# Enterohaemorrhagic *Escherichia coli* in raw beef and beef products: approaches for the provision of scientific advice

MEETING REPORT

WORLD HEALTH ORGANIZATION

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

2011

# Foreword

The Members of the Food and Agriculture Organization of the United Nations (FAO) and of the World Health Organization (WHO) have expressed concern regarding the level of safety of food at both national and international levels. Increasing foodborne disease incidence over the last decades seems, in many countries, to be related to an increase in disease caused by microorganisms in food. This concern has been voiced in meetings of the Governing Bodies of both Organizations, and in the Codex Alimentarius Commission. It is not easy to decide whether the suggested increase is real or an artefact of changes in other areas, such as improved disease surveillance or better detection methods for microorganisms in foods. However, the important issue is whether new tools or revised and improved actions can contribute to our ability to lower the disease burden and provide safer food. Fortunately new tools that can facilitate actions seem to be on their way.

Over the past decade, Risk Analysis—a process consisting of risk assessment, risk management and risk communication—has emerged as a structured model for improving our food control systems, with the objectives of producing safer food, reducing the numbers of foodborne illnesses and facilitating domestic and international trade in food. Furthermore, we are moving towards a more holistic approach to food safety, where the entire food chain needs to be considered in efforts to produce safer food. As with any model, tools are needed for the implementation of the risk analysis paradigm.

Risk assessment is the science-based component of risk analysis. Science today provides us with in-depth information on life in the world we live in. It has allowed us to accumulate a wealth of knowledge on microscopic organisms, their growth, survival and death, even their genetic make-up. It has given us an understanding of food production, processing and preservation, and of the link between the microscopic and the macroscopic world and how we can benefit from as well as suffer from these microorganisms. Risk assessment provides us with a framework for organizing all this data and information and to better understand the interaction between microorganisms, foods and human illness. It provides us with the ability to estimate the risk to human health from specific microorganisms in foods and gives us a tool with which we can compare and evaluate different scenarios, as well as to identify the types of data necessary for estimating and optimizing mitigating interventions.

Microbiological risk assessment can be considered as a tool that can be used in the management of the risks posed by foodborne pathogens and in the elaboration of standards for food in international trade. However, undertaking a microbiological risk assessment (MRA), particularly quantitative MRA, is recognized as a resource-intensive task requiring a multidisciplinary approach. Yet foodborne illness is among the most widespread public health problems, creating social and economic burdens as well as human suffering, making it a concern that all countries need to address. As risk assessment can also be used to justify the introduction of more stringent standards for imported foods, a knowledge of MRA is important for trade purposes, and there is a need to provide countries with the tools for understanding and, if possible, undertaking MRA. This need, combined with that of the Codex Alimentarius for risk-based scientific advice, led FAO and WHO to undertake a programme of activities on MRA at the international level. The Nutrition and Consumer Protection Division, FAO, and the Department of Food Safety and Zoonoses, WHO, are the lead units responsible for this initiative. The two groups have worked together to develop the

area of MRA at the international level for application at both the national and international levels.

This work has been greatly facilitated by the contribution of people from around the world with expertise in microbiology, mathematical modelling, epidemiology and food technology, to name but a few. This Microbiological Risk Assessment series provides a range of data and information to those who need to understand or undertake MRA. It comprises risk assessments of particular pathogen-commodity combinations, interpretative summaries of the risk assessments, guidelines for undertaking and using risk assessment, and reports addressing other pertinent aspects of MRA.

We hope that this series will provide a greater insight into MRA, how it is undertaken and how it can be used. We strongly believe that this is an area that should be developed in the international sphere, and have already from the present work clear indications that an international approach and early agreement in this area will strengthen the future potential for use of this tool in all parts of the world, as well as in international standard setting. We would welcome comments and feedback on any of the documents within this series so that we can endeavour to provide Member countries, Codex Alimentarius and other users of this material with the information they need to use risk-based tools, with the ultimate objective of ensuring that safe food is available for all consumers.

<div style="text-align:center">

**Mr Samuel C. Jutzi**

Officer-in-Charge
Nutrition and Consumer Protection Division
Food and Agriculture Organization of the
United Nations

**Dr M. Maged Younes**

Director
Department of Food Safety and Zoonoses
World Health Organization

</div>

# Contents

# Contributors
# (Meeting participants)

## *Experts*

| | |
|---|---|
| Wayne Anderson | Food Safety Authority of Ireland, Ireland |
| Jeppe Boel | Danish Institute for Food and Veterinary Research, Denmark |
| Sava Buncic | University of Novi Sad, Serbia |
| Francis Butler | University College Dublin, Ireland |
| John Cowden | Health Protection Scotland, United Kingdom |
| Geraldine Duffy | Teagasc, Ashtown Food Research Centre, Ireland |
| Dan Englejohn | United States Department of Agriculture Food Safety and Inspection Service, United States of America |
| Teresa Estrada | Cinvestat, Mexico |
| Seamus Fanning | University College Dublin, Ireland |
| Aamir Fazil | Public Health Agency of Canada, Canada |
| Ian Jenson | Meat & Livestock Australia, Australia |
| Micheál O'Mahony | Food Safety Authority of Ireland, Ireland |
| Mark Powell | United States Department of Agriculture Office of Risk Assessment and Cost-Benefit Analysis, United States of America |
| Emma Snary | Veterinary Laboratories Agency, United Kingdom |

## *Resource person*

Alan Reilly      Food Safety Authority of Ireland, Ireland

## *Declarations of interest*

All participants completed a Declaration of Interest form in advance of the meeting. None were considered to present any potential conflict of interest.

## *Acknowledgements*

# *Abbreviations*

| | |
|---|---|
| A/E | attaching and effacing [lesions on the surface of epithelial cells] |
| CAC | Codex Alimentarius Commission |
| CCFH | Codex Committee on Food Hygiene |
| CDC | Centers for Disease Control and Prevention [USA] |
| cfu | Colony forming units |
| EHEC | Enterohaemorrhagic *Escherichia coli* |
| FAO | Food and Agriculture Organization of the United Nations |
| FDA | Food and Drug Administration [of the United States of America] |
| FSAI | Food Safety Authority of Ireland |
| FSIS | Food Safety and Inspection Service [USDA] |
| GHP | Good hygiene practice |
| GMP | Good manufacturing practice |
| HACCP | Hazard analysis and critical control points |
| HC | Haemorrhagic colitis |
| HUS | Haemolytic uraemic syndrome |
| ICMSF | International Commission on Microbiological Specifications for Foods |
| JEMRA | Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment |
| MLA | Meat and Livestock Australia |
| MLE | Maximum likelihood estimates |
| PFGE | Pulsed field gel electrophoresis |
| QMRA | Quantitative microbiological risk assessment |
| ST | Shiga toxin |
| Stx | Shiga toxin |
| STEC | Shiga toxin producing *E. coli* |
| Teagasc | Agriculture and Food Development Authority [of Ireland] |
| TTP | Thrombotic thrombocytopaenic pupura |
| USDA | United States Department of Agriculture |
| VT | Verocytotoxin or verotoxin |
| VTEC | Verotoxigenic *E. coli* |
| WHO | World Health Organization |

# 1. Introduction

## *1.1 Background*

*Escherichia coli* O157:H7 is a member of the enterohaemorrhagic *E. coli* (EHEC) group of and was first identified as a human pathogen in 1982 when strains of a previously uncommon serotype, O157:H7, were implicated in two outbreaks of haemorrhagic colitis (HC) in the United States of America (Riley et al., 1983). Since then, outbreaks and sporadic cases of EHEC O157:H7 infection continue to be reported in a number of countries throughout the world. Outbreaks of infections from non-O157 serotypes of *E. coli*, including O26:H11, O103:H2, O104:H21, O111:H8 and O113:H21, are also regularly reported.

The symptoms of infection from this group of organisms include bloody diarrhoea and severe abdominal pain, but range from asymptomatic infection to death, with the incubation period ranging from one to eight days. Infection with EHEC may lead to further complications, most notably haemolytic uraemic syndrome (HUS). HUS is characterized by acute kidney failure and is the leading cause of renal failure in young children.

The incidence of EHEC varies by country. In 2004, the number of laboratory-confirmed cases in the European Union (17 member states) and Norway was 1.3 cases per 100 000 population (EFSA, 2006), while in the same year the incidence in the United States of America was 0.9 cases per 100 000 people (Vugia et al., 2005). In 2001, the incidence in New Zealand was reported to be 2 cases per 100 000 (Sneyd et al., 2002) and 0.2 cases per 100 000 in Australia (OzFoodNet, 2001). The frequency of EHEC, and more specifically HUS, appears to be the highest in Argentina, with estimates of approximately 22 cases of HUS per 100 000 children aged 6 to 48 months (Lopez et al., 1997). While EHEC infections have also been reported for other parts of the world, including a number of African countries, specific incidence data are not always collected or readily available. EHEC infection and associated diseases can occur in any age group; however, it seems that illness occurs most often in young children. For example, in Japan, the median age for EHEC illness has been reported to be 8 years (Kawamura, Yamazaki and Tamai, 1999). Children less than five years of age appear to be the most susceptible to HUS, while EHEC infections in the elderly lead more often to thrombotic thrombocytopaenic pupura (TTP) (Banatvala et al., 2001).

EHECs have been isolated from various domestic animals and wildlife, including cattle, sheep, swine, goats and deer. Ruminants, and in particular cattle, are considered a major reservoir of EHEC. While multiple sources and routes of transmission are now recognized, data based on outbreaks and sporadic infections indicate beef and beef products as the most frequently identified source of foodborne EHEC infection. In particular, undercooked ground beef products have emerged as an important source of foodborne infection. Other foodborne sources include milk and dairy products (e.g. unpasteurized milk, cheese from raw milk), fresh produce (e.g. sprouts, salads), drinks (e.g. apple cider or juice) and water. Dozens of new outbreaks have been reported implicating different sources and illustrating the multifaceted epidemiology of EHEC infections.

The Codex Committee on Food Hygiene (CCFH) is considering addressing the need for risk-based control of EHEC. A risk profile has been prepared as the basis for further work in this area (CCFH, 2005). The Committee has noted that no international risk assessment has

been undertaken on this issue and suggested that this could be the next step in order to move forward (CAC, 2004).

Taking into consideration the ongoing public health problem of EHEC in their member countries, the impact of this pathogen on meat trade, and the suggestion from Codex to undertake a risk assessment on this issue, FAO and WHO consider that this highlights the need for urgent attention at both national and international level to develop appropriate management interventions. Therefore, as part of JEMRA activities, FAO and WHO, together with the Food Safety Authority of Ireland (FSAI), convened an inception meeting on enterohaemorrhagic *E. coli* in raw meat and meat products from 4–7 September 2006 in Dublin, Ireland. This meeting was convened to provide guidance to FAO and WHO on the appropriate steps in the development of this activity before embarking on any risk assessment work.

As well as providing guidance to FAO and WHO in their future work on EHEC, the outcome of the meeting will be made available to CCFH to assist the committee in its efforts to develop risk management guidance documents on EHEC and to facilitate its interaction with FAO and WHO on defining and clarifying the scientific advice needed for this work.

To assist the participants in their deliberations, FAO and WHO commissioned the preparation of two background papers: one provided an update on the current state of knowledge on the bacteria and related issues at the time of the meeting, while the other gave a description and analysis of the risk assessments that had already been undertaken in a number of Member States. These two documents are appended as Annexes 1 and 2 to this report.

## *1.2 Objectives*

It has been noted that a number of risk assessments have already been undertaken on EHEC in meat and meat products and that there is experience in some countries on the risk management of this issue. The meeting was convened to review the current state of knowledge on EHEC in terms of existing risk assessments and related information. In doing so, the meeting was requested to consider the risk management actions, if any, that resulted from those risk assessments and to identify what was useful about the existing risk assessments from a risk management perspective, as well as identifying the strengths and weaknesses of the risk assessments. Based on this review and discussion, a roadmap for future FAO/WHO activities in this area would be developed to inform the process of risk-based management of EHEC in raw beef and beef products in CCFH and in member countries.

The specific objectives of the meeting were to:

- Review the existing risk assessments on EHEC in terms of (a) their fulfilling their scope and providing the basis for scientifically-based risk-management actions; and (b) their potential application (in whole or on a modular basis) to the development of a risk assessment at the international level.

- Consider the risk management actions, if any, taken to date that were based on risk assessment and identify the strengths and weakness of the risk assessments from a risk management perspective, in particular identifying when and why the risk assessments did not meet the needs of risk managers.

- Identify the key issues currently faced by risk managers in terms of addressing the problems associated with EHEC in raw beef and beef products.

- Considering the output of the above objectives and the existing data on EHEC in raw beef and beef products, provide guidance to FAO and WHO on the specific areas to be addressed in any future work on this issue, and how to address them.

## *1.3 Scope*

### 1.3.1 Hazards of concern

The hazard of concern that was the focus of this meeting was EHEC. However, throughout this document specific mention is made of one particular EHEC, verotoxin producing *E. coli* O157:H7, because of its implication in the first recognized outbreak of EHEC infection. In addition, several other major outbreaks, coupled with its unique physiology (in terms of EHEC) have resulted in *E. coli* O157:H7 being the EHEC most frequently referred to and most extensively described in the literature.

### 1.3.2 EHEC

Verocytotoxin producing *Escherichia coli* is a term used to describe strains of *E. coli* characterized by the ability to produce verocytotoxin(s) (VT), or just verotoxins that are capable of killing vero cells, a tissue culture line of monkey kidney cells. This group, often referred to as verotoxigenic *E. coli* or VTEC, includes over 100 serogroups in addition to *E. coli* O157:H7. Furthermore, it is recognized that the terms Shiga toxin producing *E. coli* (STEC) and VTEC as well as the terms Shiga toxin(s) (ST or Stx) and verocytotoxin or verotoxin (VT) are used interchangeably worldwide. A more extensive description of the relevant terms is provided in Section A2.1 of Annex 2.

EHEC is defined as a subgroup of VTEC/STEC associated with human diseases and which in addition to the verocytotoxin/shigatoxin producing capacity harbours additional genes that are important in virulence. These include the genes encoded in the pathogenicity island designated LEE (locus of enterocyte effacement), which can cause attaching and effacing lesions on the surface of epithelial cells (A/E factor, usually determined by the presence of the intimin (*eae* gene) and a 60 MDa 'virulence' plasmid (usually determined by the presence of the *ehx* gene). It should be noted that the clinical outcome of an infection might not necessarily include HC or HUS. In addition to *E. coli* O157, EHEC includes other serotypes causing foodborne illness, such as O26, O111, O113 and O121.

Due to the paucity of information on most other EHECs, the scope of this paper was limited to *E. coli* O157:H7 or VTEC O157 when discussing strain- or serotype-specific data, on the assumption that this is where most of the data are available. However, assuming that the behaviour of *E. coli* O157:H7 would be similar to other relevant EHEC serotypes, the advice provided would be applicable for most if not all EHECs. In other words, the strategy recommended on how to control EHEC is based on our knowledge of O157:H7. The meeting nevertheless recognized that such a generalization may not be appropriate as more information on other EHECs becomes available.

### 1.3.3 Food commodities of concern

It is commonly accepted that the primary reservoirs of EHEC are farm ruminants, and in particular cattle. It is, however, unclear to what extent EHEC can be considered ubiquitous in cattle, and what the reasons are for the sporadic nature of EHEC and the variation in prevalence in cattle in different regions of the world.

The main recognized routes of EHEC infection are: person-to-person transmission; contact with animals; foodborne transmission; and waterborne transmission. Only the foodborne route is considered further in this document.

Food vehicles implicated most frequently in outbreaks of EHEC infection have been raw or undercooked foods of bovine origin, especially undercooked ground beef and unpasteurized milk (CCFH, 2005). A number of case-control studies have identified ground beef as an important risk factor for EHEC infection, with current data based on outbreaks and sporadic infections indicating that consumption of ground beef remains the single most frequently identified source of foodborne EHEC infection. Dry fermented meats, as well as cooked and fermented sausages, have also been implicated in reported outbreaks of EHEC infection. However, an increasing number of outbreaks have been associated with the consumption of raw or minimally processed fruits and vegetables. Leafy green vegetables, such as lettuce, have been implicated in a number of large outbreaks of *E. coli* O157:H7, some of which had serious public health impacts (Hilborn, Mermin and Mshar, 1999; Martin et al., 1986; US FDA, 2006, 2007). These are an important source of human cases of foodborne illness due to EHEC as they are subject to contamination and eaten raw (CAC, 2002).

Thus, while recognizing the importance of other sources of foodborne EHEC infections, the meeting decided to restrict the scope of its work to raw ground beef and beef products along the entire food chain from primary production to consumption. This restriction was also motivated by the availability of data. Figure 1 illustrates the routes of transmission and products of concern considered during the meeting.
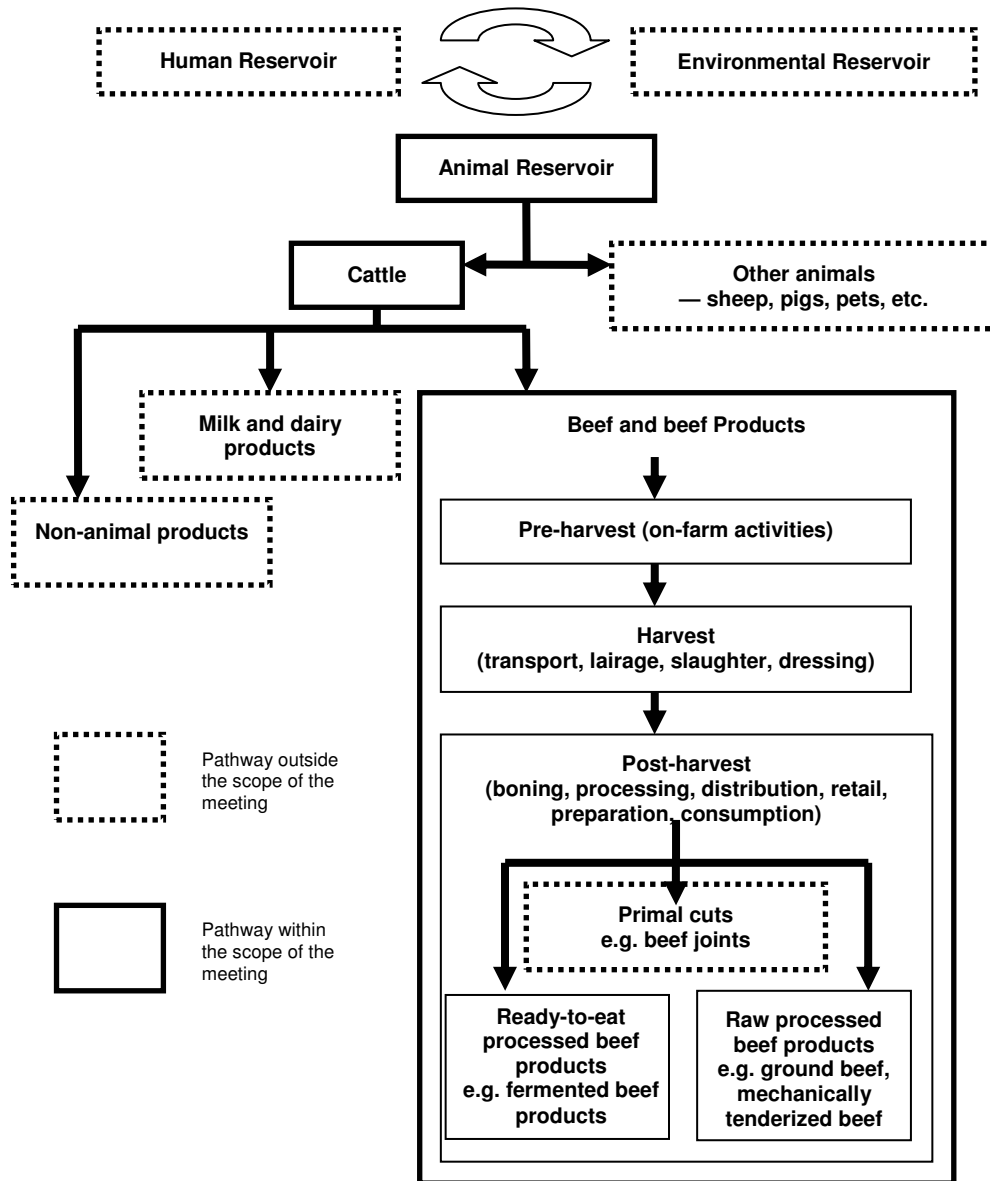
**Figure 1.** Routes of transmission of EHEC and products of concern considered during the meeting.

# 2. Review of the risk assessments undertaken to date and their application

## *2.1 Introduction*

Five risk assessments addressing the risk of EHEC, and specifically *E. coli* O157 in raw ground beef or similar comminuted products, have been undertaken to date. These are:

- Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers (Cassin et al., 1998).

- Shiga toxin-producing *E. coli* in Ground Beef Manufactured from Australian Beef: Process Improvement. Report Prepared for Meat and Livestock Australia (2000). (Meat and Livestock Australia, pers. comm.).

- Draft risk assessment of the public health impact of *Escherichia coli* O157 in ground beef (USDA-FSIS, 2001).

- Risk assessment of Shiga-toxin producing *Escherichia coli* O157 in steak tartare in the Netherlands (Nauta et al., 2001).

- *E. coli* O157:H7 in beef burgers produced in the Republic of Ireland: A quantitative microbial risk assessment (Teagasc, 2006).

These risk assessments are reflections of the published evidence and scientific opinion at the time and place of their development. This affected both the risk questions posed and also the structure and content of the risk assessment. A summary of each of the risk assessments in terms of their structure, data used, assumptions made and outputs of the model was prepared for a previous FAO/WHO activity, and is provided as Annex 3 of this report. In addition, a review of the models, particularly with regard to the dose-response models used, was prepared as a background discussion paper for this meeting and is attached as Annex 1.

These risk assessments have been developed over almost a ten-year period. So while they reflect to some extent a continuum of development, with, over time, some refinement of the approaches used, more striking is their role in building capacity and expertise in the area of risk assessment. It is important to note that all of the risk assessments consider the risk of *E. coli* O157:H7 in ground beef and ground beef products and do not consider other meat (e.g. sheep) or other food products or other EHECs. This was considered to be a reflection of the lack of information on the risk pathways for other (i.e. non-O157) EHECs and the overwhelming regulatory and scientific interest in this particular pathogen–product pair. Such information would be required before a risk assessment that specifically addresses other EHECs could be developed. Although risk managers are often interested in a holistic approach, i.e. consideration of multiple pathogens and not just EHEC, it is not surprising that none of the existing risk assessments address this. While the holistic approach is indicative of recent and ongoing changes in risk management, the meeting was not aware of any completed risk assessments for any pathogens that have taken such an approach.

In reviewing the risk assessments the meeting considered whether or not they were used as a basis for, or influenced in some way, risk management actions for the control of EHEC associated with beef and beef products in the countries in which they were undertaken. A common theme emerged in that the risk assessments were undertaken subsequent to or as a

separate exercise to risk management activities directed towards the control of EHEC. Although not yet implemented, the use of the risk assessment to re-evaluate risk management actions is planned in some countries, for example Ireland and the United States of America.

A further risk assessment regarding mechanically tenderized beefsteak (USDA-FSIS, 2002) was made known to the meeting, but was not reviewed.

## *2.2 Overview of the risk assessments*

The meeting reviewed the existing risk assessments on several levels:

- their utility in developing future risk assessments on the product-pathogen combination;
- if and how they have been used by risk managers to inform risk management actions or other activities; and
- their potential use in answering future risk management questions.

In addition, Table 1 (on the next page) provides a broad comparison of the five risk assessments under consideration, summarizing both their current use and future potential.

### 2.2.1 Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers (Cassin et al., 1998)

This was not only the first risk assessment to be undertaken on EHEC but also one of the first microbiological food safety risk assessments to be undertaken. In this regard it was ground-breaking work. Undertaken in Canada, this risk assessment was not commissioned to inform risk management actions to control EHEC, which at that time were based on existing tools such as Good Hygienic Practice (GHP) and Good Manufacturing Practice (GMP). Rather it was carried out as a research activity to develop risk assessment approaches and demonstrate how such a tool could be used to assess the risk associated with microbiological hazards in foods. However, a process is currently underway in Canada to develop public health targets for EHEC that will link to risk management activities and allow for impact assessment.

As it was undertaken as a research exercise, the risk assessors defined the scope, the risk question to be addressed and identified some hypothetical intervention scenarios for consideration. In other words, it was never intended to be a risk management tool or to address the specific needs of a risk manager. Consequently, it needs to be regarded as a research activity rather than within the context of what we now consider to be a risk analysis process, a process that implies considerable interaction between risk assessors and risk managers.

**Table1**. Broad level comparison of the risk assessments (from a risk management perspective). For a more detailed comparison, see Annexes 1 and 3

| Attribute of interest | Cassin et al. (1998) | MLA (2000) | USDA-FSIS (2001) | Nauta et al. (2001) | Teagasc (2006) |
|---|---|---|---|---|---|
| Hazard | *E. coli* O157:H7 | STEC. Presence of virulence makers Stx1, Stx2, the *eae* gene and the EHEC plasmid | *E. coli* O157 | *E. coli* O157:H7 | *E. coli* O157:H7 |
| Product | Ground beef hamburgers | Ground beef hamburgers | Any product containing ground beef | Steak tartare | Ground beef hamburgers |
| Purpose | Research exercise | Research exercise | Risk management decision | Research and capacity building exercise | Research/capacity building exercise. Risk management guidance |
| Risk questions and what-if scenarios | Identified by the risk assessors | Identified by the risk assessors | Numerous and ambitious, which led to large and highly complex model | Identified by the risk assessors | Identified by a risk management group that was formed within the research project |
| Start and end points | Start: herd prevalence and faecal contamination at farm End: probability of illness, HUS and mortality per serving | Start: herd prevalence and faecal contamination at farm End: probability of illness, HUS and mortality per serving | Start: live animal prevalence End: risk of illness, HUS and mortality per serving and per annum in the USA | Start: on farm animal prevalence End: number of human cases | Start: prevalence and contamination at point of slaughter End: probability of illness per serving |
| Data generation done in parallel with the risk assessment | None | None | None | None | Experimental data generated for both model input and model validation purposes |
| Dose-response model | Random coefficients model based on human clinical data on *Shigella dysenteriae* and *Shigella flexneri* | Same approach as used by Cassin et al. (1998) | Envelope model with upper bound based on human clinical trial data with *Shigella dysenteriae* for the upper bound and enteropathogenic *E. coli,* anchored using epidemiological data for *E. coli* O157 from ground beef | Dose response model based on Japanese outbreak data. Also used the model by Teunis, Takumi and Shinagawa (2004) | Used envelope model developed by Powell et al. (2000) as part of the USDA-FSIS risk assessment |

| Attribute of interest | Cassin et al. (1998) | MLA (2000) | USDA-FSIS (2001) | Nauta et al. (2001) | Teagasc (2006) |
|---|---|---|---|---|---|
| Model validation | No data available for the validation of the model. Model not Canadian specific | Although developed using Australian data, no data available for the validation of the model | No illness data were available to validate model as these were used in model development | Validated against epidemiological data for the burden of illness. Dose-response model changed due to overestimation of the number of cases attributable to steak tartare | Model validated and agreed to microbiological survey data at two points: prevalence of *E. coli* O157 in boxes of trim; prevalence of *E. coli* O157 at retail |
| Use in a risk management decision | Not used (commissioned as research exercise) | Not used directly (commissioned as research exercise) but guided future MLA policy re MRA | Not used directly as developed post-risk management actions, but guided some aspects of hazard control and facilitated risk communication | Not used (commissioned as research exercise) | Risk managers will consider the conclusions of the risk assessment and consider whether their current action plan for *E. coli* O157 is still valid |
| Future use and intentions | None planned | None planned | Plan to revisit the risk assessments to investigate the human health risk posed by both ground beef and mechanically tenderized beef based on new data derived from more recent outbreaks and from a nationwide baseline survey on prevalence and levels of pathogens in beef manufacturing trim | None planned | Various activities, including further data generation for use in the model and the inclusion of other EHECs |
| Generic components | Dose-response Calculation of within-herd prevalence Calculations of cross-contamination within a processing plant Growth and inactivation modelling | Dose-response Calculation of within-herd prevalence Calculations of cross-contamination within a processing plant Growth and inactivation modelling Approach for the definition of STEC | Growth and inactivation Live animal – within-herd estimates (captures the transient nature of infection) | Modelling approach used to describe mixing and partitioning | Methodology for the consideration of microbiological test sensitivity Time-temperature modelling work |

Although the risk assessment answered specific questions, its ability to answer future risk management questions could not be determined one way or the other without knowing what questions are likely to be posed. For example, if the question is "what is the impact of the reduction of *E. coli* O157:H7 in cattle faeces?", this model could be utilized. However, its use to investigate such a scenario would be more coincidental rather than a deliberate desire at the development stage to include such a scenario or intervention. The model can be used to provide in a descriptive fashion information on what risk interventions might be effective. That is, it could provide general guidance as to where to intervene, but is less fit in its ability to provide quantitative estimates regarding decrease in risk or absolute risk.

Although the risk assessment was developed for the Canadian situation, in common with many other risk assessments it was not possible to parameterize the model solely with national data.

In relation to its ability to be included as part of a risk assessment at the international level it would, most importantly, have to be noted that the Cassin et al. (1998) risk assessment is out of date, particularly in terms of the data used therein. However, it should be recognized that many of the later risk assessments were aided by the development of this risk assessment at a time when the concept of microbiological food safety risk assessment was emerging.

There are two elements of this risk assessment that were considered to be generic and could be used in the development of future risk assessments for EHEC, namely :

**The dose-response model**

While a more detailed description of this is provided in Annex 1, the meeting noted that subsequent risk assessments did not appear to substantially improve the dose-response model used by Cassin et al. (1998). While some clarifications as to its basis are still needed (see Annex 1), it was considered that this model, based on human clinical trials with *Shigella*, has the potential to be used for the development of a consensus dose-response model at international level.

**Certain approaches related to calculating prevalence and level of the hazard**

Approaches used in calculation of within-herd prevalence, cross-contamination within a processing plant, and growth and inactivation modelling may be useful in the development of future risk assessments, but in doing so consideration should be given to the scope of the risk assessment as well as features offered by some of the other risk assessments described below. For example, the live animal prevalence estimation had some useful features (e.g. considering variability among studies) but it did not consider test sensitivity or herd prevalence, which has been considered in USDA-FSIS (2001). Similarly, the growth and inactivation modelling only considered uncertainty about the predicted mean response, whereas Nauta et al. (2001) suggested an approach that could incorporate variability.

## 2.2.2 Shiga toxin-producing *E. coli* in Ground Beef manufactured from Australian Beef: Process Improvement

Report prepared for Meat and Livestock Australia (2000) (Meat and Livestock Australia, pers. comm.).

This risk assessment was commissioned by Meat and Livestock Australia (MLA). Again, rather than addressing specific risk management questions, this risk assessment was developed as a research activity and focused on the application of the approach developed and described by Cassin et al. (1998) to the situation in Australia by using data available in that country. Current risk management actions in relation to the safety of raw beef products

were not based on the findings of the risk assessment and do not focus specifically on EHEC. As in many countries, they are based on GHP. Over the two years prior to the meeting Australia had implemented an 'on-farm' HACCP programme and approximately 160 000 farms participate in this scheme. The Livestock Production Assurance scheme focuses on the supply of safe and clean cattle to slaughterhouses. The motivation for the development of an on-farm programme was the desire to have a system to underpin statutory declarations on the safety and suitability of animals for slaughter (for example, that the animal has not been fed ruminant material, or that sufficient time has elapsed between antibiotic treatments and slaughter). The development of this scheme was not driven by the risk assessment but based on HACCP principles. Few slaughterhouses have specific interventions for EHEC in their process. Control activities have developed by steady evolution and it was considered that there were no public health indications of a need for specific risk management actions for EHEC. However, an exception is the production of fermented meat products where specific production requirements and end product microbiological criteria aimed to control EHEC in these products.

Therefore, the development of the Australian risk assessment was primarily a capability building exercise that started in 1997, followed by a report in 2000. Although based on the work of Cassin et al. (1998), one important difference or addition was the consideration of the virulence markers for STEC, where the presence of virulence markers Stx1, Stx2, the *eae* gene and the EHEC plasmid were considered. Such an approach could also be adopted for any future EHEC risk assessment model. There was also an attempt to explore the use of risk assessments in assessing the equivalence of different processing systems.

The risk assessment did have some utility in shaping risk management directions and identifying data needs. MLA has made a decision not to embark on future risk assessments unless there is sufficient data available to achieve the objectives of the assessment, thus there has been more focus on collecting data in a way that is useful for risk assessment. In addition a 'process risk model' (limited to hazard identification and hazard characterization, with emphasis on the exposure assessment element and changes in hazard during processing steps) is being developed as a tool to identify issues and data gaps that might require research, and to suggest where research might best be applied towards reducing EHEC prevalence in beef products.

## 2.2.3 Draft risk assessment of the public health impact of *Escherichia coli* O157 in ground beef (USDA-FSIS, 2001)

This was the only risk assessment of the five that was specifically commissioned by a government risk management agency. It considered the risk for any food products that contain ground beef and was both time and country specific.

In the United States of America the need for action on this pathogen–product combination was driven by large outbreaks of EHEC in the late 1980s and early 1990s. Cooking practices in the catering sector, including fast-food restaurants, were changed in line with risk management recommendations. Furthermore, it was considered very important to control the levels of EHEC in product supplied to food service operators and the consumer. A risk

management decision to declare *E. coli* O157:H7 an adulterant[1] in raw ground beef was implemented as a means of driving the beef industry towards action to solve the problem and reduce the prevalence of EHEC in ground beef.

The aim of the risk assessment was to identify potential controls, and to evaluate which were most important, by considering different scenarios. Risk assessors and risk managers did not sit down beforehand to discuss and define the scope of the risk assessment. Consequently, the risk questions identified were numerous, theoretical and broadly focused. Hence, an extremely large, detailed and comprehensive risk assessment was produced. Subsequently, the needs of the risk manager were almost lost in the complexities of the risk assessment. If the risk question had been more focussed (most useful are well articulated, discrete questions), it would have been a different risk assessment. In addition, it can be said that questions relevant to risk managers were not addressed in such detail or rigour as would have been the case had the risk assessment been developed in more recent times.

As part of the risk assessment, an independent estimate of risk of illness or burden of illness was obtained using epidemiological data. Although the risk assessment and the epidemiological study estimated the same thing (i.e. risk of illness) a risk assessment was still developed to identify potential controls. The epidemiological estimate of the risk of illness was used in the model to parameterize the dose-response model.

Unlike the other risk assessments, the USDA-FSIS model identified neither live animal prevalence nor microbial load as the most important variable for risk of illness. This was due to the baseline assumption in the model that while between- and within-herd prevalence estimations were subject to uncertainty, no on-farm control measures had been demonstrated to reliably control *E. coli* O157 prevalence or levels in live animals. (See Section 3.2 below.) Nonetheless, it is intuitive that further research into control of EHEC in the animal reservoir potentially could have a substantial public health impact (Hancock et al., 2001).

Thus this baseline model can be considered more as a description of the process rather than a scenario-based model. While the model did include the ability to address 'what-if' type questions, it did so in a particularly complex manner and so the other risk assessments developed may be more appropriate as a basis for developing scenario-based models.

Similar to the Cassin et al. (1998) model, this risk assessment does have potential in terms of providing generic approaches for use in future risk assessments. It can therefore be used as a guide to methodology for countries that decide to develop a risk assessment. Just as the Cassin et al. (1998) work was used as a basis for the MLA risk assessment, this risk assessment has been used as a starting point for the risk assessment undertaken in Ireland (described in Section 2.2.5). In particular, the meeting identified the EHEC growth and inactivation components and the approach to addressing within-herd estimates in the live animals—which captures the transient nature of infection—as particularly transferable to a

---

[1]   Under the Federal Meat and Inspection Act (FMIA), 21 United States Code 601(m) (1), a product may be adulterated because it bears or contains a poisonous or deleterious substance which may render it injurious to health. Consumers in the United States of America consider ground beef to be properly cooked rare, medium rare, or medium. Thus in the case of *E. coli* O157:H7, it is not 'proper' cooking but 'thorough' cooking that is necessary to protect consumers. As *E. coli* O157:H7 can cause serious illness, including death, the courts in the United States of America have agreed that *E. coli* O157:H7 fits the definition of an adulterant under the FMIA as ground beef containing this pathogen may render the product injurious to health.

generic risk assessment for EHEC, and which could be tailored to the needs of different countries. However, it was considered that other approaches used in the risk assessment, such as the anchoring approach used in the dose-response model, would not be globally applicable as the data required are very country specific.

While this risk assessment did not lead directly to risk management actions, other benefits did ensue. The provision of a risk assessment for EHEC became the forerunner for the current United States of America system of a transparent risk analysis process whereby risk managers take decisions based on a risk assessment process with full consultation and comment. This was not common practice when many EHEC interventions were put in place in the early 1990s. From the United States of America perspective, the risk assessment proved to be a means for describing the ground beef production process using a multidisciplinary approach, and some of its findings informed subsequent HACCP plans implemented in the industry. The outbreaks of EHEC drove a greater public health focus in the federal control agency, and risk assessment was a means of linking public health outcomes to federal controls on the food industry. Data from the EHEC risk assessment helped to identify the likelihood of contamination, and this became a key part of the risk communication strategy to help with industry acceptance of federal interventions and consumer acceptance of the residual risk. The risk assessment process also helped to focus on prevalence data acquisition, which eventually led to the recognition of a more widespread prevalence than initially anticipated. The meeting noted that it was the intention of the United States of America to continue to update the EHEC risk assessment with new data and that new risk management actions or revised risk management actions may ensue. These actions will be clearly linked to public health goals. From the United States of America experience it is clear that risk assessments should not be static but should be dynamic processes that are continually updated and used to link risk management actions to public health outcomes.

The attention of the meeting was also directed to another risk assessment that was carried out on mechanically tenderized meat (USDA-FSIS, 2002). While not reviewed by the meeting, it was noted that this risk assessment was used as a basis for risk management decision-making. At the time it was undertaken, and using the available data, the risk assessment indicated that there was no increased risk associated with these products, and it was used as the basis for a decision to take no specific risk management action with regard to control of EHEC in this type of product. However, subsequent to its completion, two outbreaks in the United States of America related to EHEC in mechanically tenderized beefsteak caused the United States of America to revise its position and to issue a requirement for all regulated facilities to conduct a re-assessment of their HACCP plans in order to account for new data associated both with recent outbreaks and with failure to adequately control sanitation of the equipment. In addition the United States of America recommended, but did not require, explicit labelling of mechanically tenderized products in order to provide consumers with relevant information that could affect how these products are safely prepared. Furthermore, the risk assessment is being revised in light of this recent epidemiological information. In addition to considering more current risk management questions, more current contamination data and consumer handling data will be considered to re-evaluate the comparative risk of intact versus non-intact beef. This recent epidemiological information and subsequent data collection to re-evaluate risk estimates highlights the concept of risk assessments as evolving rather than static tools.

## 2.2.4 Risk assessment of Shiga-toxin producing *Escherichia coli* O157 in steak tartare in the Netherlands (Nauta et al., 2001)

The risk assessment by Nauta et al. (2001) was undertaken as part of a research and capacity building exercise rather than as a consequence of any risk management decision to commission a risk assessment. The risk assessment considers just one food product, steak tartare[2], a high quality comminuted beef product in the Netherlands, which is minced meat with a fat content of less than 10%. As with the work of Cassin et al. (1998), the risk assessors set the questions to be addressed in the risk assessment and decided to limit the scope to steak tartare.

Similar to the other risk assessments considered here, this one was not used to inform any risk management decisions. The risk assessors who developed it suggested three reasons for this:

- While interventions were investigated, this was not done in a very concrete way, as the scenarios selected were hypothetical. For example, the risk assessment considered the impact of an increase in the prevalence of EHEC in the animals, but did not consider any specific interventions that could lead to a decrease in such prevalence.

- It is recognized that the cost of interventions is an important factor in risk management decisions, but economic considerations were not included in this analysis.

- As the risk assessment was undertaken as a research activity, little effort was made to interact or communicate with risk managers in terms of designing the risk assessment or promoting its availability or application. In contrast, more recent risk assessments in the Netherlands, e.g. the CARMA (*Campylobacter* Risk Management and Assessment) project on risk assessment of *Campylobacter jejuni* in broilers, have been undertaken with more involvement of risk managers both during and after the risk assessment work. In addition, the CARMA project included economic considerations.

