Advancing Beef Safety through Research and Innovation

An international conference organised by *ProSafeBeef*

A European Commission Research Project
(Food CT-2006-36241)

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Advancing Beef Safety through Research and Innovation

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Editors

Geraldine Duffy,

Ashtown Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland

George J. Nychas,

Laboratory of Microbiology and Biotechnology of Foods, Dept. of Food Science and Technology, Agricultural University of Athens, Athens, Greece

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This book compiles the proceedings produced from an international conference on "Advancing Beef Safety through Research and Innovation" which was organised as part of European Framework VI Research Project ProSafeBeef (Food CT-2006-36241)

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Preface

On behalf of the conference organising committee, I am delighted to present the proceedings of an international conference on beef safety "Advancing Beef Safety through Research and Innovation" which was held at Ashtown Food Research Conference Centre, Teagasc, Dublin, Ireland on 25th to 26th March 2009. The proceedings contains extended abstracts from 14 invited speakers, 14 abstracts from selected short oral presentations and 42 abstracts from poster presentations.

The conference brought a multi-disciplinary international team of researchers together with the stakeholders from across the beef farm to fork chain to discuss the latest innovations in beef safety research and how these can applied to improve beef safety. The topics addressed at the conference ranged from detection and tracking of microbial pathogens and chemical residues in the beef chain; development and application of quantitative risk assessment models to manage beef safety; development of novel and innovative approaches to control pathogens at key stages along the farm to fork beef chain as well as consumer attitudes and perception to beef safety.

The conference was organised as part of a European Commission integrated research project ProSafeBeef "Improving the Quality and Safety of Beef and Beef Products for the Consumer in production and Processing". This is a five year integrated research project, which commenced in March 2007 and is funded within the EU VI framework programme. It is co-ordinated by Teagasc, Ashtown Food Research Centre, Dublin and involves 41 leading research and industrial organisations from Europe, North and South America, Australia and New Zealand. This conference overviews many of the latest findings from this research project.

I would like to acknowledge the support of the Food Safety Authority of Ireland, the conference exhibitors and all the speakers, poster presenters, chairpersons and delegates for contributing to the success of conference. A special thanks to the local organising committee, Edel Deane, Robert Mooney, Orla Lynch and Kaye Burgess.

Geraldine Duffy
Head Food Safety,
Ashtown Food Research Centre,
Teagasc, Ashtown, Dublin 15, Ireland.

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European Food Safety Authority (EFSA) perspectives on microbial safety of beef

Marta Hugas and Ernesto Liebana,
Biological Hazards Unit, European Food Safety Authority,
Largo N Palli 5/A, Parma, 43100, Italy.

EFSA was established in order to provide scientific advice and technical support for legislation and policies in all fields which have a direct or indirect impact on food and feed safety. EFSA has the pivotal role in food safety risk assessment, and risk communication. The Scientific Panel on Biological Hazards (BIOHAZ) deals with questions on hazards of biological nature related to food safety, food-borne diseases (FBD) including transmissible spongiform encephalopaties, food microbiology and waste management. The work the BIOHAZ panel has carried out in the field of meat hygiene / safety (excluding BSE/TSE issues) could be further classified in several categories: relevant to (1) meat hygiene inspection, (2) microbiological meat hygiene, (3) decontamination treatments at slaughter level, (4) interface of animal welfare and food safety, and (5) food as a source of antimicrobial resistance.

With reference to inspection after slaughter, the BIOHAZ Panel has answered mandates on the revision of meat inspection protocols for beef and veal, with an emphasis on hazards such as *Mycobacterium bovis* and *Cysticercus*, and in particular in reference to the subject of risk-based meat inspection without incisions. The Panel also worked on a self-task to provide an overview of different concepts in the area of microbiological criteria and targets in the food chain (Food Safety Criteria and Process Hygiene Criteria). The conclusion was that these criteria are useful for validation and verification of HACCP-based processes.

The original source of pathogens that presently cause most human FBD are farm animals that show no symptoms of illness, but which are fecal shedders. The main food-borne hazards associated with bovine farming are *Salmonella* spp., human pathogenic-verotoxigenic *Escherichia coli* (VTEC), thermophilic *Campylobacter* spp., *Taenia saginata cysticercus* and *Cryptosporidium parvum / Giardia duodenalis*. Although various foods can serve as sources of FBD, meat and meat products are an important source of human infections. In the EU in 2007, reported percentages of fresh bovine meat samples positive for the main microbial food-borne pathogens were: Campylobacter 0-2.4 %, Salmonella 0-6.7 % and VTEC 0-2.8 %. Data reported to EFSA in 2006 indicated that "unspecified" meat products were implicated in 6.7 % of the salmonellosis outbreaks, with only 1 outbreak reported as due to bovine meat; "unspecified" meat products were implicated in 23 % campylobacter outbreaks; and beef was implicated in 6.3 % of VTEC outbreaks.

Understanding in quantitative terms of the importance of meat and meat products (including beef) compared with other types of food, and other possible sources is quite limited. The BIOHAZ Panel has recently published an opinion on source attribution, and the benefits and drawbacks of different techniques (i.e. outbreak investigations, epidemiological investigations, microbiological typing, mathematical modelling of relative exposures and expert opinions) that can be considered for such attribution studies.

The BIOHAZ Panel has also adopted opinions on the relationship between welfare of cattle and food safety. It is generally considered that provision of optimal animal welfare leads to a reduction of the food safety risks associated with the resulting foods of animal origin. However, some on-farm practices beneficial for animal welfare, may increase risks of a greater survival rate of, and/or exposure to, and/or spread of, foodborne pathogens in farm animals. Further research on the quantitative relationship between on-farm factors affecting animal welfare, on one hand, and any food safety hazards associated with the resulting carcasses, on the other, should be encouraged in order to facilitate and improve quantitative risk assessment in the context of the meat chain.
Emerging of resistance to important antimicrobials (AMR) [such as quinolones, extended-spectrum cephalosporins, and meticillin] amongst zoonotic and/or human pathogens is a public health concern, foodborne aspects of which have been recently evaluated in a BIOHAZ opinion. The opinion concluded that the present extent of exposure via food to

AMR bacteria was found to be difficult to determine, and the role of food in the transfer of resistance genes insufficiently studied. Nevertheless, foodborne bacteria, including known pathogens and commensal bacteria, display an increasing, extensive and diverse range of resistance to antimicrobial agents of human and veterinary importance, and any further spread of resistance among bacteria in foods is likely to have an influence on human exposure. In the case of *Salmonella,* contaminated beef is prominent in this regard. Also, cattle are a major VTEC reservoir, and resistant strains may colonize humans via contaminated bovine meat more commonly than from other foods. In addition, beef appears to be a source of human exposure for cephalosporin resistance. Animal-derived products (including bovine meat) remain a potential source of meticillin-resistant *Staphylococcus aureus* (MRSA). Food-associated MRSA, therefore, may be an emerging problem that is currently being addressed by the BIOHAZ Panel in a self-mandate.

The role of the FVO in relation to chemical contaminants in meat

Ken Elliott, MVB (Acting Head of Unit F5, FVO)

The FVO is a Directorate (Dir. F) of the Directorate General for Health and Consumers (DG SANCO) of the European Commission. SANCO is located in three sites; Brussels, Luxembourg and Grange, near Trim, Co. Meath, Ireland, where the FVO is located.

The role of the FVO is summarised in its mission statement, which can be found on the FVO website at http://ec.europa.eu/food/fvo/index_en.cfm

The mission of the FVO is, through its evaluations, to:

- ***promote effective control systems in the food safety and quality, veterinary and plant health sectors;***
- ***check on compliance with the requirements of EU food safety and quality, veterinary and plant health legislation within the European Union and in third countries exporting to the EU;***
- ***contribute to the development of EU policy in the food safety and quality, veterinary and plant health sectors, and to inform stakeholders of the outcome of evaluations.***

To put this in context, it is important to understand the responsibilities of the various players in the food and feed production chains. These can be summarised as follows:

- It is the responsibility of the operator (primary producer, processor, transporter, wholesaler, retailer, etc.) to observe the rules;
- It is the responsibility of the Competent Authorities in Member States and Third Countries to ensure that the rules are observed and
- It is the role of the European Commission to verify that the Competent Authorities are enforcing the rules.

The FVO is organised into seven Units, under the Director:

F1: Country profiles and co-ordination of follow-up;
F2: Food of animal origin – mammals;
F3: Food of animal origin – birds and fish;
F4: Food of plant origin, plant health; Processing and distribution;
F5: Animal nutrition (inc. TSEs): Import Controls; Residues;
F6: Animal health and welfare;
F7: Quality, planning and development.

The total staff of the FVO is approximately 150, of which approximately 80 are inspectors. The professions represented include veterinarians (the largest group), agronomists, chemists, engineers, economists, environmental health officers and lawyers.

Units F2, F3, F4, F5 and F6 perform verification missions to Member States, Third Countries and to candidate countries (in preparation for accession to the EU). Unit F1 also performs missions to update the Country Profiles maintained for each Member State and to follow-up outstanding issues arising from verification missions. In addition, the FVO performs a number of other functions – of most relevance to this paper is the evaluation of Third Country annual National Residue Control Plans.

The inspection work of the FVO can be classified into the following categories:

General Audits and Specific Audits:

Regulation (EC) 882/2004 (on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules) has had a major impact on the work of the FVO. Previously, Member States were required to ensure compliance with specific requirements for each sector, but were not required to have control systems in place to do so. Regulation 882/2004 sets out the requirements for official control systems, including, *inter alia,* risk based prioritisation of checks, provision of instructions and training for control staff, reporting, verification of the effectiveness of controls (supervision and internal and/or external audit) and accreditation of laboratories. The Regulation also requires Member States to carry out their control programme on the basis of a Multi-Annual National Control Plan (MANCP) and to produce annual reports on their control activities.

Article 45 of the Regulation requires the FVO to carry out General and Specific Audits of the control systems in Member States; These commenced in 2008, following 2 pilot General Audits completed in 2007.

As stated in Article 45, the main purpose of a General Audit "shall be to verify that, overall, official controls take place in Member States in accordance with the multi-annual national control plans referred to in Article 41 and in compliance with Community law."

As implemented in the current, evolving model, *General Audits* consist of a number of *Specific Audits,* performed in a similar manner to traditional inspection missions but with an expanded scope, placing particular emphasis on the horizontal elements of the control system in the sector. These Specific Audit missions may be supplemented by additional missions to examine targeted elements of the control system (e.g. internal/ external audit, staff training programmes, etc.).

Specific Audit reports, containing the sector specific findings, conclusions and recommendations are produced, issued to the Member State for comment and, when finalised on the basis of these comments, published on the web.

General Audit reports are drafted on the basis of the control system findings from the Specific Audits together with an assessment of the MANCP, the Member State's annual report of control activities and supplementary information collected in the various meetings and additional missions to the Member State. The report contains a summary of relevant findings, conclusions and recommendations to improve the control system.

Inspection missions:
Before the advent of General Audits, these formed the bulk of missions performed. These missions are performed to verify application of sector specific requirements. All Third Country missions fall into this category.

Inspection missions are planned and executed following audit principles. Audit is defined in Regulation 882/2004 as "a systematic and independent examination to determine whether activities and related results comply with planned arrangements and whether these arrangements are implemented effectively and are suitable to achieve objectives".
An Evaluation Plan and a Pre-Mission Questionnaire are sent, in advance of the mission, to the relevant competent authority in the country to be visited. Missions begin with an opening meeting where the scope of the audit is outlined and discussed and the itinerary for the mission is confirmed.

The mission team visits a number of locations (central, regional local offices, farms, establishments, laboratories, etc.) to assess the implementation of the relevant requirements, on-the-spot. Preliminary findings and conclusions are communicated to the competent authority during a final meeting.

On return to base, a draft report is compiled, detailing the findings of the mission and the conclusions based on these. Recommendations aimed at remedying deficiencies are made. The draft report is sent to the competent authority for comments and then finalised, after which it is issued to all Member States and to the European Parliament and to stakeholders via web publication.

Competent authorities are requested to provide a plan outlining the actions intended to address the report's recommendations. This Action Plan, and any subsequent updates requested by the lead inspector, is also published.
Unit F1 then follow-up outstanding recommendations on a country basis. In the case of serious or persistent deficiencies, the matter may be referred to the Commission legal services for infringement proceedings.

Candidate Country missions:
These are missions to countries in the process of acceding to the EU and are intended to assess progress in implementation of the Community acquis in relation to controls of feed and food.

These missions are performed in the same way as other missions. As the mission reports feed into the accession negotiations, they are not published.

Fact Finding missions:
Occasionally, the FVO undertakes fact finding missions. In most cases these are intended to gather information required to inform the legislative process when new or amended legislation is being drafted.

General Review missions:
These are missions performed by Unit F1 to follow-up outstanding recommendations and to update Country Profiles (documented descriptions of the control systems in Member States and, in the future, Third countries).

In addition to the above, the FVO produces overview reports

containing findings and conclusions collated from a series of missions. Reports are produced annually of the implementation of pesticides monitoring in Member States.

The philosophy adopted when performing inspection missions on all topics can be summarised by three questions:

- Is there a system?
- Can it work?
- Does it work?

Following the introduction of Regulation 882/2004, a further question can be added: Why does it work/not work?

Residue controls:

The residue team in the FVO consists of 7 inspectors and one secretary. The size of the group has gradually increased in the past few years in response to the increasing workload from inspections and the evaluation of Third Country monitoring plans. The group has the capacity to perform approximately 15 missions per year. In addition, members of the team frequently accompany other Units carrying out sectoral inspections where residue control constitutes an important element of the overall control mechanism (e.g. aquaculture).

In 2008 – 2010, the emphasis is on Member States within c. 9 missions per year included in General Audits. When the first cycle of General Audits is completed, the team will re-focus on controls in Third Countries.

Residue missions are performed as described above. The following extracts, from the standard Evaluation Plan for Third Country missions, illustrate the mission objectives and scope and the type of on-the-spot visits included in the itinerary.

Objectives of the evaluation

The objectives of the mission will be to evaluate:

1. the national measures put in place, and their operation, aimed at the control of residues and contaminants in live animals and animal products, including;
2. the controls on the distribution and use of veterinary medicinal products and feed additives, the use of which may give rise to residues in such products.
3. the measures taken in response to the outcome of any previous FVO missions during which the above control systems were evaluated/reviewed.

Evaluation scope

The evaluation will address the ability of the competent authorities to deliver the required standards and will cover the following sectors: cattle, sheep and goats, pigs, poultry, horses, aquaculture, rabbits, farmed game, wild game, milk, eggs and honey (delete as appropriate):

- **Competent Authorities**
  - ▷ Structure and resources
  - ▷ Organisation and co-ordination of control activities
- **Residue control programmes**
  - ▷ Legal basis of national residue control plan
  - ▷ Scope of the national residue control plan
  - ▷ Procedures for the implementation of the national residue control plan
  - ▷ Data management in the national residue control plan
  - ▷ Follow-up investigations and corrective actions taken
  - ▷ Design and implementation of the hormone free cattle programme (where appropriate)
- **National Maximum Residue Limits or tolerances (if applicable) for**:
  - ▷ veterinary medicinal products (pharmacologically active substances)
  - ▷ pesticides and contaminants
- **Performance of the laboratories**
  - ▷ Accreditation and quality assurance
  - ▷ Validation of methods
- **Controls on the distribution and use of veterinary medicinal products**
  - ▷ Authorisation of veterinary medicinal products
  - ▷ Legal basis for controls on the distribution and use of veterinary medicinal products
  - ▷ Veterinary medicinal products banned for use in food producing animals
  - ▷ Off-label use of veterinary medicinal products in food producing animals
  - ▷ Prescription veterinary medicinal products and 'over the counter' veterinary medicinal products
  - ▷ Withdrawal periods for veterinary medicinal products
  - ▷ Medicines records
  - ▷ Veterinary medicinal products used for the production of medicated feedingstuffs including the use of additives with a histomonostatic or coccidiostatic action

Organisation - meetings and sites to be visited

- ✓ an ***initial meeting*** and a ***final meeting*** between the mission team and your services including the competent authorities responsible for: (a) residues

control in live animals and animal products; (b) veterinary medicinal product authorisation, manufacture, distribution and use and; (c) controls on the distribution of veterinary medicinal products and those feed additives, the use of which may result in residues in food of animal origin.

- ✓ at least two regional authority offices;
- ✓ two pharmacies and/or retailers of veterinary drugs;
- ✓ one wholesaler of veterinary;
- ✓ private veterinary practitioners;
- ✓ one feed mill authorised for the production of medicated feedingstuffs;
- ✓ two processing establishments (e.g. slaughterhouses);
- ✓ at least two farms;
- ✓ two national laboratories carrying out residue analyses under the national residue control plan.

In addition, the residue team is responsible for the evaluation of Third Country National Residue Control Programmes (NRCP). Approximately 84 countries export, to the EU, commodities for which an approved NRCP is a prerequisite. While some of these countries export a single commodity, most export multiple commodities. Plans plus the results of the previous year's programme must be submitted on an annual basis (deadline for submission 31 March).

Following evaluation, each file is submitted to the Standing Committee on the Food Chain and Animal Health, where a decision on approval is made.

Consumer perception of beef safety

Wim Verbeke[1], Lynn Van Wezemael[1], Marcia D. de Barcellos[2], Jens Kügler[2], Klaus G. Grunert[2]

1 Ghent University, Department of Agricultural Economics,
Gent, Belgium; wim.verbeke@UGent.be.

2 Aarhus University,
MAPP Centre, Aarhus, Denmark

Introduction

Perceived risks associated with beef at the consumer level relate mainly to safety and health perceptions (Verbeke and Vackier 2004; McCarthy and Henson 2005). Some recent studies have put the perceived safety risk related to beef into perspective, indicating that the intention to consume beef is gradually becoming more influenced by nutritional than by safety concerns (Verbeke et al 2007; Da Fonseca and Salay 2008). European beef producers and policy makers have done considerable efforts to recover beef consumption after the BSE crisis. The development of more safety control and traceability systems and the provision of information to consumers have been important in improving beef safety perception (Verbeke, 2001; Angulo and Gil, 2007). Consumers, however, have different ideas about food safety compared to experts, and both differ often significantly. The objective of this study is to explore consumer perceptions and expectations concerning beef safety. This includes an investigation of how consumers evaluate beef safety, who they perceive responsible for beef safety, and what kind of information they expect to receive concerning the safety of beef and beef products.

Materials and methods

Eight focus group discussions, with seven to nine participants each, were conducted in the capital cities of Germany, Spain, France and the United Kingdom during May 2008. These countries were selected because of their market volume, both in terms of beef production and beef consumption, and strategic geographical location within the EU. Participants were beef eaters (with a frequency of at least once a week) and beef shoppers without aversion to beef. Both "real" (muscle meat type) and "hidden" (minced and processed meat type) beef eaters were included. The first section of the topic guide asked consumers about their perception of and demand for beef safety, beef healthiness and related information. The participants also completed a questionnaire about socio-demographic characteristics (age, gender, marital status, children) and background attitudes to new food products and technologies. The full transcripts of the focus group discussions in the local languages were used as data for the content analysis, which was performed with NVIVO7.

Findings

Safe versus unsafe beef

Consumers defined beef safety mostly in relation to their personal health. Safe beef was perceived as beef that is not harmful for consumers' health: "It shouldn't be making me ill". Beef safety was thought of as a precondition that allowed for the consumption of beef products without the need of being concerned. Consumers acknowledged that it is hard to decide whether beef is safe or not. A major factor was the heritage of the occurrence of international food scares and the attendant media attention. Although most consumers indicated not to think too much about BSE while purchasing and consuming beef, it was still a prominent issue for some, particularly in the UK. A second source of difficulties in evaluation beef safety was the admitted lack of knowledge about beef safety. Consumers acknowledged having little objective or factual knowledge about how to evaluate the safety of beef. This caused consumers feeling uncertain and being afraid for unknown consequences when consuming beef. A third source was related to a general distrust in the beef production chain. This distrust was either related to the production system, the actors and/or the sources of information. Intermediaries, slaughterhouses, meat processing industries and beef packaging companies were generally considered not to be reliable actors and trustworthy

information sources. Independent institutions who assign quality labels and certificates could benefit from a higher degree of consumer's confidence. Brands were trusted, just as supermarkets and traditional butcher shops, which were – opposed to kebab stores, for example – thought of as reliable actors in the beef safety chain. Also consumer organisations and retailers' recommendations about beef safety were trusted. Interestingly, cattle farmers were not spontaneously mentioned as either trusted or distrusted actors responsible for beef safety. To assess beef safety, consumers indicated to use specific information cues or beef attributes. Cues or attributes perceived to signal safe beef were origin or quality labels, brands, as well as appearance and fresh or frozen beef. The most relevant cues or attributes perceived to signal rather unsafe beef were expired expiry data, foreign origin, minced or offal, further processed, as well as price (too cheap).

Beef safety information and responsibility

The amount of requested beef safety information was not fully clear from the focus group discussions. Part of the consumers felt there is not enough information about beef safety, while others stated to be faced with an overload of (sometimes conflicting) information which might increase the difficulties to assess beef safety. Also the use of beef safety information diverged between consumers. Some were looking quite actively for information, others did not pay any attention to it. The responsibility for beef safety was put mainly on actors that are situated early in the beef production chain. The conditions in which animals are born, raised and killed were thought to be important determinants of beef safety. In terms of actors, mostly farmers, veterinarians, inspectors, abattoirs and scientists were considered to be responsible for beef safety in the first place. Independent institutes should control beef safety and grant certificates and labels based on beef safety. The fact that consumers considered "available" beef as safe beef, suggests a good degree of trust by consumers in the safety regulations and controls in the current beef chain. The focus group participants indicated to trust that the control over beef safety is executed by competent people and institutions (though especially in their own country) and that government beef safety regulations and controls are adequate and trustworthy, both from the national government as from the EU.

Conclusions

This qualitative exploratory study indicates that in general, consumer trust in beef safety is relatively high, despite distrust in particular actors. Comparing the actors' perceived trust and preferable assigned responsibility for managing beef safety, it is striking that actors in the upstream parts of the beef chain (production and processing) were trusted the least, while consumers wanted them to take prime responsibility for beef safety. The more trusted actors were situated in the distribution and retailing part of the beef chain, thus closer to consumers.

Acknowledgement

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Assessing and managing the risk associated with beef pathogens using Quantitative Microbial Risk Assessment Models

Geraldine Duffy, Ashtown Food Research Centre, Teagasc, Ashtown Dublin 15, Ireland

This paper overviews the application of quantitative microbial risk assessment models (QMRA) in assessing and managing the risk attributed to pathogens in beef and describes QMRA's under development as part of the EU *Prosafebeef* project

Background

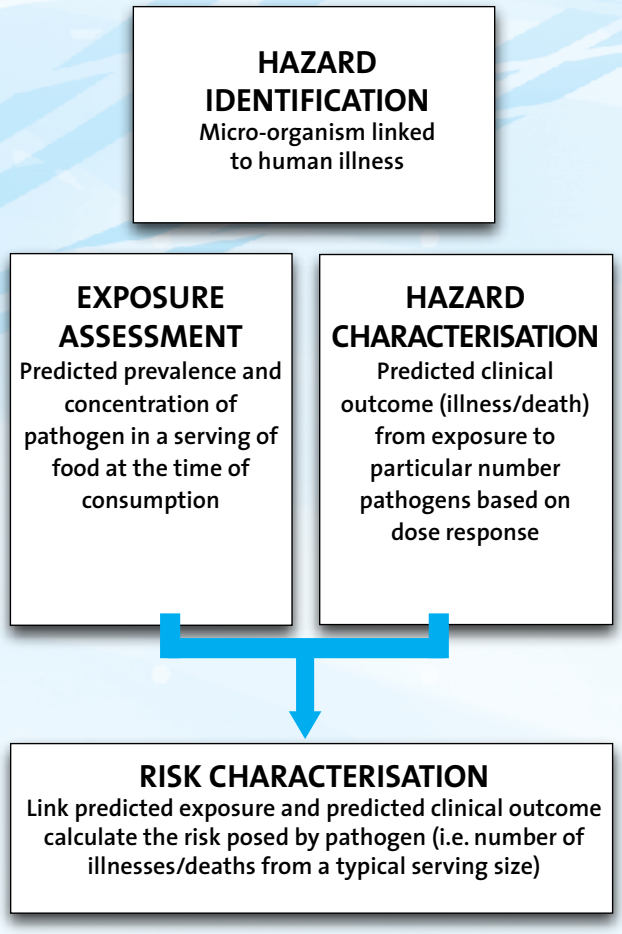
It is well recognised that food production animals including bovine animals shed a diverse range of micro-organisms in their faeces, some of which may be pathogenic. Human illness can occur as a result of direct contact with faecal material (hand to mouth route) or indirectly through consumption of faecally contaminated food or water. During beef slaughter and dressing, pathogens can potentially be transferred from faecally contaminated bovine hide or the gastro-intestinal contents onto the beef carcass. Once pathogen contamination

has occurred on the carcass, subsequent steps in beef processing, distribution, final preparation will impact on the prevalence and concentration of pathogenic bacteria on the beef at the stage of consumption and thus the actual risk to the consumer. Quantitative microbial risk assessment (QMRA) models as outlined by Codex Alimentarius Commission (Codex, 1999) can be used to measure and manage the public health risk posed by pathogens on food (Fig 1).

Published QMRA's for pathogens in beef

To date published microbial risk assessment for pathogens in beef have focused primarily on *E. coli* O157 in beef burgers (Teagasc, 2006; Ebel *et al.* 2004; Cassin *et al.*, 1998) or steak tartare (Nauta *et al.*, 2001). The developed models have in general been pathogen process models and covered the chain from either the farm or from when animals were presented for slaughter through to consumption. The outputs for models describing the risk from *E. coli* O157 in ground beef are summarised in Table 1. The models have been also been used to assess the impact of various parameters along the beef chain on overall risk (Table 2) and thus could be used to direct risk management actions. Efforts have also been directed at using the model to set performance objectives for pathogen in the chain (Duffy *et al,* 2006b),

Figure 1. Microbial Quantitative Risk Assessment (MQRA)



QMRA's under development for pathogens in beef

The is much scope to improve and extend the developed models both by incorporating improvements in modelling tools and available epidemiological data on a range of clinically significant pathogens and to use the models in a practical manner to assess and manage risk

The focus of ongoing work in *Prosafebeef* is aimed at developing a number of framework QMRA models to assess the risk and manage the risk posed by selected beef pathogens. The framework models can then be populated with specific data reflective of specific regional epidemiological data or specific practices in the chain

The microbial hazards are defined as clinically significant Verocytotoxigenic *E. coli* (*E. coli* O157, O26, O111, O145, O103), *Listeria monocytogenes, Salmonella* and *Campylobacter*. The exposure assessment will be based on data on the prevalence, concentration (CFU/g) in typical servings of selected beef and beef products. As it is recognised that not all strains of pathogens are equally infectious for humans molecular methods are being used to assess the virulence potential of strains isolated from beef and this will be factored into the risk model.

