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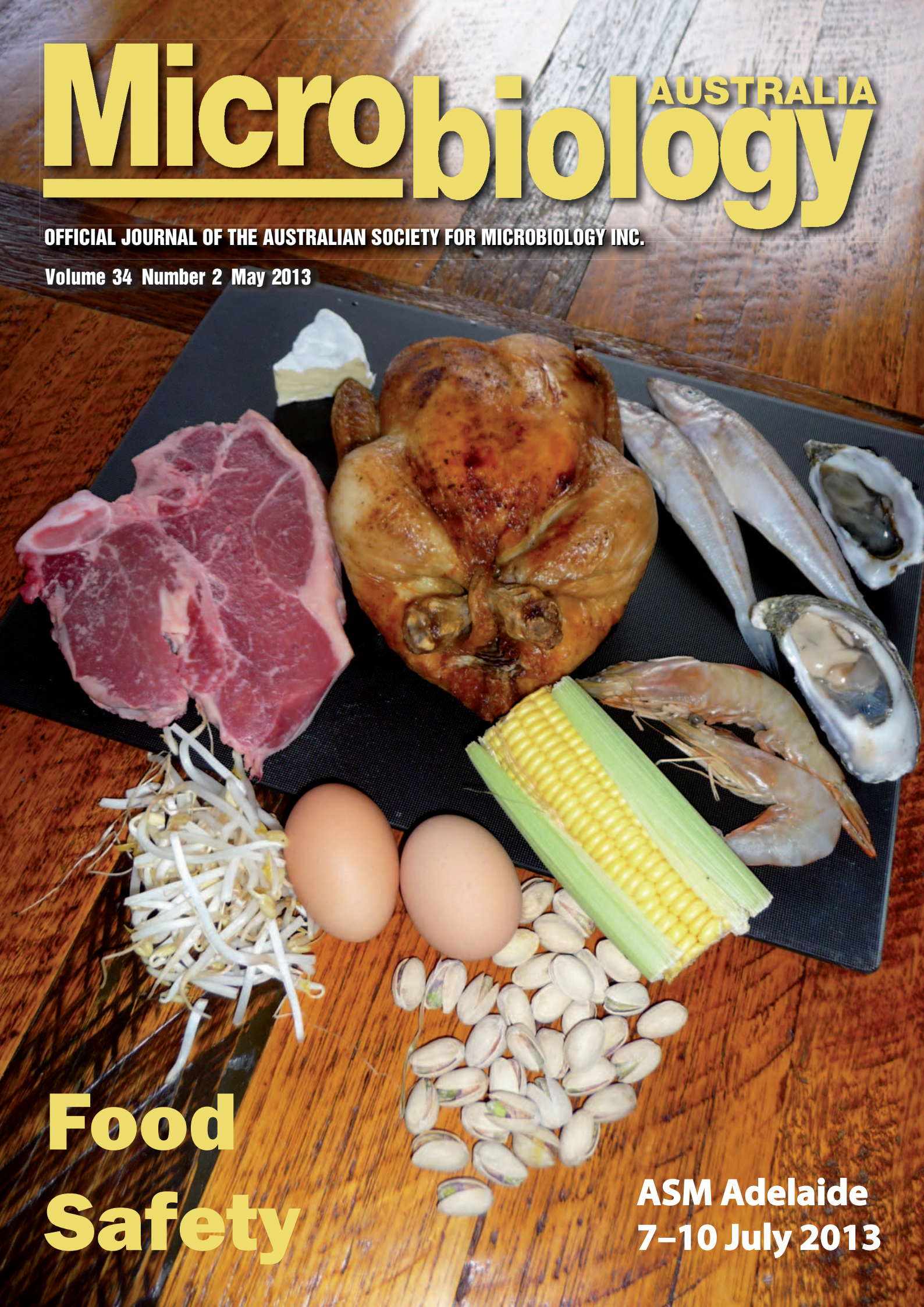

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The Australian Society for Microbiology Inc.

210/55 Flemington Road
North Melbourne, Vic. 3051
Tel: 1300 656 423
Fax: 03 9329 1777
Email: admin@theasm.com.au
www.theasm.org.au
ABN 24 065 463 274

For *Microbiology Australia* correspondence, see address below.

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Editorial correspondence
Prof. Ian Macreadie/Mrs Jo Macreadie
Tel: 0402 564 308 (Ian)
Email: ian.macreadie@gmail.com

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Publishing enquiries
Richard Hecker
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Production enquiries
Helen Pavlatos
Email: helen.pavlatos@csiro.au

Advertising enquiries
Doug Walters
Tel: 03 9662 7606
Mobile: 0419 357 779
Email: doug.walters@csiro.au

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Food safety

Narelle Fegan

Producing safe food is essential for protecting the health of consumers and for ensuring the sustainability and profitability of food industries, including primary production, food manufacture, food service and retail. Food safety hazards may result from contamination of food with microbial, physical or chemical hazards. This edition of *Microbiology Australia* focuses on microbial food safety hazards, including prions, viruses, bacteria and fungi. Technological advancements throughout history, such as pasteurisation, canning and refrigeration, have significantly improved the safety of our food supply; however, foodborne illness remains a global problem impacting on millions of people each year. A recent example is the large outbreak of *E. coli* O104:H4 that occurred during 2011 in Germany and many other countries across Europe and North America, which affected more than 4,000 people resulting in 50 deaths. The outbreak was associated with seeds used for sprouting that had originated from Egypt. The causative agent, *E. coli* O104:H4, had acquired new virulence properties in the form of toxin genes carried by bacteriophage, creating a highly pathogenic strain. This outbreak highlighted some of the difficulties faced by those involved in keeping the food we eat safe. Many of these challenges are discussed throughout the articles in this edition of *Microbiology Australia*.

The way we produce our food is constantly evolving. Changing food production practices through the application of new farming technologies, climate change and the availability of water and land resources as well as intensive rearing of animals can all impact on the presence of foodborne pathogens during primary production. Technologies are constantly being developed to improve food processing, which may in turn provide new and unpredictable ways for food pathogens to enter the food supply. Consumer preferences are also changing with an increasing demand for fresh, minimally processed foods that have limited or no interventions to reduce microbial contamination or limit their growth. Changing demographics along with an increase in ageing and immunocompromised populations require that stringent food safety measures are applied to foods served to these populations.

Probably one of the most significant challenges associated with food safety are the pathogens themselves, which are constantly adapting and changing, leading to the emergence of new pathogenic types and new modes of transmission through food. Advances in disease surveillance networks, tools for tracing outbreaks and pathogen characterisation have enabled the identification of foodborne outbreaks, which would previously have gone unnoticed. The use of standardised tests across many countries has facilitated the identification of outbreaks that may cross international borders. Food safety issues that were once restricted to a limited area may now impact on geographically diverse populations due to the globalisation of the production and trade of food in comparison to the past. This can also have a significant impact on trade with various countries requiring imported food to meet certain criteria in relation to food safety. In recent years there has also been recognition that foodborne pathogens could be used in terror attacks.

As you can see from the above challenges, keeping food safe is a complex task. It involves input from food producers, food manufacturers, consumers, researchers, clinicians, epidemiologists, politicians and regulators, and as highlighted from this edition of *Microbiology Australia*, requires skills beyond just microbiology. Understanding food safety requires knowledge of microbial ecology through the whole food chain, along with an understanding of the types of pathogens present, the virulence properties they possess and how they are transferred through food production. The availability of effective detection methods and surveillance systems is also critical for identifying outbreaks and tracing the sources of foodborne illness. Understanding the cause of outbreaks is important for preventing future occurrences. More recently, the development of new tools such as "omics" and the application of computational and mathematical science to biological/food systems has increased our fundamental knowledge on how foodborne pathogens enter, persist and behave within food systems. Such information will guide the future development of new processing technologies and underpin risk management and intervention systems to control hazards throughout the food system from farm to fork. Effective information and education around safe food handling practices and understanding risks associated with food production is important not only for those working in the food industry producing our food, but also for consumers. Having appropriate risk-based food regulation and the application of standards to the food industry is also important for limiting foodborne illness. This edition of *Microbiology Australia* covers many aspects of these complexities and highlights the challenges faced by all those who work to ensure the safety of our food supply.

Biography

Dr Narelle Fegan is a microbiologist in the Food Safety and Stability Theme and current leader of the Microbiology Group at CSIRO Animal, Food and Health Sciences. Narelle has been working at CSIRO for the past 17 years on projects studying foodborne pathogens, mostly pathogenic *E. coli* and *Salmonella*. Narelle's particular interests include understanding the epidemiology and ecology of these pathogens in foods, food animals, and in the environment.

The OzFoodNet story: 2000 to present day

Michelle Green

Department of Health and Human Services
GPO Box 125
Hobart, Tas 7001, Australia
Tel: +61 3 6222 7627
Fax: +61 3 6222 7744
Email:
michelle.green@dhhs.tas.gov.au

Gerard Fitzsimmons

Zoonoses, Foodborne and Emerging Infectious Diseases Section
Office of Health Protection
Australian Government Department of Health and Ageing
Tel: +61 2 6289 2748
Email:
gerard.fitzsimmons@health.gov.au

OzFoodNet is a network of epidemiologists whose primary interest is foodborne disease. The network is now over 10 years old and in this time, there have been many research studies and outbreak investigations undertaken by the network. A considerable body of published work exists that details the achievements and workings of the OzFoodNet network and the OzFoodNet sites. Lessons have been learnt from outbreak investigations, with improved surveillance systems developed for pathogens such as *Listeria monocytogenes*. The work of OzFoodNet plays an important part in informing food safety policy and regulations.

The origins of OzFoodNet

OzFoodNet is a collaborative program to enhance the surveillance of foodborne disease. The program was established in 2000 by the then Australian Government Department of Health and Aged Care and state and territory health departments. One of the events that led to the formation of OzFoodNet was an outbreak in 1995 of Shiga-like toxin producing *Escherichia coli* (STEC) causing haemolytic uremic syndrome (HUS) associated with mettwurst sausage[1,2]. Within months of the outbreak, Australian governments were seeking to develop new national food safety standards. However, there were challenges in the development of these standards due to the lack of comprehensive data on the incidence and causes of foodborne illness[3]. With no state or national foodborne disease surveillance system, public health practitioners and policy makers could only rely on case reports, infrequent outbreak reports, irregular and *ad hoc* summaries of foodborne disease, and overseas surveillance data to formulate food safety standards[4]. One project concluded that there was value in a population based systematic and enhanced surveillance system to better understand the epidemiology of foodborne disease. Given this finding, an 18 month trial of enhanced foodborne disease surveillance commenced in the Hunter region of NSW[5]. The trial was a proof of principle and a national work program on food safety coordinated by the Australian Government Department of Health and Ageing (DoHA) built upon this initiative in the Hunter with the formation of OzFoodNet: A national system to enhance foodborne disease surveillance[3].

What is OzFoodNet

OzFoodNet is a network of epidemiologists based in each state and territory health department and DoHA. At each site there are 1 to 3 epidemiologists, with some sites also having a supportive surveillance or administrative staff member. The network includes other organisations, including Food Standards Australia New Zealand (FSANZ), Australian Government Department of Agriculture, Fisheries and Forestry, the Public Health Laboratory Network and the National Centre for Epidemiology and Population Health (NCEPH) (Figure 1). OzFoodNet is a member of the Communicable Diseases Network Australia, a sub-committee of the Australian Health Protection Principal Committee.

OzFoodNet is a network for detecting and responding to nationally important foodborne diseases, monitoring the burden of these illnesses, and identifying the sources of specific foodborne outbreaks through enhanced communication and cooperation amongst jurisdictions. The network was modelled on the Centre for Disease Control (CDC) FoodNet model of active surveillance[6] and has evolved into a mature network. Many of the epidemiologists are long standing members of the network and some have been with OzFoodNet from its inception. The OzFoodNet network is funded through DoHA by the Australian Government.

Regular communication within the network and with the relevant stakeholders and partners has been one of the key factors in the

network's ability to function effectively. Prior to the formation of OzFoodNet, no such data sharing and communication existed between states. Through rapid communication using list servers, a fortnightly enteric disease surveillance report, regular monthly teleconferences, and face to face meetings held several times a year at different OzFoodNet sites – network members have been able to communicate in a regular and timely fashion, sharing information, data, ideas and co-operating in outbreak investigations. There is a high level of trust within the network which is one of OzFoodNet's strengths.

OzFoodNet is also partner of the WHO Global Foodborne Infections Network, which is a capacity-building program that promotes integrated, laboratory based surveillance and intersectoral collaboration among human health, veterinary and food-related disciplines[7]. Some OzFoodNet epidemiologists have participated as trainers in courses covering both the Western Pacific Region and South-East Asia region, where they are able to share their knowledge and experience in a training environment but also build relationships with international colleagues and networks interested in foodborne disease.

Achievements

In the early years of the network there was a strong emphasis on research and in developing a better understanding of foodborne disease. Several studies examined the risk factors for campylobacteriosis[8 9]; studies into particular *Salmonella* types from certain areas of Australia, such as *S.* Mississippi in Tasmania[10] and also research into the identification of risk factors for sporadic listeriosis cases[11].

OzFoodNet and NCEPH have estimated that there were 5.4 million cases of foodborne infectious gastroenteritis annually in Australia circa 2000[3]. The burden of foodborne disease in Australia is substantial, costing approximately $1.2 billion dollars annually, mainly resulting from lost productivity when people with gastroenteritis stayed home from work, or having other people staying home to look after them. Currently, an NCEPH work program is underway to provide revised estimates of foodborne disease incidence in Australia, circa 2010. Funding to revise these estimates has been provided by DoHA, Food Standards Australia New Zealand (FSANZ) and the New South Wales Food Authority.

Much of the work of OzFoodNet in recent years has been focused on foodborne outbreak investigations including between four to six multijurisdictional outbreaks annually. Working as a collaborative network involving states and territories and other Australian Government agencies has resulted in the detection and investigation of a number of notable and instructive multijurisdictional outbreaks of foodborne disease (Table 1). These have included national outbreaks of *Salmonella* Saintpaul associated with rockmelons,[12] *S.* Litchfield associated with papaya[13], a *Listeria monocytogenes* outbreak associated with chicken meat sandwiches and wraps on a domestic airline[14] , an outbreak of Hepatitis A associated with semi-dried tomatoes[15,16], and outbreaks of norovirus due to imported oyster meat[17]. At times, foodborne outbreaks have crossed international borders, with a *Shigella sonnei* outbreak in Australia and Denmark associated with imported baby corn from Thailand[18].

Multi-jurisdictional outbreak investigations coordinated by OzFoodNet are critical to maintaining a safe food supply for Australia. These investigations also assist in the development of national policies and regulation to prevent foodborne disease. OzFoodNet maintains a national Outbreak Register which is a detailed repository of data concerning outbreaks of enteric-related disease in Australia from 2001 to the present. Regulatory agencies rely on these data in order to inform risk assessment and standards development. Information from the OzFoodNet Outbreak Register has been used to inform

Table 1. Summary of selected significant multijurisdictional outbreak investigations undertaken by OzFoodNet.

Year	Outbreak	Insights and Outcomes
2006	*Salmonella* Saintpaul associated with rockmelons	• Evidence of fresh produce as vehicle for *Salmonella* transmission • Production and processing practices around rockmelon pose public health risk • Information used to inform primary production standards
2009	*Listeria monocytogenes* in chicken sandwiches and wraps	• Advancements in molecular typing of Listeria could be utilised in a surveillance system to detect outbreaks • Development and implementation of an enhanced surveillance system for Listeriosis cases
2009	Hepatitis A outbreak and semi dried tomatoes	• Public Health action and trade level recalls • Provided and example of the complex movement of food globally and related trace-back difficulties in an outbreak setting.
2012	*Listeria monocytogenes* and soft cheese	• Detection of the outbreak through enhanced surveillance • Public Health action to control and prevent further disease • National recall of implicated products

Figure 1. The recent 39th OzFoodNet Face to Face meeting held in Melbourne in February included a large number of OzFoodNet stakeholders as well as the network's epidemiologists.

food policy and contribute to food standards that are developed by FSANZ

Vehicles for *Salmonella* outbreaks have been varied – from eggs and egg products, poultry and meat products, and dishes such as sandwiches and condiments/sauces[19]. Egg-associated outbreaks have been documented frequently over the last several years[20–22] with enhanced data collection around egg-associated outbreaks being one of the network's current areas of focus[23]. In 2010, egg-associated outbreaks compromised 14% of all outbreaks investigated and 36% of all *Salmonella* outbreaks[24]. The outbreak data in relation to eggs has been one of the resources used to inform the development of the *Primary Production and Processing Standard for Eggs and Egg Products (Standard 4.2.5 by FSANZ).*

Enhanced surveillance systems

The network has developed a national approach to surveillance for some foodborne pathogens of particular interest so that consistent and comparable information is collected when needed and to detect potential outbreaks.

Following a national outbreak of *Listeria in 2009*[12], OzFoodNet undertook to improve *Listeria* surveillance and outbreak detection though the establishment of an enhanced *Listeria* surveillance system. This system uses a variety of molecular typing techniques which have been developed in recent years and been used to detect clusters of cases based on the molecular types. Information is stored on a web based database enabling real time data entry and analysis. The food histories of cases are analysed when clusters are detected to identify potential sources of infection.

A recent application of this enhanced surveillance system occurred in late 2012, when OzFoodNet began investigating an outbreak of a particular *Listeria monocytogenes* subtype. Epidemiological analysis of the case data in this enhanced surveillance system was able to quickly identify a possible association between cases and the consumption of soft cheese that led to a national recall of the suspected products thought to be associated with the outbreak (http://www.foodstandards.gov.au/consumerinformation/foodrecalls/currentconsumerlevelrecalls/jindicheesepotential5792.cfm).

Challenges

One of the strengths of the OzFoodNet work has been the ability to detect and investigate promptly potential multi-jurisdictional outbreaks of foodborne disease. Much of the work of OzFoodNet relies on the laboratories that provide typing information on a variety of isolates such as *Salmonella* and *Listeria monocytogenes*. There have been changes and improvements in molecular characterisation techniques such as multiple-locus variable number tandem repeat Analysis (MLVA) over the past few years. Due to these developments, multijurisdictional outbreaks or cluster investigations by OzFoodNet now involve detailed case definitions concerning *S.* Typhimurium phage types, MLVA types and pulse field gel electrophoresis (PFGE) patterns to include all jurisdictions and the differences in *Salmonella* typing methods that exist across Australia. The different characterisation techniques in each jurisdiction have been implemented and utilised based on the need to capture timely surveillance data for the jurisdiction. These evolving techniques provide greater discrimination of organisms which often aids outbreak investigations. However, their development has resulted in some challenges for surveillance between Australian states and territories.

Conclusions

OzFoodNet has successfully conducted surveillance and responded to outbreaks of foodborne illness since its commencement in 2000. This success has been based on continued funding support by DoHA and on the building and maintenance of strong working relationships that have fostered collaboration between many different partners, including public health units, health departments, laboratories, reference laboratories, food safety agencies, and primary industry departments.

Acknowledgements

We thank the many epidemiologists, Masters of Applied Epidemiology scholars, DoHA graduate trainees, project officers, interviewers and research assistants at each of the OzFoodNet sites. We would like to acknowledge the work of various public health professionals and laboratory staff around Australia who interviewed patients, tested specimens, typed isolates and investigated outbreaks. We would particularly acknowledge jurisdictional laboratories, the Microbiological Diagnostic Unit Public Health Laboratory, the South Australia Pathology's Australian Salmonella Reference Centre, Queensland Health Scientific Services, PathWest and the National Enteric Pathogen Surveillance Scheme for their help and guidance with foodborne disease surveillance. The quality of their work was the foundation for OzFoodNet.

Further information on the network is available from the OzFoodNet website, www.ozfoodnet.gov.au.

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Biographies

Michelle Green is the OzFoodNet epidemiologist in Tasmania, and was the OFN Epidemiologist in the Northern Territory for 6 years. Prior to her time as an epidemiologist, she spent nearly 10 years as a microbiology scientist in clinical pathology laboratories.

Gerard Fitzsimmons is the OzFoodNet Coordinating Epidemiologist in the Australian Government Department of Health and Ageing. He has worked in this role since mid-2011.

Foodborne viruses: a focus on challenges associated with detection methods

Gail E Greening

Environmental and Food Virology,
Food Group,
Institute of Environmental Science and Research
Kenepuru Science Centre
PO Box 50-348
Porirua, New Zealand
Tel: +64 4 9140 700/+61 7 3410 8794
Email: Gail.greening@esr.cri.nz

Human enteric viruses are now recognised as one of the commonest causes of foodborne disease with norovirus and hepatitis A virus (HAV) the main viruses implicated in foodborne outbreaks. Norovirus is the main cause of acute non-bacterial gastroenteritis worldwide. Foods at risk of virus contamination are bivalve shellfish, fresh produce, manually prepared ready to eat (RTE) foods and bakery products. Analysis of foods for virus presence is challenging for many reasons. Complex food matrices present processing problems for efficient recovery and detection of viruses, current molecular methods do not allow for determination of virus infectivity and low virus copy number in foods means that exquisitely sensitive methods and multiple controls are required for virus detection. There are still no international standard methods for viral analysis of foods. However significant progress towards a standard method for detection of norovirus and HAV in foodstuffs has been made by a European Committee for Standardisation (CEN) technical working group. This method is due for publication in 2013 as a two-part joint ISO/CEN Technical Specification. In later years it will be replaced by a fully validated standard.

Foodborne viral disease has emerged as a major public health problem in recent years. The globalisation of our food supply means that fresh produce may be sourced from countries where quality control and hygiene practices do not meet international standards. Food can become contaminated at either at the preharvest or post-harvest stage. Bivalve shellfish such as mussels, oysters, clams and fresh produce, including lettuce, salad greens, herbs and berry fruits, are most at risk of preharvest contamination. Food may be irrigated or washed in water containing human faecal material, or handled by field-workers with poor hygiene practices. Filter feeding bivalve shellfish grown in sewage-contaminated waters accumulate and concentrate enteric viruses and other microbial contaminants as they feed. Bacteria are eliminated from shellfish within a few days, but viruses are known to persist and retain their infectivity for several weeks or months in the shellfish gut. Although molecular methods do not determine whether these viruses are infectious, their presence and low infectious dose (<100 particles may cause infection) indicate there may be a risk of infection if shellfish are consumed either raw or lightly cooked.

Enteric viruses such as norovirus and HAV are transmitted by the faecal – oral route, excreted in human faeces and hence pass into the sewage systems. They are present in sewage effluent and discharges, septic tank leachates and also drinking, growing, irrigation and bathing waters contaminated with sewage or faecal material. These viruses are very resistant to environmental stressors and may persist in the environment for long periods.

Postharvest contamination results from unsafe foodhandling practices. Foods most at risk are uncooked or lightly cooked foods, salads, cold meats, bakery products and other RTE foods subject to extensive handling during preparation (Figure 1). Foods implicated in recent norovirus and HAV foodborne outbreaks in Australia and New Zealand include blueberries[1], semi-dried tomatoes[2,3], shellfish[4-6] and foodhandling[7,8]. In some of these outbreaks, norovirus or HAV were identified in both the implicated food and associated cases. Additional genotyping of virus strains may aid in establishing the source of outbreaks.

Analysis for pathogenic viruses is very different to bacterial analysis. Neither enrichment culture techniques nor *in vitro* culture can be used because human norovirus and wild-type HAV are not able to be grown in cell culture yet. Therefore real-time quantitative (RT-) PCR is generally used for virus detection but this technique does not determine infectivity. Detection of viruses in foods is challenging due to the following issues:

- Low virus copy number in foods
- Complex food matrices are difficult to analyse and may be inhibitory to PCR
- Efficiency of virus recovery is generally poor and can be as low as 1–10%

- Many controls are required to monitor each stage of the detection process
- Infectivity data are not provided by current molecular methods and culture is not an option for norovirus and wild-type HAV

The internationally required agreements for PCR controls for food testing are described in ISO 22174 : 2005. Many controls are required to check each stage of the viral analysis process (Table 1). These include a process control added at the start of the viral extraction procedure to provide information on virus recovery efficiency and, in the RT-PCR assay, an internal amplification control (IAC) for RT-PCR inhibition, a positive RT-PCR control, viral RNA standards, negative RT-PCR controls and an extraction blank. The IAC and process controls monitor for cross contamination, false positives, assay sensitivity and reproducibility between runs. Virus quantitation is often required for shellfish analysis. This can be achieved by establishing the limit of detection and the limit of quantitation for each assay, then using plasmid copy standard curves to determine sample quantities. The target DNA sequence is cloned into a plasmid to produce a range of standard copy numbers which are included in

Figure 1. Foods at risk of viral contamination.