The results of the model were compared to the available epidemiological data (with adjustment for under-reporting). This exercise suggested that 65% of *E. coli* O157 infections were attributable to steak tartare, which was felt to be much too high. In the baseline model, an exponential dose response function was used based on Japanese outbreak data. A hypergeometric dose response curve fitted to the same data led to a higher number of predicted cases, whereas the Beta-Poissin dose response function developed by Powell et al. (2000) led to a much lower number of predicted cases. In terms of applicability of the model, the approach for considering mixing and partitioning (Nauta, 2005) could be considered as a generic element of the risk assessment that could be used for a future EHEC model.

Currently in the Netherlands there are no risk management actions that have been specifically implemented for EHEC. The meat used in steak tartare is of a higher quality than that used for ground beef, which is the food vehicle considered by the other risk assessments. However, the hygienic practices of production and processing for both products are believed to be good in the Netherlands. Although there was an outbreak of EHEC illness related to

---

[2]   Steak tartare is a high quality product that is similar to a hamburger, but thicker. Prior to consumption, it is heated for around 10 minutes and is therefore a partly prepared and partly raw (i.e. inner) product. It is not possible to quantify the proportion of the steak tartare that is consumed raw. Steak tartare has not been linked to foodborne infections in the Netherlands.

*filet american*[3], there are currently no plans to re-visit the risk assessment or use it in any future risk management decision-making processes.

## 2.2.5 *E. coli* O157:H7 in beef burgers produced in the Republic of Ireland: A quantitative microbial risk assessment (Teagasc, 2006)

In Ireland the need for action was driven by a rise in the notification of EHEC cases and outbreaks. The risk management actions adopted in Ireland to address the EHEC hazard stemmed from a scientific report published in 1999 (FSAI, 1999). This report reviewed the scientific information available at the time and made science-based recommendations for interventions at each link in the food chain. These recommendations became the plan for risk management actions. The scientific evaluation could not be described as a risk assessment by current understanding of that term. However, a risk assessment has now been completed in Ireland and it is the intention that the relevant risk management activities would be reviewed on the basis of this work.

Although the risk assessment was developed as part of a research project, a risk management group was formed and put forward seven risk questions for consideration in the risk assessment model. The risk management questions were based on the risk management activities that were implemented following the scientific evaluation in 1999. To a great extent the model was developed with Irish-specific data and assumptions. As the most recently developed risk assessment for *E. coli* O157, the risk assessment had the advantage that the Cassin et al. (1998) and draft USDA-FSIS (2001) models were already available, and this facilitated the process, rendering the learning curve less steep.

An important element of this model is that data were collected in parallel with the development of the risk assessment. Consequently, the model was validated at two points along the food chain; these were (1) boxes of beef trim; and (2) beef products at retail. The model results and the results from the experimental work were comparable. In addition, other data types obtained from experimental work within the project were used for the estimation of model parameters.

In the model, no particular interventions were considered; rather a number of scenarios were included. For the dose-response component, it was not possible to adopt the anchoring approach, proposed by the USDA-FSIS model, using Irish data due to the small population basis for any such data. Therefore, an existing dose-response model (Powell et al., 2000) was used within the model, without further development. However, unlike Powell et al. (2000), the dose-response was not compared with epidemiological data, as such data were limited due to the small population base and thus made it impossible to validate the probability of illness predicted by the model. As with the other models, there are a number of components specific to the exposure assessment that could be used as a basis for future risk assessments. Particularly noteworthy are the methodology used for consideration of test sensitivity and the approach used to describe the impact of time–temperature storage combinations on *E. coli* O157. This is not to suggest that previous risk assessments did not adjust prevalence to account for test sensitivity. However, in this case, the sensitivity of the isolation methods was directly evaluated using inoculation studies. Other risk assessments also used models that predicted growth and inactivation based on time–temperature combinations, although the approaches differed.

---

[3] *Filet american* is a product where minced beef is mixed with mayonnaise and is eaten totally raw.

The scenario-based approach taken in developing this model broadens its application compared with, for example, the process-description approach taken in the USDA-FSIS baseline model. For example, this model has been used in an exercise that attempts to illustrate the use of risk assessment models to establish performance objectives for *E. coli* O157 (FAO/WHO, 2006; Nally et al., 2006). Although the combinations were arbitrary, i.e. not intended to represent any particular intervention, it was noted that risk assessors need to employ caution when doing exercises such as this.

In terms of its application, the risk assessment has provided a better evidence base for risk management activities that have been implemented to date in Ireland, and has illustrated at the very least that none of them were inappropriate. However, areas for improvement have been identified and work is underway to gather more data to reduce uncertainty at certain key steps in the beef production chain. Furthermore, there have been benefits arising from this risk assessment that are not necessarily captured by focusing on its utility in risk management. For example, Ireland has developed the capability to conduct quantitative microbiological risk assessment (QMRA) by funding this research activity. In addition, the identification of data gaps during the development process has led to the acquisition of data suited to risk assessment. These data may have utility in other countries. With respect to beef, the risk assessment has now provided a clearer picture of what is happening to EHEC in the beef production chain and this is due to feed into a review of current risk management practices.

## *2.3 Common features and lessons learned*

Table 1 provides a summary of each of the risk assessments in terms of their features, commonalities and potential application. Based on this review of the risk assessments, a number of issues were identified that are probably common to all risk assessments and are worth highlighting.

**Model validation**

Many of the risk assessment models were thought to have overestimated the risk of illness. However it was noted that the validation of risk assessment models is not straightforward, as there is no 'gold standard' data with which the model outputs can be compared. For example, it is difficult to compare the model outputs with human EHEC cases, as the epidemiological data will suffer from issues such as under-reporting. Likewise, in comparing the model output with microbiological sampling data, the sensitivity and statistical validity (e.g. convenience or randomized sampling) of the microbiological data need to be taken into consideration, and this is not always possible. With regard to EHEC, another important consideration is the difference in virulence among different strains of *E. coli* O157. It was not possible to directly account for this variability in any of the risk assessments that were undertaken, and it is also likely to affect efforts for validation. Although the dose response model developed by Cassin et al. (1998) did account for variability among strains, it was based on *Shigella* spp. rather than *E. coli* O157.

**Quantitative data**

Before many microbiological risk assessments were done there was an overwhelming focus on acquiring prevalence data without obtaining concentration data. Risk assessments have demonstrated that both are needed to gauge risk and link interventions to public health outcomes.

**No such thing as zero risk**

Risk assessments have demonstrated that a safe level of a pathogen does not exist. This is now more accepted than prior to risk assessment and has led to the concept of risk reduction opportunities rather than risk elimination.

**Risk assessment scope**

The importance of having a clear and focused scope for a risk assessment cannot be overemphasized. The risk assessments reviewed here differed in scope and complexity and illustrated that if something is too complex and tries to take everything into account its utility may get lost in the complexity. In other words, there is a risk of developing a risk assessment that tries to satisfy everyone by including everything, but in the end satisfies no-one.

**Application of existing EHEC risk assessments**

While the meeting explicitly considered this point and identified a number of components of each of the models that could be used in future risk assessment, it should be noted that these components have not been developed in a manner that is stand-alone and would allow them to be used directly in future risk assessments. Rather, their development and the expertise gained in their elaboration should greatly facilitate and expedite future risk assessment work.

# 3. Key management issues associated with EHEC in raw and ready-to-eat beef products

## *3.1 Introduction*

In considering the risk management issues associated with EHEC the meeting noted that to date most of these have been addressed in the absence of quantitative microbial risk assessments. The previous section describes some of the actions that have been taken in those countries that have carried out a risk assessment, irrespective of whether or not the risk assessment contributed to the risk management decision. For the purposes of identifying key issues, the meeting participants considered it useful to also look at one country where no risk assessment has been undertaken, as such an example may also reflect the situation in many other countries, especially with respect to the adoption of HACCP and GHP in slaughterhouses.

In Denmark, for example, there has not been a systematic approach to the control of EHEC (VTEC O157) and no risk assessment has been undertaken. The focus of risk managers has been on the application of GHP, with the enforcement of a low prevalence of pathogens in meat and meat products. The incidence of VTEC in Denmark was 2.8/100 000 in 2005 with 0.5/100 000 for VTEC O157. The sources for VTEC infections are generally unknown, but to date no VTEC O157 outbreaks have been associated with the consumption of beef. Although VTEC O157 are present in cattle and on carcasses at levels similar to other EU countries, this does not appear to be causing recognizable disease.

This example clearly illustrates the complexity of issues that are facing risk managers in terms of controlling foodborne, and more specifically meat-borne, EHEC. Nevertheless, from the risk manager's viewpoint, the food safety goal must be pursued realizing that absolute prevention or elimination of the pathogen cannot be guaranteed.

The meeting generated a list of key issues facing risk managers. It was not intended that this be exhaustive, but it was used by the meeting to guide subsequent deliberations on developing approaches for the provision of scientific advice.

## *3.2 Issues for consideration at pre-harvest stage*

The pre-harvest stage refers to the live animal on farm or at the feedlot and from a food safety perspective focuses on keeping the prevalence and numbers of microorganisms carried by the animal or in the animal herd to a minimum. With regard to EHEC, this requires consideration of issues such as:

- Prevalence and levels of EHEC on or in animals, hide and faeces, including issues such as 'supershedders' (Naylor et al., 2003) and seasonality, and their impact on subsequent levels in the beef or beef product.
- The relative importance of levels of EHEC in faeces and on contaminated hide.
- Age and background of the animals (i.e. whether they were dairy or meat production animals).

Although research continues, the meeting was unaware of currently available evidence that demonstrates specific on-farm measures (e.g. probiotics, competitive inhibition, stress

reduction) reliably controlling EHECs in live animals. Farmers are being encouraged to implement a clean-animal policy. However, cross-contamination in the abattoir may diminish the effect of on-farm controls. In some countries, to prevent gross contamination, food safety measures indicate that live cattle be categorized based on visible cleanliness upon arrival at the abattoir. However, the available evidence does not demonstrate the efficacy of visual inspection of live animals in lowering EHEC prevalence on carcasses. In comparison, the United States of America strategy requires that slaughter measures be designed presuming that *E. coli* O157:H7 is reasonably likely to occur on carcasses. Nevertheless it would seem to be appropriate to link on-farm controls with a logistical slaughter strategy, which would be improved if cross-contamination issues during slaughter could be addressed.

## 3.3 Issues for consideration at harvest stage (transport, lairage, slaughter and dressing)

The harvest stage is widely considered to include transport from the farm through to dressing (evisceration) of the slaughtered animal. It was noted that much of the activity at this stage related to minimizing cross-contamination and, if possible, reducing levels of EHEC on carcasses. To this end the meeting regarded key issues that required consideration to include:

• Effect of transport time, fasting and stress prior to slaughter on prevalence or levels of EHEC.
• Effect of cleaning animals prior to slaughter.
• Decontaminants and their impact and efficacy.
• Animal contact surfaces (e.g. lairage, stun box) and their role in cross-contamination.
• De-hiding processes and minimizing cross-contamination.
• Role of aerosols in cross-contamination.
• Role of workers in cross-contamination.
• Slaughterhouse design.
• Time to chilling and its impact on levels of EHEC.
• Role of high-risk cuts (e.g. head meat).

The United States of America food safety policy of declaring *E. coli* O157:H7 an adulterant in raw ground beef has resulted in substantial changes in hygienic slaughter practices designed to reduce the likelihood that the pathogen is present at detectable levels. It is reasonable to expect that the same hygienic slaughter practices have affected the occurrence and levels of other pathogens. The implementation of such a control was based on the preference of some consumers in the United States of America for lightly cooked ground beef.

The United States of America adulterant policy was fundamental in forcing a technological solution in this segment of the production chain. In the United States of America, decontaminants such as lactic acid, acidified sodium chlorate and ozonated water are treatment options for decontaminating carcasses and trim. Due to their temporary effect, such decontaminants are not considered to be food additives but rather processing aids. These chemical treatments are used with the understanding that there must be no measurable residue on the carcass and that the treatment effect is temporary. Irradiation is allowed in the United States of America, but it is primarily used by producers in response to legal liability exposure where the consumers are known to be susceptible (e.g. institutional settings), or by consumers

who prefer this additional safety treatment. In Europe, in contrast, only potable water and steam can be used for carcase decontamination, and irradiation is not permitted. However, controls are clearly seen to extend to the consumer level. This is an example of the significant differences that may exist between policies in different countries or regions.

Another control measure at this stage reflects a test-and-divert approach. The impact of such an approach can depend on the scale of the operation and the point at which the sample is taken. For example, it is more likely to detect EHEC in beef trim rather than the carcass, particularly when trim from many animals is combined. In the United States of America, the largest beef producers test large consignments of trim ('combo bins') for *E. coli* O157:H7 with high frequency (e.g. based on the ICMSF n = 60 sampling plans (ICMSF, 2002)) and divert failed product to ready-to-eat product in which a lethality step is implemented.

The meeting noted that geographical differences at this stage of the chain and differences in policy and legislation mean that the relevance of any list of key risk management issues will depend on the local situation. Nevertheless, given the extent of international trade in this product, it is pertinent for risk managers to be aware of the issues that arise, even if they are not directly applicable to their own country.

## 3.4 Issues for consideration at post-harvest stage

This is probably the most diverse stage of the process, as elements of it will vary depending on the final product that is marketed by the meat processor; the conditions of retail and of distribution; whether it is supplied in bulk to catering establishments or is purchased in small amounts for consumption in the home; and, finally, how it is prepared before final consumption. Given the diversity of this section, it was considered appropriate to address the relevant issues under three broad headings: cold chain conditions; application of lethal processes; and opportunities for and prevention of cross-contamination.

**Cold chain from slaughterhouse to consumer**

As with any fresh product, the maintenance of the cold chain is critical. The time from slaughter to chilling is an important issue. In addition, other issues to be considered in terms of control of EHEC include:

- Time and temperature of the chilling process.

- Growth and survival of EHEC under various time–temperature combinations.

- Impact of water activity and other environmental conditions on EHEC (such as freezing).

**Application of lethal processes**

Such processes can be implemented by the processor, retailer or consumer. In cases where such processes are implemented by the processor or retailer, the outcome is normally a ready-to-eat product that will not be subjected to further lethal heat treatments by the consumer. Depending on who is implementing the lethal process, some of the issues to be considered include:

- Prevalence and levels of EHEC prior to implementation of lethal process.

- Process criteria (in terms of producer practices).

- Consumer practices in terms of what they do with beef and beef products. What types of products are consumed? What are the cooking preferences and what subjective indicators of appropriate cooking are used? An important issue in terms of meat in international trade is the differences between countries in terms of their preparation practices. Thus

consumption of raw meat may be more widely accepted in some countries than others, and so may a certain level of risk associated with those products.

**Cross-contamination**

Cross-contamination can potentially occur at any point along the food chain, thus leading to a potential increase in risk. In considering relevant issues it was decided to divide cross-contamination into (a) that which can occur during commercial activities such as processing and retail, and (b) that which occurs in a domestic setting. This reflects the differences in risk management approaches needed in each situation and hence a difference in the issues to be considered. In terms of cross-contamination in commercial post-harvest settings, important issues to consider include:

- role of equipment in cross-contamination;
- presence of biofilms and survival of organisms;
- pathways of cross-contamination; and
- frequency and extent to which cross-contamination occurs.

With regard to addressing cross-contamination during domestic preparation, one of the primary tools available is consumer education. However, as a control measure its impact is difficult to measure, as knowledge does not always translate into changes in consumer behaviour. In addition, issues to be considered include:

- Role of equipment, kitchen surfaces and non-standardized equipment in cross-contamination, presence of biofilms and survival of organisms. Home kitchens are not dedicated food preparation areas.
- Pathways of cross-contamination (transport from retail to home).
- Frequency and extent to which cross-contamination occurs.

## 3.5 Other relevant issues

Apart from those issues that directly relate to the three stages of pre-harvest, harvest and post-harvest, there are a number of product-, trade- and health-specific issues that may need to be addressed by the risk manager.

### 3.5.1 Mechanically tenderized meat

It was noted that the process of mechanical tenderization of beef can introduce surface contamination into the interior of the muscle. While the United States of America considers mechanically tenderized beef to be a non-intact product, this is not necessarily the case in other countries. While not currently commonplace in many countries, this practice is increasing with the adoption of industrial beef production processes. As there are currently no requirements to label such product as mechanically tenderized, consumers are often unaware that such products need to be treated in the same manner as non-intact products. Some of the issues to be thus considered with regard to this type of product include:

- Provision of information to consumer (e.g. labelling, evidence of impact of labelling, effect on consumer behaviour).
- Cooking methods or instructions.
- Proportion of product subject to mechanical tenderizing.
- Level of hygiene and sanitation for mechanical tenderizing processes.

- Level of EHEC potentially introduced into interior muscle by the process.
- Are other tenderization processes used (e.g. injecting curing agents into meat) that could potentially introduce EHEC?

## 3.5.2 Intrinsically high risk products (risk communication)

The meeting noted that some ready-to-eat[4] meat products are not subjected to a lethal process. While it was noted that the United States of America defines ready-to-eat beef as beef subjected to an integrated 5-log reduction (e.g. a 2-log reduction via heating plus a 3-log reduction due to acidification) of EHEC, this does not represent a standard against which other approaches should be judged, and most other countries do not apply such a definition. In some European countries, for example, there is a consumer preference for some types of sausages (fermented or unfermented) or for comminuted products (such as steak tartare), which are not intended for cooking prior to consumption and which are not produced in a manner that would achieve a significant degree of *E. coli* reduction. In such cases issues that need to be considered by the risk manager include:

- Providing information to consumer (e.g. labelling, evidence of impact of labelling, effect on consumer behaviour).
- Level of risk associated with particular products.
- Product definition and method of manufacture.
- Legal requirements.

## 3.5.3 Validation, monitoring and verification, and establishment of equivalence

In addressing EHEC and other microbial pathogens, food safety risk managers face a persistent tension between the certainties of prescriptive measures versus the vagaries of performance measures. It may be difficult to determine what level of treatment is afforded by a given process. Furthermore, the resultant level of protection is dependent on the levels in incoming product. Frequently, risk managers must also make determinations of equivalence in international trade for countries that have different regulatory schemes or different levels of EHEC in incoming product. In attempting to address these issues, the meeting identified the following issues that need to be taken into consideration:

- Role of indicator organisms for process control of slaughter and processing.
- Microbiological criteria (including sampling methodology).
- Effectiveness of interventions: how this is measured or evaluated, or both.
- Evaluation of measurement and testing techniques.
- Evidence for utility of testing regime, including level of certainty of detection and level of detection, implying statistically valid sampling frequency.
- Scientific basis for performance objectives.
- Equivalence of different production and processing systems, requiring knowledge on the level of performance of a system.

---

[4] Codex defines ready-to-eat food as "Any food (including beverages) which is normally consumed in its raw state or any food handled, processed, mixed, cooked, or otherwise prepared into a form in which it is normally consumed without further processing." (CAC/GL 22-R)

- Predominance of different serotypes.

### 3.5.4 Hazard characterization (including dose-response)

While hazard characterization, and in particular dose response, are addressed in more detail in other parts of the report, it was considered that their impact in terms of risk management was worth highlighting. It was noted that, currently, most knowledge of EHEC dose-response relationships exist for *E. coli* O157, but little is known about the virulence (pathogenicity and infectivity) of different pathotypes and whether measures taken by the risk manager are also having an effect on these different types. It was considered that another important issue in this regard was that of susceptible populations and whether their consumption patterns meant that they could potentially be exposed to these pathogens.

# 4. Designing approaches for the provision of scientific advice

## *4.1 Introduction*

As indicated in Section 1, the focus of this report is risk assessment activity for beef, purchased raw or ready-to-eat. As a working hypothesis, it seems reasonable to presume that EHECs other than *E. coli* O157:H7 will respond to food safety measures in the same manner, or at least will not be made worse. The scope of issues faced by risk managers may range from production to consumption, or their authority and control may be limited to segments of the production-to-consumption continuum. Thus, their requirements for scientific advice will vary accordingly. The meeting noted the potential application of risk assessment elements of the production-to-consumption continuum to other products and routes of EHEC transmission associated with or affected by beef production, but not directly attributable to beef consumption. However, there are numerous factors that may constrain the application of a particular risk assessment, such as global food distribution and differences in processing practices. In this context, this section aims to identify approaches to the provision of scientific advice that could be applied to address a range of risk management issues.

## *4.2 Flexibility in approach to provision of advice to risk managers*

A flexible approach to risk assessment is necessary to inform risk management decisions for a wide range of products, produced and processed in a variety of ways, with different products being significant in different parts of the world. It is not possible to provide a comprehensive risk assessment for the full range of products and processes, prepared in a variety of ways and consumed by different populations.

Considerable data exists in the scientific literature on the efficacy of various risk mitigations on numbers of EHEC. These studies are useful in themselves, and provide risk managers with information about risk mitigations that might be directly applicable and cost effective. Risk management actions based on this approach may be sufficient to achieve public health goals.

Full farm-to-consumption risk assessments are huge endeavours. It may therefore be necessary to prioritize the focus of attention. This can take an iterative approach:

• prepare a risk profile (review of information);

• implement a qualitative risk assessment and simple quantitative risk assessment; and

• increase the resolution of risk assessment at key steps, in consultation with risk managers and subject to available data. Risk managers need to request modifications to generic risk assessments to cover their own circumstances.

A risk assessment is one part of a package that would allow risk managers to make risk management decisions. An assessment covering the whole chain from production to consumption was one option. However, it would not be necessary to cover the entire chain in great depth, and it would be possible to produce a detailed model only for the parts of the chain where data allowed, or where an interest in risk mitigation exists. It is possible to develop an empirical relationship to describe the input and outputs of a part of the chain, if there are few data or little interest in that part of the chain, but it is then not possible to make

more detailed inferences about the part of the chain thus treated. A risk assessment should provide an estimate of illness, but a risk manager may derive benefit from a risk assessment model that terminates at the point of exposure or some other point prior to the actual risk estimate. A general model of the entire chain, but with a low level of detail, could be developed relatively easily, but the development of a comprehensive, detailed assessment requires considerable time and expense. Once completed, a model may only be applicable for the country or geographical area, and the product selected. Risk managers on an international level (such as CCFH) may derive benefit from a general risk assessment model, but national risk managers are more likely to require detailed risk assessment models, and certainly ones based, to some extent, on data collected from their own country.

There is currently no global solution to the problems posed by EHECs. If a risk assessment is developed for a global purpose, generic components can be used from the existing risk assessments (see Table 2). The risk assessment could take a modular approach. This provides a starting point for countries that do not have a risk assessment and will provide a 'formula' into which countries can insert their own numbers. A scenario-based model could be produced, similar to the FAO/WHO *Enterobacter sakazakii* risk assessment model (FAO/WHO, 2008; for model see: http://www.mramodels.org/ESAK/default.aspx). Such a model could never fully describe country-to-country variability, and therefore some country-specific data may need to be entered into the model, unless it is deemed that the data used in the risk assessment is representative for the country of interest. However, the result of the 'what-if' analyses could provide insight into potential controls that might be applied by different countries depending upon their situation as captured in the ☐what-if' scenario.

The package of information that could be provided by scientists and assessors to risk managers might consist of:

- A critical or systematic review of aspects of the biology, ecology and risk mitigations investigated for EHEC, including non-O157. This could be based on the information provided in Annex 2 of this report. Another significant recent work is Sofos (2005).

- A risk assessment, covering three modules (pre-harvest, harvest and post-harvest) to a low level of detail, using one data set as an example and a few scenarios ('what-if' examples), which will provide risk managers with an insight into the complexities of the supply chain, where possible risk mitigations might best be applied, and the likely impact that those mitigations might have.

- Existing risk assessments (such as those reviewed in this report) that might be used to supply approaches or elements for national risk assessments.

- Consensus risk assessment model elements, that could be used in the risk assessment and consist of standard data that could be used in all risk assessments (such as dose-response functions, growth parameters, death parameters) and data that would be required to apply the basic risk assessment to another situation (e.g. data may be required on the prevalence and concentration of *E. coli* O157 in faeces at the time of slaughter, or on the times and temperatures encountered in the distribution chain).

This package of information would allow risk managers to:

- Make risk management decisions based on critically reviewed interventions, but without estimating their impact on public health outcomes.

- Collect appropriate data before commencing risk assessment activities.

- Prepare a risk assessment on a range of products of interest, to the desired level of detail consistent with the objectives of the risk manager.
- Determine an illness estimate for a particular product and population.
- Evaluate possible risk mitigations and understand the impact that they might have on disease associated with a particular product and population.

## *4.3 Asking risk management questions*

Non-specific risk management questions were a common theme in many of the O157 risk assessments and also in previous FAO/WHO work. A vague risk management question is one of the primary contributors to a less than optimal risk assessment by increasing the potential for an overly complex assessment that is not able to answer any of the risk management questions in any detail. In formulating risk questions it should also be noted that the strength of risk assessment is not primarily as an estimator of the burden of illness, as this is best done via epidemiological studies, or only estimating the risk of illness per serving. The strength of risk assessments is their ability to, *inter alia*, consider what-if scenarios and their impact on public health; measure relative risk reductions through various strategies; determine where to focus risk management attention; provide insight into where data and information is most likely to improve decisions; and to test strategies before implementation.

The risk question needs to be relevant for the activities of the risk assessment, and it is important to ensure that the risk assessment is fit for purpose, i.e. that it answers the risk question as well as possible given the resources available (time, data, staff). Depending on the risk question it may not be necessary to consider the full chain (e.g. if only interested in interventions at the consumer level, then no model, or a less detailed model, would be needed at the pre-harvest and harvest levels). However, it is often necessary to produce the full farm-to-consumption module (to some degree of complexity) since there are often multiple interventions to be considered at different points in the chain. Risk assessors need to have good communications with risk managers to provide guidance on what risk assessment can and cannot answer, i.e. to condition the expectations of the risk manager.

**Table 2.** Example of the types of risk assessments that could be undertaken to assist risk managers in controlling EHEC in beef and beef products and their potential areas of application.

| Starting point of EHEC risk assessment | Types of risk management advice | Advantages | Disadvantages | Example data or information needs |
|---|---|---|---|---|
| 1) Prevalence and concentration of EHEC in beef products exiting processing plant | Prevalence and concentration level performance targets for beef at the end of slaughter linked to a public health outcome<br><br>Risk reduction effectiveness of post-slaughter interventions<br><br>Ranking of post-slaughter interventions in terms of risk reduction potential<br><br>Risk reduction effectiveness of combinations of interventions | Ability to establish public health-linked performance targets<br><br>Ability to evaluate post-slaughter interventions in terms of risk reduction<br><br>Depending upon specific data availability, relatively fast turnaround time<br><br>Relatively low resource requirement<br><br>Minimal data and information requirements<br><br>Supports the identification and prioritization of data gaps after the processing plant<br><br>All existing EHEC risk assessments cover this scope, so moderate turnaround time due to some existing risk modelling modules | Inability to assess effectiveness of interventions prior to slaughter exit<br><br>No insight into how to achieve performance targets that might be established<br><br>Intervention options that can be implemented post-process are limited | Dose response (generic)<br><br>Consumption patterns (country specific)<br><br>Preparation and handling (country specific)<br><br>Transportation, storage, distribution (country specific)<br><br>Growth (generic)<br><br>Inactivation (generic) |

| Starting point of EHEC risk assessment | Types of risk management advice | Advantages | Disadvantages | Example data or information needs |
|---|---|---|---|---|
| 2) Prevalence of contaminated cattle and contamination level of EHEC on animals entering the slaughter and processing plant | *All of the above +*<br><br>Performance targets for prevalence of contaminated cattle entering plant linked to a public health outcome<br><br>Risk reduction effectiveness of specific interventions between slaughter and consumer<br><br>Risk reduction effectiveness of broader scope interventions such as logistical slaughter<br><br>Ranking of all interventions considered from slaughtering plant to consumer<br><br>Assessment of combinations of interventions from slaughter to consumer to determine most effective combinations | Ability to establish public health-linked performance targets at any point from process to consumer<br><br>Ability to generate guidance on how to achieve performance targets through slaughter interventions<br><br>Ability to evaluate interventions from slaughter through to consumer in terms of risk reduction<br><br>Moderate turnaround time due to some existing risk modelling modules<br><br>Supports increased understanding of how beef processing system works<br><br>Supports the identification and prioritization of data gaps through slaughter plant. Existing risk assessments cover this scope to varying degrees of detail<br><br>All existing EHEC risk assessments cover this scope, so moderate turnaround time due to some existing risk modelling modules | Inability to assess effectiveness of interventions prior to slaughter<br><br>Relatively higher resource requirements<br><br>Data requirements for intervention effectiveness through slaughter process<br><br>More time required to complete assessment model | *All of the above +*<br><br>Slaughter and processing practices and their impact on prevalence and levels of EHEC<br><br>Specific interventions (specific) used in slaughter processing house and their impact (generic) |

| Starting point of EHEC risk assessment | Types of risk management advice | Advantages | Disadvantages | Example data or information needs |
|---|---|---|---|---|
| 3) Herd Prevalence and faecal contamination levels at the farm | *All of the above +*<br><br>Performance targets can be established from farm to consumer linked to a public health outcome<br><br>Risk reduction effectiveness of specific interventions between farm and consumer<br><br>Ranking of all interventions in the farm-to-fork chain<br><br>Assessment of combinations of farm-to-fork interventions to determine multi-hurdle risk management strategy<br><br>General evaluation of farm level management practices (e.g. biosecurity) | Potential to develop risk management strategies across the entire farm-to-fork chain<br><br>Supports increased understanding of how overall beef production system works<br><br>Supports the identification and prioritization of data gaps through farm-to-fork chain<br><br>Ability to evaluate interventions at any point in the farm-to-fork chain<br><br>Existing risk assessments cover this scope in varying degrees of detail. Could therefore include some existing risk modelling modules | Inability to assess effectiveness of interventions at the farm level<br><br>High resource, time and data requirements<br><br>No inference on how to control EHEC at farm level | *All of the above +*<br><br>Prevalence and levels of EHEC in animals and herds (country specific)<br><br>Interventions used and ability to implement (country specific)<br><br>Description of cattle population (country specific)<br><br>Structure and scale of production (country specific)<br><br>Efficacy of interventions (generic)<br><br>Impact of transport and lairage on prevalence and levels of EHEC |
| 4) Routes of introduction and spread of EHEC into and within herds | *All of the above +*<br><br>Detailed evaluation of effects of farm-level management practices on public health risk (e.g. biosecurity) | Potential to develop detailed on-farm risk management strategies<br><br>Increased knowledge on the dynamics of VTEC transmission on beef farm.<br><br>Potential to link such a model to the environment (and ability to consider non-foodborne sources of VTEC) | None of the existing risk assessments cover this scope<br><br>Very high resource, time and data requirements | *All of the above +*<br><br>Increasing the knowledge of the ecology of EHECs<br><br>On-farm management of beef cattle (country specific)<br><br>Animal-level information on levels and frequency of shedding EHECs (generic)<br><br>Survival of EHECs in on-farm environments (Generic) |

**Figure 2.** Roadmap for the application of risk assessment approaches in managing the public health impact of EHEC.



Fazil, 2006
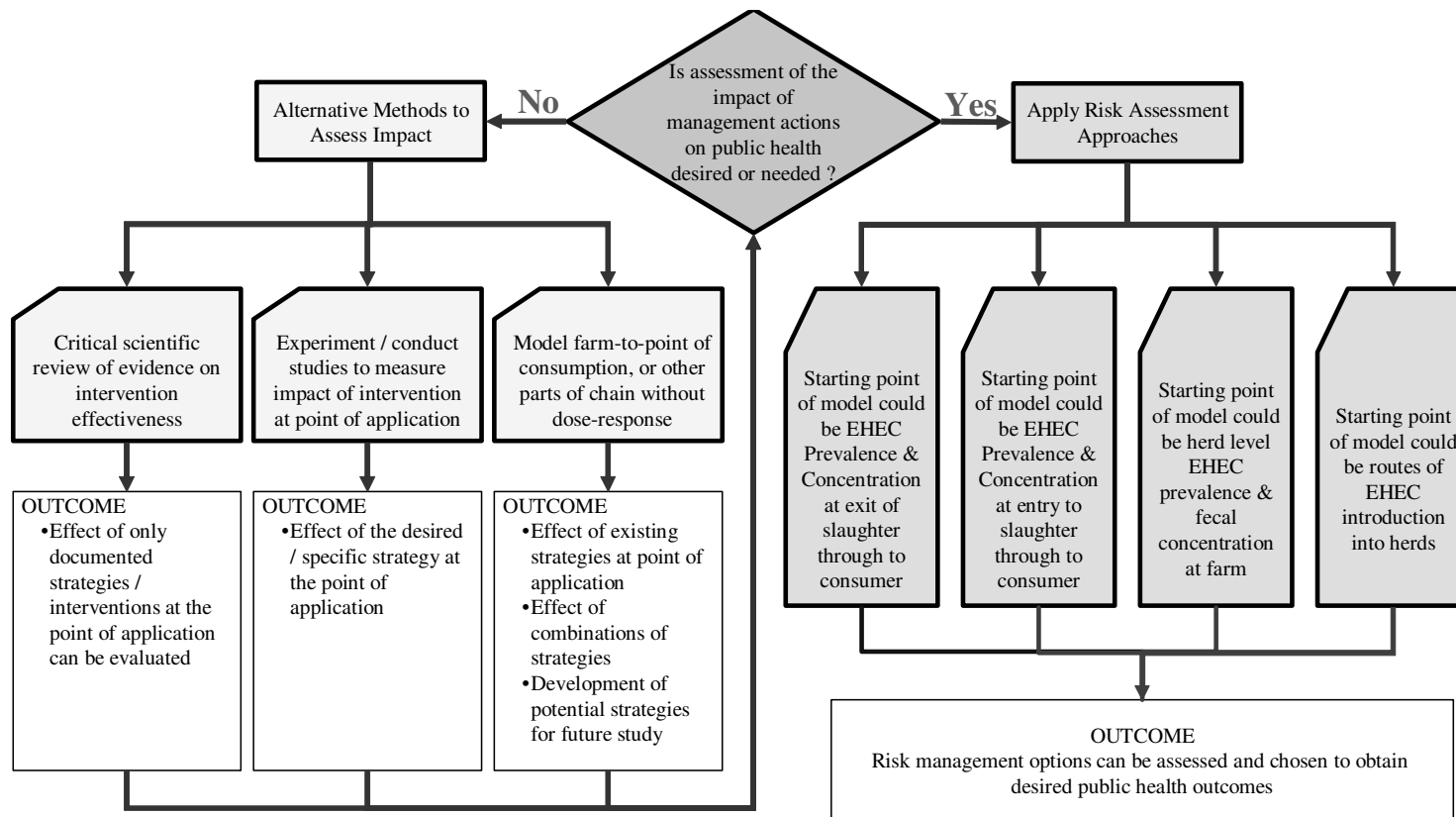
In summary, when posing the risk questions, the following should be taken into consideration:

- Risk assessors and risk managers should jointly identify the risk question(s) and revisit their relevancy during risk assessment development.
- Vague questions should be avoided. With a clear risk question a risk assessment model can be developed that is fit for purpose.
- Prioritize the risk questions and adopt an iterative approach.
- Recognize that a full farm-to-consumption risk assessment is not always necessary.
- A strength of risk assessment is its ability to investigate relative risks or 'what-if' scenarios and link them to public health outcomes.

## *4.4 Application of risk assessment*

Table 2 provides some examples of points in the chain at which an EHEC risk assessment might commence. As noted previously, it is also possible to terminate an assessment prior to determining an illness estimate. In this case, the assessment would properly be called an exposure assessment, rather than a risk assessment, but could be suitable for the purpose. As an example, the first option in the table describes the advantages, disadvantages and type of risk management advice that could be generated with a risk assessment that has as its starting point the prevalence and concentration of EHEC in ground beef exiting the slaughter plant. This type of risk assessment has the advantage of being relatively straightforward to construct (low resource demand), but can only be used to provide insight into the risk or relative risk of various contamination levels and rates exiting the slaughter plant. The assessment could not be used to measure the relative merits of interventions at points prior to the exit of slaughter, but would allow the decision-maker to establish contamination rate or contamination level targets at the exit of slaughter, which could be linked to a public health risk outcome. Risk managers need to aware that a uniform risk reduction factor across the slaughter industry as a whole is challenging, if even feasible, since there can be extensive differences between individual slaughterhouses. Thus there may be a need to initially assess individually the risks associated with different slaughterhouses and their practices.

The specific approaches that could be used to achieve those targets would be left unanswered. Specific guidance on the effect of interventions or combinations of interventions after the slaughtering plant could, however, be assessed. The other options in the table may be interpreted in a similar way.

It should be noted that the options listed in Table 2 are not exhaustive. Other risk-assessment-based approaches or elements of a risk assessment could potentially be used. For example, a model that primarily focuses on the exposure assessment segment of a risk assessment may be more applicable for use by a specific industrial producer. A roadmap for the application of risk assessment approaches in managing the public health impact of EHEC is presented in Figure 2.

## *4.5 Future application of existing risk assessments*

Existing risk assessments provide a basis for future risk assessment work in this area. Risk managers and risk assessors should be cognisant of the following issues in considering the scope of any future commissioned risk assessment.

### 4.5.1 Pre-harvest (Live animal)

Existing risk assessments all start at a point where prevalence and levels of O157 on animals is known.

All pre-harvest intervention strategies are designed to affect prevalence or concentrations, or both, of *E. coli* O157 on animals entering the abattoir.

Given data availability, some variables, such as seasonal variation in prevalence, can be relatively easily incorporated into existing risk assessment models (i.e. variables that can be easily expressed in terms of impact on prevalence or microbial load). The USDA-FSIS model incorporates seasonal effects on prevalence.

Even with available data, some variables are more difficult to incorporate into the existing risk assessment models, such as the incorporation of the effect of temporal dynamics on pathogen levels when considering the duration of fasting and its relationship with the levels of *E. coli* O157 in the animal.

There has been modelling work at the pre-harvest stage that may help in incorporating some of the more complex variables, including transmission, and recycling of organism at farm (Jordan et al., 1999a, b; Wood, McKendrick and Gettinby, 2006; Stacey et al., 2007; Turner et al., 2003. )

## 4.5.2 Harvest (transport, lairage, slaughter and dressing)

Existing risk assessments address this stage relatively comprehensively, with the exception of transport and lairage.

Existing risk assessments can be used without much modification to address intervention strategies (e.g. carcass decontamination measures) that affect levels of the organism on the carcass.

A weakness is that cross-contamination is addressed in a simplistic manner (e.g. mechanisms by which cross-contamination occur are not described). If cross-contamination is a particular area of concern, existing models would have to be modified to adequately address this, and will require specific data that may not currently exist.

Data for the harvest stage may be transferable between countries within similar slaughter processes, although differences do exist, such as permitted carcass decontamination treatments.

## 4.5.3 Post-harvest (processing, transport, storage, distribution, preparation, consumption)

Risk managers will address different stages of post-harvest separately. However, many of following comments apply to each of the stages:

- Existing risk assessments address these steps in a similar manner but there are large geographical differences in the variables, including preparation and consumption practices, and consumer practices (cooking preferences, subjective indications of appropriate cooking, acceptability of raw meat).

- Given data availability, some variables, such as storage time, can be relatively easily incorporated into existing risk assessment models (i.e. variable that can be easily expressed in terms of impact on levels). Therefore these are likely to need country- or cultural-specific survey data.

- A scenario approach could be used to consider some variables, e.g. impact of storage time.

- Cross-contamination at the retail and domestic levels has not been considered in existing models for EHEC, although it is a relevant consideration. Risk assessments for other pathogens have attempted to address this, such as those for *Salmonella* and *Campylobacter*.

- None of the quantitative risk assessments reviewed specifically address specific high-risk products, e.g. some non-heat treated ready-to-eat products. Therefore, the existing models need to be modified to include any considerations specific to these products. While the

modelling is not expected to be complex and some of the relevant predictive models exist, the relevant data need to be collected and collated.

- Current models do not consider the impact of sociological factors on food safety, including providing information to consumer or labelling.

- Risk assessment models can evaluate the impact of actions based on microbiological criteria, e.g. rejection or re-direction of non-conforming product. For instance, the USDA-FSIS risk assessment model has evaluated a specific sampling plan.