The exposure assessment models will cover the chain from animals are presented for slaughter through to consumption. The models will driven by data on the prevalence and numbers on the hides of animals presented for slaughter. This will be supported by microbial data on the impact of key steps in the chain on prevalence, concentration and molecular data on virulence potential.

Table 1. **Probability of illness from consumption of a single serving of beef contaminated with *E. coli* O157:H7**

Beef	Location	Population group	Illness	HUS	Mortality	Model
Ground beef	North America	Average adult	5.1×10^{-5}	-	-	Cassin *et al.*, 1998
Ground beef	North America	Children	3.7×10^{-5}	3.7×10^{-6}	1.9×10^{-7}	Cassin *et al.*, 1998
Ground beef	USA	Average population	9.6×10^{-7}	4.2×10^{-9}	5.9×10^{-10}	Ebel *et al.*, 2004
Ground beef	USA	Average population June – September Oct – May	 1.7×10^{-6} 6.0×10^{-7}			Ebel *et al.*, 2004
Ground beef	USA	Children	2.4×10^{-6}	-	-	Ebel *et al.*, 2004
Beef Burgers	Ireland	Average adult	1.1×10^{-6}	4.2×10^{-9}	5.9×10^{-10}	Ebel *et al.*, 2004

* note rate is for elderly people. Adapted from Duffy et al., (2006)

Table 1. Impact of various parameters along the beef chain on the probability of illness in consumed ground beef servings as determined by different risk assessment models

	Cassin *et al.*, (1998)	Ebel *et al.*, (2004)	Duffy *et al.*, (2006)
Sensitivity analysis of impact of factors on probability of illness in ascending order of importance	Concentration of pathogen in faeces	Surface area of carcass contaminated	Initial count on bovine hide
	Host susceptibility	Effectiveness of carcass chilling	Cooking temperature
	Carcass contamination factor	Max. population of *E. coli* O157 in ground beef serving	Temperature abuse during transport and storage.
	Cooking preference	Home storage temperature	Hide to carcass contamination factor
	Retail storage temperature		Hide Prevalence
	Decontamination during primary processing		Change in numbers at carcass chilling
	Growth during processing		
	Retail storage time		
	Prevalence in faeces		
	Mass ingested		

Adapted from Duffy *et al*, 2006

Work to date has focused on generating data to be included in the exposure assessment models for the four selected pathogens. A review of the literature on pathogens in the beef chain some of which is published by Rhoades *et al,* 2009 has highlighted gaps in data needed to develop the models. Gaps identified include a lack of qualitative (prevalence) and quantitative (concentration) data on the pathogens on the bovine hides of animals presented for slaughter and the rate of pathogen transfer from hide to carcass. Previously developed models have estimates this transfer rate using a calculated cross contamination factor (Cummins *et al* 2006). There is also a lack of data on the human virulence of some pathogens transmitted by beef. To address these gaps microbiological and molecular risk assessment studies (virulence potential) have been undertaken. Using a sponge swab technique, an area 400cm^2 on the brisket area of the bovine hide of animals presented for slaughter were examined for prevalence and concentration of the four pathogens. The carcasses of the same animals were also examined, after dehiding and before chilling, for the same pathogens to estimate the rate of pathogen transfer. Standard ISO protocols are being used to examine the samples for *Salmonella* (ISO 6579), *L. monocytogenes* (11290-1, 11290-2)

and *Campylobacter* (ISO 10272-1, 10272-2) while a PCR based approach is being used to detect VTEC serogroups (McCann et al, 2008). Molecular sub-typing tools (PFGE pulsenet protocols, MLST and MLVA) are being used to genomically compare isolates and to categorically assess the patterns of cross contamination. Isolates recovered from beef are being assessed for the presence of known and putative virulence genes using PCR.

The presentation will overview some of the data generated on pathogen prevalence, concentration on tracked bovine hide and carcasses and the virulence potential of pathogens isolated from beef. This data is also presented in a number of abstracts in this proceedings (Chiarini *et al,* poster 8; Denis *et al,* poster 10; Ioannis *et al,* poster 18; Khen *et al,* poster 26 and Thomas *et al,* poster 37). A noteworthy finding was that O157 remains the most dominant VTEC serogroup on beef though emerging serogroups were isolated. Strains of serogroup O157 were in general virulent, containing *eae* and *vt*2 and *vt*2C profile which is often linked to haemolytic ureamic syndrome (HUS). Many of the emergent serogroups lacked virulence genes and in those strains which were virulent the predominant profile was *vt*1 with *eae* which is generally associated with less serious illness.

The data will be used to generate distributions to populate the exposure assessment model. The aim is to use the developed models in a practical manner to assess the impact of novel interventions on overall reduction of microbial risk and to set performance objectives in the chain.

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The Use of Predictive Microbiology in Risk Assessment

Kostas Koutsoumanis, Dept. of Food Science & Technology, Lab. Of Food Microbiology and Hygiene. School of Agriculture, Aristotle University of Thessaloniki,
P.O. Box 256, Thessaloniki GR-541 24, Greece.
e-mail: kkoutsou@agro.auth.gr,
Tel-Fax. + 30 2310 – 991647

Quantitative microbiological risk assessment (QMRA) is a newly effective tool in food safety management. Assessing health risk from food-borne pathogens requires the determination of the number of the pathogen at the time of consumption (exposure assessment). Predictive microbiology allows estimation of pathogen numbers in foods based on known levels at the starting point of the risk assessment and the conditions during processing, storage and distribution. In QMRA however, models of bacterial growth need to be applied in a probabilistic way, in order to predict the probability that a critical concentration is reached within a certain amount of time. For this the uncertainty and variability of factors affecting pathogen growth should be taken into account and incorporated in mathematical models. In addition, since in "real" conditions contamination of foods with pathogens occurs at very low levels (close to one cell) the variability in single-cell behaviour should be taken into account. Finally, interaction of pathogens with the natural microflora of foods as well as the probability of spoilage before consumption should be considered in risk assessments so that risk is not overestimated. This review presentation discusses the principles of QMRA and predictive microbiology and the effective approaches and pitfalls in the application of bacterial growth modelling in microbial food safety risk assessment

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Quantitative risk assessment of *E. coli* O157:H7 in processed beef products in Ireland

E. Cummins[1], F. Butler1, P. Nally[1], S. O'Brien[1] and G. Duffy[2]
[1]UCD School of Agriculture, Food Science and Veterinary Medicine, Belfield Dublin 4, Ireland.
[2]Ashtown Food Research Centre, Teagasc, Dublin, Ireland.

A qualitative risk assessment model was developed to model the prevalence and counts of *E. coli* O157:H7 in Irish minced beef from the abattoir through to human consumption. The model was developed in a spreadsheet (Microsoft Excel 97) with the @Risk add-on package (Palisade Software, Newfield, N.Y.). The Monte-Carlo simulation model combined all the input parameters and was run for 100,000 iterations of the model. The mean simulated prevalence in fresh burgers was 2.9% and the mean simulated prevalence for frozen burgers was 2.2%. These results compare favourably with results of a retail survey (mean value 2.3%). The simulated counts in frozen burgers (mean of -0.22 $\log_{10}$ CFU/g) are less than those for fresh burgers (mean of 1.96 $\log_{10}$ CFU/g), this is mainly due to the greater probability of temperature abuse of the fresh burgers on retail display. The simulated counts compare favourably with results of a retail survey where counts varied from 0.51 Log_{10} CFU/g – 4.03 Log_{10} CFU/g. Transposing this exposure data through a dose response model yields an estimate of the probability of illness caused by exposure to *E. coli* O157:H7 in beef burgers. The simulated mean probability of illness from a contaminated serving of fresh beef was –5.94 log (i.e. 10-5.94 = approximately 1 in 1 million chance). A sensitivity analysis indicated the initial count on bovine hides and the initial hide prevalence as important parameters indicating the importance of minimising contaminated bovine hides entering the slaughter plant. Cross contamination at the hide removal stage was also an important parameter indicating were producers might focus efforts to reduce risk. Consumer behaviour in terms of cooking temperature and temperature abuse during transport and storage also plays an important part in dictating the final risk value, indicating the important role consumers have to play in ensuring their food is safe for consumption.

Prevalence and virulence determinants of Verocytotoxigenic *Escherichia coli* in Brazilian bovine hides and carcasses

Verônica S. Fogo[2], Kathelin M.S. Lascowski[1], Gabriela A. Siqueira[1], Eb Chiarini[2], Mariza Landgraf[2], Maria Teresa Destro[2], Bernadette D.G.M. Franco[2], Beatriz E.C. Guth[1]

[1] Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo, SP, Brazil;

[2] Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil

The prevalence and virulence profiles of *Verocytotoxigenic E. coli* (VTEC) from cattle hides and on corresponding beef carcasses in a processing plant in Brazil were determined. Hides and pre-chilled carcasses sponges were collected from cattle (n=198) slaughtered at an abattoir located in the northwest region of São Paulo State, Brazil, between January and December 2008. The samples were enriched and PCR was used as an initial screening for detecting *vt*1, *vt*2 and *eae* sequences. All the samples were submitted to IMS using Dynabeads for O157 followed by plating onto selective media. *vt* positive samples were further submitted to IMS O26, O103, O111 and O145 serogroups. Presumptive colonies were confirmed for vt and by serogroup specific PCR. Isolates were confirmed as *E. coli* by biochemical assays, and tested for *eae* and *ehx*A. The use of enrichment broth followed by PCR detected *vt* in 61% (120/198) of the animals, 57% (113/198) originated only from hide and 4% (7/198) from pairs of hide and carcass. Using the enrichment broth followed by isolation and PCR, a total of 56 VTEC strains were recovered from 40 animals, corresponding to 33% (40/120) of the *vt* positive samples. Thirty one isolates belonged to serogroup O157 and were recovered from 26 different animals (hide = 21, carcass = 2, hide + carcass = 3). Twenty-five non-O157 strains were recovered from 17 animals (hide = 14, hide + carcass = 3). Most (77%) of the strains carried only *vt*2, but *vt*1 *vt*2 and *vt*1 genotypes were also identified. All O157 strains carried eae and *ehx*A. Among the non-O157 isolates, 84% carried *ehx*A and none of the isolates were positive for *eae*. The presence of VTEC strains on hides and/or carcass of cattle identified in this study pose a risk to public health.

Challenges and opportunities for transfer of knowledge from research to the beef industry

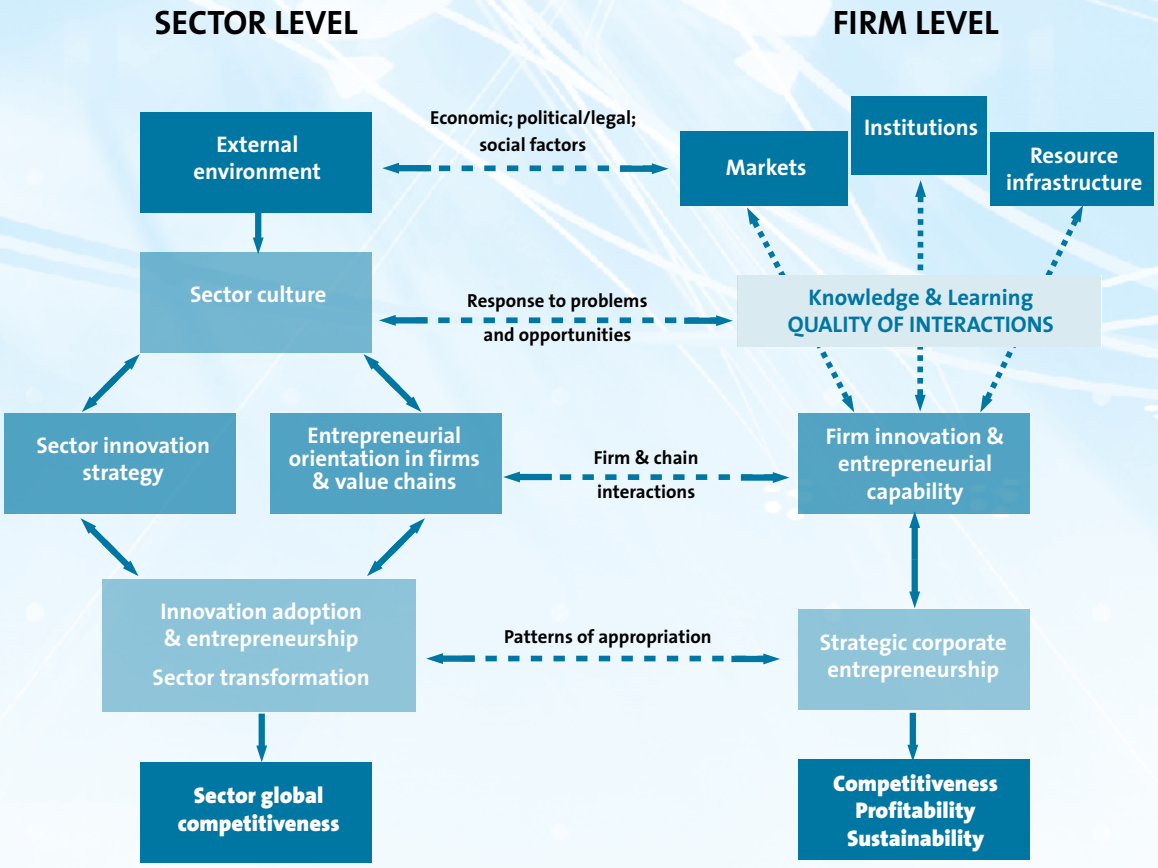
Ian Jenson,
Meat & Livestock Australia

A prevailing paradigm in science research is that it should be applied for the benefit of individuals, corporations and societies. In the context of the beef industry, and funding of applied research associated with beef production, processing or value-adding, researchers and funding agencies have a responsibility to ensure that the research conducted will provide information that is required to fill a significant knowledge gap and furthermore, that the results will be applied to meet some industry need. The development of research strategies and choice of subjects for research projects is beyond the scope of this paper which will concentrate on how research results can be successfully applied to industry needs, using a beef industry example.

Failures in innovation systems

The traditional approach to the dissemination of knowledge by scientists is to regard other scientists as the primary target for knowledge transfer, accomplished through publication of research reports in the academic literature. While this form of knowledge dissemination has its place, it cannot be relied upon as a means of transferring knowledge to the industry. Even if industry-oriented publications are produced, they may still not be effective in reaching an industry that is not print-oriented, generally does not value knowledge divorced from practise, may not perceive a need for the information being provided, or has an inability to make use of the information.

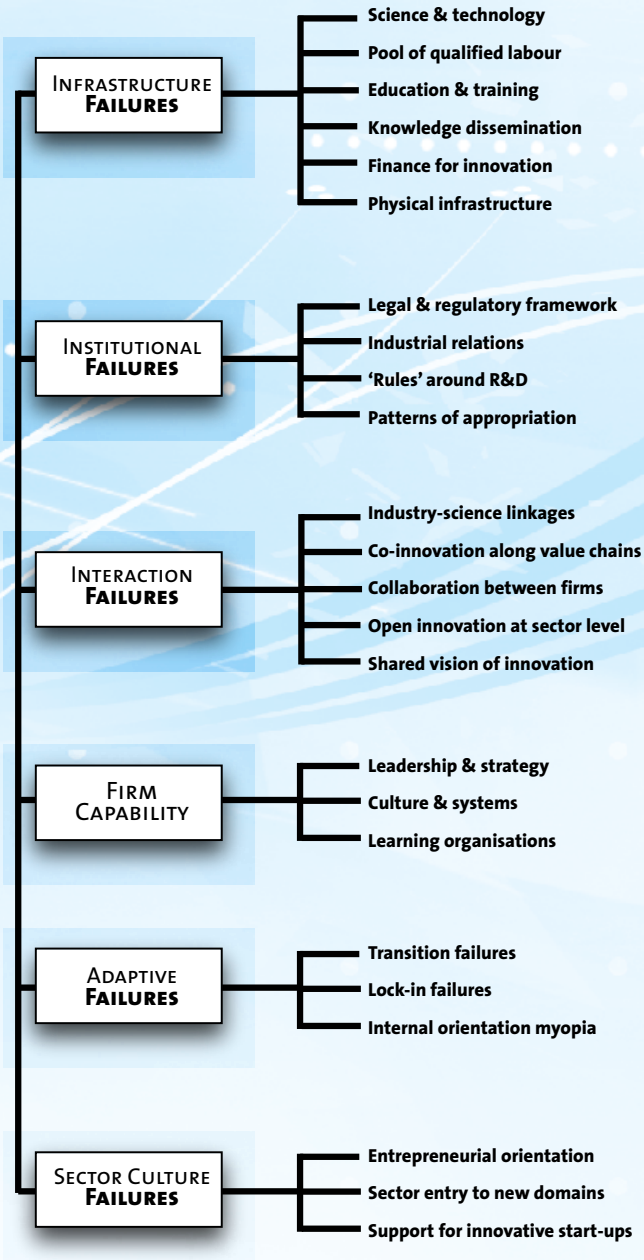
Figure 1. Sectoral innovation and entrepreneurship system



Transfer of knowledge must be understood in the context of the industry and may be usefully considered in the context of the "innovation system" of the industry (1,2). Innovation is considered at the level of the industry sector, of the individual firm and the interactions between the two (figure 1). The innovation system model, which is in an early stage of development, provides a framework for considering innovation failures (figure 2), not all of which may be relevant to a particular innovation scenario.

Failure of innovation within a sector or within a firm is viewed, by this model, as a failure of the innovation system, which can be addressed through identification and correction of the relevant system element(s).

Figure 2. System failure analytical framework



Innovations in the Australian red meat industry

Concurrently with the development of the innovation system model, and to an extent, informing its development, the model's developer was a senior manager with Meat & Livestock Australia (MLA), responsible for off-farm R&D. MLA is the R&D management (and marketing support) organisation for the Australian red meat industry (3). Research is conducted in a number of areas relevant to red meat processing, in cooperation with universities, government research organisations and consultants. A key role for MLA is ensuring that the industry benefits from the R&D conducted. MLA is therefore concerned, not just with the transfer of knowledge, but the application of knowledge for industry benefit. Contribution to the development of industry's innovation strategy and evaluation of the impact of innovation are also part of MLA's remit

From Science to Application: the refrigeration index

The refrigeration index (RI) is a tool for assessing the microbial response to a meat chilling process based on predictive microbiology (4). The predictive microbiology underpinning the RI was developed with funding from MLA, on the basis that predictive microbiology was an emerging technology relevant to food safety, and could be applied to microorganisms relevant to the red meat sector. The industry faces many challenges every day, some of them of a technical nature. One problem, for which predictive microbiology appeared to offer some insight, was the safety of the hot boning process (removing primals from a hot/warm carcase and chilling them). The competent authority was concerned about the growth of pathogenic microorganisms on the relatively large surface area, that sometime chilled relatively slowly, compared to traditional carcase chilling. MLA convened an 'expert' panel, comprising representatives of the competent authority, technical managers of hot boning processors, the industry representative body, food safety specialists and predictive microbiologists. The purpose of this group was to determine how hot boning could be safely conducted. The panel chose to utilise an *Escherichia coli* predictive model, using time and temperature as the only variables (pH, water activity and lactate were set to realistic 'worst case' values) and defining acceptable values for the resultant predicted growth (called the refrigeration index- actually the predicted growth of *E. coli,* expressed as $\log_{10}$) based on meeting acceptable industry-wide microbiological criteria. The competent authority then chose to apply the RI to all chilling processes, which on one hand placed a burden on the industry to verify their process control, but also offered the whole industry opportunities to innovate with their

processes, in ways that were not possible when prescriptive process criteria were applied.

The success (5, 6) of this example of transferring and applying knowledge to the industry can be attributed to addressing (in an empirical, intuitive manner) a number of the failures (or potential failures) identified by the innovation system model:

Infrastructure

- good quality science in peer-reviewed journals of high standing
- initial training was provided by face-to-face training of both industry and competent authority personnel in mixed groups
- development and implementation of personnel competency standards for use of the RI, together with training materials to ensure ongoing supply of competent personnel (7)
- provision of "RI Calculator" software that was simple to use and tailored in approach (8)

Institutions

- competent authority involvement in the panel process allowed the science to be tailored to the regulatory need
- legal and regulatory framework provided through the Export Control (meat and meat products) Orders
- industry involvement in the panel ensured that the approach taken covered all scenarios and would not have unintended consequences

Interaction

- the panel process provided an informal way for industry (and regulators) to explore the science, and mould the scientific outputs to meet their needs
- the panel process ensured that the output would meet the needs of the whole industry, not just one sector

Firm Capability and Adaptability

- the compulsory nature of the Export Orders, training and provision of the RI Calculator minimised the capability and adaptability required to implement the RI as a regulatory tool. Further capability in a firm would be required to utilise the RI for process innovation.

Sector Culture

- the sector culture was sufficient to provide funding for the scientific work to commence

Implications

As noted by Pitt (1), following a similar approach with other food safety innovations has not always been (as) successful. The dimensions of innovation failure are not simply present or absent, but vary by degree as well as significance to a particular innovation. It is also likely that failures interact to make innovation in particular areas more difficult than would otherwise seem to be the case.

An implication of the sectoral innovation model for researchers and funding bodies, is that considerable effort needs to be made if there is to be successful translation of research results (knowledge) into industry practice (innovation). Innovation cannot be achieved by one party acting alone, but a concerted effort needs to be made in the required areas by the appropriate parties.

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Meat spoilage, methods for controlling and assessing spoilage

George - John E. NYCHAS, and Efstathios Z. PANAGOU,
Laboratory of Microbiology and Biotechnology of Foods, Dept. of Food Science & Technology, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

From the scientific point of view, meat spoilage can be considered as an ecological phenomenon that includes the changes of the available nutrients, during the prevailing of a particular microbial association, the so called Specific Spoilage Organisms (SSO). In fact spoilage of meat depends on an even smaller fraction of SSO, called Ephemeral Spoilage Organisms (ESO). These ESO are the consequence of factors that dynamically persist or imposed during processing, transportation and storage in the market. Spoilage so far is a subjective judgment by the consumer, which may be influenced by cultural and economic considerations and background, as well as by the sensory acuity of the individual and the intensity of the change. In general most consumers would agree that gross discoloration, strong off-odors, and the development of slime would constitute the main qualitative criteria for meat rejection. For the authorities' point of view meat evaluation ***still relies heavily on regulatory inspection and sampling regimes.*** This approach, however, seems inadequate because it cannot sufficiently guarantee consumer protection, since 100% inspection and sampling is technically, financially and logistically impossible. In addition, the following issues need to be considered: *(i)* more than 50 chemical, physical and microbiological methods have been proposed for the detection and measurement of bacterial safety or spoilage in meat, *(ii)* most of these methods are time-consuming and provide retrospective information and thus they can not be used on- or at-line, *(iii)* the lack of general agreement on the early quality changes, and *(iv)* the changes and development in technologies for food processing and preservation [e.g. vacuum packaging (VP), modified atmosphere packaging (MAP), active packaging, etc] make it evident that the important and urgent task of ***identifying safety and spoilage indicators*** is a complicated proposition. On the other hand, the meat industry needs rapid analytical methods or tools for quantification of these indicators in order to determine and select suitable processing procedures for their raw material and predict the remaining shelf life of their products. Furthermore, ***inspection authorities*** need reliable methods for control purposes, while the ***wholesale and retail*** sectors need these valid methods to ensure the freshness and safety of their products and to resolve potential disputes between buyers and sellers. Methods and approaches are desirable for reliable indication of the safety and quality status of meat at retail and through consumption. It is, therefore, crucial to have ***valid methods to monitor*** freshness and safety in order to be able to ***ensure*** quality, irrespective of perspective (i.e. that of the consumer, the industry, the inspection authority, or the scientist). Thus, identification and quantification of the most influential metabolic compounds associated with meat safety and spoilage, and development and application of practical means such as ***analytical methods or devices*** and ***robust model systems*** are of great importance in assuring consumers that food safety and quality are ensured. For such procedures and tests be used widely by the meat processing industry, they must be ***rapid and simple*** to perform on a large number of samples at relatively low cost and, if possible, to be applicable on-line and involve non-destructive sampling. Moreover, uniform and effective methods of meat inspection, applicable throughout the Community, need to be developed and validated on the basis of meat raw materials and products from different geographical regions within the Community.

Blown pack spoilage: protecting the European beef industry

Declan J. Bolton[1], GalatiosMoschonas[1], James J, Sheridan[1] and David McDowell[2]
[1] Food Safety Department, Teagasc-Ashtown Food Research Centre, Dublin 15, Ireland,
[2]Biomedical Sciences Research Institute, School of Health Sciences,
University of Ulster, Jordanstown, N. Ireland

Blown pack spoilage (BPS) is the biggest food safety/quality issue facing beef processors. BPS emerged in the USA in 1989 and was quickly followed by incidences in the UK, New Zealand and Ireland. Each spoilage event occurred in correctly chilled batches (0 to 2°C) of vacuum packaged meat after 2 to 4 weeks and was characterised by the production of large volumes of gas, a putrid smell (H_2S) with a metallic sheen on the meat. Meat spoiled in this way has no commercial value and blown pack spoilage represents a considerable loss to meat processors estimated to be €375,000 per incident. Furthermore, large scale spoilage may result in a failure to meet a customer order thus jeopardising future contracts. Prior to this research it was believed that there were 2 psychrophilic Clostridia spp. (*Clostridium estertheicum* and *Clostridium gasigenes*) responsible for BPS and there were no known control measures. This project initially undertook an epidemiological traceability study in beef abattoirs to identify sources before focusing on the development of control measures. A new BPS Clostridia spp. was discovered and is currently being characterized for deposition in an international culture collection and publication in the *International Journal of Systematic and Evolutionary Microbiology*. The research team also developed and patented a Real Time Polymerase Chain Reaction (RT-PCR) assay based on unique fragments of the 16SrRNA gene. Spore inoculation studies identified thermal shrinkage (a common practice during meat vacuum packaging) as effecting spore activation and reduced shelf-life and formed the basis for an intervention control measure. This presentation will introduce BPS, introduce the newly discovered Clostridial species including the patented detection technology and discuss the control measure developed and delivered to the European beef processing industry.