Table 1. Controls used by ESR in real-time RT-qPCR assays for viral analysis of foods.

	Procedure			
Control #	**Food processing**	**RNA extraction**	**RT**	**PCR**
1	Murine norovirus	Murine norovirus	Murine norovirus	Murine norovirus
2		Positive NoV or HAV control	Positive NoV or HAV control	Positive NoV or HAV control
3		Extraction blank	Extraction blank	Extraction blank
4		Armored RNA	Armored RNA	Armored RNA
5			Positive RNA standards	Positive RNA standards
6			Reagent blank	Reagent blank
7				NoV or HAV DNA plasmid

each run. As these DNA transcripts do not provide quality control for the RT step, RNA standards and the IAC are included to monitor RT efficiency and detect inhibition. Analysis of foods for viruses is costly because of the number of replicate samples tested, the number of controls and standards required per sample and the controls required for each PCR run.

There are currently no international standard methods for virus detection in foods and shellfish. However, in 2004 the European Committee for Standardization (CEN) established a technical advisory group (CEN/TC275/WG6/TAG4) comprising 30 members from 13 countries to develop a standard method for detection of norovirus and HAV in foodstuffs - namely bivalve shellfish, salad crops, soft fruits, bottled water and hard surfaces[9]. This method is to be published in 2013 as Technical Specification CEN ISO/TS 15216; Microbiology of food and animal feed – Horizontal method for detection of hepatitis A virus and norovirus in food using real-time RT-PCR (TS). Methods for virus detection in RTE foods are not included in the CEN method and there are few published methods[10–12]. The range of RTE food matrices is extensive (e.g. pasta, potato salad, dairy and bakery products, sliced meats) and so virus recovery processes need to be adapted for each matrix.

The CEN method is currently undergoing validation and, when completed, the TS will be replaced with a fully validated CEN/ISO standard in 2018. Despite the lack of formal validated methods, a European National Reference Laboratory (NRL) ring trial for detection and quantitation of norovirus and HAV in lenticules and shellfish tissue is carried out annually with over 25 laboratories worldwide participating, including New Zealand, Australian, Asian and North American laboratories. Several laboratories use their own methods in combination with components of the CEN method. The results show that overall the CEN methods are consistently performing well. The EU plans to introduce legislation for regulatory testing once the method is complete (*personal communication Dr J Lowther, CEFAS, UK*).

Since 2007, the ESR Environmental and Food Virology Laboratory (EFVL) has gained 100% accuracy in the European NRL trial using its ISO 17025 accredited method for detection of norovirus in shellfish[13]. This method, based on the CEN method, has been used for analysis of over 700 Australasian and Asian shellfish samples referred to the EFVL laboratory from water and shellfish quality monitoring programmes, outbreak investigations, product clearances, export screening and following sewage discharge events (Figure 2). Norovirus was detected in varying concentrations from low (<80 copies/g of gut tissue) to high (>10,000 copies/g) in 368/709 (52%) of

Figure 2. Dissecting out shellfish digestive tissue for viral analysis.

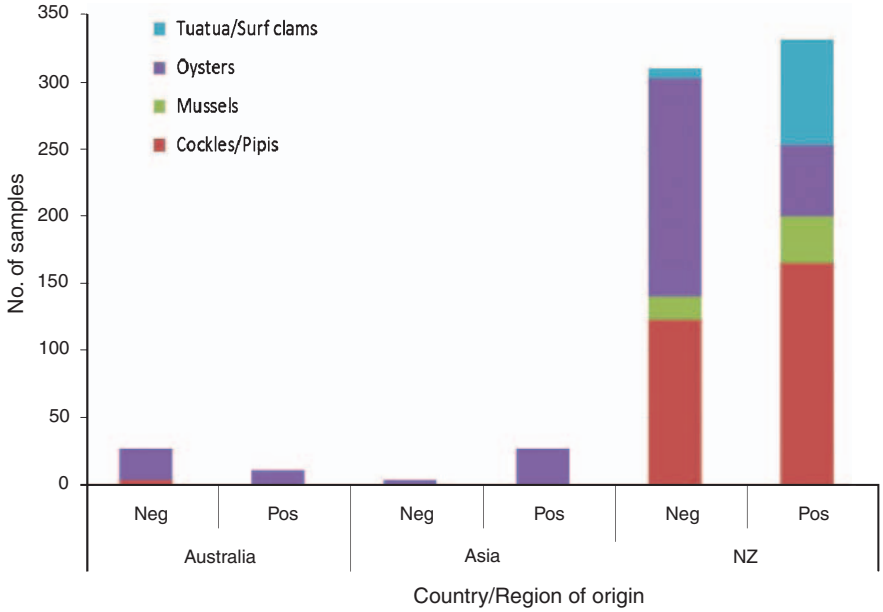

Figure 3. Detection of norovirus in shellfish submitted to ESR for analysis from 2006–2011 ($n = 709$).

shellfish analysed (Figure 3) including 48/74 (65%) shellfish associated with outbreak investigations.

The main drawback of molecular methods is their inability to provide information on virus infectivity. Recently, new approaches to differentiate between inactivated and infectious viruses have been evaluated. The principal hypothesis is that non-infectious viruses have damaged capsids and so may be more susceptible to stressors and may also be unable to bind to cellular antigens. Capsid damage may expose the RNA genome and allow it to be degraded. Researchers have evaluated pre-treatment or exposure of samples to different agents, including heat, chlorine/hypochlorite, UV, RNAse, proteinase K, oxidative damage and propidium monoazide (PMA) prior to RT-PCR[14–17]. Pretreatment by enzymes may damage capsids and/or viral RNA[14,15]. Oxidative damage to capsid is measured by biotin labeling of the damaged capsid[16]. PMA is a light activated intercalating chemical which can damage RNA once capsid damage has occurred [17].

In a different approach, long target (~3kb) and short target (~100bp) RT[18] or PCR[19] were used to distinguish between infectious and inactivated viruses based on the premise that long genome sequences could be amplified in infectious viruses, but only short sequences could be amplified in inactivated viruses where the RNA genome may be partially degraded.

All of these approaches have been carried out using culturable viruses so that the methodologies can be compared with conventional cell culture. They have shown that it may be possible to distinguish between infectious and inactivated viruses by molecular methods but further research to validate these methods is required. As some of these approaches are technically complex, they may more useful in research applications rather than for routine analysis.

Current molecular detection and quantification methods for viruses are used in many applications. These include food and water quality control and monitoring programmes, outbreak investigations, prevalence studies, and for tracing contamination sources. In conjunction with real-time RT-PCR methods, where culture methods are available for culturable HAV and surrogate viruses, virus persistence, survival and inactivation studies can be carried out to develop appropriate intervention and control strategies.

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Biography

Dr Gail Greening is a Science Leader/Consultant in Environmental and Food Virology for the Institute of Environmental Science and Research, New Zealand (and now based in Queensland). Her research interests include development of molecular virology methods for clinical, environmental and food applications, molecular epidemiology of noroviruses and occurrence of human enteric viruses in shellfish, foods and the environment.

Campylobacter survival through poultry processing

Lesley Duffy

Food Safety and Stability
CSIRO Animal, Food & Health Sciences
39 Kessels Road, Coopers Plains
Qld 4053, Australia
Tel: +61 7 3214 2055
Fax: +61 7 3214 2062
Email: Lesley.Duffy@csiro.au

Australia has recorded around 100 cases of campylobacteriosis per 100,000 population, each year, since the mid-1990's. *Campylobacter jejuni* and *C. coli* are recognized as the main species isolated from clinical cases. Approximately 30% of cases have been linked to poultry. Through poultry processing, from slaughter to packaging, the prevalence and concentration of *Campylobacter* can be reduced. Published Australian data on the effect of current processing conditions are minimal. Data from other countries suggests that the stages of scalding and immersion chilling can have significant impact on the prevalence and concentration of *Campylobacter*. Understanding the complexities of these processing stages (physical, chemical and microbiological) and their effect on *Campylobacter* species may lead to improved control during processing and hence improved public health outcomes.

Campylobacter spp. are the leading cause of bacterial gastroenteritis in Australia and most of the western world. While most cases are sporadic in nature rather than outbreak related, poultry has been associated with 30% of all cases in Australia[1]. Poultry are the natural host of this organism with *C. jejuni* and *C. coli* considered the predominant species. Flocks can become contaminated from as early as 14 to 21 days of age[2]. Once *Campylobacter* enters a flock during the rearing period, it spreads rapidly such that flocks can be contaminated at high levels at slaughter, dependent on age[3]. Poultry are slaughtered and prepared for sale through a multistage process (Figure 1). This process can be described in stages: 1. stunning, either electrical or gas; 2. bleeding, severing of the carotid artery and jugular vein; 3. scalding, at temperatures from 53°C to 58°C for approximately two to three minutes to loosen feathers; 4. defeathering, removal of feathers; 5. evisceration, removal of the viscera; 6. washing, both inside and outside of the chicken carcase to remove gross organic contamination; 7. chilling, water immersion from 30 min to 3 h or air chilling from 60 to 80 min, to drop the temperature of the carcase and 8. packaging or further processing. *Campylobacter* can survive each of these processing steps and subsequent storage through to retail and food preparation for poultry to be a source of human infection. Although there is no specific processing step that will kill *Campylobacter* spp., good control of both scalding and chilling can significantly reduce the concentration of *Campylobacter* spp.[4]. Studies have been published in a number of countries that examine the change in prevalence and on the concentration of *Campylobacter* spp. at the various processing stages. A reduction in the concentration of *Campylobacter* spp. by 2 $\log_{10}$ can lead to a reduction in the number of human cases by up to 30 times[5].

A systematic review of the prevalence of *Campylobacter* through poultry processing was published by Guerin[4]. This review of 29 separate published studies covering different stages of the process, highlights the highly variable nature of the effects of various poultry processing stages. Scalding decreased the prevalence of *Campylobacter* anywhere between 20 and 40% while defeathering increased the prevalence between 10 and 72% from four studies. A decrease in prevalence of between 10 and 100% was found after chilling in 6 of the 9 studies which examined this stage, while there was an increase in prevalence after chilling up to 27% in the other three studies. The process of immersion chilling has been demonstrated to lead to cross contamination events which may in part explain an increase in prevalence after chilling. A recent Australian study of four flocks found no decrease in prevalence from pre-scald to pre-chill and reductions in prevalence within two flocks after chill of 10 and 20%[6]. More important than prevalence alone, knowledge on the effect on the concentration of *Campylobacter* at each processing stage is more limited although both scalding and chilling stages are frequently reported to result in a decrease in concentration of *Campylobacter*[4]. Scalding temperature affects the extent of reduction in *Campylobacter* spp. concentration as does the equipment with counter-flow multi stage scalding tanks decreasing the level of contamination. In countries where chlorination of the chilling water is allowed, significant reductions can be made with improved control of chlorine and pH levels within the chilling tanks. The

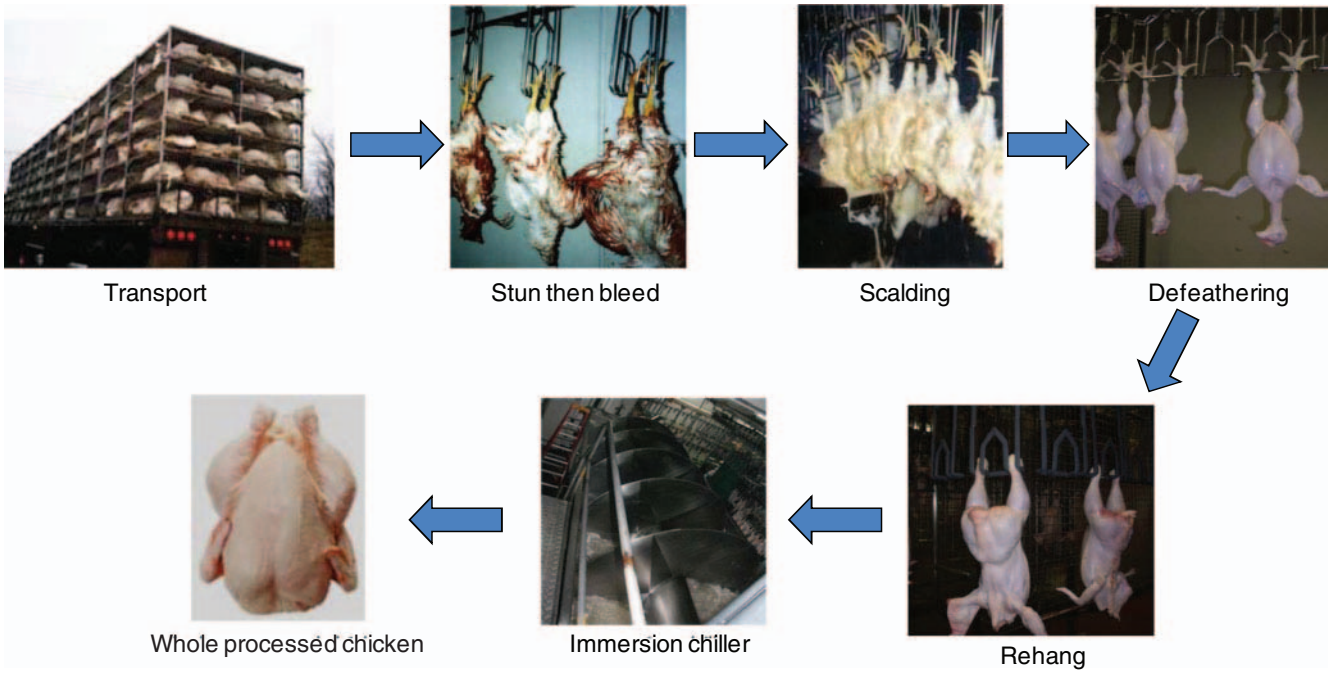

Figure 1. Schematic diagram of poultry processing stages.

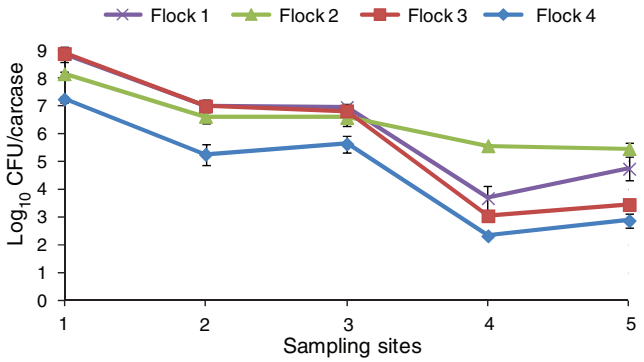

Figure 2. Concentration (log_{10} CFU/carcase) at each sampling site for four flocks. Sampling sites 1. Before scald; 2. After scald; 3. Before chilling; 4. After chilling; 5. After packaging. Flock 1 and 3 were processed at Abattoir A and flock 2 and 4 were processed at abattoir B.

decrease in the concentration of *Campylobacter* within New Zealand processed chickens has in part been attributed to the better control of these parameters in processing[7]. Chlorine dissolves in water to form hypochlorous acid and hypochlorite ion[8]. Hypochlorous acid is the most biocidal form although the formation of these two compounds is pH dependent. The acid form is very reactive being both an oxidizing and halogenating species and therefore the level of free available chlorine in conjunction with pH and contact time will determine the effectiveness of chlorine as a disinfectant on poultry[8].

An Australian study measured the concentration of *Campylobacter* spp. at each stage during processing[6] (Figure 2). Significant reductions were achieved after scalding and again after chilling. No significant changes in concentration were noted after evisceration or after packaging. A few studies have examined the effect of scalding temperatures and chlorine levels under laboratory conditions on the decimal reduction times (D values). A single strain of *Campylobacter* had D_{55C} values in scald tank water of 0.2 min for planktonic grown cells compared with 2.2 min for cells attached to chicken skin. Sub-populations were noted that had increased D_{55C} values of 13.9 min in water and 19.4 min attached to skin. These sub-populations may indicate a level of resistance within the *Campylobacter* population. When the same strain was subjected to chlorine at 50ppm, D_{50ppm} values were recorded of 0.5 min in water compared to 73.0 min when attached to chicken skin with no sub-population detected. New Zealand *Campylobacter* isolates from poultry do not have unusual heat resistance and have similar heat resistance in the planktonic state as those belonging to the sub-populations mentioned above (D_{55C} 8.5 – 17.0 min)[9]. No heat or chlorine resistance data are available on Australian isolates.

The factors that influence the effectiveness of the immersion chiller in the Australian situation where chlorine is a permissible processing aid, are numerous and complex. Examining chickens from two separate flocks, processed at the same abattoir with the same measured pH and chlorine levels in the immersion chiller does not always produce a similar decrease in the concentration of *Campylobacter*[6]. Clearly other aspects of poultry production at the chilling stage, both physical and chemical, can have a significant impact on the survival of *Campylobacter*. Consideration must also be given to the strain to strain variation common in *Campylobacter* studies and the extensive variation in the genetic makeup of this organism compared to other enteric bacteria previously noted by Park[10]. The genotypic variation within the *Campylobacter* genus

may allow specific genotypes to occur or be selected for, when encountering environmental stresses[11].

Understanding the changes that *Campylobacter* spp. undergo when subjected to typical processing temperatures and chilling (chlorine and pH) conditions in conjunction with an understanding of how these are applied within the technical aspects of poultry production, may be key to ensuring future declines in both prevalence and concentration of *Campylobacter* spp. on poultry products. This may lead to improved public health outcomes.

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Biography

Lesley Duffy is a Research Microbiologist with CSIRO. Her research projects have included the ecology of *E. coli* O157 and *Salmonella* in red meat production systems including beef, sheep and goat; source tracking of *Listeria* in cooked meat and ready-to-eat food production facilities; and the survival of *E. coli* O157 during the manufacture of fermented meat products. Lesley's current research project examines the ecology of *Campylobacter* during poultry processing and the selection and survival of specific genotypes through this process.

Mycotoxins and food

Nai Tran-Dinh

CSIRO Animal, Food and Health Sciences
Riverside Corporate Park
11 Julius Avenue, North Ryde
NSW 2113, Australia
Tel: +61 2 9490 8473
Email: Nai.Tran-Dinh@csiro.au

Mycotoxins are toxic secondary metabolites produced by filamentous fungi that may occur in almost all food commodities but particularly in cereals, oilseeds and nuts. They are recognised as an unavoidable risk and are found in the world's most important food and feed crops, including maize, wheat, and barley. When present in foods in sufficiently high levels, mycotoxins pose a significant food safety risk and health hazard. Besides negative health impacts, mycotoxin contamination of food and feeds has a major worldwide economic impact. Mycotoxin contamination of foods is the subject of increasing international importance due to a number of worldwide issues, including globalisation of food trade, global food security and climate change. Innovative strategies to meet the menace of mycotoxin contamination are required, and a greater understanding of the ecology of mycotoxigenic fungi and the molecular regulation of mycotoxin production may aide in the development of such strategies.

Mycotoxigenic fungi: occurrence, costs and effects

Fungi are ubiquitous in nature and are a normal part of the microflora of worldwide food supplies. They can colonise food throughout the food chain from preharvest to storage wherever favourable conditions prevail. Some of these fungi are able to produce mycotoxins; the most important mycotoxigenic fungi belong to the genera *Aspergillus, Fusarium* and *Penicillium*. Thousands of mycotoxins have been identified, but only a few are a food safety risk and have an impact on global agriculture[1]. Major mycotoxin classes are aflatoxins, produced by *Aspergillus flavus* and *Aspergillus parasiticus*; fumonisins, produced by *Fusarium verticillioides*; trichothecenes, most importantly deoxynivelanol, produced by *Fusarium graminearum*; and ochratoxins, produced by *Aspergillus ochraceus, Aspergillus carbonarius* and *Penicillium verrucosum*. A wide range of commodities can be contaminated by these mycotoxins (Table 1). Exposure to mycotoxins may cause diverse and powerful toxic effects leading to both acute and chronic disease, ranging from liver and kidney damage, cancer, immunosuppression and childhood stunting (Table 1). These diseases are referred to as mycotoxicoses and the symptoms are dependent upon the type of mycotoxin, the concentration and length of exposure, and characteristics of the individual exposed, such as genetics, age, health and gender. Other factors contributing to disease development include synergies with other diseases and mycotoxin co-contamination food and feed[2]. The main route of exposure to mycotoxins is through ingestion of plant derived contaminated foods, however exposure may also occur through carryover of mycotoxins and their metabolites in animal products such as milk, meat and eggs or through inhalation of air and dust containing toxins[3,4].

The true cost of mycotoxin contamination is difficult to estimate due to the complexity of the issue and its effect throughout the food chain on numerous stakeholders. Obvious costs include health impacts, crop losses and reduced animal productivity. Aflatoxins alone may cause up to 150,000 deaths worldwide per annum from liver cancer, and many more when the synergistic effect of hepatitis B virus is taken into account[5]. Other costs from mycotoxin contamination are incurred through efforts by producers to improve production, storage and handling to minimise the risk of mycotoxin contamination[6]. With over 100 countries having regulations regarding levels of mycotoxins in the food and feed industry[7], there are significant costs associated with monitoring, enforcing and analysing at-risk commodities. There are also social costs associated with the loss of consumer confidence in the safety of food products.

In developed countries, stringent food safety regulations and monitoring ensures low levels of mycotoxin exposure in the population, however this is not the case in developing countries. In developing countries, the lack of infrastructure, the prominence of subsistence farming systems, the lack of irrigation, and inadequate drying and storing facilities results in chronic exposure to mycotoxins in the diet and the risk of serious health problems. The costs of these health problems include mortality and morbidity, as well as the more intangible costs of pain, suffering, anxiety, and reduction in quality of life.

Table 1. Mycotoxins, associated commodities, toxic effects and producing fungal species.

Mycotoxin	Commodities	Toxic effect	Fungal species
Aflatoxins	Peanuts, maize, tree nuts, cottonseed	Hepatotoxicity, cancer, probable immunosuppression and childhood stunting, reduced growth in livestock	*Aspergillus flavus, Aspergillus parasiticus*
Ochratoxin A	Cereal grains grown in cool climates, coffee, cocoa, dried vine fruits, wines	Nephrotoxicity, urinary tract tumours, porcine nephropathy; various symptoms in poultry	*Aspergillus ochraceus, A. carbonarius, Penicillium verrucosum*
Fumonisins	Maize	Equine leukoencephalomalacia, porcine pulmonary edema, possible human oesophageal cancer	*Fusarium verticillioides, Fusarium proliferatum*
Trichothecenes (Deoxynivalenol)	Maize, wheat, barley	Inhibition of protein synthesis, human intestinal upsets, porcine feed refusal	*Fusarium graminearum*

Globalisation of food trade has several potential impacts on mycotoxin contamination in food and feeds. It possibly can extend the impact of mycotoxin contamination in food supplies beyond local communities. Mycotoxin exposure in humans and other animals, previously dictated by local factors such as crop production, climatic conditions and agronomic practices, is now affected by international food trade potentially distributing mycotoxin contaminated crops globally. The use of strict mycotoxin regulations on commodities for importation by developed countries reduce exposure risks in the importing countries, however the complexity and volume of international trade, importing corporations lacking accountability, greater opportunities for intentional fraud and the lack of enforcement tools, mean that there remains a potential mycotoxin food safety risk[8]. These strict mycotoxin regulations also have a significant economic impact on developing countries[9]. For example, a World Bank study estimated that the European Union regulations on aflatoxins cost Africa $750 million each year in exports of cereals, dried fruit and nuts[10]. Strict international mycotoxin regulations may also inadvertently result in higher exposure in developing countries because only the best quality foods are exported, leaving poorer quality, mycotoxin contaminated commodities for local consumption[11].