## 4.5.4 Assessment of equivalence

The existing risk assessments provide a starting point in terms of establishing performance objectives, although this is still a developing area and one being tackled in other FAO/WHO consultations. Nevertheless, the meeting considered that the use of risk assessment is the only transparent and objective way of showing equivalence between processing systems in terms of public health outcomes. An attempt has been made to explore this direction in the Australian risk assessment (MLA, pers. comm.) but it is an area that still needs more work. Equivalence is not only an issue for internationally traded beef and beef products. Domestically, a risk manager faces issues of equivalence between different plants and between different inspection systems.

It is also important to consider the range of organisms that constitutes an EHEC. Differences in the prevalence of serotypes of importance in different countries may be significant to public health outcomes (See Section 3.5.3). If the prevalence and concentration of a relevant serotype in animals is known to be lower than levels of O157, then a risk assessment based on *E. coli* O157 may be adequate. It may be safe to assume that data on the behaviour of *E. coli* O157 in response to interventions will be the same as for other serotypes, but if microbiological testing is used as a risk management option then that approach may be insensitive to serotypes other than *E. coli* O157.

## 4.5.5 Dose response

The application of a common dose response relationship in risk assessments facilitates consistency and comparison. Use of different dose response relationships in an assessment outcome may introduce more variability than other parameters. While there is uncertainty in the dose-response relationship, there is still sufficient information for estimation of relative risk. There are questions about the applicability of the *E. coli* O157 dose response relationship to other serotypes, but there is little or no evidence that this dose response would not be conservative for other types of EHECs.

# 5. Data gaps and future research needs

The objective of this section is to identify the data gaps and other information and resource needs in order to improve and expand the existing quantitative risk assessments. Quantitative risk assessments are not a substitute for good data or information acquisition, and quantitative risk assessments cannot be conducted in the absence of data required for the development of the model. It was the view of the meeting that data gaps will continue to change and are dependent on the specific risk management questions being addressed, which also limits the range of the data gaps. For example, if the focus of the risk assessment is on harvest issues, then pre-harvest data gaps have no relevance. The data relevant to risk assessment need to be constantly updated, and historical data (on prevalence, consumer practice, etc.) runs the risk of no longer being valid for inclusion in a risk assessment. The ideal would be similar to the Irish risk assessment, where data gathering was carried out in parallel with the quantitative risk assessment.

In the background paper prepared by Sava Buncic, and included as Annex 2 of this report, a review outlines the main scientific knowledge gaps where improvement is needed. In addition, the meeting thought it appropriate to highlight broad categories of information gaps that a risk manager would have to address, depending on the category of risk assessment being undertaken. Table 2 sets out four broad categories of possible risk assessments that could be undertaken and their associated data gaps. The categories are not exhaustive and other risk assessments are conceivable. Likewise, the lists of data requirements for each category of risk assessment are also not exhaustive. The risk assessments may not always need to terminate with an estimate of illness, and this will be determined by the risk management issue being addressed.

The meeting distinguished between information gaps that were universally transferable and country-specific data gaps that will have to be addressed locally to ensure that any future country or regional risk assessment is relevant. The group considered that it was better to describe information gaps that were universally transferable as research priorities, and to highlight these research priorities so that they could be incorporated into international and national research funding programmes. Accordingly, in Table 2, the data gaps are divided into generic and universal issues versus country-specific requirements.

## *5.1 Other information gaps*

### 5.1.1 Non-O157 EHECs

There is a significant lack of information on non-O157 EHECs and consequently this report focuses only on *E. coli* O157:H7. However the narrow focus on one serotype (for example, O157) in a risk assessment and in subsequent control strategies may result in insufficient control of other serotypes.

*E. coli* O157 could well be a useful surrogate for process control as long it can be demonstrated that the behaviour of *E. coli* O157 is representative of the major organism of concern. However, microbial surveillance based only on *E. coli* O157 may well result in other serotypes being missed. This could cause difficulties in trade if the control is based only on *E. coli* O157. However control of EHECs is an emerging control issue and if other serogroups are shown to be a public risk, then policy will be changed accordingly.

### 5.1.2 Dose response

The meeting concluded that although the dose-response component is one of the most generic components of an *E. coli* O157:H7 risk assessment (i.e. applicable to risk assessments developed

in any country), having considered the available models it would also appear to be the most uncertain component of a production-to-consumption risk assessment. While uncertainty remains relating to the appropriateness of the model used by Cassin et al. (1998) it was considered that this could be reduced if the basis of the model were further explained (see Annex 1), and that this might be a good basis for a consensus dose response model at the international level. Although the resultant confidence intervals for this model were wider than the other dose-response curves, it should be noted that this is because it is the only one that considers variability across studies and strains. It should be noted that although there is high uncertainty about the ability of the existing dose-response models to estimate risk of infection and illness, it is not such an issue when the aim of the risk assessment is to identify relative risks, which is one of the strengths of risk assessment.

Sources of data for dose-response models have typically been old data from feeding trials, which are now considered unethical to perform, and outbreak data. It was noted that laboratory models could also be used to provide inference on the impact of gastric fluids and variability in virulence between strains. The applicability of such data needs to be considered further. An identified knowledge gap was whether strains of *E. coli* O157 isolated as part of outbreak investigations are different from those isolated in sporadic cases.

Although outbreaks of *E. coli* O157 are a regular occurrence, the primary role of outbreak investigators is to identify the source and to control the outbreak. Their primary role is not to increase the knowledge base on dose-response mechanisms. Following extensive discussion the meeting agreed that three pieces of information are required to gain dose-response data from outbreaks, and it was suggested that these be communicated within any FAO/WHO-produced documents relating to this area. The three data requirements, which would provide one data point on the dose-response curve, are:

- dose consumed (number of *E. coli* O157 per gram and number of grams of food vehicle consumed);
- number of people exposed; and
- number of people with a response (e.g. illness).

In terms of obtaining such data, it was considered that those countries that have a requirement for large catering establishments (e.g. schools, care homes) to keep samples of all meals for a period of time after serving to assist their investigations in the event of an outbreak occurring were the most likely source of this type of information. It was suggested that were an international risk assessment on EHEC to be commissioned, then FAO and WHO should approach these particular countries with the abovementioned requirement in place to establish whether they have any new EHEC information that could be shared and hence used in the dose-response model.

No dose-response relationships are available for non-O157 EHECs, which is a significant data gap. Consequently there is high uncertainty on the infectivity and virulence of these non-O157 pathotypes, and how representative the current O157 dose-response models are for other EHECs. Certainly, it seems that *E. coli* O157 with *vt2* and *eae* genes are the pathotypes more often associated with cases of HUS.

In conclusion, while there are a number of dose response estimates available, it was considered by the meeting that all are in need of improvement. There is consensus required to develop a standardized dose response module that can be bolted on to new risk assessments at international and national level. The existing dose response models were developed for *E. coli* O157 and it is unknown how applicable they are to other EHECs. However, in the absence of other knowledge, and reflecting the uncertainty associated with the current dose response models,

they can possibly be applied to other EHECs. It should be recognized that while there is considerable uncertainty in the existing dose response models, it does not invalidate their use for comparative purposes. There was agreement that there is a requirement to develop a consensus dose response so that outputs from different exposure assessments are put through the same dose response model and so the results of the risk assessments can be compared.

## 5.1.3 Emerging issues

The meeting recognized that processes and practices are continually changing and the pathogens of concern also continue to evolve. Changing practices in the food chain could impinge on the validity of a risk assessment. Therefore, existing risk assessments may need to be updated prior to use in informing future risk management actions. This will require systematic studies of new practices, which will need to be structured to meet the needs of risk assessment. The same applies to the development of new risk assessments. To be useful they must take into account the most up-to-date situation and data. Certain data loses its value with time and needs to be updated. However, it was also noted that data collection can be expensive and not always feasible. At the same time, some data can be considered to be shelf-stable, such as basic relationships for predictive microbiology. Thus the application of tools such as predictive microbiology and the sharing of new data as it becomes available are critical to ensure that risk assessments can be developed and updated in a way that addresses new issues as they emerge.

# 6. Conclusions and recommendations

Many of the risk management decisions made to date to address the problems associated with EHECs, and specifically *E. coli* O157:H7, were of a GHP nature and not explicitly based on risk assessment. Apart from the United States of America, which had witnessed a decline in the incidence of EHEC cases at the time of the meeting, all the other countries represented in this meeting had not seen a significant public health effect from any of the interventions that might have been taken along the production chain. Foodborne EHEC remains an important public health problem. Predominantly, in the past, corrections to risk management decisions have been led by single or independent scientific evaluations. For example, initially washing hides seemed like a good intervention but subsequently scientific studies showed that aerosols can spread contamination from hides to carcasses. This, however, did not directly demonstrate that washing hides would increase the level of contamination on carcasses. This correction did not necessarily look at the effect on risk but rather focused on the effect on hazard alone. Thus it was concluded that while risk managers can undertake common sense risk management activities without risk assessment, when more difficult decisions are made or complex interventions throughout the food chain are needed, the risk assessment is more likely to help. This appears to be a common thread in many countries.

The risk assessment performed in Ireland is, to the best knowledge of the meeting, the only EHEC risk assessment designed using an iterative process of collaboration with risk managers. It is also the only EHEC risk assessment where the model can be validated and the effects of controls measured. Nevertheless, the other EHEC risk assessments developed provide a valuable resource for risk managers and those planning risk assessment work on EHEC in ground beef and related products.

While the existing risk management actions to address EHEC were designed taking into consideration available science, the meeting concluded that more widely applicable EHEC risk assessment would permit future risk management actions to be analysed based on their quantifiable impact on public health, while also taking into consideration other priorities, resources, etc.

Risk assessments may generate universally applicable information in terms of decision-making advice, but country-specific risk management actions need to be predicated on supplemental country-specific data. There is no such thing as a 'one size fits all' risk assessment, but certain elements of specific risk assessments may be widely applicable. In some cases it may be adequate to supplement a risk assessment model with data representative of the situation being assessed, but in other cases more substantive changes to the model may be required (for example due to differences in processing steps among countries).

As always, knowledge gaps inhibit the construction of risk assessments. In particular the lack of agreement on the dose-response relationship limits the comparability of different models for estimating the probability and magnitude of illness. There are several dose response models available, and while no single model is perfect, the adoption of a consensus model would allow for consistency and comparability of national risk assessments. The meeting recommended that the development of a consensus dose response model at the international level would be a valuable asset to future risk assessments in this area.

As the development of risk assessments can be resource intensive, the value of sharing models and expertise cannot be underestimated. However, as risk assessments are not currently developed in a format that can be easily and readily made available, the meeting recommended

that, in the design of future risk assessments, consideration be given as to how generic modules in particular could be easily shared with other users. It was acknowledged that simply making the whole risk assessment available on the internet does not necessarily make it more accessible, and therefore efforts in this direction requires incorporation of user-friendly explanatory elements into the model. While microbial risk assessment should strive for transparency, it should also be recognized that the technical nature of the model presents real barriers to meaningful broad participation.

Although this deliberation addressed the control of risk associated with *E. coli* O157 in beef, there is a broader need to address risks associated with this and other EHECs in the environment and on other meats and foods. The meeting made the assumption that EHECs other than *E. coli* O157:H7 would respond to many food safety measures in the same manner, or at least would not be made worse. It further concluded that controls for EHEC would also affect other pathogens.

In terms of developing future risk assessment work on this issue, it was recommended that FAO and WHO:

- develop a flexible risk assessment tool with generic modules that could be adapted for use at national level;
- develop a consensus module for dose-response;
- provide a template of how such generic modules could be used to illustrate the application of the tool at national level and also to provide a basis for some broad or widely applicable risk management actions in this area; and
- recognize that the interventions applicable in some countries might not be the same as those for other countries and take these into consideration in developing the model so that the model would be relevant and adaptable for all countries.

With regard to the scientific basis for future risk management on the control of EHEC in raw beef and beef products, the meeting recommended that risk managers at both national and international level consider the approach provided in this report and in particular Table 2 in its deliberation on what kind of scientific advice they need and what issues they want to have addressed.

# 7. References

Banatvala, N., Griffin, P.M., Greene, K.D., Barrett, T.J., Bibb, W.F., Green, J.H. & Wells, J.G. 2001. The United States National Prospective Hemolytic Uremic Syndrome Study: microbiologic, serologic, clinical, and epidemiological findings. *Journal of Infectious Diseases*, 183(7): 1063–1070.

Cassin, M.H., Lammerding, A.M., Todd, E.C.D., Ross, W. & McColl, R.S. 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *International Journal of Food Microbiology,* 41: 21–44.

CAC [Codex Alimentarius Commission]. 2002. Risk profile for enterohaemorrhagic *E. coli* including the identification of the commodities of concern, including sprouts, ground beef and pork. Doc. no. CX/FH 03/5-Add.4.

CAC. 2004. Report of the Thirty-Sixth Session of the Codex Committee on Food Hygiene, Washington, DC, United States of America, 29 March–03 April 2004. Report ALINORM 04/27/13. Available at http://www.codexalimentarius.net/download/report/615/al04_13e.pdf

CCFH [Codex Committee on Food Hygiene]. 2005. Discussion paper on guidelines for the application of the general principles of food hygiene to the risk based control of enterohaemorrhagic *Escherichia coli* in ground beef and fermented sausages. Doc. no. CX/FH 05/37/11. Available at ftp://ftp.fao.org/codex/ccfh37/fh37_11e.pdf

EFSA [European Food Safety Authority]. 2006. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004. Available at http://www.efsa.europa.eu/en/efsajournal/pub/310ar.htm

FSAI [Food Safety Authority of Ireland]. 1999. The prevention of *E. coli* O157:H7 infection – A shared responsibility. Available at http://www.lenus.ie/hse/bitstream/10147/44797/1/6358.pdf

FAO/WHO. 2006. The use of microbiological risk assessment outputs to develop practical risk management strategies: metrics to improve food safety. [Report of the] FAO/WHO Expert Meeting on the Development of Practical Risk Management Strategies Based on Microbiological Risk Assessment Outputs. FAO/WHO in collaboration with the Federal Dairy Research Centre, Institute for Hygiene and Food Safety, Kiel, Germany, 3–7 April 2006. Available at http://www.who.int/foodsafety/micro/jemra/meetings/2005/en/index.html

FAO/WHO. 2008. *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered formulae: Meeting report. *Microbiological Risk Assessment Series*, No. 15.

Hancock, D.D., Besser, T.E., Lejeune, J., Davis, M. & Rice, D. 2001. The control of VTEC in the animal reservoir. *International Journal of Food Microbiology*, 66(1-2): 71–78.

Hilborn, E.D., Mermin, J.H. & Mshar, P.A. 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. *Archives of Internal Medicine,* 159(15): 1758–1764.

ICMSF [International Commission on Microbiological Specifications for Foods]. 2002. *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management*. Kluwer Academic/Plenum Publishers, New York, USA.

Jordan, D., McEwan, S.A., Lammerding, A.M., McNab. W.B. & Wilson, J.B. 1999a. A simulation model for studying the role of pre-slaughter factors on the exposure of beef carcasses to human microbial hazards. *Preventive Veterinary Medicine*, 41: 37–54.

Jordan, D., McEwan, S.A., Lammerding, A.M., McNab, W.B. & Wilson, J.B. 1999b. Pre-slaughter control of *Escherichia coli* O157 in beef cattle: a simulation study. *Preventive Veterinary Medicine*, 41: 55–74.

Kawamura, N., Yamazaki, T. & Tamai, H. 1999. Risk factors for the development of *Escherichia coli* O157:H7 associated with haemolytic uremic syndrome. *Pediatrics International*, 41: 218–222.

López, E.L., Contrini, M.M., Sanz, M., Viñas, M.R., Parma, A., De Rosa, M.F. & Cleary, T.G. 1997. Perspectives on shiga-like toxin infections in Argentina. *Journal of Food Protection,* 60: 1458–1462.

Martin, D.L., Gustafson, T.L., Pelosi, J.W., Suarez, L. & Pierce, G.V. 1986. Contaminated produce – a common source for two outbreaks of shigella gastroenteritis. *American Journal of Epidemiology,* 124(2): 299–305.

Nally, P., Cummins, E., Butler, F., O'Brien, S., Carney, E., Duffy, G. & Sheridan, J.J. 2006. Evaluation of microbiological performance objectives for the beef industry. pp. 301–302, *in:* D. Troy, R. Pearce, B. Byrne and J. Kerry (editors). Proceedings of the 52nd International Congress of Meat Science and Technology. Wageningen Academic Publishers, The Netherlands.

Nauta, M. 2005. Microbiological risk assessment models for partitioning and mixing during food handling. *International Journal of Food Microbiology,* 100: 311–22.

Nauta, M.J., Evers, E.G., Takumi, K. & Havelaar, A.H. 2001. Risk assessment of Shiga-toxin producing *Escherichia coli* O157 in steak tartare in the Netherlands. RIVM report 257851003. RIVM, Bilthoven, The Netherlands.

Naylor, S.W., Low, J.C., Besser, T.E., Mahajan, A., Gunn, G.J., Pearce, M.C., McKendrick, I.J., Smith, D.G.E. & Gally, D.L. 2003. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infection and Immunity*, 71: 1505–1512.

OzFoodNet, 2001. Annual report. Enhancing foodborne disease surveillance across Australia in 2001: the OzFoodNet Working Group. Available at http://www.health.gov.au/internet/wcms/publishing.nsf/Content/cda-cdi2603-pdf-cnt.htm/$FILE/cdi2603d.pdf

Powell, M., Ebel, E., Schlosser, W., Walderhaug, M. & Kause, J. 2000. Dose-response envelope for *Escherichia coli* O157:H7. *Quantitative Microbiology,* 2: 141–163.

Riley, L.W., Remis, R.S., Helgerson, S.S., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., Blake, P.A. & Cohen, M.L. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine*, 308: 681–685.

Sneyd, E., Lopez, L., Eglinton, M., McDowell, R. & Margolin, T. 2002. Annual surveillance summary 2001. Client Report FW0165. ESR, Kenepuru Drive, Porirua, New Zealand.

Sofos, J.N. (editor). 2005. *Improving the Safety of Fresh Meat*. CRC/Woodhead Publishing, Cambridge, UK. 780 p.

Stacey, K.F., Parsons, D.J., Christiansen, K.H. & Burton, C.H. 2007. Assessing the effect of interventions on the risk of cattle and sheep carrying *Escherichia coli* O157:H7 to the abattoir using a stochastic model. *Preventive Veterinary Medicine*, 79(1): 32–45.

Teagasc [Agriculture and Food Development Authority of Ireland]. 2006. *E. coli* O157:H7 in beef burgers produced in the Republic of Ireland: A quantitative microbial risk assessment. Ashtown Food Research Centre, Dublin, Ireland.

Teunis, P., Takumi , K. & Shinagawa, K. 2004. Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. *Risk Analysis,* 24: 401–407.

Turner, J., Begon, M., Bowers, R.G. & French, N.P. 2003. A model appropriate to the transmission of a human foodborne pathogen in a multigroup managed herd. *Preventive Veterinary Medicine*, 57(4): 175–198.

US FDA [United States of America Food and Drugs Administration]. 2006. *Update: E. coli O157:H7 outbreak at Taco bell restaurants likely over. FDA traceback investigation continues.* Retrieved 1/6, 2009, from http://www.fda.gov/bbs/topics/NEWS/2006/NEW01527.html

US FDA. 2007. *FDA and states closer to identifying source of E. coli contamination associated with illness at Taco John's restaurants.* Retrieved 1/6, 2009, from http://www.fda.gov/bbs/topics/NEWS/2007/NEW01546.html

USDA-FSIS [United States Department of Agriculture, Food Safety and Inspection Service]. 2001. Draft risk assessment of the public health impact of *Escherichia coli* O157:H7 in ground beef. USDA, Washington, DC, USA. Available at http://www.fsis.usda.gov/Science/Risk_assessments/index.asp#e.coli

USDA-FSIS. 2002. Comparative risk assessment for intact (non-tenderized) and non-intact (tenderized) beef: Technical report. USDA, Washington, DC, USA. Available at http://www.fsis.usda.gov/Science/Risk_assessments/index.asp#e.coli

Vugia, D., Cronquist, A., Hadler, J. and 13 others. 2005. Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food – 10 Sites, United States, 2004. *Morbidity and Mortality Weekly Report,* 54(14): 352–356.

Wood, J.C., McKendrick, I.J. & Gettinby, G. 2006. A simulation model for the study of the within-animal infection dynamics of *E. coli* O157. *Preventive Veterinary Medicine,* 74(2-3): 180–193.

# Annex 1 – Background paper 1
# Overview of existing risk assessments on enterohaemorrhagic *Escherichia coli* in meat and meat products

Prepared by Mark Powell,
USDA/ORACBA, Washington, DC, United States of America.

---

DISCLAIMER

This summary paper was prepared in response to a request from FAO/WHO stating specific Terms of Reference, which determined the main scope of the paper, as follows:

- Provide a summary and analysis of existing microbiological risk assessments (MRA) of *E. coli* O157 in different meat and meat products done at national level. In doing so, the author will in particular present an analysis of the strong and weak elements of each relevant MRA with regards to what they can and cannot provide in terms of risk estimation and scenario analysis.

- Identify and discuss the elements of these risk assessments that would particularly be useful in developing an international risk assessment, as the meeting will consider whether it is necessary or possible to develop such a risk assessment to address risk management of this hazard at an international level.

    In addition to the above, and also due to the limited timeframe in which this paper was prepared, the author, FAO and WHO do not warrant that the information contained herein is complete and shall not be liable for any damage incurred as a result of its use.

    The views expressed herein are those of the authors and do not necessarily represent those of FAO or WHO or the United States Department of Agriculture (USDA). Reference herein to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation or favouring by the United States Government.

---

## *A1. Introduction*

Butler et al. (2006) provide a summary of several available risk assessments of EHEC in meat and meat products: Cassin et al. (1998); Lammerding et al. (1999); USDA-FSIS (2001); Nauta et al. (2001); and Duffy et al. (2005). (Note: USDA-FSIS has also conducted a risk assessment of *E. coli* O157:H7 in blade-tenderized beef products. However, that risk assessment was not included in the review charge, and USDA-FSIS plans to update that risk assessment based on more recent information.) Therefore, this background paper focuses primarily on available dose-response analyses. Other risk assessment topics that may generalize internationally include a working definition of EHEC, evaluation of microbiological methods for EHEC identification, EHEC growth and inactivation (by fermentation or heat), the efficacy of well-specified controls or treatments in removing EHEC contamination that may be present in raw product, and case ratios (e.g. proportion of EHEC infected individuals that progress to more severe illnesses).

## *A1.1 EHEC dose-response analyses*

### A1.1.1 Cassin et al., 1998

Cassin et al. (1998) used what they refer to as a Beta-Binomial dose-response model for *E. coli* O157:H7. However, based on the available documentation, the dose-response model does not appear to be based on the beta-binomial likelihood function, which allows for extra-binomial dispersion among replicates (Haas, Rose and Gerba, 1999: 299–300). Instead, the dose-response relationship appears to be based on a random coefficients model developed by Ross (1995) that accounts for random variability among studies. Given the substantial variability in infectivity observed among EHEC strains and studies, a random coefficients dose-response model may be preferable to the more conventional, non-hierarchical dose-response models used in other EHEC risk assessments. The latter limit inferences to the strains and studies observed.

As shown in Figure A1.1 below, the dose-response model specified by Cassin et al. (1998, Table A5) differs from that presented by Cassin et al. (1998, Figure 2). The latter is actually the corresponding beta-Poisson (BP) model. The expected value of the dose-response (not shown) is the same for both curves, but the confidence intervals for the Beta-Binomial (BB) are broader.

**Figure A1.1.** Comparison of Cassin et al. (1998) dose-response models
NOTES: BP = Beta-Poisson; BB = Beta-Binomial.
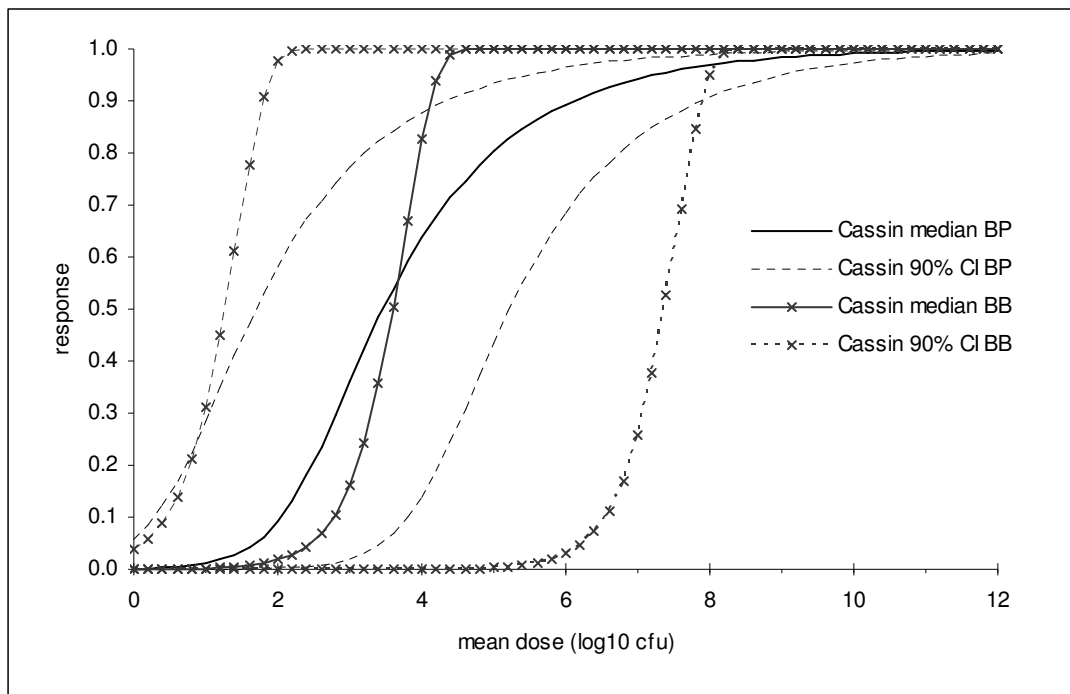


In developing the dose-response model, Cassin et al. (1998) used human clinical data on *Shigella dysenteriae* and *S. flexneri* reported in Crockett et al. (1996). However, Crockett et al. (1996) only found a significant fit to the beta-Poisson model for the pooled *S. dysenteriae* and *S. flexneri* data after removing a suspected outlier (the $10^7$ colony forming units (cfu) dose group for *S. flexneri*). Therefore, it is unclear exactly which dose-response data were used by Cassin et al. (1998). Furthermore, Crockett et al. (1996) omit clinical trial data available for *S. dysenteriae* Strain A-1. (Data are available for two *S. dysenteriae* Strain A-1 dose groups: $2 \times 10^2$ cfu, with 1 out of 4 subjects ill; and $10^4$ cfu, with 2 out of 6 subjects ill. Levine et al. (1973), cited by Crockett *et al.* (1996), is the source for both the Strain A-1 and Strain M131 data.)

Based on the conventional beta-Poisson dose-response model (Haas, Rose and Gerba, 1999), combining the *S. dysenteriae* Strain A-1 and Strain M131 data provides a statistically significant fit. Although there are insufficient degrees of freedom to statistically assess variability between strains because there were only two dose groups for *S. dysenteriae* A-1, Figure A1.2 below suggests that the omission of the *S. dysenteriae* Strain A-1 clinical trial data may have a practical impact on the estimated dose-response relationship for *S. dysenteriae*. In Figure A1.2, the beta-Poisson model parameter maximum likelihood estimates (MLEs) were obtained by minimizing the residual deviance (Regli et al., 1991), and the confidence intervals were obtained by parametric bootstrapping. (See Appendix for details.) Similar to the findings of Crockett et al. (1996), however, pooling all of the *S. dysenteriae* (A-1 and M131) and *S. flexneri* data results in a statistically insignificant beta-

Poisson model fit, but removing the suspect outlier ($10^7$ cfu dose group for *S. flexneri*) results in a statistically significant fit to the beta-Poisson model.

**Figure A1.2.** Beta-Poisson dose-response models for *S. dysenteriae* strains
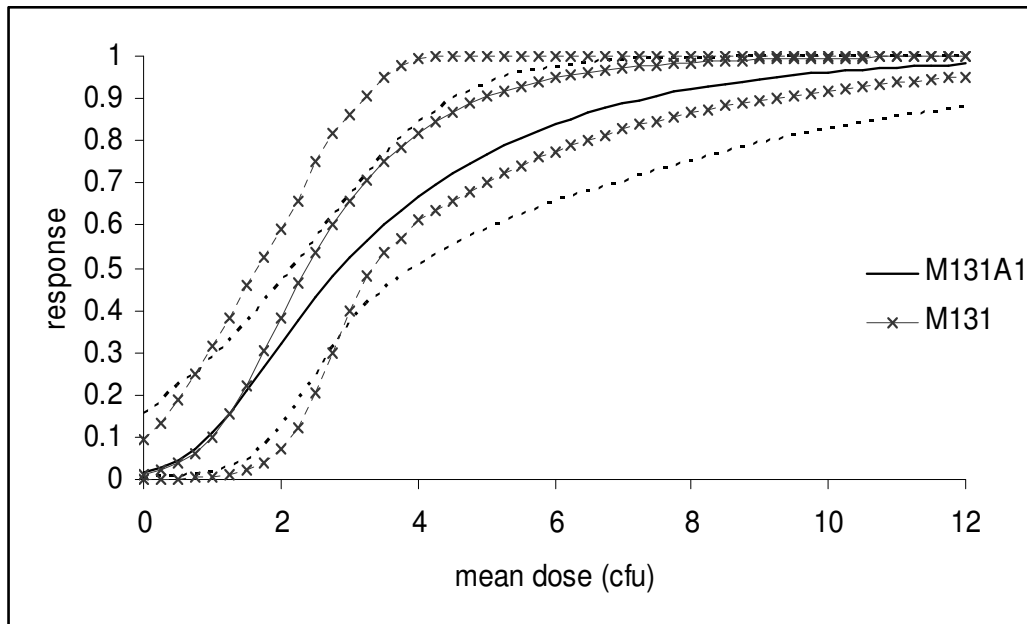


## A1.1.2 Lammerding et al., 1999

Lammerding et al. (1999) used the same dose-response model and parameters used by Cassin et al. (1998). However, Lammerding et al. (1999) only mentions the use of *S. dysenteriae* as a surrogate; there is no mention that the model used by Cassin et al. (1998) also is derived from *S. flexneri*.

## A1.1.3 USDA-FSIS 2001

USDA-FSIS (2001) used a dose-response envelope model described by Powell et al. (2000) to estimate the dose-response relationship for *E. coli* O157:H7. The upper bound of the envelope was the MLE of the beta-Poisson model for *S. dysenteriae* (M131 and A-1) (solid black line in Figure A1.2). The lower bound of the envelope was the MLE of the beta-Poisson model based on human clinical trial data for four enteropathogenic *E. coli* (EPEC) strains. Within these bounds, a 'best estimate' of the dose-response relationship was obtained by 'anchoring,' or calibrating the dose-response model parameters such that the product of the exposure assessment output distribution and the dose-response model agreed with an epidemiologically-based estimate of the annual number of cases of *E. coli* O157:H7 illness in the United States of America attributable to ground beef consumption.

The motivation for specifying a single most likely value within the bounds of the envelope was to provide a point estimate and an uncertainty range for each percentile of the variability distribution, consistent with two-dimensional Monte Carlo modelling approaches that seek to separate uncertainty and variability. There are several disadvantages to the anchoring

approach, however. First, the specification of the 'best estimate' of the dose-response curve is subjective; the median was assumed to be the 'best estimate', but the mode or average could have been used. Second, the 'best estimate' of the dose-response curve changes with each update or revision of the exposure assessment model or epidemiologically-based estimate of the burden of illness. For example, the 'most likely value' reported by Powell et al. (2000) was not identical to the 'best estimate' presented by USDA-FSIS (2001), since the latter reflected revisions to the exposure model. Finally, an expert panel that reviewed USDA-FSIS (2001) concluded that although the 'anchoring' technique is "well founded in health risk assessment and the related field of environmental modelling ... the ability to validate the model through comparison with observed events or the output of other *E. coli* O157:H7 risk assessments is compromised" (Institute of Medicine, 2002: 10). Institute of Medicine (2002) recommended removing the algorithms for calibrating the dose-response parameters and replacing them with model elements based on evidence that is independent of *E. coli* O157:H7 epidemiologic data to allow for a stable evidence base and provide for some validation of model estimates with epidemiologic data.

Institute of Medicine (2002) also supported the decision to use *Shigella* as an upper bound for the EHEC dose-response function, but found the use of EPEC as the lower bound problematic because epidemiologic data do not support the relevance of EPEC as a surrogate for EHEC. Institute of Medicine (2002: 81) recommended several candidates for a more rational source of a lower limit to bracket the EHEC dose-response relationship: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), diffusely adherent *E. coli* (DAEC), *Campylobacter jejuni, Salmonella enterica* serovar Typhi, and *V. cholerae* O1, O139 and non-O1/non-O139. It is worth noting, however, that the lower confidence limit for the dose-response model used by Cassin et al. (1998) based on *Shigella* spp. is comparable with the EPEC dose-response function used by USDA-FSIS (2001) as the lower bound for *E. coli* O157:H7 for mean doses less than $10^5$ cfu (Figure A1.3). This suggests that the expert panel (Institute of Medicine, 2002) may have underestimated the full range of uncertainty in the EHEC dose-response relationship.

**Figure A1.3.** Comparison of Cassin et al. (1998) *Shigella* spp. and USDA-FSIS (2001) EPEC dose-response models
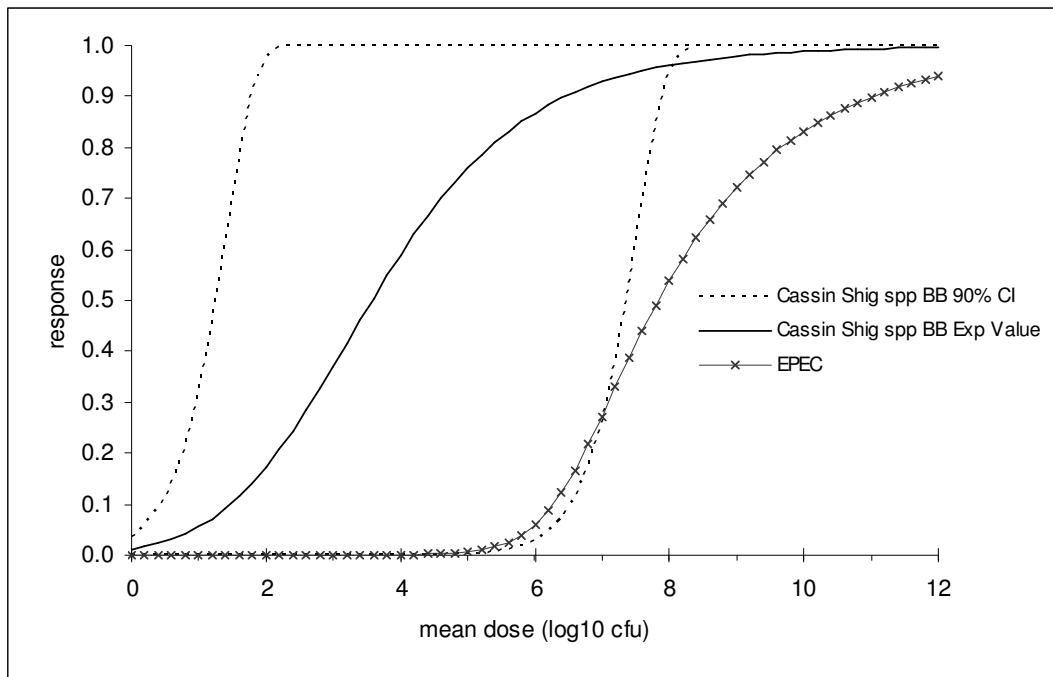


## A1.1.4 Nauta et al., 2001

Nauta *et al*. (2001) based their dose-response analysis on a 1996 *E. coli* O157:H7 outbreak in Morioka, Japan. A significant advantage of this approach is that the model is based on a well-documented outbreak of *E. coli* O157:H7 in humans. Given the rather unique outbreak data, including stool samples collected from all exposed individuals, the dose-response endpoint is the probability of infection, rather than the symptomatic illness that is more commonly reported. This provides potentially important insights about attack rates and case ratios. Samples of the suspected foods were also available for quantitative analysis, allowing uncommonly accurate estimation of the bacterial concentration for an outbreak exposure reconstruction. The primary limitation is that the outbreak provides a single data point with which to estimate the entire dose-response function.

A noteworthy observation based on the outbreak report alone is that the infection rates in children (208 of 828) and adults (7 of 43) are not significantly different. In Figure A1.4 uncertainty about the infections rates is characterized by beta distributions using the method of matching moments. This suggests the possibility that children may be no more susceptible than adults to infection (i.e. colonization) with *E. coli* O157:H7, but may be more susceptible to more severe health outcomes caused by infection.

**Figure A1.4.** Comparison of infection rates in children and adults from the 1996 *E. coli* O157:H7 outbreak in Morioka, Japan
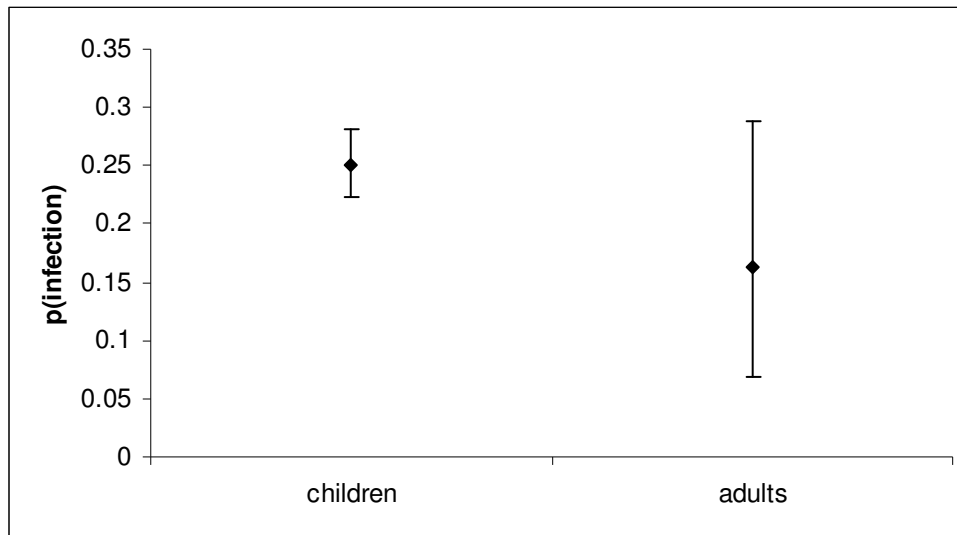


Figure A1.5 compares the exponential dose-response function calculated based on the Japan 1996 outbreak data reported by Nauta *et al.* (2001), for adults and children and adjusted for the reported 55% case ratio (p(illness|infection)), with an exponential dose-response function calculated for the 1992–93 multi-state outbreak in the United States of America Pacific Northwest. Powell et al. (2000) provided a crude reconstruction of the 1992–93 outbreak to provide starting values for the dose-response 'anchoring' algorithm (shown as an open diamond). Figure A1.5 presents an exponential dose-response function calculated using a slightly more refined estimate for the 1992–93 Pacific-Northwest outbreak (solid diamond, using the mean consumed dose, a more precise estimate of the number of servings rendered free of *E. coli* O157:H7 by cooking, and adjusting for under-reporting of illness according to USDA-FSIS (2001)). Compared on the basis of a common endpoint—cumulative probability of illness—the difference in the location of the two exponential curves is well within the range reasonably expected given the large parameter uncertainty and the variability among consumers and EHEC strains. Although crude, this comparison suggests the potential for considering additional outbreak data in formulating a dose-response analysis for EHEC in meat and meat products.