Spectroscopic imaging as a real-time solution for the detection of faecal contamination on carcasses

M.R.F. Lee[1], V.J. Theobald1, M. K. Theodorou[1], A. Veberg Dahl[2], F. Lundby[2] and J. P. Wold[2]
[1]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Gogerddan Campus, Aberystwyth, Ceredigion, UK, SY23 3EB
[2]Nofima Mat Ås, Osloveien 1, N-1430, Ås, Norway

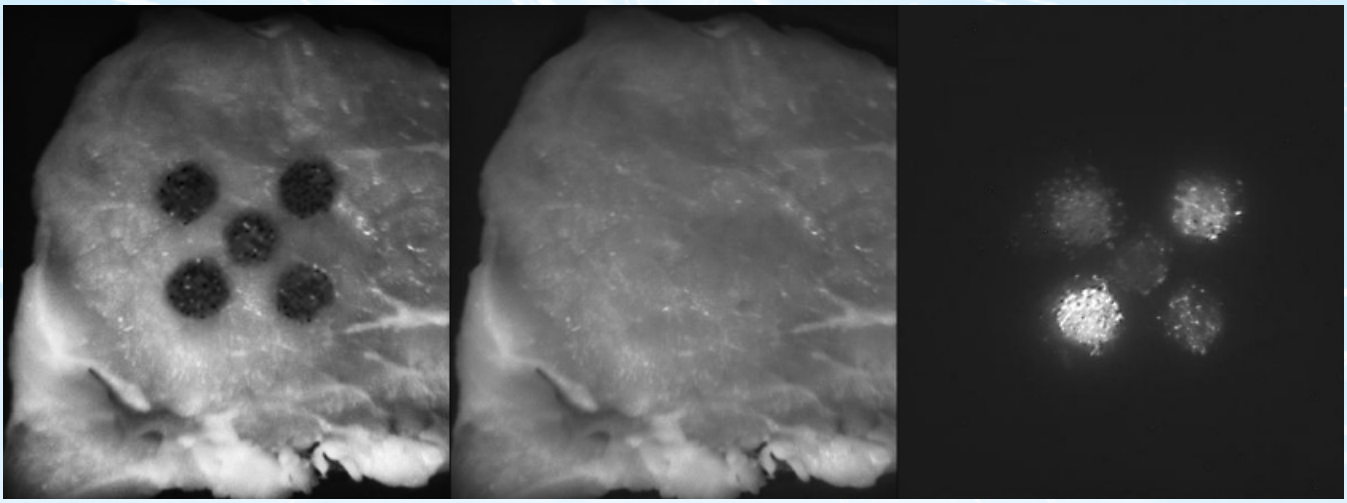
Beef production within the EU region is an activity of major economic importance, valued at €75 billion. However, reforms to the Common Agricultural Policy, increased globalisation, reduced commodity prices and an increasingly sophisticated, health-conscious consumer are requiring the industry to produce beef and beef products that are convenient, traceable, nutritious and of consistent quality. Alongside these considerations, today's consumer demands assurances regarding food safety and health, which is of paramount importance given the serious impact of beef related health scares. In order to boost consumer trust and invigorate the industry, a Framework 6 European Union Project - *ProSafeBeef* is examining new ways of reducing contaminants in the beef chain from 'farm to fork' as well as enhancing quality, choice and diversity.

Cleanliness in the abattoir is of the utmost importance and numerous practices are carried out on farm to ensure that the animals arrive at the abattoir with limited faecal matter clinging to the hide. Such strategies include: change to hay and cereal based diets before slaughter to encourage 'dry' faeces; cleaning the animals before they travel; reducing stress on the animals during transport and at the abattoir to limit pathogen shedding. However, even with these strategies in place, a major source of contamination in the abattoir is from small traces of faeces still associated with the hide coming into contact with the dressed carcass. Currently carcasses are checked by 'eye' and washed with chemical sprays or dissected to remove contaminated areas. Unfortunately small areas of faecal contamination may not be visible to the eye and may harbour millions of pathogenic bacteria. Spectroscopic imaging is a rapidly evolving research area, with the potential to provide real-time solutions for the detection of faecal contamination on carcasses. Chlorophyll is ubiquitous in green plants and thus livestock diets. During digestion in the gut, chlorophyll is only partially degraded to coloured and fluorescent intermediates: the phaeophytin, chlorophyllide, phaeophorbide and pyrophaeophorbide derivatives of chlorophylls a and b. In a preliminary investigation we investigated the best chlorophyll breakdown products for use as markers of faecal contamination on a range of diets. Eight Cheviot sheep were fed either: i) fresh grass and clover, ii) grass silage, iii) hay or iv) concentrate and barley straw. Each diet was offered to two sheep for a period of two weeks before diet change over in a duplicate 4 x 4 Latin Square design. Samples of feed were collected during the whole feeding period and bulked whilst samples of faeces were taken at the end of the period. Samples were measured for chlorophyll and its derivatives using HPLC. Fluorescence emission spectra were measured directly on the faeces. The samples were placed into sample cuvettes, which exposed a flat circular surface with a diameter of 5 cm for the measurements. The fluorescence emission spectra were measured with excitation at 382 and 430 nm, using an optical bench system, suitable for solutions and solid samples. The excitation light was generated by a 300 W Xenon light source (Oriel 6258, Oriel Corporation, Stratford, CT) and passed through a 10 nm bandwidth interference filter (Oriel 59920) and (Oriel 59295). The light was directed onto the samples at an angle of about 45°. The spectra were collected by an imaging spectrograph (Acton SP-150, Acton Research Corporation, Acton, MA) connected to a sensitive charge coupled device (CCD-camera) (Roper Scientific NTE/CCD-1340/400-EMB, Roper Scientific, Trenton, NJ). Cut-off filters at 400 nm (for the 382 nm excitation) (Melles Griot 03FCG049) and 475 nm (for the 430 nm excitation) (Melles Griot 03FCG068) were positioned in front of the spectrograph slit to suppress excitation light reflected from the samples. Exposure time was 10 and 5 sec for excitation at 382 and 430 nm, respectively. The temperature of the samples was 4 °C. All the samples were measured twice and an average was used in the analysis. The field of illumination was not perfectly homogenous, so the samples were rotated 90° between each measurement to even out sample heterogeneity. To ensure stable illumination, the emission intensity at 440 nm at excitation 382 nm was measured from a stable fluorescence standard of washable plastic (Ciba, Basel, Switzerland) before and after the measurements. Fluorescence images were

collected with the same system, slightly modified. A Nikon 102 mm photographic lens was mounted on the imaging spectrograph, the spectrograph slit was removed and the grating was exchanged with a mirror. Spectral images were created by placing a 40 nm bandwidth interference filter in front of the lens. Samples were illuminated by 400 nm (10 nm bandwidth filter, Melles Griot 03FIV026) light and images were captured at 685 nm (10 nm bandwidth). Exposure time for each image was 60s.

Not surprisingly animals offered the fresh grass and clover diets had a greater concentration of fluorescent compounds in their faeces and subsequent fluorescence than animals on conserved forages and on concentrate based diets. Consequently the accuracy of the spectral imaging detection of faecal contamination depended on the diet of the animal. This is why previous similar techniques such as 'Verifeye®' have not been universally accepted. In a second experiment we investigated natural markers which can be added to the diet before slaughter which will increase the intensity of the fluorescence.

Ten Cheviot sheep were offered a concentrate and barley diet and split into five treatment groups during a duplicate 5 x 5 Latin square design where each period lasted 2 weeks. Four of the groups received a different chlorophyll based marker at a rate of 1 g per day for the second week of the experimental period. The last group received no supplement and was used as the control. At the end of each period faeces were collected to be analysed as in the first experiment (HPLC and fluorescence) before the animals changed treatment. The results are shown in figure 1 where each of the markers significantly increased the fluorescence intensity of the faeces over the control. The use of markers in pre-slaughter diets would thus improve the accuracy of faecal detection as a result of greater fluorescence and pin pointing the excitation wavelengths of the marker to help with visualisation. Further work is being continued to identify the most suitable marker and feeding regime to supply the marker.



A **B** **C**

Figure 1.

Picture A – Meat sample with five spots of faeces from animals on a concentrate diet. The central spot is the control where animals were not fed a marker the surrounding spots animals supplemented with four different markers for 1 week.

Picture B – The faeces were washed off the meat with water.

Picture C – Is the same as Picture B but under ultra violet light showing the potential of two of the markers. Note the control sample can hardly be seen.

Analysis of physiology of *Escherichia coli* O157:H7 in bovine digestive content by transcriptomic profiling

Yolande Bertin[1], Jean-Pierre Girardeau[1], Josée Harel[2], and Christine Martin[1]
[1]INRA, UR454 Unité de Microbiologie, F-63122 Saint-Genès Champanelle, France.
[2]University of Montreal, Faculty of Veterinary Medicine, Ste-Hyacinthe, Qc, Canada.
cmartin@clermont.inra.fr

Introduction

Enterohaemorrhagic *Escherichia coli* (EHEC) are Shiga-toxin-producing *E.coli* (STEC) that causes a spectrum of human illnesses such as haemorrhagic colitis or haemolytic-ureamic syndrome. *E.coli* O157:H7 is the most prevalent serotype associated with severe disease and large outbreaks. Cattle and other ruminants are the principal reservoir of EHEC strains, and outbreaks have been associated with the consumption of meat, raw milk and dairy products, water, and fruits or vegetables contaminated with ruminant manure. Clearly, a reduction in the carriage of EHEC in healthy ruminants will lead to a decline in the incidence of human EHEC infections. As such, understanding of EHEC ecology and physiology in the ruminant gastrointestinal tract (GIT) is critical for limiting EHEC shedding and consequently contamination of food products. However, little is known about the nutrients preferentially used by EHEC for growth in the bovine intestine, which is thought to be the main site of EHEC colonization, the metabolic pathways required in this digestive compartment, or the stress response and virulence factors whose expression is activated.

Methods

We investigated EHEC gene expression in the ruminant gut. Cattle intestinal content was harvested at the slaughterhouse from conventional healthy cattle fed grassland hay, and then the commensal microbiota was removed by centrifugation and filtration. *E.coli* O157:H7, strain EDL933, was cultured in this medium to early stationary phase. Cultures were performed at 39°C without any nutrient supplementation and without aeration to mimic the physico-chemical conditions of the cattle GIT tract. Transcriptional profiling was performed by using DNA microarrays covering the genome of two O157:H7 EHEC strains (EDL933 and Sakai) and of *E.coli* K12 MG1655. The reference medium used to determine gene expression ratios was the M9-minimal medium supplemented with glucose.

Results

Microarray analysis indicates that 12% of EDL933 genes were up regulated and 10% down regulated in the cattle intestinal content in comparison to M9-glucose. Genes involved in energy metabolism, protein synthesis, transport and binding, were the most numerous to be differentially expressed (Fig.1).

Cell wall structure

Expression of a high number of genes involved in cell wall structure and cell division was altered in the intestinal content. *glmU*, encoding the enzyme catalyzing the two last steps of UDP-*N*-acetyl-D-glucosamine biosynthesis, was up-regulated. UDP-*N*-acetyl-D-glucosamine is the common precursor to three major cell wall components, i.e. the peptidoglycan, the lipidA, and the enterobacterial common antigen (ECA). Furthermore, genes involved in KDO-lipidA, ECA and colonic acid biosynthesis are also up-regulated, whereas genes involved in peptidoglycan synthesis were down-regulated, as well as genes essential for cell division, or required for integrity of the bacterial cell envelope and for the normal shape of the cells.

Nutrient metabolism

The mammal intestine is a complex ecosystem with an abundant microbiota composed of a high diversity of bacterial species. According to the Freter's nutrient niche theory, bacteria can coexist as long as each member of the microbiota is able to utilize one or a few limiting nutrients better than the other members. Therefore proliferation of EHEC strains in the gut of ruminants should involve competition with the resident microbiota for a variety of carbon, nitrogen and energy sources. Analysis of the microarray data indicates that metabolic pathways involved in the catabolism of three sugars present in the intestinal mucus, i.e. fucose, mannose, and galactose, were up-regulated in the intestinal content, as well as metabolic pathways required for utilization of metabolites produced

by the commensal microbiota, such as glycerol, lactate, and succinate. Therefore these compounds are good candidates as EHEC carbon sources in the bovine gut. The nitrogen source could be provided by amino acid catabolism, but we focused our analysis on ethalomine utilization, encoded by the *eut* operon which was highly up-regulated in the intestinal content. We showed that ethanolamine was actually present in the cattle gut content, probably due to release from plant, intestinal, and bacterial cell walls, and was entirely consumed by EHEC as a nitrogen source. An isogenic *eutB* mutant demonstrated a reduced growth during co-culture with the wild-type strain in the intestinal content (Fig.2), suggesting that the ability to metabolize ethanolamine confers a growth advantage to *E. coli* O157:H7 in the GIT of ruminants. Furthermore, the *eutB* gene is less expressed in commensal non-pathogenic *E. coli* strains than in EDL933, suggesting that ethanolamine utilisation by commensal *E. coli* strains is less efficient; *in silico* analyses revealed that most of the bacterial species constituting the autochthonous digestive microbiota do not possess this operon. Taken together, these data suggest that ethanolamine utilisation as a nitrogen source represents an ecological niche that confers a competitive advantage for EHEC strains to persist and develop in the bovine digestive content.

Stress response

The most obviously up-regulated genes involved in stress response encode multi-drug resistance systems. They included 4 members of the Major Facilitator Superfamily, *ydhC, bcr, emrD,* and *yidY,* that mediate drug extrusion with different specificities. The three component multi-drug efflux system AcrAB-TolC, belonging to the RND family that facilitates drug extrusion directly into the internal medium, was also up-regulated, as well as its positive regulator, AcrR. Other up-regulated drug resistance genes included *ybjG, mdaA, ompW* and *marA*. The stress signals that induced such multi-drug efflux systems in the bovine gut remains to be identified since the animals were not fed antibiotics, but they could be produced by the autochthonous microbiota.

In contrast, several genes involved in acid-resistance were down-regulated in the intestinal content, such as the cfa gene that encodes the Cyclopropane Fatty Acid synthase. Cyclopropane fatty acid (CFA) formation is a post-synthetic modification of the membrane lipid bilayer that contributes to protect E. coli from acid shock. Other genes included asr, that plays a role in survival under acidic conditions, and genes carried by the Acid Fitness Island (AFI), encoding the acid-resistance regulators GadE and GadX, the chaperones HdeA and HdeB, the glutamate-dependent acid-resistance system GadA and GadC, Slp and the transcriptional regulator YhiF, involved in protection against organic acids at low pH, and HdeD conferring acid-resistance at high cell densities (Fig.3).

Virulence factors

The locus of enterocyte effacement (LEE) is responsible for the EHEC-induced characteristic attaching-effacing lesions on the intestinal epithelium in humans. It encodes a type three secretion system (TTSS), effector proteins translocated by this TTSS, an adhesine mediating intimate attachment to the epithelial cells and its own receptor, and transcriptional regulators. All of the LEE genes were among the highest up-regulated in the intestine content (Fig. 4). A number of genes encoding non LEE-encoded effector proteins secreted by the TTSS were up-regulated as well. In addition, genes encoding the fimbrial adhesin F9 were also up-regulated. LEE and F9 genes have been previously identified as influencing colonization of the bovine GIT. LEE proteins would be required for colonization of the terminal rectal mucosa while F9 would be rather required for colonization at other intestinal locations. Another up-regulated gene encodes a poorly characterized Sfa-related fimbriae. In contrast, the gene encoding the major subunit of the long polar fimbriae 1, which has been shown to influence EHEC colonization in lambs, was down-regulated, as well as genes involved in curli biogenesis.

Conclusion

Microarrays data give a general view of the physiology of EHEC and allow identifying colonisation factors and metabolic pathways expressed in the ruminant intestinal content. The knowledge generated by these experiments should help to develop intervention strategies to limit EHEC carriage by ruminants.

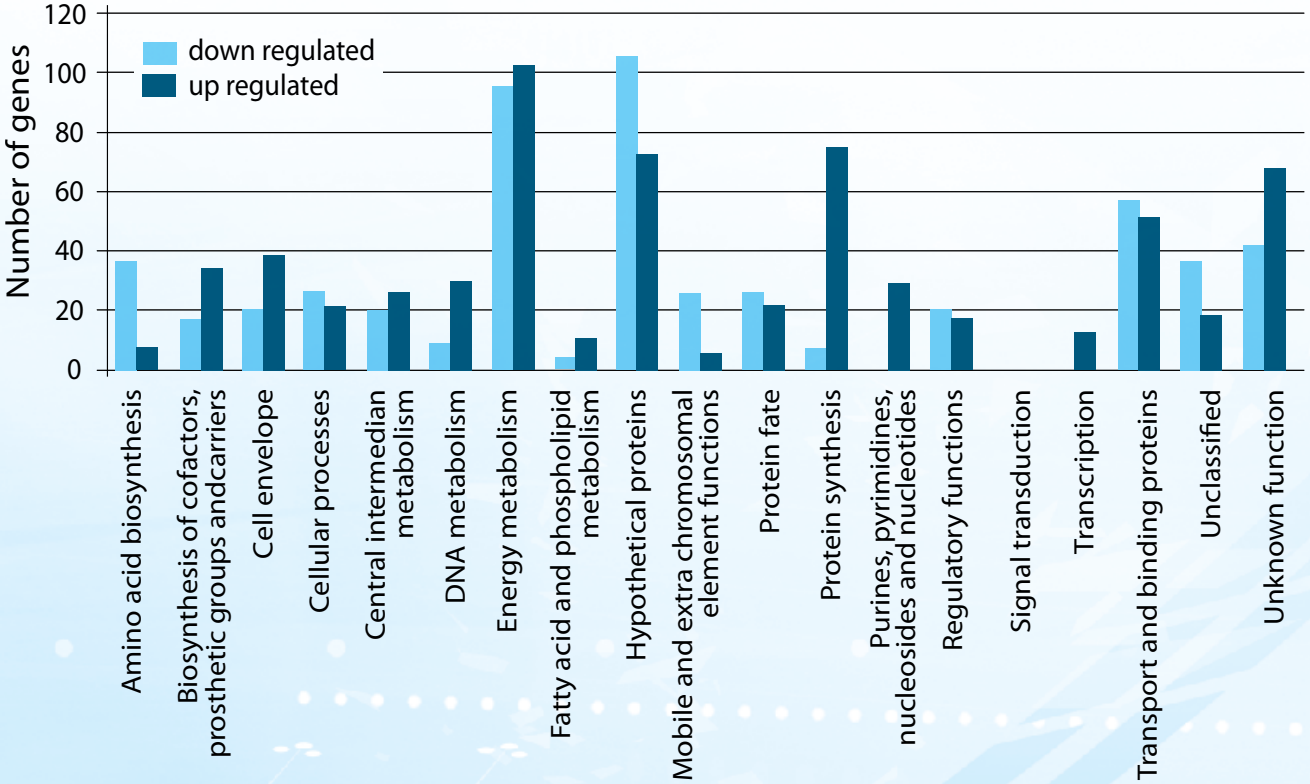


Figure 1. Number of genes with altered expression in bovine intestinal content versus M9-glucose

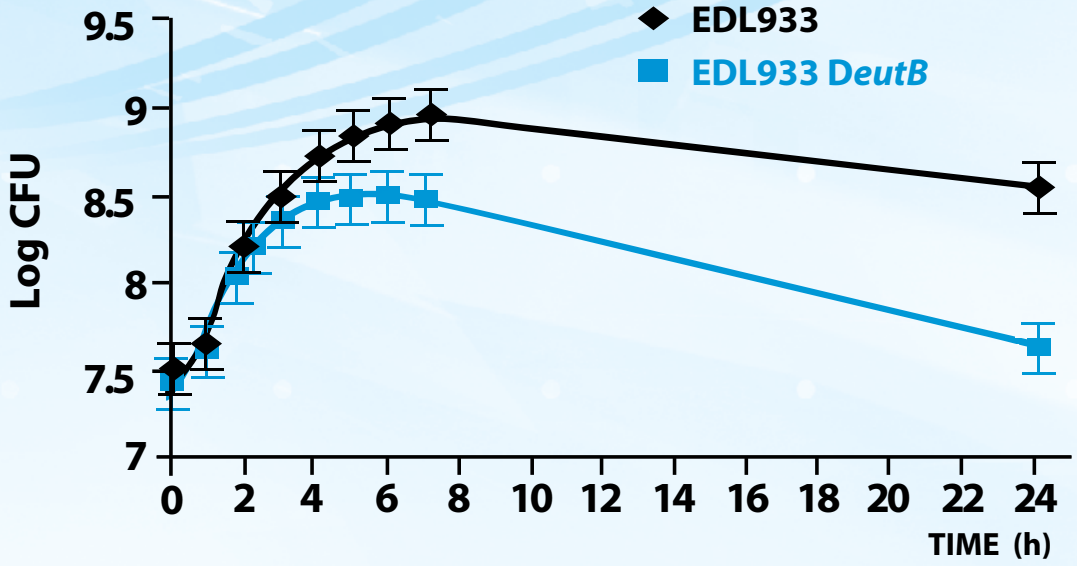


Figure 2. A mutant unable to use ethanolamine has a growth defect in bovine intestinal content

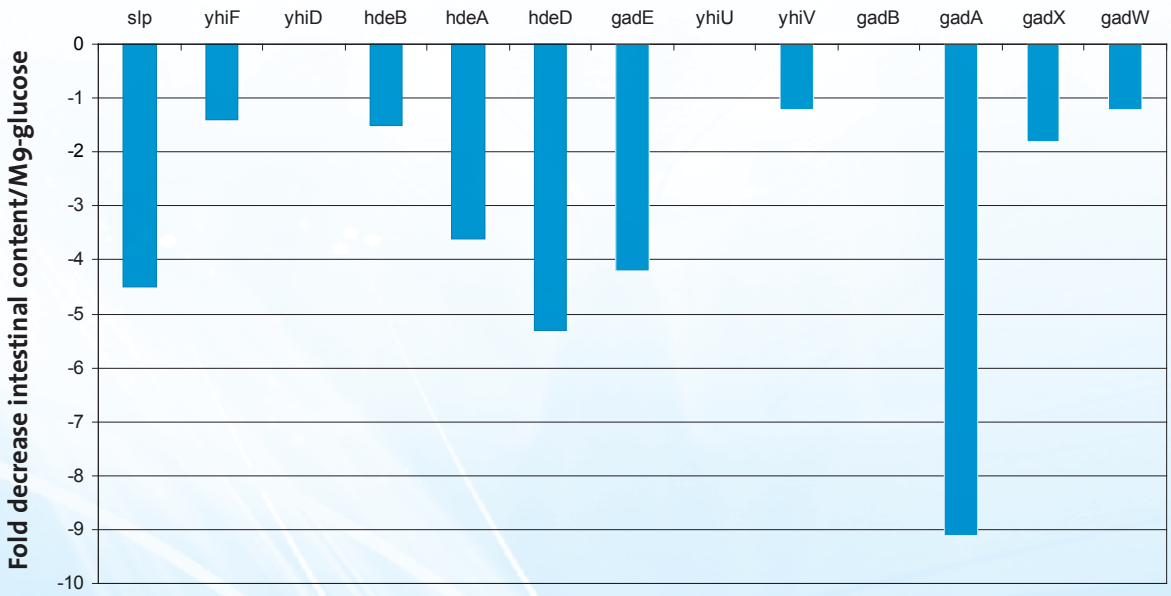


Figure 3. Genes carried by the AFI, involved in acid resistance, are down-regulated in bovine intestinal content

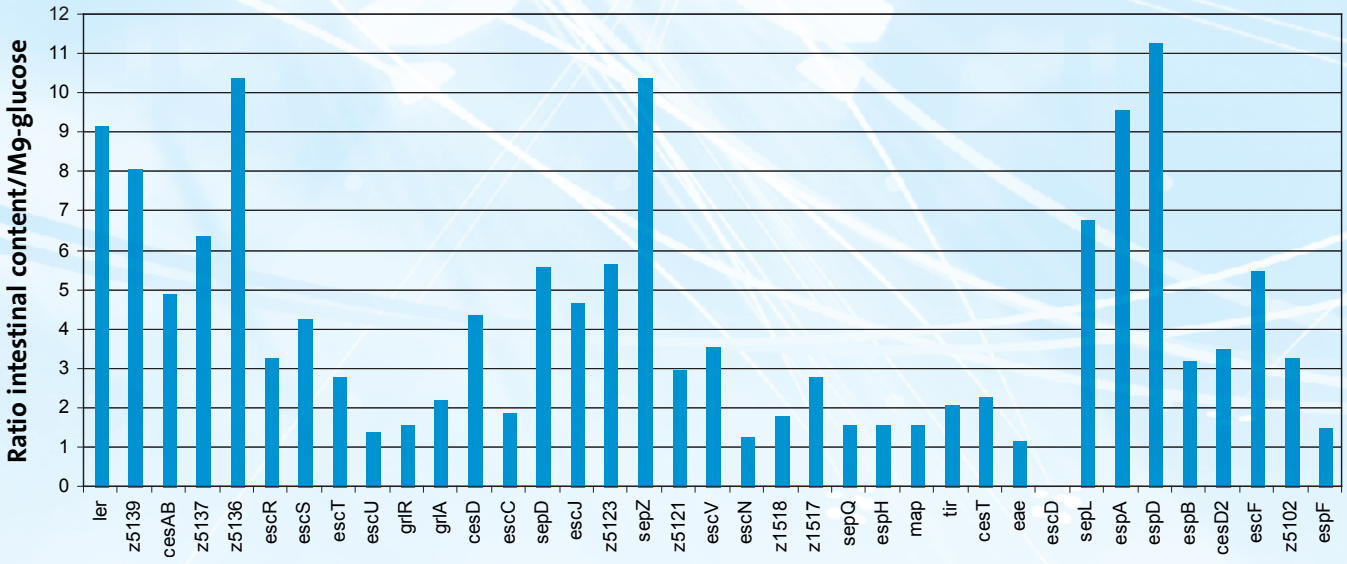


Figure 4. LEE genes are up-regulated in bovine intestinal content

Contribution of -*omics* in the Microbiology of Meat

Samoilis[1], G., Paramithiotis[2], S., Skandamis[2], P., & Nychas[1], G
[1]Agricultural University of Athens, Department of Food Science and Technology, Laboratory of Microbiology and Biotechnology of Foods, Athens, 11855
[2] Agricultural University of Athens, Department of Food Science and Technology, Laboratory of Food Hygiene and Quality Control, Athens, 11855

The rapid technological advances achieved in the post-genomic era have provided the researcher with a great number of molecular "tools" for high-throughput and in-depth analyses of biological systems. Approaches such as proteomics and transcriptomics are now routinely being applied in many research laboratories with the aim to reveal the molecular basis underlying specific phenotypes. Moreover, it is now obvious that research is turning towards the upcoming field of Systems Biology in which, every question or problem is being examined in a more global manner within the frames of the biological system being studied. The use of *–omics* approaches in this case is particularly important, as these techniques can provide us with a global overview of the molecules being analyzed, i.e. proteins in proteomics, mRNAs in transcriptomics etc. In the Department of Food Science and Technology of the Agricultural University of Athens, a master plan has been developed which aims to utilize such techniques in order to reveal the molecular basis of particularly important phenomena in the microbiology of meat. Specifically, these phenomena include: (a) biofilm formation and their role in the contamination of industrial surfaces, (b) the effect, at the molecular level, of various stresses and interventions that a microorganism will go through during preparation and packaging of meat and its products, and (c) the communication and interaction between microorganisms in terms of "quorum sensing". Every intervention applied to the microorganism and/ or the system being studied will have an effect at the molecular level that could possibly be expressed in the phenotype too. Therefore, in all cases mentioned above comparative proteomics and trancriptomics studies are potentially able to reveal those molecular differences that come up between different treatments of our sample, both qualitatively and quantitatively. In this present study, the results from a series of experiments are being presented, in which we have used the basic "tool" in proteomics two-dimensional electrophoresis (2-DE), to analyze total protein (proteome) from cells that have or not gone through interventions, i.e application of conditions that are different from their physiological ecological niche.