Mycotoxin production on a food commodity is greatly influenced by environmental factors, most importantly temperature, relative humidity, insect attack, and stress conditions of the crop[12]. Global climate change with warmer temperatures and more extreme rainfall and drought events are likely to increase the threat of mycotoxins to human health[12–14]. Climate change effects on fungal colonisation and mycotoxin production should be assessed on a case-by-case basis, as optimum temperature and relative humidity for growth and mycotoxin formation vary between fungi[12]. In general, however, warmer temperatures with greater extremes in rainfall and drought events will increase plant stress, predisposing crops to fungal infection and mycotoxin contamination. Additionally, warmer temperatures may increase insect activity facilitating the establishment of mycotoxigenic fungi, through altered insect population growth rates, increased insect voltinism, altered crop-pest synchrony, and altered geographical ranges of important pest species[14].

The costs of mycotoxin contamination of food commodities will significantly hamper the world's ability to address the challenge of global food security. As defined by the World Health Organization, global food security exists "when all people at all times have access to sufficient, safe, nutritious, food to maintain a healthy and active life"[15]. Clearly, mycotoxin contamination of food commodities will affect the provision of safe and nutritious foods. The Food and Agricultural Organization (FAO) estimates that 25% of the world's food crops are significantly contaminated with mycotoxins and that in the range of 1 billion tonnes of food is lost worldwide due to mycotoxins[16].

Future considerations

Mycotoxin contamination of food and feeds remains a food safety risk of worldwide significance that has major economic impacts in both developed and developing countries. Various approaches have been put forward to reduce the impact of mycotoxins in food, and have had varying degrees of success: biocontrol by competitive exclusion, plant breeding and genetics, improved agricultural practices, increased irrigation, improved sorting, drying and storage

techniques, dietary interventions including specific clays and antioxidants and, specifically for aflatoxin, immunisation against hepatitis B[6]. A greater understanding of the interactions between mycotoxigenic fungi and their host plants, and the use of genomic and transcriptomic information may assist in improving existing intervention strategies and may also lead to other interventions. Whole-genome sequences are available for *A. flavus*, *F. verticillioides* and *F. graminearum*[17–19], and research is at a point that allows an examination of the similarities and differences in molecular mechanisms that regulate mycotoxin biosynthesis[20]. These whole-genome sequences have provided reference databases for genomic, transcriptomic and proteomic analyses that have revealed complex transcriptional and epigenetic regulation of mycotoxin biosynthesis.

It is important that strategies aimed at reducing the risk of mycotoxin contamination of human food and animal feed be implemented, especially in the light of global issues such as international food trade, food security and climate change.

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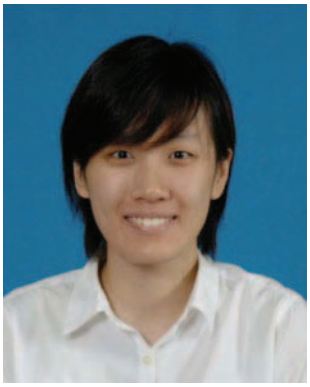
Biography

Nai Tran-Dinh joined CSIRO in 2002 as a postdoctoral fellow and is currently a project leader with research interests in mycotoxigenic moulds. He has expertise in microbiology and molecular biology, and has applied these skills in a variety of food safety/spoilage research areas. He has worked on and led projects investigating the physiology, ecology, taxonomy, biochemistry, mycotoxigenic potential and understanding relationships between strains of fungi from the agriculturally important fungal genera of *Aspergillus*, *Fusarium* and *Alternaria*. He has worked extensively in the area of *Aspergillus flavus/parasiticus* infection and aflatoxin contamination in peanuts and other crops. This work has included investigating infection cycles, fungal population surveys from crops and soils, mycotoxin production, biological control of aflatoxin contamination and differentiation and phylogeny of strains using molecular markers.

Antimicrobial resistance in food associated *Salmonella*




Boey Y Cheng, Amy HT Teh, Patric TC Chua and Gary A Dykes

School of Science, Monash University, Jalan Lagoon Selatan, 46150 Bandar Sunway, Selangor, Malaysia
Tel: +60 3 5514 4964, Fax: +60 3 5514 6364, Email: gary.dykes@monash.edu

***Salmonella enterica* is one of the leading causes of foodborne disease worldwide. Infection with *Salmonella* results in symptoms ranging from mild gastroenteritis through to severe complications such as septicemia and even death. These infections place a significant financial and health burden on the economies of both developed and developing countries. The emergence of antibiotic resistance in bacteria is of current international concern and has added an extra dimension to the issue of foodborne salmonellosis. Understanding and controlling the spread of antibiotic resistance among foodborne *Salmonella* is a goal that requires a global approach but one that needs to be tailored to local scenarios.**

Food associated *Salmonella* strains which are resistant to multiple antibiotics are widely distributed and their prevalence has been increasing[1]. Of particular concern is the emergence of resistant strains which are also particularly virulent and appear to persist well throughout the food supply chain. The *S.* Typhimurium DT104 strain is one such strain of concern and which was first isolated in the 1990's. This strain is characterised by chromosomally encoded resistance to ampicillin, chloramphenicol, sulphonamides, streptomycin and tetracycline. Many of these antibiotics are used therapeutically to treat salmonellosis and the resistance of this strain may enhance its morbidity and mortality. In addition many other infections are also treated with these antibiotics and it is feared that this strain may act as a reservoir for the transfer of antibiotic resistance genes to other bacteria. This strain is also reportedly more virulent than many other strains, although this claim is contentious[1]. The *S.* Typhimurium DT104 strain is widely spread and has been isolated from the food supply chain in countries across the globe. Human infections with this strain have been associated with the consumption of a range of foods including chicken, beef, pork and unpasteurised cheese[2]. Understanding how strains such as these emerge, and preventing this from happening, is an important public health goal.

The presence of antibiotic resistant strains in the food supply chain is widely suggested to be due to the selective pressure imposed on bacteria by the frequent use of antibiotics as therapeutics, prophylatics or for growth promotion in farm animals. An association with the use of antibiotics in human medicine is also likely to play a role in resistance among foodborne bacteria[3]. Different countries have different approaches to the regulation and enforcement of the use of antibiotics in animals and humans. These differing approaches lead to a variety of local scenarios that may influence the prevalence of antibiotic resistant foodborne pathogens, such as *Salmonella*, on food. With an increasingly globalised food supply, however, the presence of high levels of antibiotic resistant bacteria on food in one region is likely to be felt in another. In developing countries the issue of antibiotic resistant bacteria in food is often complicated by a lack of general hygienic practice throughout the food supply chain. In particular high levels of contamination of food with bacteria from various sources (including wild animals and humans) confound

attempts to establish a link between on-farm practice and antibiotic resistant bacteria in food.

Surveys of the prevalence of antibiotic resistant *Salmonella* in foods of animal origin in developed and developing countries illustrate this issue. Retail surveys indicate that the prevalence of *Salmonella* in developed countries range from 0-10% on red meat[4–7] and from 12–20% on poultry[2,8]. In these same surveys *Salmonella* strains resistant to two or more antibiotics made up between 0.6–48% of the isolates from red meat and 50–100% of the isolates from poultry. By contrast, retail surveys of the prevalence of *Salmonella* in developing countries range from 17–64% on red meat[9–11] and 54–57% in poultry[8–11]. In these same surveys *Salmonella* strains resistant to two or more antibiotics made up between 6–70% of the isolates from red meat and 57–70% of the isolates from poultry. A similar scenario for *Salmonella* from foods of plant origin is likely although there is a lack of data in this area, particularly from developing countries. These data effectively demonstrate the key differences between developed and developing countries with respect to *Salmonella*. In developing countries at retail there is an overall higher prevalence of *Salmonella* on muscle-based foods but this prevalence is not necessarily correlated with a higher prevalence of resistance to multiple antibiotics than in developed countries. In order to understand the scenario in developing countries an approach which entails identifying as many sources of *Salmonella* as possible which result in contamination of food is required. This will aid in establishing the potential contribution of these sources to both the prevalence of antibiotic resistant strains and the diversity of antibiotics to which they are resistant. This would include assessing contributions both from direct sources, such as farm animals and humans, as well as from other potential sources, such as cross-contamination and vectors including insects and wild animals. In developed countries the lower *Salmonella* prevalence and better levels of hygiene and traceability mean that a more direct approach is possible.

In summary, reducing antibiotic resistance in food associated *Salmonella* is an important global public health goal. Approaches to understanding and controlling the prevalence of antibiotic resistant strains in developed and developing countries must necessarily be different. In particular, in developing countries a focus on promoting general food hygiene and establishing the relative contribution of all sources of antibiotic resistant bacteria, rather than making assumptions based on the situation in developed countries, is required.

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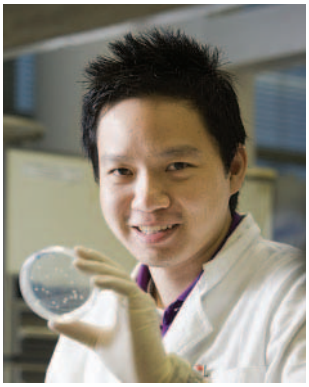
Biographies

Boey Cheng, **Amy Teh** and **Patric Chua** hold BSc (Hons) degrees and are currently research assistants in the School of Science at Monash University's Sunway Campus in Malaysia. All have a strong interest in food microbiology and plan to pursue their PhD studies in the area in the near future. **Gary Dykes** holds a PhD and is currently Professor and Discipline Head for Food Science and Technology in the School of Science at Monash University's Sunway Campus in Malaysia. He has a strong research interest in food microbiology with a focus on adhesion, survival and persistence of foodborne pathogens.

Listeria monocytogenes: illuminating adaptation with proteomics






John P Bowman, Rolf E Nilsson, Chawalit Kocharunchitt and Tom Ross

Food Safety Centre, Tasmanian Institute of Agriculture, University of Tasmania, College Road, Sandy Bay, Tas 7005, Australia
Tel: +61 3 6226 6380, Fax: +61 3 6226 2642, Email: john.bowman@utas.edu.au

With increased consumption of minimally processed ready-to-eat foods the potential for exposure to *Listeria monocytogenes* has increased. Thus, there is a need to maintain a balance between food convenience and safety. *L. monocytogenes* is not a homogenous species; certain strains are more resilient to stressful conditions while others are potentially more virulent. To understand the basis of these differences we are applying proteomics to determine the molecular mechanism of adaptations of *L. monocytogenes* in food-relevant environments. The goal is to define how this species grows, behaves and survives thus allowing us to fine tune food safety risk management, especially when developing new minimal food processes or considering introduction of unpasteurised food such as raw milk cheeses.

Listeria monocytogenes causes listeriosis, a serious disease primarily acquired by food consumption, and that mainly impacts immunocompromised people, the elderly, and neonates. Listeriosis also occurs in livestock and was originally discovered in animals. Though the prevalence of listeriosis in Australia is relatively low it remains a major concern for the food industry. A case in point is the January 2013 brie and camembert cheese-associated outbreak that occurred in south-east Australia, associated with 3 deaths and one miscarriage. *L. monocytogenes* mainly occurs as an environmental contaminant and can enter food anywhere along the industrial food supply chain including within domestic settings. Despite being relatively nutritionally fastidious this tendency to be a frequent contaminant is due to the species inherent hardiness since it is low water activity and acid tolerant and able to grow at refrigeration temperatures. Fortunately it is readily eliminated by standard pasteurisation or by cooking so that the threat is limited to certain ready-to-eat foods, typically those with long refrigerated shelf-lives.

L. monocytogenes mainly causes disease by invading gastrointestinal epithelial cells by encouraging endocytosis[1]. Once inside host cells other proteins aid intracellular survival, mobility, and cell-to-cell spread. *L. monocytogenes* is adept at evading and surviving within cellular and humoral immune systems. A fascinating aspect of *L. monocytogenes* is its ability to shift from an environmental saprophytic state where virulence genes are turned off to a parasitic state within animal or human hosts[2]. At 37°C, if carbohydrate levels are low, many virulence genes are activated. This switch is mainly controlled by temperature sensitive small RNAs[3]. The ability to respond to stress is also intertwined in this transitive process. The many sequenced genomes of *L. monocytogenes* are rich in transcriptional regulators controlled in overarching regulons by various "master" regulators, which functionally overlap in a complex network. This network allows *L. monocytogenes* to rapidly respond to changing environments, including switching on and off stress defence and virulence genes[4].

Using comprehensive proteomics we are attempting to understand more holistically the mechanistic basis of *L. monocytogenes*' adaptation to different situations. State-of the art proteomics is now a very powerful tool and is becoming more cost-effective. Employing gel-free and label-free liquid chromatography (operated in either one or two dimensional modes) and sensitive, high resolution ion trap mass spectrometry, it is possible to take complex protein mixtures digested by a peptidase such as trypsin and identify and quantify peptides en-masse after bioinformatic comparison to proteome databases. This is possible due to better separation of individual

peptides and highly accurate mass estimations to error levels of less than 1 part per 2 million. By counting each individual peptide spectrum (a spectral count) one can estimate the protein abundance of most proteins detectable within the proteome of a bacteria. This has major cost and labour advantages over gel-based proteomics. For *L. monocytogenes* which encodes some 2900–3000 proteins such an approach is very efficient with moderate depth peptide surveys able to detect >40% of its proteome. Though some limitations occur with this approach, such as accurately determining the abundance of inefficiently extracted proteins (e.g. proteins with several transmembrane helical domains) it is still readily possible to generate a large amount of data that can be used comparatively to

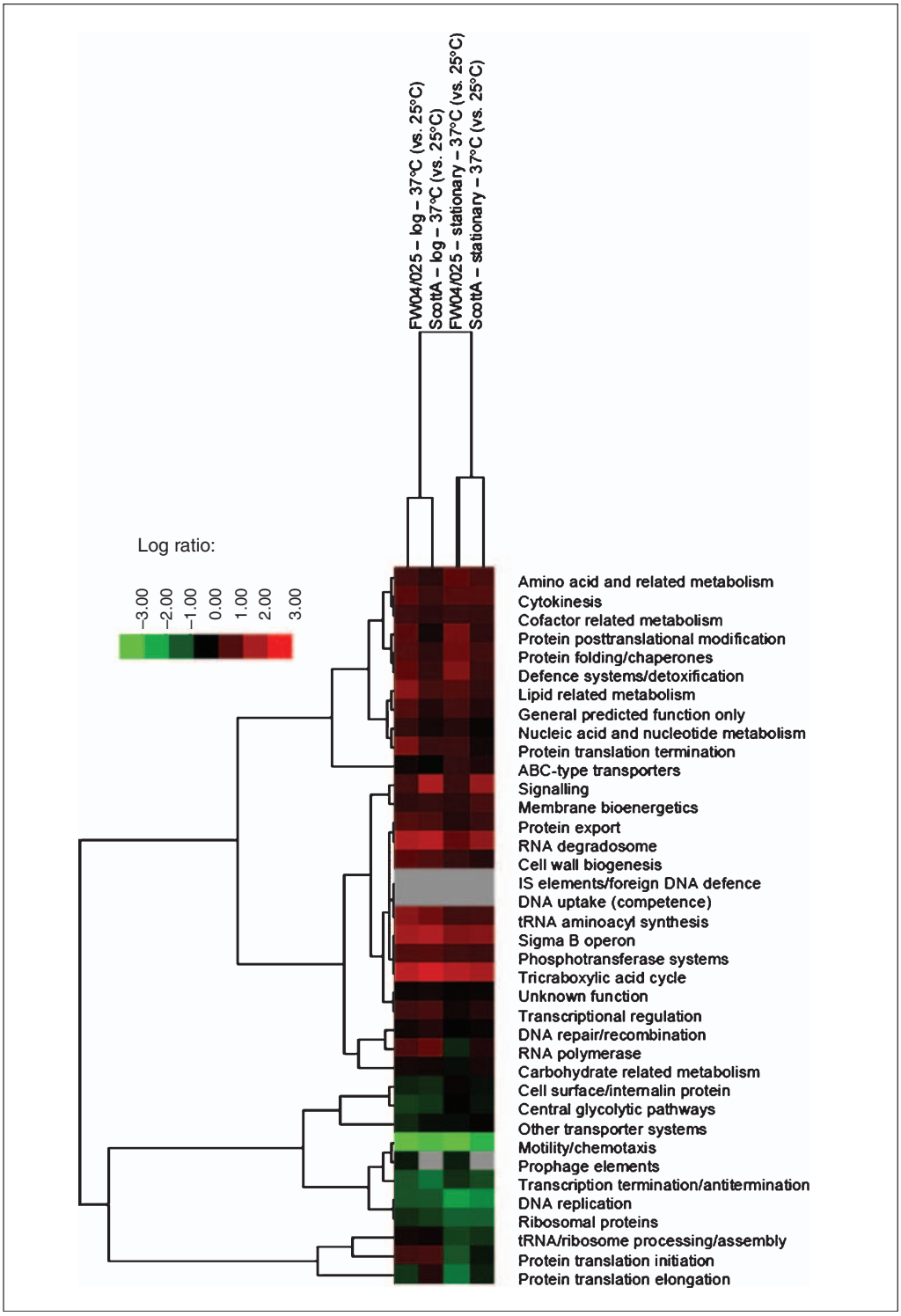


Figure 1. Heat map showing comparative abundance ratios of proteins grouped in functional classes for two genome sequenced *L. monocytogenes* strains grown at 37°C and 25°C. The strains FW04/0025 (serotype 1/2a, food isolate) and ScottA (serotype 4b, clinical strain) were grown in brain heart infusion broth and proteins were extracted during exponential (log) and stationary growth phases with ~1200 proteins for each strain identified via LC/tandem MS/MS. Functional groups are based on gene ontology and calculated from accumulated spectral counts. Groups shown in grey did not have enough spectral counts for a calculated log ratio.

"dissect" specific genomic functions and phenotypes. Details on the typical LC/MS methodology used has been reviewed[5]. A number of software- and statistical approaches for assessing protein abundance via spectral counting have been devised that have improved validation of sample comparisons[6,7] and also have improved absolute protein quantitation in highly complex samples[8].

We have used gel-free proteomics to investigate a variety of stresses and phenotypes of *L. monocytogenes* and *E. coli* O157:H7 strains, including water activity, cold, acid and alkaline stresses all relevant to either food or the food processing environment[9–11]. Essentially any prokaryote or eukaryote for which a proteome is available could be studied in a similar fashion across a myriad of scenarios as long as the proteins can be obtained in sufficient quantities. As mentioned above *L. monocytogenes* pathobiology is strongly controlled by temperature. As an example, we compared the proteomes of genetically different strains at two different temperature, 25°C and 37°C. Cells at these temperatures behave quite differently. At 25°C cells are actively swimming via peritrichous flagella while at 37°C cells lack flagella, become hydrophobic and better attach to surfaces. As can be seen in the accompanying heat map (Figure 1) when the proteins (covering 40-42% of the strain proteomes) are organised on the basis of their essential cellular function large differences between the temperatures occur, above and beyond growth phase- and strain-dependent changes. One of the obvious hallmarks of the temperature effect at 37°C is the suppression of motility and chemotaxis proteins with the most suppressed protein at 37°C being flagellin (reduced >300-fold). The most induced protein (40-60 fold) in both strains at 37°C is an OsmC family protein similar to the organic hydroperoxide resistance protein OhrA of *Bacillus subtilis*. Organic peroxides are toxic metabolites that accumulate during metabolism[12] thus it makes sense that at a more rapid rate of metabolism enhancement of peroxide detoxification is needed. Several other stress response proteins are also enhanced at 37°C including glutamate decarboxylases required for survival against acid shocks, such as gastric passage, as well as superoxide dismutase, which protects against reactive oxygen radicals. Such protein changes likely also contribute to gastrointestinal, intracellular and external environmental survival. Hundreds of other protein changes also occur, including that of many involving uncharacterised proteins. Individual protein abundances within a given proteome differ by 4-5 orders of magnitude and, thus, defining and especially *interpreting* proteomic-level responses consistent at the species level as well as between strains represent substantial challenges to overcome.

In summary, proteomics is rapidly emerging as an accessible approach that can capture large amounts of functionally relevant proteomic, and by inference genomic information. The range of applications within microbiology itself is enormous in terms of understanding bacterial behaviour, physiology and pathogenesis.

Acknowledgements

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Biographies

Tom Ross is the Associate Professor in Food Microbiology and Graduate Research Coordinator at the Food Safety Centre, Tasmanian Institute of Agriculture at the University of Tasmania.

John Bowman is the Associate Professor in Microbiology at the Food Safety Centre, Tasmanian Institute of Agriculture at the University of Tasmania.

Chawalit Kocharunchitt is a postdoctoral research associate at the Food Safety Centre, Tasmanian Institute of Agriculture at the University of Tasmania.

Rolf Nilsson is a postdoctoral research associate at the Food Safety Centre, Tasmanian Institute of Agriculture at the University of Tasmania.

Baseline studies for pathogens in meat

Ian Jenson

Meat & Livestock Australia
Locked Bag 991, North Sydney
NSW 2059, Australia
Tel: +61 2 9463 9264
Fax: +61 2 9463 9182
Email: ijenson@mla.com.au

Baseline microbiological studies, seeking to take a "snapshot" of the quality or safety of product produced across the nation at a point in time, are a valuable adjunct to other sources of information on quality or safety of foods. They have been used by the Australian red meat industry as a point of reference, to promote trade, and as a starting point for further research.

Australia is amongst the world's largest exporters of beef and sheep meat, exporting more than half of production with the total industry valued at over $10 billion[1]. The Australian red meat industry has conducted four baseline surveys (beef and sheep meat) since 1995 through Meat & Livestock Australia, the industry's research and development corporation. They are called a 'baseline' because they provide a baseline, or benchmark, against which the performance of individual processors, or the quality of individual samples may be judged. Baseline studies became prevalent in the USA in the 1990s, around the time of introduction of the pathogen reduction and Hazard Analysis Critical Control Point (HACCP) rules by the US Department of Agriculture[2].

The industry conducted the last of four baselines of beef and sheep meat in 2011 resulting in peer reviewed publications[3,4] as well as a comprehensive report for the industry and customers[5]. Publication provides all stakeholders with access to information that can be used for various purposes. It is not the purpose of this article to attempt to summarise the results, but it is worth noting that frozen boneless beef had a mean bacterial count of 166 cfu/g and frozen boneless sheep meat had a mean bacterial count of 631 cfu/g. These are extraordinarily good results for a raw agricultural product and suggest a high degree of safety considering that meat is intended to be cooked prior to consumption.

Having data that can be compared to those of major trading partners is one very good reason for conducting baseline studies. All over the world (including Australia) there is a continuing suspicion that imported food products are inferior to domestic product, so providing a comprehensive data set can allay those fears. In effect, a well-conducted baseline study provides validation that the entire system (for example, sourcing of animals, processing methods, quality assurance and regulatory oversight) is resulting in microbiological quality (and therefore, safety) that is acceptable to the importing country. These data should be accepted as a basis for determining that World Trade Organisation rules on technical barriers to trade are not being breached.

However, there is great difficulty in conducting a survey that is comparable to other surveys due to differences in design, sampling and testing methods. The approaches taken in these surveys have attempted to align with international approaches, but there is not always a consensus. There has been a high degree of consistency taken through the four Australian surveys but, for various reasons, there has been a change in the microorganisms examined, and in the most recent surveys, there has been a change in the products sampled.

Baseline studies provide an opportunity to examine meat for microorganisms not routinely examined. Routine testing includes Total Viable Count (same as Standard Plate Count, except that incubation is performed at 25°C for 4 days), *E. coli* and *Salmonella*. Baselines have provided the opportunity to survey for the prevalence and concentration of microbes that may be significant to food safety (such as *Clostridium perfringens*, *Staphylococcus aureus*) or are potential issues in trade (*Listeria monocytogenes*).

The type of sample collected has shifted in the most recent survey towards packed product. Previous surveys had always tested chilled carcases; which is only part way through the process (carcases are subsequently cut into smaller pieces, or primals), but of great interested to veterinarians, and of importance in quality control. Reliable data for carcases are now available for export establishments through the Department of Agriculture Fisheries and Forestry, which reduces the need to collect these data in a baseline survey. In the last survey, for the first time, primals were sampled, which represent the product that is exported, or often shipped to retailers in Australia, for final cutting.