**Figure A1.5.** Comparison of exponential dose-response functions for *E. coli* O157:H7 based on Nauta *et al.* (2001) analysis of 1996 Japanese outbreak and analysis of the 1992-93 Pacific-Northwest outbreak
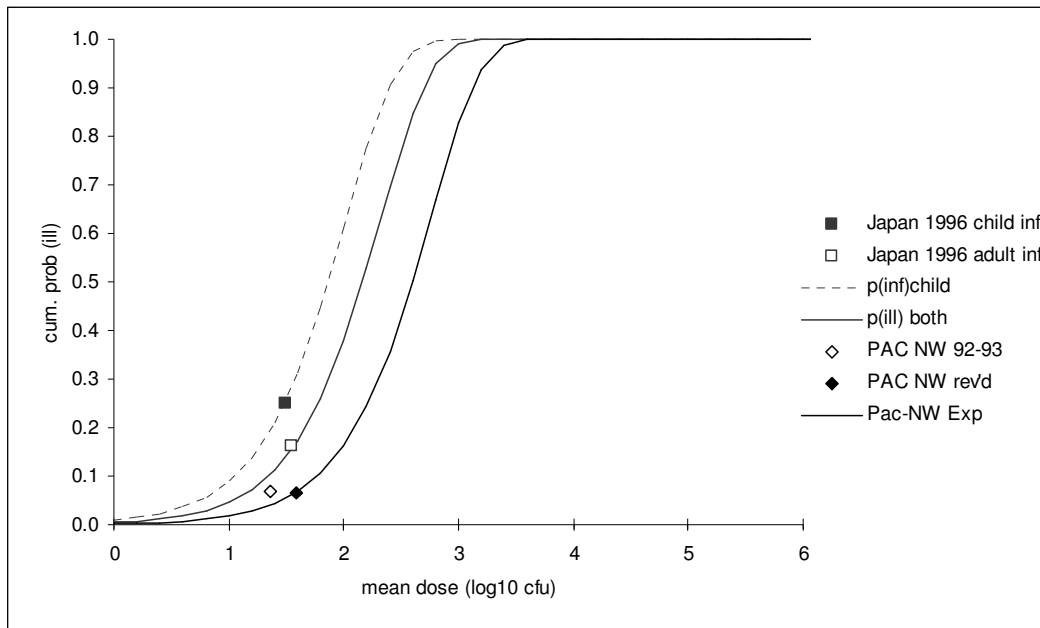


Nauta et al. (2001) do not provide sufficient documentation to reproduce the hypergeometric (beta-Poisson) dose-response model results. Because a single outbreak data point is used to update the model, the prior distribution for the parameters ($\alpha$, $\beta$) must dominate the posterior distribution. The functional forms of the prior distributions for $\alpha$ and $\beta$ are specified, but there is no mention of the values. However, Teunis, Takumi and Shinagawa (2004) present a more detailed description of the hypergeometric (beta-Poisson) dose-response analysis of the 1996 outbreak in Morioka, Japan. The latter paper includes a detailed specification of the diffuse prior on the dose-response model parameters. Unsurprisingly, therefore, Teunis, Takumi and Shinagawa (2004: Figure 1) indicate large uncertainty surrounding the posterior dose-response relationship. Although comparing infection and illness rates clouds the analysis to some extent, like Nauta et al. (2001), Teunis, Takumi and Shinagawa (2004) found the responses from both children and adults in the 1996 Japanese outbreak consistent with the human clinical trial data for *Shigella dysenteriae*, but strongly discordant with the *E. coli* O157:H7 infant rabbit model data and the human clinical trial data for EPEC.

## A1.1.5 Duffy et al., 2005

Duffy et al. (2005) report using the *E. coli* O157:H7 dose-response model reported by Powell et al. (2000). However, only the estimated 'most likely value' reported by Powell et al. (2000), rather than the entire dose-response envelope, was used in the model developed by Duffy et al. (2005).

## A1.1.6 Strachan et al., 2005

Duffy et al. (2006) note that Strachan et al. (2005) evaluated additional *E. coli* O157:H7 outbreak data relevant to dose-response analysis. Specifically, Strachan et al. (2005) discuss eight outbreaks that suggest a very broad scatter in the observed interaction between the pathogen and human host. Figure A1.6 represents the outbreak data presented by Strachan et al. (2005), as well as their estimated dose-response curve.

**Figure A1.6.** Outbreak data and dose-response curve estimated by Strachan et al. (2005)
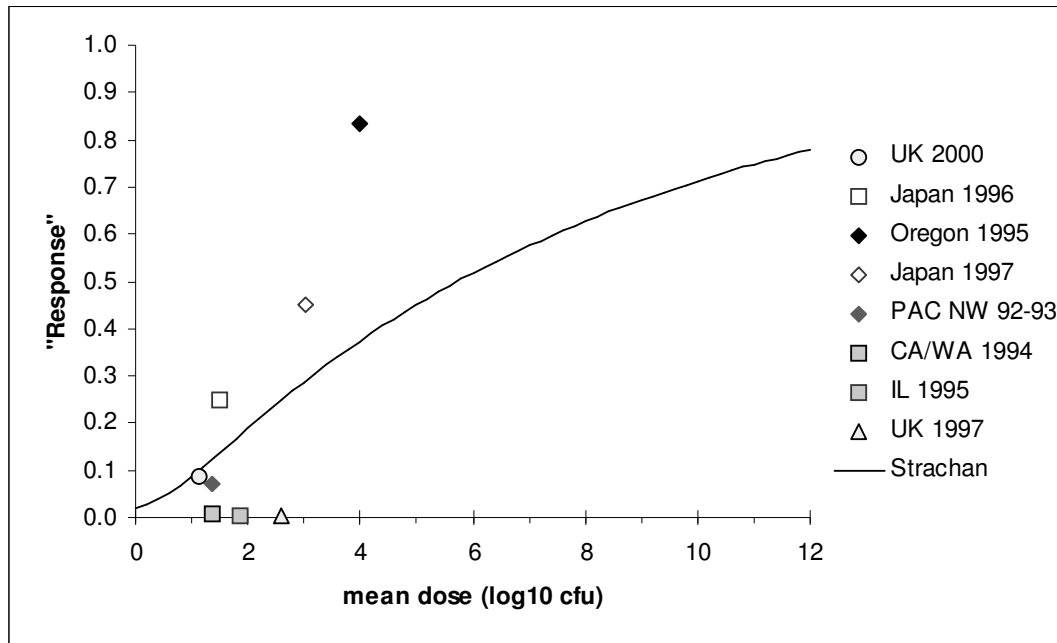


The important point to note from Figure A1.6 is the large variability among outbreaks; however, this is clouded by the use of an inconsistent definition of 'response' among the outbreaks. In some outbreaks, the observed response was illness, whereas in other outbreaks the observed response was infection. In addition, in some cases, the estimated outbreak attack rate was adjusted for under-reporting, while in other outbreaks the estimated rate reflects only reported cases. Especially in outbreaks involving large numbers of exposed consumers, the proportion of unreported illness may be substantial. Furthermore, while Strachan et al. (2005) demonstrate a useful analytical approach that may be applied to outbreak data analysis, the maximum likelihood estimate of the dose-response relationship obtained by Strachan et al. (2005) was not statistically significant. It may be possible to refine the analysis by adopting a consistent response definition and incorporating any additional EHEC outbreak data where information is available about the attack rate (including the number of exposed consumers who did not become ill) and the ingested dose.

Finally, despite the inconsistency in the definition of response, it is useful to consider the outbreak data presented by Strachan et al. (2005) in the light of the previously available dose-response analyses. Although limited inferences may be drawn from what appears to be a tangled bowl of pasta (Figure A1.7), it appears that the random coefficients *Shigella* dose-

response model developed by Ross (1995) (and used by Cassin et al., 1998) may be reasonable, at least as a first approximation.

**Figure A1.7.** *E. coli* O157:H7 dose-response 'Pasta Model'
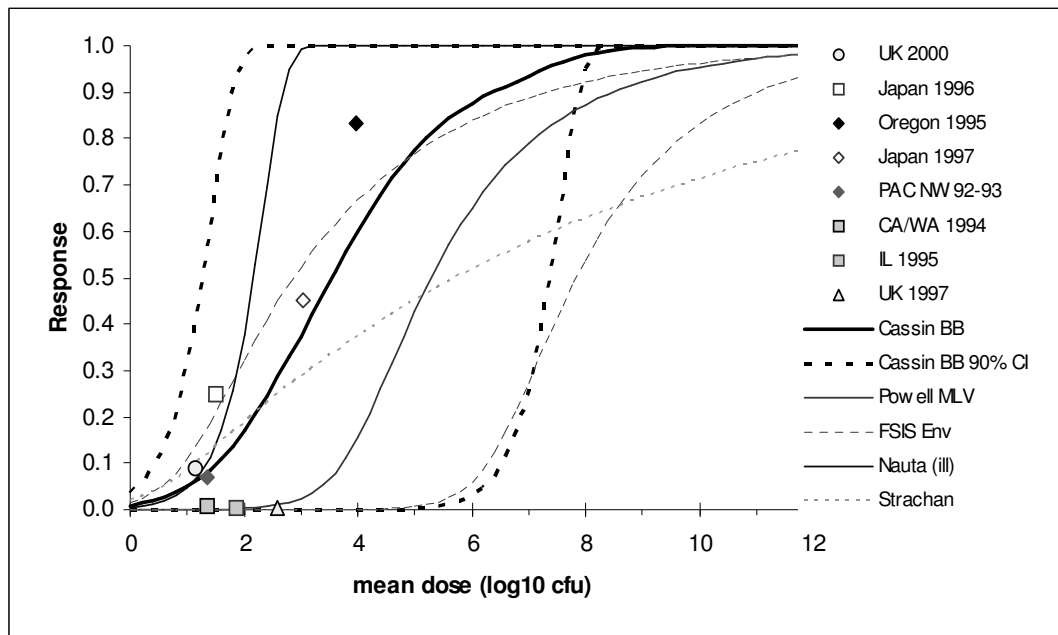


## *A1.2. Other modelling issues*

### A1.2.1 Addressing uncertainty and variability

The discussion by Duffy et al. (2005) suggests that there may be some confusion regarding the meaning of second-order risk analysis models. In predictive microbiology, a primary model describes changes in microbial numbers over time, while a secondary model predicts changes in primary model parameters based on environmental conditions. In risk analysis, a two-dimensional (2D) or second-order Monte Carlo simulation model attempts to separate uncertainty and variability. Separation of uncertainty and variability has been widely touted as a principle of good risk assessment practices (Burmaster and Anderson, 1994). However, it is frequently difficult in practice to completely or unambiguously separate uncertainty and variability. Furthermore, the need to distinguish uncertainty and variability depends on the decisional criteria (Burmaster and Anderson, 1994). For example, it would be necessary if the food safety tolerance must be defined as X% confidence that the $Y^{th}$ percentile of the risk variability distribution is less than a threshold of concern, Z.

**Figure A1.8.** Illustration of model-dependence of separating uncertainty and variability. Two models are based on the same data (Panel A). Model 1: $\log_{10}(y) = b_0+b_1X_1+e_1$, with $R^2 \cong 0.8$. Model 2: $\log_{10}(y) = b_0+b_1X_1+b_2X_2+e_2$, with $R^2 \cong 0.9$. Both models are evaluated at $X_1=10$ (vertical reference line). A portion of the residual variability unaccounted for by Model 1 is shifted to parameter uncertainty under Model 2 (Panel B). CL = 90% confidence limits for each variability distribution.
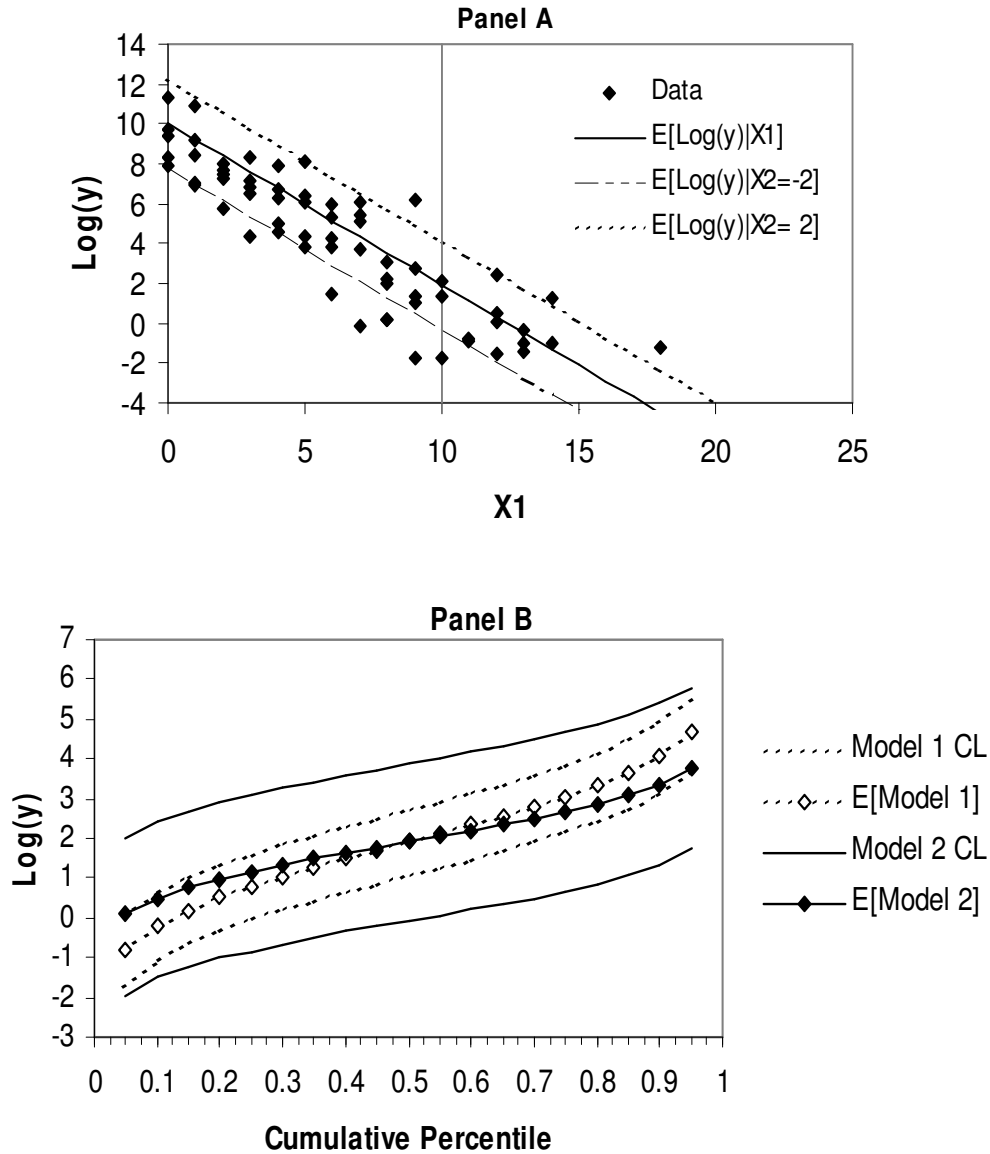
This hypothetical example illustrates that what appears to be random variation given one state of knowledge may, in fact, be deterministic. Unexplained variation may become predictable as knowledge increases, or it may remain unpredictable. Consequently, the classification of unexplained variation as 'epistemic' uncertainty or variability ('aleatory' uncertainty) can be ambiguous. This is not to suggest that attempts to separate uncertainty and variability cannot provide useful information for decision-making, only that the limitations of the approach must be recognized. Nauta et al. (2001), for instance, provide a useful and pragmatic example of distinguishing between uncertainty and variability. Each scenario considered by Nauta et al. (2001) is modelled as a variability-only Monte Carlo simulation. Nauta et al. (2001) felt that uncertainty could not be adequately quantified, due to its complex nature and the level of uncertainty in the food pathway, the models and the data. Therefore, a single-dimension variability model with most-likely parameter values provided a baseline risk distribution, and the effect of uncertainty in individual parameters was explored under alternative scenarios.

The use of secondary predictive microbiology models illustrates an important aspect of considering the impact of both uncertainty and variability. Typically, reported secondary models for microbial growth only provide confidence intervals about growth model parameters such as lag time, maximum specific growth rate, and maximum population density. This parameter uncertainty, however, only translates to uncertainty about the *expected* (mean) growth response. To capture the natural scatter of data about the mean response, an estimate of the variability in growth under replicated environmental conditions is required.

## A1.2.2 Detecting differences

The discussion by Duffy et al. (2005) suggests that there may be some confusion about detecting differences in distributions generated by Monte Carlo simulation. In some cases where two distributions overlap, the inference is that there is no statistically significant difference between the distributions. In other cases, two distributions overlap, but the inference is that they differ. To some extent, this confusion may stem from the fact that conventional statistical methods for hypothesis testing are not directly applicable to Monte Carlo simulation. Under conventional hypothesis testing, two variability distributions may overlap to a considerable degree such that if we take a single observation (X) at random, we would be unable to say with much confidence from which distribution the value was drawn. However, if the sample size is large enough, the means of the two distributions can be arbitrarily close but we nevertheless may be able to have confidence that the two samples represent populations with different means. In the Monte Carlo context, there is no 'uncertainty' about the average value of a particular simulation; it is directly calculable, and the only constraint on the precision of the estimate (convergence) is computer run time. In general, distributions may overlap, but they nevertheless may differ in terms of location, spread or shape in ways that have important implications for decision-making.

Consider, for example, two post-lethality concentration distributions (treatments A and B) where the pre-lethality prevalence is 3%. Because neither treatment A nor B has any effect on 97% of the product, the two post-lethality distributions have a dominant modal value of zero. If we randomly sampled a serving from the two streams and found no contamination, there would be no basis for inferring that the sample came from A or B. Nevertheless, there may be an important difference in the location of the second modes of the distributions that represent

the post-lethality concentration of the contaminated product. For example, treatment A may have a second mode of 0.5 log and treatment B may have a second mode of 2 logs.

In contrast, when dealing with surveillance data, standard statistical methods based on random sampling error are applicable. For example, the seasonality inferred by Duffy et al. (2005: 19) in the prevalence of *E. coli* O157:H7 at retail would appear to be indistinguishable from a random pattern given the limited sample sizes. Discerning temporal patterns would require a statistical test for trend or correction for multiple comparisons to maintain an overall type I error rate.

## A1.2.3 Thorough documentation

At a minimum, transparency in risk assessment requires that the analysis be reproducible by a qualified analyst given sufficient time and resources. The existing quantitative EHEC risk assessments, however, provide numerous examples of incomplete model documentation.

## A1.2.4 Evidence vs intuition

Many of the 'risk management' documents reviewed contained examples of recommendations based on intuition and practical experience rather than documented data and analysis. For example, conventional wisdom considers cheek and head meat suspect. Thus Food Safety Authority of Ireland (1999: 22) states: "Of particular concern is the risk of contamination of meat taken from the head of the carcass shortly after slaughter." CCFH (2005: 7) notes "The head and cheek meat, which typically are used in the manufacture of ground beef, have been identified as sources of *E. coli* O157:H7 in manufacturing trimmings." Butler et al. (2006), discussing control of *E. coli* O157:H7 in the United States of America state "Pre-chilled meat from the head and the oesophagus, removed before applications of carcass interventions, are now inspected for evidence of contamination, and industry has begun to apply decontamination treatments to the head, cheek and weasand (oesophagus) meat, and testing this material for the presence of *E. coli* O157:H7." Practical experience and intuition suggest that due to the configuration and practices of some slaughter facilities, head meat represents a 'plausible worse-case scenario.'

Nevertheless, experimental data and surveillance evidence may not support conventional wisdom. For example, as shown in Figure A1.9, Duffy et al. (2005: 17–18) report surveillance data that provide no statistical basis for distinguishing among trim, carcass and head in the terms of prevalence of O157. Other data may exist, however, that support the identification of high-risk products based on verifiable evidence, rather than intuition or anecdote.

**Figure A1.9.** Percent of samples with O157 recovered by Duffy et al. (2005)
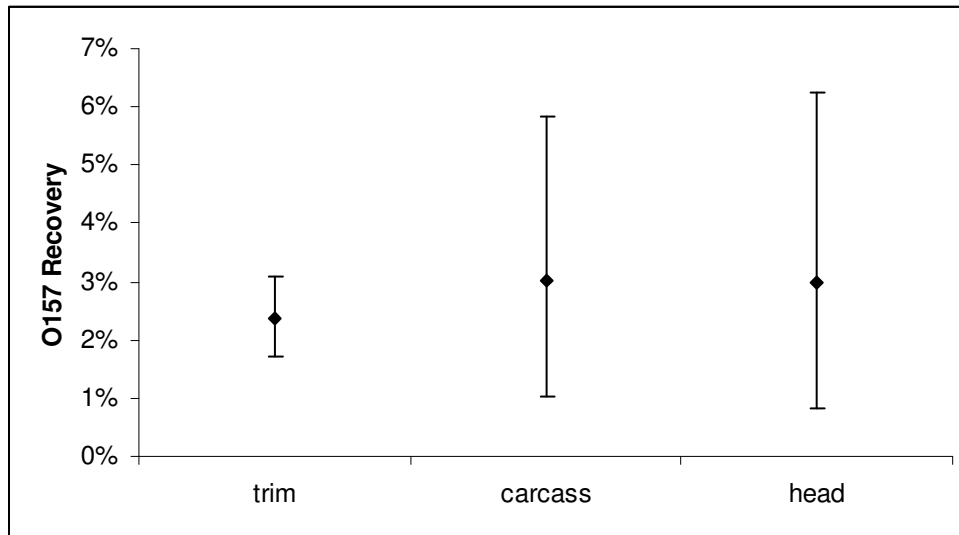
## *A1.3 References*

Butler, F., Duffy, G., Engeljohn, D., Lammerding, A.M. & Tompkin, R. 2006. Background Paper. Case study: *Escherichia coli* O157:H7 in fresh raw ground beef. Joint FAO/WHO Expert Consultation on Development of Practical Risk Management Strategies based on Microbiological Risk Assessment Outputs, Kiel, Germany, 3–7 April 2006. Available at http://www.fao.org/ag/agn/agns/jemra/Ecoli.pdf

Burmaster, D.E. & Anderson, P.D. 1994. Principles of good practice for the use of Monte Carlo techniques in human health and ecological risk assessments. *Risk Analysis,* 14(4): 477–481.

Cassin, M.H., Lammerding, A.M., Todd, E.C.D., Ross, W. & McColl, R.S. 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *International Journal of Food Microbiology,* 41: 21–44.

CCFH [Codex Committee on Food Hygiene]. 2005. Discussion paper on guidelines for the application of the general principles of food hygiene to the risk based control of enterohaemorrhagic *Escherichia coli* in ground beef and fermented sausages. Doc. no. CX/FH 05/37/11. Available at ftp://ftp.fao.org/codex/ccfh37/fh37_11e.pdf

Crockett, C.S., Haas, C.N., Fazil, A., Rose, J.B., Gerba, C.P. 1996. Prevalence of shigellosis in the US: Consistency with dose-response information. *International Journal of Food Microbiology,* 30(1-2): 87–99.

Duffy, G., Butler, F., Cummins, E., O'Brien, S., Nally, P., Carney, E., Henchion, M., Mahon, D. & Cowan, C. 2005. *E. coli* O157:H7 in minced beef produced in Ireland: a quantitative risk assessment (Draft Report). Irish National Food Centre, Ashtown, Dublin, Ireland.

Duffy, G., Cummins, E., Nally, P., O'Brien, S. & Butler, F. 2006. A review of quantitative microbial risk assessment in the management of *Escherichia coli* O157:H7 on beef. *Meat Science*, 74: 76–88.

Haas, C.N., Rose, J.B. & Gerba, C.P. 1999. *Quantitative Microbial Risk Assessment*. John Wiley & Sons, New York, USA.

Institute of Medicine. 2002. *Escherichia coli* O157:H7 in ground beef: review of a draft risk assessment. National Academies Press, Washington, DC, USA.

Lammerding, A.M., Fazil, A., Paoli, G., Deschmarchelier, P. & Vanderlinde, P. 1999. Shiga toxin-producing *E. coli* in ground beef manufactured from Australian beef: Process improvement. Report prepared for Meat and Livestock Australia.

Levine, M.M., Dupont, H.L., Formal, S.B., Hornick, R.B., Takeuchi, A., Gangaros, E.J., Snyder, M.J. & Libonati, J.P. 1973. Pathogenesis of *Shigella dysenteriae* 1 (Shiga) dysentery. *Journal of Infectious Diseases*, 127(3): 261–270.

Nauta, M.J., Evers, E.G., Takumi, K. & Havelaar, A.H. 2001. Risk assessment of Shiga-toxin producing *Escherichia coli* O157 in steak tartare in the Netherlands. RIVM report 257851003. RIVM, Bilthoven, The Netherlands.

Powell, M., Ebel, E., Schlosser, W., Walderhaug, M. & Kause, J. 2000. Dose-response envelope for *Escherichia coli* O157:H7. *Quantitative Microbiology,* 2: 141–163.

Regli, S., Rose, J.B., Haas, C.N. & Gerba, C.P. 1991. Modeling the risk from Giardia and viruses in drinking-water. *Journal of the American Water Works Association*, 83(11): 76-84.

Ross, W.M. 1995. Accounting for uncertainty in the synthesis of dose-response data. *In:* S. Bartlett and D. Krewski (editors). Proceedings of Uncertainty in Toxicological Risk Assessment Workshop, 18–19 October 1995. Environmental Health Directorate, Health Canada, Ottawa, Ontario, Canada.

Strachan, N.J.C., Doyle, M.P., Kasuga, F., Rotariu, O. & Ogden, I.D. 2005. Dose response modelling of *Escherichia coli* O157 incorporating data from foodborne and environmental outbreaks. *International Journal of Food Microbiology*, 103(1): 35–47.

Teunis, P., Takumi, K. & Shinagawa, K. 2004. Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. *Risk Analysis,* 24: 401–407.

USDA-FSIS [U.S. Department of Agriculture, Food Safety and Inspection Service]. 2001. Draft risk assessment of the public health impact of *Escherichia coli* O157:H7 in ground beef. USDA, Washington, DC, USA.

# Appendix to Annex A1

The conventional beta-Poisson dose-response model (Haas, Rose and Gerba, 1999) is:

$$p = 1 - \left(1 + \frac{d}{b}\right)^{-a}$$

where $p$ = probability of response, $d$ = dose, and $a$ and $b$ are model parameters.

Beta-Poisson model parameter maximum likelihood estimates (MLE) may be obtained by minimizing the residual deviance (Regli et al., 1991):

$$\min Y = -2 \sum_{i=1}^{j} \left[ P_i \ln\left(\frac{p_i}{p_i^o}\right) + (T_i - P_i) \ln\left(\frac{1-p_i}{1-p_i^o}\right) \right]$$

where $Y$ is the residual deviance, $j$ is the number of dose groups, $P_i$ is the observed number of positive responses at the $i$th dose, $p_i$ is the response estimated by the beta-Poisson model for the $i$th dose, $p_i^o$ is the observed proportion of responses at the $i$th dose, and $T_i$ is the total number of subjects in the $i$th dose group. Confidence intervals may be obtained by parametric bootstrapping using the following SAS$^©$ PROC NLP code:

**Beta-Poisson Bootstrapping Routine**

```
data bootdata;
set sasuser.shigspp;
itrns = 1000; /*itrns = number of bootstraps desired*/
do j = 1 to itrns;
    possim=ranbin(0,subj,pobs);
    pobssim=possim/subj;
    output;
end;
run;
proc sort data=bootdata out=bootdata2;
by j dose pobs possim subj;
run;
proc nlp data=bootdata2 noprint outvar=estimates maxiter=1000;
min Y;
parameters a b = 0.157 9.169; /*Use MLEs as initial values*/
bounds 0.01<a, 0.1<b; /*a,b>0, but bounds > 0 avoid convergence problems for very
small parameter values*/
phat = 1-(1+(dose/b))**-a;
dev_th = (-possim*log((phat+1e-9)/(pobssim+1e-9))+
    (-(subj-possim)*log((1-phat+1e-9)/(1-pobssim+1e-9))));
```

```
      /*small increment (e.g., 1e-9) added for zero pobs values*/
  s=2*dev_th;
  Y=s;
  by j;
  run;
  data parms;
  set estimates;
  if _TYPE_ eq 'PARMS' then output;
  run;
  proc print data=parms;
  run; /*this is the end*/
```

The bootstrapped beta-Poisson parameter pairs (*a*, *b*) output from this procedure may then be exported to an @Risk spreadsheet, with Monte Carlo simulation performed by selecting discrete pairs with uniform probability (RiskDuniform (a:b, lookup)).


**Reference cited:**

Haas, C.N., Rose, J.B. & Gerba, C.P. 1999. *Quantitative Microbial Risk Assessment*. John Wiley & Sons, New York, USA.

# Annex 2 – Background paper 2
# An overview of *Escherichia coli* O157 along the meat chain

Prepared by Sava Buncic
Professor of Meat Hygiene and Safety, University of Novi Sad, Serbia.

---

DISCLAIMER

This summary paper was prepared in response to a request from FAO/WHO with specific Terms of Reference that determined the main scope of the paper:

• Prepare a literature review summarizing the current state of knowledge on *E. coli* O157 in meat and meat products [other serogroups and foods or sources were therefore not dealt with];

• in particular, focus on information relevant for the Hazard Characterization and Exposure Assessment steps of a risk assessment [the Risk Characterization step was therefore not addressed]; and

• identify knowledge gaps with regard to risk assessment work on *E. coli* O157 in meats.

   Because of its condensed nature, and also because both the time available for its preparation and its target size were pre-determined and limited, the paper is meant to be a guide through the topic and related literature, and should not be taken as an all-inclusive source of data or details *per se*.

   The author, FAO and WHO do not warrant that the information contained herein is complete and shall not be liable for any damage incurred as a result of its use.

   The views expressed herein are those of the author and do not necessarily represent those of FAO or WHO

---

# A2.1. Introduction

*Escherichia coli* O157 is a potential foodborne pathogen, a variant of the bacterial species *E. coli*. Generic (non-toxigenic) *E. coli* are normally harmless bacteria found most frequently in the gastrointestinal tract of numerous animal species, including humans. There, they can be beneficial for the host through competition with pathogenic microorganisms and by helping digestion. Faecally excreted, they spread to the environment, water or foods that are directly or indirectly contaminated by the faecal material. In contrast, *E. coli* O157 is a toxin-producing serogroup that—after ingestion—can cause severe damage to the intestinal lining and, in some cases, other internal organs of the human host.

   First reports of the *E. coli* O157:H7 serotype as a foodborne pathogen date from the early 1980s, with undercooked meat as the source of infection. The public largely perceives *E. coli* O157:H7 infections as associated with consumption of meat and meat products ('the hamburger bug'), although numerous outbreaks and sporadic cases of *E. coli* O157:H7 illness have been caused via non-meat vectors: milk and dairy products, water, fresh produce (vegetables, fruit) or contact with patients.

*E. coli* O157 emerged as one of the most significant pathogens of public health relevance not because of the incidence of the illness, which is much lower than that of other foodborne pathogens such as *Campylobacter* or *Salmonella*, but because of the severity of the symptoms and the low infectious dose. Associated with its high public health profile and related consumer sensitivities, other significant *E. coli* O157 concerns include the potential for large financial losses to the meat industry due to closures of facilities and product recalls associated with meat-related outbreaks.

Therefore, advancement of relevant knowledge and development of efficient control measures for *E. coli* O157 are, and will remain for the foreseeable future, one of the priorities for both researchers and regulators in the area of meat safety. In the context of a risk assessment-based, longitudinal and integrated approach to meat safety assurance that is now widely accepted in all relevant circles (i.e. scientific, regulatory and commercial) (Buncic, 2006), availability and quality of relevant data at different points in the meat chain are key pre-requisites.

# A2.2. Hazard identification

## A2.2.1 Characteristics of Escherichia coli *O157*

### A2.2.1.1 Genus *Escherichia* and enterohaemorrhagic *E. coli*

*Escherichia coli*, a prokaryote commonly found in gastrointestinal tracts of humans and animals, is a member of the family Enterobacteriaceae, which also includes the genera *Salmonella, Shigella, Klebsiella* and *Enterobacter*. *E. coli* and the four species of *Shigella* are a single genus on the basis of DNA-relatedness (Brenner et al., 1972, 1973). Brenner (1984) noted that the original classification into two genera was based on *Shigella* being pathogenic and *Escherichia* being apathogenic. However, Enterobacteriaceae, including *E. coli,* range in behaviour from relatively harmless commensals to fully pathogenic for humans and animals. Therefore, although this family classification may be useful for identification purposes, it serves little purpose in helping to define the organisms, establishing their relatedness, or identifying their ability to cause human disease.

*E. coli* is a Gram-negative straight rod, facultatively anaerobic, oxidase negative and catalase positive, and usually motile. Several hundred antigenic types of *E. coli* are differentiated via 171 O (somatic lipopolysaccharide in the cell wall) and 56 H (flagellar protein) antigens, producing a range of serovars (Ørskov, 1984). O-antigens classify *E. coli* isolates into serogroups, while the H-antigens define serovars (Doyle and Padhye, 1989).

*E. coli* pathogenic for humans can be faecally shed by humans and animals; they can be divided into different groups, including:

- enteropathogenic *E. coli* (EPEC), associated with infantile diarrhoea;
- enteroinvasive *E. coli* (EIEC), causing dysentery-like disease;
- enterotoxigenic *E. coli* (ETEC), producing enterotoxins and diarrhoea;
- enteroaggregative *E. coli* (EAEC), expressing aggregative adherence;
- diffusely adherent *E. coli* (DAEC), adhering to the surface of epithelial cells;

- enterohaemorrhagic *E. coli* (EHEC, including serotype O157), producing Verocytotoxin (VT) or Shiga-like toxin (ST), and causing haemorrhagic colitis (HC) in humans; and

- others?

When considering primary sources of pathogenic *E. coli* contaminating foods, it is useful to keep in mind that human shedders are the primary reservoir for pathogenic *E. coli* belonging to the first five groups, whilst for those belonging to the sixth group (e.g. *E. coli* O157) the primary reservoir is farm ruminant shedders, i.e. cattle.

*E. coli* O157:H7 was recognized as a distinct group of *E. coli* in 1983 after two outbreaks of a distinct illness in the United States of America were associated with this particular serovar (Riley et al., 1983). The gastrointestinal illness, dubbed haemorrhagic colitis (HC), was characterized by severe abdominal pain, with watery diarrhoea followed by bloody diarrhoea, and little or no fever. However, sporadic cases of illness with haemolytic uraemic syndrome (HUS) (Karmali et al., 1983) or thrombotic thrombocytopaenic pupura (TTP) manifestations were also associated with the presence of cytotoxin and cytotoxin-producing *E. coli* in human faeces. *E. coli* O157 typically does not grow well or at all in the temperature range 44 to 44.5°C (Doyle and Schoeni, 1984), does not ferment or only slowly ferments sorbitol, and does not produce the enzyme β-glucuronidase (Doyle and Padhye, 1989). These characteristics enabled *E. coli* O157 to be distinguished relatively easily from other *E. coli* serogoups by their isolation on related culture media (e.g. containing sorbitol) and probably contributed to the fact that most studies have focused on this particular serotype. Nevertheless, cases of HUS caused by sorbitol-fermenting *E. coli* O157:H-- (Ammon, Petersen and Karch, 1999) and *E. coli* O157 (SCIEH, 2002) have been reported.

## A2.2.1.2 Definitions associated with *E. coli* O157

### VTEC

In Europe, most commonly, the cytotoxin produced by *E. coli* serotype O157 has been called verotoxin (verocytotoxin) due to its lethal *in vitro* effects on Vero cells. Hence, a group of *E. coli* producing verotoxin has been called verotoxigenic *E. coli* (VTEC) and currently over 100 serogroups, including O157, are recognized within VTEC (Neill, 1997). However, although all VTEC produce verotoxin, they do not always have other virulence factors that enable them to cause human disease (Neill, 1997; Nataro and Kaper, 1998; Pradel et al., 2000). Therefore, not all VTEC strains are, nor can be, considered pathogenic or able to cause foodborne disease, so the use of term VTEC to denote *E. coli* O157 causing foodborne infections is imprecise from a food safety perspective. Furthermore, VTEC includes not only O157 but also some other non-O157 serotypes causing illness.

### VTEC O157

This usually denotes verotoxin-producing *E. coli* of O (somatic) serogroup O157, but with either an 'unspecified' or 'undetermined' H (flagellar) serovar. In addition, it can include *E. coli* O157 determined as non-motile, so of H-- serovar. The term VTEC O157 is imprecise from a food safety perspective as it does not indicate whether the strains produce other virulence factors (apart from verotoxin) necessary for causing foodborne illness.

### VTEC O157:H7

This denotes verotoxin-producing *E. coli* of the O157 serogroup and of H (flagellar) 7 serovar. The term VTEC O157:H7 is imprecise from a food safety perspective as it does not

indicate whether the strains produce other virulence factors (apart from verotoxin) necessary for causing foodborne illness.

**STEC**

In United States of America, most commonly, the cytotoxin produced by *E. coli* O157 has been called Shiga toxin (Stx). Hence, a group of *E. coli* producing Shiga toxin has been called Shiga-toxigenic *E. coli* (STEC; including O157). However, for the same reasons as indicated for the term VTEC above, the use of term STEC to denote *E. coli* O157 causing foodborne infections is imprecise from a food safety perspective. Furthermore, STEC includes not only O157 but also some other non-O157 serotypes that cause illness.

**EHEC**

Those VTEC that cause enterohaemorrhagic colitis (i.e. a subset of VTEC) have been called enterohaemorrhagic *E. coli* (EHEC; including O157), particularly in the medical domain. In addition to possessing a particular 60 MDa plasmid coding for verotoxigenicity, EHEC posses other necessary virulence factors, such as the A/E factor causing attaching and effacing lesions on the surface of epithelial cells (Neill, 1997; Nataro and Kaper, 1998). However, some EHEC strains do not cause enterohaemorrhagic colitis as the only clinical manifestation of the human infection; either instead of, or in addition to, they cause other manifestations, i.e. HUS or TTP. Therefore, because it inherently relates only to one of the possible clinical manifestations (i.e. HC), the use of the term EHEC to denote *E. coli* O157 causing foodborne infections is imprecise from a food safety perspective. Furthermore, EHEC includes not only O157 but also some other non-O157 serotypes causing foodborne illness.

**HP-VTEC**

More recently, the use of the term Human pathogenic verotoxigenic *E. coli* (HP-VTEC; including *E. coli* O157) has been proposed (EU, 2003) in an attempt to cover both key aspects, namely the ability to cause illness of 'any' clinical manifestation in humans, and the ability to produce verotoxin. However, the use of the term HP-VTEC has not yet been widely adopted, and also HP-VTEC includes not only O157 but also some other non-O157 serotypes causing foodborne illness.

The use of various terms to define *E. coli* O157 causing human food (meat)-borne illness is confusing and creates significant difficulties in the interpretation of related data and reports, as well as in between-report comparisons. For reporting *E. coli* O157 in the context of food safety and foodborne disease, as well as for developing a risk assessment for the pathogen in meat and meat products, a more precise and appropriate term(s) for the target microorganism has yet to be defined. In this paper, when quoting various reports or sources, in general the original terminology of the source will be used so as to avoid possible misrepresentation of the reports and sources.

## A2.2.1.3 Other (i.e. non-O157) serotypes of *E. coli* that can cause human foodborne illness

*E. coli* O157 is notably prevalent in the Canada, Japan, United Kingdom and United States of America. Because of the prominence of *E. coli* O157 in these countries, specific methods for its detection and confirmation have been developed, making this serogroup one of the most frequently looked for and, indeed, the most frequently found.

However, HUS and HC can also be caused by many other serogroups of *E. coli*. In mainland Europe, O26, O103, O113, O118 and O145 are relatively frequently isolated from patients (Caprioli and Tozzi, 1998), while O26, O103, O111 and O121 commonly cause human disease in the United States of America (Griffin et al., 2001). It is important to keep in mind that a large proportion of cases of human infections caused by *E. coli* serogroups other than O157, even those that occurred in outbreaks, could not be proven to be of foodborne origin. One example of a microbiologically clearly confirmed non-O157 foodborne outbreak was that caused by *E. coli* serogroup O111 (H--) in Australia (CDC, 1995a; Paton et al., 1996). There were 21 cases, with one death, and the confirmed food vehicle was semi-dry fermented sausage 'mettwurst' (Paton et al., 1996). Examples of microbiologically unconfirmed non-O157 foodborne illness include HC cases in the United States of America, caused by non-sorbitol fermenting *E. coli* O104:H21, in which the implicated food vehicle, putatively pasteurized milk, was identified by epidemiologic evidence only (CDC, 1995c). Other examples of non-O157 *E. coli* foodborne illnesses include HUS cases in France caused by *E. coli* O119, possibly from unpasteurized goat milk cheese (Deschenes et al., 1996) and in Germany caused by *E. coli* O22 (possibly from mayonnaise) or O101 (possibly from raw milk) (Bülte, 1995).