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Keywords: 2-DE, biofilm, intervention, proteomics, quorum sensing, two-dimensional electrophoresis, stress, -omics

Detection of Anthelmintic Residues in food using Rapid Polarity switching UPLC MS/MS combined with QuEChERS technology

Martin Danaher[1], Michelle Whelan1,[2], Brian Kinsella[2], Helen Cantwell[1], Martin McCormack[1], Patrick Byrne[1], Kevin Cooper[3], Glenn Kennedy[4], Andrew Cannavan[5], Alfredo Montes Nino[6], Rodrigo Granja[6], Gemma Trigueros[7], Ester van Asselt[8], Ambrose Furey[2], Steve Lehotay[9]

[1]Ashtown Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland
[2]Team Elucidate, Department of Chemistry, Cork Institute of Technology, Bishopstown, Cork, Ireland
[3]Queen's University Belfast, School of Biological Sciences, Belfast, Northern Ireland
[4]Veterinary Sciences Division, Agri-Food & Biosciences Institute, Belfast, Northern Ireland
[5]International Atomic Energy Agency, Wagramer Strasse 5, P.O. Box 100, A-1400, Vienna, Austria
[6]Microbioticos, P.O. Box 6175, 13083-970, Av. Santa Isabel, Campinas, Brazil
[7]OCU Ediciones, 28037, Calle Albarracin 21, Madrid, Spain
[8]RIKILT Institute of Food Safety, P.O. Box 230, 6700 AE Wageningen, The Netherlands
[9]U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA

Anthelmintics are one of the most important classes of veterinary drugs used in animal production. They are widely available as over-the-counter medicines for the prophylactive control of nematode, cestode and trematode infections in food producing animals. This has led to concerns that anthelmintics are often unnecessarily administered to animals, leading to an increased risk of residues occurring in food and due to the build-up of resistance to the dwindling numbers of useful drugs. The majority of anthelmintic drug residues are detected in food using a suite of laborious high performance liquid chromatography methods that often require derivatisation of residues. Methods frequently do not cover the complete range of key metabolites or rarely tested flukicide residues. In recent years, there has been intense focus on the application of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to measure individual drug groups in this class, which offer improvements in sensitivity, selectivity and throughput.

Teagasc researchers in collaboration with the U.S. Department of Agriculture have addressed this problem through the development of a new residue technology that enables the detection of 35 major anthelmintic drug residues in muscle, liver and milk by LC-MS/MS. The method uses a novel QuEChERS sample preparation method, which has been widely applied in the area of pesticide residue analysis. The technology has been improved as part of the *ProSafeBeef* project through the inclusion of deuterated internal standards, introduction of a sample concentration step and the application of ultra high performance LC-MS/MS (UPLC-MS/MS). The UPLC-MS/MS system used in this application can rapidly switch polarity to allow sensitive analysis of both negatively and positively charged ions after a single injection with a 12 minute chromatographic run time.

This new technology offers advantages over existing methods including improvements in sample throughput (leading to reduced sample turnaround times), residue coverage, sensitivity and data processing. One major advantage of the technology is that it has resulted in a five fold reduction in the solvent usage in the residue laboratories at Teagasc. The technique is also a very useful tool for monitoring low levels residues of veterinary drug residues in food, which can also be used to identify what veterinary drug treatment strategies that are being used on farms. A comprehensive evaluation of the method has been carried out of the method at Teagasc where it has been applied to approximately 1000 samples of milk, muscle and liver across animal species. The limit of quantitation of the method is 2 µg/kg for 34 analytes and 25 µg/kg for four analytes (namely, clorsulon, oxyclozanide, morantel and bithionol). The method has been validated according to 2002/657/EC criteria.

In *ProSafeBeef*, the new residue technology will be applied to investigate the safety of beef products collected in a survey of five EU member states (representative of different climatic conditions) and Brazil. A thousand samples of beef are being purchased during the course of the project and will be analysed for residue content. The survey will be used to identify the seasonal pattern of usage of anthelmintic drug residues in beef in the EU and INCO countries. The results of the survey can be used to support the development of a risk based approach for the control of anthelmintic residues in food, which takes climatic, production and epidemiological factors into consideration. In 2009, the technology developed on the project will be transferred to the Community Reference Laboratory in Berlin. It is planned to carry out training for different EU member states in May 2009 at the CRL.

Dioxin contamination of Irish pork and beef

Rhodri Evans
Food Safety Authority of Ireland

In December 2008, the Irish Authorities ordered the largest recall of food products ever seen in the State following the discovery of dioxin contamination of pork and beef products. Close collaboration between the authorities in Ireland and colleagues in other Member States allowed the rapid identification of a common source of contamination, this being feed produced in one plant from recycled bread manufactured using a direct heating process. This rapid identification in turn meant contaminated meat and meat product could be removed from sale very quickly and thus consumer protection was ensured, both in Ireland and in other countries.

Whilst only approximately 8% of the national pig herd was exposed to contaminated feed, the accepted level of traceability in the pork processing industry, which complies with the minimum legal requirements, meant that it was not possible to distinguish between contaminated and non-contaminated pork in production representing 98% of Irish pork entering the food chain. Therefore a decision was taken to recall all Irish pork and pork products produced from 1 September 2008 up to the date of the recall in December. By way of contrast, only 0.02% of the national beef herd was exposed to contaminated feed and traceability in the beef sector meant that all of this product could be traced and withdrawn from the food chain.

Risk assessments carried out by FSAI and EFSA confirmed that as a result of the swift action taken to recall product, there had been no unacceptable risk to consumers' health arising from the contamination incident.

Work is continuing to identify the actual source of the contamination, thought to be recycled transformer oil, and in addition a number of experiments are planned using tissue from contaminated animals which will help to develop our understanding of the ways in which these contaminants are metabolised by food producing animals.

Rapid Methods for Dioxins and DL-PCBS in meat

Peter A. Behnisch
BioDetection Systems BV (BDS), Sciencepark 406, 1098 XH Amsterdam, The Netherlands

Introduction

Last year several new "dioxin and dl-PCBs crisis" in meat occurred e.g. in Italy ('mozzarella buffalo crisis'), Chile ('animal feed') or Ireland ('PCB transformer oil in drying process') showing the needs for rapid, high capacity, easy-to-learn and cost-efficient screening methods to support National Food Monitoring programs as well as traceability systems for the feed/food industry[1, 2, 3]. To permit bioassays to be used for screening foodstuffs for the presence of dioxins/PCBs and for further ISO 17025 accreditation of laboratories, the EU has laid down specific requirements for cell-based screening tests[4]. One of the best evaluated screening tests is the DR CALUX® bioassay (see FP6 Project Difference, www.dioxins.nl; as well as "Ten EC country intercalibration test" by the JRC), which has full standardised methods for extraction, clean-up and measurement of all EC required food/feed items[5]. Several round robin studies have been performed to evaluate the DR CALUX® technology for feed and food[6]. Within the last decade the service laboratory of BDS has analyzed all kinds of feed and food by DR CALUX® method for Total TEQ. In this paper we present exemplary the results of around 500 food samples and 100 feed samples. In the cases of food samples only 6.5% of the samples were found to exceed the EU guideline for only dioxins, while in case of feed samples 17% exceeded these EU limits.

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BioCop: Biomarker profiling as a screening tool to ensure beef safety

Mark H. Mooney & Chris T. Elliott
Centre for Food: *ASsured, SafE and Traceable food* (ASSET), Institute of Agri-Food and Land Use, School of Biological Sciences, Queen's University Belfast, Northern Ireland

Due to the economic benefits which accrue from their use, the illegal administration of growth promoters in animals destined for beef production persists and continues to be an enormous challenge to regulatory authorities charged with enforcing their ban. Despite many recent analytical advances the effectiveness of approaches currently used to detect banned growth promoter administrations is compromised by the continued use of endogenous hormones and low dose chemical cocktails, and also by the limited number of samples which can be routinely analysed for drug residue presence. Alternative testing strategies with high-throughput screening capabilities may offer the potential for targeted confirmatory analytical analysis on only those samples suspected of being derived from illicitly treated animals. With this in mind attention has focused on developing novel testing methodologies based not on the direct detection of specific drug residues but rather on indirect evidence centred on perturbations to physiological activity arising from growth promoter administrations. BioCop is an EU 6th framework project aimed at developing new screening technologies to identify chemical contaminants in food and work on specific strands within this project has attempted to identify markers within plasma which are representative of altered biological activity. Through the comparative analysis of plasma from experimentally treated and non-treated animals a range of biomarkers have been revealed which are reflective of changes induced by growth promoter administrations. Biosensor assay technology has been developed to enable measurement of a panel of these biomarkers whose profiles can be used to distinguish between control and treated animals. It is proposed that such a biomarker approach may find use as a tool aiding the identification of herds illegally treated with growth promoting agents thus helping to overcome new challenges which currently circumvent existing testing methodology and promoting confidence in the integrity of the beef supply chain.

Meat Decontamination: Needs, Approaches. Benefits, Concerns

John N. Sofos
Colorado State University
E-mail: john.sofos@colostate.edu

Undercooked ground beef patties were involved in an outbreak of *Escherichia coli* O157:H7 in the western United States in 1992-1993 and resulted in illness of several hundred people and four deaths. Major public scrutiny associated with this outbreak led to a major change in the meat inspection system which was in place since 1906. The United States Department of Agriculture Food Safety and Inspection Service (FSIS), first, reinforced a policy requiring absence of visible soil on carcasses ("zero tolerance") before washing. In addition, FSIS declared *E. coli* O157:H7 an "adulterant" in ground beef, in 1994, as well as in all non-intact beef products made with pieces of beef, injected with marinades or needle/blade tenderized, in 1999. Then in 1996, the United States Meat and Poultry Inspection Regulations were revised to require: (i) establishment of formal sanitation standard operating procedures; (ii) implementation of the hazard analysis critical control point (HACCP) system of process control; and, (iii) compliance with performance criteria for *Escherichia coli* biotype I to verify process control (done by the processor), and *Salmonella* as a verification of HACCP and for tracking pathogen reduction (done by FSIS). Additional FSIS Directives of the past 5-10 years include re-evaluation of HACCP plans, and testing of ground beef raw materials for *E. coli* O157:H7. In addition, the industry has imposed microbiological specifications and other requirements on their suppliers of raw beef. In response to these regulatory and commercial requirements, as well as being aware of its responsibility to provide safe products to consumers, the industry has adopted strategies to improve the microbiological quality of carcasses and fresh meat.

The best strategy for minimizing contamination on raw meat is based on application of technologies that: (i) reduce sources, levels, access and transfer of contamination on the animal and in the processing environment; (ii) reduce contamination on animals before slaughter; (iii) minimize transfer of microorganisms to carcasses or meat; and, (iv) through decontamination interventions, reduce pathogen prevalence and levels of microorganisms on carcasses or meat. Hundreds of research studies, published in the past 10-15 years, provided information based on which the industry has developed decontamination strategies some of which have been approved and are included in HACCP programs.

Decontamination interventions are applied extensively by large beef, pork and poultry operations in the United States and include animal cleaning, spot-cleaning before evisceration by knife-trimming or steam-vacuuming, and exposure of carcasses to hot water, steam, and chemical solutions immediately after hide removal and at the end of the dressing process, as well as after carcass chilling or immediately before boning. Hide contamination may be reduced by washing with water or chemical solutions such as chlorine, cetylpyridinium chloride, sodium hydroxide, trisodium phosphate, and acids. Use of an online, specially designed, hide-washing cabinet is employed by at least one beef processor in the United States. The process is applied after animal exsanguination and involves washing with a sodium hydroxide solution (1.5%), followed by rinsing with a chlorine solution (1 ppm), and, as the carcass exits the cabinet, steam-vacuuming, along the hide cutting pattern lines, to remove excess liquid. Chemical dehairing, which was developed for application at this stage to remove hair and associated external contaminants, has been found effective but it is not used because of cost and the need for disposal of hydrolyzed hair and dehairing chemical residues.

Beef carcass decontamination interventions are applied immediately after dehiding with the objective to remove bacterial cells before they attach strongly to the carcass surface. Research has indicated that pre-evisceration washing may alter the physical characteristics (e.g., contact angle and surface free energy) of the carcass surface, resulting in less attachment of contaminants. Under the "zero tolerance" policy for visible carcass contamination in the United States, cutting with a knife (knife-trimming) is required to remove visible contamination on carcasses before any washing or other decontamination treatment is applied. An alternative to cutting with a knife is to use steam-vacuuming for removal of fecal

and ingesta contamination spots of ≤ 2.5 cm in diameter. This is accomplished with hand-held equipment applying hot water and/or steam to loosen soil and inactivate bacteria, followed by removal of contaminants by vacuum. The effectiveness of knife-trimming and steam-vacuuming in reducing microbial contamination depends on employee diligence of application and the operational status of equipment. Before opening for removal of the viscera, the whole carcass may be sprayed with water and possibly organic acid (i.e., lactic or acetic) solution to reduce microbial contamination acquired during the hide removal process.

Following evisceration and carcass splitting with a saw, carcass sides (halves) that have passed the "zero tolerance" inspection are washed with water sprays of various pressures to remove bone dust and blood at the end of dressing. Following washing, carcasses are generally decontaminated with hot water or steam and/or chemical solutions. Hot water (≥ 74°C) may be applied through immersion or dipping, deluging, rinsing at low pressures, or spraying at higher pressures in special cabinets, depending on type of animal. A patented steam process for carcass decontamination (the Frigoscandia Steam Pasteurization System™) has been approved and is used in the United States.

Decontamination with organic acid (i.e., lactic or acetic) solutions (2-5%) is also applied to reduce the bacterial load of meat and poultry carcasses. The effectiveness of acids in decontamination of meat is enhanced when the temperature of the solution is 55oC. In general, organic acid spraying is used extensively in beef carcass decontamination after treatment with hot water or steam. Additional chemical solutions approved and used in the decontamination of meat and poultry include a mixture of hydrochloric and citric acids, peroxyacetic acid-based preparations, acidified sodium chlorite, trisodium phosphate, activated lactoferrin, chlorine, and chlorine dioxide. A variety of other chemical compounds have been tested with various rates of success for the decontamination of meat and poultry.

The use of more than one treatment may lead to synergistic or additive decontamination effects. This multiple hurdle fresh meat decontamination approach may involve simultaneous (e.g., warm acid solutions) or sequential (e.g., hide cleaning, steam vacuuming, pre-evisceration washing, hot water or steam treatment, organic acid rinsing) application of interventions. The effectiveness of these combined treatments in reducing contamination is affected by conditions such as pressure, temperature, chemicals used and their concentration, duration of exposure, and method and stage of application.

Concerns associated with decontamination include potential undesirable effects on color and/or flavor of the products. However, such effects are only slight and reversible at concentrations, temperatures and exposure times (<10 sec) employed. Proper selection and adjustment of factors (e.g., pressure, nozzle type, configuration and distance) affecting the efficacy of decontamination prevents contamination spreading and redistribution or cell penetration or embedding concerns. In addition, use of interventions that inactivate (e.g., hot water, steam, chemical solutions) rather than remove contamination, as in multiple intervention systems, prevents such issues. Approved decontamination chemicals are classified by the United States Food and Drug Administration (FDA) as generally recognized as safe (GRAS) and are approved by FSIS for use in meat and poultry, if effective, and only at levels that do not change the properties or identity of the product; if a change occurs, the product needs to be labeled accordingly. Proper use of the multiple hurdle decontamination system is considered effective in preventing potential antimicrobial resistance selection issues.

Reduction of pathogen prevalence on animals pre- and post-slaughter leads to a reduced probability that errors occurring in subsequent parts of the food chain will lead to foodborne illness. Proper application of the processes described above should yield products that should be safe for consumption following proper cooking and serving.

Foodborne pathogens on cattle hides and their control in the context of beef safety

Buncic S
University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Trg D. Obradovica 8, 21000 Novi Sad, Serbia

Modern microbial beef safety concept is based on a farm-to-fork principle that involves a wide range of coordinated control measures applied at all relevant steps in the beef chain. At abattoirs, certain microbial contamination of bovine carcasses during slaughter and dressing operations is inevitable and occurs at abattoirs in spite of adherence to process hygiene principles. Consequently, cattle carcass decontamination interventions have been considered, and widely used in beef abattoirs in some countries (e.g. USA), as a means to eliminate foodborne pathogens from carcasses. This approach could be seen as "reactive", as it deals with the problems after they occurred. In any case, the ultimate effectiveness of antimicrobial treatments, when assessed through the levels of surviving microflora remaining on treated substrate, depends on the initial microbial load to a great extent. Better ultimate results of the antimicrobial treatment are achieved when applied to cleaner substrate. Therefore, preventative approach, using hygiene-based measures, is universally and mandatorily used in the beef chain including at abattoirs, but can be complemented with the "reactive" approach in some situations within the regulatory frame.

Bovine carcasses may become contaminated with enteric pathogens during slaughter and dressing operations primarily from faecal material originating from the gut content or the hide. Today, in modern abattoirs, spillage of the gut content onto the carcass during evisceration operation occurs only rarely. However, based on numerous published studies, it has been widely recognised that the hide is the main source of direct and/or indirect microbial contamination of bovine carcasses. This has been supported by research findings that the occurrence of microbial pathogens (e.g. *E. coli* O157) on cattle hides and dressed bovine carcasses can be significantly correlated both quantitatively and with respect to relatedness of particular molecular subtypes of bacteria. Although a relatively small proportion (<0.1%) of hide microflora is transferred onto meat during direct contact, because very high microbial levels on hide exist (6-10 log/cm2), the counts of bacteria remaining on meat post-contact are still very significant. Therefore, hide-meat contact must be either totally prevented during, or pathogens must be eliminated from hide before, skinning of carcasses.

Data from published studies indicate that levels of general microflora on hides are relatively high and highly variable. Approximately, total count of bacteria can vary between 4 and >10 log per cm2 of hide, *Enterobacteriaceae* between 2 and 5 log, and generic *Escherichia coli* between 1.5 and 5.5 log. Furthermore, reported incidences of main bacterial foodborne pathogens on cattle hides are also relatively high and variable e.g. *E. coli* O157 between 7.5% and 100%, *Salmonella* between 3.3% and 100%, *Campylobacter* between 2.1% and 13%, and *Listeria monocytogenes* between 0.8% and 47.9%. A number of pre-slaughter factors differing between studies contribute to such a high variability of reported hide microflora including on-farm bacterial incidences/prevalences, animal production systems and animal husbandry, seasons, visual animal cleanliness, sample sizes, and sampling and microbiological methods. Furthermore, animal transport and lairaging (TL phase) lead to increased faecal shedding and/or levels of foodborne pathogens in animals including on hides, and microbial cross-contamination occurs via animal-to-animal and/or animal-to surfaces-to animal routes. The main factors contributing to the increasing occurrence of pathogens in animals during TL phase include mixing of animals of different origin, stress, extended TL duration, visual dirtiness of animals, and dirtiness of transport vehicles and lairage pens. Understandably, pre-slaughter controls of pathogens on hides including during TL phase are focused on effective measures to prevent/minimize these contributing factors. In particular, effective sanitation of vehicles and pens is essential to reduce T-L-related cross-contamination of hides, but naturally-occurring pathogens (e.g. *Salmonella, E. coli* O157) often persist on surfaces even after routine sanitation. Overall, these measures applied alone seem insufficiently effective; and even visually clean hides harbour high levels of microorganisms that often include pathogens.

More recently, additional hide-related controls have been considered including various treatments of hides (i.e. decontamination) applied after slaughter but before skinning of cattle to eliminate (kill) pathogens. The reported reductions in microbial and foodborne pathogens' counts on hides achievable by various treatments (e.g. singeing, commercial sanitizers or disinfectants, organic acids, sodium hydroxide, electrolysed water) under experimental conditions range from 1 log to 4-5 log, but under commercial abattoir conditions they are most commonly around 2-3 log reduction levels.

Most recently, our research has been focused on a novel approach to pre-skinning hide treatment so that it includes microbial fixation (immobilisation) on the hair. With this approach, the hide microflora including foodborne pathogens does not need necessarily to be entirely killed. Rather, the hide can be treated with some compounds that would just prevent microflora detaching from the hair, and thus prevent contamination of the carcass meat during skinning. A range of potential microflora-hide fixation/immobilisation compounds were considered. The results gathered to-date indicate that some natural resins commercially available as a glazing agent in food (e.g. candy or fruit) industry appear very promising. For example, treatment of hide with a solution of 30% Shellac (an insect-produced natural resin-based glazing agent) in ethanol resulted in up to several log reduction of natural total viable count and in >3-fold prevalence reduction of naturally-occurring *E. coli* O157.

In conclusion, as a meat safety measure, post-slaughter but pre-skinning hide treatment can be considered as a "proactive" approach to beef safety as it deals with the cause of microbial contamination of bovine carcasses i.e. with potential beef safety problems before they occur. It can be routinely used either alone or in combination with the end-product (dressed carcass) decontamination. It can be expected that, in the latter case, overall improvement of the microbiological status of the meat would be determined by a combination of microbial reductions achieved by both treatments, and very likely exceeding the improvements presently achievable by skinned carcass decontamination alone.

Effect of recipe and process parameters on reduction of enterohaemorrhagic *Escherichia coli* in Norwegian type dry fermented sausage

Even Heir[1], Askild Holck[1], Ingrid Måge[1], Ole Alvseike[2], Mohamed K. Omer[2], Lars Axelsson[1]
[1]Nofima Mat AS, Oslovn. 1, N-1430 Ås, Norway;
[2]Animalia–Meat and Poultry Research Centre, N- 0503 Oslo, Norway.

Several outbreaks have shown that dry fermented sausages can be a risk product with regard to enterohaemorrhagic *E. coli* infections. In a Norwegian EHEC outbreak in 2006, cases were linked to consumption of dry fermented sausage where 10 of 18 reported cases developed HUS and one child died. This has emphasized the need for strategies to obtain enhanced microbial safety of this type of product. To obtain fermented sausages with enhanced safety, strategies can be implemented at various stages in the production process, e.g. on raw ingredients, in the production process and post processing on finished fermented sausages.

We applied fractional factorial design to study how variations in key recipe and process parameters affect survival of EHEC in the sausage fermentation process. Conditions tested included variations in NaCl, nitrite, final pH and fermentation temperatures on two types of fermented sausages. After fermentation and maturation, effect of post process strategies for reduction of EHEC in finished fermented sausage was investigated. These strategies included storage at various temperatures, combined freezing, thawing and storage, mild heat treatments and high pressure processing.

The results indicate that changes in levels of standard process and recipe parameters in general have a limited effect on EHEC reduction during the production process (approx. 1-2 log reductions obtained). In the production process, increased reductions were obtained at higher fermentation temperatures (34°C versus 20°C). Further reductions in EHEC were obtained be applying post process strategies on finished sausages. High pressure processing (400/600 MPa), storage at room temperature, mild heat treatments and combined freezing, thawing and storage had effect on reduction of EHEC. Implementation of one or a combination of these strategies can be an approach to obtain Norwegian type dry fermented sausages with enhanced safety.

Translocation and Thermal Inactivation of Shiga-Toxin Producing *Escherichia coli* in Non-Intact Beef

Luchansky, J. B., L. Huang, C. Sommers, B. Shoyer, J. E. Call, and A. C. S. Porto-Fett.
United States Dept of Agriculture, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA 19038 USA

We compared translocation of genetically-marked strains of O157:H7 *Escherichia coli (ECOH)* to non-O157:H7 Shiga-Toxin producing *Escherichia coli (STEC)* following blade tenderization of beef subprimals and the subsequent lethality of these pathogens following cooking of steaks prepared from tenderized subprimals. In Phase I, subprimals were inoculated on the lean side to a target level of ca. 6.0 log CFU/g and then passed once through a mechanical blade-tenderizer with the lean side facing upwards. Ten core samples were removed from each and cut into six segments starting from the inoculated side: segments 1 to 4 comprised the top four cm and segments 5 and 6 comprised the deepest four cm. Ten cores were also obtained from control (inoculated, non-tenderized) subprimals, but only the topmost 1 cm (segment 1) was sampled. Each segment was macerated, and the resulting fluid was plated onto Sorbitol-MacConkey plus kanamycin (10 µg/ml) for *STEC* or plus rifampicin (10 µg/ml) for *ECOH*. Levels of *STEC* and *ECOH* recovered from segment 1 were 5.26 and 5.96 log CFU/g, respectively, for the control subprimals and 4.99 and 5.74 log CFU/g, respectively, for tenderized subprimals. In Phase II, lean side inoculated and single-pass tenderized subprimals were cut into 1.5 inch thick steaks and cooked on a commercial gas grill to internal temperatures ranging from 120° to 160°F. In general, *STEC* were more recalcitrant to heat than *ECOH* at <140°F. Over the range of temperatures tested, however, we observed about a 2.5- to 5.0- and 4- to 4.5-log CFU/g reduction of *STEC* and *ECOH,* respectively. These results confirm that blade tenderization transfers the majority of E. coli cells into the topmost 1 cm of non-intact suprimals. Our data also demonstrate that cooking results in an appreciable reduction of these pathogens that may become internalized into whole muscle cuts of meat via mechanical tenderization.

Alternative methods to control pathogens

Mansel Griffiths
University of Guelph, Canada

An effective strategy to control pathogens must include interventions at all points along the food chain. These include methods to prevent dissemination of pathogens in the environment, control of pathogens at the processing site and, ideally, protection of the consumer. To achieve this holistic approach to pathogen control, we are developing packaging materials based on immobilized bacteriophage that can be used to prevent growth of pathogens on meat and designing self-decontaminating surfaces that can reduce the incidence of pathogens in the environment. We are also investigating bioactive molecules that down-regulate genes encoding proteins of Type 3 secretory systems in Gram negative enteric pathogens with a view to prevent shedding of bacterial pathogens by animals and to act as prophylactic agents to protect against enteric infections.