Baseline studies of Australian red meat have demonstrated that Australian beef and sheep meat has a high level of microbiological

quality and therefore, safety. There is a trend towards setting regulatory limits for microorganisms in food, especially for meat, and these studies provide valuable data for assessing risks, considering how control should be exerted, and how industry and regulators should use microbiological criteria to control processes[6].

Baselines have provided data sets that provide answers in other projects or suggest further research and development activities. The relationship between the counts of various indicators has been evaluated[7]. The 2004 survey data were used as the basis for investigating differences in processing factors that contributed to high or low microbial counts on beef carcases[8], and a process assessment tool was developed to assist processors who wished to improve their process hygiene[9]. The surveys for *Campylobacter* and *Cl. perfringens* in the 2004 survey were essential in assessing foodborne disease risks from beef and sheep meat products. The 2011 survey found that some indicators had deteriorated compared to the previous survey and investigations strongly suggested an association with rainfall – el Niño v. la Niña, which needs to be further investigated[5].

Despite the complexity, and the expense, of conducting periodic baseline studies, the collection of data from well-designed surveys, provides tremendous value to trade, avoids complacency and provides an ability to respond to issues that may arise.

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Biography

Ian Jenson holds a B.Sc. in Microbiology and Biotechnology and a M.Sc. in Bioprocess Engineering, both from University of New South Wales. He is a Fellow of the Australian Society for Microbiology and a Professional Member of the Australian Institute of Food Science and Technology. Ian has worked as a microbiologist and food safety specialist in the food and fermentation industries throughout his career, working for Australian companies with international operations and markets. He has experience in fermented dairy products, beverages and bakery products, as well as quality management, including systems, standards and process control. He has published widely in risk assessment, meat microbiological quality and risk management, as well as actively participating in international food safety activities.

Foodborne pathogenic *E. coli* (focus on STEC)

Robert S Barlow and Glen E Mellor

Food Safety and Stability
CSIRO Animal, Food and Health Sciences
PO Box 745, Archerfield BC, Qld 4108, Australia
Tel: +61 7 3214 2035
Fax: +61 7 3214 2050
Email: robert.barlow@csiro.au

Foodborne pathogenic *E. coli* continue to emerge and evolve as significant human pathogens. With cattle and other ruminants acting as natural reservoirs, they contaminate food directly via contamination of animals at slaughter or indirectly via the use of contaminated manure or water during food production. *E. coli* O157 remains the predominant disease causing serotype although additional serotypes such as O26 and O111, along with *E. coli* possessing novel combinations of virulence genes, highlight the increasing complexity associated with reducing the prevalence of foodborne pathogenic *E. coli*. Variability in the severity of disease caused by different *E. coli* provides insight into the significance of virulence factors thereby enabling the design of possible control methods such as vaccines. The continuing burden of foodborne pathogenic *E. coli* presents a challenge for food producers and researchers to overcome to ensure an ongoing supply of safe and healthy food.

What is STEC?

Escherichia coli is a member of the gut microbiome of the majority of warm blooded animals, including humans. *E. coli* generally exist harmlessly within the gut performing physiological activities that benefit themselves, their host, and the associated microbiome. However, *E. coli* is perhaps most well known for its ability to cause disease in humans. Shiga toxin-producing *E. coli* (STEC) were first identified as a foodborne pathogen in 1982 and are characterised by the ability to produce Shiga toxin[1]. STEC are an important public health concern as they have caused large foodborne outbreaks, are particularly dangerous to small children where acute renal failure is often observed, which can sometimes lead to death.

How does *E. coli* become pathogenic?

Enterohaemorrhagic *E. coli* (EHEC) are a subset of STEC that have acquired additional virulence traits, such as the locus of enterocyte effacement (LEE), that enhances the capacity to attach to intestinal cells and subsequently cause disease. Conversely there are many STEC that are considered non-pathogenic to humans because they lack specific additional virulence traits[2]. Even within the disease causing STEC or EHEC variability in virulence between different serotypes may be attributed to specific virulence factors encoded on large, horizontally acquired gene cassettes[3]. Furthermore, differences in the types of Shiga toxins present and the point at which the Shiga toxin-bearing phage insert in the chromosome have also been associated with isolates that cause varying disease symptoms in humans[4].

How does food become contaminated with pathogenic *E. coli*?

Cattle and other ruminants (Figure 1) are able to harbor and shed populations of STEC and EHEC often without deleterious effects to the animal's health. Transmission of pathogenic *E. coli* to foods can occur directly, particularly when beef or dairy cattle are slaughtered and the exterior surface of the carcase becomes contaminated, or indirectly through the use of contaminated cattle manure or water during horticulture production. The contamination of beef carcases is an issue when ground beef is produced. In this scenario, contamination that is on the surface of the meat is mixed and distributed throughout the product and thorough cooking to the centre of the product then becomes the only point of control. Similarly, contamination of horticulture products is a risk to human health as pathogenic *E. coli* can adhere to the surface of these

Figure 1. Sources of pathogenic *E. coli.*

products and can be extremely difficult to remove. Moreover, many horticulture products are consumed raw and consequently emphasis must be placed on ensuring contamination events during production are eliminated. There are many other ways in which food can become contaminated with pathogenic *E. coli* (Figure 2) and producers should therefore be aware of the inputs to their production system and, where possible, implement strategies to reduce the risk of contamination.

Outbreaks – changing paradigm?

Since the first description of STEC in 1982 *E. coli* O157 has been the most commonly implicated serotype in disease outbreaks. Consequently, STEC-related research initially progressed with absolute focus on the O157 serotype to the exclusion of most other serotypes. Between 1982 and 2002 the USA recorded 350 outbreaks of *E. coli* O157 resulting in 8,598 cases[5]. While in Australia sporadic cases of *E. coli* O157 infection are more likely to occur than outbreaks, there were at least four outbreaks attributed to the serotype between 2001 and 2009[6]. Analysis of the USA outbreaks indicated that food remains the predominant transmission route accounting for 52% of outbreaks with ground beef and produce accounting for 41% and 21% of foodborne outbreaks, respectively[5]. Similar trends have been observed in Europe, Japan and South America.

Despite *E. coli* O157 remaining the dominant serotype implicated in sporadic cases and outbreaks of STEC-associated disease, it has become apparent over the last 10-15 years that a group of additional serotypes of STEC are responsible for a much greater proportion of disease than originally estimated. Australian STEC outbreak data from 2001 to 2009 determined that seven of the 11 outbreaks that occurred during that time were attributable to serotypes other than O157[6]. STEC notifications in Australia between 2001 and 2008 revealed that non-O157 serotypes were substantial contributors to the overall incidence of STEC-associated infections observed in the Australian health system (Figure 3[7]). In the USA, STEC surveillance data analysis determined that the ratio of non-O157 to O157 infections is approaching two to one with 63,000 *E. coli* O157 and 110,000 non-O157 infections estimated to occur annually[8]. Although the occurrence of serious diseases and death remains more common with *E. coli* O157 infections than with non-O157, the burden of non-O157 infections on health systems is evident. As surveillance and methods for non-O157 isolation improve we can expect greater implication of non-O157 STEC in outbreaks and sporadic cases of human disease.

In addition to the materialisation of non-O157 STEC serotypes as major human pathogens, 2011 saw the emergence of a novel pathotype of *E. coli* that was ultimately responsible for the most devastating *E. coli* outbreak to date. The three month long outbreak was caused by a hybrid *E. coli* of serotype O104:H4 that harbored virulence traits common to STEC as well as enteroaggregative *E. coli*. A total of 4321 cases were recorded with 852 cases

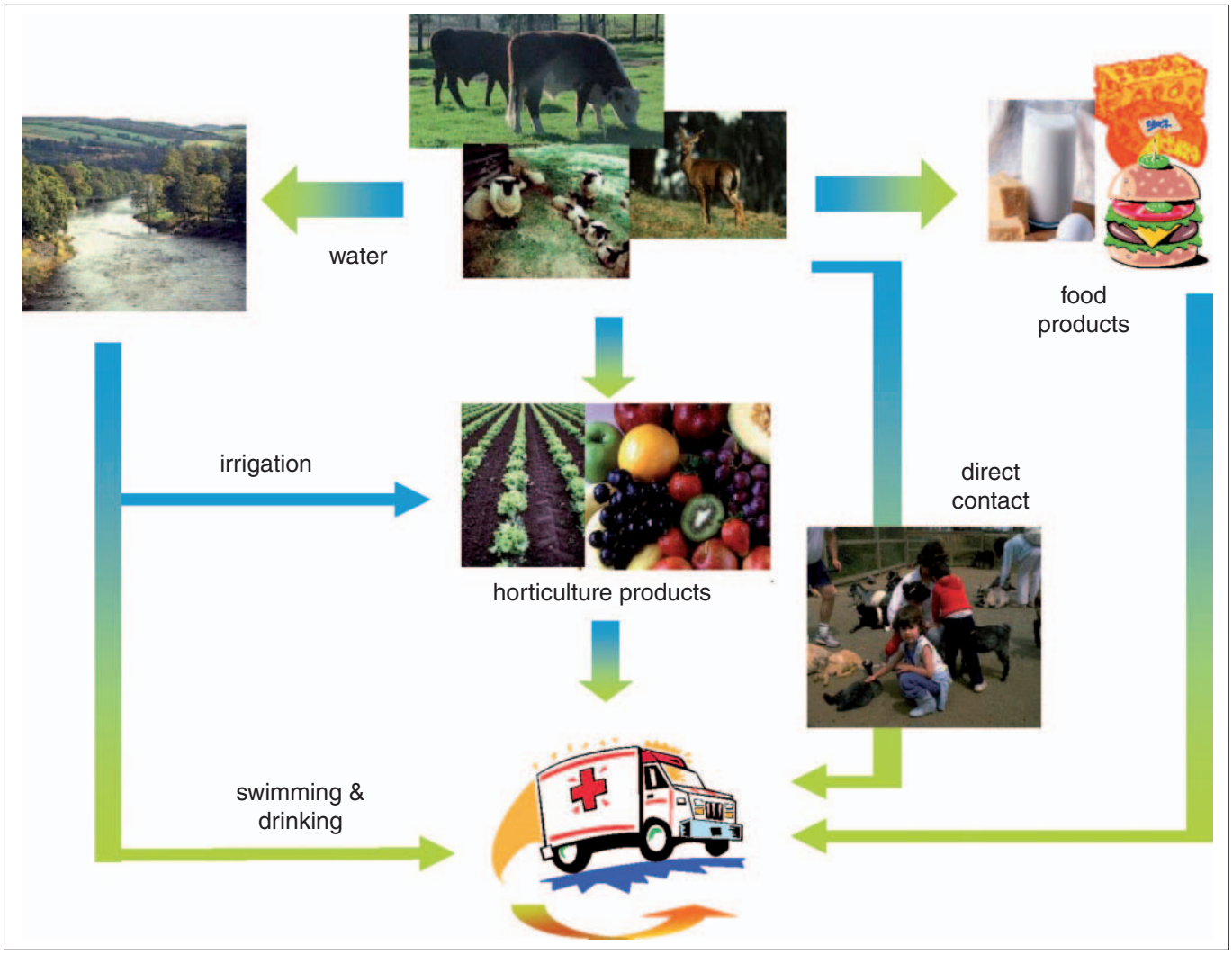

Figure 2. Contamination pathways for pathogenic *E. coli*.

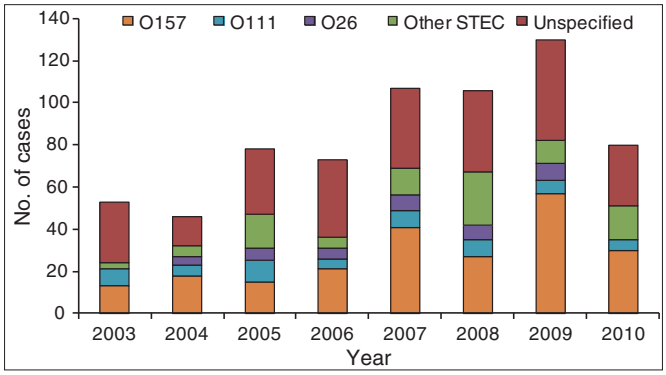

Figure 3. *E. coli* serotype breakdown of STEC notifications in Australia 2003–2010.

progressing to haemolytic uraemic syndrome and 50 deaths were reported[9]. Investigation of the outbreak concluded that contaminated fenugreek sprouts was the food vehicle responsible. The novel nature of the *E. coli* strain involved in this outbreak highlights the complexity associated with producing safe food and the difficulty in preparing for the emergence of novel *E. coli* pathotype. At the time of the outbreak the O104:H4 strain would not have been detected using conventional testing approaches for O157 and non-O157 STEC.

How can we prevent contaminated foods entering commerce?

There are generally two ways we can prevent foods contaminated with pathogenic *E. coli* from entering commerce: put hurdles in place to restrict food becoming contaminated or find ways to identify and treat foods that have become contaminated. Researchers have demonstrated that vaccines and probiotics such as *Lactobacillus* species can be effective in reducing pathogenic *E. coli* loads in cattle[10,11], however constraints relating to overall efficacy as well as timing and cost of application have limited their implementation at a commercial level. Consequently, the current focus for preventing STEC-related foodborne illness revolves around implementing practices that reduce the likelihood of releasing contaminated food products into commerce. Examples of practices include: carcase

decontamination during cattle slaughter, the use of properly composted manure in agriculture, and mechanical interventions like pasteurisation and irradiation to name a few. At the consumer level, cooking remains the only effective control mechanism, however, it is of little use to foods destined to be consumed raw. Washing of raw fruits and vegetables may be useful in reducing microbial loads but it can be problematic and it will have no effect if the organisms have become internalised during growth[12]. Testing product prior to its release into commerce can assist in reducing the likelihood of exposure to consumers. Interestingly, despite a surge in produce-related STEC outbreaks in the last decade there is not a concerted push to use testing as a way to improve the safety of these products. However, as the STEC paradigm shifts, both in terms of the food vehicles involved and the range of *E. coli* pathotypes involved, incorporation of testing or more substantial control measures may be required.

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Biographies

Robert Barlow is a research microbiologist with CSIRO Animal, Food & Sciences. He did his PhD on integron-associated antimicrobial resistance in beef production systems and has spent over 15 years conducting research into foodborne pathogenic *E. coli* and antimicrobial resistant bacteria in beef destined for export. Robert routinely assists food production industries and SMEs by providing advice and guidance on matters relating to food safety.

Glen Mellor is a research microbiologist with CSIRO Animal, Food & Health Sciences. Glen has extensive knowledge relating to the typing and virulence of pathogenic *E. coli* strains. In recent years, he has investigated the global genotypic diversity of *E. coli* O157 from Australia, Argentina and the USA and has been a key researcher in large-scale surveys within the red meat and poultry industries.

Microbiological risk assessment: making sense of an increasingly complex world

Duncan Craig

Food Standards Australia New Zealand
55 Blackall Street, Barton
ACT 2610, Australia
Tel: +61 2 6271 2222
Fax: +61 2 6271 2278
Email:
Duncan.Craig@foodstandards.gov.au

As our understanding of microbiological pathogens and their interaction with hosts expands, the complexity of assessing the risks posed by these hazards is also increasing. This is compounded by the extension of food production pathways, with multiple processes and/or new technologies used to produce the food that consumers desire. While based on principles developed for assessing toxicological and carcinogenic hazards, microbiological risk assessment throws up many challenges due to the ability of some microorganisms (bacteria) to multiply, or become inactivated, as food moves through the production to consumption continuum.

In addition, microorganisms themselves are not static entities but are constantly changing through natural selection and exchange of genetic material.

Food Standards Australia New Zealand's (FSANZ) primary role in the food regulatory system is to develop food standards covering the composition and labelling of food sold in Australia and New Zealand and Australia-only food standards, including those that address food safety and primary production and processing.

Food standards are a tool to facilitate the management of microbiological risks. FSANZ utilises the widely accepted framework of risk analysis[1], which is a structured way of examining and incorporating the wide variety of factors that impact on a decision-making process. This framework – comprised of risk assessment, risk management and risk communication – is described in detail in the FSANZ publication *Analysis of Food Related Health Risks*[2].

Microbiological risk assessment

The general risk assessment approach can be applied to the assessment of microbiological risks[3]. It is a structured process of organising and examining information to understand the interaction between microorganisms, foods and human illness. Its objective is to provide an overall statement of the nature (severity) and likelihood of harm resulting from human exposure to the hazard (bacterial, viral, protozoal, fungal organisms, or their metabolites) in food, and identify factors that may influence this risk throughout the supply chain. This information is used by decision makers to identify interventions that can lead to the greatest reduction in risk and provides a basis to weigh risk management options. Just as importantly, risk assessment can also help target research to fill data gaps that would have the greatest effect of reducing the level of uncertainty in the risk estimate.

Risk assessments can be qualitative (descriptive analysis and/or categorical descriptions of risk such as 'low', 'medium' and high') through to quantitative, which express risk in numerical terms (e.g. probability of illness per serve). Quantitative risk assessments involve mathematically describing the behaviour of microorganisms through the supply chain using the principles of predictive microbiology and combining with dose-response models to estimate the likelihood of illness at a given level of exposure. Quantitative assessments require extensive resources and expertise from multidisciplinary teams, however, the outputs can be extremely valuable to risk managers to quickly, and transparently, compare risk management options. Probabilistic risk assessments take this one step further and incorporate the underlying variability and uncertainty associated with model inputs, and describes the influence these have on the overall risk estimate. For example, this type of assessment was utilised by FSANZ for assessing the risk of illness from consumption of raw cow milk[4].

The type of risk assessment utilised is influenced by many factors, including the extent and availability of data, time and resources available, but most importantly the risk management question – that is, what information is required to make necessary risk management decisions. For example, during food safety incidents, a risk assessment may need to be completed in a short amount of time, with limited availability of data – therefore a quantitative microbiological risk assessment may not be feasible.

Assessing microbiological hazards from 'paddock to plate'

In 2001, FSANZ was given the mandate to develop food standards that cover the whole supply chain, from paddock-to-plate (Chapter 4 of the *Australian New Zealand Food Standards Code*)[5]. In progressing the primary production and processing standards, FSANZ

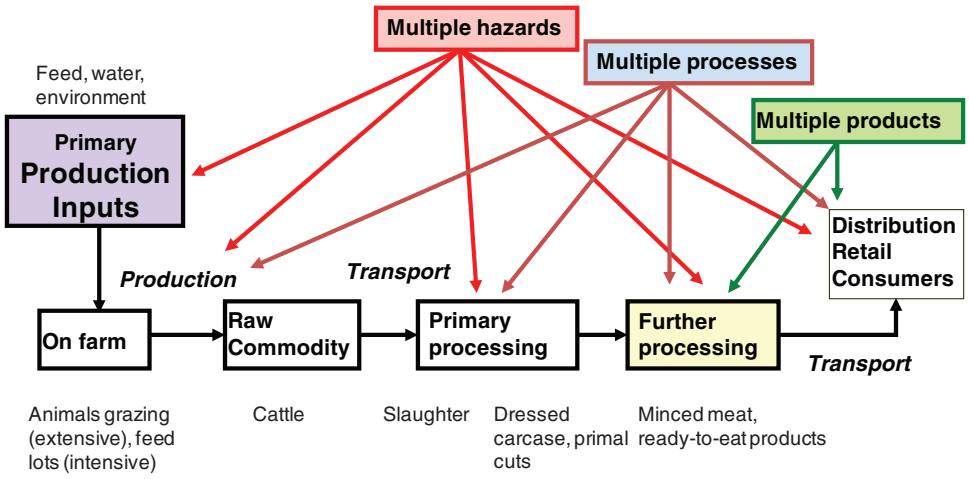

Figure 1. Example primary production pathway.

has been required to undertake a number of complex through-chain risk assessments for key commodity areas. These have included the seafood, poultry meat, dairy, egg and egg products, meat, and seed sprout industries and the associated reports are available on the FSANZ website (www.foodstandards.gov.au). While primarily undertaken to inform the development of food standards, these risk assessments can also be a useful resource for the food industry when developing Hazard Analysis Critical Control Point (HACCP) food safety management systems, particularly for hazard analysis and determination of critical limits.

While traditional risk assessments have considered single hazard: commodity pairs, these through-chain risk assessments often need to consider multiple hazards, across different production pathways, processes, and end products. As illustrated in Figure 1, this quickly increases the complexity of the assessment, with the ability of microbiological hazards to be introduced at each step of the supply chain (from animal, human and environmental sources) as well as increase or decrease in numbers due to potential growth and inactivation.

The changing environment

The changing environment in which assessing microbiological food safety hazards now occurs was exemplified in the outbreak of *Escherichia coli* O104:H4 in Europe in 2011. Over 3,800 cases of illness were notified to public health authorities in Germany, with 845 cases of HUS during the outbreak period of 1 May through 4 July 2011[6]. Cases were also reported in up to 15 other countries, mostly in people who had travelled to northern Germany during the outbreak period.

Following extensive epidemiological investigation by the authorities, seed sprouts were identified as the likely source of the outbreak. The detailed traceback investigations clearly illustrated the complex distribution of these types of food products at each stage of the supply chain (i.e. multiple seed suppliers, sprouters, food service, retail) and across many different countries[7].

This outbreak also exhibited quite a different epidemiological profile compared to previous outbreaks of Shiga-toxin producing *E. coli* (STEC). Rather than the high rates of HUS typically observed in children (predominantly seen for infections of serotype O157:H7), 88% of *E. coli* O104:H4 cases occurred in adults[6]. It is not clear if this changed profile was due to differences in host susceptibility or was representative of the exposure patterns (i.e. consumption of foods containing raw seed sprouts).

The highly virulent *E. coli* O104:H4 was also unusual in that it had virulence features that were common to the enteroaggregative *E. coli* pathotype. It carried the gene for Shiga-toxin 2 variant (stx_{2a}), however other genes typically observed in STEC such as *stx1*, *eae* and *ehx* were missing[8]. The exchange of virulence factors by means of horizontal gene transfer (e.g. prophage- and plasmid-mediated), and changing epidemiological profile for previously well-established hazards demonstrates the need for microbiological risk assessment to systematically collect and analyse all available information specific to the hazard and commodity in question, rather than making decisions on previous assumptions and experiences alone.

Future directions

As our understanding of the nature and behaviour of microorganisms in the environment and their interactions with the host increases, the tools available to undertake microbiological risk assessment have also evolved. There is a push for more quantitative assessments of microbiological risks, involving the application of predictive microbiology and mathematic modelling to describe the behaviour of microorganisms throughout the supply chain in an effort to determine the overall risk of causing human illness. As the complexities of microbiological risk assessment increase, there is also a desire for more user-friendly tools for risk managers/industry to utilise the outputs of risk assessment, such as the development of web-based tools (for example, those developed by FAO/WHO, which are available at http://www.mramodels.org/).

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Biography

Dr Duncan Craig is the Principal Microbiologist and manager of the Risk Assessment – Microbiology Section at Food Standards Australia New Zealand. His research interests are microbiological food safety and host-pathogen interactions.

Bovine spongiform encephalopathy and food safety

Rosalind Dalefield and Scott Crerar

Food Standards Australia New Zealand
PO Box 7186, Canberra BC
ACT 2610, Australia
Tel: +61 2 6271 2222
Fax: +61 2 6271 2278
Email: scott.crerar@foodstandards.gov.au

Bovine spongiform encephalopathy (BSE) is a fatal disease of cattle, caused by infective proteins known as prions. A prion ($PrP^{Sc)}$ is a mis-folded isoform of the glycoprotein PrP^{C}, which is highly expressed in the nervous system. Prions replicate by coercing PrP^{C} to refold into PrP^{Sc}. The BSE epidemic was propagated by rendering dead cattle to produce meal which was then included in cattle feed. Consumption of BSE PrP^{Sc} from contaminated beef resulted in over 200 human cases of variant Creutzfeldt-Jakob (vCJD) disease, which is invariably fatal. There were rare cases of person-to-person vCJD transmission by blood transfusion. Variant CJD is now very rare, due to adoption of measures that prevent the feeding of ruminant protein to ruminants and the contamination of beef with the tissues that harbour PrP^{Sc}. Beef from countries with these control systems are safe for human consumption.

