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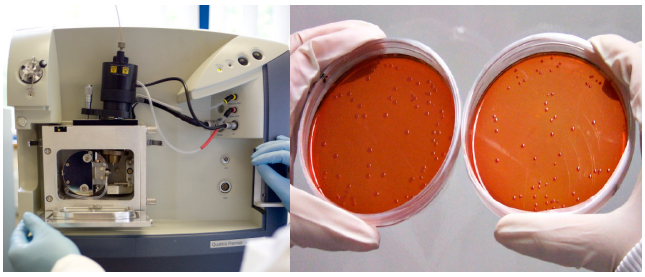
ProSafeBeef: Assessment of microbiological and chemical safety of beef



Key external stakeholders:
Beef sector, Regulators, FSAI

Practical implications for stakeholders:
The study indicated that the risk posed by the microbial pathogens and chemical residues examined in beef in this study was generally low. Nonetheless the study showed that the hide was an important vehicle of microbial pathogen contamination into the abattoir and would thus be a key target for risk reduction measures. A new technology for anthelmintic drug residues was developed and is now in use by the Irish national reference laboratory where it is used for the control and monitoring of food of animal origin for such residues according to EU legislation ensuring beef and food safety.

This research was carried out as part of a multi-national EU Framework project, *ProSafeBeef* which focused on research and innovation to improve beef safety and quality. Research on beef safety at Teagasc focused on the risk posed by microbial pathogens and chemical residues in beef.

Main results:

- Overall, the occurrence of the four pathogens examined (verocytotoxigenic *E.coli, Listeria monocytogenes, Campylobacter* and *Salmonella*) in the beef chain was low, though many of the isolates that were recovered had traits similar to those seen in human illness causing strains highlighting the need for continued vigilance in risk management of such pathogens along the beef chain (farm to fork).
- Verocytotoxigenic *E.coli* are a human health concern with new serotypes of these pathogens being linked to human illness in recent years. In this study *E. coli* 0157, the most common type of VTEC in human illness, was also the most commonly recovered VTEC from beef. Emergent serogroups were recovered at a lower prevalence, and the majority of these isolates did not have the combination of virulence genes typically seen in human disease causing strains.
- During slaughter, it was shown by genetic finger-printing that the source of pathogens on a carcass could be from an animal's own hide or from hide of another animal being slaughtered on the same day, highlighting that the hide is a key target in the chain for interventions.
- A new state-of-the art Mass Spectroscopy (UHPLC-MS/ MS) method was developed for the detection of 38 anthelmintic drug residues. The method was validated according to Commission Decision 2002/657/EC and accredited to ISO 17025 standard. The method was then applied to assess occurrence of anthelmintic residues in 1061 retail beef samples from across Europe over a two year period. Results showed that the risk of exposure to EU consumers from anti-parasitic drug residues in beef was negligible.

Opportunity / Benefit:

The study showed that the hide was an important vehicle of microbial pathogen contamination into the abattoir and would thus be a key target for risk reduction measures. A new technology for anthelmintic drug residues was developed and has been transferred to a number of EU laboratories, thus harmonising the approach of residue control for beef consumed by EU consumers. This research underpins the safe image of EU beef, ensuring consumer confidence and safeguarding international investment in the sector.

Collaborating Institutions:

42 national and international collaborators: see page two of the full Technology Update

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External collaborators: Collaborating researchers in the project included: Institut National de la Recherche Agronomique; Aberystwyth University; Nofima Mat AS, The Norwegian Food Research Institute; Agricultural University of Athens; University College Dublin; Ghent University; University of Bristol; Agricultural University of Poznan, University of Veterinary Medicine Austria; Aristotle University of Thessaloniki; Aarhus School of Business; Danish Meat Research Institute; University College Cork; RIKILT, Institute of Food Safety; Queen's University Belfast; International Atomic Energy Agency; British Nutrition Foundation; University of Novi Sad; Institute of Agro-Food Research and Technology, Spain; Universidad Federale de Sao Paulo; Universidade de Sao Paulo; USDA, Western Regional Research Centre; University of Guelph; Institute of Environmental Science and Research, New Zealand.