Biocontrol of Bacterial Pathogens on Meat by Bacteriophages

J. Andrew Hudson, Craig Billington, Lynn McIntyre, Teresa Bigwood, Aruni Premaratne and Stephanie Brandt

ESR, Food Safety Programme, PO Box 29-181, Christchurch 8540, New Zealand

Biocontrol is the use of one species, or a product derived from it, to control another. An example of a biocontrol approach (albeit originally unintentional) to the preservation of a meat product that has been used for hundreds of years is salami manufacture, where lactic acid bacteria not only impart desirable organoleptic qualities but also act as protective cultures inhibiting pathogens and preventing spoilage.

There are many possible ways that biocontrol could be used. For example Nisin, a bacteriocin, has approval for use in a number of foods. Other, less well developed, options include bacteriophages (phages), quorum sensing-disruption, and the use of plant extracts (e.g. essential oils). We are involved in a programme (IMPACT- Improved PAthogen Control Technologies) with AgResearch and Massey University in New Zealand to investigate and optimise the potential use of two of these possibilities, phages and quorum sensing inhibition, to control *Escherichia coli* O157:H7 and *Campylobacter jejuni* on meat products; the potential of phages will be discussed here.

The clinical use of phages to control of human infections started in the early 1900s and was subsequently experimentally applied to control veterinary infections. More recently, the potential to control pathogenic bacteria in foods has been recognised. Phages are viruses that can specifically infect and kill bacteria, and infection by virulent phages results in the destruction of the cell with the release of around 100-300 progeny phages. Under these conditions the concentration of the biocontrol agent increases as a result of its action, and this has been cited as an advantage of phage biocontrol. However, the use of virulent phages replicating *in situ* requires host cells to be growing and this is not usually the case with pathogens in refrigerated foods (except for *Listeria monocytogenes* and *Yersinia enterocolitica* which can grow at <5°C). In addition, it would be expected that pathogens will be present at low concentrations and so the contribution to the phage titre made by, maybe, a few hundred cells will be negligible. It is now being recognised that the control which has been shown in numerous papers results from the infection of cell *in situ* in the absence of host growth with lysis occurring on the resumption of growth. High concentrations of phages are required to achieve this efficiently.

Other properties of phages could be harnessed for effective control. For example many phages may be added to destroy cell walls without infection (lysis from without), or phage-encoded enzymes could be cloned, expressed and purified to destroy target bacterial cell walls.

Phages could be used at many points in the farm to fork chain. For example, they could be used as a means of removing pathogens from animal faeces or hides. Phages have been used to control *Escherichia coli* O157:H7 in bovine gastrointestinal tracts with mixed success, but there are no published data on the use of phages to decontaminate bovine hides.

The potential to use phages infecting *Salmonella* and *Campylobacter* to inactivate target pathogens on raw and cooked beef surfaces has been studied by our group. Pathogens were applied at two concentrations (approximately 102 and 104 CFU cm-2) and phages also at two phage:host (P:H) ratios (102 and 104), and samples incubated at 5°C or 24°C to simulate chilled and room temperature storage. Experiments were initially for 24 h, but then extended to 8 days at 5°C to simulate typical extended meat storage conditions. Over 24 h, phages produced varying reductions of both pathogens, with the greatest reductions occurring at 24°C where both the host concentration and phage:pathogen ratio were high. Over 8 days both phages reduced pathogen populations, with reductions greatest on raw meat with hosts and phages at the highest concentration used. These reductions were not, however, as large as those observed in the initial short-term experiments.

In published work concerning phages infecting *E. coli* O157:H7, host cell lysis was found to occur at high phage concentrations

on beef at 37°C and 10°C. Control of *L. monocytogenes* has been demonstrated on a cooked processed meat product (hot dogs), again with the use of high phage concentrations, but results with raw beef cubes were less encouraging, although in this case phages were applied at low numbers.

Phage endolysins have been the subject of considerable interest in the USA as possible means of countering terrorist activity involving the use of Gram-positive pathogens such as *Bacillus anthracis*. We are in the process of cloning endolysins from phages infecting *Campylobacter* but there are technical problems to overcome because of the presence of an outer membrane in Gram negative bacteria which acts to keep the enzyme away from the cell wall.

While we, and others, have found phages to be effective in the control of pathogens in foods there remain technical and other challenges that may need to be overcome in certain circumstances. For example:

i) The presence of high concentrations of non-target bacteria or other material may interfere with the ability of phages to find their targets. This will be important in sites such as the gastrointestinal tract of food animals.
ii) Resistant mutants may survive phage attack to re-grow, but this can be prevented through the use of phage cocktails, preferably with the phages recognising different cell wall structures during adsorption. The use of cocktails will also enable mixtures to be formulated which will kill all, or a vast majority of, target cell subtypes.
iii) Several safety concerns have been raised but these can be accommodated. In one recent study subjects consumed coliphage T4 and suffered no ill effects. The regulatory approvals which have been granted reflect the acceptability of phage treatments, as does research into their application to human therapeutics.
iv) There is little information on consumer acceptance of phage control of pathogens. One report indicated that focus groups considered them to be "green" alternatives to chemical preservatives. Since phages are naturally present in many foods, as well as in/on the consumer, the addition of phages to foods only changes the concentration present, rather than introducing a "foreign" substance.

For phages to become a mainstream control of pathogens in food the mistakes of the past need to be avoided. In the first half of the 20th century the use of phages for human treatment fell into disrepute because of a lack of understanding of the processes involved, coupled with the inability to produce phage preparations consistently. For food applications we need to understand the science behind the observations so that the use of phages can be optimised and success assured. Encouragingly, there is a growing list of regulatory approvals for the application of phages to food control. Two phage preparations for the control of *L. monocytogenes* have been granted GRAS status in the USA and so can be applied to foods on which the pathogen might be present. One commercial phage preparation for the control of *L. monocytogenes* has very recently been approved by the EPA as a decontamination product for processing plants, and two phage-based hide decontamination products (one for *Salmonella E. coli* O157:H7) are now approved by the USDA.

Control of *E coli* O157 and *Salmonella* in harvest-ready cattle using siderophore receptors and porin proteins (SRP)-based vaccine technology

Guy H Loneragan[1], Daniel U Thomson[2], Thomas S Edrington[3], Doug T Burkhardt[4], Mindy M Brashears[5]

[1]Department of Agricultural Sciences, College of Agriculture, Science and Engineering, West Texas A&M University, Canyon, Texas 79016, USA

[2]Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas 66506, USA

[3]Southern Plains Agricultural Research Center, United State Department of Agriculture Agricultural Research Service, College Station, Texas 77845, USA

[4]Epitopix, LLC, 3735 West First Ave, Willmar 56201, Minnesota, USA

[5]International Center for Food Industry Excellence, Texas Tech University, Lubbock 79409, Texas, USA

Introduction

Because iron is an essential nutrient of pathogenic bacteria, immunity against SRP may limit their relative fitness within the complex gut environment. Using identical technological platforms, but pathogen-specific SRP, vaccines were developed to aid in the control of *Salmonella* (conditionally licensed) and *E.coli* O157 (pre-licensure).

Objective

Evaluate pre-harvest control of *E.coli* O157 and *Salmonella* using pathogen-specific SRP-based vaccines

Methods

Study 1: Nine dairies were enrolled; feces were collected from cull cows intended for harvest. Samples were pre-enriched in TT and RV broth then cultured for *Salmonella* on selective agar.

Study 2: 1,284 steers were allocated to vaccinate or nonvaccinate cohorts and housed in 20 (10/cohort) pens within a feedlot. Vaccine (or placebo) was administered on days 0, 21, and 42. Feces were collected on days 0, 42, and 98 from 30 animals/pen. Samples were cultured for *E.coli* O157 using standard methods. Concentration of *E.coli* O157 in d98 samples was estimated using an MPN method.

Results

Study 1: *Salmonella* was recovered from 32.6% of 706 cows culled from 9 dairies. Dairy-level prevalence varied from 4.4 to 86.3%. Dairies (n=3) that practiced whole-herd vaccination with the *Salmonella*-SRP vaccine had a lower prevalence of *Salmonella* than herds that did not (7.6 vs. 39.2%; P<0.01).

Study 2: *E.coli* O157 was recovered from 7.5% of samples. Animals vaccinated with the *E.coli* O157-SRP vaccine had a lower prevalence (1.5 vs. 10.4%; P<0.01) and a 98.2% reduction in concentration of *E.coli* O157 on d98 (0.80 vs. 2.54 log MPN/g; P<0.01).

Conclusions

While not eliminating pathogen risk, administration of pathogen-specific SRP-based vaccines may be an effective pre-harvest strategy to control *Salmonella* and/or *E.coli* O157. These products may serve as another hurdle within a multihurdle system of interventions designed to control pathogens in the farm-to-fork continuum.

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Development and application of a novel packaging technology for fresh meat based on the incorporation of a Lactobacillus sakei protective culture into a an Na-caseinate edible film

Gialamaç Haralamos[1], Biliaderis Costas[2] and Koutsoumanis Kostas[1]
[1]Laboratory of Food Hygiene & Microbiology, Department of Food Science and Technology, School of Agriculture, Aristotle University Thessaloniki, 541 24 Thessaloniki, Greece.
e-mail: kkoutsou@agro.auth.gr
[2]Laboratory of Food Chemistry & Biochemistry, Department of Food Science and Technology, School of Agriculture, Aristotle University Thessaloniki, 541 24 Thessaloniki, Greece.

A novel packaging technology was developed based on the incorporation of a *Lactobacillus sakei* protective culture into a Na-caseinate edible film. The incorporation of the culture was based on the addition of the cells in the initial film solution during preparation or on surface spraying of the culture to the final film. In both cases the final concentration of *L. sakei* in the film was 10^6 cfu/cm^2, approximately. The analysis performed included the effect of the culture on the structural integrity, thermal and mechanical properties of the film, the survival of *L. sakei* during storage of the film at room and chill temperature conditions and the antimicrobial activity of the polymeric film against *Listeria monocytogenes* on model food (agar) and fresh beef. The results showed that the addition of the culture did not affect the mechanical properties of the film. The addition of sorbitol in the film enhanced the survival of cells during storage. In particular, the survival of the culture exceeded 90% after one month storage at both room and chill temperature conditions. Application of the developed film to both food model and beef samples inoculated with *L. monocytogenes* resulted in a significant inhibition of the pathogen compared to the control samples (no film). The above result indicate that the developed film can be used as an effective packaging technology for improving food safety.

A Systems Approach to Effective Control of *E coli* O157 using Lactic Acid Bacteria as a Pre- and Post-harvest Intervention

Mindy M Brashears[1], Guy H Loneragan[2], and D Ware[3]

[1]International Center for Food Industry Excellence, Texas Tech University, Lubbock 79409, Texas, USA

[2]Department of Agricultural Sciences, College of Agriculture, Science and Engineering, West Texas A&M University, Canyon, Texas 79016, USA

[3]Nutrition Physiology Corporation, LLC Nutrition Physiology Company ,LLC. 97533 Franklin Ridge, Chapel Hill, NC 27517

Introduction

Escherichia coli O157:H7 causes a severe bloody diarrheal illness which can lead to kidney failure or death. Cattle are the primary reservoir for the pathogen with beef products being commonly associated with outbreaks of the illness. Lactic acid bacteria (LAB), and specifically *Lactobacillus acidophilus* NP51, produce inhibitory compounds that can potentially control pathogens in multiple environments if properly managed.

Objective

Determine the reduction of *E. coli* O157:H7 in beef feedlot cattle using NP51 when fed as a direct-fed microbial and in beef products as a direct additive.

Methods

Study 1: A meta-analysis was conducted on 13 studies evaluating cultures containing a 1 x 10^9 dose of the LAB NP51 for the reduction of either shedding of *E. coli* O157:H7 in the feces and/or the prevalence on the hides.

Study 2: Warm and cold beef surfaces and raw ground beef was inoculated with a 1 x 10^5 dose of *E. coli* O157:H7 supplemented with a 1 x 10^9 cfu either per cm2 or per g and then held at 5oC over a 48 hour period. Samples were collected at 0, 6, 12, 24 and 48 hours to determine pathogen reductions compared to a control containing no NP51 supplementation. *E. coli* O157:H7 was recovered on MacConkey's agar using a thin layer of trypic soy agar to allow for cell recovery.

Results

Study 1: The fecal carriage in the beef was reduced from 26.5% in the animals not exposed to the NP51 to 12.7% in animals fed a diet supplemented with NP51. The hide carriage in animals not exposed to the NP51 was at 20% while it was significantly lower in the animals exposed to the NP51 at 11.3%.

Study 2: In all beef products there was a 90% reduction after 6 hours exposure to the NP51 compared to the controls. After 24 hours, the reductions were up to 99% in the ground beef with a final reduction of 99.9% observed after 48 hours. There appeared to be no residual effect in the hot and cold surface applications. Application of LAB to beef products resulted in no adverse sensory parameters.

Conclusions

LAB NP51 can be used in both pre and post-harvest environments as a key component within a systems approach to control *E. coli* O157:H7 and thereby improve the overall safety of the beef supply in a farm to fork approach.

The use of carvacrol for the inhibition of *Escherichia coli* O157:H7 in a model rumen system

L. Rivas[1], M. J. McDonnell[1,2], C. M. Burgess[1], S. Fanning[2] and G. Duffy[1]

[1]Food Safety Department, Ashtown Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland.
[2]School of Agriculture, Food Science, and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland.

Escherichia coli O157:H7 is a food borne pathogen that causes serious illness in humans. Reducing the risk of contamination and transfer of *E. coli* O157:H7 from animals to food can reduce the risk of infection. There is a potential to use essential oil components such as carvacrol to control *E. coli* O157:H7 in food animals prior to slaughter. The aim of this study was to evaluate the activity of carvacrol against *E. coli* O157:H7 in a laboratory model of the rumen system. The model was composed of rumen fluid collected from a fistulated bovine animal, rumen buffer and substrates (grass silage:concentrate). A non-toxigenic *E. coli* O157:H7 rendered resistant to 1,000µg/ml streptomycin sulfate and 50µg/ml nalidixic acid (nas) was used in the experiment. *E. coli* O157:H7 was added to the rumen model at a 'low' (10^3 cfu/ml) and 'high' (10^{ff}cfu/ml) inoculums level. Carvacrol was added to the rumen model at concentrations of 0.0125, 0.025, 0.05, 0.1, 0.2 and 0.5% (v/v). The number of surviving *E. coli* O157:H7 over 24h was determined using direct plating onto sorbitol macconkey agar with nas (SMAC-nas) or tryptone soya agar overlaid with SMAC-nas. An *in vitro* gas production method was used to determine the effects of the carvacrol on rumen fermentation. All results were compared using ANOVA. Carvacrol at concentrations of $\geq$ 0.05% significantly ($p<0.05$) reduced inoculum levels of *E. coli* O157:H7 to undetectable levels within 0.5-1h and remained undetected after 24h incubation. Concentrations of $\leq$ 0.025% did not significantly ($p>0.05$) reduce *E. coli* O157:H7 numbers compared to the control. The in vitro gas production method showed that carvacrol can significantly ($p<0.05$) reduced the total gas production of the rumen model. The data suggests that carvacrol can inhibit/kill *E. coli* O157:H7 in a model rumen system. However, the concentrations required to inhibit *E. coli* O157:H7 can also affect the rumen microflora and fermentation and further evaluation is required to determine the appropriate levels of carvacrol which can be safely administered to bovine animals.

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Biocontrol of *Escherichia coli* O157:H7: Evaluation of Two Anti-*E coli* O157:H7 Bacteriophages in the Cattle Rumen

Brid Coffey[1,2], Lucy Rivas[3], Mary J McDonnell[3], Aidan Coffey[2], Geraldine Duffy[3], R. Paul Ross[1] and Olivia McAuliffe[1]

[1]Teagasc, Moorepark Food Research Centre, Fermoy, Co. Cork.
[2]Department of Biological Sciences, Cork Institute of Technology, Cork.
[3]Teagasc, Ashtown Food Research Centre, Castleknock, Dublin 15.

Escherichia coli O157:H7 is a highly virulent food-borne pathogen that can cause a variety of severe diseases including haemolytic uremic syndrome. It is known that healthy ruminants harbour the human pathogen *E. coli* O157:H7 in their gastrointestinal tract and can shed the organism in their faeces. Contamination from the hide and/or faeces onto the carcass can occur during slaughtering and processing. Reducing the risk of contamination and transfer of *E. coli* O157:H7 from animals to food can lessen the risk of infection. Two virulent anti-*E. coli* O157:H7 lytic bacteriophages, e11/2 and e4/1c, were isolated previously from bovine slurry samples after an extensive screening project. These phages have the potential to be used as biocontrol agents to reduce intestinal levels and possibly faecal shedding of *E. coli* O157:H7 in cattle. The objective of this work was to determine the ability of the phages to decrease numbers of *E. coli* O157:H7 in a model rumen system. Both phages were tested separately in the model rumen which contained rumen fluid obtained from fistulated animals, rumen buffer and feed. High (10^6 cfu/ml) and low inoculums (10^3 cfu/ml) of a non-toxigenic strain of *E. coli* O157 were added to the rumen assay. In two separate experiments, phages e11/2 and e4/1c were added at an MOI (multiplicity of infection) of 100 and samples were taken and plated to enumerate *E. coli*O157 over a 24 h period. Phage e11/2 was very effective at reducing *E. coli* numbers; *E. coli* O157:H7 was undetectable after 1 h and remained undetectable for 24h. After addition of phage e4/1c however, while *E. coli* 0157 numbers were reduced in comparison to the control, the results were not as significant as seen with phage e11/2. Results from this work are promising and suggest that these phages may have an application in the biocontrol of *E. coli*O157 in beef.

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P1

Distribution of microflora on cattle hides

Antic D.[1], Blagojevic B.[1], Ducic M.[1], Mitrovic R.[2], Nastasijevic I.[2] and Buncic S.[1]
[1]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Trg D. Obradovica 8, 21000 Novi Sad, Serbia
[2]Institute of meat hygiene and technology, Kacanskog 13, 11000 Belgrade, Serbia

Microbial contamination of dressed beef carcasses with food-borne pathogens occurs when they are transferred from hide onto meat during skinning on the slaughterline. Therefore, the main aim of research in this study was to characterise the nature, composition and distribution of bacterial microflora on cattle hides at slaughter.

To determine levels of TVC and *Enterobacteriaceae*, as well as prevalence of generic *E. coli* and *Salmonella*, a total of 200 swabs were taken on the slaughterline from five areas of hide of each of 40 cattle in one commercial abattoir in Serbia. In addition, a second study was conducted to determine the occurrence of *E. coli* O157 on the same areas of cattle hides, with 355 swabs taken from 71 cattle. Another study was performed to investigate the "vertical distribution" of microflora (TVC and *Enterobacteriaceae*) on cattle hair, collected by clipping the pieces of visually clean hides.

Overall, the highest level of TVC, *Enterobacteriaceae* and prevalence of generic *E. coli* was found on brisket (7.1 log cfu/cm^2, 4.4 log cfu/cm^2 and 65%, respectively) and metacarpus (7.0 log cfu/cm^2, 4.5 log cfu/cm^2 and 75%, respectively) areas of cattle hides. The hide on neck, rump and flank areas was significantly less microbiologically contaminated. With respect to *E. coli* O157, the occurence was, in decreasing order: metacarpus (11.3%), brisket (8.4%), rump (7%), neck (4.2%) and flank (2.8%). *Salmonella* was not found on any cattle hide examined.

There were no significant differences in the mean TVC and *Enterobacteriaceae* count between the hair's top (7.8 and 3.3, respectively) and lower (7.6 and 2.9, respectively) layers on cattle hides.

The obtained information on the composition, nature and distribution of microflora on hides of slaughtered cattle is a pre-requisite for further studies aimed on development of effective hide decontamination treatments.

Keywords: hides, meat, microbial contamination, *Salmonella, E. coli* O157, faecal indicator organisms, beef

P2

Microbial transfer from cattle hides onto beef via direct contact

Antic D.[1], Blagojevic B.[1], Ducic M.[1], Mitrovic R.[2], Nastasijevic I.[2] and Buncic S.[1]
[1]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Trg D. Obradovica 8, 21000 Novi Sad, Serbia
[2]Institute of meat hygiene and technology, Kacanskog 13, 11000 Belgrade, Serbia.

Today, based on a number of published studies, it is widely accepted that the key source of microbial contamination of bovine carcass meat is the hide. Hide-to-meat microbial cross-contamination during skinning (dehiding) of slaughtered cattle can occur:
a) through accidental contact between loosened parts of hide and underlying meat ("hide inrolling");
b) via hide-contaminated equipment/tools; and
c) via aerosols generated during dehiding.

In this study, transfer ratios of general microflora from hide onto meat following direct contacts, as well as the effects of selected factors (dry/wet hide, lean/fatty meat, lower/higher pressure, contact duration and friction), were investigated.

A relatively low proportion (<0.1% on average; from 0.5% to 0.0002%) of the hide TVC was transferred onto the meat via direct contact. On average, around 0.01% of *Enterobacteriaceae* counts and 0.001% of generic *E. coli* counts were transferred from hide onto the meat, but the transfer was observed only in 60% and 10% of contacts, respectively. An overall tendency of enhanced microbial hide-to-meat transmissions was associated with higher contact pressure and wet hide. On the other hand, the effects of differences in meat fattiness, contact duration and hide-meat friction on the hide-meat transfer ratios were highly variable causing their statistical insignificance.

In conclusion, although a relatively small proportion of hide microflora is transferred onto meat via direct contact, because very high microbial levels on hide exist (6-10 log/cm^2), the counts of bacteria remaining on meat post-contact are still very significant. Therefore, hide-meat contact must be either totally prevented during, or hide must be decontaminated before, skinning of carcasses.

Keywords: Hide; meat; skinning; carcass cross-contamination

P3

Evaluation of "microbial fixation" treatments on cattle hides – a proof of concept study

Antic D.[1], Blagojevic B.[1], Ducic M.[1], Mitrovic R.[2], Nastasijevic I.[2] and Buncic S.[1]
[1]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Trg D. Obradovica 8, 21000 Novi Sad, Serbia
[2]Institute of meat hygiene and technology, Kacanskog 13, 11000 Belgrade, Serbia.

Recently, much research has been published on elimination of foodborne pathogens from the main source of beef carcass contamination – hide – before skinning (dehiding) of the slaughtered bovines.

In the present study, a novel, alternative approach to hide treatments has been explored: microbial fixation on the hair. Amongst a range of potential microflora-on-hide fixation compounds, an insect-produced natural resin (Shellac) and a hair spray for cosmetic purposes (both commercially available) were experimentally evaluated.

With respect to general, natural microbiota on uninoculated hides, up to 6.6 $\log_{10}$CFU/cm^2 reductions in recoveries of total viable count of bacteria, *Enterobacteriaceae* and generic *E. coli* were achieved by spray-treatment of hides with Shellac solution (30% in ethanol). Comparably smaller reduction effects on these groups of microorganisms were obtained by the hair spray treatment (up to 5.9 log reduction), or by the control rinse-vacuum treatment with a quaternary ammonia compound-based sanitizer (up to 4.9 log reduction).

With respect to pathogens, the Shellac treatment reduced the prevalence of naturally-occurring *E. coli* O157 recovered from uninoculated hides by 3.7-fold. Furthermore, Shellac treatment reduced (by 2.0 logs) the counts of *E. coli* O157 recovered from artificially inoculated hides. Nevertheless, the study demonstrated that inoculated-hide model does not satisfactorily simulate the conditions of the naturally-occurring microflora on untreated hide.

The results of the study provided a "proof of concept" i.e. that a novel, alternative approach to hide decontamination – i.e. microbial fixation treatment – is promising for reduction of microflora transferable from hide to carcass during dressing.

Keywords: Hide; hide decontamination; *Escherichia coli* O157; beef; carcass

P4

Validation of a DNA and a RNA real time assay for detection of *Salmonella* enterica on fresh beef carcasses

E. Mc Cabe[1], C. Burgess[1], Edel O'Regan[3], Sheila Mc Guinness[2], T. Barry[2] and S. Fanning[3], G. Duffy[1]
[1]Food Safety Department, Ashtown Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland;
[2]Department of Microbiology, National University of Ireland, Galway, Ireland;
[3]School of Agriculture, Food Science, and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland.

A major challenge for the European beef industry is meeting the requirements of the new EU hygiene regulations (852/2004, EC 853/2004, EC 854/2004) and regulation on the microbiological criteria for foodstuffs (EC 2073/2005) which came into force in January 2006. The criteria require the implementation of a HACCP system in all beef business operations (from slaughter to butcher). These criteria include new directives to test for *Salmonella* on beef carcasses and processed beef products.

Salmonella continues to be a major food-borne pathogen and in many countries and is a leading cause of food-borne outbreaks and infections. In order to ensure beef safety, the availability of reliable test systems to detect the presence or absence of these pathogens is becoming increasingly important for the beef industry, as well as for legislative control. Standard methods, such as ISO 6579:2002 (ISO 6579:2002), for detecting *Salmonella* spp. in foods are time-consuming, having up to 4–6 days isolation procedure. In recent years, nucleic acid amplification technologies have offered the potential for improved detection of *Salmonella* in beef, providing greater sensitivity and rapidity. The objective of this study was the validation of a DNA and RNA diagnostic PCR methods for the detection of food-borne *Salmonella* on beef carcasses. Both real time PCR assays were compared with the standard bacteriological ISO 6579 reference method (ISO 6579:2002) in selectivity and sensitivity studies. The inoculation of *Salmonella* free swab samples with six different levels of inocula and with five different typed strains of *Salmonella* enterica was carried out where, data on the inoculated sample swabs indicated that the PCR methods were able to detect after enrichment < 10 CFUs per beef carcass swab. The DNA method was further validated in a collaborative inter-laboratory trial. This study addresses the increasing demand for alternative diagnostic methods for the specific and sensitive detection of *Salmonella* enterica on fresh beef carcasses.

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P5

Serogroups and virulence genes in verocytotoxigenic *Escherichia coli* on beef farms and in the beef abattoir

Declan J. Bolton, Ciara Ennis, Brain Byrne and Aine Monaghan
Food Safety Department, Ashtown Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland.

