna

2014 **Technical:** Sungyul Yoo
Posters: Cristina Rodriguez and Renáta Kugler
2015 **Technical:** Bernhard Merget
Poster: Hend Al Gahmi
2016 **Posters:** Cristina Rodriguez and Ifigeneia Makariti
2017 **Technical:** Marcia Boura
Poster: Ifigeneia Makariti
2018 **Technical:** Lena Fritsch
Poster: Aurelien Maillet
2019 **Technical:** Krishna S. Gelda
Poster: Beatriz Nunes Silva

PAST LOCATIONS

2005 Prague, Czech Republic
2006 Barcelona, Spain
2007 Rome, Italy
2008 Lisbon, Portugal
2009 Berlin, Germany
2010 Dublin, Ireland
2011 Ede, The Netherlands
2012 Warsaw, Poland

2013 Marseille, France
2014 Budapest, Hungary
2015 Cardiff, Wales
2016 Athens, Greece
2017 Brussels, Belgium
2018 Stockholm, Sweden
2019 Nantes, France
2020 Meeting Cancelled

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