1. 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 including, verocytotoxigenic *E. coli* (VTEC), *Listeria monocytogenes, Salmonella* and *Campylobacter.* Such pathogens can persist and circulate in the farm environment posing a risk for contamination of the food and water chain while during beef slaughter and dressing, these pathogens can potentially be transferred from contaminated bovine hide or the gastrointestinal contents onto the beef carcass. While it is known that food pathogens are circulating in the beef chain, few studies have comprehensively tracked or quantified these pathogens in the beef chain or assessed the human clinical significance of strains transmitted by this vehicle. Research at Teagasc aimed to address this gap in knowledge at key stages of the beef chain, farm, beef slaughter and retail.

Additional research from a chemical beef safety perspective focused on anti-parasitic drugs which are important for the control and treatment of helminths such as roundworm, tapeworm and fluke infections in beef-producing animals. Many of these products are licensed for use and are safe for use if product labels are adhered to. However, undesirable levels of residues may be detected in beef if the incorrect dosage is administered; if the drug is not licensed for use in that species; or if withdrawal periods are not adhered. To address this concern, the aim of research at Teagasc was to develop a method for the determination of anti-parasitic drug residues and to then apply the methods to assess levels of these residues in retail beef.

2. Questions addressed by the project:

- What is the occurrence and transmission of *Salmonella*, *Campylobacter* and VTEC, including *E. coli* O157, at key stages in the beef chain (farm, slaughter and retail) ?
- What is the human virulence potential of pathogen recovered from beef ?
- What is the risk posed by anti-parasitic drug residues in beef ?

3. The experimental studies:

- Field studies were performed on 10 Irish beef farms to examine the incidence and spread of *Salmonella*, *Campylobacter*, VTEC and *E. coli* O157. Bovine faecal samples were examined for the pathogens and all isolates were assessed for human virulence potential.
- Samples of bovine hides, and carcass (pre chill) of the same tracked animal were collected from beef abattoirs over a 3 year period. Ground beef samples were collected during the same time period from retail outlets. All samples were examined for prevalence and concentration of four pathogens (verocytotoxigenic *E. coli, Listeria monocytogenes, Campylobacter* and *Salmonella*). Isolates recovered were characterised, genetically finger printed and assessed for human virulence potential.
- A new state-of-the art UHPLC-MS/ MS method was applied to the detection of 38 anthelmintic drug residues. The method was validated according to Commission Decision 2002/657/EC and accredited to ISO 17025 standard and then applied to assess the occurrence of these residues in a two year study of 1061 retail beef samples from across Europe.

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4. Main results:

- In farm faecal samples, 2% and 3% of samples were positive for *Salmonella*, and *Campylobacter*, respectively. All of the *Salmonella* detected were *S.* Typhimurium. *Campylobacter* species included *C. jejuni* (4 farms). This particular serotype of *Salmonella* and species of *Campylobacter* are the most common types seen in human illness.
- Verocytotoxigenic *E. coli* isolates was recovered on all farms examined with a wide diversity of serotypes recovered including *E. coli* O157 and emergent serogroups. Apart from the *E. coli* O157 isolates few of the emergent serotypes had the combination of virulence genes typically seen in human disease-causing strains.
- VTEC were also recovered from beef at slaughter, on hides (17%) and on carcass (1%). *E. coli* 0157 was the most common serogroup recovered. It was noted that as for farm isolates, only a small proportion of the non O157 VTEC had the combination of virulence genes typically seen in human disease-causing strains.
- The prevalence of *Listeria monocytogenes* ranged from 14% on carcass to 29% on ground beef, highlighting that cross contamination and growth of this pathogen can occur in the beef chain. The most common serotypes recovered were 1/2a followed by 4b (the most common serotype in human illness) and the majority of isolates contained a key virulence gene (*lmo*2821) seen in human illness strains.
- The prevalence of *Campylobacter* on hides was high (51%) but was very low on ground beef samples (1%) indicating that the pathogen did not survive the environmental conditions experienced in the beef chain (chilling, drying etc.). *Campylobacter jejuni (*the most common species in human illness) was also the most common species recovered from beef
- The prevalence of *Salmonella* was low with a diversity of serogroups recovered
- Genetic finger-printing showed the source of pathogens on a carcass could be its own hide or the hide of another animal slaughtered on the same day, highlighting that the hide is a key target for risk reduction measures.
- A mass spectroscopy method (UHPLC-MS/ MS) was developed for the detection of 38 different anthelmintic drug residues. The method was validated according to Commission Decision 2002/657/EC and the technology was transferred to the Irish national reference laboratory for anti-parasitic drugs where it is used for the control and monitoring of food of animal origin according to EU legislation ensuring food safety
- Research showed that 26 of the 1061 beef samples analysed contained residues of the anti-parasitic drugs, and of these 26, only one was at a non-compliant level. The non-compliant sample contained low levels of ivermectin, however, as there was no maximum residue limit (MRL) for ivermectin in muscle, any presence of the drug is deemed non-compliant. These results indicate that the risk of exposure to EU consumers from anti-parasitic drug residues in beef is negligible.