VTEC strains belonging to the serotype O157:H7 are responsible for most of haemolytic uraemic syndrome (HUS) cases and outbreaks worldwide. However, serogroups O26, O111, O103 and O145 are also considered to be clinically important and other VTEC serogroups are increasingly associated with human illness. The farming environment is a critical stage for the development of new VTEC as well as the dissemination of existing strains. VTEC are found on virtually all farms, at least intermittently, with a strong seasonal peak in late Summer-early Autumn. Carriage in the gastrointestinal tract of individual animals is asymptomatic and transient (2 months or less) with faecal shedding occurring in sharp peaks separated by long periods of low prevalence. This study determined the prevalence of VTEC on beef farms, on cattle hides and on beef carcasses in Ireland and characterised the isolates by serogroup and virulence factors. Faecal and soil samples were obtained from 20 farms throughout Ireland over a 12 month period. In a related study, faecal and slurry samples were obtained from 12 farms on the same water course over a similar timescale. In the abattoir, 450 bovine hides and their corresponding carcasses were also tested. The analytical techniques used included a preliminary PCR screen for *vt1, vt2, eaeA* and *hlyA* before plating onto TBX agar. All isolates were serotyped and PCR screened for a range of virulence factors including *vt1, vt2, TIR, hlyA, eaeA, saa, IpfA, katP, etpD, espF, espP, espB,* and *espA* and the *vt2* gene variants were further investigated. A range of different VTEC serogroups were discovered with many of these being associated with clinical disease in humans. The virulence gene profiles varied with isolate and 15% of abattoir isolates had VT2c, a variant associated with a higher risk of HUS in humans. These reults and data on farm and abattoir VTEC will be presented and discussed.

P6

ISO 16140 validation of a real time PCR method for the simultaneously detection of *E. coli* O157:H7 and *Salmonella* spp. in beef in 10 hours

Sirine Assaf, Sylvie Hallier-Soulier, and Patrice Chablain
GeneSystems, Bruz, France.

We have developed a molecular method for simultaneous detection of *Salmonella* spp and shigatoxin-producing *E. coli* (STEC) and the associated-virulence factors in beef in 10h. This method was recently AFNOR validated according to ISO 16140 standard.

Briefly, 25g of food sample are enriched in BPW for 8h at 41.5°C or for 16h at 37°C. Bacterial DNA is extracted from the enrichment, then analyzed with the GeneDisc Cycler, a PCR-based technology allowing the amplification of specific sequences for:
(i) *Salmonella* spp (*iroB*),
(ii) *E. coli* O157 (*rfbE*),
(iii) *stx* genes and
(iv) *eae* gene.

When the *rfbE* PCR and/or STEC PCR is positive, the DNA extract is further analyzed using a second PCR assay targeting the presence of the flagella H7 (*flicH7*) and of the main *E. coli* serovars (O26, O103, O111 and O145), described in Europe as being implicated in haemolytic uremic syndrome in humans.

GeneSystems method has been compared to ISO 6579 and ISO 16654 for *Salmonella* spp. and *E. coli* O157, respectively. Accuracies, specificities and sensitivities have been evaluated with 70 meat samples. Results obtained using standard and GeneSystems methods are shown not to differ (α=0.05). The limits of detection of PCR methods are 0.1-1.0 and 0.1-1.2 CFU/25g for *Salmonella* and *E. coli* O157:H7, respectively. Finally, 50 targeted strains and 32 other strains were analysed for specificity.

The second part of the validation was a ring trial (12 independent laboratories) using ISO 16140 guidelines. Three discordant assays were obtained amongst a total of 576 analyses. The variability of the PCR and ISO methods were shown to be equivalent.

This AFNOR approved method is a promising tool for faster and more reliable results in routine food controls onsite. In addition, the combination of multiparametric assays allows the discrimination of STEC and EHEC using the detection of *stx* and *eae* genes on the same device.

P7

Effects of a yeast based probiotic and an essential oil on *Escherichia coli* O157 and *Listeria innocua* intestinal populations in sheep

M.R.F. Lee, M.K. Theodorou, V.J. Theobald, N.D. Scollan and C.J. Newbold
Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, Ceredigion, UK, SY23 4LL

Pathogenic bacteria *E. coli* O157 and *L. monocytogenes* are two of the most common agents of food borne illness' in humans. Essential oils have been shown to have antimicrobial activity against food borne agents, whereas certain strains of yeast (*Saccharomyces cerevisiae*) have been shown to reduce pathogen survival (*E. coli* and *L. innocua*) in the rumen. To further investigate these findings this experiment examined the effect of a yeast based probiotic (SC20) and an essential oil (CF-SD) on gastro-intestinal tract (GIT) populations of *E. coli* and *L. innocua* in experimentally infected sheep.

Twenty-four sheep were blocked according to sex, liveweight, and background faecal pathogen counts. They were then allocated to one of three groups: control (C), essential oil (EO) and yeast (Y). Animals received grass silage at maintenance either with no additive (C), 2ml/d CF-SD (EO) or 0.5g/d of SC20 (Y), on top of the silages for a period of 3 weeks. Animals were then inoculated with 1.8 x 10^8 CFU/ml of each pathogen (*E. coli* and *L. innocua*) contained in broth medium. Animals were then offered silage and humanely killed 24h after inoculation. Four sections of the GIT were isolated: Rumen (Ru); small intestine (SI, distal duodenum – terminal ileum); large intestine (LI, ascending colon – caudad descending colon) and rectum and anus (ReAn). Luminal contents were collected for bacterial plate enumeration. There were no significant differences between treatments in any of the GIT sections with mean numbers of 15903 ± 5193.6, 77913 ± 37259.0, 54583.6 ± 18767.8, 3605.7 ± 14716.5 CFU/gDM for *L. innocua* and 454807 ± 311191.4, 1904415 ± 548770.3, 1757669 ± 433729.7, 1585674 ± 363253.8 CFU/gDM for *E. coli* in the Ru, SI, LI and ReAn, respectively. The results suggest that regulation of pathogens in *vivo* through dietary supplementation of yeast probiotics and plant essential oils was unsuccessful.

P8

Exposure assessment to microbial pathogens in Brazilian bovine hides and carcasses

Eb Chiarini[1], Angela P. Azevedo[1], Graciela V. Lopes[1], Kathelin M.S. Lascowski[2], Janaina T. Lopes[1], Verônica S. Fogo[1], Gabriela A. Siqueira[2], Beatriz E. C. Guth[2], Mariza Landgraf[1], Maria Teresa Destro[1], Sueli A. Fernandes[3], Bernadette D. G. M. Franco[1]

[1]Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil;

[2]Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo, SP, Brazil;

[3]Bacteriology Section, Adolfo Lutz Institute, São Paulo, SP, Brazil.

The study aimed at determining the prevalence, level of contamination and characteristics of *Listeria monocytogenes, Campylobacter jejuni/coli, Salmonella* spp and *Verocytotoxigenic Escherichia coli* (VTEC) in bovine hides and carcasses in an abattoir located in São Paulo State, Brazil. This abstract reports results of 85 animals (hides and the correspondent carcasses). For detection, enumeration and characterization of pathogens, appropriate ISO methods were used. When necessary, molecular methods were applied. Screening for antibiotic resistance of *Salmonella* spp was done using the disk diffusion method. *Salmonella* spp was detected in eleven hides (13%) and four carcasses (5%). Positive carcasses derived from hide-positive animals. Counts of *Salmonella* spp were usually low both in hides and carcasses (< 0.01 CFU/cm^2), only one hide presented counts of 95 CFU/cm^2. *Salmonella* isolates were sensitive to all twenty tested antimicrobials, except one strain that was resistant to nalidixic acid. The serotypes detected were: Typhimurium (two isolates), Give (seven isolates), *enterica* (three isolates), Agona (one isolate) and Dublin (one isolate). O157 VTEC and non-O157 VTEC were detected in 20 hides (23.5%) and 3 carcasses (3.5%). The *vt2* genotype was identified in most of the VTEC strains, followed by *vt1* and *vt2*. *Campylobacter jejuni* was detected in seven samples (8%) of hides, but was absent in the carcasses. Counts of *C. jejuni* were also lower than 0.01 CFU/cm^2, and no *C. coli* was found. None of the samples (hides and carcasses) contained *L. monocytogenes*. Two hides were simultaneously positive for *Salmonella* spp and *C. jejuni*, and one hide contained three pathogens simultaneously: *C. jejuni*, O157 VTEC and non-O157 VTEC. One hide contained both O157 VTEC and non-O157 VTEC. The three carcasses containing non-O157 VTEC were derived from animals with positives hides for this pathogen. One of the carcasses containing non-O157 contained O157 VTEC as well. Although this study is still ongoing and further sampling will generate new results, the data obtained so far indicates that in this specific abattoir, responsible for 75% of the beef exported to EU, the incidence of these pathogens in the carcasses is low, evidencing that GHP and GMP are being followed.

P9

Impact of pediocin PA-1 in combination with plant essential oils on microbiological and sensory profiles of minced beef

Julien Lonchamp, Kieran Germaine, Jorge Gutierrez, Patricia Nobmann, Catherine Barry Ryan, Paula Bourke

School of Food Science and Environmental Health, Dublin Institute of Technology, Dublin 1, Ireland

Current technologies for preservation and shelf life extension of meat products, including chemical preservatives, heat processing, modified atmosphere packaging, vacuum packaging or refrigeration, do not eliminate food borne pathogens from these products or delay microbial spoilage entirely. Alternative preservation techniques such as novel non-thermal technologies and naturally derived antimicrobial ingredients need to be assessed and developed. In this study, the antimicrobial efficacy of oregano and thyme essential oils (EO's) and the bacteriocin pediocin PA-1, both alone and in combination, against food-borne pathogens and key spoilage bacteria was evaluated in raw minced beef. The impact of the addition of EO's on the sensory and physiochemical profiles of the products was also assessed. Oregano (0.15%), thyme (0.15%) and their combinations with pediocin (500 ng/ml) were investigated as preservatives in a raw beef model, challenged with *L. innocua*. Pediocin alone or in combination with oregano were the most effective treatments for initial reduction of *Listeria*. Combining oregano with pediocin gave the best initial reduction in Total Viable Counts, and maintained the initial decrease for Enterobacteria over storage. Oregano and thyme EO's were accepted by the sensory panel at respective concentrations of 0.15% and 0.30% in raw samples, and 0.10% and 0.15% in cooked samples. Thus, pediocin in combination with oregano or thyme has potential for practical application as a natural and safe tool to preserve the safety and quality of meat products. Ongoing studies are evaluating the antimicrobial efficacy of pediocin PA-1, both alone and in combination with oregano and thyme EO's, in cooked minced beef using both culture-based methods and the non-culture based Denaturating Gradient Gel Electrophoresis (DGGE) technique.

P10

Prevalence of selected pathogens on hide and carcass of cattle slaughtered in Poland

Edyta Denis, Kinga Wieczorek, Jacek Osek
National Veterinary Research Institute, Partyzantow 57, 24-100 Pulawy, Poland

Introduction

Verotoxin-producing *Escherichia coli* (VTEC), *Campylobacter* spp., *Listeria monocytogenes,* and *Salmonella* spp. strains are responsible for food poisoning in humans and associated with a wide spectrum of illness. Cattle are recogized as a primary reservoir of VTEC and several outbreaks of VTEC infections were epidemiologically linked to undercooked beef products. There are studies reporting that VTEC have been recovered from the bovine hide and it has also been established that there is a link between the incidence of the pathogens on hides and on derived carcasses. However, there are very few information concerning the prevalence of the other mentioned pathogens, both on bovine hide and carcass. Thus, the aim of this study was to investigate cattle as a potential carrier of the four pathogenic bacteria responsible for human food infections.

Materials and methods

Two hundred seventy six cattle slaughtered in the eastern part of Poland were used during the study. All samples were collected from the brisket area (400 cm^2) using a swab method. The hide samples were obtained after exsanguination whereas the carcass swabs were taken after the corresponding carcass spliting. The sponges were placed into 200 ml of MRD and stomached for 3 min. Then, after the centrifugation, the pellets were resuspended in 100 ml of appropriate for each pathogen enrichment broth and incubated in the relevant culture conditions. Further, the ISO standard methods with some modifications and molecular approaches were used.

Results

The presence of at least one tested pathogen was found at 95 of 276 (34.4%) hides, 14 (5.1%) of carcasses and 33 (12.0%) both hides and the corresponding carcasses. In case of 276 hides analyzed, 68 (24.6%) were positive for *Campylobacter* spp. (*C. jejuni* – 34 and *C. coli* – 31 samples) 28 (10.1%) were contaminated with *L. monocytogenes,* 6 (2.2%) were positive for *Salmonella* spp., and 54 (19.6%) for VTEC. When the corresponding carcasses were tested 8 (2.9%) were *Campylobacter*-positive (*C. jejuni* – 4 and *C. coli* – 4), *L. monocytogenes* was found in 7 (2.5%) samples, *Salmonella* spp. in 5 (1.8%) carcasses, whereas VTEC was identified in 30 (10.9%) samples. It was also found that some bovine hides were contaminated with more then one pathogen – *Campylobacter* and VTEC (12 samples), *Campylobacter* and *L. monocytogenes* (7 samples) or VTEC and *Listeria* (4 samples). On the other hand, only 3 carcasses contained more then one pathogen – VTEC and *L. monocytogenes* (2 carcasses) and *Campylobacter* + VTEC (1 sample).

Conclusion

Several pathogenic bacteria can be present in slaughtered cattle, therefore the bovine carcasses may be a potential source of food-borne infections due to either *Campylobacter, L. monocytogenes, Salmonella* or VTEC.

P11

Molecular characterization of pathogens identified on hide and carcass of cattle slaughtered in Poland

Kinga Wieczorek, Edyta Denis, Jacek Osek
National Veterinary Research Institute, Partyzantow 57, 24-100 Pulawy, Poland

Introduction

Verotoxin-producing *Escherichia coli* (VTEC), *Campylobacter* spp., and *Listeria monocytogenes*, strains are responsible for food poisoning in humans and associated with a wide spectrum of illness. These bacteria have been isolated from bovine hide and carcass in several countries. It has been established that there is a link between the incidence of these pathogens on hide and on derived carcass. Thus, it is important to analyze whether the bacteria isolated from this part of food chain may be potentialy pathogenic for humans. The aim of this study was to investigate the main virulence genetic factors of VTEC, *Campylobacter* spp., and *L. monocytogenes* isolated from cattle slaughtered in Poland.

Materials and methods

One hundred fourty six cattle slaughtered in the eastern part of Poland were used during the study. All samples were collected from the brisket area (400 cm2) using a swab method. The hide samples were obtained after exsanguination whereas the carcass swabs were taken after the corresponding carcass spliting. The ISO standard methods with some modifications and molecular approaches for identification of virulence marker genes were used. For VTEC the *eaeA* (intimin), *stx* (Shiga toxin), and *hlyA* (enterohemolysin) genes were identified whereas *Campylobacter* spp. were tested for the presence of *cadF, flaA, cdt, virB11,* and *iam* genes. Moreover, molecular serotyping of *L. monocytogenes* isolates were performed.

Results

Six and 5 VTEC strains were isolated from bovine hides and carcasses, respectively. All but one the hide isolates possessed the *eaeA* gene whereas only 2 out of 5 carcass strains had this virulence marker. The enterohemolysin gene was found in 4 and 2 VTEC recovered from hides and carcasses, respectively. Most of the isolates possessed the *stx2* together with *stx2c* genes (5 strains from hide and carcass) whereas no VTEC had the *stx1* marker. Altogether, 39 *Campylobacter* spp. strains were recovered during the study (35 from hide and 4 from carcass). It was found that 19 of them were *C. coli*, 18 *C. jejun*i and 2 classified as *Campylobacter* spp. The molecular analysis of the isolates revealed that the vast majority of strains had the *cadF* (37 isolates, 94.9%), *flaA* (35, 89.7%), and *iam* (31, 79.5%) genes. On the other hand, only 22 (56.4%) *Campylobacter* possessed the *cdtA* marker. None of the isolates tested had *the virB11* virulence gene. Twenty four *L. monocytogenes* recovered from hides and carcasses were serotyped with the PCR method and it was found that most of them (21, 87.5%) were of the 1/2a serogroup. The serotypes 1/2b, 1/2c, and 4b were only identified on single strains, respectively.

Conclusion

Pathogenic bacteria identified in slaughter cattle in Poland possessed several virulence marker genes, therefore they may be potentially pathogenic for beef consumers.

P12

Bacterial adhesion and biofilm formation: assessment of novel and standard microtiter plate assays

Chamot S.[1], Hannevik S.[2], Wold Åsli A.[2], Chavant P.[3], Bernardi T.[3], Desvaux M.[1], Heir E.[2] and Hébraud M.[1]

[1]UR454 Microbiologie, Equipe QuaSA, INRA de Clermont-Ferrand/Theix, F-63122 Saint-Genès Champanelle, France;

[2]Nofima Food, Matforsk, Osloveien 1, N-1430 Ås, Norway;

[3]BioFilm Control, Biopôle Clermont-Limagne, F-63360 Saint Beauzire, France.

Biofilms consist of cooperative communities of microorganisms attached to biotic or abiotic surfaces and each other and generally embedded in a self-produced matrix of extracellular polymeric substances. Many bacterial species are able to form biofilms with different architectures going from more or less confluent aggregates to complex three-dimensional structures. This mode of growth constitutes the most widespread in natural and man-made ecosystems. It is a key factor for microorganisms to persist and resist in diverse environments with sometimes hostile conditions. When biofilms are constituted of pathogens, it becomes a crucial problem for public health. Regarding the relevant interest represented by these microbial communities in different environmental contexts, the studies considerably increased these last years to understand their physiology and the mechanisms of adhesion and growth in biofilm.

Some methods to evaluate and to get information on the ability of bacterial species to adhere or on the bacterial susceptibility to biocides and antimicrobial agents are available since several years. However, these methods are generally fastidious, time-consuming and consequently not adapted to large scale experiments. One of the most usually used techniques to evaluate bacterial adhesion consists in a colorimetric method with crystal violet in microtiter plate. Its main disadvantages are the low sensitivity and the lack of reproducibility.

We have tested this classical method after standardisation of the different steps of the procedure and the *BioFilm Ring Test*® which is a new rapid and easy-handling method based on the immobilisation of magnetic microbeads by bacterial cells forming biofilm. The evaluation of the two devices has been carried out on a panel of foodborne pathogen strains, namely verocytotoxin producing *Escherichia coli* (VTEC) and *Listeria monocytogenes*. The efficiency of the two devices in term of rapidity, reproducibility and high throughput potential and the relevance of the results compared to their biological significance are discussed.

P13

Meat quality evaluation: an inexpensive and effective protocol

P. Cornale, S. Isoppo, G. Sala, M. Prandi, S. Barbera
Dipartimento di Scienze Zootecniche, Università di Torino, Via L. Da Vinci 44,
10095 Grugliasco (TO), Italy

Meat quality evaluation is as important in meat production as meat safety assessment. To reduce costs and improve effectiveness we have defined a protocol, useful to evaluate some fresh-meat quality parameter perceived in a very important by consumers. The protocol analyzes on raw and cooked meat: color, marbless, cooking effects on meat, flavour, tenderness, juiciness, WHC, water cooling loss. Our protocol involves 5 technicians working, in temporal sequence, on the same sample. Cooking effects are evaluated measuring Meat Cooking Shrinkage (difference between the raw and cooked area) by the MCSInstrument, based on video image analysis. Moreover it measures color, marbling and cooking loss. Flavor is analysed by Electronic Nose (EN) on raw, during and after cooking in oven, to measure the flavor as it is perceived by consumer in everyday life. Tenderness is analysed by a TPA using compression. On raw meat residues is measured the Water Holding Capacity trend, a dinamic measurement of water release applying video image analysis, and some physical parameters. A *Longissimus dorsi* 1-cm thick circular sample is used as meat source. The raw sample is measured by the Electronic Nose, then weighed and measured by the MCS- Instrument. Afterwards sample is put in oven (165°C) for 600s while Electronic Nose analyses aroma. After 600 s cooked steak flavor is measured again. Sample, after 20' cooling at room temperature, is weighed and measured by the MCS-Instruments. Three cylinders are obtained from this steak to measure tenderness. On the same time homogenised residues are used for WHCtrend and others analysis.

This procedure includes innovative, objective and quick methods to evaluate, on a 1cm thick sample, more than 10 parameters related to consumer expectations, to improve correlations among measured parameters and finally to reduce analysis costs.

P14

The use of carvacrol for the inhibition of *Escherichia coli* O157:H7 in different environmental conditions using a model broth system

M. J. McDonnell[1,2], L. Rivas[1], C. M. Burgess[1], S. Fanning[2] and G. Duffy[1]

[1]Food Safety Department, Ashtown Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland.
[2]School of Agriculture, Food Science, and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland.

Escherichia coli O157:H7 is a major food safety concern due to the severity of disease that it can cause. Reducing the risk of contamination and transfer of *E. coli* O157:H7 from animals to food can reduce the risk of infection. Essential oils have been identified to have antimicrobial properties. Carvacrol is one of the active components of oregano and thyme oil. Investigations into the application of carvacrol to control *E. coli* O157:H7 at key stages of the beef chain is ongoing. The aim of this study was to evaluate the inhibitory activity of carvacrol against *E. coli* O157:H7 in different environmental conditions using a model broth system (Tryptone Soya Broth with 0.05% agar). Environmental conditions included various temperatures (37°C, 16-18 h, 15°C, 2 d or 4°C for 10 d), pH (2-7), NaCl (0.5-2.5%) concentration, water activity (aW; 0.99-0.87) and the presence of microflora (consisting of a cocktail of typical meat flora microorganisms). Minimum inhibitory concentration (MIC) values were determined using a microtitre plate method. *E. coli* O157:H7 (380-94) was exposed to various concentrations (0.004-0.2% v/v) of carvacrol in combination with one of the environmental conditions above. The MIC was defined as the lowest concentration of carvacrol that resulted in no visible growth after incubation. The number of surviving *E. coli* O157:H7 following exposure to the different carvacrol concentrations was determined for each of the environmental conditions tested. All cell counts were compared using ANOVA. The MIC values varied significantly ($p<0.05$) for all environmental conditions ranging from 0.025-0.2%. Some environmental conditions alone affected the growth and survival of *E. coli* O157:H7, for example pH >4 or 10°C. The number of surviving E. coli O157:H7 in the presence of carvacrol (0.025-0.2%) varied significantly ($p<0.05$) at each environmental condition in comparison to the controls. For all environmental conditions tested, ≥ 0.05-0.1% carvacrol was bactericidal (kills) against *E. coli* O157:H7. The antimicrobial activity of carvacrol against *E. coli* O157:H7 was not affected by different NaCl concentrations or the presence or absence of microflora. The data suggests that carvacrol can inhibit *E. coli* O157:H7 in a range of environmental conditions. Further work is required to determine whether carvacrol can be applied to control *E. coli* O157 at critical points in the beef chain.

P15

A novel application of count data distributions and their zero-modified counterparts for modelling hygiene indicator organisms of beef carcasses

Ursula Gonzales Barron[1]; Ilias Soumpasis[1]; James J. Sheridan [2]; Francis Butler[1]
[1]Biosystems Engineering, UCD School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4. Ireland.
[2]Food Safety Department, Teagasc, Ashtown Food Research Centre, Dublin 15, Ireland.

In many cases, microbial data may be characterised by the presence of large numbers of negative samples or zero counts. This occurs with some hygiene indicator organisms and pathogens and complicates statistical treatment of the data under the assumption of a log normal distribution. The objective of the present work was to introduce an alternative distribution framework, capable of representing this type of data without incurring in loss of information. The negative binomial (NB), zero-inflated Poisson (ZIP), zero-inflated negative binomial (ZINB), hurdle Poisson (HP) and hurdle negative binomial (HNB) distributions were fitted to actual data consisting of total coliforms (n=590) and *Escherichia coli* (n=677) present on beef carcasses sampled from nine Irish abattoirs. Due to a heterogeneity parameter that accounts for the large variance of the data, improvement over the simple Poisson was shown by the NB, although it overestimated the zero counts and underestimated the first few counts. Whereas the ZIP could not cope with the data over-dispersion ($p<0.001$ for χ^2 GOF), the ZINB collapsed into a NB in both data sets due to the non-significance of its logit component. Addressing both the large variance and the excess of zero counts, the HNB predicted the observed count data slightly better than the NB, and was capable of depicting, with a comparable degree of accuracy, data of ~13% zero counts ($\chi^2=70.15<\chi^2_t$ for coliforms) as well as of ~42% zero counts ($\chi 2=69.30<\chi^2_t$ for *Escherichia coli*). Because a (two-component) hurdle model consists of a logit model that determines whether or not contamination on a carcass is detected (zero versus non-zero), and a count model that determines the numbers of CFUs, the HNB distribution has an interesting interpretation for stochastic risk assessment applications. Thus, bacterial data of pathogens in beef (*Salmonella*, verotoxigenic *Escherichia coli*) consisting of a considerable amount of negative samples can be accurately represented using modified count data distributions.

Keywords: Count distribution, Poisson, negative binomial, zero-inflated, hurdle, *Escherichia coli*, coliforms, beef

P16

A stochastic modeling approach for taking into account spoilage in Risk Assessment: Application for *Escherichia coli* O157:H7 in ground beef

Koutsoumanis Kostas
Laboratory of Food Hygiene & Microbiology, Department of Food Science and Technology, School of Agriculture, Aristotle University Thessaloniki, 541 24 Thessaloniki, Greece.

This study presents a stochastic modeling approach for taking into account spoilage in risk assessment. The aim of the proposed approach is to identify products that have been spoiled before consumption. The principles of the proposed approach are presented through an exposure assessment application, which modifies the assessment for *E. coli* O157:H7 in ground beef published by Cassin et al., (1998) by incorporating data and models for ground meat spoilage. The results of the application showed that based on the time-temperature scenarios for retail storage used in the latter study, the level of pseudomonads exceeded the spoilage level in 9.3% of packages at the end of retail storage (before cooking). This means that during preparation for cooking these products would show obvious spoilage and hence would not be consumed. It needs to be stressed that the concentration of *E. coli* O157:H7 in spoiled packages at the end of retail storage was found to be significantly higher compared to unspoiled ones. This was observed by a clear positive correlation between the levels of pathogen and pseudomonads at the end of retail storage. In particular, the level of *E. coli* O157:H7 in spoiled packages was about 3 logs cfu/g (on average) higher compared to unpoiled packages. The above results indicate that ignoring spoilage in exposure assessment may lead to significant overestimation of the actual risk since the contribution of the spoiled products to the calculated probability of illness can be significant. The stochastic modeling approach presented in this work allows the identification of products with acceptable quality at the time of consumption by integrating spoilage modeling with the model for pathogen and leads to more realistic risk assessments.