The infectious agent

Bovine Spongiform Encephalopathy (BSE) is one of a number of diseases known collectively as Transmissible Spongiform Encephalopathies (TSEs) of which scrapie of sheep, chronic wasting disease (CWD) of deer and elk and Creutzfeldt-Jakob disease (CJD) in humans are also members. TSEs are caused by a mis-folded isoform of the prion glycoprotein (PrP). The mis-folded pathogenic isoform is known as a 'prion', a contraction of the words 'proteinaceous' and 'infectious'[1]. By convention, normal PrP is represented as PrP^{C}, while the prion is represented as PrP^{Sc}. Prions replicate themselves by binding to PrP^{C} and acting as a template that coerces PrP^{C} to refold into PrP^{Sc}[2,3].

In mammals, PrP^{C} is present in a wide variety of tissues but is highly expressed in the nervous system[2,4,5]. The physiological function of PrP^{C} remains obscure and mice modified to express no PrP^{C} show only subtle, non-lethal differences to wild-type mice[3,6].

Three strains of BSE exist, which exhibit differences in prion distribution, histopathology, incubation time and clinical signs[7] as well as the appearance of the prions on western blots. Only one strain, classical BSE, was responsible for the BSE epidemic and the associated epidemic of vCJD[8]. The atypical H-type and L-type strains typically occur in cattle over eight years of age, and appear to arise spontaneously[9,10].

Diseases

Cattle

The incubation period of BSE is estimated to be from 30 months to 8 years and clinical disease usually occurs in cattle of four to five years of age. The course of clinical disease is generally less than 6 months[11]. Clinical signs in cattle include abnormal posture, incoordination, and changes in temperament[9,11].

Humans

Consumption of BSE prions in contaminated beef resulted in over 200 human cases of variant CJD (vCJD)[7]. The great majority of patients were residents in the United Kingdom (UK) during the period 1985-1996[12]. Patients ranged in age from 17–42 years[5]. Variant CJD is distinct from the most common human prion disease, sporadic CJD (sCJD), which occurs spontaneously in people,

including lifelong vegetarians[8], between 55 and 70 years old[12]. Both forms of CJD are fatal.

Pathogenesis

The first tissues in which PrP^{Sc} can be detected in BSE are those of the nervous system supplying the intestine[13] although it is not clear how infection reaches the nerves from the intestinal lumen. Infection ascends to the brain via the autonomic nerves[2]. The routes of spread of prions from cell to cell within the nervous system are not fully understood[14,15], and the mechanisms of cerebral damage are unknown. Depletion of PrP^{C} does not appear to be a factor. On the contrary, depletion of PrP^{C} in mice has been shown to reverse early degeneration and prevent progression to clinical disease[7].

Transmission and incidence of disease

Animals

The epidemic of BSE was first recognized in 1986 in the UK and was propagated by the rendering of dead cattle to produce meat-and-bone meal (MBM) which was then included in feed for cattle[8]. The infection was spread elsewhere in the world by exports of cattle and contaminated MBM[9]. There is no evidence that BSE can be transmitted between living cattle. This is in marked contrast to the horizontal infectivity of scrapie in sheep and CWD in deer[16–18].

More than 184,000 cases of BSE have been diagnosed in cattle. At the peak of the epidemic 1,000 cases were being diagnosed each week in the UK[18]. The feeding of MBM to cattle was banned in the UK in 1988, but because of the long incubation period and initially ineffective implementation of the feed ban, clinical incidence continued to rise, peaking in 1992. The incidence has steadily declined since, and the disease is now very rare[19].

A number of zoo animals, including *Bovidae*, *Felidae* and non-human primates, developed TSEs at the same time as the BSE epidemic[20]. Cases of TSE were also diagnosed in two domestic goats[20] and a number of domestic cats[8,18]. All these cases were attributed to ingestion of BSE prions in beef or processed feed.

The epidemic is believed to have been amplified from a single common source[7], which remains unknown. Sporadic cases of BSE occur in cattle, although to date only the atypical L- and H-type strains have been found. It is possible that classical BSE may also occur spontaneously. Although it has sometimes been suggested that BSE arose from rendering of scrapie-infected sheep, encephalopathy induced in cattle by intracerebral inoculation with scrapie prions does not resemble BSE, and experimental BSE in sheep does not resemble scrapie. Furthermore, cattle are resistant to oral infection with scrapie or CWD[8].

Humans

Since the first ten cases were reported in April 1996, over 200 vCJD cases have been identified[7,21]. The epidemic of vCJD is attributed to consumption of beef contaminated with central nervous system tissue containing BSE PrP^{Sc}. BSE PrP^{Sc} and vCJD PrP^{Sc} have identical biochemical properties and cause identical lesions in mice, and on a country-by-country basis the incidence of vCJD in humans generally correlates with the prevalence of BSE in cattle[7]. The infective dose of bovine PrP^{Sc} to human beings is unknown[12].

Four cases of person-to-person vCJD transmission by blood transfusion have been reported in the UK[13]. Iatrogenic transmission of vCJD remains a concern because retrospective analysis of tonsil and appendix specimens suggests that up to 1 in 4000 persons exposed during the UK epidemic may be a sub-clinical carrier[8,22]. Internationally, blood donations are generally not accepted from people who lived in the UK between 1980 and 1996, or who received a blood transfusion in the UK since 1980. These precautions are in place in Australia[23] and in New Zealand[24].

A polymorphism at position 129 of the PrP^{C} amino acid sequence has been identified in humans, which appears to affect susceptibility to TSEs. Approximately 40% of Caucasians are homozygous for methionine (Met) at position 129, 10% are homozygous for valine (Val) and 50% are Met/Val heterozygotes. To date, all confirmed clinical cases of vCJD have been Met/Met homozygotes[12]. However, PrP^{Sc} was found in the spleen of a Met/Val heterozygote who died of unrelated causes five years after receiving a blood transfusion from a person incubating vCJD[2,12], and PrP^{Sc} was also found in anonymous postsurgical samples of appendices from two Val/Val homozygotes. Thus, lymphoid tissue of all three genotypes may become infected[8,12,25]. It is not yet clear whether the Met/Val and Val/Val genotypes prevent or only delay neurological infection with vCJD[12]. Besides vCJD, the only other orally acquired TSE known in humans is kuru, a historical disease of some communities in Papua New Guinea who practiced funerary cannibalism. The mean incubation period of kuru is 12 years, but the incubation period has exceeded 50 years in some individuals[13]. Retrospective analysis of samples has shown that the majority of those with unusually long incubation periods were Met/Val heterozygotes[12]. Some authors have predicted a late peak of vCJD cases affecting Met/Val heterozygotes[7,25].

Food safety and controls

A key component of prevention of both BSE in cattle and vCJD in humans is the prohibition on feeding mammalian-derived protein to food animals. Feeding of mammalian-derived proteins, other than dairy proteins, to livestock has been prohibited in the UK since 1996

and throughout the European Union since 2001[26,27]. Numerous other countries worldwide have enacted similar legislation. Enforcement of this ban includes[27]:

- Registration and regular auditing of feed producers
- Mandatory physical separation of ruminant feed production, storage and transport from production, storage and transport of non-ruminant feed that may contain animal proteins
- Testing of raw materials and finished feeds for presence of mammalian proteins
- Warning labels on feed bags
- Education of livestock keepers.

Throughout the EU, cattle are individually and permanently identified, and each country has an electronic database recording the details, locations, movements and fate of all cattle. Cattle that die or require emergency slaughter on farms are rendered and then incinerated to ensure that infective material is destroyed[27] and cannot be used in animal feed.

As a result of these measures (also see Figure 1 for a summary of the critical BSE control points) the BSE epidemic was controlled, and classical BSE is now very rare [19]. Surveillance programs for BSE are in place in many countries throughout the world, and occasionally detect isolated cases of atypical BSE[28].

The risk of oral infection of humans with vCJD can be eliminated by preventing contamination of the food supply with the animal tissues know to harbour infectivity, which are known as specific risk materials (SRM). SRM include the brain, spinal cord, eyes, palatine tonsils and gastrointestinal tract. Slaughter and processing procedures have been implemented throughout Europe and other countries to prevent contamination of beef with SRM. Following removal, SRM are transported to rendering plants under strict controls, and are rendered and incinerated under conditions known to destroy infectivity[27]. Beef and beef products from countries with these control systems are safe for human consumption.

Australia and New Zealand are among eleven countries internationally recognized as being at 'negligible risk' for BSE. Live cattle may not be imported into Australia from BSE-affected countries. A ban on the importation of MBM from countries other than New Zealand was in place almost two decades before the UK BSE outbreak, as a measure to prevent importation of anthrax spores. Should infected material enter Australia, propagation to the national cattle herd would not occur, because the feeding of ruminants with MBM is prohibited. Information on Australia's approach to BSE may be found on the website of the Department of Agriculture, Fisheries and Forestry[29].

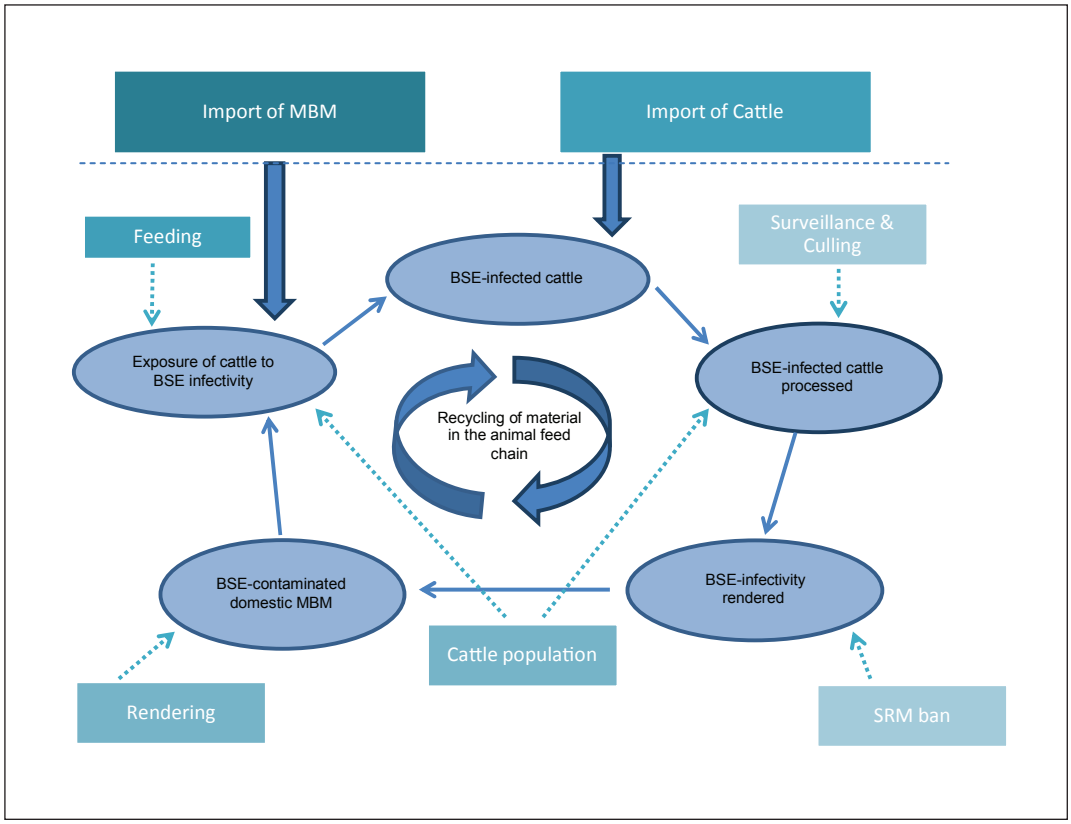

Figure 1. The critical points within the cattle production system that BSE controls need to be implemented to prevent introduction and recycling of the BSE agent.

With respect to the importation of beef products, Australia implemented a revised policy in 2010 whereby any country wishing to export beef to Australia must undergo a rigorous risk assessment of their BSE-related control measures[30]. The risk assessment, consistent with the principles outlined by the World Organisation for Animal Health, is undertaken by Food Standards Australia New Zealand who also visits each country to verify the effectiveness of the control measures. Countries are given a BSE category that establishes the conditions under which they may import beef products into Australia[31].

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Biographies

Dr Scott Crerar manages the implementation of Australia's BSE risk assessment policy with respect to the safety of imported beef and beef products at Food Standards Australia New Zealand (FSANZ). Dr Crerar has worked in food regulation and safety for 15 years and previously coordinated imported foods and food recall operations at FSANZ. He has also worked in Hong Kong and New Zealand in food regulation.

Dr Rosalind Dalefield participates in the implementation of Australia's BSE risk assessment policy with respect to the safety of imported beef and beef products at Food Standards Australia New Zealand (FSANZ).

Listeria surveillance in Australia from the laboratory perspective

Geoff Hogg and Agnes Tan

Microbiological Diagnostic Public Health laboratory
Email: ghogg@unimelb.edu.au

Joy Gregory

Department of Health Victoria and OzFoodNet

***Listeria monocytogenes* surveillance requires robust laboratory support in detection and organism characterisation. Such laboratory support includes ensuring all relevant isolates are secured and uniformly typed to allow detection of clusters and attribution to potential source. Different typing have different strengths. The move toward sequencing of the whole genome and its subsequent analysis although presenting new challenges to laboratories and practitioners receiving the outputs alike is proving of great utility by providing information of genetic distance between isolates. Australia has established surveillance integrating laboratory typing with epidemiological information relating to human cases. Extending this model to include the potential sources of *L. monocytogenes* does and has led to early detection of sources thus mitigating the risk to all stakeholders and vulnerable consumers.**

Primary diagnostic and jurisdictional public health laboratories from all sectors provide crucial information informing *Listeria monocytogenes* surveillance in Australians.

- Listeria spp. are Gram positive, facultative, non-sporing bacteria which we commonly find in the environment (soil, water) and in food processing premises.
- Infections can result in gastrointestinal or invasive disease (septicaemia, meningitis). *Listeria monocytogenes* is the species most commonly associated with disease although there are reports of infections caused by *L. ivanovii*, *L. seeligeri*, *L. welshimeri* and, in recent times, *L. innocua*.
- Listeriosis is a fairly uncommon infection affecting elderly, the immune-compromised, pregnant women and their fetuses, and, neonates. Listeriosis is not a common infection. The rate of listeriosis in Australia is estimated at 3 per 100,000 (OFN 2010 annual report). However, the case-fatality rate for listeriosis is high, at 20–30%[1].
- Foodborne transmission of *Listeria monocytogenes* was established in the early 1980s[2]. Since then, a variety of foods ranging from ready to eat sliced meats, dairy products, seafood, fruit and vegetables, and even butter[3] have been implicated as vehicles for transmission – especially when multiply handled.
- Foods associated with high profile outbreaks in Australia include pate, chicken wraps (2009) and soft cheese (2013).
- Food manufacture and distribution practices means that food produced by a single factory and perhaps of the same batch, can be distributed widely. A contamination event may have wide geographic impact over a period of time.

Monitoring of *Listeria monocytogenes* strains – laboratory aspects

Listeria monocytogenes infections of humans are notifiable as are detections in food in some jurisdictions. Human strains are uniformly referred to jurisdictional public health (PH) laboratories for further characterisation. Coverage is complete. Non-human strains are sourced from targeted initiatives, sporadic isolations and investigations. Coverage is opportunistic.

Methods used for typing include PFGE, serotyping (antisera or "molecular"), binary typing, MLVA, MLST, Riboprinter ribotyping and more recently next generation/whole genome sequencing (NGS/WGS). There are other variations on these tests but they are

alternatives rather than additional. Of these, it is PFGE which has had the greatest Public Health surveillance utility – such that MDU has so-typed over 3,500 isolates since the early 1990s.

Binary typing is readily and rapidly performed but shows less discrimination than PFGE. Similarly, molecular serotyping. Binary typing and serotyping may be useful in ruling particular strains "out" of a particular investigation, but usually do not definitively rule as "in".

MLST is time consuming, relatively expensive and more useful at a broad level rather than in finalising views on particular attributions. Riboprinter use is insensitive and largely discontinued locally. PFGE for *Listeria monocytogenes* has proved an excellent tool for discrimination and attribution. Centralising testing has not been problematic as numbers are relatively low. MLVA performs similarly to PFGE and may prove easier to standardise – although this can be harder than it seems.

It seems inevitable that using NGS to determine phylogenetic relationships will replace all of the above – with some of the existing parameters utilised still being derivable. MDU has now sequenced over 200 isolates and NGS does indeed look promising. The NGS issues will include, for example, for SNP analysis, choosing comparators within lineages, accessing bioinformatics expertise, analytical algorithms and presenting the data to public health practitioners. As numbers of cases of *Listeria monocytogenes* are relatively low, we now believe we have these issues in hand. It may be that the laboratory analysis and reporting will focus more on cluster and attribution identification via comparative reporting rather than isolate by isolate reporting. NGS is particularly useful in addressing "when is the same the same?" – as needed in attribution assessments. An understanding of the rate and nature of changes over time of the apparently relatively stable *Listeria* genome under different selection pressures has been gleaned to address the all-important question during or preceding investigations of "when is different different"? When strains are "different", they do not need inclusion in the relevant investigation. This question still proves challenging in day-to-day considerations and it remains critical to always interpret typing in the particular epidemiological context.

Monitoring of *Listeria monocytogenes:* epidemiologic aspects

Typing results are made known to senders, jurisdictions and OzFoodNet (OFN). Analyses of these results takes place at laboratory, jurisdictional, industry and national levels. This is best done when a One-Health approach is used.

Human data (epidemiology and laboratory) is centrally collated by OFN who regularly analyse and feed-back to jurisdictional stakeholders. An exposure history using a standardised national questionnaire is performed for each case where possible with data being entered into a national database. This is a very sensitive national strategy – even challengingly sensitive as very small clusters (3 or 4) may be identified, and identifying which are "over" and which "the start of something" is constantly under review.

Recent food recalls including those involving multiple jurisdictions have been identified by these means. When there is a problem evident and it is confined to that jurisdiction, the investigation is conducted locally. When problems are multi-jurisdictional then OFN instigate a structured "Multi Jurisdictional Outbreak Investigation" (MJOI), which informs next steps at the various food safety management levels, including internationally where relevant.

Challenges

We know what we know and we know there are things which would be helpful to know, but we don't know them – e.g. which strains normally reside in which industry sectors. The competing interests of risk assessment, privacy, resource allocation and desired approach (collaboration vs regulation) are always to the fore.

Currently human data are comprehensive and uniform. Timeliness is challenging given the potentially long incubation before disease (especially in maternal/foetal cases). A strong case can be made that every isolate made from a food which reflects potential human exposure, whether the food is recalled or not, should be forwarded for typing to detect early leakage into humans. A case can similarly be made with respect to isolates from high-risk foods' production environments. It is helpful to know strain prevalence by sector as this information can ensure that results are not over-interpreted.

Given the human, financial and reputational cost of contamination events and associated recalls, further development of collaborations across sectors backed up by Regulation and Industry Guidelines – when necessary or helpful in mitigating adverse outcomes – continue to be the optimal approach.

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Biographies

Geoff Hogg is Director of the Microbiological Diagnostic Unit Public Health Laboratory. His role includes ensuring that laboratory services meet the needs of all those relying on them. He has a diverse background leading to a firm belief that where organisms and the diseases they cause involve multiple sectors, such as is evident for Listeria and Listeriosis, that a One-health approach is required to meet the needs of all stakeholders, including mitigating risks to patients.

Agnes Tan is a Senior Scientist at the Microbiological Diagnostic Unit. She has assisted public health colleagues in outbreak investigations over many years.

Joy Gregory is the OzFoodNet epidemiologist employed by the Victorian Department of Health. She has been with OzFoodNet since its inception in 2000. Joy has a keen interest in surveillance and epidemiology of enteric diseases, especially those with a foodborne mode of transmission, and has been involved in investigating numerous foodborne outbreaks including multijurisdictional outbreaks of listeriosis. Joy has a strong commitment to working in multifaceted teams during investigations to achieve the best public health outcomes.

Food poisoning due to *Clostridium perfringens*

John Bates

Public Health Microbiology
Laboratory Queensland Health
Forensic & Scientific Services
Tel: +61 7 3274 9101
Fax: +61 7 3274 9175
Email:
john_bates@health.qld.gov.au

"No other food poisoning bacterium is so uniquely poised to take advantage of the slackness of human beings involved in food preparation." (John Bates – Food Poisoning lectures). *Clostridium perfringens*, a Gram-positive anaerobic spore-forming bacillus, has been responsible for a large number of food poisoning events in Australia. Frequently, these are spectacular outbreaks involving large numbers of diners at catered events. While the organism is perhaps better known in clinical laboratories as an agent of gas gangrene and deep tissue infections, in Public Health laboratories it is recognised as one of the major causes of food poisoning in Australia. While these outbreaks can cause high morbidity with severe abdominal cramps and diarrhoea lasting 24 hours, generally, fatal cases are rare, except in the very young and the elderly. For this reason, outbreaks in nursing homes or elderly patients in hospitals need to be identified quickly and their symptoms managed to avoid severe dehydration and shock.

Classical Type A *Clostridium perfringens* are typically β-haemolytic on Horse Blood Agar (HBA) and the spores are oval and subterminal with no swelling of the sporangium. The bacilli are 1–1.5 µm wide and 4–8 µm long. The organism has complex nutritional requirements for growth, and therefore their frequent involvement in meat and curry-type dishes is hardly surprising. Depending on which text is consulted, the incubation period for *Cl. perfringens* food poisoning can vary from 6 to 24 hours following consumption of contaminated food. However, in practice, cases usually manifest in 8–22 hours with a mean of around 15 hours. Unlike Staphylococcal or *Bacillus cereus* emetic food poisoning, the enterotoxin of *Cl. perfringens* is not preformed in the food during multiplication. Rather, the food, laden with high numbers of vegetative cells, has to be ingested first, and after passing through the acid barrier of the stomach to the alkaline environment of the jejunum, the vegetative cells start to sporulate, and as the spores are released into the lumen of the gut, enterotoxin is also released. The enterotoxin binds to brush border membrane receptors of intestinal epithelial cells, which then induces a calcium ion-dependant breakdown of permeability, leading to a massive influx of fluid into the gut and producing the profuse diarrhoea associated with this syndrome. The peak for enterotoxin production is just before lysis of the cell sporangium. This is why the time intervals for onset of symptoms are considerably longer, and it also means that testing the food for enterotoxin is not warranted.

The main criteria for diagnosis of food poisoning caused by *Cl. perfringens* include the detection of at least 1×10^5 vegetative cfu/g in incriminated food, and/or a faecal spore count of at least 1×10^6 cfu/g in faeces from ill complainants. However, high counts need to

Figure 1. Storage of curries in a Bain Marie.

be interpreted very carefully in geriatric patients, as counts of at least 1×10^5 cfu/g are common in this age group. There is a Reversed Passive Latex Agglutination (Oxoid PET-RPLA, Basingstoke, England) kit available for detection of enterotoxin in faeces, but it is essential to test samples within 24–48 hours of onset of symptoms as the toxin is rapidly eliminated from the gut. Of more use is a molecular approach which can determine the serotype of the organism and the presence of the *cpe* (enterotoxin) gene, and a separate PCR to determine if the *cpe* gene is carried on a plasmid or chromosomally (see below)[1].

The literature is replete with reports of large outbreaks of food poisoning resulting from catered events. Most outbreaks occur in large eating establishments where large quantities of food are prepared. In particular, *Cl. perfringens* was a leading cause of food poisoning in hospitals in the United Kingdom up until the late 1980s[2]. Careful reading of many of these reports will usually reveal that food had been kept warm (<47°C) for extended periods of time, allowing germination of spores and subsequent proliferation of vegetative cells. A recent article from the United States estimates that *Clostridium perfringens* is the second most common bacterial cause of food poisoning[3]. Likewise in Australia, *Clostridium perfringens* is a common cause of food poisoning and outbreaks are often quite large because they occur at mass-catering events[4–8].