5. Opportunity/Benefit:

The study showed that the hide was an important vehicle of microbial pathogen contamination into the abattoir and would thus be a key target for risk reduction measures. A new technology for anthelmintic drug residues was developed and has been transferred to a number of EU laboratories, thus harmonising the approach of residue control for beef consumed by EU consumers. This research underpins the safe image of EU beef, ensuring consumer confidence and safeguarding international investment in the sector

6. Dissemination:

Main publications:

Bolton DJ, Ennis C, McDowell D. (2013)Occurrence, Virulence Genes and Antibiotic Resistance of Enteropathogenic *Escherichia coli* (EPEC) from Twelve Bovine Farms in the North-East of Ireland. 10.1111/zph.12058. [Epub ahead of print]

Ennis C., McDowell D, Bolton DJ. (2012). The prevalence, distribution and characterisation of Shiga toxin-producing *Escherichia coli* (STEC) serotypes and virulotypes from a cluster of bovine farms. *J Appl Microbiol*. 113(5):1238-1248.

Rhoades, J.R., Duffy, G., and Koutsoumanis, K. (2009) Prevalence and concentration of verocytotoxigenic *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes* in the beef production chain: A review. *Food Microbiology, Volume* 26, I 4, 357-376

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Thomas, K.M., McCann, M., Collery, M.M., Logan, A., Whyte, P., McDowell, D.A., and Duffy, G. (2012). Tracking Verocytotoxigenic *Escherichia coli* 0157, 026,0111, 0103 and 0145 in Irish Cattle. *Int J. Food Micro* 15(153):288-296

Cooper, K. M., Kennedy, D. G., & Danaher, M. (2012a). ProSafeBeef and anthelmintic drug residues - A case study in collaborative application of multi-analyte mass spectrometry to enhance consumer safety. Analytical and Bioanalytical Chemistry, 404(6-7), 1623-1630.

Cooper, K. M., Whelan, M., Kennedy, D. G., Trigueros, G., Cannavan, A., Boon, P. E., Wapperom, D. and Danaher, M. (2012) Anthelmintic drug residues in beef: UPLC-MS/MS method validation, European retail beef survey, and associated exposure and risk assessments. Food Additives & Contaminants: Part A, 29, 746-760.

Cooper, K. M., Whyte, M., Danaher, M., & Kennedy, D. G. (2012). Emergency slaughter of casualty cattle increases the prevalence of anthelmintic drug residues in muscle. Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 29(8), 1263-1271.

Cooper, K.M., Whelan, M., Danaher, M., Kennedy, D.G. Stability during cooking of anthelmintic veterinary drug residues in beef (2011) Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 28 (2), pp. 155-165.

Whelan, M., Kinsella, B., Furey, A., Moloney, M., Cantwell, H., Lehotay, S.J., Danaher, M. Determination of anthelmintic drug residues in milk using ultra high performance liquid chromatography-tandem mass spectrometry with rapid polarity switching (2010) Journal of Chromatography A, 1217 (27), pp. 4612-4622.

Kinsella, B., Whelan, M., Cantwell, H., McCormack, M., Furey, A., Lehotay, S.J., Danaher, M. A dual validation approach to detect anthelmintic residues in bovine liver over an extended concentration range (2010) Talanta, 83 (1), pp. 14-24.

Popular publications:

Tracking key pathogens in the beef chain. Prosafebeef TechKnowledge Stakeholder Digest No. 7. www.prosfebeef.eu

Methods for the Detection of Anti-Parasitic Drug Residues in Beef. Prosafebeef TechKnowledge Stakeholder Digest No. 10. www.prosfebeef.eu

7. Compiled by: Geraldine Duffy, Martin Danaher, Declan Bolton and Kaye Burgess

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