P17

Effect of Thawing Practices of Ground Beef on the Heat Tolerance of *Listeria monocytogenes* and *Salmonella* Enteritidis

Lianou Alexandra and Koutsoumanis Kostas
School of Agriculture, Department of Food Science and Technology,Laboratory of Food Microbiology and Food Technology, Aristotle University of Thessaloniki, Thessaloniki, PC 54124.

Pathogen proliferation and/or stress hardening encountered during thawing of raw meat items may compromise their or other products' safety under certain circumstances, such as undercooking or cross-contamination events. The heat tolerance of *Listeria monocytogenes* Scott A and *Salmonella* Enteritidis PT4, as affected by freezing and thawing of ground beef artificially inoculated with the pathogens, was evaluated. Raw ground beef (300-g portions) was inoculated with *L. monocytogenes* or *S.* Enteritidis (~7 log cfu/g), frozen-stored (-27.2 ± 2.8°C) for approximately 3 days, and then thawed by one of the following practices: (i) at 5°C for 15 h, to simulate thawing under refrigeration (R); (ii) at 25°C for 12 h, to simulate overnight countertop thawing (C); or (iii) microwave defrosting until completely thawed. Portions of thawed product's homogenates were exposed to 57°C (in culture broth) for up to 80 and 20 min for *L. monocytogenes* and *S.* Enteritidis, respectively. Fresh ground beef portions inoculated with the pathogens on the day of the heat tolerance assays served as controls. Total surviving mesophilic microbial populations were enumerated on tryptone soy agar, while uninjured *L. monocytogenes* and *S.* Enteritidis populations were determined on PALCAM agar and XLD agar, respectively. Freezing and thawing of ground beef did not alter considerably the pathogens' populations. Regarding the heat tolerance assays, the thermal inactivation of *S.* Enteritidis was not affected ($P \geq 0.05$) by freezing/thawing or thawing practice. The heat inactivation of *L. monocytogenes* under treatment R was similar ($P \geq 0.05$) to that observed for the control, while treatment C resulted, overall, in the slowest inactivation of the pathogen, followed by treatment M. However, significant ($P<0.05$) differences among thawing practices were only observed between treatments C and R, and only at 20 and 40 min of the heat challenge trials; treatment C resulted in mean *L. monocytogenes* reductions (log cfu/ml) of 0.95 and 2.88 at 20 and 40 min, respectively, while the corresponding reductions for treatment R were 2.04 and 3.79, respectively.

P18

A survey study on the prevalence and concentration of pathogens and hygiene "indicators" bacteria on beef hides and carcasses in Greek slaughterhouses

Tzavaras Ioannis, Skidina Christina, Boston-Smite Natalie and Koutsoumanis Kostas
School of Agriculture, Department of Food Science and Technology,Laboratory of Food Microbiology and Food Technology, Aristotle University of Thessaloniki, Thessaloniki, PC 54124, Tel/Fax +302310-991647

Microbiology of fresh meat and meat products is important not only from the aspect of public health and consumer safety but also for the quality of meat products. Slaughter is one of the most important stages of the meat chain since the level of contamination during this step, determines the level of risk of the final product. The present survey includes visits in Greek beef slaughterhouses and collection of samples from the hide (post exsanguinations) and from the carcass (post evisceration). The sampling points were: rump, flank, brisket and neck. The number of samples was representative of each season. All samples are taken using the swab method. Samples were analyzed for the detection of *Salmonella* spp, and *E.coli* O157:H7. The populations of total aerobic mesophilic bacteria, total coliforms and *E.coli* were also monitored. Additionally, all information on the processing steps, hygiene conditions, implementation of HACCP system etc. were recorded with the use of questionnaires. So far, the results of the sampling of 166 beef hides and carcasses have shown that the prevalence of *Salmonella* spp was low (1,2% both in hide and carcass). The prevalence of *E.coli* O157:H7 was 6,6% (6% in the hide 7,2% in the carcass). In most of the cases, carcasses which were found to be contaminated with *E.coli* O157:H7 derived from animals whose hide was also contaminated with *E.coli* O157:H7. As regards to the hygiene indicators bacteria, the results have shown that it is urgent to improve the hygiene conditions of the slaughterhouses in Greece in order to meet the criteria of the European legislation which have been established since 2005 with the implementation of the new European regulation 2073. The continuation of the survey in a higher number of samples, will lead to the development of a database for the microbiological status of beef in Greece which can be used as a base for risk assessment studies.

P19

Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef

Zinoviadou Kyriaki[1], Koutsoumanis Kostas[2] and Biliaderis Costas[1]

[1]Laboratory of Food Chemistry & Biochemistry, Department of Food Science and Technology, School of Agriculture, Aristotle University Thessaloniki, 541 24 Thessaloniki, Greece.

[2]Laboratory of Food Hygiene & Microbiology, Department of Food Science and Technology, School of Agriculture, Aristotle University Thessaloniki, 541 24 Thessaloniki, Greece.

Antimicrobial films were prepared by incorporating different levels of oregano oil (0.5%, 1%, 1.5% w/w in the film forming solution) into sorbitol-plasticized whey protein isolate (WPI) films. The moisture uptake behavior and the water vapor permeability (WVP) were not affected by the addition of oregano oil at any of the concentrations used. A reduction of the glass transition temperature was caused by addition of oil into the protein matrix. A decrease of Young modulus (E) and maximum tensile strength (σ_{max}) accompanied with an increase in elongation at break (%EB) were observed with increasing oil concentration up to a level of 1% (w/w). Wrapping of beef cuts with the antimicrobial films resulted in smaller changes in total color difference (ΔE) and saturation difference (Δ_{chroma}) during storage under refrigeration. The maximum specific growth rate (μ_{max}) of Total Viable Count (TVC) and pseudomonads was significantly reduced with the use of antimicrobial films containing 1.5% oil, while the growth of Lactic Acid Bacteria (LAB) was completely inhibited.

P20

Validation of a quantitative real time PCR assay for enumerating total viable counts on beef carcasses

CM Burgess[1], A Dolan[2], T Barry[3], S Fanning[4] and G Duffy[1]

[1]Dept of Food Safety, Teagasc Ashtown Food Research Centre, Ashtown, Dublin, Ireland.
[2]RCSI Education and Research Centre, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland.
[3]Dept of Microbiology, National University of Ireland, Galway, Ireland.
[4]School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Ireland.

During beef carcass dressing, transfer of bacteria from the hide surface to the carcass is unavoidable due to the nature of the hide removal process. From a microbiological stand point, muscle foods have a particularly unique nutritional profile, with intrinsic factors such as a neutral pH, high water content, a high protein content and fat providing an excellent platform for microbial growth. The degree of contamination and the microbiological profile of a product will determine the length of time before the onset of spoilage. According to EC regulation 2073/2005 the total viable count of meat carcasses should be tested using a plate count method according to ISO4833:2003, or another method if shown to be equivalent according to ISO16140:2003. The plate count method takes three days to obtain a result; a costly delay for the food industry. In order to reduce the length of time needed to determine the TVC of a product a quantitative real time PCR assay was developed using two sets of primers which targeted either Gram positive or Gram negative genera. To show equivalence of the assay with the conventional method sixty beef carcases were swabbed and the TVC was enumerated using both methods. The novel assay and the conventional plate count method showed a correlation of R^2 =0.93. This indicates that the PCR assay used in this study can enumerate the total bacteria on beef carcasses and has the potential to be applied to various sample types as an alternative rapid method.

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P21

Prophage gene deletion identified in verocytotoxigenic *E. coli* isolated from the Irish beef chain

CM Burgess[1], C Parker[2], S Huynh[2], R Mandrell[2] and G Duffy[1]

[1]Dept of Food Safety, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland.

[2]US. Department of Agriculture, Agricultural Research Service, Produce Safety and Microbiology Unit, 800 Buchanan Street, Albany, CA 94710, USA

Verocytotoxigenic *Escherichia coli* (VTEC) is a serious pathogen of significant public health concern worldwide. Infection is usually characterised by bloody diarrhoea and can be life threatening due to the subsequent development of haemolytic-uremic syndrome (HUS), which is mediated by verocytotoxins. These infections are associated with strains belonging to a number of serogroups but the most predominant include O157, O26, O103, and O145. They are foodborne pathogens of zoonotic origin, with cattle being a main reservoir. These pathogens can be diverse in their genomic content, and particularly in their virulence gene profile. This may play a role in their ability to survive through the farm to fork chain and subsequently cause illness. In order to investigate genomic diversity among VTEC a number of strains isolated from the Irish beef chain were selected for whole genome analysis using a microarray approach. These were analysed in comparison with a number of VTEC isolates from the United States of America and EDL933, a sequenced *E. coli* O157 strain. *E. coli* O157 isolates from Ireland were found to cluster separately from those isolated in the USA; this was in the main part due to the deletion of a number of genes in one section of the genome in the Irish isolates. On further analysis it was found that this deletion encompasses approximately 25 genes from the prophage which encodes the verocytotoxin 2 gene. Many studies have demonstrated the ability of *vt* - encoding bacteriophage to move by transduction into other strains. The deletion observed in the Irish strains may have implications for the ability of the prophage to replicate and transduce effectively.

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P22

Effect of diet on the survival of *Salmonella* in bovine rumen fluid and faeces

S. Kelly[1,2], M. Lenahan[1], S. Fanning[2] and D. Bolton[1]
[1]Teagasc, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland.
[2]School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland.

This study investigated the use of animal diet to limit the excretion of *Salmonella* in faeces from cattle. In order for *Salmonella* shedding to occur, it must first pass through the bovine gastrointestinal tract. Animal diet has been shown to alter rumen characteristics such as pH and volatile fatty acid (VFA) concentrations. A reduced rumen pH can induce the Acid Tolerance Response (ATR) in *Salmonella* resulting in a more acid resistant organism better able to withstand subsequent passage through the abomasum (pH 2.5). Increased survival through the abomasum inevitably results in increased numbers being shed in the faeces leading to an increased risk of contamination. Five diets were fed to cattle as follows: 100% grass, grass + concentrate, 100% grass silage, 100% hay and 73% maize & grass silage + 27% concentrate. Faeces and rumen fluid were inoculated with non-acid and acid adapted Salmonella. Survival was monitored in rumen fluid at 37°C and in faeces at 6 and 15°C for up to 84 days. The rumen fluid pH ranged from 5.77 – 6.6 and faecal pH ranged from 6.86 – 7.06. Non-acid and acid adapted *Salmonella* survived for 84 days in faeces at both temperatures and for up to 21 days in rumen fluid. The survival of Salmonella was not significantly different in rumen fluid at 37°C between the majority of the five diets. The survival of acid and non-acid adapted *Salmonella* was significantly reduced ($P<0.05$) in faeces at 15°C from diet 5 compared to the other diets. No significant differences were detected in non-acid and acid adapted *Salmonella* in faeces from the five diets incubated at 6°C. Results indicate that *Salmonella* survival was not significantly altered by the pH and VFA profiles generated by the feeding of different diets, and the development of dietary management controls for the shedding of *Salmonella* requires further investigation.

P23

Emerging non-O157 Verocytotoxigenic *Escherichia coli* (VTEC) on Irish Cattle Farms

B. Byrne[1], A.M. Monaghan[1], D.A. McDowell[2], S. Fanning[3]. T. Sweeney[3], and D.J. Bolton[1]

[1]Food Safety Department, Ashtown Food Research Centre (AFRC), Teagasc, Ashtown, Dublin 15, Ireland.

[2]Department of Food Studies, University of Ulster, Jordanstown, Newtownabbey, Co. Antrim, Northern Ireland, UK.

[3]University College Dublin (UCD), School of Agriculture, Food Science and Veterinary Medicine, Belfield, Dublin 4, Ireland.

Aim

The objective of this study was to investigate the incidence of non-O157 verocytotoxigenic *E. coli* (VTEC) on Irish farms over the course of one year.

Method

Faecal and soil samples were obtained from 20 farms on 6 different occasions over the course of 12 months. A preliminary polymerase chain reaction (PCR) screen was carried out on the each farm sample and the VTEC positive samples were further analysed using mTBX and PCR. All isolates were serotyped and PCR screened for a range of virulence factors including *vt1, vt2, TIR, hlyA, eaeA, saa, lpfA, katP, etpD, espF, espP, espB,* and *espA*.

Results

107 VTEC (non-O157) isolates were cultured from 1800 farm samples. The farm study discovered 17 different non-O157 VTEC serogroups (16 faeces and 7 soil). The most predominant non-O157 VTEC serogroup on Irish farms was O113 (29% of all VTEC isolates) followed by O26 (13%), O2 (12%), O168 (9%), O174 (7%), O119 (6%), O116 (6%), O-Untypable (6%), O171 (4%), O136 (3%), O6 (1%), O20 (1%), O86 (1%), O109 (1%) and O145 (1%). VTEC O26 and O145 were found to be the most virulent serotypes, with all of their isolates carrying genes encoding a range of different virulence factors *vt1* and/or *vt2, eaeA* and hlyA commonly associated with HUS in humans.

Conclusion

This study found a wide range of non-O157 VTEC serogroups on Irish farms. The data obtained from this study suggests VTEC O113 has the potential to become a public health risk in the future and should be targeted in future control and monitoring programs.

P24

Validation of a method for the determination of Ractopamine in urine using Surface Plasmon Resonance (SPR) according to Commission Decision 2002/657/EC

L. Walshe, D. McEvoy, S. McGettrick, L. Scott and P. Rafter
Central Meat Control Laboratory, Department of Agriculture, Fisheries and Food, Backweston Campus, Co Kildare, Ireland.

Ractopamine belongs to a class of drugs known as β-agonists. Ractopamine can enhance the growth rate of treated cattle by promoting muscle synthesis while decreasing the amount of fat accumulation. It is licensed for use in many countries worldwide but banned within the European Union under Council Directive 96/22/EC. In order to monitor illegal use, screening test methods must be able to detect residues present below the recommended concentration of 1ppb as set by the Community Reference Laboratory and also be validated in accordance with the criteria outlined in Section 3 of Commission Decision 2002/657/EC. The objective of this study is to evaluate the suitability of a screening method for the determination of Ractopamine in urine by SPR using a number of factors; CCα, CCβ, recovery, repeatability, reproducibility, ruggedness, stability and cross reactivity. Twenty negative porcine, bovine and ovine urine samples were obtained and spiked at levels of interest. The Biacore Q and the Biacore Q flex kits were used to carry out the analysis. ANOVA was used to analyse the data for repeatability and reproducibility. The CCβ was 0.69ng/ml, 0.8ng/ml, and 0.73ng/ml for bovine porcine, and ovine urine respectively. Coefficient of variation for repeatability and reproducibility was 7.3% and 14.4% respectively. The method was shown to be robust as the percentage coefficient of variation was significantly less than the standard deviation of the method carried out under laboratory reproducibility conditions. Ractopamine was shown to be stable up to 9 weeks in storage –10 to -30˚C. To analyse cross reactivity, eight substances that are likely to interfere with the assay were examined and showed little or no interference.

The results obtained from this study show that this method is fit for purpose and is validated in accordance with the criteria from Commission Decision 2002/657/EC.

P25

A novel application of count data distributions and their zero-modified counterparts for modelling hygiene indicator organisms of beef carcasses

Ursula Gonzales Barron[1], James J. Sheridan [2], Francis Butler[1]

[1]Biosystems Engineering, UCD School of Agriculture, Food Science and Veterinary Medicine, University College Dublin. Belfield. Dublin 4, Ireland.

[2]Food Safety Department, Teagasc, Ashtown Food Research Centre, Dublin 15, Ireland.

In many cases, microbial data may be characterised by the presence of large numbers of negative samples or zero counts. This occurs with some hygiene indicator organisms and pathogens and complicates statistical treatment of the data under the assumption of a log normal distribution. The objective of the present work was to introduce an alternative distribution framework, capable of representing this type of data without incurring in loss of information. The negative binomial (NB), zero-inflated Poisson (ZIP), zero-inflated negative binomial (ZINB), hurdle Poisson (HP) and hurdle negative binomial (HNB) distributions were fitted to actual data consisting of total coliforms (n=590) and *Escherichia coli* (n=677) present on beef carcasses sampled from nine Irish abattoirs. Due to a heterogeneity parameter that accounts for the large variance of the data, improvement over the simple Poisson was shown by the NB, although it overestimated the zero counts and underestimated the first few counts. Whereas the ZIP could not cope with the data over-dispersion ($p<0.001$ for χ^2 GOF), the ZINB collapsed into a NB in both data sets due to the non-significance of its logit component. Addressing both the large variance and the excess of zero counts, the HNB predicted the observed count data slightly better than the NB, and was capable of depicting, with a comparable degree of accuracy, data of ~13% zero counts ($\chi^2=70.15<\chi^2_t$ for coliforms) as well as of ~42% zero counts ($\chi^2=69.30<\chi^2_t$ for *Escherichia coli*). Because a (two-component) hurdle model consists of a logit model that determines whether or not contamination is detected (zero versus non-zero), and a count model that determines the numbers of CFUs, the HNB distribution has an interesting interpretation for stochastic risk assessment applications. Thus, bacterial data of pathogens in beef (*Salmonella*, verotoxigenic *Escherichia coli*) consisting of a considerable amount of negative samples can be accurately represented using modified count data distributions.

Keywords: Count distribution, Poisson, negative binomial, zero-inflated, hurdle, *Escherichia coli*, coliforms, beef

P26

Tracking *Listeria, Campylobacter* and *Salmonella* spp. on bovine hides and carcasses

Bimal Kumar Khen[1, 2], Ó. A. Lynch[1], J. Carroll[1], D. McDowell[2] and G. Duffy[1]
[1]Ashtown Food Research Centre, Teagasc, Dublin, Ireland
[2]University of Ulster, Jordanstown, Northern Ireland

Microbial pathogens on cattle hides can potentially be transferred to underlying carcass tissue during the de-hiding process and quantitative risk assessments have identified the bovine hide as a key source of microbial contamination on the carcass. The aim of this study was to establish qualitative and quantitative data on pathogens on bovines hides and the potential for cross contamination onto the carcass. Beef cattle (n=300) animals presented for slaughter at an export abattoir were examined for *Listeria monocytogenes, Salmonella* and *Campylobacter*. The brisket area of the bovine hide (400 cm^2) and the brisket area of the carcass (400 cm^2) following dehiding but before chilling, were swabbed with a polyurethane sponge and examined for the presence and numbers of the three pathogens using ISO cultural methods (ISO 11290-1, 1290-2, 6579, 10272-1 and 1072-2). PCR based approaches were used to confirm, speciate, serotype and screen for virulence genes in recovered isolates. *Campylobacter* was detected in 55% and 16% of hides and carcasses respectively. Cross contamination of the carcass occurred in 9% of animals tested. The numbers present ranged from Log_{10} 0.1 cfu cm^2 – Log_{10} 2.52 cfu cm^2. Molecular characterisation showed that *C. jejuni* was the most prevalent species, followed by *C. lari* and *C. coli*. Screening of isolates for the *cdt* virulence genes demonstrated that *cdtB* (100%) was the principal subunit present, followed by *cdtC* (97%) and *cdtA* (51%). *Listeria monocytogenes* was detected in 16% and 2.5% of hides and carcasses respectively, with cross contamination of the carcass occurring in 1.5 % of animals. The numbers present ranged from Log_{10} 0.65 cfu cm^2 – Log_{10} 1.16 cfu cm^2. Serotyping confirmed that 1/2a / 3a was the most predominant serogroup present (66%). *Salmonella* was detected in 1% of hides at a level of Log_{10} 0.69 cfu cm^2. For the three pathogens, a key finding was that the rate of transfer of pathogen form hide to carcass was very low.

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P27

The potential of HPLC analysis of organic acids on predicting the self life of minced beef stored under conventional and active packaging conditions

Anthoula A. Argyri [1,2], Agapi I. Doulgeraki [1,2], Vasiliki A. Blana [1,2] and George-John E. Nychas [1]
[1]Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Technology, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.
[2]Laboratory of Applied Microbiology, Cranfield Biotechnology Centre, Cranfield Health, Cranfield University, College Road, Cranfield, Bedfordshire MK43 0AL, United Kingdom.

In this study, the potential of using the metabolic profile of organic acids on predicting the self life of minced beef was evaluated, performing in parallel microbiological analysis, sensory analysis and pH measurements. The shelf life of minced beef stored aerobically, under Modified Atmosphere Packaging (MAP) and MAP with the presence of the volatile compounds of oregano essential oil (MAP + OEO) at 0, 5, 10, and 15 °C was assessed, monitoring the microbial association of meat and the biochemical changes occurring in the meat substrate. Microbiological analysis that implicated counts of TVC, *Pseudomonas* spp., *Brochothrix thermosphacta*, lactic acid bacteria (LAB), *Enterobacteriaceae*, yeasts and molds was performed at the same time with sensory analysis, pH measurements and HPLC analysis. The spectral data collected from HPLC were subjected to statistical analysis, including Principal Components Analysis (PCA) and Factorial Discriminant Analysis (FDA), revealing qualitative discrimination of the samples concerning their spoilage status, whilst quantitative predictions of the TVC, *Pseudomonas* spp., *Br. thermosphacta*, LAB, *Enterobacteriaceae*, yeasts and molds where conducted using PLS-R models. The FDA provided qualitative classifications of the samples regarding their spoilage status, giving a correct classification of the samples 93.3% (fit of the model) and a cross validation of 88.0%. The values of bias (*Bf*) and accuracy factors (*Af*), describing the performance of the TVC PLS-R model, were 0.993 and 1.100, respectively, whilst the 90.8% of predictions was within the 20 %RE (Relative Error) zone indicating good agreement between observed and predicted bacterial counts. Similar results were obtained for *Pseudomonas* spp., *Br. thermosphacta*, LAB, *Enterobacteriaceae*, yeasts and molds PLS-R models, with the LAB PLS-R model being the most accurate. These results demonstrate that the metabolic profile of organic acids as attributed from the HPLC analysis may be considered as a method to evaluate the spoilage and the microbial status of a meat sample regardless the storage conditions (e.g. packaging and temperature).

Keywords: Minced meat, Spoilage, HPLC, organic acids, metabolites

Acknowledgement: This work was co-funded by the projects ProSafeBeef *and S*ymbiosis.

P28

Effect of marination as a new processing method on beef safety/spoilage stored at refrigeration/abuse temperatures

Nisiotou, A., Iliopoulos, V., Gounadaki, A.,Panagou, S., & Nychas G-J.E
Agricultural University of Athens, Department of Food Science and Technology, Laboratory of Microbiology and Biotechnology of Foods, Athens, 11855

Four different marinade solutions were tested for their effectiveness to control the growth of *Listeria monocytogenes* and to extend the shelf-life of beef fillets. Beef slices were inoculated with a coctail of 4 *L. monocytogenes* strains and subjected to marinating by immersion into lactic acid (1% v/v), calcium chloride (1% v/v), oregano essential oil (0.5% v/v)-based marinades or their combinations, for 24 h at 4°C. *L. monocytogenes* counts as well as total viable counts, pH and sensory characteristics were followed for an 11-day period of aerobic storage at 10 °C. No significance effects on the growth of *L. monocytogenes* were recorded for oregano essential oil- or calcium chloride-based marinades. Lactic acid marinades were the most effective against *L. monocytogenes*, although they caused browning of the samples probably due to oxidation. The impact of lactic acid marinades on the growth of *Escherichia coli* O157:H7 and *Salmonella* Enteritidis in beef slices stored at various temperatures under aerobic or modified atmosphere (MAP) was further evaluated. Beef cuts were inoculated with S. Enteritidis or E. coli O157:H7 and marinated by immersion in 0.5% or 1% v/v lactic acid. The growth of *S.* Enteritidis and *E. coli* O157:H7 were followed for 21 days at 5 or 15 °C under air or MAP (40% CO_2, 30% O_2 and 30% N_2). Marination in 0.5% or 1% lactic acid decreased the population of both *S.* Enteritidis and *E. coli* O157:H7 by 0.7 or 1.5 and 0.7 Log_{10} CFU/g, respectively. By the end of storage, both marinades reduced the levels of *S.* Enteritidis to below detection limit (<1 Log_{10} CFU/g), except for marination in 0.5% lactic acid where the population was decreased by 1.8 and 3 Log_{10} CFU/g at 5 and 15 °C under MAP, respectively. In the case of *E. coli*, 1% lactic acid was the most effective marinade, reducing population levels by 1.7 - 3.6 Log_{10} CFU/g, depending on the particular storage condition. Present results show that lactic acid-based marinades are efficient from a safety and shelf life point of view, although they may cause browning probably due to ferric myoglobin oxidation. Thus, combined with antioxidant additives, marination in lactic acid could serve as a promising processing method for beef.

P29

Potential implication of extracellular signals produced by *Hafnia alvei* on the early stages of biofilm development by *Salmonella enterica* serovar Enteritidis on stainless steel

N.G. Chorianopoulos, E.D. Giaouris and G.-J.E. Nychas
Department of Food Science and Technology, Laboratory of Microbiology and Biotechnology of Foods, Agricultural University of Athens, Athens, Greece.

Aims

To investigate biofilm formation by *Salmonella enterica* serovar Enteritidis PT4 on stainless steel coupons in the presence of extracellular signals produced by a *Hafnia alvei* strain, capable of producing *N*-acylhomoserine lactones (AHLs).

Methods and Results

*S.*Enteritidis was left to develop biofilm on stainless steel coupons for 72 h at 18oC. For this, coupons were statically incubated in inoculated with *Salmonella* Brain Heart Infusion (BHI) broth (ca. 10^7 CFU/mL), which contained three different concentrations (0, 20 and 50 %) of *Hafnia alvei* cell-free culture supernatant (CFS). Biofilm development on coupons was monitored during four samplings, at the 12, 24, 48, and 72 hours of incubation period. Direct enumeration of detached biofilm cells, obtained by bead vortexing, interestingly revealed that incubation of coupons in the high *H. alvei* CFS concentration (50 %) resulted in about 1 log (CFU/cm^2) reduction in the number of biofilm cells the first 24 h of incubation, compared to when coupons were incubated in BHI broth containing 0 % or/and 20 % *H. alvei* CFS. However, no significant differences in the quantity of biofilm production were observed at the end of incubation period (72 h). Conductance measurements, used to assess indirectly biofilm populations without detaching any cells, confirmed agar plating results, since significant delays in detection times were observed in the cases of less biofilm formation (as assessed by agar plating). Thin-layer chromatography revealed the existence of signalling compounds, in the form of AHLs, in the growth media containing *H. alvei* CFS, during whole incubation period.

Conclusions

Obtained results indicate that *H. alvei* cell-free culture supernatant, containing AHLs among other metabolites, negatively influences the early stages of biofilm formation by *S.* Enteritidis on stainless steel.