Studies on food poisoning strains have demonstrated that chromosomal carriage of the *cpe* gene actually confers heat resistance on the organism, a handy trait for an organism that grows in cooked food. One of the key features of food poisoning strains is their ability to grow at elevated temperatures. The optimum growth temperature ranges from 43°C to 47°C. For this reason, many of the outbreaks in Australia are associated with the use of Bain Maries (Figure 1), particularly in restaurants serving curries and other meat dishes, where the Bain Marie is turned down to prevent the food from drying out too much. With a generation time of only seven minutes at 43°C, foods do not have to be temperature-abused for long before the vegetative cell count becomes significant. In particular, the practice of using the Bain Marie to heat the food from scratch is highly prone to proliferation of this organism. Investigation of outbreaks will invariably uncover a critical control point (CCP) that has not been implemented correctly. In the case of some restaurants in Brisbane, curries have been cooked and then placed in bins to allow them to cool down slowly out the back of the restaurant. This is particularly a problem with food preparation personnel who translate their home food practices to a catering premise where the standards of food handling are necessarily expected to be much higher. Often, a change in practices resulting from the breakdown of a key piece of equipment used to keep food hot will be uncovered. In some cases, outbreaks will occur simply because the food has not been cooked properly in the first place and is then kept warm for an extended period, allowing ample time for spores to germinate and proliferate, eg. spit-roast caterers.

Other manifestations of toxigenic *Clostridium perfringens*

In addition to its traditional role of food poisoning, *Cl. perfringens* has also been associated with a couple of different syndromes. In Papua New Guinea, the highlanders suffered for many years from a frequently fatal human necrotic enteritis syndrome called Pig-Bel, which was associated with consumption of Type C *Cl. perfringens* in pig feasts. The highlanders' staple diet throughout the year is the sweet potato, which contains a trypsin inhibitor which predisposes them to intoxication by this strain[9].

More recently, there have been outbreaks in nursing homes in a couple of Australian States where person to person spread over a number of days is suspected, rather than the traditional point-source outbreaks associated with food poisoning. These outbreaks are caused by Antibiotic-Associated Diarrhoea (AAD) strains of *Cl. perfringens*, in which the *cpe* gene that codes for enterotoxin production is carried on a plasmid, rather than chromosomally as seen with food poisoning strains[10].

There have also been reports in the literature of fatal cases of *Cl. perfringens* food poisoning in elderly patients in psychiatric hospitals who have been medicated with anti-cholinergic drugs. These drugs have been linked to faecal impaction in the large bowel, leading to retention of toxin in cases of food poisoning and massive necrosis to the lining of the colon, resulting in death[11,12].

Conclusion

Food poisoning caused by *Cl. perfringens* is a preventable condition. If the right checks and balances are built into the food preparation process, then the organism should not have an opportunity to germinate and proliferate. Even if this does occur, thorough reheating of the food at temperatures in excess of 60°C will eliminate the organism and prevent an outbreak of food poisoning. This highlights the importance of appropriate education and compliance of personnel involved in food preparation so that they understand what can happen when they mishandle food or essential equipment breaks down.

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Biography

John Bates is the Chief Scientist in charge of the QHFSS Public Health Microbiology laboratory. This laboratory provides a support arm to the Queensland Health Public Health Units in the investigation of outbreaks of human, water and foodborne disease, as well as providing phenotypic and genotypic data on a wide range of notifiable bacteria in Queensland.

Salmonella and egg-related outbreaks

Cameron RM Moffatt and Jennie Musto*

*Communicable Disease Control Section
Australian Capital Territory Health Protection Service
25 Mulley Street, Holder
ACT 2611, Australia
Tel: +61 2 6205 1734
Fax: +61 2 6205 1739
Email: cameron.moffatt@act.gov.au

Non-typhoidal *Salmonella* infections are a significant public health issue in Australia, with record numbers of both disease notifications and outbreaks being reported in recent years[1,2]. Epidemiology plays an important role in *Salmonella* outbreak investigation, helping to identify raw and minimally cooked eggs as an increasingly common cause for these events. Of particular relevance to disease caused by Australian eggs is *Salmonella enterica* subsp. *enterica* serovar Typhimurium. A crucial element in demonstrating this serovars presence throughout the food chain is the ability to trace suspect eggs to their source. High product turnover makes this challenging but through the adoption of integrated surveillance practices and harmonised laboratory methods, a more effective response may emerge.

Epidemiological investigations of egg-related outbreaks and salmonellosis

Determining the source of infection for a case of salmonellosis is difficult because the volume of cases, coupled with competing public health priorities, means that most cannot be investigated. Consequently much of our understanding of disease causation comes from outbreak investigation. To determine if an outbreak might be attributed to eggs, investigators use both descriptive and analytical epidemiology, findings from environmental health and food safety investigations and the results from microbiological testing of human, food and environmental samples[3].

In outbreaks where the exposure or food vehicle is not immediately apparent, analytical epidemiology may be used to examine disease occurrence and risk. These analyses involve making comparisons between groups of people, e.g. those with a *Salmonella* infection and those without (i.e. case control study) or those who have eaten a particular food and those who not (i.e. retrospective cohort study). Statistical inferences can then be made as to whether a case was more likely to have eaten a particular food or whether having exposure to a particular food carried increased risk of illness. For those cases unable to be investigated by epidemiological or microbiological means, the same attribution to a potential source cannot be as easily made, hence the focus on outbreak cases.

Between 2001–2009, 1,025 foodborne outbreaks were reported in Australia, with *Salmonella* being identified as the causative agent in one-third of these[4]. Among *Salmonella* outbreaks, eggs and egg containing foods were the most commonly identified causes. *S.* Typhimurium was responsible for 90% of these outbreaks, which resulted in some 2300 cases, including over 500 hospitalisations[4]. In the period 2010–2011, this significant morbidity has further increased, with OzFoodNet identifying another 60 egg-related outbreaks, involving over 1000 cases and 180 hospitalisations (*personal communication OzFoodNet Egg Working Group*).

Egg-related outbreaks occur in varied settings, with restaurants and cafes being most frequently identified[1,5,6]. Raw or minimally cooked egg containing sauces (aioli and hollandaise) and desserts (mousse, fried ice cream and tiramisu) are food vehicles commonly involved in these outbreaks[1,5,7]. Such settings are identified because large numbers of people become unwell in a short time, enabling surveillance systems to detect increases in case numbers above expected thresholds or because community members are more likely to alert health officials to issues within commercial premises. Outbreaks in private residences are also common but usually involve fewer cases and are not reported as often. Finally, outbreaks linked to takeaways and bakeries[8,9] are also frequently identified, often being characterised by large case numbers, spread over a wide geographic area. Such characteristics highlight how outbreaks might be more or less likely to be identified due to the setting, potentially biasing our understanding of foodborne illness settings. Nonetheless, outbreak reporting analysis remains our best source of information about foodborne disease while also reducing concerns related to publication bias[10].

During 2001–2009[4], investigators conducted analytical studies for one third of reported outbreaks, with nearly three quarters of these showing an association with eggs or an egg-containing food. Laboratory confirmation of *Salmonella* in an egg-containing food was obtained in 39% of outbreaks. Trace back was conducted in 62% of all outbreaks, with around 70% conducted to the farm level. For farms and processing facilities where on-site testing was conducted, 50% had the outbreak strain recovered in the farm or processing environments.

It is reasonable to assume that for some serovars, the food sources identified during outbreaks will also be responsible for causing sporadic disease[11]. Since 2007, New South Wales (NSW) has used Multi-locus Variable number of tandem repeats Analysis (MLVA) as the primary typing method for S. Typhimurium. MLVA is able to discriminate further within an S. Typhimurium phage type, allowing a better attribution of the source of infection for both sporadic and outbreak cases. For example, during 2012 NSW Health investigated a restaurant outbreak of *S.* Typhimurium MLVA 3-9-9-12-523, an uncommon MLVA pattern. The investigation determined the food vehicle as a raw egg-containing dessert. The New South Wales Food Authority (NSWFA) traced the eggs used back to a specific farm, isolating the outbreak strain from the farm environment and grading facility. Retrospectively, another 30 cases were able to be linked to the egg farm, either via further outbreaks at the restaurant or through consumption of products from a bakery using eggs from the same farm.

Salmonella Typhimurium, *Salmonella* Enteritidis and their control

The epidemiology of egg-related salmonellosis and the efforts to control contamination in laying hens, farm and processing environments differs between Australia and elsewhere internationally. In Australia, *S.* Typhimurium causes the majority of infections, being frequently identified as a cause for foodborne outbreaks, particularly where raw or minimally cooked eggs have been used[1]. In North America and Europe, control efforts have been in response to a problematic and costly *S.* Enteritidis epidemic. Importantly this serovar is not endemic in Australian layer flocks[12]. *S.* Enteritidis differs from other serovars in its capacity for trans-ovarian transmission, i.e. an ability to infect the egg's internal contents. However,

S. Typhimurium has also been shown to colonise the reproductive tracts of infected hens, though the significance of this remains unclear[13]. Furthermore uncertainty exists around whether vertical or horizontal transmission is more important in *S.* Enteritidis with studies showing shell contamination exceeding that of the internal contents[14].

International poultry control programs have resulted in significant decreases in egg-related salmonellosis[15,16]. These programs employ measures including: on-farm monitoring, diverting contaminated eggs for processing, culling infected flocks, cleaning and disinfection of sheds, through chain refrigeration of eggs, and vaccination of flocks[15,16]. In the United States, these measures apply to *S.* Enteritidis infected flocks whereas the European Union Regulation addresses monitoring and control for both *S.* Enteritidis and *S.* Typhimurium[17]. The absence of *S.* Enteritidis in Australian flocks is fortunate however other serovars still cause egg-related disease. The Australian standard for egg production and processing[18] was developed due to Australia having unacceptably high numbers of cases linked to eggs and inadequate regulatory and non-regulatory measures to prevent illnesses[18].

Eggs, public health and food safety

Egg-related outbreaks result from breakdowns in control measures along the farm to fork continuum. When eggs are epidemiologically associated with illness, the use of food prepared with raw egg is frequently confirmed via observation or interviews with restaurant staff. There is however no strong evidence suggesting any sudden change in preparation and preferences that might explain the increase in outbreaks. What is known is that both egg production and consumption have soared[19]. Given shell egg production could never be *Salmonella* risk free, it is plausible that the volume of contaminated eggs in circulation has risen. While campaigns targeting consumer and food service practices help reduce the incidence of disease, reduction of bacterial contamination in the egg production system would be more effective, as shown with international *Salmonella* control in poultry.

Public health authorities are particularly concerned when *Salmonella* serovars that commonly cause human disease are recovered from egg-laying, grading or processing environments. The Food Standards Australia and New Zealand (FSANZ) Primary Production Processing Standard for Eggs and Egg Products[18] requires producers and processors to identify and control hazards, prohibits the sale of cracked and dirty eggs (unless pasteurised) and requires individual egg stamping to enable tracing. If, as outbreak investigations suggest, increased numbers of contaminated eggs are entering the retail and commercial market supply chains, further evidence of pathogen reduction on-farm, at the grading and at the processing level is required. Prevalence data for contaminated eggs in Australian are limited: estimates were last published in 2005[20] and were based on work from over 10 years ago, well before the observed increase in outbreaks.

Trace back in outbreak investigations

Trace back is a method of determining the source and distribution of a product associated with an outbreak, in addition to identifying the points where contamination could have occurred[21]. It is often difficult to achieve as investigations commence after an initial contamination event. The FSANZ Primary Production Standard for Eggs and Egg Products[18] requires the stamping of all eggs, *theoretically* allowing trace back to individual farms. However in reality this is unlikely to be as useful as eggs and their packaging will likely have been used or discarded before an investigation commences.

Table 1. Laboratory characterisation of *Salmonella* in Australian states and territories.

State/Territory	Serotyping	Phage typing	MLVA	PFGE
Australian Capital Territory	–	–	–	–
New South Wales	✓	–	✓[A]	–
Northern Territory	–	–	–	–
Queensland	✓	–	✓[A]	–
South Australia	✓	✓[A]	✓	–
Tasmania	–	–	–	–
Victoria	✓	✓[A]	✓	–
Western Australia	✓	–	–	✓[A]

[A]Primary method for *S.* Typhimurium sub-classification.

Currently small producers are more likely to have on farm investigations conducted because they have localised operations and less complex supply chains. If an investigation determines 'Brand X' eggs were used by a cafe and those eggs were produced on a single farm, regulators can have greater confidence in conducting on-farm testing for the outbreak strain. However, if Brand 'Z' eggs (produced by a larger company) were used, the trace back investigation can become more difficult. In large operations packaging and processing facilities may be supplied with eggs from dozens of smaller farms. In theory egg stamping will allow trace back to farm level but because of high product turnover in the food sector, leftover eggs, cartons or packaging with bar coding and other identifiers will have been long discarded. Regulators are left in a difficult position: suspicion exists that contaminated eggs have been produced but they are unable to verify this due to a lack of traceability and documentation. OzFoodNet data shows where trace back has been possible and undertaken in a timely manner, the outbreak strain will frequently be found in the farm and processing environments[4].

Integrated surveillance: sharing data from farm to beyond the fork

There is a growing appreciation of the need to integrate surveillance data from on-farm through to the point of sale, including laboratory data derived from human, environmental and animal surveillance[22]. This will require a new level of trust between government and industry, including recognition that reducing foodborne illness is not just a role for health agencies. While OzFoodNet works where possible with regulators and food safety agencies to compare data on human pathogens with other sources, there are drawbacks to the informality of the approach: a lack of access to test results conducted in private laboratory settings and, more worrying, a reluctance to sometimes share information between government agencies, even within the same jurisdiction. The sharing of data for public health purposes and collaboration between government and industry has underpinned the success of the EU *Salmonella* control programs[23].

The role of the microbiology laboratory

The contribution of reference laboratories in the testing of clinical, food and environmental samples is vital. However, the adoption by some but not all jurisdictions, of MLVA typing for the characterisation of *S.* Typhimurium has impeded the ability to rapidly compare data nationally. The predominance of *S.* Typhimurium, particularly in south-eastern Australia, and its responsibility for large numbers of outbreaks, including those due to eggs[7], highlights the value of a coordinated and harmonised laboratory response. Currently smaller jurisdictions forward isolates to their larger neighbours for serotyping. For *S.* Typhimurium, further classification may result in an MLVA profile or a phage type, depending on the typing method used by the receiving reference laboratory. Such issues relating to laboratory characterisation of human isolates are inevitably addressed through OzFoodNet's strong collaborative ties with reference laboratories and related stakeholders. Table 1 shows typing methods employed in Australian jurisdictions.

Conclusion

Salmonella *Typhimurium* is the serovar most responsible for Australia's ongoing '*epidemic* of egg-related outbreaks'. These outbreaks occur most frequently in restaurants and cafés, with raw or minimally cooked egg-containing sauces and desserts being identified as responsible food vehicles, reinforcing the need for ongoing consumer and food service industry education. Although a number of comprehensive and guiding documents exist in Australia[18,24], stronger trace back and regulatory capacity is required. This is unlikely to be achieved through egg stamping; rather improvements in retailer and producer documentation are needed. Nevertheless trace back investigations have provided evidence that *Salmonella* has entered into the wider food chain, reinforcing the need for public health authorities, regulators and those in industry, to develop systems for data sharing and integrated surveillance. Underpinning this should be a goal of targeted reductions in on-farm pathogen prevalence. Finally, while acknowledging laboratories invaluable contribution, the differences in techniques should be flagged as being potentially problematic but by no means insurmountable.

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Biographies

Cameron Moffatt is a communicable diseases epidemiologist currently working with the OzFoodNet network. His particular interests include outbreak investigation and the epidemiology of *Salmonella* infections, notably their link with eggs. Cameron is a graduate of the Australian National University's field epidemiology training program.

Jennie Musto is an infectious diseases epidemiologist currently employed as Team Leader, Enterics and Zoonoses in Communicable Diseases Branch, Health Protection, NSW, Australia. Jennie has been working in foodborne diseases epidemiology since 2002. Jennie's interests are enteric and zoonotic disease epidemiology, and outbreak investigations.

Foodborne campylobacteriosis in Australia

Russell Stafford

Disease Surveillance, Prevention and Control
Communicable Diseases Unit
Chief Health Officer Branch
Department of Health, Queensland
Tel: +61 7 3328 9733
Fax: +61 7 3328 9740
Email:
Russell_Stafford@health.qld.gov.au

Thermophilic *Campylobacter* are an important cause of human illness worldwide. *Campylobacter* reservoirs include a wide variety of wild birds, poultry, farm animals, domestic pets and natural water systems. In Australia, infection is mainly associated with foodborne transmission, though other routes of exposure including waterborne and direct zoonotic transmission are not uncommon. Most cases of infection appear to be sporadic in nature, with outbreaks rarely reported. Epidemiological and microbiological evidence suggests chicken meat is the principal source of infection among cases. A recent study estimated there are more than 50,000 cases of *Campylobacter* infection attributed to chicken meat each year in Australia. When outbreaks are detected, they are most often associated with the consumption of poultry, contaminated water and occasionally unpasteurised milk. The lack of recognised foodborne outbreaks of campylobacteriosis could be due to organism-related factors such as the inability of thermophilic *Campylobacter* to multiply on food left at room

temperature, their microaerophilic nature and their susceptibility to drying. However, it is likely that outbreaks of *Campylobacter* infection are also under-reported in developed countries due to the current lack of a suitable phenotypic or genotypic typing method for routine surveillance and outbreak detection. The future development of improved typing methods for detecting outbreaks should enable further sources and risk factors for *Campylobacter* infection to be determined.

Campylobacter is the leading cause of gastrointestinal illness in Australia among all the notified enteric pathogens[1]. There are more than 15,000 cases of *Campylobacter* enteritis notified through surveillance systems in Australia each year; excluding cases from New South Wales where the disease is not notifiable. However, notified cases represent only a fraction of all cases of infection occurring in the community and after adjusting for under-reporting and incomplete population coverage, recent estimates indicate approximately 225,000 (1180/100,000 population) *Campylobacter* infections occur in Australia each year[2]. The majority of infections are sporadic illnesses with community outbreaks infrequently reported, partly due to the lack of an efficient standardized typing system for routine surveillance. Most infections are caused by two species, *C. jejuni* and *C. coli*[3]. Foodborne transmission appears to be the most common method of transmission of *Campylobacter* infection to humans causing an estimated 75% to 80% of sporadic infections[4–6]. While the majority (98% to 99%) of cases of campylobacteriosis lead to a self-limiting episode of acute gastroenteritis, antimicrobial therapy may be indicated in prolonged or complicated illness. Occasionally, more severe disease outcomes occur, particularly in patients with immune deficiency, notably hypogammaglobulinaemia and AIDS. Chronic carriage of *Campylobacter* with recurrent enteritis and bacteraemia are typical problems among this group. Post-infectious complications associated with *Campylobacter* infection include Guillain-Barré syndrome, an autoimmune disorder of the peripheral nervous system causing acute flaccid paralysis (0.01–0.1%) and reactive arthritis (1–5%)[7].

Sources of infection

The intestinal tract of a wide range of wild and domestic birds and warm-blooded animals have been identified as major reservoirs of *Campylobacter* in the environment[8–10]. Colonisation of the intestinal mucosa may be as a commensal or as an asymptomatic transient infection[11,12]. Farm animals, in particular, are major reservoirs for this organism including beef cattle, dairy cows, sheep, pigs and poultry[13–15]. Consequently, this organism is frequently found in foods of animal origin including raw meat and raw milk[16]. The organism is ubiquitous in the environment, probably as a result of faecal contamination by birds and animals, and is often detected in natural water sources including coastal seawater, rivers, streams, lakes, ponds and groundwater[17].

At the retail level, *Campylobacter* are more frequently isolated from poultry meat than from red meats. Prevalence studies conducted in Australia and overseas of raw poultry meat, in particular raw chilled chicken, often show frequencies in excess of 50%. Furthermore, contamination levels in excess of 10^5 organisms per carcass at retail level have been reported[18–22]. Prevalence studies conducted at the retail level on raw red meats have generally shown the frequency of contamination to be considerably lower than that seen in raw poultry. Australian surveys of beef, pork and lamb have shown a prevalence range of 0% to 8%[23,24]. The lower prevalence of *Campylobacter* seen in red meat as opposed to white meat is thought to be due to differences in slaughtering processes and the extended forced-air chilling of red meat carcasses (most chicken carcasses in Australia are subjected to immersion chilling)[25]. Offal, on the other hand, is not subjected to forced-air chilling and consequently the prevalence of *Campylobacter* contamination tends to be higher than for whole cut meats. A recent Australian retail study reported contamination frequencies of 13% and 23% for raw lamb kidneys and livers respectively[23]. The prevalence of *Campylobacter* in poultry livers is considerably higher[26].

The intestinal tract of poultry, including laying hens, is a common reservoir for *Campylobacter*; however, shell eggs are not considered to be a high risk food for transmission as the organism does not survive well on the shell surface due to desiccation[27]. Although *Campylobacter* have been detected on the surface of faecally contaminated eggs that are not of commercial quality, penetration studies indicate the organism does not penetrate readily through the egg shell membrane[28]. A recent study also suggests that vertical transmission of *Campylobacter* through the egg yolk is likely to be rare[29].

Risk factors for sporadic infection

Risk factors for *Campylobacter* infection have generally been identified either through case-control studies of laboratory-confirmed sporadic infections or from investigation of disease outbreaks. The vast majority of case-control studies have been conducted in developed countries including the United States, Canada, the United Kingdom, Norway, Denmark and New Zealand[30–36]. The majority of studies have demonstrated that poor handling and/or consumption of raw or undercooked chicken was the single most important risk factor for infection, being reported in no less than 20 case-control studies. Other meats identified as potential risk factors for sporadic

infection include pork and beef, though these foods are less frequently reported in case-control studies as risk factors than poultry. Raw milk is another regularly identified foodborne risk factor among case-control studies for sporadic infection, more so in those countries where raw milk consumption is relatively common[30,35,36].

The association between chicken consumption and *Campylobacter* infection has been extensively reported in the literature and this risk factor appears to be the major source of infections in Australia as well. A large multi-centre case-control study conducted by OzFoodNet among persons aged five years or older identified the consumption of undercooked chicken and offal as independent risk factors for infection[37]. This study showed that almost one-third of *Campylobacter* infections that occur in Australia each year can be attributed to chicken meat, either through the consumption of undercooked chicken or from poor food handling of raw chicken and subsequent cross-contamination to cooked or ready-to-eat foods. The population attributable risk proportions from this study indicated that more than 50,000 cases of *Campylobacter* infection could be attributed to chicken meat annually in Australia among persons aged 5 years and older[38]. Similarly, there are an estimated 3,500 cases of *Campylobacter* infection each year in Australia attributed to eating offal.

Consumption of chicken has not been identified as a risk factor for *Campylobacter* infection in children ≤4 years of age in Australia, despite three case-control studies which have examined risk factors for infection in this age group[39–41]. Two studies conducted outside of Australia in other developed countries have also failed to identify chicken consumption as a risk factor for infection in this age group[42,43]. Regardless of these findings, it is likely that foodborne transmission from chicken is a risk factor for infection in young children, albeit of less importance. Contact with pets such as puppies and young chickens have been identified as important risk factors for infection in young children[39,40].

Outbreaks of *Campylobacter* infection

Foodborne transmission is the predominant route of infection for outbreaks of *Campylobacter*. In Australia, 27 (82%) of the 33 *Campylobacter* outbreaks reported between 2001 and 2006 were foodborne or suspected foodborne, three (9%) were waterborne, one (3%) was due to person to person transmission and two (6%) outbreaks had unknown transmission routes[44]. A food vehicle was confirmed for 16 (59%) of the 27 foodborne outbreaks; poultry (chicken or duck) was associated with 11 (41%) outbreaks, unpasteurised milk and salads were associated with two (7%) outbreaks each. Seven (44%) of the 16 outbreaks with identified food vehicles were attributed to consumption of the contaminated raw product (undercooked food) while four (25%) were attributed to consumption of a ready-to-eat food that was cross-contaminated from a raw food product. The contributing factors were unknown for the other five outbreaks. Although the sale of unpasteurised milk for consumption to the public is illegal in Australia, occasional outbreaks still occur. The two outbreaks reported above were associated with the consumption of raw milk during school excursions to dairy farms.