Because the Terms of Reference of this review clearly state that an overview of *E. coli* O157 is required, non-O157 serogroups will not be dealt with further in this paper.

## A2.2.2 Overview of E. coli *O157 infections*

### A2.2.2.1 Incidence

**Incidence in Europe**

The EU system for monitoring and collection of information on zoonoses, including *E. coli* O157, was previously based on the Council Directive 92/117/ECC and included data collected in 2004. Reporting according to the new rules in Zoonoses Directive 2003/99/EC, entering into force on 12-12-2003, and to be applied from 12 June 2004, would start with data collected during 2005 (EU, 2003).

In 2004, a total of 4 143 laboratory-confirmed cases of all VTEC infections in humans were reported in 17 EU states and in Norway; the EU-incidence was 1.3 cases per 100 000 population (EFSA, 2006). The large majority of the VTEC infections had diarrhoea as clinical manifestation (i.e. were non-HUS), whilst VTEC infections with HUS manifestations were markedly less frequent (Table A2.1).

**Table A2.1**. Cases of laboratory confirmed VTEC infections in humans in Europe in 2004 (countries that did not report them are not shown) (Adapted from EFSA, 2006)

| Country | VTEC infections with manifestations other than HUS* | | VTEC infections with HUS manifestations* | |
|---|---|---|---|---|
| | VTEC cases per 100 000 population | % caused by O157 | VTEC cases per 100 000 population | % caused by O157 |
| Austria | 0.6 | 29 | 0.12 | 90 |
| Belgium | 0.3 | 56 | <0.1 | 100 |
| Czech Republic | 17.1 | 18 | — | — |
| Denmark | 3.0 | 27 | <0.1 | 60 |

| | | | | |
|---|---|---|---|---|
| Estonia | 0 | 0 | 0 | 0 |
| Finland | 0.2 | 40 | — | — |
| France | — | — | 0.1 | 82 |
| Germany | 1.1 | 10 | <0.1 | 67 |
| Greece | — | — | 0.76 | — |
| Hungary | <0.1 | 71 | <0.1 | 0 |
| Ireland | 1.4 | 88 | 0.1 | 50 |
| Italy | 0 | 100 | <0.1 | 24 |
| Poland | 0.2 | 99 | — | — |
| Portugal | — | (3 cases) | — | (1 case) |
| Slovakia | 0.3 | 0 | — | — |
| Slovenia | 0.1 | — | — | — |
| Sweden | 1.7 | — | <0.1 | 100 |
| Netherlands | 0.2 | 100 | <0.1 | 100 |
| United Kingdom | 1.5 | 99 | <0.1 | 95 |
| EU-Total | 1.3 | | <0.1 | |
| Norway | 0.3 | 7 | <0.1 | 100 |

NOTES: * = EU total incidence is based on population in reporting countries.

When considering only countries that reported VTEC infections, on the whole, around 50% of the cases were caused by *E. coli* O157. However, the participation of *E. coli* O157 in all VTEC infections (regardless of whether with non-HUS or HUS manifestations) at individual country level was extremely variable (ranging from 0% to 100%); there is no clear understanding of the underlying reasons for this phenomenon, but it is likely to be multifactorial. Furthermore, no information on the serotype distribution was available for many of the countries. Also, because the laboratory-diagnostic methods used by the countries to detect VTEC are usually specific for *E. coli* O157, this may lead to overestimation of the participation of O157 serotype, or underestimation of the participation of non-157 serotypes, or both, in all VTEC infections in the countries.

### Incidence in United States of America

National surveillance for EHEC began in 2001. Surveillance categories for EHEC include (1) EHEC O157:H7; (2) EHEC, serogroup non-O157; and (3) EHEC, not serogrouped. During 1994–1999, reported infections with the most well known pathogen in this group, *E. coli* O157:H7, increased annually, to a peak of 4 744 cases in 1999. During 2004, cases of EHEC were reported from 50 States, the District of Columbia, and Puerto Rico. Of these, 80% were classified as EHEC O157:H7; 10% as EHEC, serogroup non-O157; and 10% as EHEC, not serogrouped (Vugia et al., 2005). The overall incidence in 2004 was 0.9 per 100 000 population (Table A2.2).

**Table A2.2.** Incidence of *E. coli* O157 in United States of America in 2004 (Adapted from Vugia et al. 2005)

| State | *E. coli* O157 incidence per 100 000 population | Population in surveillance* (million) |
|---|---|---|
| California | 0.8 | 3.2 |
| Colorado | 0.8 | 2.5 |
| Connecticut | 0.9 | 3.5 |
| Georgia | 0.3 | 8.7 |
| Maryland | 0.4 | 5.5 |
| Minnesota | 2.2 | 5.1 |
| New Mexico | 0.5 | 1.9 |
| New York | 1.3 | 4.3 |
| Oregon | 1.7 | 3.6 |
| Tennessee | 0.8 | 5.8 |
| Overall | 0.9 | 44.1 |

NOTES: * Populations indicated are either of the whole state or selected counties.

**Examples of incidence in other countries**

In Canada, VTEC infection first became nationally notifiable in 1990. The annual incidence of infection ranged from 3 to 5.3 cases per 100 000 population during the 1990–1994 period (Wilson et al., 1997). The large majority of cases were caused by *E. coli* O157:H7, reflecting its predominance in more severe forms (bloody diarrhoea and HUS) as well as the reliance of diagnostic laboratories on the specific method.

In New Zealand, VTEC infections were made notifiable in 1996; the incidence in 2001 was 2.0 per 100 000 population (Sneyd et al., 2002), with *E. coli* O157 causing 90% of cases.

In Australia, the incidence of VTEC reported for 2001 was 0.3 cases per 100 000 (Blumer et al., 2003), whilst the incidence of HUS (excluding the epidemic cases) during the 1994–1995 period was 0.62 cases per 100 000 children under 16 (Desmarchelier, 1997); the majority of the VTEC isolates were non-O157 serotypes. Active surveillance of HUS was initiated in 1994.

In Argentina, the frequency of HUS appear to be the highest in the world (250–300 cases/year); in Buenos Aires, the risk of HUS was estimated as approximately 22 cases per 100 000 children aged 6–48 months (Lopez et al., 1997). The majority of the cases were caused by non-O157 serotypes; *E. coli* O157 was associated with only 2% to 18% and 4.5% to 7% of children with HUS and bloody diarrhoea, respectively.

In Japan, according to a report by Takeda (1999), 6 outbreaks (946 cases) of *E. coli* O157 and 4 outbreaks (445 cases) of non-O157 infections occurred during 1984–1995. However, subsequently, in just one year (1996), a huge surge of 23 outbreaks of *E. coli* O157 (8456 cases) and 1 outbreak of non-O157 (6 cases) occurred. After that, EHEC infections in 1997 and 1998 decreased to 1532 and 1380, respectively.

## A2.2.2.2 Routes of infection

**'Proving' the source-case association**

Much knowledge of food- and waterborne *E. coli* O157 infections has accumulated due to study of disease outbreaks affecting more than one person. Outbreaks are easier to detect, and provide more useful epidemiological data than isolated sporadic cases, where only one person is affected. Hence, most of the published data concentrates on outbreaks rather than sporadic cases. Sub-serovar typing methods are used to study the epidemiology of *E. coli* O157 disease and measure relatedness of isolates. Generally, more than one sub-serovar typing method should be used in epidemiologic investigations (Grif et al., 1998). Phage typing is based on the abilities of bacteriophages to lyse individual isolates of *E. coli* O157:H7 (Ahmed et al., 1987). Pulsed field gel electrophoresis (PFGE), the current 'gold standard' of genetic typing techniques visualizes differences in the sizes of high molecular weight fragments produced after bacterial chromosomes are digested with rare-cutting endonucleases. Restriction Fragment Length Polymorphism (RFLP) determines differences in multi-copy genes (e.g. ribosomal RNA). Plasmid profiling shows differences in plasmid size and copy number among different isolates.

**Person-to-person transmission**

The faecal-oral route of infection of *E. coli* O157 appears to be commonly occurring in patients' homes, pre-schools, geriatric homes and hospitals (Bell et al., 1994; CDC, 1995b; Chapman et al., 1997a; Paunio et al., 1999; Rangel et al., 2005). An Argentinean prospective

study showed 34/87 (39%) of household contacts of HUS patients had free faecal Stx (López et al., 1997).

**Contact with animals**

*E. coli* O157 was found in a range of animal species, including farm animals (primarily cattle, but also sheep and pigs), companion animals (horses, rabbits) and wild animals (gulls, rats, flies). Contact with faecally contaminated animals or animal-related environments can lead to the faecal/oral route of infection. For example, three children developed HC or HUS after animal contact on an open farm during a school visit in England (Milne et al., 1999). The three matching patient isolates were identical by PFGE typing to isolates from a goat paddock and from one cow. Animal-to-person transmission was also confirmed by PFGE typing in Canada (Louie et al., 1999). Also, some cases in Belgium were more likely than controls to have had farm animal contact (Piérard et al., 1999). Patients (51) with symptoms of vomiting, HC or HUS caused by *E. coli* O157 were associated with a farm in the United States of America, where the patient matching strains (identified using PFGE) were isolated from 28/216 (13%) of cattle rectal swabs and a rail surface (CDC, 2001). Hand-mouth contact, nail biting and purchasing food at the site were significantly associated with disease (CDC, 2001).

**Foodborne**

This is considered, overall, to be the main infection route, at least in the outbreaks. The implicated sources of foodborne infections include:

- meats (e.g. meat patties, fermented sausages, deer jerky);
- milk or dairy (e.g. unpasteurized milk, heat-treated milk, cheese from raw milk);
- produce (e.g. potato, alfalfa or radish sprouts);
- drinks (e.g. apple cider); and
- water (well water, reservoir water, mains water supply).

Information on the relative relevance of the foodborne route *vs* other routes for *E. coli* O157 infections is scarce. In the UK during the 1995–2004 period, within O157 infections with identified infection routes, the foodborne route was the most common and was responsible for 25% to 40% of the cases (Smith, 2004). In the United States of America, over a 20-year period, 52% of outbreaks were foodborne, amongst which ground beef was implicated as a food vehicle in 41% of outbreaks (Rangel et al., 2005). However, the situation regarding routes of foodborne diseases can—and do—vary markedly between countries, and also both spatially and temporally within a given country.

In this paper, only the foodborne route of infection with meat or meat products as the sources will be dealt with; other routes and sources will not be addressed.

# A2.3. Hazard characterization

## *A2.3.1* E. coli *O157 infections*

### A2.3.1.1 Basic mechanism of *E. coli* O157 infection

**Infection process in humans**

After ingestion and incubation of around 4 (range 3 to 9) days, *E. coli* O157 is thought to be non-invasive, presumably colonizing the gastrointestinal tract (large intestine, i.e. colon) by adhering to the external surface of gut epithelial cells (mediated by the Locus of Enterocyte Effacement - LEE). The establishment of the pathogen in the host gastrointestinal tract comprises the following stages: (i) loose adherence to the mucosal lining via bundle-forming pili; (ii) production and translocation into the host cell of a number of virulence-associated proteins; and (iii) enterocyte effacement and intimate attachment. It seems that the exact location associated with A/E lesions in the gut is not fully understood, possibly because human colon biopsy specimens are collected relatively late in the disease process, and lesions would only be visible during the early stages of infection (Nataro and Kaper, 1998). However, Phillips et al. (2000) found one *E. coli* O157 isolate did form microcolonies and attaching/effacing (A/E) lesions on human Peyer's patches *in vitro*, but not on proximal or distal small intestine nor on colon tissue. Once established in the gastrointestinal tract, *E. coli* O157 cells do not move from the gut lumen, but produce one or more verotoxins in the large intestine (Griffin, Olmstead and Petras, 1990). The verotoxins bind to the globotriasylceramide (Gb$_3$) receptor on the host cells and are translocated to the kidney and central nervous system, which are the sites of active cell damage (Nataro and Kaper, 1998). The Gb$_3$ receptor is found on most host cells but in varying amounts; kidney and brain cells are particularly rich in it. The damage or death of the affected cells manifests in different clinical forms as considered below.

**Pathogen growth vs toxin production in the gastrointestinal tract**

It is not known whether, or to what extent, attached bacteria need to proliferate in the gastrointestinal tract in order for disease to occur, although one could assume that if large numbers are excreted in stools following initial ingestion of small numbers of pathogen cells, then growth could have occurred. Also, given the low infectious dose (see below), it is possible that intestinal growth is needed to produce sufficient quantities of verotoxin to induce a clinical effect. Alternatively, the production of verotoxin in the gastrointestinal tract by very few cells of *E. coli* O157 may potentially be dramatically enhanced by yet unknown conditions in the gut. Cornick et al. (2000) speculated that in their animal (pig) model system, the pathogen adhered to pig intestinal epithelia had enhanced production, release or activation of verotoxin. If this indeed occurs, then environmental conditions in the large intestine or the status of the organism on arrival in the large intestine, or both, could have a major impact on its ability to cause clinical disease.

**Observations in animals or tissues**

A/E lesions occurred in lambs after experimental inoculation (Wales et al., 2001), so colonization of ruminants may occur, even though inoculation studies reveal inconsistent faecal shedding. One VTEC O139 isolate (which causes oedema disease of pigs) grew after

initial fimbrial attachment in pig small intestine (Cornick et al., 2000). *E. coli* O157 growth and A/E lesions occurred with pedestal formation or intimate attachment in adult steer epithelial mucosal tissues (Baehler and Moxley, 2000), and in bovine ileum, where the cells involved were probably epithelial cells (Phillips et al., 2000). Recent studies indicate that the caudal end of the bovine gastrointestinal tract, just inside the anus, may be a focal area for *E. coli* O157 in cattle (Grauke et al., 2002; Naylor et al., 2003; Rice et al., 2003). The rectal-anal junction of inoculated calves harboured microcolonies of *E. coli* O157, as did the same area of one unrelated, naturally infected calf (Naylor et al., 2003). The presence of microcolonies may indicate that the organism has proliferated *in situ*.

Nevertheless, it is important to keep in mind that normally animals are asymptomatic carriers of *E. coli* O157.

## A2.3.1.2 Manifestations of human infection

### Hemorrhagic colitis (HC)

HC is usually accompanied by abdominal cramps causing severe pain. It may start with non-bloody diarrhoea that progresses to bloody diarrhoea within 2 to 3 days, and may be accompanied by vomiting and sometimes relatively mild fever (Nataro and Kaper, 1998). Most cases are self-limiting (Park, Worobo and Durst, 1999). In a number of *E. coli* O157 infection outbreaks, all cases had bloody diarrhoea (Hilborn et al., 2000). Nevertheless, *E. coli* O157 infection also can cause non-bloody diarrhoea (Ackman et al., 1997; Chapman et al., 1997a; Trevena et al., 1999; Pebody et al., 1999); in some outbreaks it was 20 to 50% of cases. It is not clearly understood if or which factors contribute to development of non-bloody diarrhoea compared with bloody diarrhoea, or whether this is merely an anomaly of the reporting or diagnostic procedures used in the different outbreaks.

### Haemorrhagic uraemic syndrome (HUS)

HUS is characterized by acute kidney failure; it is the leading cause of kidney failures, overall, in children (Park, Worobo and Durst, 1999). Verotoxin causes endothelial damage in kidney glomeruli and arterioles, and in cases involving haemolytic anaemia, erythrocytes burst during passage through occluded vessels (Park, Worobo and Durst, 1999). In many cases of HUS, a triad of clinical symptoms is actually present: kidney failure, thrombocytopaenia and acquired haemolytic anaemia. It is important to keep in mind that HUS can be caused also by pathogens other than *E. coli* O157, such as *Shigella* and *Campylobacter.*

### Thrombotic thrombocytopaenic pupura (TTP)

In adults, *E. coli* O157 infection may result in thrombotic thrombocytopaenic pupura (TTP). This disease is similar in course to HUS, but the central nervous system is involved in addition to the kidneys. Neurological complications occur in about 25% of HUS patients (Mead and Griffin, 1998). Precursor diarrhoea may not occur, but patients can develop blood clots on the brain, and death is relatively frequent (Park, Worobo and Durst, 1999).

### Manifestations in outbreaks vs sporadic cases

The percentage of *E. coli* O157:H7 infections which progress to HUS appears to vary between sporadic cases and those associated with outbreaks. *E. coli* O157:H7 infection progresses to HUS in 3% to 7% of sporadic cases, and ≥20% of outbreak-associated cases (Mead and Griffin, 1998). In addition, it seems that the severity of HUS illness also may differ between sporadic cases and those associated with outbreaks, with the latter more often

having a shorter diarrhoeal prodrome, a higher rate of bloody diarrhoea and severe HC (Elliott et al., 2001).

**Mortality**

Approximately 30% to 45% of the *E. coli* O157 infection cases are hospitalized. Long-term complications are possible in patients that have recovered from the acute infection, particularly in case of HUS (e.g. chronic renal sequelae). Fatalities are usually associated with HUS and the mortality rate is usually between 2 and 7% (Siegler et al., 1994; Banatvala et al., 2001; Tarr and Hickman, 1987; Mahon et al., 1997; Roberts and Upton, 2001).

**Asymptomatic carriers**

Humans can be asymptomatic carriers of *E. coli* O157 (Curnow, 1999). In a summary of human cases and carriage (both sporadic and outbreak) that were detected in Wales from 1990 to 1998, 15% were asymptomatic (Chalmers et al., 1999). In Japan, Takeda (1999) reported 632 and 624 asymptomatic 'infections' in 1997 and 1998, respectively. It is also known that asymptomatic carriers can be detected during family surveillance of clinical cases. However, the proportion of exposed individuals who shed *E. coli* O157 without developing the illness is unknown.

## A2.3.1.3 Meats associated with *E. coli* O157 infections

**Outbreaks**

The meat-borne outbreaks of *E. coli* O157 shown in Table A2.3 totalled 1898 cases. If a very simplified calculation is applied to the shown data, it appears that around 52% of these cases were associated with ground beef (minces, burgers or patties), around 33% from other meats, around 12% from various fermented sausages, and around 0.5% from dried venison. Although these *ad hoc* obtained percentages cannot be taken as definitive parameters of the relative importance of different types of meats in meat-borne *E. coli* O157 infections, they illustrate the dominant role of raw meats intended for cooking, particularly ground beef, followed by ready-to-eat sausages (i.e. fermented salamis).

**Table A2.3.** Examples of confirmed *E. coli* O157 cases in meat-borne outbreaks

| Implicated meats (country) | Cases (deaths) | Reference |
|---|---|---|
| Ground beef (USA) | 26 | Wells, Davis and Wachsmuth, 1983. |
| Ground beef (USA) | 21 | Wells, Davis and Wachsmuth, 1983. |
| Ground beef (USA) | 34 (4) | Ryan et al., 1986. |
| Ground beef (USA) | 51 | Pavia et al., 1990. |
| Ground beef (USA) | 54 | Belongia et al., 1991. |
| Ground beef (USA) | 732 (4) | Meng et al., 2001., Bell et al., 1994., CDC, 1993. |
| Ground beef (USA) | 22 (1) | CDC, 1999. |
| Ground beef (USA) | 8 (1) | CDC, 2000. |
| Ground beef (USA) | 19 | CDC, 2003. |
| Ground beef (USA) | 28 | Barrett et al., 1992. |
| Beef tacos (USA) | 13 | Conway, 1995. |
| Beef ('seeme rolle') (USA) | 11 | Werber et al., 2002. |
| Beef (roast) (USA) | 70 | CDC, 1991. |
| Cooked meat (Scotland) | 496 (20) | Pennington, 1998. |
| Cooked meat (UK) | 30 | Rajpura et al., 2003. |
| Meat balls, coleslaw (USA) | 13 | Meng et al., 2001. |
| Salami (USA) | 20 | CDC, 1995b. |
| Genoa salami (Canada) | 39 | Williams et al., 2000. |
| Hungarian-style sausage (Canada) | 150 (?) | Health Canada, 2000. |
| Sausages (mortadella and teewurst) (Germany) | 28 (3) | Ammon, Petersen and Karch, 1999. |
| Cold-smoked sausage | 30 | Sartz et al., 2008. |
| Venison jerky (USA) | 11 | Keene et al., 1997. |

## Sporadic cases

The major characteristics of sporadic meat-borne cases are that they are categorized as caused from undercooked meats, and that they lack laboratory confirmation.

**Table A2.4.** Examples of possible *E. coli* O157 sporadic cases (case-control; laboratory-unconfirmed)

| Likely implicated meat | Likely risk factor | Reference |
|---|---|---|
| Ground beef | Consumption of cooked meat with 'pink centre' | Slutsker et al., 1998. |
| Ground beef | Consumption of cooked meat with 'pink centre' | Kassenborg et al., 2004. |
| Ground beef | Consumption of meat cooked 'rare' | MacDonald et al., 1988. |
| Ground beef | Consumption of 'undercooked' meat | Le Saux et al., 1993. |

## *A2.3.2 Dose-response relationship*

Assumption that exposure to a relatively low number of *E. coli* O157 cells can lead to the development of the illness is generally accepted, although it appears to be inferred from retrospective analysis of foods and the occurrence of animal-to-person and person-to-person spread, rather than from clear experimental data. If the infectious dose is very low, the consequence would be that infection may occur without pathogen growth occurring in contaminated food (Anon., 1999).

### A2.3.2.1 Infectious dose concept

Based on a retrospective analysis of foods involved in outbreaks, the capability of person-to-person transmission, and the ability of the pathogen to tolerate acidic conditions, which enables survival in the acidic environment of the stomach, Doyle et al. (1997) estimated the infectious dose of *E. coli* O157:H7 to be less than a few hundred cells, and even <10 cells ingested. Some other studies (FSIS, 1993; Willshaw et al., 1994) have indicated that as low as <2 cells per 25 gram of foodstuff were sufficient to cause infection. A comparable estimate of infectious dose has been proposed by CAST (1994).

The retrospectively calculated infectious dose for patients who consumed known quantities of contaminated dry-fermented salami was only 2 to 45 colony forming units (cfu) (Tilden et al., 1996). Furthermore, contaminated hamburger patties sampled during one outbreak contained 0.9 to 4.3 cfu per patty (Johnson et al., 1995), but the authors did not extrapolate this information to estimate the actual average cfu dose consumed by patients. Similarly, in another United States of America outbreak, the uncooked hamburger patties contained 67 to 670 cfu per patty, suggesting an infectious dose of fewer than 700 cfu (Tuttle et al., 1999). However, although it is reasonable to assume that the heat-treated patties (at the time of consumption) contained fewer organisms than the uncooked, the actual number of the ingested cells is not known.

### A2.3.2.2 'Probability of infection' concept

More recently, the concept of estimating the probability of infection from exposure to differing numbers of cells has been introduced.

An estimate of the dose response for *E. coli* O157:H7 using a beta-Poisson model gives a value of $1.9 \times 10^5$ cells as the median dose (50% exposed become symptomatic), with a probability of 0.06 ($6 \times 10^{-2}$) of infection when exposed to 100 cells (Powell et al., 2000).

With respect to non-O157 serotypes, Haas, Rose and Gerba (1999) developed dose-response relationships for *E. coli* O111 and O55 using human volunteers. The relationship gave a $2.6 \times 10^6$ organism dose for infection of 50% of the exposed population; and a $3.5 \times 10^{-4}$ risk for consumption of 100 organisms.

### A2.3.2.3 Factors affecting dose-response

**Pathogen-related factors 1 – Virulence factors**

*Verotoxins*

Among the most important virulence characteristics of *E. coli* O157 is the ability to produce one or two verotoxins (i.e. verocytotoxins, Shiga toxins (Stx) or Shiga-like toxins) (Mead and Griffin, 1998). Verotoxin 1 (VT1) is indistinguishable from Shiga toxin produced by *Shigella dysenteriae* type 1. However, verotoxin 2 (VT2) has only 56% amino acid homology with

Shiga toxin 1. The majority of *E. coli* O157 strains produce VT2, whilst the proportion of strains producing VT1 can vary from <25% (in Europe) to >80% (in North America and Japan) (Nataro and Kaper, 1998). Both toxins are encoded on a temperate bacteriophage (inserted into the pathogen's chromosome) and are composed of five B subunits and a single A subunit. The B subunit binds to globotriaosylceramide ($Gb_3$). The A subunit, after endocytosis, activates the 60S ribosomal subunit which blocks protein synthesis. Production of VT1 or VT2, or both, is not in itself sufficient to cause disease.

*Enterohaemolysin*

Nearly all strains of *E. coli* O157 produce a haemolysin (termed enterohaemolysin) that is encoded on the 60-MDa plasmid. Enterohaemolysin belongs to the RTX toxin family, members of which are expressed by uropathogenic *E. coli*, *Pasteurella haemolytica* and other pathogens (Bauer and Welch, 1996). Patients with HUS develop antibodies to enterohaemolysin (Schmidt, Beutin and Karch, 1995), but it is still unclear whether or how it is involved in pathogenesis of disease.

*Intimin*

The genes encoding the A/E histopathology are contained on a 35.6 kb pathogenicity island called the Locus of Enterocyte Effacement (LEE) (Frankel et al., 1998) the complete sequence of which has been determined in *E. coli* O157 strain 933. The intimin (94- to 97-kDa outer membrane proteins), encoded by the *eae* gene, is an adherence factor that plays a role in intestinal colonization of *E. coli* O157 *in vivo* and in animal models (Nataro and Kaper, 1995). In conventional and gnotobiotic piglets, *E. coli* O157:H7 strains produce extensive A/E lesions in the large intestine, whilst strains specifically mutated in the *eae* gene neither produce A/E lesions nor colonize the intestinal site. The role of the intimin is further supported by the anti-intimin immune response seen in HUS patients (McKee and O'Brien, 1996).

*Other intestinal adherence factors*

Some adherence factors other than intimin have been reported for *E. coli* O157:H7 but they have been neither well characterized nor specifically demonstrated *in vivo*. For example, a 94-kDa outer membrane protein (distinct from intimin) mediated adherence to Hep-2 epithelial cells (Sherman et al., 1991) but it was not further characterized.

*pO157 plasmid*

All *E. coli* O157:H7 strains contain a 93.6–104 kb plasmid, designated pO157 (Schmidt, Karch and Beutin, 1994) that, in addition to enterohaemolysin and adherence factors mentioned above, encodes a catalase-peroxidase with unknown function. The plasmid is widely distributed among human EHEC isolates, but its role in the pathogenesis of disease is not yet determined and the results of *in vivo* and *in vitro* studies have been conflicting (Nataro and Kaper, 1995).

*Iron transport*

*E. coli* O157:H7 strains contain an iron transport system (a 69-kDa protein encoded by the *chu*A gene) allowing the use haeme or haemoglobin as an iron source (Torres and Payne, 1997), which possibly aids infection as it stimulates the growth of the pathogen.

*EAST1*

Many strains of *E. coli* O157:H7 possess the *ast*A gene encoding EAST1 (Savarino et al., 1996), the role of which in pathogenesis of disease is not known although it may be involved in non-bloody diarrhoea.

## Pathogen-related factors 2 – Strain-diversity effects on virulence

*Overall variability of verotoxin production*

A number of reports indicate that *in vitro* verotoxin production pattern of *E. coli* O157 (and other VTEC) can significantly differ between different strains (Köhler, Karch and Schmidt, 2000; Tilden et al. 1996; Wagner et al. 2001; Whiting and Golden 2002; Yokoyama et al. 2000).

*Virulence of human vs animal strains*

VTEC strains from an international collection, including *E. coli* O157:H7 and O157:H–, were analysed statistically (univariate and multivariate analyses) for correlation of virulence factors with source, bovine or human clinical (Boerlin et al., 1999). Bovine isolates were more likely to carry $stx_1$ and *espP* (a gene encoding for a protease) than isolates from humans. Additionally, $stx_2$ and *eae* were most likely to occur in isolates from cases of severe human disease. The authors conclude that VTEC isolates from humans are a distinct sub-population of VTEC isolates from bovines (Boerlin et al., 1999). Also, Baker, Moxley and Francis (1997) found that *E. coli* O157 isolates from humans were more virulent in their gnotobiotic pig model than isolates from cattle. Furthermore, Ritchie et al. (2003) found significantly greater *in vitro* VT2 production by *E. coli* O157 isolates from HUS cases than by bovine isolates. In addition, an *in vitro* study was conducted using 123 strains of *E. coli* O157 of known origin (from animals, human cases and meats) and sub-types (PFGE-type and phage-type) that were stored at 4°C, subsequently anaerobically grown at 37°C, followed by examination of the VT2 produced in the broths; the human isolates were significantly more likely to produce *in vitro* VT2 (Avery, 2003).

## Pathogen-related factors 3 – Virulence-marker characteristics of strains from animal and meat

It is known from a number of studies from different countries that animals excrete a diversity of VTEC strains, which differ not only with respect to serotype but also regarding virulence patterns (Table A2.5).

**Table A2.5.** Examples of virulence-marker diversity in VTEC strains (including, but not limited to, O157) associated with the meat chain (adapted from Desmarchelier, 2001)

| Source | VT1 (% strains) | VT2 (% strains) | VT1+VT2 (% strains) | *eae* (% strains) | *ehx* (% strains) |
|---|---|---|---|---|---|
| Cattle | | | | | |
|   Faeces | 36 | 34 | 31 | 16 | 34 |
|   Carcass | 11 | 49 | 40 | 49 | 69 |
|   Ground beef retail | 2 | 54 | 43 | 2 | 67 |
| Sheep | | | | | |
|   Faeces | 13 | 3 | 84 | 1 | 58 |
|   Carcass | 38 | 4 | 58 | 3 | 33 |
|   Lamb meat retail | 11 | 9 | 80 | 5 | 55 |

It is clear that not all *E. coli* O157 isolated at various points of the meat chain possess all the known virulence genes. Childs et al. (2006) determined the distribution of *E. coli* O157 virulence genes *eae*$A_{O157}$, VT1, VT2, *hly*A, *rfb*$E_{O157}$ and *fli*$C_{H7}$ among samples collected longitudinally from feedlot to slaughter. Different groups of isolates differed with respect to which genes they possessed and which they did not, but relatively few possessed all the genes investigated: 0–9 isolates (out of 65) from various samples collected at feedlot level; 1–7 isolates (out of 19) at transport level; 0–6 (out of 16) at lairage level; and 3–24 isolates (out of 224) at slaughtered animal level (hides or colon).

**Pathogen-related factors 4 – Sub-cultivation effects on virulence determination**

A study by Karch et al. (1992) indicated that verotoxin genes in human clinical isolates of VTEC, including *E. coli* O157, were frequently lost during their sub-cultivation. Such phenomena could affect both establishing and reporting of dose-response data for isolates.

**Pathogen-related factors 5 – Overall relevance of *E. coli* O157 strain diversity regarding virulence**

One of the most relevant potential implications of the strain diversities of *E. coli* O157 (indicated above) is that not all the *E. coli* O157 strains present in animals or meats would necessarily have the same dose-response relationship if ingested by humans. However, it is unclear whether these differences indeed occur in real life.

**Host-related factors 1 – Gastric juice effects on virulence**

Survival rates of ingested *E. coli* O157 during gastric passage within the host can be presumably reflected in the dose-response relationship. The survival of *E. coli* (one non-pathogenic strain and one enterohaemorrhagic strain) during passage through the stomachs of young and elderly people has been investigated using mathematical modelling, and a fermenter that mimicked the human gastric pH (Takumi, de Jonge and Havelaar, 2000). On average 20–80% of the ingested *E. coli* were estimated to arrive at the small intestine without inactivation by low pH. This was attributed to the temporary increase in gastric pH after consumption of food, as well as acid tolerance of *E. coli*. To illustrate this last point, the *E. coli* O157:H7 isolate tested showed no decline in numbers after incubation for two hours at pH 2.5, and 26% of the cells survived when the pH was 2.0. Furthermore, McKellar and Knight (1999) reported significantly higher survival rates of outbreak strains of enterohaemorrhagic *E. coli* (serotype O157:H7 was included in, but not separated from, these) to acid (pH 2.0) as compared with non-outbreak strains.

On the basis of the results it could be speculated that outbreak strains would have survived gastric juice to a greater extent than other strains, which would affect the dose-response relationship.

**Host-related factors 2 –  Host susceptibility**

Infection with *E. coli* O157 can occur in any population and age group. Nevertheless, it is assumed that the illness most often occurs in infants (<4 years) and the elderly (>65 years); children under five years are considered as the most susceptible to HUS whereas the elderly are more likely to develop TTP (Baker et al., 1999).

*Children*

In Japan, the median age of both HUS and non-HUS cases of *E. coli* O157 infections was 8 years (Kawamura, Yamazaki and Tamai, 1999). In the United States of America, one report showed that the majority (55.4%) of patients with HUS were younger than 5 years, whilst

approximately one-third (32.5%) were 5 to 17 years old (Banatvala et al., 2001). Another report showed that 35.3% of reported HUS cases occurred in 1–10-year olds and 17.6% in 10–20-year olds (CDC, 1999). The young-age-dependency in children can be also seen from reported overall incidences of HUS (cases per 100 000 population):

- Data for 'children younger than 15 years': 0.7 (United States of America), 0.65 (Austria) and 0.64 (Australia) (Elliott et al., 2001);

- Data for 'children younger than 5 years': 1.4 (United States of America) and 1.35 (Australia) (CDC, 1999).

*Elderly*

Studies in the United States of America indicated that, among patients with HUS (both children and adults), as high as 80% of the adult patients also developed TTP, with 25% mortality (Banatvala et al., 2001), whilst the children patients had no fatalities. It seems that >60 year olds are more affected (CDC, 1999).

**Food matrix effects on virulence**

Current knowledge on whether or how food-matrix-related factors affect virulence and pathogenicity of *E. coli* O157 is very poor.

**Growth phase**

It has been reported that expression of A/E activity on HeLa epithelial cells by enteropathogenic *E. coli* (EPEC) depends on a previous *in vitro* growth phase and on temperature, with early-logarithmic-phase EPEC grown at 37°C eliciting particularly strong A/E activity (Rosenshine et al., 1996). However, this cannot be extrapolated directly to the A/E activity of *E. coli* O157 in the host following exposure to different growth conditions and temperature in foods.

**Food treatments**

Some *in vitro* studies indicated that verotoxin production by *E. coli* O157 can vary depending whether or not the pathogen was previously exposed to acid environment alone (Duffy et al., 2000) or to an acid shock-heat shock combination (Buncic and Avery, 1998). Again, the results cannot be extrapolated directly to the situation when *E. coli* O157 is pre-exposed to real foods and subsequently produces verotoxin within the host.

**Food composition**

Generally, fat from fatty foods can protect foodborne pathogens from the bactericidal effects of the gastric fluid during their passage through the stomach (Blaser and Newman, 1982), but whether or how much this factor plays a role in the process of *E. coli* O157 illness is unclear.

# A2.4. Exposure assessment

## *A2.4.1 Introduction*

At present, there is no single point along the meat chain at which *E. coli* O157 can be reliably eliminated so as to entirely prevent exposure of consumers to the pathogen, apart from sufficient heat treatment and reliable post-heating control of contamination. However, these are inapplicable or unachievable universally. Therefore, a longitudinal and integrated approach to the meat chain, including reduction of the pathogen at multiple points, is necessary to reduce the risk of *E. coli* O157of infections occurring via meats.

As early as 1980, the World Health Organization (WHO, 1980) formulated a meat-chain approach to the control of *Salmonella* in meat, comprising three lines of defence: the food producing animal; slaughter and further processing of meat; and final preparation of the food. In general agreement with that, and for the purpose of this document, the meat chain will be approached through its three global phases (EFSA, 2006), covering:

- pre-harvest: the on-farm part of the chain;
- harvest: the part of the chain beginning with the transport of the slaughter animals from the farmgate (normally controlled by abattoirs), the lairage phase, slaughtering itself, up to the cooling of the carcasses; and
- post-harvest: the part of the chain comprising meat cutting, meat processing, and retail and consumer levels.

With respect to the importance of individual farm animal species with respect to *E. coli* O157, literature data confirms that cattle are the most important reservoir and source. Therefore, not surprisingly, most published data relate to *E. coli* O157 in the beef chain; hence it will dominate in the sections below.

## *A2.4.2 E. coli O157 along the meat chain – Prevalence and incidence data*

### A2.4.2.1 Pre-harvest phase

**Fate in the farm environment**

The intermittent nature of shedding in cattle (see below) produced the suspicion that animals are possibly re-infected regularly from environmental sources as, of course, animals may infect the environment (e.g. pasture, slurry ponds and water sources) and *vice versa*. However, more quantitative data concerning the actual spread of the pathogen from one environmental source to another and subsequent re-infection of livestock (i.e. on-farm 'recycling' of pathogens) are lacking.

**Slurry used as fertilizer**

*E. coli* O157 was found in slurry collected from cattle feedlots (Cízek et al., 1999) and a dairy farm (Porter et al., 1997). Additionally, *E. coli* O157 inoculated into slurry or animal faeces or occurring naturally in manure piles from inoculated animals can survive for very extensive periods, ranging from a few weeks to 21 months (Kudva, Blanch and Hovde, 1998; Bolton et al., 1999; Fukushima, Hoshina and Gomyoda, 1999; Himathongkham et al., 1999).

**Soil**

*E. coli* O157 survived up to 99 days in soil (Bolton et al., 1999), or proliferated in various soil types, including silt loam, sandy loam and clay loam (Gagliardi and Karns, 2000).

**Feed**

Commercial stock feeds sampled at retail and on-farm contained *E. coli*, but not *E. coli* O157 (Lynn et al., 1998). It seems that proper silage fermentation reduces and, depending on initial levels, can even eliminate *E. coli* O157. However, the organism proliferated in some commercial stock feeds (Lynn et al., 1998) and in improperly fermented silage. If silage is made from grass where animals have been shedding *E. coli* O157, or which was irrigated with contaminated water, the organism could spread between the environment and animals.

**Vectors**

Potential for 'mechanical' spread of *E. coli* via vectors (e.g. rodents, birds, flies, vehicles, workers, visitors, feeds) exist on farms, in the same (well known) way as with other foodborne pathogens, such as *Salmonella*.

**Presence in animals on farms**

The relative relevance of the main red meat species with respect to *E. coli* O157 can be illustrated by data from the UK showing the presence of the pathogen in animal faeces at slaughter: in 4.7% of cattle, 1.8% of sheep and 0.16% of pigs (Synge and Paiba, 2000). It is accepted that cattle represent main reservoir and source of *E. coli* O157 in the food chain, so most published data relate to cattle, as summarized in Table A2.6.

It is important to keep in mind that healthy cattle can harbour *E. coli* O157 in the digestive tract and intermittently shed the organism without any symptoms.

The reported occurrences of faecal shedding are variable, and the reasons why shedding is only intermittent in cattle, and what contributes to a burst of shedding from an animal, are not clear, in spite of much research in this field. Factors, or their combinations, that can affect on-farm prevalence of *E. coli* O157:H7 in cattle are considered below (not in order of relevance):

### Age

Prevalence is usually higher in younger animals (Synge, 2000; Cray and Moon, 1995; Hancock et al., 1997; Mechie, Chapman and Siddons, 1997; Van Donkersgoed, Graham and Gannon, 1999).

### Weaning status

Unweaned calves may be protected against *E. coli* O157 colonization (Garber et al., 1995; Zhao et al., 1995).

### Individual variability in shedding patterns

According to the 'supershedder' concept, individual animal shedding patterns within cattle herds can be divided into three categories: non-shedders, low-level shedders and 'supershedders' (those shedding >10 000 cfu/g faeces) (Grauke et al., 2002; Naylor et al., 2003). Obviously, the presence of supershedders exacerbates in-herd and on-farm spread of the pathogen.