Significance and Impact of the Study

Our data suggest that extracellular signals / metabolites produced by *H. alvei* are implicated in the initial stages of biofilm development by *S.* Enteritidis on a food contact surface, possibly through quorum sensing processes (AHL perception). The possibility of governing interspecies communication in *Salmonella* could lead to the control and prevention of its biofilm formation.

P30

Growth and survival of *Escherichia coli* O157:H7 in minced beef stored under different packaging and temperature conditions

Alexandros Doukas, Athina Grounda, Anastasios Stamatiou, Efstathios Panagou and George-John Nychas

Laboratory of Microbiology and Biotechnology of Foods, Dept. of Food Science and Technology, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.

The aim of the present study was to investigate the kinetic characteristics e.g. survival and /or growth, of the pathogen *Escherichia coli* O157:H7 on naturally contaminated minced beef in conjunction with the spoilage flora of meat. To evaluate this, the meat was stored at three different packaging conditions: Aerobic storage, Modified Atmosphere Packaging (MAP, 40%CO_2 & 30%O_2 & 30%N_2) and MAP with the presence of the volatile compounds of Oregano Essential Oil (MAP+OEO). Each packaging system was stored at 0°, 5°, 10°, 15°C. The initial level of the pathogen's inoculum was 4.22, 4.21 and 3.99 $\log_{10}$cfu/g for each type of packaging, respectively. In samples stored under aerobic conditions the pathogen exhibited a significant growth rate in all temperatures, reaching the highest population of 9.25 $\log_{10}$cfu/g at 15°C in the end of storage. Under MAP conditions, the effect of gas composition suppressed the growth of the pathogen by 0.5 $\log_{10}$cfu/g at low temperatures. At 10°C no increase of the pathogen was observed in contrast with 15°C where an increase of 2.0 $\log_{10}$cfu/g was recorded. Additionally, when beef was stored under MAP+EO, no significant growth was observed at 0°, 5°, 10°C due to the antimicrobial effect of the essential oil but at 15°C the pathogen reached 7.92 $\log_{10}$cfu/g at the end of storage. As far as the spoilage flora is concerned, pseudomonads were the main spoilage organisms, followed closely by *Br. thermosphacta* and Lactic Acid Bacteria at all aerobic storage temperatures. In MAP Lactic Acid Bacteria were the main spoilage organisms at all storage temperatures. Changes in microbial association were observed between *Br. thermosphacta* and *Pseudomonas*. At 0° and 5°C *Br. thermosphacta* was in highest number than *Pseudomonas*, the opposite was observed at 10° and 15°C.

P31

Quorum Sensing compounds and the role of lactic acid bacteria in beef spoilage

Doulgeraki, A., Blana, V., Panagou, E., Sofos, J., & Nychas G-J
Laboratory of Microbiology and Biotechnology of Foods, Dept. of Food Science and Technology, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.

The contribution of lactic acid bacteria in packaging under modified atmospheres which has been proved very effective in extending the shelf life of meat is well established. Indeed, lactic acid bacteria (LAB) are the dominant spoilage-causing microorganisms in such meat ecosystems. In different food ecosystems, the presence of low-molecular-weight signalling compounds, known as autoinducers, has been reported. The role of these compounds in food spoilage is not considered. In principle, this process, called quorum sensing, allows bacteria to monitor their environment for the presence of other bacteria and respond by adapting specific strategies (e.g. competition for nutrients). These quorum-sensing circuits can involve both intraspecies (autoinducer-1 mediated [AI-1]) and interspecies (autoinducer-1 or/ and -2 mediated [AI-2]) communication mechanisms. In order to exploit the contribution of the AI-2-like activity to the diversity of LAB and to the shelf life of meat under modified atmosphere packaging, minced beef was stored at 0°, 5°, 10° and 15°C in a package consisted of 40% CO_2-30% O_2-30% N_2. The changes among the LAB population were screened with PFGE, whilst the AI-2-like activity of the isolates was screened with Vibrio harveyi BB-170 as reporter strain.

The AI-2-like activity was evident among LAB strains isolated from the 'abuse' temperatures (10°and 15°C), whereas only a small fraction of isolates from chill temperatures (0° and 5°C) found to produce QS compounds. In the latter case, the majority of strains was characterised as *Lactobacillus sakei*. In conclusion, further studies are needed to explore that storage temperature strongly affects the expression of genes encoding the AI-2 activity that concomitantly affects the diversity of the LAB population.

Keywords: Autoinducer-2, lactic acid bacteria, temperature

P32

Psychrotrophic bacteria from meat and their spoilage potential *in vitro* and in beef

Danilo Ercolini[1], Federica Russo[2], Antonella Nasi[2], Pasquale Ferranti[2] and Francesco Villani[2]
Department of Food Science, University of Naples Federico II, Via Università 100, 80055 Portici, Italy;
[1]School of Biotechnological Sciences and
[2]School of Agriculture.

Psychrotrophic populations from refrigerated meat were identified in this study and the spoilage potential of microbial isolates in packaged beef was evaluated by analysing the release of volatile organic compounds (VOC) by GC-MS. Fifty mesophilic and 29 psychrotrophic isolates were analyzed by RAPD-PCR and representative strains were identified by 16S rRNA gene sequencing. *Carnobacterium maltaromaticum* and *C. divergens* were the species most frequently found. *Acinetobacter baumannii, Buttiauxella* spp. and *Serratia* spp. were identified among the mesophilic isolates, while *Pseudomonas* spp. were commonly identified among the psychrotrophs. The isolates were further characterized for their growth at different temperatures and their proteolytic activity in vitro on meat proteins extracts at 7°C. Selected proteolytic strains of *S. proteamaculans*, *P. fragi* and *C. maltaromaticum* were used to examine their spoilage potential *in situ*. Single strains of the above species and their mixture were used to contaminate beef chops that were packed and stored at 7°C. At time intervals up to 1 month, viable counts were determined and VOC were identified by GC-MS. Generally the VOC concentrations increased during storage and the profiles of the analyzed meat changed dramatically depending on the contaminating microbial species. About 100 volatiles were identified arising from the different contaminated samples. Among the detected volatiles, some specific molecules were identified only when the meat was contaminated by a specific microbial species. Compounds such as 2-ethyl-1-hexanol, 2-buten-1-ol, 2-hexyl-1-octanol, 2-nonanone, 2-ethylhexanal were detectable only for *C. maltaromaticum*. The highest number of alcohols and ketons were detected in the headspace of meat samples contaminated by *P. fragi,* while the highest concentrations of some alcohols, such as 1-octen-3-ol, and some esters, were produced by *S. proteamaculans*. In conclusion, different microbial species can contribute to meat spoilage with release of different volatile compounds that concur to the overall quality decrease of spoiling meat.

Acknowledgements: This work was partly funded by the project Symbiosis *www.symbiosis-eu.net*

P33

Potential Use of Fourier Transform Infrared spectroscopy (FT-IR) to assess beef spoilage

Argyri, A. Tarantilis P.A., Pappas, C., Panagou, E., Ammor, S., Polysiou, M., & Nychas G.-J
Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.

Quality is a subjective and sometimes elusive term. Freshness of meat muscles are generally considered the most important contributors to quality. It is therefore crucial to have valid methods to monitor freshness and quality. Indeed, methods should be valid for application by industry and consumers in order to obtain reliable information on freshness status when merchandizing and purchasing products. Fourier Transform Infrared (FT-IR) spectroscopy is a rapid, non-destructive analytical technique with considerable potential for application in the food and related industries. FT-IR has been tested for several muscle food analyses and recent studies on meat tissues, stored at ambient temperature, correlate microbial spoilage of meat with biochemical changes within the meat substrate.

Beef fillets were stored aerobically at five different temperatures (0, 5, 10, 15 and 20°C) and the microbiological analysis (Total Viable Counts) was performed in parallel with FT-IR analysis, pH measurements and sensory analysis. The spectral data collected from FT-IR were subjected to principal component analysis (PCA) to investigate differences between samples and thus reduce the size of the data set. A second PCA with the selected variables (wavenumbers) revealed the principal components (PCs) that significantly contributed to the variance of the data set. These PCs were further subjected to factorial discriminant analysis (FDA) in order to predict the quality of a sample that was pre-characterized as Fresh (F), Semifresh (SF) or Spoiled (S) from the sensory analysis. A corresponding procedure was followed in order to qualitatively predict the storage temperature of a sample.

The FDA exhibited a correct classification of 98.68% of samples regarding their spoilage status (F, SF, S) and 93.42% regarding their storage temperature. These data revealed a good correlation between sensory detection of spoilage status and that of chemical metabolites according to storage temperature, as detected from FT-IR. On the other hand, sensory evaluation of spoilage was not always correlated with the same microbial load at the time of the early sensorial detection of spoilage (meat characterized as SF) which was increased with temperature (e.g. 4.01 cfu cm^{-2} at 0°C and 7.17 cfu cm^{-2} at 20°C).

Keywords: Chemometrics, FTIR-ATR, meat, metabolites, spoilage

P34

Contribution of lactic acid bacteria population in meat stored under modified atmospheres

Doulgeraki, A., Nisiotou, A., & Nychas, G-J.
Laboratory of Microbiology & Biotechnology of Foods, Dept of Food Science & Technology, Iera Odos 75, Athens 11855, Greece.

Lactic acid bacteria (LAB) are members of the microbial association that can contribute in meat spoilage while their role in meat stored under modified atmosphere (MA) is detrimental, despite the fact that MA packaging system has been proved to be very effective in extending shelf life of meat.

In order to exploit the influence of package and storage temperature on LAB population during storage of minced beef, samples were stored at 0, 5, 10 and 15°C aerobically and in a package consisted of 40% CO_2-30% O_2-30% N_2. Molecular methods like pulse field gel electrophoresis (PFGE) and polymerase chain reaction (PCR) were used to identify and distinguish LAB community that developed in minced beef during spoilage.

PFGE identification demonstrated that the fluctuations of LAB populations depended on the packaging conditions (package, temperature), although similar viable counts were found. Also, the diversity in genera seemed to increase at lower temperatures and in minced beef stored under MA. These findings strengthen the position that viable counts alone may not be enough to highlight the shift of the bacterial communities according to environmental changes and consequently, the spoilage. The assessment of microbial species dominating in meat spoilage will be fundamental for unearthing – improving the weak link of the chill chain for chilled meat products.

Keywords: *Lactic acid bacteria, meat spoilage, PFGE*

P35

Scientific sYnergisM of nano-Bio-Info-cOgni Science for an Integrated system to monitor meat quality and Safety during production, storage, and distribution in the EU

Nychas, G-J., Ercolini, D., Bessant, C., Aarts, H., Dufour, E., Carstensen, J.M., On., S., Sofos,J., Spais, V., Schiavo,L., Tassou, C., Goodacre,R., Papadopoulos, A., & Del Perci, G.,

The SYMBIOSIS-EU project will bring together 14 partners from 6 EU countries (plus one each from NZ and US) to study meat safety & quality. The overall aim is to identify and quantitatively evaluate practical and easy to use chemical, biochemical and molecular indices and establish their applicability as quality monitors for inspection of meat safety and quality. The project will apply a multidisciplinary system-wide approach relying on converging technologies (bioinformatics, nanotechnology, modelling) to obtain knowledge for meat safety that will be translated into simple devices and practical indicators of quality and safety. The main objectives are
(i) to develop and/or validate easy to use chemical/biochemical methods (e.g. biosensors, Videometer, fluorescence, FT-IR), molecular methods (DNA microarrays),
(ii) to develop a suitable software platform for data sharing and integration,
(iii) to apply multivariate statistical methods and machine learning (neural networks, fuzzy logic, genetic algorithms) to identify robust multiple compound quality indices,
(iv) integration of the sensors and information platform and development of a system to automatically transform data acquired from a sample into a "diagnosis" of meat safety and quality.

The project plan designed to meet these objectives comprises 3 pillars:
1 Microbial status and major metabolomic and molecular profiling of spoilage bacteria
2 Development of an easy to use integrated system to monitor meat safety and quality
3 Development of protocols for simple, effective and cheap evaluation of meat quality and safety in industry, based on new indices of quality and safety relying on detection of metabolites by simple sensors, driven by user friendly software that facilitates practical use of the developed methods.

The project will be of benefit to the EU meat industry, providing useful tools and fundamental knowledge of the spoilage and hazard. It will also impact on the research and informatics communities.

The project is funded from SYMBIOSIS-EU (Contract No. 211638)

Keywords: bioinformatics, meat quality, meat safety, modelling nanotechnology, SYMBIOSIS-EU project

P36

Prediction of beef quality using a machine learning approach based on spectral data from FTIR.

Panagou, E ., Argyri, A. Tarantilis P.A., Polysiou, M., & Nychas G-J.
Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.

In the present study, the applicability of artificial neural networks (ANNs) was investigated to differentiate the quality of beef samples and predict their microbiological load based on FTIR metabolic fingerprinting. Beef fillets were stored aerobically at five different storage temperatures (0, 5, 10, 15, and 20˚C) from freshness to spoilage (*ca*. 3 to 15 days). Duplicate packages from each storage temperature were sampled at appropriate time intervals and subjected to FTIR measurements in the spectral range of 4.000 to 400 cm^{-1}. Additional samples were analyzed to allow for the determination of total viable counts (TVC) and sensory characteristics. Sensory evaluation was based on a three point hedonic scale classifying samples as fresh (F), semi-fresh (SF), and spoiled (S). Spectral data collected between 1700 and 1000 cm^{-1} were initially subjected to principal components analysis (PCA) for dimensionality reduction and the data of the first five principal components, explaining *ca*. 98% of data variability, were employed in further analysis. A three layer network was developed with seven nodes in the input layer (one for temperature, sampling time, and each one of the five principal components) and two nodes in the output layer (one for class and total viable counts). The number of neurons in the hidden layer was empirically determined based on the performance of the network. ANN training was based on the steepest-descent gradient learning algorithm and validation was carried out using the leave-1-out cross validation method. Network performance was compared with the experimental data with both graphical plots and statistical indices namely bias factor (Bf), accuracy factor (Af), percent relative error (% RE), and root mean squared error (RMSE). The overall correct classification of the network was 90.5% in a 74-sample population (24 F, 16 SF, 34 S). Classification accuracies were 91.7%, 81.2%, and 94.1% for fresh, semi-fresh, and spoiled beef samples, respectively. No fresh sample was misclassified as spoiled and vice versa indicating the good discriminating potential of the network between these two classes. The network was able to predict the microbiological load (TVC) of beef samples quite satisfactorily. Specifically, the values of bias and accuracy factors were 0.991 and 1.123, respectively, indicating good agreement between observed and predicted bacterial counts. The average differences between predictions and observations were 12.3% as inferred by the value of the accuracy factor. The % RE values fall within the ± 20% zone for 75%, 87.5%, and 97.1% for fresh, semi-fresh, and spoiled beef samples, respectively.

Keywords: artificial neural networks, FTIR, meat, metabolites, spoilage

Acknowledgements: This work was co-funded by the projects ProSafeBeef *and* Symbiosis.

P37

Tracking verocytotoxigenic *Escherichia coli* (O157, O111, O26, O103 and O145) in the beef chain

K.M Thomas[1,3], M.S. McCann[1], A. Logan[2], P.Whyte[2], D.A. McDowell[3] and G. Duffy[1]
[1]Teagasc, Ashtown Food Research Centre, Ashtown, Dublin 15, Ireland.
[2]University College Dublin, Belfield, Dublin 4, Ireland.
[3]Food Microbiology Research Unit, School of Health and Life Sciences, University of Ulster, Newtownabbey, Co. Antrim.

Verocytotoxigenic (VTEC) *E. coli* have emerged as highly significant zoonotic threats to public health. While many serogroups of VTEC are circulating in the food chain, those most often linked to human illness in Europe include O157, O111, O26, O103 and O145, however not all VTEC are virulent for humans. Illness depends on particular virulence factors (genes) including those which encode for the production of verotoxins (*vt1, vt2*) and the eae gene, which encodes for an attaching and effacing lesion in the large intestine.

This research aimed to investigate the role of cattle in carriage and transmission of clinically significant VTEC. Individual cattle were tracked and faecal samples, hide and carcass swabs were analysed for verotoxin (*vt1* & *vt2*) genes using a duplex real-time PCR assay. Positive samples were screened for the five serogroups of interest by Real-time PCR. Samples positive by PCR for both verotoxin and serogroups genes were culturally examined to recover an isolate. Isolates were subject to a full virulence screen (*vt1, vt2, eaeA* and *hlyA*) by PCR.

VTEC O157 (with *eae*) was the most common isolate recovered from hide (16.0%), faeces (2.3%) and pre/post wash carcass (0.6%) samples. The other four serogroups were much less common with VTEC O26 and O145 (with *eae*) isolated from 0.7% and 1.4% of faeces samples but no other sample type. Serogroup O103 was highly prevalent in all sample types (27.1% hide), however only a small proportion of these carried vt genes and *eae*, thus were clinically significant (0.3% of hide and 0.7% of faeces). *E. coli* O111 was not detected in any samples. For all serogroups the rate of transfer from hide to carcass was low. This study shows that while emergent non-O157 VTEC are being carried by cattle presented for slaughter in Ireland, serogroup O157 remains the most dominant.

P38

The occurrence of *Escherichia coli* O157 in/on faeces, carcasses and fresh meats from cattle

Nastasijevic I.[1], Mitrovic R.[1] and Buncic S.[2]
[1]Institute of meat hygiene and technology, Kacanskog 13, 11000 Belgrade, Serbia.
[2]University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Trg D. Obradovica 8, 21000 Novi Sad, Serbia.

The aim of this study was to investigate whether *E. coli* O157 is present in/on raw beef in Serbia. The *E. coli* O157 method incorporated selective enrichment, immunomagnetic separation, selective media-plating, biochemical confirmation and latex-agglutination test. Correlated faecal and carcasses samples from 115 slaughtered cattle plus 26 uncorrelated carcass samples were examined.

The *E. coli* O157 occurrences were 2.6% in faeces and 2.8% on carcasses. In positive animals, the pathogen was found either in faeces or on carcasses, i.e. no tested animal had the pathogen in both. The *E. coli* O157 occurrences were 0%, 6.2% and 2.1%, respectively, in 106 samples of beef trimmings, 48 samples of minced beef (without additives and spices) and 48 samples of batter intended for production of raw, fermented sausages (with additives and spices).

The results confirmed a key role of faecal contamination and/or the likely hide-mediated cross-contamination for the occurrence of *E. coli* O157 on beef carcasses. Furthermore, the present study revealed the occasional presence of the pathogen in raw materials used for producing raw, fermented beef sausages.

Keywords: *Escherichia coli* O157; beef; faeces; carcass; trimmings; fermented sausages

P39

Development of a microbial Time Temperature Indicator (TTI) for monitoring microbiological quality of fresh meat products

Vaikousi Hariklia[1], Biliaderis Costas[1], and Koutsoumanis Kostas[2]
[1]Laboratory of Food Chemistry & Biochemistry, Department of Food Science and Technology, School of Agriculture, Aristotle University Thessaloniki, 541 24 Thessaloniki, Greece.
[2]Laboratory of Food Hygiene & Microbiology, Department of Food Science and Technology, School of Agriculture, Aristotle University Thessaloniki, 541 24 Thessaloniki, Greece.

Temperature conditions that usually take place in the distribution chain are one of the weakest links in fresh-refrigerated food products management affecting both their safety and overall quality. Since temperature is the most important factor determining the rate of microbial activity, the continuous monitoring of the full time temperature history of chilled products would be of critical importance for an effective product quality optimization. Time Temperature Indicators or Integrators (TTIs) are simple and inexpensive devices that respond with an easily measurable time-temperature dependent colour change, based on the temperature history and quality status of the product they are attached to. The development of a microbial TTI system was based on the microbial growth and metabolism of a lactic acid bacteria strain in a model substrate system (glucose consumption, lactic acid production and the following pH drop, progressively leading to the color change of a chemical chromatic indicator which also exists in the system). The study of the TTI kinetics (under isothermal storage conditions) showed that the pH and colour change of the TTI followed closely bacterial growth. The end point of the TTI coincided with a bacterial population similar to that observed at spoilage levels and with activation energy almost identical to the one of the lactobacillus strain growth. Furthermore, the desired end point of the TTI at a certain temperature can be achieved by appropriate adjustment of the initial microbial concentration. The developed microbial TTI system was successfully applied to monitor the quality status of minced beef packed under modified atmosphere with the end point of TTI coinciding with the time of sensory rejection point of the product during its storage at either isothermal chilled temperature conditions or periodic temperature changing protocols.

P40

Growth of *Salmonella* Enteritidis and *Salmonella* Typhimurium in the presence of quorum sensing signalling compounds produced by spoilage and pathogenic bacteria

Dourou, D., Ammor, M.S., Skandamis, P., & Nychas, G-J.E.

The effect of acylated homoserine lactones (AHLs) and autoincuder-2 (AI-2) signalling molecules produced by *Pseudomonas aeruginosa* 108928, *Yersinia enterocolitica*-like GTE 112, *Serratia proteamaculans* 00612, *Y. enterocolitica* CITY650 and *Y. enterocolitica* CITY844 on the growth kinetic parameters (i.e. lag phase and growth rate) of two *Salmonella* Enteritidis and *S.* Typhimurium strains, respectively, has been assessed by using conductance measurements. AHLs and AI-2 in the cell-free culture supernatants (CFS) of these microorganisms were assayed using different bacterial biosensors and/or thin layer chromatography (TLC). Except from *P. aeruginosa* 108928, which was not found to produce AI-2, all other strains produced both AHLs and AI-2. Thereafter, aliquots (20% in the final volume) of these CFS were transferred in NZ Amine broth inoculated with 10^3-10^4 CFU/ml of 18h cultures of *S.* Enteritidis and *S.* Typhimurium strains, respectively. Changes in conductance of the medium were monitored and detection time (Tdet) was recorded. While *P. aeruginosa* 108928 induced a shorter detection time, i.e. start of the metabolic activity, the other microorganisms enlarged the detection time of *Salmonella* serotypes compared to control samples. Results obtained provide evidence of the effects of QS signalling compounds from other bacterial species on the growth of *Salmonella* and confirm the complexity of bacterial interaction and communication in food spoilage or poisoning processes.

Keywords: *Salmonella enterica,* quorum sensing, acylated homoserine lactones, autoinducer-2, conductance

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P41

Detection of cold tolerant clostridia other than *Clostridium estertheticum* associated with spoilage of vacuum-packed chill-stored meat.

Ana L. Renteria, Janet E.L.Corry, Laura Cavill and Christopher R. Helps
University of Bristol, Department of Clinical Veterinary Science, Langford, Bristol, BS40 5DU, UK.

Spoilage of vacuum-packed chill-stored red meat (beef, lamb and venison) is often caused by *Cl. estertheticum*. We first detected spoilage caused by this bacterium in January 1994 in vacuum-packed beef from South Africa, and then in beef (possibly also of S. African origin) from a UK plant in December 1994. Following spoilage problems due to this bacterium in an Irish boning plant from 1996 onwards, we developed a PCR-based detection test (Helps et al. 1999). Since then we have devised a quantitative (real-time) PCR test which we used last year to test 7,700 meat and processing environment samples for *Cl. estertheticum*, with positive samples from the UK, Ireland, New Zealand, Brazil, Chile and Argentina. Occasionally *Cl. estertheticum* is not detected in a blown pack, suggesting that other species of clostridia could be causing the spoilage.

This study aimed to develop a PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism) test to detect and discriminate between species of psychrophilic and psychrotolerant clostridia other than *Cl. estertheticum*, and subsequently use it to screen *Cl. estertheticum*-negative samples from meat.

The PCR-RFLP was designed to amplify 16S rDNA from *Cl. algidicarnis, Cl. bowmanii, Cl. frigidicarnis, Cl. frigoris, Cl. gasigenes* and *Cl. putrefaciens*.

PCR products were only obtained from the target *Clostridium* spp. tested and not from any of 14 strains of competitive flora. *Cl. algidicarnis* and *Cl. putrefaciens* could not be distinguished, but all the other species could be identified using appropriate restriction enzymes.

From 265 commercial samples from spoiled vacuum-packed meat negative for *Cl. estertheticum*, 27 had intense bands, indicating the presence of relatively high numbers of *Clostridium* sp. and therefore probably the cause of spoilage. Of these 27 samples, three had restriction patterns identical to *Cl. algidicarnis/putrefaciens*, 10 to *Cl. bowmanii*, four to *Cl. frigidicarnis*, five to *Cl. frigoris* and three to *Cl. gasigenes*. The remaining two were not identified to species level.

Helps et al. 1999 *Int. J. Food Microbiol.* **52**, 57-65.

P42

A Multi-Residue Isotope Dilution LC-MSMS Method to Support Risk Assessment for Anthelmintic Drug Residues in Beef

J. Sasanya[1], M. Islam[1], A. Kist[1], M. Danaher[2], M. Whelan[2], R. Granja[3] and A. Cannavan[1]
[1]Agrochemicals Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, Joint FAO/IAEA Programme, International Atomic Energy Agency, Vienna, Austria.
[2]Ashtown Food Research Center, Teagasc, Ashtown, Dublin 15, Ireland.
[3]Microbioticos Laboratories, Campinas, Brazil.

A key priority of the EU Sixth Framework project *ProSafeBeef* is the development and application of quantitative risk assessment models to strategically manage microbial and chemical contamination in the beef chain. To adequately safeguard the European consumer, as well as their own populations, countries that wish to export beef to the EU should have the capability to apply equivalent risk management strategies to those in place in the EU.

A LC-MSMS method for the determination of residues of thirty eight veterinary anthelmintic drugs in beef has been adapted from the UPLC-MSMS method developed and validated in Ashtown Food Research Centre (AFRC), and optimized for transfer, initially to Microbioticos Laboratories in Brazil and thereafter to other INCO countries. The method covers a wide range of anthelmintics, including flukicides, macrocyclic lactones, benzimidazoles, imidazothiazoles and others. Sample preparation is by a modification of the QuEChERS method and extracts are analysed using a Waters Alliance 2695-series HPLC with a C18 Atlantis® analytical column connected to a Waters Quattro-Micro triple-quadrupole mass spectrometer. Multiple reaction monitoring is used with electrospray ionization and polarity switching to facilitate the analysis of a range of compounds with varying physico-chemical properties. Eight deuterated internal standards are employed in an isotope dilution format to improve method precision by compensating for matrix effects. Locally purchased beef samples were used to optimize and perform preliminary validation of the method. Although less expensive, 'lower end' instrumentation is used, careful optimization in collaboration with AFRC has resulted in method performance similar to that of the AFRC method, with reporting limits typically between10 and 25 µg/kg. The method is currently being transferred to Microbioticos Laboratories and is suitable for use in other developed and developing countries wishing to collect risk assessment data for safeguarding public health, facilitating international trade and improving food production practices.