In recent years, both Australia and the United Kingdom have reported an increase in the number of outbreaks of *Campylobacter* associated with poultry liver dishes[45,46]. Seven outbreaks associated with poultry liver have been recorded in the OzFoodNet outbreak register since 2001, with six (86%) of these occurring between 2008 and 2011. All seven outbreaks involved commercial food venues with either chicken (5) or duck (2) liver dishes prepared on site. Undercooking of the poultry liver dishes was the likely contributing factor for these outbreaks.

Conclusion

Foodborne campylobacteriosis is a major cause of bacterial enteritis in Australia. The incidence of disease in our community provide a strong argument for both government and industry to focus efforts into reducing contamination of chicken carcasses with *Campylobacter* either through improved on-farm control or interventions during processing. In addition, the figures justify the need for government to continue educating consumers and foodhandlers about the risks associated with the handling of raw chicken and the potential for cross-contamination in the kitchen. Improved surveillance and detection of *Campylobacter* outbreaks will increase our knowledge on the epidemiology of this organism and help inform prevention and control strategies.

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Biography

Russell Stafford (MPH, PhD) is an epidemiologist with the Communicable Diseases Unit in Queensland Health and has been a member of the OzFoodNet Working Group since its inception in 2001. He has a special interest in the epidemiology of Campylobacter, Salmonella, Shiga toxin-producing *E. coli* and Listeria.

Food safety plans: three problems to address when analysing microbiological hazards

Prue Bramwell

School of Applied Science
RMIT
PO Box 71, Bundoora
Vic 3083, Australia
Email: p.bramwell@rmit.edu.au

Food safety programs set out how safe food is produced. The primary objective of designing food safety plans is to ensure food is safe and suitable for human consumption. However if the design of the food safety plan is affected by lack of knowledge of the biological, chemical and physical (BCP) hazards of the food production process, then food safety may not be assured. This paper focuses on three problems when analysing the microbiological hazards that can affect the quality of the food safety plan and which may result in unsafe food and a false sense of security for the food manufacturer.

The seven principles of Hazard Analysis Critical Control Point (HACCP) (Table 1)[1,2], used to design a food safety plan, are well known by the food industry worldwide and an overview of the principles can be taught in one training session.

However, this does not mean that the HACCP team (which may be only one person in a small food company) has the expertise to actually identify all the BCP hazards that could occur or understand whether the hazards pose a significant risk, as is required in Principle 1 (and is necessary for effective design of Principles 2 to 7). What will be the effect on safety if, due to lack of knowledge and education, a hazard is not identified and is therefore left out of the plan, or a potentially serious hazard is classified as not significant?

In order to identify potential hazards a process flow diagram (PFD) should be constructed showing each step in the process from raw material purchase and receival to finished product storage and delivery[1,2]. The *first problem* can arise when designing the PFD. The PFD can be very complicated as food can be multicomponent. If the HACCP team, *overlooks a step* when drawing the PFD, such as thawing of frozen ingredients, a cooling step or delay after mixing, the hazards for these steps such as growth of pathogens and the associated time and temperature controls for safety will not even be considered.

Once the PFD is accurate and complete, the BCP hazards at each step of the PFD must be identified. Principle 1 states that all the potential hazards that are reasonably likely to occur must be listed[1,2]. The *second problem* is therefore when the team *does not recognize a potential hazard* at a step. When considering the microbiological hazards, the HACCP team's knowledge of the likely foodborne pathogens will be critical for safety. Table 2 lists the most common microbial pathogens present in food and also lists common chemical and physical hazards[3].

However it is important to keep microbial pathogens in perspective. Not all pathogens can be found in all foods. Not all foods can support pathogen growth and survival. Some pathogens will enter the food in the raw material. Other pathogens will enter the food during processing, such as handling and packaging or from the factory environment. Figure 1 shows an example of this by illustrating important biological hazards that should be considered at various steps in the production of sushi containing seafood[4–12]. This figure shows that specific scientific knowledge of the ecology of foodborne pathogens is essential.

Once all the hazards have been identified, the next question that must be considered under Principle 1 is whether the hazard is significant for safety of the food. To determine whether a hazard is significant an assessment should be made of the level of probability of the occurrence and the level of severity if the hazard is present[1,2]. How will the HACCP team determine the significance of the hazard or quantify it? Again, knowledge of the ecology of foodborne pathogens is essential to answer these questions and if the answers are not known, the HACCP team must know how to source or research the information. The *third problem* is therefore related to a lack of knowledge about the level of severity or probability of the hazard occurring, in which case *the significance of the hazard may be wrongly evaluated*.

Examples of questions for consideration by the HACCP team to identify evaluate and control a potential microbiological hazard are: does the pathogen produce spores or a toxin; can the pathogen, its spores or a toxin enter via an ingredient or contaminate the food

Table 1. The seven principles of HACCP, used to identify, evaluate and control food safety hazards.

Principle
1 Conduct a hazard analysis
2 Determine critical control points
3 Establish critical limits
4 Establish a system to monitor control of critical control points
5 Determine corrective actions
6 Verify the system
7 Establish documentation and record keeping

during storage, handling, processing, packaging or distribution; what are the characteristics of the food or an ingredient (such as pH, water activity, nutrient content) that could allow the pathogen or its toxin to grow or be produced in this food or an ingredient; what effects will temperature, production environment, equipment and treatment of the food during processing and packaging have on the survival or growth of the pathogen or toxin; is there a past history of the pathogen causing foodborne disease in this food and if so what is the frequency; is there an estimate of the minimum infectious dose; would the effects of the hazard be moderate, serious, severe or critical for the general population or for vulnerable people if exposed to it; and is there an established Food Safety Objective (FSO) for this pathogen in this food[1,2,13,14]? These questions indicate the importance of the HACCP team's knowledge of how to access resources such as scientific literature, information from specific food industries, government and international organizations and experts in the field, to determine if a pathogen is a significant hazard and therefore needs a critical control point for safety .

There are many examples of weakness in the hazard analysis of a HACCP plan resulting in foodborne disease outbreaks. One example, as described by Dillon[15], was highlighted when a brand of infant formula was recalled due to suspected *Salmonella* cases. Contamination levels were previously found below the accepted adult

Table 2. Examples of biological, chemical and physical (BCP) hazards that are commonly considered in a food.

B = Biological	C = Chemical	P = Physical
Bacteria	Pesticides	Glass
Listeria monocytogenes	Machine lubricants and inks	Packaging materials
Clostridium botulinum/perfringens	Cleaner and sanitiser residues	Pieces of machinery
Pathogenic E. coli	Antibiotics	Wood splinters
Staphylococcus aureus	Heavy metals	Bristles
Bacillus cereus	Allergens	Plastic
Campylobacter jejuni/coli	Veterinary residues	
Vibrio parahaemolyticus/cholerae/ vulnificus	Plastics additives	
Yersinia enterocolitica		
Cronobacter sakazakii		
Shigella spp.		
Other biological		
Viruses		
Fungi		
Algae		
Protozoa		
Parasites		

Figure 1. Important biological hazards for consideration in the production of sushi containing seafood.

infectious dose and the possibility of susceptibility of infants had not been adequately considered in the product formulation or the initial HACCP study. Another example was snack salami with finger thick dimensions (1 cm diameter). In this case the method of production continued as for normal salami (7–8 cm diameter) but the surface area to mass ratio was critically different. The snack salami therefore dried much faster resulting in the growth of the fermenting bacteria being suppressed sooner and production of acidity being incomplete. This allowed *Salmonella* to survive and cause food poisoning[15].

In a national survey of over 1000 food businesses undertaken by the Food Safety Authority of Ireland, 40% of food industry personnel stated that the major barrier to implementing HACCP plans was lack of knowledge, but only 20% thought that understanding food microbiology and the ecology of microorganisms in food was important[16]. This highlights a lack of understanding of the importance of education in this area for food safety.

This article has described challenges when identifying and evaluating microbiological hazards that may affect the quality of the HACCP plan. Many national and international companies have already successfully invested resources in this area and, as a result, these companies have ensured identification of existing hazards necessary for control. It is acknowledged that this may be harder for smaller food companies or those in countries with access to fewer resources. However, the knowledge gained by investing in quality education in microbial ecology of food will ultimately benefit food businesses, consumers and the industry, as it will be an added insurance of safe food.

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Biography

Prue Bramwell is a Senior Lecturer in the School of Applied Science at RMIT University. She has over 20 years' experience in food microbiology and has been an educator in the fields of food microbiology and HACCP for over 15 years. Her research interests are in methods for the isolation and identification of foodborne microbes.

Microbiological testing of foods: what, why, how

Agnes Tan

Microbiological Diagnostic Unit (MDU) Public Health Laboratory
Melbourne, Vic, Australia
Tel: +61 3 8344 5701
Fax: +61 3 8344 7833
Email: agct@unimelb.edu.au

Numani Weerasuriya

Standards Australia
Sydney, NSW, Australia
Tel: +61 2 9237 6131
Fax: +61 2 9237 6010
Email: Numani.Weerasuriya@standards.org.au

Complexity in food testing arises from the food (matrix), the need to detect low numbers of target microorganisms in the presence of potentially similar background microflora, the potential use of testing to demonstrate compliance and the high cost (not just financial) of getting it wrong. Microbiological criteria for food specify the method of analysis[1] because "test results are dependent on the analytical method used"[2]. Several bodies are involved in the development of standardised methods, and laboratories may have to run several methods for the same target to meet client needs. The current review of Standard 1.6.1 of the Food Standards Code[3] and the July 2012 collaboration agreement struck between the International Organization for Standardization (ISO) and the Association of Analytical Chemists (AOAC International)[4] should hopefully reduce the workload for food laboratories.

Testing food

Microbial contamination is not uniform throughout a food[5] and test results may not paint the right picture if an unrepresentative sample is examined. Mitigation of the risk of reporting on an unrepresentative sample includes the use of at least 10 g sample for testing and, when the results are used for assessing the quality of a batch of product, the use of a sampling plan which requires the removal of a number of samples for testing.

From a food safety perspective, there is a desire to detect low levels of pathogens in a food because of the potential for multiplication in the time between production and consumption. A minimum of 25 g is routinely taken for the examination for pathogens such as *Salmonella* and *Listeria monocytogenes*.

Food microbiologists must also consider the need to optimise the recovery of their target. Processing of foods may cause sub lethal injury and although the microorganisms are viable (and pose a potential health risk), they may not be culturable. The use of a resuscitation step in pathogen testing is designed to overcome, at least in part, this problem. Some food ingredients may inhibit the growth and subsequent isolation of the target organism. For example, the antibacterial compounds in cocoa containing products,

such as chocolate, must be neutralised by skim milk when testing for *Salmonella*[6].

Tests for indicator organisms and enumeration of bacteria are very much a feature of food (and water) microbiology. Indicator organisms are used to assess the potential for the presence of pathogenic bacteria. Tests for indicator organism/s seek out those microorganisms that are universally present in human faeces in greater numbers than faecally transmitted pathogens. Such tests must be reasonably easy and cheap. Non-specific bacterial counts, such as Standard Plate Counts provide a measure of the amount of bacterial load in a food, and are used to provide some measure of its microbiological quality.

Context for testing

The context for testing dictates what tests are appropriate. When testing is part of quality assurance programs, the aim is to verify that products have been suitably processed and that hygienic production conditions have prevailed. Tests for indicator organisms and bacterial count are common.

Testing is also used to meet product specification/s in trade agreements and to verify compliance with microbiological criteria set by regulators. A combination of tests for indicator organisms, Standard Plate Counts and pathogens is normally used. Such results can have financial and legal ramifications and it is important that they stand up to independent scrutiny. That is, results must be valid (do they provide a true picture of the item examined?) and reproducible.

Standard methods

It was recognised back in the 1890s, that results generated by different laboratories could not be compared "because of the substantial lab to lab variation in methods"[7] and that there was a need for standardised methods.

Standard methods are consensus methods and aim to be the best practicable. That is, they must have acceptable test performance characteristics, be able to generate a timely result (results must be available before foods are consumed or past their shelf-life), do not need specialised equipment or special training of the analyst, and the cost of analysis must not be so prohibitive as to prevent its widespread use[1,8]. The first manual of standard methods (for water analysis) was published in 1905[7,8].

Standard methods: who does what

Today, a large number of organisations, such as the International Organization for Standardization (ISO), the European Committee for Standardization (CEN), the North American based Association of Analytical Chemists (AOAC International) and Standards Australia are charged with developing standard methods. The decision on which standard method to follow is largely driven by trade agreements and regulatory compliance. ISO works closely with CEN and their joint ISO/EN standard methods are referenced in European Commission regulation[2]. Thus, ISO/EN methods are widely used in Europe and AOAC methods, in North America; and their trading partners follow suit.

Method standardisation in Australia

Standards Australia is recognised by the Australian Government as the peak non-government standard organisation. In the early days of method standardisation, Standard methods were developed along industry lines. Thus, there were the AS 1142 series for eggs and egg products; AS 1095 series for the dairy industry and AS/NZS 1766 series for food. Methods were developed by technical committees in response to requests from stakeholders, mostly regulatory agencies. This was obviously duplicitous and, commencing in 1987, the egg and dairy methods were transferred to the AS 1766 series.

Around 2001, the Standards Australia Food Microbiology Committee proposed to adopt international standards whenever possible, in line with Australia's obligation under the World Trade Organization Treaty on (the reduction of) Technical Barriers to Trade (WTO TBT)[9]. A restructure of Standards Australia Food Technology committees was then undertaken so that the local committee structure aligned with those in ISO/TC 34 (Food Products) and a new technical committee, FT-024-01 (renamed FT-035 in 2011) was constituted to mirror ISO/TC 34/SC 9, Microbiology and ISO/TC 34/SC 5, Milk and Milk Products (dairy microbiological test methods)[8]. The AS 5013 series commenced with this change. Most of the standards in this series are ISO clones, some with Australian Annexes which either clarify requirements in the standard or document variations that apply in Australia.

Microbiological criteria/Food Standards

Microbiological criteria (referred to, in Australia, as Food Standards) are set to protect public health. Standard 1.6.1 of the Australian New Zealand Food Standards Code "lists the maximum permissible levels of foodborne microorganisms that pose a risk to human health in nominated foods, or classes of foods"[10]. This Standard prescribes the methods of analysis – AS/NZS 1766 for food and AS 4276 for packaged water in line with Codex Alimentarius recommendations[1]. Alternative methods may be used, but they must be demonstrated (using AS/NZS 4659) to be equivalent to that prescribed.

Methods are prescribed in legislation[1,2,10] as that ensures that the same measures are used to assess compliance. However, at times the legislative tool lags behind changes in the standardisation

community. This has been the case in Australia where the current methods referred to in the Food Standards are no longer available: since 2004, the AS/NZS 1766 methods have been gradually migrated across to the AS 5013 series. This is expected to be rectified soon as Standard 1.6.1 is currently under review[3].

Looking forward

Many of us have realised that the ISO methods did not meet our needs and we hope to influence the development of ISO standards by attendance at the annual plenary meeting and more importantly, through participation in method development working groups. Australia is currently represented in 6 working groups: for meat and meat products, method validation, *Cryptosporidium* and *Giardia* in foods, General requirements and guidance for microbiological examinations and psychrotrophic microorganisms.

At the international level, ISO is collaborating on an AOAC project [International Stakeholder Panel on Alternative Methods (ISPAM)] on the harmonisation of the microbiological criteria for alternative methods (i.e. validation and verification requirements) and in June 2012, ISO and AOAC International signed a cooperation agreement that will allow them to jointly develop and approve common standards. This will hopefully eliminate the need for laboratories with a wide client base to run several methods for the one target.

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Biographies

Agnes Tan is a Senior Scientist at the Microbiological Diagnostic Unit. She is a member of several Standards Australia committees and is the recipient of the 2004 Standards Award for Outstanding Service – Community and Materials. She chairs the *Legionella* methodology (FT020-01) and Food Microbiology (FT035) committees and has represented Standards Australia on ISO TC34/SC9 – the mirror committee for food microbiology, since 2007.

Numani Weerasuriyais a Projects Manager of Standards Australia. She is responsible for a broad range of technical committees in Standards Australia. Until recently, Numani was managing the FT-035 – Food Microbiology Committee. Numani facilitates the development of international consensus publications by coordinating Australian participation in the standardisation process in ISO/TC 34 and ISO TC 147/SC 4.

Advanced food preservation technologies

Roman Buckow

CSIRO, Animal, Food and Health Sciences, Werribee, Vic. 3030, Australia.

Michelle Bull

CSIRO, Animal, Food and Health Sciences, North Ryde, NSW 1670, Australia.

Food preservation has been practiced by humans for millennia through fermentation, salting and drying. The industrialisation of food manufacture brought processes like canning and freezing to control microbial safety and enzymatic spoilage of foodstuffs. However, this often comes at the expense of nutritional and sensorial quality attributes and, thus, novel food processing technologies continue to be developed to serve the increasing demand for healthy and eco-friendly food products. In contrast to thermal processing, these new technologies make use of physical stressors other than just heat to kill microorganisms, using high pressure, electric fields, cool plasma or ultraviolet irradiation. The underlying inactivation mechanisms, efficiencies and limitations of these technologies are currently still under investigation and will be highlighted in this paper.

High pressure processing

High pressure processing (HPP) is a way to modify and preserve food without using heat. HPP normally involves subjecting food to hydrostatic pressures of 300 to 700 MPa for periods of a few minutes. This treatment inactivates vegetative microorganisms and some enzymes at room temperature, whilst valuable low molecular constituents, such as vitamins, colours and flavourings, remain largely unaffected. Therefore, HPP is increasingly used by the food industry to produce safe and fresh-like food with enhanced nutritional and functional properties and extended shelf life. Currently, there are approximately 200 industrial HPP systems installed worldwide, producing more than 300,000 tons of food per annum. In the Australian market, HPP food includes small goods, fruit juices, vegetable purees, and wet salads.

The efficacy of HPP is governed by Le Chatelier's principle, which states that reactions or phase transitions associated with a decrease in volume are favoured, whilst those accompanied with a volume increase are inhibited. Low molecular weight molecules in food such as peptides, lipids and saccharides are rarely affected by HPP because of the very low compressibility of covalent bonds at high pressures[1]. On the other hand, macromolecules, such as proteins and starches, can change their native structure during HPP, in a manner analogous to thermal treatments[2].

The viability of vegetative microorganisms is affected by inducing structural changes at the cell membrane or by the inactivation of enzyme systems which are responsible for the control of metabolic actions[3]. At pressures higher than 300 MPa, significant inactivation of vegetative bacteria, yeasts and viruses has been observed at ambient temperature. The rate and magnitude of microbial inactivation is dependent on the applied pressure and temperature as well as environmental factors such as pH, water activity, salts and other antimicrobials. Foodborne pathogens such as enterohemorrhagic *Escherichia coli* and *Listeria monocytogenes*, and food spoilage organisms including *Lactobacillus* spp. (in acidic food), often exhibit high pressure tolerance compared with other bacteria; possibly because of their relatively higher tolerance to other physical and chemical stressors such as heat or acid. Bacteria may also develop increased resistance to pressure due to their prior growth history, e.g. growth of *L. monocytogenes* at higher temperatures[4] or stationary phase cells being more pressure resistant[5].

High pressure thermal processing

Low-acid food (LAF) that is microbiologically safe and stable is not obtainable by HPP at low or ambient temperature. High pressure thermal (HPT) processing can inactivate bacterial spores through high-pressure treatment at 600 MPa with initial temperatures above 60°C[6]. Accelerated and homogeneous heating and cooling of food occurs during HPT processing from the increase in temperature

accompanying the physical compression of the product. This facilitates uniform heating of all food packs and also reduces the need for excessively long heating times. HPT products have improved food quality attributes, such as flavor, texture, nutrient content and color, compared with thermal processing, as they receive less heat damage[7].

Of particular interest for ambient stable LAF is the ability of a HPT process to inactivate spores of the major bacterial spore-forming pathogens of concern, which are proteolytic strains of *Clostridium botulinum*. HPT processed LAF with extended chilled shelf-life will need to have demonstrated safety with respect to psychrotrophic *C. botulinum*. HPT processing conditions for the inactivation of non-proteolytic *C. botulinum* spores are more moderate than required for inactivation of proteolytic *C. botulinum*[8].

The combination of high pressure and heat is often more effective than under equivalent heat-only conditions, i.e. synergistic, for various species, including *C. botulinum* (psychrotrophic and non-psychrotrophic strains), and relevant spoilage-associated sporeformers[6,9,10]. The amount of synergy observed, however, is affected by both the product and the bacterial strain under observation.

The mechanism of spore inactivation has been primarily studied in *Bacillus subtilis*; high pressure initiates spore germination via at least two mechanisms dependent on the magnitude of pressure applied[11]. At moderately high pressure (50–300 MPa), the spore nutrient receptors are activated and germination proceeds down the nutrient-triggered pathway[12,13]. Very high pressures (400-800 MPa), however, trigger the release of calcium dipicolinic acid (DPA), possibly by opening the DPA channels in the inner membrane or via another action on the inner membrane, and subsequent germination and heat sensitivity[12,13].

Pulsed electric field processing

Pulsed electric field (PEF) processing involves the application of very short, high voltage pulses to a food which is placed between or pumped through two electrodes. Typically, several thousand volts per cm applied for 20 to 1000 µs are required for effective microbial inactivation. The sensitivity of microorganisms to PEF depends on cell characteristics such as structure and size[14]. In addition, extrinsic factors such as product pH, water activity, soluble solids and electrical conductivity affect the decontamination efficiency of the technology.

Although the underlying mechanisms are not yet fully explained on a molecular basis, PEF treatment disturbs and perforates microbial cell membranes[15]. It is likely that the loss of cell membrane functionality through PEF is due to formation of hydrophilic pores in the membrane and the forced opening of protein channels. The applied electrical field causes changes in the conformation of phospholipids, leading to rearrangement of the membrane and formation of hydrophilic pores.

PEF, when combined with low to moderate temperatures (<50°C), effectively inactivates microbial cells but does not significantly change flavour or nutrients. This makes it a promising alternative to conventional thermal preservation processes for liquid food that contains heat labile bioactive or volatile components such as fruit and vegetable juices. Currently, PEF is commercially used in Europe to extend the chill-stability of fresh fruit juices and smoothies from 6 to 21 days[16].

Cool plasma

Cool plasma is an ionised gas state, generated from gas or liquids treated with a power source such that it becomes temporarily excited to the point of partial ionisation. Interest in cool plasma for food processing has increased with technology breakthroughs allowing processing at larger scale and at atmospheric pressures. For food applications, nitrogen, air or oxygen are typically used.

Cool plasma is only suitable for surface treatments; however, it has advantages over most other methods of decontamination as it does not require water or chemicals, leaves no chemical residues, and may be applied to thermally sensitive materials. Cool plasmas consist of a number of components affecting biological systems, including charged particles (electrons and ions) as well as free radicals, excited state atoms and molecules, other reactive species, ultraviolet (UV) photons and transient electromagnetic fields[17].

There are a number of chemical and physical mechanisms, probably acting synergistically, by which cool plasma treatment may inactivate microorganisms. Microbial nucleic acids (DNA and RNA) damage may be induced by direct UV radiation; cell membranes may be damaged by diffusing free-radicals or excited state molecules; unstable compounds may be formed at the microbial surface through adsorption of radicals; membranes may be disordered through the electrostatic tension of plasma electrons and ions accumulated at the cell surface; or plasma ions may induce oxidation reactions within the cell causing inactivation[18].

Possible applications for cool plasma treatment include food contact surface decontamination, where it can be very effective for the inactivation of microorganisms, including bacterial spores, on glass, stainless steel and plastics[19]. Cool plasma treatment of more complex surfaces, including food, is more challenging due to the limited

penetrative capacity of plasmas; however, sufficient inactivation of pathogens has been observed on meat and produce surfaces[20–22]. Research on the influence of factors such as microbial load, microbial growth history, biofilms and the role of critical processing parameters on cool plasma effectiveness is still ongoing.