**Table A2.6.** Examples of *E. coli* O157 occurrences found in individual adult cattle on-farm (adapted from Buncic, Avery and De Zutter, 2004; Avery and Buncic, 2005)

| Occurrence prevalence (%) | Reference |
|---|---|
| Faeces | |
| 0.00 | Numerous studies, but data not included in this table |
| 0.50 | Rahn et al., 1997. |
| 0.50 | Buncic and Avery, 1997. |
| 1.70 | Minihan et al., 2003. |
| 4.40 | Conedera et al., 2001. |
| 4.51 | Faith et al., 1996. |
| 4.60 | Vold et al., 2001. |
| 5.50 | Rahn et al., 1997. |
| 6.50 | Heuvelink et al., 1998b. |
| 9.50 | Barham et al., 2002. |
| 16.00 | Lahti et al., 2003. |
| 18.00 | Minihan et al., 2003. |
| 22.70 | Smith et al., 2001. |
| Faeces - Average* 8.17 | |
| Faeces - Median* 5.05 | |
| Hides | |
| 18.00 | Barham et al., 2002. |
| Animal-related surfaces | |
| 24.60 | Lahti et al., 2003. |

NOTES: *Average and median occurrences are calculated from positive findings only.

### Season

Both measured shedding prevalences in cattle (Chapman et al., 1997b; Mechie, Chapman and Siddons, 1997; Heuvelink et al., 1998a; Garber et al., 1999; Van Donkersgoed, Graham and Gannon, 1999) and disease in people (López et al., 1997; Simmons, 1997; WHO, 1997; Michel et al., 1999; Decludt et al., 2000) appear to be seasonally distributed, as both are more common in warmer months.

### Diet

One study claimed that inoculated cattle eating hay shed *E. coli* O157:H7 for significantly longer than grain-fed cattle (>39 days and 4 days, respectively), but type of feed did not affect numbers of *E. coli* O157:H7 shed (Hovde et al., 1999). A dramatic increase in ovine shedding inoculated *E. coli* O157 was reported in a study where lamb diet changed from low

to high fibre (Kudva, Hatfield and Hovde, 1995), although housing and weaning also changed during this study and could have contributed to this result. Adult sheep inoculated with *E. coli* O157 feeding on hay shed the organism for significantly longer (and tended to shed higher levels) than animals eating a corn-alfalfa mixture (Kudva et al., 1997).

**Fasting**

Some studies indicated that fasting can lead to proliferation in, or higher shedding of generic *E. coli* from, or both, the caudal end of the gastrointestinal tracts of fasting cattle (Reid et al., 2002a; Naylor et al., 2003).

**Water**

Water troughs may be a route for animal-to-animal transmission (Rahn et al., 1997; Hancock, Besser and Rice, 1998; Barham et al., 2002). *E. coli* O157 survives in waters for significant periods of time, although numbers generally decrease (Wang and Doyle, 1998; Muniesa, Lucena and Jofre, 1999).

**Husbandry**

Some reports indicate that prevalence of *E. coli* O157 is significantly higher in housed cattle than in unhoused (Synge et al., 2001); in such cases the underlying reasons may include close proximity of animals promoting cross-contamination, but also are likely to be multi-factorial. McGee et al. (2004) examined the transmission of *E. coli* O157 within groups of cattle held in pens; into each group an *E. coli* O157-inoculated animal was introduced. Within 48 hours, the pathogen extensively contaminated hides of cohort animals and pen surfaces. Within 3 days, positive faecal samples also started to be found in cohort animals.

**Anti-*E. coli* O157 'treatments'**

The effectiveness of on-farm (i.e. before transport to abattoir) treatments of cattle was evaluated in a recent study (Woerner et al., 2006). The treatments comprised: controls (no treatment), Bovamine (Bov; a Lactobacillus dietary product), NEOMIX (feeding Neo; neomycin sulphate), an anti-*E. coli* O157 vaccine (Vac), and all combinations of the single treatments. The result showed reduced prevalences in animals with all treatments. Animals treated with the Bov-Vac-Neo combination had *E. coli* O157 prevalence of 2.7% and 6.7% in faeces and on hides, respectively, whilst control animals had 45.8% and 40.3%, respectively.

**Sampling and isolation methods for *E. coli* O157**

Sampling methods (e.g. faecal pats *vs* rectal swabs) and the amount of faecal material collected will obviously affect the results. Also, generally, less sensitive microbiological methods (e.g. plating, enrichment-plating) were used in early studies, whilst more sensitive (e.g. enrichment-immunomagnetic separation-plating) were used in recent studies. These factors probably account for some of the variability seen in on-farm *E. coli* O157 prevalence data.

## Pre-harvest controls

Currently, there are no on-farm measures that can be relied upon to guarantee complete absence of *E. coli* O157 carriage in individual cattle or herds. This is very difficult, even if herds are subjected to an on-farm testing regime, because of the intermittent nature of the shedding. Due to the multi-factorial nature of the *E. coli* problem on-farm, the efficacies of individual control measures or their combinations at pre-harvest level are difficult to quantify and involve many uncertainties. Therefore, when cattle destined to enter the human meat chain are transported from farms for slaughter, their *E. coli* O157-carriage status is largely

unknown. Currently, on-farm control measures could only reduce *E. coli* O157 incidences and prevalences, and only to uncertain extents. The main considerations related to on-farm controls include:

- Prevention of pathogen recycling, through:
  - land management (animal wastes, irrigation, etc.);
  - limiting vectors (rodents, wildlife, workers, mechanical vectors, etc.); and
  - animal husbandry (GFP, GHP, animal cleanliness).

- Prevention of ingestion of the pathogen, by:
  - feed treatments;
  - water treatments; and
  - animal interactions (suckling, licking, etc)

- Suppression of the ingested pathogen by:
  - dietary manipulation;
  - probiotics, pre-biotics, competitive exclusion, etc.; and
  - phage therapy.

- Modification of animal response, through:
  - vaccination.

## A2.4.2.2 Harvest phase

**Transport, livestock markets and lairage**

During transport and lairage, *E. coli* O157 can be present in or on both animals and related environments (Table A2.7).

*Transport*

Whilst transport may not necessarily increase *E. coli* O157 in the cattle gastrointestinal tract (i.e. faeces; Minihan et al., 2003), significant spread of the pathogen contaminating animal coats during transport can

**Table A2.7.** Examples of *E. coli* O157 occurrences found during transport and lairage of cattle

| Occurrence (%) | Reference |
|---|---|
| Transporter surfaces | |
| 5.0 | Childs et al., 2006. |
| 7.3 | Barham et al., 2002. |
| Faeces post-transport but pre-lairage | |
| 1.7–13.0 | Minihan et al., 2003. |
| Lairage surfaces | |
| 13.0 | Rivera-Betancourt et al., 2004. |
| 6.7–50.0 | Small et al., 2002) |
| 50.0 | (Tutenel et al., 2003b) |

occur. This occurs through the same mechanisms of animal-to-animal and animal to surfaces to animal cross-contamination taking place during lairage (see below). Using PFGE characterization, Childs et al. (2006) confirmed transport trailers as an important source of *E. coli* O157 found on cattle hides at slaughter, as the *E. coli* O157 isolates from the trailer and the hides matched genotypically.

*Livestock markets*

Information on the prevalence and fate of *E. coli* O157 in and on cattle during the livestock market phase is lacking. Nevertheless, a recent study showed that the prevalence of marker organisms (including generic *E. coli*) inoculated onto the hides of cattle entering the market process increased 2- to 5-fold on those animals post-market (Collis et al., 2004).

*Lairage*

The lairage-to-dressing environment plays an important role in the spread of *E. coli* O157 in cattle at slaughterhouses through animal-animal and animal-environment-animal contacts (Avery and Buncic 2005; Buncic, Avery and De Zutter, 2004). PFGE typing identified the pre-dressing slaughterhouse environment (e.g. surfaces in lairage pens or stunning boxes) as the most likely source of a predominant *E. coli* O157 clone found on 24.7% of hides of slaughtered cattle (Avery et al., 2002). The cattle had originated from 18 different farms and arrived at the slaughterhouse via 17 different hauliers, so had no epidemiological link apart from the same lairage-to-stunning box environment (Avery et al., 2002). Similarly, Bonardi et al. (2001) found indistinguishable *E. coli* O157 isolates in the faeces of cattle slaughtered on the same day in a single slaughterhouse, but which had originated from different farms, suggesting that pre-slaughter cattle mixing served as a vector. In contrast, Tutenel et al. (2003a) found differing *E. coli* O157 types in the lairage-to-dressing environment and on the hides of slaughtered cattle, on two of three days they studied. However, on the third day, a predominant *E. coli* O157 clone existed in the environment, and on the hides (Tutenel et al., 2003a).

*Survival of* E. coli *O157 on transport and lairage surfaces*

The pathogen survives very well on environmental surfaces; decimal reduction times (*D*-values) on hide, concrete, metal or straw ranged between 3 and 15 days (Small, Reid and Buncic, 2003) although significant surface-cleanliness- and strain-related variability exists (Avery and Buncic, 2003). An additional meat safety concern is that naturally-occurring *E. coli* O157 can persist on surfaces even after routine washing; not only in lairage areas (Small et al., 2002; Tutenel et al., 2003b), but also on surfaces on-farm (Lahti et al., 2003) and in transporters (Barham et al., 2002). It should be noted that, unfortunately, cattle lairage washing rarely includes treatments with detergents or sanitizers (Small, Reid and Buncic, 2003). Therefore, carry-over of *E. coli* O157 contamination on lairage surfaces from one day to subsequent days seems likely. When considering the relative meat safety relevance of different sites along the progression from lairage to dressing at slaughterhouses, the most relevant sites are those through which all animals are funnelled (e.g. pen and corridor gates). One of the most important sites is the stunning box, the surfaces of which all animals slaughtered during the day contact in succession (Small et al., 2002; Avery et al., 2002).

**Controls during transport-livestock market-lairage phase**

A summary of main considerations related to controls of *E. coli* O157 during this phase include:

- Transport (in sanitized vehicles) with minimal duration because: stress increases shedding; and cross-contamination.

- Avoid livestock markets because of: mixing of animals from different farms; and environment-mediated cross-contamination.

- Lairage (in sanitized pens) with minimal duration because of: carry-over of pathogens on surfaces from one day to another; cross-contamination; and animals lying on contaminated floor.

- Sanitation of stun boxes, because of: surface-mediated cross-contamination of consecutively stunned animals.

One of the biggest meat safety implications of the transport and lairage findings is that animal-to-animal and animal-to-surface-to-animal mediated cross-contamination occurring during transport and lairaging may ultimately lead to carcasses even from "pathogen-free" farms becoming contaminated at abattoirs. In other words, it could negate any successful control of *E. coli* O157 achieved on individual farms.

## Slaughter and dressing

### *Sources, routes and frequency of contamination along slaughterline*

The main sources and routes of microbial contamination along the slaughterline, i.e. during slaughter of animals and dressing of carcasses, can be summarized as in Figure A2.1.

**Figure A2.1**. Main sources and routes of microbial contamination along the slaughterline
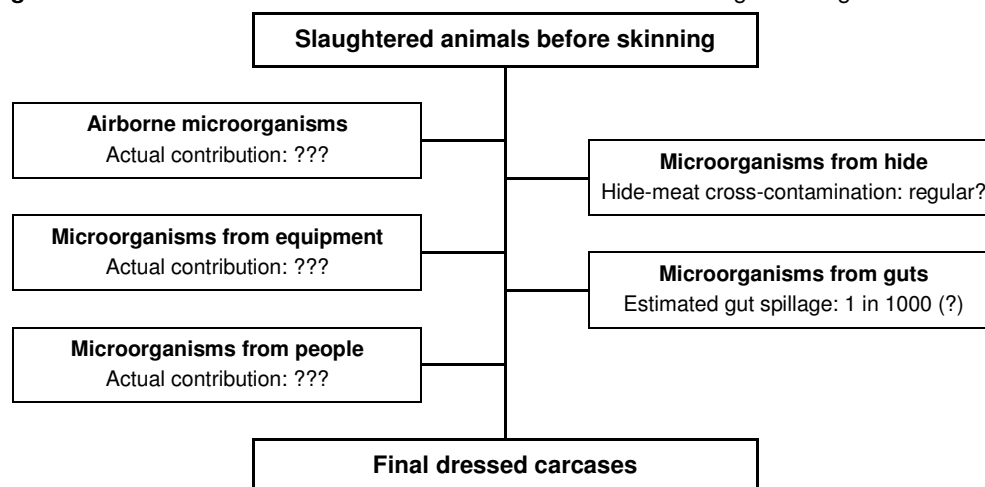


Both levels and composition of microflora found on carcasses at the end of the slaughterline depend on microbial loads brought in or on incoming animals, as well as on how hygienic is the abattoir process technology. However, it should be kept in mind that, even in the best abattoirs, total prevention of microbial contamination of carcasses is unachievable under commercial conditions. In abattoirs applying good hygienic practice (GHP), direct faecal contamination from animal gastrointestinal tracts should be rare. However, based on a number of recent studies, it is now widely accepted that animal coats are the key source of carcass contamination, including with *E. coli* O157, in ruminants. A summary of published occurrences of *E. coli* O157 along the slaughterline is given in Table A2.8.

**Table A2.8.** Examples of *E. coli* O157 occurrences found during slaughter and dressing (adapted from Buncic, Avery and De Zutter, 2004; Avery and Buncic, 2005)

| Occurrence (%) | Reference | Occurrence (%) | Reference |
|---|---|---|---|
| Hides at skinning (cattle) | | Carcases during dressing (cattle) | |
| 4.50 | Barham et al., 2002. | 1.10 | Lahti et al., 2003. |
| 10.70 | Elder et al., 2000. | 3.20 | McEvoy et al., 2003. |
| 18.00 | Ransom et al., 2002. | 6.70 | Tutenel et al., 2003b. |
| 22.20 | Reid et al., 2002b. | 11.10 | McEvoy et al., 2003. |
| 25.00 | Tutenel et al., 2003b. | 12.00 | Bonardi et al., 2001. |

| Occurrence (%) | Reference | Occurrence (%) | Reference |
|---|---|---|---|
| 28.80 | Small et al., 2002. | 43.40 | Elder et al., 2000. |
| 32.90 | Avery et al., 2002. | Carcass dressing – Average* 12.92 | |
| 56.00 | Tutenel et al., 2003b. | Carcass dressing – Median* 8.90 | |
| Hide – Average* 24.76 | | | |
| Hide – Median* 23.60 | | | |
| 5.5 (Sheep pelt) | Small et al., 2002. | | |
| Faeces at evisceration (cattle) | | Final carcases (cattle) post-decontamination and/or post-chill | |
| 0.00 | Minihan et al., 2003. | 0.00 | Minihan et al., 2003. |
| 2.40 | McEvoy et al., 2003. | 0.00 | Minihan et al., 2003. |
| 5.50 | Barham et al., 2002. | 0.00 | Ransom et al., 2002. |
| 6.20 | Cízek et al., 1999. | 1.40 | Chapman et al., 2001. |
| 6.70 | Ransom et al., 2002. | 1.80 | Elder et al., 2000. |
| 7.50 | Van Donkersgoed, Graham and Gannon, 1999. | 3.20 | McEvoy et al., 2003. |
| 8.30 | Tutenel et al., 2003b. | 5.50 | McEvoy et al., 2003. |
| 10.60 | Heuvelink et al., 1998a. | Final carcass – Average* 2.98 | |
| 12.00 | Minihan et al., 2003. | Final carcass – Median* 2.50 | |
| 12.90 | Chapman et al., 2001. | | |
| 15.70 | Chapman et al., 1997. | | |
| 17.00 | Bonardi et al., 2001. | | |
| 27.80 | Elder et al., 2000. | | |
| Faeces - Average* 11.05 | | | |
| Faeces - Median* 9.45 | | | |

NOTES: *Average and median occurrences are calculated from positive findings only.

Obviously, *E. coli* O157 occurrences associated with the slaughter-dressing phase vary markedly between different studies. These variations are due to correspondingly large variations that exist between individual studies in terms of the pre-slaughter microbiological status of animals, abattoir process hygiene, and study design, including sampling and examination methods used. Nevertheless, when considering the data in the *E. coli* O157 occurrences (Table A2.8), some general points are important to be noted:

- Hides at skinning > faeces at evisceration (i.e. hides are the key source).

- Carcasses during dressing < hides at skinning (i.e. significant reductions are achievable via hygienic skinning).

- Faeces at evisceration ~ carcases during dressing (Direct correlations? Uncertainty whether carcasses were contaminated from guts or hides).

- Final carcases < carcases during dressing (i.e. significant reduction via carcass treatments are achievable).

*Controls along the slaughterline*

Significant control measures for *E. coli* O157 (and, indeed, other pathogens) during slaughter and dressing include:

- efficient cleaning and sanitation of the slaughter-hall environment;

- minimizing microbial contamination through application of GHP and HACCP principles; and
- decontamination treatments, including pre-emptive decontamination of hides before skinning and reactive decontamination of carcasses.

**Cleaning and sanitation** Although there is no doubt that effective cleaning and sanitation of the slaughterline environment is necessary and beneficial for microbial safety of the meat, quantitative information is lacking on the relative contribution of contaminated slaughterline environmental surfaces (i.e. those not in direct contact with meat) to incidences of *E. coli* O157 on dressed carcasses.

**GHP and HACCP** A range of measures aimed at prevention or reduction of microbial contamination during slaughter and dressing are used, including slaughtering only visually clean animals (i.e. rejection of dirty ones), mechanical skinning, bagging of anus and tying ('rodding') of oesophagus before evisceration, and regular hot water or steam 'sterilization' of all tools and equipment coming in direct contact with meat.

**Decontamination treatments** It seems clear that washing live animals with a power hose does not reduce *E. coli* O157:H7 carriage on the carcases after slaughter (Byrne et al., 2000). Therefore, hide decontamination can be used and is applied on animals post-exsanguination but pre-skinning (Table A2.9). Furthermore, decontamination treatments of dressed carcasses (e.g. steam vacuuming, steam pasteurization, hot water washes, organic acid washes) and their combinations can be used (Table A2.9). Carcass decontamination is a mandatory critical control point for abattoirs in the United States of America, whilst it is not used in the EU at present.

Overall, as meat safety measures, decontaminations can be effective and routinely used as a part of or in addition to (but not instead of) GHP- and HACCP-related hygiene-based measures. Where both hide- and carcass-decontamination treatment are used, it is expected that overall improvement of the microbiological status of the meat is determined by a combination (possibly synergistic) of microbial reductions achieved by both treatments. Nevertheless, it should be borne in mind that *E. coli* O157 reductions achieved under experimental conditions probably exceed those actually achievable under routine, commercial abattoir conditions.

**Table A2.9.** Examples of the effects of decontamination treatments on *E. coli* O157 on hide or meat

| Treatment | Anti-*E. coli* O157 effects achieved (approx.) | Reference |
| --- | --- | --- |
| Hide decontamination | | |
| Sodium sulphide-hydrogen peroxide combination (chemical dehairing) | 5 log reduction | Castillo et al., 1998a. |
| Steam (condensing at 80 °C; sub-atmospheric pressures) | 4 to 6 log reduction | McEvoy et al., 2001. |
| Sodium hydroxide wash plus chlorinated (1 ppm) water rinse | Prevalence reduced from 44% to 17% | Bosilevac et al., 2005. |
| Meat decontamination | | |
| Hot water (74–80 °C) | 3.7 log reduction | Castillo et al., 1998b. |
| Steam pasteurization (above atmospheric pressure) | 3.7 to 4.4 log reduction | Phebus et al., 1997. |

| Trisodium phosphate (TSP) spray at 55°C | 0.8 to 1.3 logs reduction | Dickson, Cutter and Siragusa, 1994. |
|---|---|---|
| Organic acid sprays (e.g. acetic, lactic, citric) at potable water temperature | No significant reductions | Brackett, Hao and Doyle, 1994. Cutter and Siragusa, 1994a. Uyttendaele et al., 2001. |
| Other treatments (e.g. organic acid sprays at 55°C, combinations of nisin with acids or chelating agents) | Inhibitory or some reducing effects | Cutter and Siragusa, 1994b. Carneiro, Cassar and Miles, 1998. |

## A2.4.2.3 Post-harvest phase

### *E. coli* **O157 in or on meats at post-harvest**

The pathogen can be present in meats at retail level with varying incidences (Table A2.10). The highest incidences are reported for beef, whilst sheep-, pig- and poultry-meat can also contain the pathogen, but to significantly lesser extents. With respect to the risk of meat-borne *E. coli* O157 infections in humans, based on epidemiological data, the most important categories of meats are:

- fresh beef preparations (e.g. ground beef, hamburger) that are frequently eaten undercooked ('pink centre'); and

- raw or mildly heated fermented sausages, particularly those containing beef (e.g. salami; these are ready-to-eat).

For other meats (e.g. sheep, goat, venison, pig or poultry), information is much more limited. In addition, any ready-to-eat foods can become cross-contaminated from fresh meat. For example, cross-contamination between raw beef and processed meats coupled with inadequate refrigeration was thought to be the cause of a Scottish VTEC outbreak (Pennington Group Report, 1997).

**Table A2.10.** Examples of *Escherichia coli* O157 occurrences on beef at processing and retail level (adapted from Buncic, Avery and De Zutter, 2004; Avery and Buncic, 2005)

| Occurrence (%) | Reference | Occurrence (%) | Reference |
|---|---|---|---|
| Beef at retail | | Lamb/mutton at retail | |
| 0 | Tarr, Tran and Wilson, 1999. | 2.0 | Doyle and Schoeni, 1987. |
| 0 | Atalla et al., 2000. | 2.9 | Chapman et al., 2000. |
| 0 | Brooks et al., 2001. | < 6 (beef and mutton) | Duffy et al., 2001 |
| 0 | Fantelli and Stephan 2001. | Pork at retail | |
| 0 | Silveira et al., 1999. | 0.3-1.3 | Heuvelink et al., 1999. |
| 0 | Uhtil et al., 2001. | 1.5 | Doyle and Schoeni, 1987. |
| 0.18 | Tutenel et al., 2003a. | 1.5 | Duffy et al., 2001. |
| 1.10 | Chapman et al., 2000. | Poultry at retail | |
| 1.10 | Heuvelink et al., 1999. | 1.5 | Doyle and Schoeni, 1987. |
| 2.00 | Stampi et al., 2004. | 4 | Duffy et al., 2001. MISSING |
| 3.70 | Doyle and Schoeni 1987. | Fermented sausage ('salami') | |
| 3.80 | Chinen et al., 2001. | 0 | Little and de Loubois, 1998 |
| 4.20 | Vuddhakul et al., 2000. | 0 | Levine et al., 2001 |

| 5.00 | Blanco et al., 1996. | 0.3 | Heuvelink et al., 1999 |
| 5.00 | Zhou et al., 2002. | 3.3 | Chinen et al., 2001 |
| 9.00 | Suthienkul et al., 1990. | | |
| 36.00 | Radu et al., 1998. | | |
| Average* 6.5 | | | |
| Median* 3.8 | | | |

NOTES: *Average and median occurrences are calculated from positive findings only.

## Meats at processing and retail level

### *Fresh meats and preparations*

After chilling, carcasses are cut into different parts. Meat cutting and de-boning operations involve relatively intensive manipulation and handling of meat, which increases the risk of microbial cross-contamination via hands and utensils (knives, saws, etc.) and transfer of bacteria from the meat surface to the internal parts. Fresh meat can be ground and sold as such, or can be used for meat preparations comprising raw ground (minced) meat and additives (salts, spices), such as for hamburgers or meat patties. Although such meat preparations are commonly cooked before consumption, they may be undercooked, or in some cultures can even be eaten raw.

### *Further processing of meat*

Fresh meat also can be used for further processing into a large number of different meat products. Generally, meat processing techniques can involve various treatments including salting or curing based on the addition of salt (sodium chloride) alone or together with other additives (e.g. sodium nitrite, potassium nitrate or a combination), smoking, drying, fermentation, with or without a heat treatment. These treatments can be used in various combinations to produce a very large number of different types of meat products in different countries; due to their large numbers, it is not possible to consider various meat products individually in this paper.

### *Meats at retail level*

At the retail level, meats and meat products are further extensively handled, including slicing into individual parts (e.g. ham, sausages, pâtés) and packaging, which can lead to cross-contamination. The nature of food safety problems associated with *E. coli* O157 in meats at retail level, as well as related control measures, do not differ significantly from those associated with other foodborne pathogens. The retail-level issues have been recently summarized in the form of brief guidelines (Bolton and Maunsell, 2006).

## Meats at catering and consumer levels

Food safety problems associated with *E. coli* O157 in meats at catering and consumer levels are similar; they relate to final preparation of food for consumption. The catering-level issues have been recently summarized in the form of brief guidelines (Bolton and Maunsell, 2004). At consumer level, epidemiological data from Europe (Tirado and Schmidt, 2000), North America, Australia and New Zealand indicate that substantial proportions of foodborne disease can be attributed to food preparation practices used in the domestic environment. Major risk factors include:

- cross-contamination from raw to cooked foods via refrigerators, contaminated hands, cutting boards and kitchen towels;
- inadequate refrigeration;

- improper cooking; and
- inadequate post-cooking handling, including slow cooling and re-contamination.

However, quantitative contributions of these factors, or their combinations, specifically to meat-borne *E. coli* O157 infections have yet to be determined. A more general overview of factors contributing to red-meat-borne outbreaks in England and Wales (Smerdon et al., 2001) indicated that inappropriate storage was implicated in 32%, inadequate heat treatment in 26% and cross-contamination (most commonly, raw-to-cooked) in 25% of those outbreaks.

**Controls during the post-harvest phase**

Principles for control of *E. coli* O157 in meats during the post-harvest phase are largely based on GHP and HACCP principles, and include:

- effective cleaning and sanitation in related premises;
- prevent cross-contamination during cutting, de-boning and further processing;
- include a bactericidal step (e.g. heating) in the process;
- prevent recontamination of the heated products during further handling (e.g. slicing, packaging);
- apply a 'hurdle' concept for non-heated products;
- maintain the cold chain at all steps of the post-harvest phase; and
- prevent cross-contamination of ready-to-eat products from raw meats (and other raw ingredients) during food preparation.

With respect to the two types of meats that are most relevant regarding *E. coli* O157 infections, examples of recommended specific controls are indicated below (Table A2.11).

**Table A2.11.** Examples of recommended controls of *E. coli* O157 in 'higher risk' meat products

| Meat | Recommended controls | Reference |
|---|---|---|
| Ground meats and hamburgers | – Cooking to an internal temperature of 66ºC for 1 min, 68ºC for 15 sec, or 70ºC for <1sec.<br><br>– Consumers to use a thermometer to ensure that ground beef is cooked to 71ºC | FDA, 1999.<br>USDA, 2003. |
| Fermented sausages | The five options :<br><br>1) Utilize a heat process equal to 63°C for 4 minutes<br><br>2) Include a validated 5D inactivation treatment<br><br>3) implement a 'hold and test' programme for finished product<br><br>4) Propose other approaches to assure at least a 5D inactivation<br><br>5) Initiate a HACCP system that includes raw batter testing and a 2D inactivation. | Reed, 1995. |

NOTES: D-value (Decimal reduction time) is the time required for a 10-fold reduction in viable numbers of organisms at a given temperature. 2D and 5D are the time required for 2-log and 5-log reductions, respectively.

## A2.4.3 E. coli *O157 along the meat chain – Concentration data*

The large majority of currently available published quantitative data on *E. coli* O157 along the meat chain is in the form of incidences or prevalences. In contrast, quantitative data on concentrations of *E. coli* O157 cells in positive samples is scarce (Table A2.12).

**Table A2.12.** Examples of concentrations of *E. coli* O157 in positive samples along the beef chain

| Samples | E. coli O157 (Log$_{10}$ cfu) | Reference |
|---|---|---|
| Bovine | | |
| Faeces | <2.0 to 6.0/g | Zhao et al., 1995; Robinson et al., 2004; Fegan et al., 2004; Lahti et al., 2003; Rice et al., 2003; Shere, Bartlett and Kaspar, 1998; Shere et al., 2002. |
| Hide | 0.13 to 4.24/100 cm$^2$ | O'Brien et al., 2005. |
| Carcasses | 0.70 to 1.41/g | Carney et al., 2006. |
| De-boned meats: | | O'Brien et al., 2005. |
| - Trimmings | 0.70 to 1.61/g | |
| - Head meat | 0.70 to 1.00/g | |
| Retail beef (minced, burger) | 0.52 to 4.03/g | Cagney et al., 2004. |
| Ovine | | |
| Retail lamb (burger) | <0.3 to 90/g | Chapman et al., 2001. |

As indicated before, the main reason for the lack of concentration data is the fact that *E. coli* O157 is usually present at very low levels, so is difficult to determine by direct plating, i.e. without a previous enrichment step. The lack of concentration data is particularly associated with earlier studies. More recently, some direct plating media (e.g. chromogenic) for *E. coli* O157 have been developed and become commercially available. From currently available concentration data (Table A2.12), it appears that the range of *E. coli* O157 counts in comparable samples at any given point of the meat chain can be very wide.

Further concentration data for *E. coli* O157 is needed, particularly for the purpose of developing quantitative microbial risk assessments, as well as for more accurate quantifying the control measure effects and efficacies.

## A2.4.4 Microbial ecology of E. coli *O157 in meats*

Published information on various aspects of the effect of intrinsic or extrinsic, or both, factors in foods on the related behaviour of *E. coli* O157 is voluminous. A recent overview of *E. coli* O157 in foods including meats, one that also addressed the issues of microbial ecology of the pathogen in meats, was been produced in the EU (SCVMPH, 2003).

**Chilling**

Chilling is probably the most widely used method for meat preservation. Meat chilling to temperatures below 7°C prevents growth of *E. coli* O157, but allows survival of the pathogen (Dykes, Moorhead and Roberts, 2001; Barkocy-Gallagher, Kang and Koohmaraie, 2002). Some isolates can grow at 7°C, but not at 4°C (Barkocy-Gallagher, Kang and Koohmaraie, 2002).

**Freezing**

During frozen storage of meat, the number of *E. coli* O157 declines, but for substantial reductions (e.g. 2 log) long storage periods (>15 months) are required (Conner and Hall, 1996).

**pH**

Fresh red meats and most meat products are relatively mildly acidic environments (e.g. pH in beef normally 5.4–5.8, and the majority of meat products 6.0–6.5). Only fermented products (salamis) are significantly more acidic, as discussed below. Generally, low concentrations (<2%) of organic acids did not reduce *E. coli* O157 levels on meats (Park, Worobo and Durst, 1999), but there is conflicting evidence on the efficacy of various acids against the pathogen (Davidson, 2002; Buchanan, Whiting and Golden, 2002). Although different *E. coli* O157 isolates had a range of acid resistances (Benjamin and Datta, 1995; Lin et al., 1996; Duffy, Grau and Vanderlinde, 2000), on average, *E. coli* O157 did not appear to be more acid resistant than generic *E. coli* (Lin et al., 1996; Duffy, Grau and Vanderlinde, 2000).

**Curing**

The combination of salt and nitrites is used in many meat products and can have an inhibitory or even bactericidal effect on *E. coli* O157. This is especially the case in products with acid pH and high salt concentrations (Riordan et al., 1998; Casey and Condon, 2000).

**Fermentation**

Fermented sausages rely on both a reduced pH (4.6 to 5.3) and a reduced water activity ($a_w$) of <0.95 for microbial stability (ICMSF, 1998). Alternatively, either a pH <4.5 or $a_w$ <0.91 may achieve the same result (Ross and Shadbolt, 2001). If the moisture reduction during drying is less than 15%, smoking and mild heat treatment may be used as additional steps to restrict microbial growth. It was demonstrated that *E. coli* O157:H7 can survive but not grow during fermentation, drying and storage (for 2 months) of salami (Glass et al., 1992). Some types of fermented sausages that are microbiologically less stable, such as those with relatively higher pH or $a_w$, or both, are additionally pasteurized. The dynamics of changes of *E. coli* O157 counts during processing and pasteurization and storage of various types of fermented sausages have been described in a number of papers (Glass et al., 1992; Hinkens et al., 1996; Clavero and Beuchat, 1996; Faith et al., 1997, 1998a, b; Riordan et al., 1998; Calicioglu et al.,1997, 2001)

**Cooking**

Adequate cooking is currently the only bactericidal step in the meat chain by which any level of *E. coli* O157 can be reliably and completely eliminated. Some studies showed that the pathogen is not particularly heat-resistant and that usual heat treatments designed for the elimination of other vegetative pathogens would eliminate *E. coli* O157 (Juneja, Snyder and Marmer, 1997; Duffy et al., 1999; Ryu and Beuchat, 1999). With respect to hamburger cooking, some data on consumers' preferences are available (Table A2.13).

**Table A2.13.** Internal temperature achieved by cooking hamburgers according to various doneness preferences (in Cassin et al., 1998)

| Cooking preference | Percent of population (McIntosh, Christensen and Acuff, 1994) | Internal temperature (ºC) (Jackson, Hardin and Acuff, 1996) |
|---|---|---|
| Rare | 3.0% | 54.4 |
| Medium rare | 16.1% | 58.6 |
| Medium | 17.9% | 62.7 |
| Medium well | 23.4% | 65.6 |
| Well | 39.6% | 68.3 |

**Irradiation**

In the United States of America, legislation allows use of irradiation for decontaminating minced meat and poultry, in doses suitable for *E. coli* O157 inactivation. Irradiation is not permitted for red meats in the EU at present, although permitted for certain herbs and spices (in UK: Satin, 2002).

Again, a relatively large body of information on the microbial ecology of *E. coli* O157 in meats has accumulated, but the reported data are variable, and even conflicting in some cases, probably due to significant differences in the experimental conditions between the studies and the many strains involved. Nevertheless, ecological effects most relevant for the fate and control of *E. coli* O157 in foods are summarized in Table A2.14.

## A2.4.5 Global trends in meat production and consumption

One of the most important characteristic of both meat production and consumption patterns is their very high variability and changeability, due to the specific effects of meat product, country, culture, population group, economic development, and time. Therefore, both gathering and interpreting meat production and consumption data are difficult, unless it is for a very specific set of conditions. Even in the case of very specific conditions, the data validity is likely to change over time. Furthermore, most consumption data—where available—are collected for nutritional and not for microbial food safety purposes. For these reasons, and also because of space limitations for this paper, only global trends will be briefly commented on here.

### A2.4.5.1 Trends in meat production

Data for the volume of meat production per year are shown in Table A2.15. The United States of America is the world's major beef producing country, producing 65% more than all the EU countries together. China produces more than double the amount of sheep and pig meat than the EU countries, and the United States of America produces double the amount of poultry meat.

**Table A2.14.** A summary of the effects of main ecological factors on *E. coli* O157 in foods

| Food-related factors | Effects on *E. coli* O157 |
|---|---|
| **Growth or survival** | |
| Temperature | - Optimum 37°C (range 7°C to 46°C) <br> - Generation time at 37°C approximately 0.4 hours <br> - Survives freezing well: e.g. -20°C for 9 months in meat hamburgers |
| pH | - Optimum: 6–7 (range 4.4 to 9.0) <br> - Survives in acidic environments (e.g. pH 3.6) |
| Atmosphere | - Can grow with or without $O_2$ <br> - Growth occurred in vacuum-packed meat at 8 to 9°C <br> - Growth prevented in meat packed under 100% $CO_2$ at 8–9°C |
| Water activity ($a_w$) | - Optimum for growth: $a_w = 0.995$ <br> - Minimum for growth: $a_w = 0.950$ |
| Salt | - Growth retarded: 2.5% NaCl <br> - Growth prevented: 8.5% NaCl at 37°C <br> - Growth prevented: 5% NaCl at 12°C |
| 'Hurdle' concept <br><br> (salamis are a typical example of the 'Hurdle' technology) | - 'Hurdle' technologies: Preservation of foods using 'mild' techniques enabling the concerted and sometimes synergistic effects of a number of preservative factors ('hurdles') used at levels that are lower than if only one hurdle were used. <br> - In typical uncooked salamis, *E. coli* O157 does not multiply but can survive in reduced numbers; reductions can vary 0.9 to 2.1 log during production and up to 3.4 if subsequent storage is included. <br> (for cooked salamis types, see additional heat effects below) |
| **Elimination (inactivation)** | |
| Heating | - Inactivated rapidly by 71°C (USA: recommended hamburger cooking conditions = 70°C for 2 minutes) <br> - $D_{54.4°C} = 40$ minutes <br> - $D_{60.0°C} = 4.95$ minutes <br> - $D_{64.3°C} = 0.16$ minutes <br> - $D_{70°C} = 3$ seconds <br> - Reductions in heat-treated salamis (e.g. 54°C in centre) range 3.2 to 7 log, depending on other intrinsic factors (e.g. pH) |
| Irradiation | - Inactivated by 2 to 3 kGy <br> - D value = approx. 0.31 kGy (frozen beef) <br> - D value = approx. 0.24 kGy (chilled beef) |

**Table A2.15.** Total meat production ('000 tonne) in different countries (Wood, 2006)

| Meat type | Largest producer worldwide | EU production (15 countries; pre-2004) | UK production | Highest EU producer |
|---|---|---|---|---|
| Beef and veal | USA 12 311 | 7 445 | 700 | France 1 755 |
| Sheep and goat | China 2 654 | 1 149 | 392 | UK 392 |
| Pigmeat | China 43 053 | 17 606 | 901 | Germany 3 864 |
| Poultry | USA 16 471 | 8 802 | 1 526 | France 2 255 |

## A2.4.5.2 Trends in meat consumption

An illustration of global meat consumption patterns is shown in Figure A2.2. Meat consumption is very variable between countries; for example, the average EU annual per capita consumption of pigmeat is currently 43 kg, but in some countries it is much higher, e.g. 66 kg in Spain and 64 kg in Denmark (Wood, 2006). There are many factors influencing meat consumption other than price and availability. Some of the important ones include: popularity of high protein rather than high carbohydrate foods (e.g. the 'Atkins' diet); vegetarianism; food safety scares (especially BSE); animal welfare issues (e.g. intensification); and nutritional value (red meats being high in saturated fat). For all these many reasons, meat consumption patterns have changed significantly over time. Processed meats and ready-to-eat meals supply the increasing demand for meals prepared simply and quickly, i.e. convenience food. In developed countries, people today are less prepared to spend the time required to cook a traditional meat meal, especially during the week. In addition to the effects of consumers' preferences, another force driving processed meats supply is their higher economic value (Wood, 2006).

In 2000 and 2003, the retail market for fresh beef remained constant, but that for fresh processed beef increased by 17%. Even more marked were the increases in the food service sector, reflecting an increase in the number of meals eaten outside the home. For example between 2000 and 2003, food service sales of beef increased by 56% and 52% for the fresh/frozen and processed sectors, respectively (Wood, 2006).

**Figure A2.2.** Meat consumption (kg/head population/year) by species (Wood, 2006)
NOTES: black columns are Britain; shaded columns are EU average.
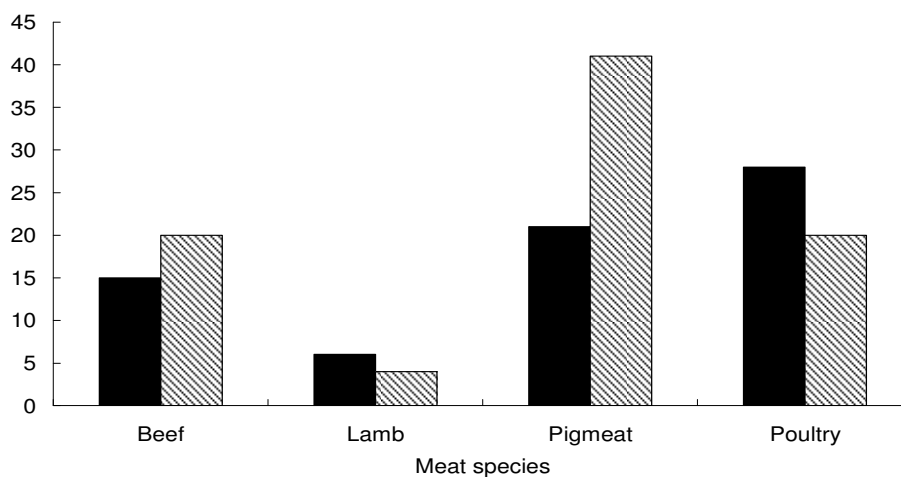
# A2.5. Knowledge gaps and improvements needed

Overall trends of *E. coli* O157 occurrence along the meat chain (using the beef chain as an example) are indicated in Figure A2.3 for illustrative purposes, based on average values calculated from published data.