Ultraviolet light processing

UV light (200–310 nm) has been widely used in the food industry for disinfection of food and surfaces such as packaging materials or bottles. Similar to cool plasma, UV light, especially wavelengths around 250 nm, damages microbial DNA preventing microorganisms from replicating their genetic material. The sensitivity of microorganisms to UV light is dependent on their cell wall structure and thickness, their ability to repair UV damage, and the environment such as pH or the presence of UV absorbing proteins. In general, Gram-positive bacteria are more resistant to UV light than Gram-negative bacteria, however, the difference between vegetative bacteria like *E. coli* K-12 and *Listeria innocua* was not considerable[23]. Protozoa and algae are very UV resistant, possibly because of enhanced DNA repair mechanisms[24]. The efficacy of UV light to decontaminate food and food surfaces is dependent on its penetration capabilities which may be affected by food composition including the presence of colour compounds, organic solutes and suspended matter. For example, UV absorption of milk is approximately 10 and 10^5 times higher than clear apple juice or water, respectively.

Degradation of food quality can occur as a result of photochemical reactions during UV light processing. The following nutrients are considered "light sensitive": vitamins, tryptophan, and unsaturated fatty acid residues in oils, solid fats and phospholipids. Thus, UV processing is not suitable for most dairy products but has potential to extend shelf-life of clear fruit juices and wines with minimal effects on their colour and flavours.

Conclusions

The advanced food preservation technologies presented here represent many opportunities for the food industry to meet contemporary retail and consumer desires for convenient food that is fresh tasting, reduced in (chemical) additives, microbiologically safe and have an extended shelf life. Technological breakthroughs, advances in equipment design and methodologies for measuring the critical process factors will improve our ability to assess and control the performance of novel processes. Continued research into inactivation kinetics and the mechanisms of microbial inactivation will contribute to the validation of these processes and, therefore, possible applications and uptake by the food industry.

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Biographies

Roman Buckow holds a PhD in Engineering from the Berlin Institute of Technology, Germany. In 2006, Roman joined Food Science Australia (now CSIRO) to complete his postdoctoral research fellowship in the area of novel nonthermal food processing technologies. Roman currently leads the Process Engineering Science Research Group of CSIRO which focuses on process systems engineering, separations science and delivery systems to enable sustainable transformation of agri-food materials into safe and healthy food ingredients and products. Roman's research interests include designing new food structures and enhancing the nutritional value and safety of processed foods by novel food preservation technologies and processes, including high pressure, pulsed electric field, ultrasound and extrusion processing. In addition, he is investigating new opportunities to increase the efficiency and sustainability of conventional and novel food processing technologies. Roman has published more than 40 papers in high impact scientific journals and delivered over 100 presentations at international conferences.

Michelle Bull holds a PhD in Microbiology from the University of Sydney and is a Research Projects Officer within the Microbiology Program of CAFHS. Michelle contributes to multidisciplinary research projects utilising advanced food preservation technologies to enhance the safety and stability of a range of foods. Michelle's current research interest is in understanding the response of pathogens to high pressure thermal processing, from single cell to population level.

Cooking meat at home

Patricia Desmarchelier and Juliana Madden

Food Safety Principles
558 Pullenvale Road, Pullenvale
Qld 4069, Australia
Tel: +61 7 3374 4530
Email: tdesmarchelier@bigpond.com

One of the five keys to safer food promoted by the World Health Organization for consumers is "cook thoroughly" as cooking food properly kills almost all dangerous microorganisms[1]. While this simple message is similarly promoted throughout Australia, beliefs and self-reported behaviours among consumers concerning cooking can vary. Here we describe consumer surveys on cooking meat as an example.

Preparing food at home remains a common practice for most Australians. In 2009, when 1,421 people were interviewed about the dinner meal they had the previous night, more than 7 out of 10 meals were prepared at home and common food items were vegetables (92%) and meats, including fish and poultry (90%; MLA, 2011)[2]. In Australia, between 2001 and 2009, 9.8% of 1,025 reported foodborne outbreaks were located in private residences[3]. There are multiple factors within and/or outside the home that could have contributed to these outbreaks although this information is limited. For the consumer and home food preparer, practising basic food safety measures will help to prevent foodborne illness and these include: Clean (wash hands, utensils and surfaces), Separate (prevent cross-contamination), Cook (cook to proper temperature), and Chill (refrigerate promptly to the right temperature)[4].

The Food Safety Information Council (FSIC) is an independent, not-for-profit group supported by Federal, State and Local government agencies, professional, industry and community organisations, and individual members. It provides food safety information for Australian consumers through its online resources, media releases,

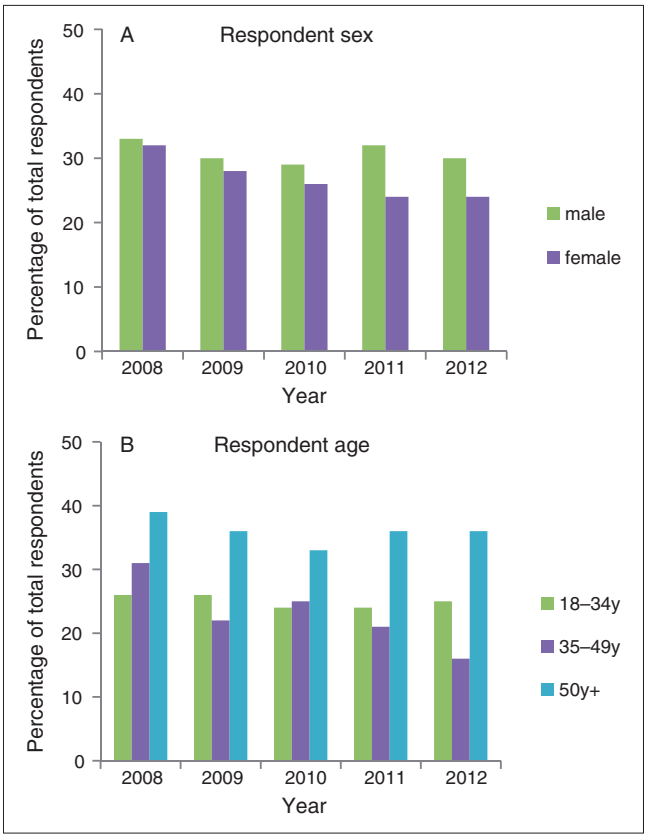

Figure 1. Percentage of respondents in surveys between 2008–1012 that believed steak cuts of beef need to be cooked right through to be safe categorised by (A) sex and (B) age of the respondent.

Figure 2. A thermometer used to determine the adequate cooking temperatures are reached when cooking meat.

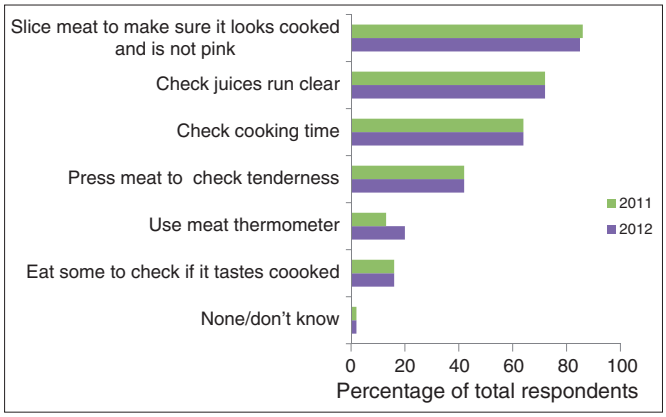

Figure 3. Survey respondents' choice of methods to determine when chicken is cooked and safe to eat in 2011 and 2012.

television and radio community service announcements and Australian Food Safety Week. Each year after Food Safety Week the FSIC contracts a public opinion polling agency to conduct a nationwide survey of consumer knowledge and self-reported behaviours related to its key food safety messages. The survey is conducted by telephone among persons over 18 y (>1,000) selected by a random process including capital and non-capital cities and subdivisions, telephone numbers and household positions; return calls for those frequently away; and, to reflect population distributions results are post-weighted using Australian Bureau of Statistics data. Here, we present some results of FSIC surveys related to cooking, one of the key food safety measures, and cooking meat, one of the most common foods cooked.

Between 2002 and 2012, respondents have been asked which of a selection of meats and meat products must be cooked all the way through to avoid food poisoning. Respondents have consistently believed chicken (>97%) was most important followed by sausages (overall range 86–92%) and hamburgers (overall range 78–84%). The belief hamburgers should be thoroughly cooked had a downward trend to 79% in 2012 mainly among younger adults, males, university educated and highest income groups. This trend should be monitored as consuming hamburgers has been identified as a risk factor for Shiga toxin-producing *Escherichia coli* in Australia[5]. About a third of respondents believed beef steaks should be thoroughly cooked with a progressive and significant downward trend among females and 35–49 y olds (Figure 1). This practice is safe unless steaks are not intact through tenderising, or, if marinade is internalised during vacuum tumbling. Pork was included in 2011 with 85–86% believing this meat required thorough cooking, particularly older respondents, non-city dwellers, and households with children.

This may reflect continuing misbeliefs about parasites present in pork (DAFF, 2004)[6].

Rolled roast meats need to be thoroughly cooked to inactivate internalised vegetative cells and were included in 2011 and 2012. Responses were consistent, though varied, with meat species: 87–86% believed boneless rolled turkey and 44–43% believed boneless rolled lamb roasts needed to be cooked through; about half of respondents over 50 y believed the latter. Further investigation is required as consumers may differentiate cooking requirements based on the meat species, although they may not understand the requirements for different preparations e.g. when internalising surfaces and/or ingredients not directly exposed to heat.

The surest way to determine when meats reach safe cooking temperatures is using a thermometer[7] (Figure 2). In 2011 and 2012, 23–25% respondents claimed to have a meat thermometer in their home. Ownership was negatively linked with low household income and those over 65 y. Of those owning a thermometer in 2012, 44%, 35% and 10% claimed to have used it ≤1, 2–12 and >12 months ago, respectively. Thermometer use has been actively promoted for domestic use more recently in Australia. In the United States of America thermometers have been promoted for longer and ownership was reported to have increased to 70% and linked with socio-economics[7]. In the USA self-reported usage varies with meats e.g. roasts, followed by chicken and hamburgers. Continuing promotion of usage and retail availability of appropriate thermometers should be encouraged.

Chicken dishes have been commonly attributed in foodborne illness outbreaks in Australia[3]. Thermometers are recommended to test that safe temperatures are reached during cooking. Ensuring the meat is not pink and the juices run clear has been widely recommended, although this is not always a reliable indicator of reaching safe temperatures or "doneness". Pink colour can be due to characteristics of the bird, storage conditions, ingredients or marinades. Respondents were surveyed from 2011 on how they check if poultry is cooked and safe to eat (Figure 3). Most respondents, both years, claimed to test for colour change in flesh (85%) and juices (72%) and 62% used both; a lesser number used cooking time (64%) and tenderness (42%). Of concern is the 16%, mainly males, and lower income and education level groups, who "eat some to see if it tastes cooked". These results flag an ongoing need to inform consumers on the hazards of consuming undercooked chicken and safe cooking practices. On the other hand, the use of a meat thermometer increased from 13% to 20% indicating a promising increased awareness of this more safe method.

The results presented are beliefs and self-reported behaviours that have not been validated by observations. However, the value of these results is a consistent methodology, following food safety campaigns over 10 years, providing trends linked with demographic data. Just one of the surveyed issues is presented here to illustrate how food safety messages are perceived and practiced, and this could guide consumer information messages.

Acknowledgements

The FSIC acknowledges Newspoll who helped design and conduct the surveys and all previous FISC Executive members who initiated and have sustained the activity. Also acknowledged are the Australian Government Department of Health and Ageing, the major sponsor, and other Australian, State and Territory Government Agencies, commercial enterprises, Professional Associations, Local Government and individual members who support the FSIC.

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Biographies

Dr Trish Desmarchelier is a consultant in food safety specialising in bacterial foodborne pathogens and food safety risk management. She is the current Convenor of the Food Safety Information Council's Technical Group.

Ms Juliana Madden is a science communications consultant specialising in food issues. She has been the Executive Officer of the Food Safety Information Council since 2007.

ASM NZ Postgraduate Research Travel Award, 2012

Jayne Manning

Thank you for the great opportunity to attend my first international scientific meeting, the New Zealand Society for Microbiology 2012 conference, and for the chance to visit the laboratory of my collaborator Professor John Tagg, co-discoverer of the probiotic bacterial strain that is the focus of my PhD project.

My time in New Zealand was spent at The University of Otago in Dunedin, where I presented a poster at the 4 day conference and was then based in the laboratories of BLIS Technologies Ltd, located in the Centre of Innovation at the University, for 8 days.

BLIS Technologies Ltd, of which Professor Tagg is a principal scientific consultant, holds the rights to unique collection of bacteriocin-like inhibitory substance (BLIS)-producing bacteria, including *Streptococcus salivarius* K12, which was originally isolated by Professor Tagg and colleagues at the University's Department of Microbiology and Immunology. BLIS Technologies Ltd produce K12 in the form of a probiotic throat lozenge aimed to prevent pharyngitis and halitosis (bad breath), available commercially for more than a decade.

Together, The University of Otago and BLIS Technologies Ltd have published numerous studies investigating and characterising BLIS produced by various bacteria. Their findings show strain K12 has the ability to inhibit pathogenic respiratory bacteria such as *Streptococcus pyogenes* via several megaplasmid-encoded bacteriocins (salivaricins). Fellow commercial probiotic *S. salivarius* strain M18 also harbours megaplasmid-encoded salivaricins. In our laboratory K12 has been shown to inhibit colonisation of epithelial cells by *Streptococcus pneumoniae* (the pneumococcus) *in vitro*. The primary focus of my PhD is to investigate the mechanism of this inhibition. Experiments performed in the BLIS laboratories allowed me to investigate whether these *S. salivarius* megaplasmids are required for production of BLIS active against pneumococci. I became competent in the technique of deferred antagonism testing, analysing the BLIS activity of 10 *Streptococcus salivarius* strains against pneumococcal isolates shipped to New Zealand from our laboratory for this purpose.

Jayne Manning with Professor John Tagg.

My results showed *S. salivarius* strains harbouring known salivaricins all produced BLIS capable of inhibiting the growth of all pneumococcal isolates tested, suggesting salivaricins are required for *in vitro* BLIS activity against pneumococci.

K12 and M18 megaplasmid-negative strains did not produce BLIS activity against pneumococci (Fig. 1), suggesting these megaplasmids mediate BLIS production in these strains, likely due to their encoded salivaricins.

Furthermore, K12 strains harbouring only salivaricin A2 (A234) or salivaricin B (NR) were tested to investigate the specificity of the K12 salivaricins. While each salivaricin was capable of producing BLIS activity against all pneumococcal strains tested, salivaricin B showed significantly stronger inhibition (data not shown).

Overall, these results add to already published evidence that *S. salivarius* bacteriocins are important for the *in vitro* inhibition of respiratory pathogens, and suggest that for strains K12 and M18 *in vitro* pneumococcal inhibition is megaplasmid-mediated. I am excited to observe if these megaplasmids are also necessary to inhibit pneumococcal colonisation in the tissue culture pharyngeal cell adhesion model I am currently developing.

In addition to the laboratory-based experience and results I gained in New Zealand, I was able to practice my networking and presentation

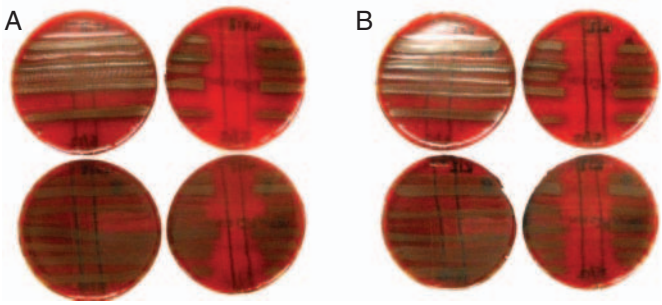

Figure 1. Deferred antagonism test. Example of deferred antagonism test of 9 pneumococcal isolates. (A) K12 (right) and K12 megaplasmid negative (left); (B) M18 (right) and M18 megaplasmid negative (left). Briefly, *S. salivarius* strains were inoculated vertically on human blood agar plates, culture was removed following overnight incubation and plates were treated with chloroform. Pneumococcal isolates were then inoculated horizontally across the plate and incubated overnight. Inhibition of pneumococcal growth in the area where the *S. salivarius* inoculum was present was interpreted as the *S. salivarius* strain producing BLIS active against the pneumococci.

Jayne with the BLIS team.

skills at both the conference and during a company presentation given at BLIS Technologies Ltd. I also had the opportunity to hear about a diverse range of interesting microbiology research while at the conference. Aside from Professor Tagg, I had the opportunity to meet with five scientists specifically interested in my field from several university departments and biotechnology companies including John Hale and Philip Wescombe from BLIS Technologies Ltd, Nick Heng from the Oral Sciences Department, Otago University Dental School and Dan Power from Life Technologies, who generously found time to meet and discuss my project, enlightening me to information outside of the scope of my current literature searches. I also gained valuable insight into the structure of a biotechnology company, extending my knowledge of possible post-doctoral career paths.

I thank Professor Tagg and the team at BLIS Technologies (especially John Hale and Philip Wescombe) for having me in their lab and sharing their wealth of *S. salivarius* knowledge, and I look forward to continuing our future collaborations. Finally, I thank the ASM once again for this much appreciated opportunity.

Biography

Jayne Manning is completing her PhD in the Pneumococcal Research Group at the Murdoch Childrens Research Institute.

ASM, Parasitology and Tropical Medicine SIG

Master Class 2013 Adelaide 1–2 March 2013

Andrew Butcher

National Convenor 2012–2013
ASM, Parasitology and Tropical Medicine SIG
Email: andrew.butcher@health.sa.gov.au

The ASM, Parasitology and Tropical Medicine Special Interest Group held the 4th Master Class in Adelaide on 1–2 March 2013. The Master Class coincided with the Adelaide Fringe, Arts Festival and Clipsal 500 V8 car race but more importantly with the visit to Australia of Lynne Garcia, who was in Melbourne to present at the RCPA Update 2013 program. Lynne's visit to Australia was kindly sponsored by the RCPA. The Parasitology SIG would like to acknowledge the on-going support of the RCPA and ASM as we partner in the on-going education of technicians, scientists and medical trainees in diagnostic parasitology. We also acknowledge the generous support of Thermofisher, BioPharm, SA Pathology and University of SA who provided financial and facility support to host the Master Class.

Lynne Garcia was the keynote speaker for the Master Class. Lynne was the Manager of the UCLA Clinical Microbiology Laboratory before her retirement a few years ago. Now she is the Director of LSG & Associates, providing training, teaching, and consultation for Diagnostic Medical Parasitology and Health Care Administration. With over 400 presentations (international, national, and local), over 175 peer-reviewed manuscripts, book chapters, and books, including *Diagnostic Medical Parasitology* (5th edn, 2007) and *Practical Guide to Diagnostic Parasitology* (2nd edn, 2009), Lynne is considered to be one of the world-leading experts in diagnostic parasitology.

The Master Class program consisted of a full day of interactive seminars with a second day devoted to a hands-on wet workshop. During the seminar program Lynne presented a range of topics related to diagnostic parasitology. In the opening sessions she discussed the importance of

Parasitology Master Class morning tea break with Lynne Garcia.

correct test ordering, specimens collection and preservation along with the various test methods available to ensure the highest quality results. The development of a new non-formalin-based fixative (Total Fix) for the preservation of faecal specimens was presented. The fixative is suitable for a range of permanent stain faecal smear methods as well as commonly used rapid lateral flow EIA tests for *Giardia* and *Cryptosporidium*. Following the morning tea break, Lynne gave a comprehensive review of human intestinal protozoa and free living amoeba. After lunch Lynne reviewed malaria diagnosis highlighting the morphological characteristics of the various species and the common pitfalls in the misdiagnosis of malaria. To complete the picture she then reviewed other common blood parasites.

To complement Lynne's presentations a number of young and emerging scientists with a special interest in parasitology were invited to present short case studies or papers on their research and development work. The first presenter was Tania Sadlon from SA Pathology who detailed her work on the development of an in-house multiplex PCR assay for the routine screening of faecal samples for *Giardia*, *Cryptosporidium*, *Dientamoeba fragilis* and *Entamoeba histolytica*. Tania presented the validation of the method and comparison with microscopy. The multiplex PCR significantly increased the detection rates of all parasites in the multiplex assay when compared with microscopy. This was highlight by a case study of locally acquired Entamoeba histolytica in two families. Tamalee Roberts from St Vincent's hospital Sydney presented a case report on of microsporidial myositis involving a lung transplant recipient infected with *Anncaliia* (*Brachiola*) *algerae*. Tamalee summarised the diagnostic feature of microspordia and the opportunistic infections seen in immuno-compromised patients. She used the case of microsporidial myositis to demonstrate the importance of accurate diagnosis. Shirley Chong from Fremantle Hospital in Western Australia presented a case study of malaria in an African male returning to Australia after a visit to his homeland. The case report helped emphasize the importance of accurate diagnosis and patient history as discussed by Lynne Garcia during her session on malaria. To continue with the malaria theme Franca Azzato from Victorian Infectious Diseases Reference Laboratory presented the versatility of a PCR assay for malaria. Franca presented the development and use of a malaria PCR assay to eliminate any uncertainty in diagnosis. The assay has provided a rapid, sensitive and accurate confirmatory test to assist in the diagnosis of mixed infections; to determine the effectiveness of treatment; and to aid in the detection of any other discrepancies in diagnosis, especially in cases of low parasitaemia. The final case study in the seminar session was presented by Thuy Phan from Concord Hospital in Sydney. Thuy presented a case study of a family with unexplained eosinophilia with the only parasite detected was *Dientamoeba fragilis*. She detailed the family's history and clinical presentation to discuss various aspects of the family's life style which exposed them to the risk of parasitic infections.

The final session of the day 1 seminar series was presented by Harsha Sheorey a consultant Microbiologist from St Vincent's Hospital in Melbourne. Harsha presented a scheme for the identification of common medically important arthropod. He discussed the classification of arthropods, important morphological features and the infestations caused by these organisms. The identification scheme provided by Harsha will greatly assist in the accurate classification of arthropods submitted to laboratories for identification.

Parasitology Master Class wet workshop (Uni SA Lab).

The second day of the Master Class was a hands-on wet workshop held in the University of South Microbiology teaching laboratory. The first session of the morning involved the preparation of faecal smears and concentrations ready for staining and microscopic examinations. Two faecal egg concentration techniques were compared with participants counting egg recovery from each technique. After a relaxing morning tea on the plaza deck of the Uni SA laboratories it was back to the microscope to view a series of case study specimens. This included a range of specimens from protozoa, helminth and arthropod infections. Participants were provided with stained slides or wet mount material to view the target organisms. They were assisted and guided by expert faculty and the projection of microscope-camera live images of the target organisms to facilitate discussion about the case, parasite life cycle and diagnostic morphology.

Expert faculty consisted of Lynne Garcia on the microscope-camera to provide expert comments about the parasite morphology, life cycle, characteristics and diagnostic pitfalls. On the floor, assisting the participants at the bench and presenting discussion on each case were the "old heads" Harsha Sheorey, Norbert Ryan, Gary van Arkadie and Andrew Butcher who were excellently supported by the "young heads" Tamalee Roberts, Shirley Chong, Thuy Phan. It was fabulous to see the competency and enthusiasm of the next generation of parasitology scientists as they presented in the seminar and wet workshop series.

The Parasitology and Tropical Medicine SIG held the AGM at the end of day one. There were a number of new initiatives which have progressed including a Facebook page, shared space on a Google G-Drive to upload images for tele-diagnosis, SIG web presence hosted by the ASM and the establishment of a master class scholarship to support disadvantaged scientist attending future master classes. Participants were encouraged to be a member of ASM and the SIG to access these facilities. Also, ASM members with an interest in Parasitology and Tropical Medicine were encouraged to update their profiles to be an active member of the SIG to ensure they receive the latest news.

As we finish one master class the planning for the next starts. The intention is to hold the next master class in Perth in late 2014 or early 2015 with a focus on Tropical Medicine. Stay tuned for more information as the program develops over the next 12 months or so.

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