**Figure A2.3.** Overall trends in *E. coli* O157 occurrence (%) along the beef chain
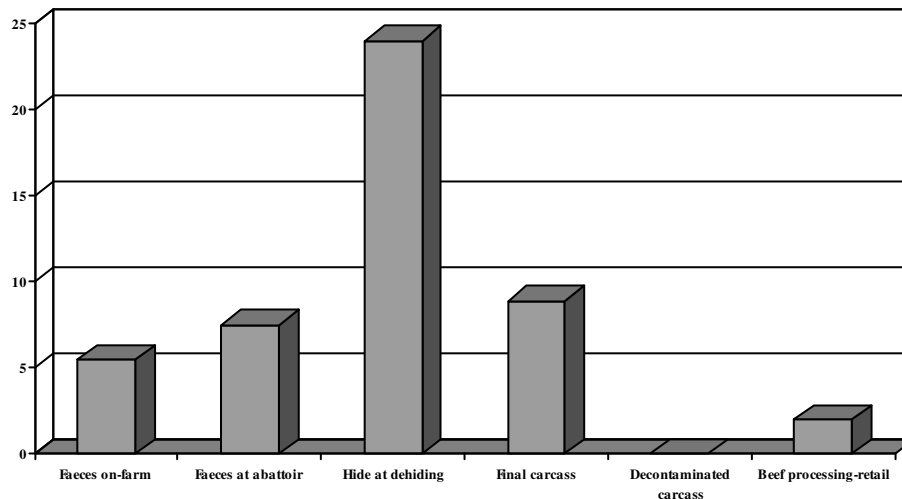


Whilst such an illustration may be useful as an indication of global trends, they are significantly affected by combinations of high degrees of variability, incomparability and non-reproducibility of data, which make their quantitative analysis very difficult, as indicated below.

## A2.5.1 Hazard identification

- The use of different terminology for the targeted organism (*E. coli* O157) is confusing, and needs to be defined and standardized to enable direct data comparability between different sources.
- Notification–confirmation–reporting systems for *E. coli* O157 infections are very variable, so the reported data are difficult to compare between countries, regions and reports.
- Sampling protocols and methods, as well as isolation-characterization methods for *E. coli* O157, vary significantly between studies, even for the same type of sample, which significantly affects the reported data and their comparability.

## A2.5.2 Hazard characterization

- Overall, available *E. coli* O157 concentration data are insufficient, compared with prevalence-incidence data.

- The contribution of different routes to human *E. coli* O157 infection (e.g. foodborne *vs* others; meat-borne *vs* other foodborne; higher risk meats *vs* lower risk meats) is difficult to quantify because a relatively large proportion of cases (e.g. sporadic) are unconfirmed.

- Whilst raw beef (particularly ground) and raw fermented sausages appear to be the most relevant meats with respect to meat-borne *E. coli* O157 infections, the relative contribution of other meat species and meat products is unclear.

- The infectious dose of meat-borne *E. coli* O157 is not clearly determined.

- Clear definition is lacking of the combination or set of virulence factors necessary for *E. coli* O157 isolates to be considered as able to cause foodborne illness.

- Strain diversity with respect to *E. coli* O157 virulence is significant, but the food safety relevance of observed differences between human isolates from outbreaks and 'ordinary' isolates from animals-meats is insufficiently understood.

- The relevance of extrinsic or intrinsic factors, or both, acting in meats and potentially contributing to the infectious dose, and their effects on different *E. coli* O157 strains, is not sufficiently understood.

## A2.5.3 Exposure assessment

- Overall, available *E. coli* O157 concentration data are insufficient, compared with prevalence-incidence data.

### A2.5.3.1 Pre-harvest

- The contribution of environmental recycling of *E. coli* O157 (e.g. animal waste fertilizers, contaminated pasture and contaminated plants for feed production) to the occurrence in farm animals and further along the meat chain are very difficult to evaluate and quantify.

- Effects of various interrelated on-farm variables (farming system, diet, husbandry, geographical region, etc.) on *E. coli* O157 occurrence and shedding patterns in meat animals are very difficult to evaluate and quantify.

- Effects of various on-farm controls (dietary manipulations, probiotics, etc.) on *E. coli* O157 occurrence and shedding patterns in meat animals are very difficult to evaluate and quantify.

- The intermittent nature of faecal shedding raises serious questions about the comparability of *E. coli* O157 occurrences in meat animals determined during different timeframes.

### A2.5.3.2 Harvest

- The effect of cross-contamination during transport on *E. coli* O157 occurrence in meat animals, and its relevance for the occurrence in meat, is difficult to quantify.

- The effect of cross-contamination during lairaging on *E. coli* O157 occurrence in meat animals, and its relevance for the occurrence in meat, is difficult to quantify.

- The effect of cross-contamination via stun-box surfaces on *E. coli* O157 occurrence in meat animals, and its relevance for occurrence in meat, is difficult to quantify.

- The effect of significant variability related to process hygiene between abattoirs (related to slaughter and dressing) on *E. coli* O157 occurrence on final carcasses is difficult to quantify.

- The eliminating or reducing effects of decontamination treatments on *E. coli* O157 on hides or carcasses determined under experimental conditions are variable and dependent on a number of factors; and are difficult to extrapolate to commercial conditions.

## A2.5.3.3 Post-harvest

- The nature and the extent of *E. coli* O157 cross-contamination occurring during the initial stages of meat processing (e.g. cutting, boning, mincing) is highly variable and processor specific, so its contribution to the occurrence of the organism in the final product is difficult to quantify.

- An extremely large variety of meat products exist, and these differ between regions, countries and cultures—even within a given type of meat product—so *E. coli* O157 data obtained with one meat product cannot be directly extrapolated to other (even nominally similar) meat products.

- The nature and the extent of *E. coli* O157 cross-contamination occurring during retailing and food preparation is highly variable, so its contribution to the *E. coli* O157 ingested by consumers is difficult to quantify.

- Data (those that are microbial food safety orientated) on consumption of meat and meat products are scarce and highly variable, and are subject to both spatial and temporal changes, which results in significant difficulties in dose-response analysis.

# A2.6. References

Ackman, D., Marks, S., Mack, P., Caldwell, M., Root, T. & Burkhead, G. 1997. Swimming-associated haemorrhagic colitis due to *Escherichia coli* O157:H7 infection: evidence of prolonged contamination of a freshwater lake. *Epidemiology and Infection*, 119: 1–8.

Ahmed, R., Bopp, C., Borczyk, A. & Kasatiya, S. 1987. Phage typing scheme for *Escherichia coli* O157:H7. *Journal of Infectious Diseases*, 155: 806–809.

Ammon, A., Petersen, L.R. & Karch, H. 1999. A large outbreak of haemolytic uraemic syndrome caused by an unusual sorbitol-fermenting strain of *Escherichia coli* O157:H--. *Journal of Infectious Diseases*, 179: 1274–1277.

Anon[ymous]. 1999. The prevention of *E. coli* O157:H7 infection: a shared responsibility. Food Safety Authority of Ireland, Dublin, Ireland.

Atalla, H.N., Johnson, R., McEwen, S., Usborne, R.W. & Gyles, C.L. 2000. Use of a Shiga toxin (Stx)-enzyme-linked immunosorbent assay and immunoblot for detection and isolation of Stx-producing *Escherichia coli* from naturally contaminated beef. *Journal of Food Protection*, 63: 1167–1172.

Avery, S.M. 2003. Assessment of genotype and phenotype diversity of *Escherichia coli* O157 in the context of the meat chain. PhD Thesis. University of Bristol, UK. See pp. 100–103.

Avery, S.M. & Buncic, S. 2003. *Escherichia coli* O157 diversity with respect to survival during drying on concrete. *Journal of Food Protection*, 66: 780–786.

Avery, S.M. & Buncic, S. 2005. Presence, spread and tracing of *Escherichia coli* O157 in cattle from farm to carcass dressing at abattoirs. *Food Science Central* [Online].– see www.foodsciencecentral.com/fsc/ixid13916.

Avery, S.M., Small, A., Reid, C.-A. & Buncic, S. 2002. Pulsed-field gel electrophoresis characterization of Shiga toxin-producing *Escherichia coli* O157 from hides of cattle at slaughter. *Journal of Food Protection*, 65: 1172–1176.

Baehler, A.A. & Moxley, R.A. 2000. *Escherichia coli* O157:H7 induced attaching-effacing lesions in large intestinal mucosal explants from adult cattle. *FEMS Microbiology Letters*, 185: 239–242.

Baker, M., Eyles, R., Bennett, J. & Nicol, C. 1999. Emergence of verotoxigenic *Escherichia coli* (VTEC) in New Zealand. *New Zealand Public Health Report*, 6: 9–12.

Baker, D.R., Moxley, R.A. & Francis, D.H.. 1997. Variation in virulence in the gnotobiotic pig model of O157:H7 *Escherichia coli* strains of bovine and human origin. *Advances in Experimental Medicine and Biology*, 412: 53–58.

Banatvala, N., Griffin, P.M., Greene, K.D., Barrett, T.J., Bibb, W.F., Green, J.H. & Wells, J.G. 2001. The United States National Prospective Hemolytic Uremic Syndrome Study: microbiologic, serologic, clinical, and epidemiological findings. *Journal of Infectious Diseases*, 183(7): 1063–1070.

Barham, A.R., Barham, B.L., Johnson, A.K., Allen, D.M., Blanton, J.R. & Miller, M.F. 2002. Effects of the transportation of beef cattle from the feedyard to the packing plant on prevalence levels of *Escherichia coli* O157 and *Salmonella* spp. *Journal of Food Protection*, 65: 280–283.

Barkocy-Gallagher, G.A., Kang, D.H. & Koohmaraie, M. 2002. Fate of field-isolated *Escherichia coli* O157 in ground beef at different storage temperatures. *Journal of Food Protection*, 65: 1106–1109.

Barrett, T.J., Kaper, J.B., Jerse, A.E. & Wachsmuth, I.K. 1992. Virulence factors in Shiga-like toxin-producing *Escherichia coli* isolated from humans and cattle. *Journal of Infectious Diseases*, 165: 979–980.

Bauer, M.E. & Welch, R.A. 1996. Characterization of an RTX toxin from enterohaemorrhagic *Escherichia coli* O157:H7. *Infection and Immunology*, 64: 167–175.

Bell, B.P., Goldoft, M., Griffin, P.M., Davis, M.A., Gordon, D.C., Tarr, P.I., Bartleson, C.A., Lewis, J.H., Barrett, T.J., Wells, J.G., Baron, R. & Kobayashi, J. 1994. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhoea and hemolytic uremic syndrome from hamburgers. *Journal of the American Medical Association*, 272: 1349–1353.

Belongia, E.A., MacDonald, K.L., Parham, G.L., White, K.E., Korlath, J.A., Lobato, M.N., Strand, S.M., Casale, K.A. & Osterholm, M.T. 1991. An outbreak of *Escherichia coli* O157:H7 colitis associated with consumption of precooked meat patties. *Journal of Infectious Diseases*, 164: 338–343.

Benjamin, M.M. & Datta, A.R. 1995. Acid tolerance of enterohemorrhagic *Escherichia coli*. *Applied and Environmental Microbiology*, 61: 1669–1672.

Blanco, J.E., Blanco, M., Mora, A., Prado, C., Rio, M.,Fernandez, L., Fernandez, M.J., Sainz, V. & Blanco, J. 1996. Detection of enterohaemorrhagic *Escherichia coli* O157:H7 in minced beef using immunomagnetic separation. *Microbiologia*, 12: 385–394.

Blaser, M.J. & Newman, L.S. 1982. A review of human salmonellosis: I. Infective dose. *Reviews of Infectious Diseases,* 4(6): 1096–1106.

Blumer, C., Roche, P., Spencer, J., Lin, M., Milton, A., Bunn, C., Gidding, H., Kaldor, J., Kirk, M., Hall, R., Della-Porta, T., Leader, R. & Wright, P. 2003. Australia's notifiable diseases status, 2001: Annual report of the National Notifiable Diseases Surveillance System. *Communicable Disease Intelligence*, 27(1):1-78.

Boerlin, P., McEwen, S.A., Boerlin-Petzold, F., Wilson, J.B., Johnson, R.P. & Gyles, C.L. 1999. Associations between virulence factors of shiga toxin-producing *Escherichia coli* and disease in humans. *Journal of Clinical Microbiology*, 37: 497–503.

Bolton, D.J. & Maunsell, B. 2004. Guidelines for food safety control in European restaurants. Tegasc, Dublin, Ireland.

Bolton, D.J. & Maunsell, B. 2006. Guidelines for food safety control in retail establishments. Tegasc, Dublin, Ireland.

Bolton, D.J., Byrne, C.M., Sheridan, J.J., McDowell, D.A. & Blair, I.S. 1999. The survival characteristics of a non-toxigenic strain of *Escherichia coli* O157:H7. *Journal of Applied Microbiology*, 86: 407–411.

Bonardi, S., Maggi, E., Pizzin, G., Morabito, S. & Caprioli, A. 2001. Faecal carriage of verocytotoxin-producing *Escherichia coli* O157 and carcass contamination in cattle at slaughter in northern Italy. *International Journal of Food Microbiology*, 66: 47–53.

Bosilevac, J.M., Nou, X., Osborn, M.S., Allen, D.M. & Koohmaraie, M. 2005. Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. *Journal of Food Protection,* 68(2): 265–272.

Brackett, R., Hao, Y. & Doyle, M. 1994. Ineffectiveness of hot acid sprays to decontaminate *Escherichia coli* O157:H7 on beef. *Journal of Food Protection,* 57: 198–203.

Brenner, D.J. 1984. Family I. *Enterobacteriaceae*. pp. 408–420, *in*: J.G. Holt and N.R. Krieg (editors). *Bergey's Manual of Systematic Bacteriology.* Vol I. Williams and Wilkins, Baltimore, MD, USA.

Brenner, D.J., Fanning, G.R., Skerman, F.J. & Falkow, S. 1972. Polynucleotide sequence divergence among strains of *Escherichia coli* and closely related organisms. *Journal of Bacteriology*, 109: 953–965.

Brenner, D.J., Fanning, G.R., Miklos, G.V. & Steigerwalt, A.G. 1973. Polynucleotide sequence relatedness among *Shigella* species. *International Journal of Systematic Bacteriology*, 23: 1–7.

Brooks, H.J.L., Mollison, B.D., Bettelheim, K.A., Matejka, K., Paterson, K.A. & Ward, V.K. 2001. Occurrence and virulence factors of non-O157 Shiga toxin-producing *Escherichia coli* in retail meat in Dunedin, New Zealand. *Letters in Applied Microbiology*, 32: 118–122.

Buchanan, R., Whiting, R. & Golden, M. 2002. Modelling acid inactivation of foodborne microorganisms. pp. 461–477 (Ch. 18), *in:* V. Juneja and J. Sofos (editors). *Control of Foodborne Microorganisms.* Food Science and Technology series, Vol. 114. Marcel Dekker, New York, USA.

Bülte, M. 1995. Enterohemorrhagic *Escherichia coli* strains (EHEC) – topicality in the Federal Republic of Germany. 1. Potential pathogenicity of EHEC strains – significance as foodborne pathogen. *Fleischwirtschaft*, 75: 1430–1432.

Buncic, S. (editor). 2006. *Integrated Food Safety and Veterinary Public Health.* CABI International, Oxon, UK. See pp. 3–4, 361–364.

Buncic, S. & Avery, S.M. 1997. *Escherichia coli* O157:H7 in healthy dairy cows. *New Zealand Veterinary Journal*, 45: 45–48.

Buncic S. & Avery, S.M. 1998. Effects of cold storage and heat-acid shocks on growth and verotoxin 2 production of *Escherichia coli* O157:H7. *Food Microbiology*, 15: 319–328.

Buncic S., Avery, S.M. & De Zutter, L. 2004. Epidemiology of *Escherichia coli* O157 in cattle from farm to fork. pp. 67–84, *in:* B. Maunsell, J. Sheridan and D.J. Bolton (editors). *Food Pathogen Epidemiology: Microbes, Maladies and Methods.* Proceedings of an International European Union Risk Analysis Information Network (EU-RAIN) Conference, Padua, Italy, 2–3 December 2004. Teagasc – The National Food Centre, Dublin, Ireland.

Byrne, C.M., Bolton, D.J., Sheridan, J.J., McDowell, D.A. & Blair, I.S. 2000. The effects of pre-slaughter washing on the reduction of *Escherichia coli* O157:H7 transfer from cattle hides to carcasses during slaughter. *Letters in Applied Microbiology*, 30: 142–145.

Cagney, C., Crowley, H., Duffy, G., Sheridan, J.J., O'Brien, S., Carney, E., Anderson, W.A., McDowell, D.A. & Blair, I.S. 2004. Prevalence and numbers of *Escherichia coli* O157:H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *Food Microbiology*, 21: 203–212.

Calicioglu, M., Faith, N.G., Buege, D.R. & Luchansky, J.B. 1997. Viability of *Escherichia coli* O157:H7 in fermented semidry low-temperature-cooked beef summer sausage. *Journal of Food Protection*, 60: 1158–1162.

Calicioglu, M., Faith, N.G., Buege, D.R. & Luchansky, J.B. 2001. Validation of a manufacturing process for fermented semidry Turkish Soudjouk to control *Escherichia coli* O157:H7. *Journal of Food Protection*, 64: 1156–1161.

Caprioli, A. & Tozzi, A.E. 1998. pp. 38-48, *in:* J.B. Kaper and A.D. O'Brien (editors). Escherichia coli *O157:H7 and other shiga toxin-producing* E. coli. American Society for Microbiology Press, Washington, DC, USA.

Carneiro, D., Cassar, C. & Miles, R. 1998. Trisodium phosphate increases sensitivity of Gram-negative bacteria to lysozyme and nisin. *Journal of Food Protection*, 61: 839–843.

Carney, E., O'Brien, S.B., Sheridan, J.J., McDowell, D.A., Blair, I.S. & Duffy, G. 2006. Prevalence and level of *Escherichia coli* O157 on beef trimmings, carcasses and head meat at a beef slaughter plant. Food Microbiology,. 23(1): 52–59.

Casey, P. & Condon, S. 2000. Synergistic lethal combination of nitrite and acid pH on a verotoxin-negative strain of *Escherichia coli* O157. *International Journal of Food Microbiology*, 55: 255–258.

Cassin, M.H., Lammerding, A.M., Todd, E.C.D., Ross, W. & McColl, R.S. 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *International Journal of Food Microbiology,* 41: 21–44.

CAST [Council for Agricultural Science and Technology]. 1994. Foodborne pathogens: risks and consequences. Task Force Report No. 122.

Castillo, A., Dickson, J.S., Clayton, R.P., Lucia, L.M. & Acuff, G.R. 1998a. Chemical dehairing of bovine skin to reduce pathogenic bacteria and bacteria of fecal origin. *Journal of Food Protection*, 61: 623–625.

Castillo, A., Goodson, L.L., Savell, K. & Acuff, G. 1998b. Use of hot water for beef carcass decontamination. *Journal of Food Protection*, 61: 19–25.

CDSC [Communicable Disease Surveillance Centre]. 2001. Verocytotoxin-producing *E. coli* O157: 1999 and 2000. *Communicable Disease Report Weekly.* Available as Web Publication. http://www.hpa.org.uk/cdr/archives/2001/cdr1901.pdf. Last accessed 29 July 2011.

CDC [Centers for Disease Control and Prevention]. 1991. Foodborne outbreak of gastroenteritis caused by *Escherichia coli* O157:H7 – North Dakota. *Morbidity and Mortality Weekly Report*, 40: 265–267.

CDC. 1993. Update, multistate outbreak of *Escherichia coli* O157:H7 infections from hamburgers – western United States, 1992–1993. *Morbidity and Mortality Weekly Report*, 42: 258–263.

CDC. 1995a. Community outbreak of hemolytic uremic syndrome attributable to *Escherichia coli* O111:NM – South Australia, 1995. *Morbidity and Mortality Weekly Report*, 44: 550–551, 557-558.

CDC. 1995b. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami – Washington and California, 1994. *Morbidity and Mortality Weekly Report*, 44: 157–160.

CDC. 1995c. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21 – Helena, Montana, 1994. *Morbidity and Mortality Weekly Report*, 44: 501–503.

CDC. 1999. Summary of Notifiable Diseases, United States, 1998. *Morbidity and Mortality Weekly Report*, 47(53): i-xvi, 1–94.

CDC. 2000. Surveillance for outbreaks of *Escherichia coli* O157:H7 infection: Summary of 1999 data. See http://www.cdc.gov/ncidod/dbmd/diseaseinfo/files/ecoli_99summary.pdf.

CDC. 2001. Outbreaks of *Escherichia coli* O157:H7 infections among children associated with farm visits – Pennsylvania and Washington, 2000. *Morbidity and Mortality Weekly Report*, 50: 293–297.

CDC. 2003. Summary of notifiable diseases – United States, 2001. *Morbidity and Mortality Weekly Report*, 50(53): i–xxiv, 1–108.

Chalmers, R.M., Parry, S.M., Salmon, R.L., Smith, R.M.M., Willshaw, G.A. & Cheasty, T. 1999. The surveillance of vero cytotoxin-producing *Escherichia coli* O157 in Wales, 1990 to 1998. *Emerging Infectious Diseases*, 5(4). Web Publication. http://www.cdc.gov/ncidod/EID/vol5no4/chalmers.htm. Last accessed 2011-05-20.

Chapman, P.A., Siddons, C.A., Cerdan Malo, A.T. & Harkin, M.A. 1997a. A one-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. *Epidemiology and Infection*, 119: 245–250.

Chapman, P.A., Siddons, C.A., Manning, J. & Cheetham, C. 1997b. An outbreak of infection due to verocytotoxin-producing *Escherichia coli* O157 in four families: the influence of laboratory methods on the outcome of the investigation. *Epidemiology and Infection*, 119: 113–119.

Chapman, P.A., Siddons, C.A., Cerdan Malo, A.T. & Harkin, M.A. 2000. A one-year study of *Escherichia coli* O157:H7 in raw beef and lamb products. *Epidemiology and Infection*, 124: 207–213.

Chapman, P.A., Cerdan Malo, A.T., Ellin, M., Ashton, R. & Harkin, M.A. 2001. *Escherichia coli* O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. *International Journal of Food Microbiology*, 64: 139–150.

Childs, K.D., Simpson, C.A., Warren-Serna, W., Bellenger, G., Centrella, B., Bowling, R.A., Ruby, J., Stefanek, J., Vote, D.J., Choat, T., Scanga, J.A., Sofos, J.N., Smith, G.C. & Belk, K.E. 2006. Molecular characterization of *Escherichia coli* O157:H7 hide contamination routes: feeedlot to harvest. *Journal of Food Protection*, 69: 1240–1247.

Chinen, I., Tanaro, J.D., Miliwebsky, E., Lound, L.H., Chillemi, G., Ledri, S., Baschkier, A., Scarpin, M., Manfredi, E. & Rivas, M. 2001. Isolation and characterization of *Escherichia coli* O157:H7 from retail meats in Argentina. *Journal of Food Protection*, 64: 1346–1351.

Cízek, A., Alexa, P., Literák, I., Hamrík, J., Novák, P. & Smola, J. 1999. Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and Norwegian rats from a large-scale farm. *Letters in Applied Microbiology*, 28: 435–439.

Clavero, M.R.S. & Beuchat, L.R. 1996. Survival of *Escherichia coli* O157:H7 in broth and processed salami as influenced by pH, water activity, and temperature, and suitability of media for its recovery. *Applied and Environmental Microbiology*, 62(8): 2735–2740.

Collis, V.J., Reid, C.-A., Hutchison, M.L., Davies, M.H., Wheeler, K.P.A., Small, A. & Buncic, S. 2004. Hide contamination with marker bacteria in cattle in a simulated livestock market and at an abattoir. *Journal of Food Protection*, 67: 2397–2402.

Conedera, G., Chapman, P.A., Marangon, S., Tisato, E., Dalvit, P. & Zuin, A. 2001. A field survey of *Escherichia coli* O157 ecology on a cattle farm in Italy. *International Journal of Food Microbiology*, 66: 85–93.

Conner, D. & Hall, G. 1996. Temperature and food additives affect growth and survival of *Escherichia coli* O157:H7 in poultry meat. *Dairy, Food and Environmental Sanitation*, 16: 150–153.

Conway, P. 1995. Microbial ecology of the human large intestine, pp. 1–24, *in*: G.T. Macfarlane and G.R. Gibson (editors). *Human Colonic Bacteria: Role in Nutrition, Physiology, and Pathology*. CRC Press, London, UK.

Cornick, N.A., Matise, I., Samuel, J.E., Bosworth, B.T. & Moon, H.W. 2000. Shiga toxin-producing *Escherichia coli* infection: temporal and quantitative relationships among colonization, toxin production, and systemic disease. *The Journal of Infectious Diseases*, 181: 242–251.

Cray, W.C. & Moon, H.W. 1995. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Applied and Environmental Microbiology*, 61: 1568–1590.

Curnow, J. 1999. *Escherichia coli* O157 outbreak in Scotland linked to unpasteurized goat's milk. *Eurosurveillance Weekly*, 3(24). Web Publication. http://www.eurosurveillance.org/ew/1999/990610.asp#2.

Cutter, C.N. & Siragusa, G.R. 1994a. Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcase tissue using pilot-scale model carcass washer. *Journal of Food Protection*, 57: 97–103.

Cutter, C.N. & Siragusa, G.R. 1994b. Decontamination of beef carcass tissue with nisin using a pilot scale model carcass washer. *Food Microbiology*, 11(6): 481–489.

Dalla-Costa, L.M., Irino, K.,Rodrigues, J., Rivera, I.N.G. & Trabulsi, L.R. 1998. Characterization of diarrhoeagenic *Escherichia coli* clones by ribotyping and ERIC-PCR. *Journal of Medical Microbiology*, 47: 227–234.

Davidson, P.M. 2002. Control of microorganisms with chemicals. pp. 165–190 (Ch. 6), *in:* V. Juneja and J.N. Sofos (editors). Control of Foodborne Microorganisms. Journal of Food Science and Technology series, volume 114. Marcel Dekker, New York, USA.

Decludt, B., Bouvet, P., Mariani-Kurkdjian, P., Grimont, F., Grimont, P.A.D., Hubert, B., Loriat, C. and the Société di Néphrologie Pédiatrique. 2000. Haemolytic uraemic syndrome and Shiga toxin-producing *Escherichia coli* infection in children in France. *Epidemiology and Infection*, 124: 215–220.

Deschenes, G., Casenave, C., Grimont, F., Desenclos, J.C., Benoit, S., Collin, M., Baron, S., Mariani, P., Grimont, P.A. & Nivet, H. 1996. Cluster of cases of haemolytic uraemic syndrome due to unpasteurized cheese. *Pediatric Nephrology*, 10: 203–205.

Desmarchelier, P.M. 1997. Entrerohaemorrhagic *Escherichia coli* – The Australian perspective. *Journal of Food Protection*, 60: 1447–1450.

Desmarchelier, P.M. 2001. STEC in meat and milk production. pp. 1–4, *in:* G. Duffy, P. Garvey, J. Coia, Y. Wasteson and D.A. McDowell (editors). Verocytotoxigenic *E. coli* in Europe: 5. Epidemiology of Verocytotoxigenic *E. coli*. Teagasc, Dublin, Ireland.

Dickson, J., Cutter, C. & Siragusa, G. 1994. Antimicrobial effect of trisodium phosphate against bacteria attached to beef tissue. *Journal of Food Protection*, 61: 1602–1608.

Doyle, M.P. & Padhye, V.V. 1989. *Escherichia coli*. pp. 235–281, *in:* M.P. Doyle (editor). *Foodborne Bacterial Pathogens.* Marcel Dekker, New York, USA.

Doyle, M.P. & Schoeni, J.L. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. *Applied and Environmental Microbiology*, 48: 855–856.

Doyle, M.P. & Schoeni, J.L. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. *Applied and Environmental Microbiology*, 53: 2394–2396.

Doyle, M.P., Zhao, T., Meng, J. & Zhao, S. 1997. *Escherichia coli* O157:H7. pp. 171–191, *in:* M.P. Doyle, L.R. Beuchat and T.J. Montville (editors). *Food Microbiology Fundamentals and Frontiers*. American Society for Microbiology Press, Washington, DC, USA.

Duffy, L.L., Grau, F.H. & Vanderlinde, P.B. 2000. Acid resistance of enterohaemorrhagic and generic *Escherichia coli* are associated with foodborne disease and meat. *International Journal of Food Microbiology*, 60: 83–89.

Duffy, G., Riordan, D.C.R., Sheridan, J.J., Eblen, B.S., Whiting, R.C., Blair, I.S. & McDowell, D.A. 1999. Differences in thermotolerance of various *Escherichia coli* O157:H7 strains in a salami matrix. *Food Microbiology*, 16: 83–91.

Duffy, G., Riordan, D.C.R., Sheridan, J.J., Call, J.E., Whiting, R.C., Blair, I.S. & McDowell, D.A. 2000. Effect of pH on survival, thermotolerance and verotoxin production of *Escherichia coli* O157:H7 during simulated fermentation and storage. *Journal of Food Protection*, 63(1): 12–18.

Duffy, G., Garvey, P., Wasteson, Y., Coia, J.E. & McDowell, D.A. 2001. Epidemiology of Verocytotoxigenic *E. coli.* A technical booklet produced for an EU Concerted Action (CT98-3935). ISBN 1 84170 206 4.

Dykes, G.A., Moorhead, S.M. & Roberts, S.L. 2001. Survival of *Escherichia coli* O157:H7 and *Salmonella* on chill-stored vacuum or carbon dioxide packaged primal beef cuts. *International Journal of Food Microbiology*, 64: 401–405.

EFSA [European Food Safety Authority]. 2006. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004. Available at http://www.efsa.europa.eu/en/efsajournal/pub/310ar.htm

Elder, R.O., Keen, J.E., Siragusa, G.R., Barkocy-Gallagher, G.A., Koohmaraie, M. & Laegreid, W.W. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157:H7 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proceedings of the National Academy of Sciences of the USA*, 97: 2999–3003.

Elliott, E.J., Robins-Browne, R.M., O'Loughlin, E.V., Bennett-Wood, V., Bourke, J., Henning, P., Hogg, G.G., Knight, J., Powell, H. & Redmond, D. 2001. Nationwide study of hemolytic uraemic syndrome: clinical, microbiological, and epidemiological features. *Archives of Diseases in Childhood,* 85: 125–131.

EU [European Union]. 2003. Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. *Official Journal of the European Union*, L 325/31 of 12.12.2003. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:325:0031:0040:EN:PDF [Accessed 2011-05-23]

Faith, N.G., Shere, J.A., Brosch, R., Arnold, K.W., Ansay, S.E., Lee, M.S., Luchansky J.B., Kaspar, C.W. 1996. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. *Applied and Environmental Microbiology*, 62: 1519–1525.

Faith, N.G., Parniere, N., Larson, T., Lorang, T. & Luchansky, J.B. 1997. Viability of *Escherichia coli* O157:H7 in pepperoni during the manufacture of sticks and the subsequent storage of slices at 21, 4 and –20°C under air, vacuum and $CO_2$. *International Journal of Food Microbiology*, 37: 47–54.

Faith, N.G., Parniere, N., Larson, T., Lorang, T., Kaspar, C.W. & Luchansky, J.B. 1998a. Viability of *Escherichia coli* O157:H7 in salami following conditioning of batter, fermentation and drying of sticks, and storage of slices. *Journal of Food Protection*, 61: 377–382.

Faith, N.G., Wierzba, R.K., Ihnot, A.M., Roering, A.M., Lorang, T.D., Kaspar, C.W. & Luchansky, J.B. 1998b. Survival of *Escherichia coli* O157:H7 in full- and reduced-fat pepperoni after manufacture of sticks, storage of slices at 4°C or 21°C under air and vacuum, and baking of slices of frozen pizza at 135, 191 and 246°C. *Journal of Food Protection*, 61: 383–389.

Fantelli, K. & Stephan, R. 2001. Prevalence and characteristics of shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. *International Journal of Food Microbiology*, 70: 63–69.

FDA [Food and Drug Administration]. 1999. Food Code. US Department of Health and Human Services, Public Health Service, Food and Drug Administration. United States Department of Agriculture, Washington, DC, USA.

Fegan, N., Higgs, G., Vanderlinde, P. & Desmarchelier, P. 2004. Enumeration of *Escherichia coli* O157 in cattle faeces using most probable number technique and automated immunomagnetic separation. *Letters in Applied Microbiology*, 38: 56–59.

Frankel, G., Phillips, A.D., Rosenshine, I., Dougan, G., Kaper, J.B. & Knutton, S. 1998. Enteropathogenic and enterohaemorrhagic *Escherichia coli*: more subversive elements. *Molecular Microbiology*, 30(5): 911–921.

FSIS [Food Safety and Inspection Service]. 1993. Report on the *E. coli* O157:H7 outbreak in the Western states. Food Safety and Inspection Service, United States Department of Agriculture.

Fukushima, H., Hoshina, K. & Gomyoda, M. 1999. Long-term survival of shiga toxin-producing *Escherichia coli* O26, O111, and O157 in bovine feces. *Applied and Environmental Microbiology*, 65: 5177–5181.

Gagliardi, J.V. & Karns, J.S. 2000. Leaching of *Escherichia coli* O157:H7 in diverse soils under various agricultural management practices. *Applied and Environmental Microbiology*, 66: 877–883.

Garber, L.P., Wells, S.J., Hancock, D.D., Doyle, M.P., Tuttle, J., Shere, J.A. & Zhao T. 1995. Risk factors for fecal shedding of *Escherichia coli* O157:H7 in dairy calves. *Journal of the American Veterinary Medical Association,* 207: 46–49.

Garber, L.P., Wells, S., Schroeder-Tucker, L. & Ferris, K. 1999. Factors associated with fecal shedding of verotoxin-producing *Escherichia coli* O157 on dairy farms. *Journal of Food Protection*, 62: 307–312.

Glass, K.A., Loeffelholz, J.M., Ford, J.P. & Doyle, M.P. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. *Applied and Environmental Microbiology*, 58: 2513–2516.

Grauke, L.J., Kudva, I.T., Yoon, J.W., Hunt, C.W., Williams, C.J. & Hovde, C.J. 2002. Gastrointestinal tract location of *Escherichia coli* O157:H7 in ruminants. *Applied and Environmental Microbiology*, 68: 2269–2277.

Grif, K., Karch, H., Schneider, C., Daschner, F.D., Beutin, L., Cheasty, T., Smith, H., Rowe, B., Dierich, M.P. & Allerberger, F. 1998. Comparative study of five different techniques for epidemiological typing of *Escherichia coli* O157. *Diagnostic Microbiology and Infectious Diseases*, 32: 165–176.

Griffin, P.M., Olmstead, L.C. & Petras, R.E. 1990. *Escherichia coli* O157:H7-associated colitis: a clinical and histological study of 11 cases. *Gastroenterology*, 99: 142–149.

Griffin, P.M., Mead, P.S., Van Gilder, T., Hunter, S.B. & Stockbrine, N.A. 2001. Epidemiology of Shiga toxin-producing *E. coli* infections in the United States: update, successes and challenges. pp. 56–57, *in*: G. Duffy, P. Garvey, J. Coia, Y. Wateson and D.A. McDowell (editors). Concerted Action CT98-3935. Verocytotoxigenic *E. coli* in Europe. 5. Epidemiology of Verocytotoxigenic *E. coli*. The National Food Centre, Teagasc, Duinsea, Castlenock, Dublin, Ireland.

Haas, C.N., Rose, J.B. & Gerba, C.P. 1999. *Quantitative Microbial Risk Assessment*. John Wiley & Sons, New York, USA.

Hancock, D.D., Besser, T.E. & Rice, D.H. 1998. Ecology of *Escherichia coli* O157:H7 in cattle and impact of management practices. pp. 85–91, *in:* J.B. Kaper and A.D. O'Brien (editors). *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* strains. American Society for Microbiology, Washington, DC, USA

Hancock, D.D., Besser, T.E., Rice, D.H., Herriott, D.E. & Tarr, P.I. 1997. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. *Epidemiology and Infection*, 118: 193–195.

Hancock, D.D., Besser, T.E., Rice, D.H., Ebel, E.D., Herriott, D.E., & Carpenter, L.V. 1998. Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the northwestern USA. *Preventive Veterinary Medicine*, 35: 11–19.

Hancock, D.D., Besser, T.E., Lejeune, J., Davis, M. & Rice, D.H. 2001. The control of VTEC in the animal reservoir. *International Journal of Food Microbiology*, 66: 71–78.

Health Canada. 2000. Interim guidelines for the control of Verotoxinogenic *Escherichia coli* including *E. coli* O157:H7 in ready to eat fermented sausages containing beef or a beef product as an ingredient. Available online through http://www.hc-sc.gc.ca/index-eng.php

Heuvelink, A.E., van den Biggelaar, F.L.A.M., de Boer, E., Herbes, R.G., Melchers, W.J.G., Huis In't Veld, J.H.J. & Monnens, L.A.H. 1998a. Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 strains from Dutch cattle and sheep. *Journal of Clinical Microbiology*, 36: 878–882.

Heuvelink, A.E., van den Biggelaar, F.L.A.M., Zwartkruis-Nahuis, J.T.M., Herbes, R.G., Huyben, R., Nagelkerke, N., Melchers, W.J.G., Monnens, L.A.H. & de Boer, E. 1998b. Occurrence of verocytotoxin-producing *Escherichia coli* O157 on Dutch dairy farms. *Journal of Clinical Microbiology*, 36: 3480–3487.

Heuvelink, A.E., Zwartkruis-Nahuis, J.T.M., Beumer, R.R. & de Boer, E. 1999. Occurrence and survival of verocytotoxin-producing *Escherichia coli* O157 in meats obtained from retail outlets in the Netherlands. *Journal of Food Protection*, 62: 1115–1122.

Hilborn, E.D., Mshar, P.A., Fiorentino, T.R., Dembek, Z.F., Barett, T.J., Howard, R.T. & Cartter, M.L. 2000. An outbreak of *Escherichia coli* O157:H7 infections and haemolytic uraemic syndrome associated with consumption of unpasteurized apple cider. *Epidemiology and Infection*, 124: 31–36.

Himathongkham, S., Bahari, S., Riemann, H. & Cliver, D. 1999. Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in cow manure and cow manure slurry. *FEMS Microbiology Letters,* 178: 251–257.

Hinkens, J.C., Faith, N.G., Lorang, T.D., Bailey, P., Buege, D., Kaspar, C.W. & Luchansky, J.B. 1996. Validation of pepperoni processes for control of *Escherichia coli* O157:H7. *Journal of Food Protection*, 59: 1260–1266.

Hovde, C.J., Austin, P.R., Cloud, K.A., Williams, C.J. & Hunt, C.W. 1999. Effect of cattle diet on *Escherichia coli* O157:H7 acid resistance. *Applied and Environmental Microbiology*, 65: 3233–3235.

ICMSF [International Commission on Microbiological Specifications for Foods]. 1998. Microorganisms in Food 6. Microbial Ecology of Food Commodities. 1st ed. Blackie Academic & Professional. 615 p.

Jackson, T.C., Hardin, M.D. & Acuff, G.R. 1996. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. *Journal of Food Protection*, 59(3): 230–237.

James, C, Goksoy, E.O. & James, S.J. 1997. Past, present and future methods of meat decontamination. [Report of a] MAFF Fellowship in Food Process Engineering, University of Bristol.

Johnson, J.L., Rose, B.E., Sharar, A.K., Ransom, G.M., Lattuada, C.P., & McNamara, A.M. 1995. Methods used for detection and recovery of *Escherichia coli* O157:H7 associated with a food-borne disease outbreak. *Journal of Food Protection*, 58: 597–603.

Juneja, V.K., Snyder, O.P. & Marmer, B.S. 1997. Thermal destruction of *Escherichia coli* O157:H7 in beef and chicken: determination of D- and z-values. *International Journal of Food Microbiology*, 35: 231-237.

Karch, H., Meyer, T., Rüssmann, H. & Heesemann, J. 1992. Frequent loss of Shiga-like toxin genes in clinical isolates of *Escherichia coli* upon subcultivation. *Infection and Immunity*, 60: 3464–3467.

Karmali, M.A., Petric, M., Steele, B.T. & Lim, C. 1983. Sporadic cases of haemolytic uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet*, i, 619–620.

Kassenborg, H.D., Hedberg, C.W., Hoekstra, M., Evans, M.C., Chin, A.E., Marcus, R., Vugia, D.J., Smith, K., Ahuja, S.D., Slutsker, L. & Griffin, P.M. 2004. Farm visits and undercooked hamburgers as major risk factors for sporadic *Escherichia coli* O157:H7 infection: data from a case-control study in 5 FoodNet sites. *Clinical Infectious Diseases*, 38: Supplement 3, S271–278.

Kawamura, N., Yamazaki, T. & Tamai, H. 1999. Risk factors for the development of *Escherichia coli* O157:H7 associated with haemolytic uremic syndrome. *Pediatrics International*, 41: 218–222.

Keene, W.E., Sazie, E., Kok, J., Rice, D.H., Hancock, D.D., Balan, V.K., Zhao, T. & Doyle, M.P. 1997. An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. *Journal of the American Medical Association*, 277: 1229–1231.

Köhler, B., Karch, H. & Schmidt, H. 2000. Antibacterials that are used as growth promoters in animal husbandry can affect the release of Shiga toxin2-converting bacteriophages and Shiga toxin 2 from *Escherichia coli* strains. *Microbiology*, 146: 1085–1090.

Kudva, I.T., Blanch, K. & Hovde, C.J. 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology*, 64: 3166–3174.

Kudva, I.T., Hatfield, P.G. & Hovde, C.J. 1995. Effect of diet on the shedding of *Escherichia coli* O157:H7 in a sheep model. *Applied and Environmental Microbiology*, 61: 1363–1370.

Kudva, I.T., Hunt, C.W., Williams, C.J., Nance, U.M. & Hovde, C.J. 1997. Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding by sheep. *Applied and Environmental Microbiology*, 63: 3878–3886.

Lahti, E., Ruoho, O., Rantala, L., Hanninen, M.-L. & Honkanen-Buzalski, T. 2003. Longitudinal study of *Escherichia coli* O157 in a cattle finishing unit. *Applied and Environmental Microbiology*, 69: 554–561.

Le Saux, N., Spika, J.S., Friesen, B., Johnson, I., Melnychuck, D., Anderson, C., Dion, R., Rahman, M. & Tostowarky, W. 1993. Ground beef consumption in non-commercial settings is a risk factor for sporadic *Escherichia coli* O157:H7 infection in Canada. *Journal of Infectious Diseases*, 167: 500–502.

Levine, P., Rose, B., Green, S., Ransom, G. & Hill, W. 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. *Journal of Food Protection,* 64(8): 1188–1193.

Lin, J., Smith, M.P., Chapin, K.C., Baik, H.S., Bennett, G.N. & Foster, J.W. 1996. Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. *Applied and Environmental Microbiology*, 62: 3094–3100.

Little C.L. & de Loubois, J. 1998. The microbiological examination of butchery products and butchers' premises in the United Kingdom. *Journal of Applied Microbiology*, 85: 177–186.

López, E.L., Contrini, M.M., Sanz, M., Viñas, M.R., Parma, A., De Rosa, M.F. & Cleary, T.G. 1997. Perspectives on shiga-like toxin infections in Argentina. *Journal of Food Protection*, 60: 1458–1462.

Louie, M., Read, S., Louie, L., Ziebell, K., Rahn, K., Borczyk, A. & Lior, H. 1999. Molecular typing methods to investigate transmission of *Escherichia coli* O157:H7 from cattle to humans. *Epidemiology and Infection*, 123: 17–24.

Lynn, T.V., Hancock, D.D., Besser, T.E., Harrison, J.H., Rice, D.H., Stewart, N.T. & Rowan, L.L. 1998. The occurrence and replication of *Escherichia coli* in cattle feeds. *Journal of Dairy Science*, 81: 1102–1108.

MacDonald, K.L., O'Leary, M.J., Cohen, M.L., Norris, P., Wells, J.G., Noll, E., Kobayashi, J.M. & Blake, P.A. 1988. *Escherichia coli* O157:H7, an emerging gastrointestinal pathogen. Results of a one-year, prospective, population-based study. *Journal of the American Medical Association*, 259: 3567–3570.

Mahon, B.E., Griffin, P.M., Mead, P.S. & Tauxe, R.V. 1997. Hemolytic uremic syndrome surveillance to monitor trends in infection with *Escherichia coli* O157:H7 and other shiga-producing *E. coli*. *Emerging Infectious Diseases*, 3: 409–412.

McEvoy, J.M., Doherty, A.M., Sheridan, J.J., Blair, I.S. & McDowell, D.A. 2001. Use of steam condensing at subatmospheric pressures to reduce *Escherichia coli* O157:H7 numbers on bovine hide. *Journal of Food Protection*, 64: 1655–1660.

McEvoy, J.M., Doherty, A.M., Sheridan, J.J., Thomson-Carter, F.M., Garvey, P., McGuire, L., Blair, I.S. & McDowell, D.A. 2003. The prevalence and spread of *Escherichia coli* O157:H7 at a commercial beef abattoir. *Journal of Applied Microbiology*, 95: 256–266.

McGee, P., Scott, L., Sheridan, J.J., Earley, B. & Leonard, N. 2004. Horizontal transmission of *Escherichia coli* O157:H7 during cattle housing. *Journal of Food Protection*, 67: 2651-2656.

McIntosh, W.A., Christensen, L.B. & Acuff, G.R. 1994. Perceptions of risks of eating undercooked meat and willingness to change cooking practices. *Appetite*, 22(1): 83–96.

McKee, M.L. & O'Brien, A.D. 1996. Truncated enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 intimin (EaeA) fusion proteins promote adherence of EHEC trains to Hep-2 cells. *Infection and Immunity*, 64: 2225–2233.

McKellar, R.C. & Knight, K.P. 1999. Growth and survival of various strains of enterohemorrhagic *Escherichia coli* in hydrochloric and acetic acid. *Journal of Food Protection*, 62: 1466–1469.

Mead, P.S. & Griffin, P.M. 1998. *Escherichia coli* O157:H7. *Lancet,* 352: 1207–1212.

Mechie, S.C., Chapman, P.A. & Siddons, C.A. 1997. A fifteen month study of *Escherichia coli* O157:H7 in a dairy herd. *Epidemiology and Infection*, 118: 17–25.

Meng, J., Doyle, M.P., Zhao, T. & Zhao, S. 2001. Enterohemorrhagic *Escherichia coli*. pp. 193–213, *in:* M.P. Doyle, L.R. Beuchat and T.J. Montville (editors). *Food Microbiology: Fundamentals and Frontiers*. American Society for Microbiology Press, Washington, DC, USA.

Michel, P., Wilson, J.B., Martin, S.B., Clarke, R.C., McEwen, S.A. & Gyles, C.L. 1999. Temporal and geographical distributions of reported cases of *Escherichia coli* O157:H7 infection in Ontario. *Epidemiology and Infection*, 122: 193–200.

Milne, L.M., Plom, A., Strudley, I., Pritchard, G.C., Crooks, R., Hall, M., Duckworth, G., Seng, C., Susman, M., Kearney, J., Wiggins, R.J., Moulsdale, M., Cheasty, T. & Willshaw, G.A. 1999. *Escherichia coli* O157 incident associated with a farm open to members of the public. *Communicable Disease and Public Health*, 2: 22–26.

Minihan, D., O'Mahony, M., Whyte, P. & Collins, J.D. 2003. An investigation on the effect of transport and lairage on the faecal shedding prevalence of *Escherichia coli* O157 in cattle. *Journal of Veterinary Medicine Series B – Infectious Diseases and Veterinary Public Health*, 50: 378–382.

Muniesa, M., Lucena, F. & Jofre, J. 1999. Comparative survival of free shiga toxin 2-encoding phages and *Escherichia coli* strains outside the gut. *Applied and Environmental Microbiology*, 65: 5615–5618.

Nataro, J.P. & Kaper, J.B. 1998. Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews*, 11: 142–201.

Naylor, S.W., Low, J.C., Besser, T.E., Mahajan, A., Gunn, G.J., Pearce, M.C., McKendrick, I.J., Smith, D.G.E. & Gally, D.L. 2003. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infection and Immunity,* 71: 1505–1512.

Neill, M.A. 1997. Overview of verotoxigenic *Escherichia coli*. *Journal of Food Protection*, 60: 1444–1446.

O'Brien, S.B., Duffy, G., Carney, E., Sheridan, J.J., McDowell, D.A. & Blair, I.S. 2005. Prevalence and numbers of *Escherichia coli* O157 on bovine hide at a beef slaughter plant. *Journal of Food Protection*, 68: 660–665.

Ørskov, F. 1984. Genus I. *Escherichia*. pp. 420–423, *in:* J.G. Holt and N.R. Kreig (editors). *Bergey's Manual of Systematic Bacteriology*. Vol. I. Williams and Wilkins, Baltimore, MD, USA.

Park, S., Worobo, R.W. & Durst, R.A. 1999. *Escherichia coli* O157:H7 as an emerging foodborne pathogen: a literature review. *Critical Reviews in Food Science and Nutrition*, 39: 481–502.

Paton, A.W., Ratcliff, R.M., Doyle, R.M., Seymour-Murray, J., Davos, D., Lanser, J.A. & Paton, J.C. 1996. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with shiga-like toxin-producing *Escherichia coli*. *Journal of Clinical Microbiology*, 34: 1622–1627.

Paunio, M., Pebody, R., Keskimäki, M., Kokki, M., Ruutu, P., Oinonen, S., Vuotari, V., Sütonen, A., Lahti, E. & Leinikki, P. 1999. Swimming-associated outbreak of *Escherichia coli* O157:H7. *Epidemiology and Infection*, 122: 1–5.

Pavia, A.T., Nichols, C.R., Green, D.P., Tauxe, R.V., Mottice, S., Greene, K.D., Wells, J.G., Siegler, R.L., Brewer, E.D. & Hannon, D. 1990. Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. *Journal of Pediatrics*,116: 544–551.

Pebody, R.G., Furtado, C., Rojas, A., McCarthy, N., Nylen, G., Ruutu, P., Leino, T., Chalmers, R., deJong, B., Donnelly, M., Fishery, I., Gilham, C., Graverson, L., Cheasty, T., Willshaw, G., Navarro, M., Salmon, R., Leinikki, P., Wall, P. & Bartlett, C. 1999. An international outbreak of vero cytotoxin-producing *Escherichia coli* O157 infection amongst tourists; a challenge for the European infectious disease surveillance network. *Epidemiology and Infection*, 123: 217–223.

Pennington Group Report. 1997. Report on the circumstances leading to the 1996 outbreak of infection with *Escherichia coli* O157:H7 in Central Scotland, the implications for food safety and the lessons to be learned. The Stationery Office, Edinburgh. Her Majesty's Stationary Office, Norwich, UK.

Pennington, H. 1998. Factors involved in recent outbreaks of *Escherichia coli* O157:H7 in Scotland and recommendations for its control. *Journal of Food Safety*, 18: 383–391.

Phebus, R.K., Nutsch, A.L., Schafer, D.E., Wilson, R.C., Riemann, M.J., Leising, J.D., Kastner, C.L., Wolf, J.R. & Prasai, R.K.. 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. *Journal of Food Protection*, 60(5): 476–484.

Phillips, A.D., Navabpour, S., Hicks, S., Dougan, G., Wallis, T. & Frankel, G. 2000. Enterohaemorrhagic *Escherichia coli* O157:H7 target Peyer's patches in humans and

cause attaching and effacing lesions in both human and bovine intestine. *Gut*, 47: 377–381.

Piérard, D., Crowcroft, N., De Bock, S., Potters, D., Crabbe, G., Van Loock, F. & Lauwer, S. 1999. A case-control study of sporadic infection with O157 and non-O157 verocytotoxin-producing *Escherichia coli*. *Epidemiology and Infection*, 122: 359–365.

Powell, M.R., Ebel, E., Schlosser, W., Walderhaug, M. & Kause, J. 2000. Dose response envelope for *E. coli* O157:H7. *Quantitative Microbiology,* 2: 141–163.

Porter, J., Mobbs, K., Hart, C.A., Saunders, J.R., Pickup, R.W. & Edwards, C. 1997. Detection, distribution and probable fate of *Escherichia coli* O157 from asymptomatic cattle on a dairy farm. *Journal of Applied Microbiology*, 83: 279–306.

Pradel, N., Livrelli, V., De Champs, C., Palcoux, J.-B., Reynaud, A., Scheutz, F., Sirot, J., Joly, B. & Forestier, C. 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *Journal of Clinical Microbiology*, 38: 1023–1031.

Radu, S., Mutalib, S.A., Rusul, G., Ahmad, Z., Morigaki, T., Asai, N., Kim, Y.B., Okuda, J. & Nishibuchi, M. 1998. Detection of *Escherichia coli* O157:H7 in the beef marketed in Malaysia. *Applied and Environmental Microbiology*, 64: 1153–1156.

Rahn, K., Renwick, S.A., Johnson, R.P., Wilson, J.B., Clarke, R.C., Alves, D., Lior, H. & Spika, J. 1997. Persistence of *Escherichia coli* O157:H7 in dairy cattle and the dairy farm environment. *Epidemiology and Infection*, 119: 251–259.

Rajpura, A., Lamden, K., Forster, S., Clarke, S., Cheesbrough, J., Gornall, S. & Waterworth, S. 2003. Large outbreak of infection with *Escherichia coli* O157 PT21/28 in Eccleston, Lancashire, due to cross-contamination at a butcher's counter. *Communicable Disease and Public Health*, 6: 279–284.

Rangel, J.M., Sparling, P.H., Crowe, C., Griffin, P.M. & Swerdlow, D.L. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerging Infectious Diseases*, 11: 603–609.

Ransom, J.R., Belk, K.E., Bacon, R.T., Sofos, J.N., Scanga, J.A. & Smith, G.C. 2002. Comparison of sampling methods for microbiological testing of beef animal rectal/colonal feces, hides and carcasses. *Journal of Food Protection*, 65: 621–626.

Reed, C.A. 1995. Approaches for ensuring the safety of dry and semi-dry fermented sausage products. Letter to plant managers of August 21, 1995. U.S. Department of Agriculture, Food Safety Inspection Service, Washington, DC, USA.

Reid, C.-A., Avery, S.M., Warriss, P. & Buncic, S. 2002a. The effect of feed withdrawal on *Escherichia coli* shedding in beef cattle. *Food Control*, 13: 393–398.

Reid, C.-A., Avery, S.M., Warriss, P. & Buncic, S. 2002b. Presence of food-borne pathogens on cattle hides. *Food Control*, 13: 411–415.

Rice, D.H., Sheng, H.Q., Wynia, S.A. & Hovde, C.J. 2003. Rectoanal mucosal swab culture is more sensitive than fecal culture and distinguishes *Escherichia coli* O157:H7-colonized cattle and those transiently shedding the same organism. *Journal of Clinical Microbiology*, 41: 4924–4929.

Riley, L.W., Remis, R.S., Helgerson, S.S., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., Blake, P.A. & Cohen, M.L. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine*, 308: 681–685.

Riordan, D.C.R., Duffy, G., Sheridan, J.J., Eblen, B.S., Whiting, R.C., Blair, I.S. & McDowell, D.A. 1998. Survival of *Escherichia coli* O157:H7 during the manufacture of pepperoni. *Journal of Food Protection*, 61: 146–151.

Ritchie, J.M., Wagner, P.L., Acheson, D.W.K. & Waldor, M.K. 2003. Comparison of Shiga toxin production by hemolytic-uremic syndrome-associated and bovine-associated Shiga toxin-producing *Escherichia coli* isolates. *Applied and Environmental Microbiology,* 69: 1059–1066.

Rivera-Betancourt, M., Shackelford, S.D., Arthur, T.M., Westmoreland, K.E., Bellinger, G., Rossman, M., Reagan, J.O. & Koohmaraie, M. 2004. Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in two geographically distant commercial beef processing plants in the United States. *Journal of Food Protection*, 67(2): 295–302.

Roberts, J.A. & Upton, P.A. 2001. The socio-economic impact of *E. coli* O157:H7. pp. 85–97, *in:* G. Duffy, P. Garvey, J. Coia, Y. Wasteson and D.A. McDowell (editors). *Verocytotoxigenic* E. coli *in Europe: 5. Epidemiology of Verocytotoxigenic* E. coli. Teagasc, Dublin, Ireland.

Robinson, S.E., Wright, E.J., Hart, C.A., Bennett, M. & French, N.P. 2004. Intermittent and persistent shedding of *Escherichia coli* O157 in cohorts of naturally infected calves. *Journal of Applied Microbiology*, 97: 1045–1053.

Rosenshine, I., Ruschkowski, S. & Finlay B.B. 1996. Expression of attaching/effacing activity by enteropathogenic *Escherichia coli* depends on growth phase, temperature, and protein synthesis upon contact with epithelial cells. *Infection and Immunity*, 64(3): 966-973.

Ross, T. & Shadbolt, C.T. 2001. Predicting *Escherichia coli* inactivation in uncooked comminuted fermented meat products. Meat and Livestock Australia, Sydney, Australia.

Ryan, C.A., Tauxe, R.V., Hosek, G.W., Wells, J.G., Stoesz, P.A., McFadden, H.W. Jr., Smith, P.W., Wright, G.F. & Blake, P.A. 1986. *Escherichia coli* O157:H7 diarrhoea in a nursing home: clinical, epidemiological, and pathological findings. *Journal of Infectious Diseases*, 154: 631–638.

Ryu, J.-H. & Beuchat, L.R. 1999. Changes in heat tolerance of *Escherichia coli* O157:H7 after exposure to acidic environments. *Food Microbiology*, 16: 317–324.

Sartz, L., De Jong, B., Hjertqvist, M., Plym-Forshell, L., Alsterlund, R., Löfdahl, S., Osterman, B., Ståhl, A., Eriksson, E., Hansson, H.B. & Karpman, D. 2008. An outbreak of *Escherichia coli* O157:H7 infection in southern Sweden associated with consumption of fermented sausage; aspects of sausage production that increase the risk of contamination. *Epidemiology and Infection*, 136(3): 370–380.

Satin, M. 2002. Use of irradiation for microbial decontamination of meat: situation and perspectives. pp. 3-8, *in:* Proceedings of the 48th International Congress on Meat Science and Technology, Rome, 25–30 August 2002. Vol. 1.

Savarino, S.J., McVeigh, A., Watson, J., Cravioto, A., Molina, J., Echeverria, P., Bhan, M.K., Levine, M.M. & Fasano, A. 1996. Enteroaggregative *Escherichia coli* heat-stable enterotoxin is not restricted to enteroaggregative *Escherichia coli*. *Journal of Infectious Diseases,* 173(4): 1019–1022.

Schmidt, H., Beutin, L. & Karch, H. 1995. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. *Infection and Immunology*, 63: 1055–1061.

Schmidt, H., Karch, H. & Beutin, L. 1994. The large-sized plasmids of enterohemorrhagic *Escherichia coli* O157 strains encode hemolysins which are presumably members of the *E. coli* alpha-hemolysin family. *FEMS Microbiology Letters*, 117: 189–196.

SCIEH [Scottish Centre for Infection and Environmental Health]. 2002. Sorbitolfermenting, verotoxigenic *E. coli* O157. *SCIEH Weekly Report*, 43: 281. See: http://www.documents.hps.scot.nhs.uk/ewr/pdf2002/0243.pdf [Accessed 2011-05-21]

SCVMPH [Scientific Committee on Veterinary Measures relating to Public Health]. 2003. Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on Verotoxigenic *E. coli* (VTEC) in Foodstuffs. European Commission, Health & Consumer Protection Directorate General. Available at http://ec.europa.eu/food/fs/sc/scv/out58_en.pdf

Shere, J.A., Bartlett, K.J. & Kaspar, C.W. 1998. Longitudinal study of *Escherichia coli* O157:H7 dissemination on four dairy farms in Wisconsin. *Applied and Environmental Microbiology,* 64: 1390–1399.

Shere, J.A., Kaspar, C.W., Bartlett, K.J., Linden, S.E., Norell, B., Francey, S. & Schaefer, D.M. 2002. Shedding of *Escherichia coli* O157:H7 in dairy cattle housed in a confined environment following waterborne inoculation. *Applied and Environmental Microbiology*, 68: 1947–1954.

Sherman, P., Cockerill, F., Soni, R. & Brunton, J. 1991. Outer membranes are competitive inhibitors of *Escherichia coli* O157:H7 adherence to epithelial cells. *Infection and Immunology*, 59: 86–94.

Siegler, R.L., Pavia, A.T., Christoferson, R.D. & Milligan, M.K. 1994. A 20-year population-based study of post-diarrheal haemolytic uremic syndrome in Utah. *Pediatrics*, 94: 35–40.

Silveira, N.F.A., Silva, N., Contreras, C., Miyagusku, L., Baccin, M.D.F., Koono, E. & Beraquet, N.J. 1999. Occurrence of *Escherichia coli* O157:H7 in hamburgers produced in Brazil. *Journal of Food Protection,* 62(11): 1333–1335.

Simmons, N.A. 1997. Global perspectives on *Escherichia coli* O157:H7 and other verocytotoxic *Escherichia coli* spp.: UK views. *Journal of Food Protection*, 60: 1463–1465.

Slutsker, L., Ries, A.A., Maloney, K., Wells, J.G., Greene, K.D. & Griffin, P.M. 1998. A nationwide case-control study of *Escherichia coli* O157:H7 infection in the United States. *Journal of Infectious Diseases*, 177: 962–966.

Small, A., Reid, C.-A. & Buncic, S. 2003. Conditions in lairages at abattoirs for ruminants in Southwest England and *in vitro* survival of *Escherichia coli* O157, *Salmonella kedougou*, and *Campylobacter jejuni* on lairage-related substrates. *Journal of Food Protection*, 66: 1570–1575.

Small, A., Reid, C.-A., Avery, S.M., Karabasil, N., Crowley, C. & Buncic, S. 2002. Potential for the spread of *Escherichia coli* O157, *Salmonella*, and *Campylobacter* in the lairage environment at abattoirs. *Journal of Food Protection*, 65: 931–936.

Smerdon, W.J., Adak, G.D., O'Brien, S.J., Gilespie, I.A. & Reacher, M. 2001. General outbreaks of infectious intestinal disease linked with red meat, England and Wales, 1992–1999. *Communicable Disease and Public Health*, 4(4): 259–267.

Smith, G. 2004. VTEC infections in UK. Paper presented at Zoonoses Conference, Veterinary Laboratory Agency, Weybridge, UK.

Smith, D., Blackford, M., Younts, S., Moxley, R., Gray, J., Hungerford, L., Milton, T. & Klopfenstein, T. 2001. Ecological relationships between the prevalence of cattle shedding *Escherichia coli* O157:H7 and characteristics of the cattle or conditions of the feedlot pen. *Journal of Food Protection*, 64: 1899–1903.

Sneyd, E., Lopez, L., Eglinton, M., McDowell, R. & Margolin, T. 2002. Annual surveillance summary 2001. Client Report FW0165. ESR, Kenepuru Drive, Porirua, New Zealand.

Stampi, S., Caprioli, A., De Luca, G., Quaglio, P., Sacchetti, R. & Zanetti, F. 2004. Detection of *Escherichia coli* O157 in bovine meat products in northern Italy. *International Journal of Food Microbiology*, 90: 257–262.

Suthienkul, O., Brown, J.E., Seriwatana, J., Tienthongdee, S., Sastravaha, S. & Echeverria, P. 1990. Shiga-like toxin-producing *Escherichia coli* in retail meats and cattle in Thailand. *Applied and Environmental Microbiology*, 56: 1135–1139.

Synge, B. 2000. Verocytotoxin-producing *Escherichia coli*: a veterinary view. *Journal of Applied Microbiology*, 88: 31S–37S.

Synge, B. & Paiba, G. 2000. Verotoxin-producing *E. coli* O157. *Veterinary Record*, 147: 27.

Synge, B.A., Gunn, G.J., Ternet, H.E., Hopkins, G.F., Thomson-Carter, F., Foster, G., Chase-Topping, M. & McKendrick, I. 2001. Prevalence and factors affecting the shedding of verocytotoxin-producing *Escherichia coli* O157 in beef cattle in Scotland. pp. 98–103, *in:* G. Duffy, P. Garvey, J. Coia, Y. Wasteson and D.A. McDowell (editors). *Verocytotoxigenic* E. coli *in Europe: 5. Epidemiology of Verocytotoxigenic* E. coli . Teagasc, Dublin, Ireland.

Takeda, Y. 1999. Enterohaemorrhagic *Escherichia coli* infection in Japan. *Pediatrics International*, 41: 198–201.

Takumi, K., de Jonge, R. & Havelaar, A. 2000. Modelling inactivation of *Escherichia coli* by low pH: application to passage through the stomach of young and elderly people. *Journal of Applied Microbiology*, 89(6): 935–943.

Tarr, P.I. & Hickman, R.O. 1987. Hemolytic uremic syndrome epidemiology: a population-based study in King County, Washington, 1971–1980. *Pediatrics*, 80(1): 41–45.

Tarr, P.I., Tran, N.T. & Wilson, R.A. 1999. *Escherichia coli* O157:H7 in retail ground beef in Seattle: results of a one-year prospective study. *Journal of Food Protection*, 62: 133–139.

Tilden, J., Young, W., McNamara, A.-M. Custer, C., Boesel, B., Lambert-Fair, M.A., Majkowski, J., Vugia, D., Werner, S.B., Hollingsworth, J. & Morris, J.G. 1996. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. *American Journal of Public Health*, 86: 1142–1145.

Tirado, C. & Schmidt, K. (editors). 2000. WHO surveillance programme for control of foodborne infections and intoxications in Europe, 7th report, 1993–1998. BGVVFAO/WHO Collaborating Centre for Research and Training in Food Hygiene and Zoonoses. Available at http://www.who.int/foodsafety/publications/foodborne_disease/dec2000/en/

Torres, A.G. & Payne, S.M. 1997. Haem iron-transport system in enterohaemorrhagic *Escherichia coli* O157:H7. *Molecular Microbiology*, 23: 825–833.

Trevena, W.B., Willshaw, G.A., Cheasty, T., Domingue, G. & Wray, C. 1999. Transmission of vero cytotoxin producing *Escherichia coli* O157 infection from farm animals to humans in Cornwall and West Devon. *Communicable Disease and Public Health*, 2: 263–268.

Tutenel, A.V., Pierard, D., Van Hoof, J., Cornelis, M. & De Zutter, L. 2003a. Isolation and molecular characterization of *Escherichia coli* O157 isolated from cattle, pigs and chickens at slaughter. *International Journal of Food Microbiology*, 84: 6369.

Tutenel, A.V., Pierard, D., Van Hoof, J. & De Zutter, L. 2003b. Molecular characterization of *Escherichia coli* O157 contamination routes in a cattle slaughterhouse. *Journal of Food Protection*, 66: 1564–1569.

Tuttle, J., Gomez, T., Doyle, M.P., Wells, J.G., Zhao, T., Tauxe, R.V. & Griffin, P.M. 1999. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. *Epidemiology and Infection*, 122: 185–192.

Uhtil, S., Jakšic, S., Petrak, T. & Botka-Petrak, K. 2001. Presence of *Escherichia coli* O157:H7 in ground beef and ground baby beef meat. *Journal of Food Protection*, 64: 862–864.

USDA [United States Department of Agriculture]. 2003. Color of cooked meat as it relates to doneness. Food Safety and Inspection Service, United States Department of Agriculture, technical publication. Rev. April 2003. Available at: http://www.fsis.usda.gov/Fact_Sheets/color_of_cooked_ground_beef/index.asp.

Uyttendaele, M., Jozwik, E., Tutenel, A., De Zutter, L., Uradzinski, J., Pierard, D. & Debevere, J. 2001. Effect of acid resistance of *Escherichia coli* O157:H7 on efficacy of buffered lactic acid to decontaminate chilled beef tissue and effect of modified atmosphere packaging on survival of *Escherichia coli* O157:H7 on red meat. *Journal of Food Protection*, 64: 1661–1666.

Van Donkersgoed, J., Graham, T. & Gannon, V. 1999. The prevalence of verotoxins, *Escherichia coli* O157:H7 and *Salmonella* in the feces and rumen of cattle at processing. *Canadian Veterinary Journal*, 40: 332–338.

Vold, L., Sandberg, M. Jarp, J. & Wasteson, Y. 2001. Occurrence and characterization of *Escherichia coli* O157 isolated from cattle in Norway. *Veterinary Research Communications*, 25: 13–26.

Vuddhakul, V., Patararungrong, N., Pungrasamee, P., Jitsurong, S., Morigaki, T., Asai, N. & Nishibuchi, M. 2000. Isolation and characterization of *Escherichia coli* O157 from retail beef and bovine feces in Thailand. *FEMS Microbiology Letters*, 182: 343–347.

Vugia, D., Cronquist, A., Hadler, J. and 13 others. 2005. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food – 10 Sites, United States, 2004. *Morbidity and Mortality Weekly Report*, 54(14): 352–356.

Wagner, P.L., Neely, M.N., Zhang, X., Acheson, D.W., Waldor, M.K. & Friedman, D.I. 2001. Role for a phage promoter in Shiga toxin 2 expression from a pathogenic *Escherichia coli* strain. *Journal of Bacteriology*, 183: 2081–2085.

Wales, A.D., Pearson, G.R., Skuse, A.M., Roe, J.M., Hayes, C.M., Cookson, A.L. & Woodward, M.J. 2001. Attaching and effacing lesions caused by *Escherichia coli* O157:H7 in experimentally inoculated neonatal lambs. *Journal of Medical Microbiology*, 50: 752–758.

Wang, G. & Doyle, M.P. 1998. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. *Journal of Food Protection*, 61: 662–667.

Wells, J.G., Davis, B.R. & Wachsmuth, I.K. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rare *Escherichia coli* serotype. *Journal of Clinical Microbiology*, 18: 512–520.

Werber, D., Fruth, A., Liesegang, A., Littmann, M., Buchholz, U., Prager, R., Karch, H., Breuer, T., Tschape, H. & Ammon, A. 2002. A multistate outbreak of Shiga toxin-producing *Escherichia coli* O26:H11 infections in Germany, detected by molecular subtyping surveillance. *Journal of Infectious Diseases*, 186: 419–422.

Whiting, R.C. & Golden, M.H. 2002. Variation among *Escherichia coli* O157:H7 strains relative to their growth, survival, thermal inactivation, and toxin production in broth. *International Journal of Food Microbiology*, 75: 127–133.

WHO, 1980. Report of the WHO/WAVFH Round Table Conference on the Present Status of the *Salmonella* Problem (Prevention and Control), Bilthoven, the Netherlands, 6–10 October 1980. WHO/VPH/81.27. Available as: http://whqlibdoc.who.int/hq/pre-wholis/VPH_81.27.pdf

WHO, 1997. Prevention and control of enterohaemorrhagic *Escherichia coli* (EHEC) infections: Report of a WHO consultation. Geneva, Switzerland. Available at http://whqlibdoc.who.int/hq/1997/WHO_FSF_FOS_97.6.pdf

Williams, R.C., Isaacs, S., Decou, M.L., Richardson, E.A., Buffet, M.C., Slinger, R.W., Brodsky, M.H., Ciebin, B.W., Ellis, A. & Hockin, A. 2000. Illness outbreak associated with *Escherichia coli* O157:H7 in Genoa salami. *Canadian Medical Association Journal*, 162: 1409–1413.

Willshaw, G.A., Thirlwell, J., Jones, A.P., Parry, S., Salmon, R.L. & Hickey, M. 1994. Verocytotoxin-producing *Escherichia coli* O157 in beefburgers linked to an outbreak of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome in Britain. *Letters in Applied Microbiology*, 19: 304–307.

Wilson, J.B., Johnson, R.P., Clarke, R.C., Rahn, K., Renwick, S.A., Alves, D., Karmali, M.A., Michel, P., Orrbine, E. & Spika, J.S. 1997. Canadian perspectives on verocytotoxin-producing *Escherichia coli* infection. *Journal of Food Protection*, 60: 1451–1453.

Woerner, D.R., Ransom, J.R., Sofos, J.N., Scanga, J.A., Smith G.C. & Belk, K.E. 2006. Preharvest processes for microbial control in cattle. *Food Protection Trends*, 26: 393–400.

Wood, J. 2006. Trends in the organization of the meat industry. pp. 97–104, *in:* S. Buncic (editor). 2006. *Integrated Food Safety and Veterinary Public Health.* CABI International, CABI International Publishing, Oxon, UK.

Yokoyama, K., Horii, T., Yamashino, T., Hashikawa, S., Barua, S., Hasegawa, T., Watanabe, H. & Ohta, M. 2000. Production of Shiga toxin by *Escherichia coli* measured with reference to the membrane vesicle-associated toxins. *FEMS Microbiology Letters*, 192: 139–144.

Zhao, T., Doyle, M.P., Shere, J. & Garber, L. 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Applied and Environmental Microbiology*, 61: 1290–1293.

Zhou, Z., Nishikawa, Y., Zhu, P., Hong, S., Hase, A., Cheasty, T., Smith, H.R., Zheng, M. & Haruki, K. 2002. Isolation and characterization of Shiga toxin-producing *Escherichia coli*

O157:H7 from beef, pork and cattle fecal samples in Changchun, China. *Journal of Veterinary Medicine and Science*, 64: 1041–1044.

# Annex 3
# Summary of existing risk assessments

*Note:* This summary was prepared by a working group consisting of Anna Lammerding, Canada; Geraldine Duffy, Ireland; Dan Englejohn, United States of America; and Bruce Tompkin, United States of America, as part of a case study on *Escherichia coli* O157:H7 in fresh raw ground beef to examine how existing risk assessments could be used as a basis for risk management actions.

## A3.1. Quantitative risk assessment for Escherichia coli O157:H7 in ground beef hamburgers (Cassin et al., 1998).

**Model description**

A farm-to-fork process risk model for the production of beef trim in 5 kg packs for grinding at retail, and final preparation and consumption of hamburgers in the home.

**Initial data inputs**

- Prevalence of *E. coli* O157:H7 in cattle: 0–1.6 % (published data from survey years 1984–1994 in Canada and the United States of America);

- estimated distribution of numbers of *E. coli* O157:H7 shed in faeces of positive animals, based on one study of 13 animals, ranging from <2.0–5.0 $\log_{10}$ cfu/g (Zhao et al., 1995);

- distribution of hamburger cooking practices based on United States of America survey data indicating approximately 20% of consumers prepared rare or medium rare, corresponding to mean internal temperatures of 58.6°C or less.

**Key assumptions**

A 'carcass contamination factor' was derived from data on concentration of Biotype 1 *E. coli* counts in faeces and resulting counts on carcass surfaces after de-hiding. The reduction of counts of *E. coli* O157:H7 on the carcass due to decontamination treatments was aggregated into a single parameter and assumed to be a 1–2.5 log reduction; retail and home storage temperatures distribution assumed a minimum of 4°C, mode 10°C, to a maximum of 15°C. The dose-response model was a modification of one based on *Shigella* feeding studies. Probability of illness for susceptible populations was assumed to be the same as the general population, although the model considered increased severity of outcomes in children and the elderly (i.e. HUS and mortality) based on epidemiological data.

**Intermediate outputs**

Mean prevalence of contaminated retail packages (300–1000 g) of fresh ground beef: 2.9%. Predicted number of O157:H7 in contaminated packages: 87% <10 cfu/package; 90% <1000 cfu.

**Risk estimate**

Average probability of illness from a single meal: $5.1 \times 10^{-5}$ (range $10^{-2}$ to $10^{-22}$).

**Key findings**

Importance analysis ranked the following factors as the most important predictors of risk: Concentration of pathogen in faeces; host susceptibility; carcass contamination factor; cooking preference; retail storage temperature; reductions due to decontamination during primary processing; growth during processing.

Potential targets for risk management were assessed using hypothetical assumptions for effectiveness of interventions: (i) pre-slaughter treatment of cattle to reduce maximum concentration of pathogen shed in faeces such that levels higher than 4 log cfu/g were eliminated resulted in a 25% reduction in risk; (ii) retail storage temperature mode reduced to 8°C from 10°C resulted in an 80% reduction in risk; and (iii) an information campaign targeting consumers resulting in a shift from 18.6% consuming rare or medium rare ground beef to 12% resulted in a 16% reduction in number of illnesses.

## A3.2. Shiga toxin-producing E. coli *in Ground Beef manufactured from Australian Beef: Process Improvement.*

Report prepared for Meat and Livestock Australia (2000). (Meat and Livestock Australia, pers. comm.)

### Model description and data inputs

The process risk model was an adapted model of Cassin et al. (1998), substituting data for prevalence of STEC (Shiga-toxin (Stx)-producing *E. coli*) in Australian cattle (35.4–53.4%) and with a modification to account for the proportion of 'potentially pathogenic' STEC, based on the presence of virulence markers Stx1, Stx2, the *eae* gene and the EHEC plasmid. Weekend chilling for a portion of carcasses was also considered and assumed to allow greater proliferation of the pathogen than overnight chilling.

### Risk estimate

Expected probability of illness was $6.4×10^{-4}$ per serving for adults and $4.6×10^{-4}$ for a child under the age of 5 years.

### Key observations

Scenario analysis showed that use of hot water decontamination (expected 1- to 4-log reduction in STEC numbers on carcasses) resulted in a predicted 99.7% reduction in risk of illness; irradiation of de-boned and frozen trimmings with 1 kGy (expected reduction in STEC numbers of 1.3 to 1.8 logs) gave a 97% reduction of illnesses; eliminating or implementing stricter temperature controls for over-weekend chilling such that the maximum proliferation was limited to the same as overnight chilling resulted in a 20% decrease in risk.

## A3.3. Draft risk assessment of the public health impact of Escherichia coli *O157 in ground beef (USDA-FSIS, 2001)*

### Model description

Exposure assessment considers farm, slaughter, production of trim for grinding, and preparation factors that influence likelihood of consumption in ground beef servings; multiple 2000 lb (909.1 kg) combo bins or 60 lb (27.27 kg) boxes produced for grinding; and ground beef servings as patties, meatballs and meatloaf.

### Initial data inputs

*E. coli* O157-H7 prevalence data published 1994–2001 were used, adjusted to estimate true prevalences, namely breeding (cull) cattle: herd mean 63%; and within-herd 3% during low prevalence season (October–May), 4% during high prevalence season (June–Sept). Feedlot (beef) cattle: 88% herd prevalence; within-lot animal prevalence during low and high prevalence seasons of 9% and 22%, respectively. Carcass pathogen densities were based on 1994 baseline data: positive carcasses ranged from <0.03 to 3.0 cfu/cm$^2$. Fresh ground beef

prevalence was predicted by the model adjusted by 'anchoring' to actual survey data. Cooking practices were based on United States of America survey data (approximately 20% prepared rare or medium rare).

**Key assumptions**

The collective effect of interventions for decontamination (First decontamination step after de-hiding: trim/wash/vacuum; Second decontamination step after final wash: hot water, steam pasteurization) was estimated to be 1.5 log reduction. The dose-response function was derived from (i) epidemiological data for *E. coli* O157:H7 illnesses; (ii) predicted number of contaminated ground beef servings; and (iii) bounded by upper and lower dose-response curves from surrogate pathogens *Shigella dysenteriae* and enteropathogenic *E. coli* (EPEC), respectively (Powell et al., 2000).

**Intermediate outputs**

For the combo bins of manufacturing trim derived from dairy cattle, an estimated average of 6% in the low-prevalence season and 8% in the high-prevalence season contained at least 1 or more *E. coli* O157:H7 organism (Figure A3.1). Similarly, for the combo bins of manufacturing trim derived from steers and heifers, an estimated average of 23% and 43% of combo bins contained at least 1 or more *E. coli* O157:H7 organisms during the low and high prevalence seasons, respectively (Figure A3.2). Estimates also were made on the likely counts of *E. coli* O157:H7 in combo bins of manufacturing trim, ranging from 2 and 3 organisms in trim from dairy cattle in the low and high prevalence season, respectively. For combo bins of manufacturing trim from steers and heifers, the likely counts of *E. coli* O157:H7 organisms ranged from 13 and 41 organisms in the low and high prevalence seasons, respectively. For the combo bins of ground beef composed of combinations of manufacturing trim from both dairy cattle and steers and heifers in order to attain a specified fat content in the resulting finely comminuted beef, an average of 68% and 86% of combo bins contained at least 1 or more *E. coli* O157:H7 organisms during the low and high prevalence seasons, respectively (Figure A3.3). The model predicted that 0.018% of prepared servings consumed during June through September (high prevalence season) and 0.007% of servings consumed during the remainder of the year are contaminated with one or more *E. coli* O157:H7.
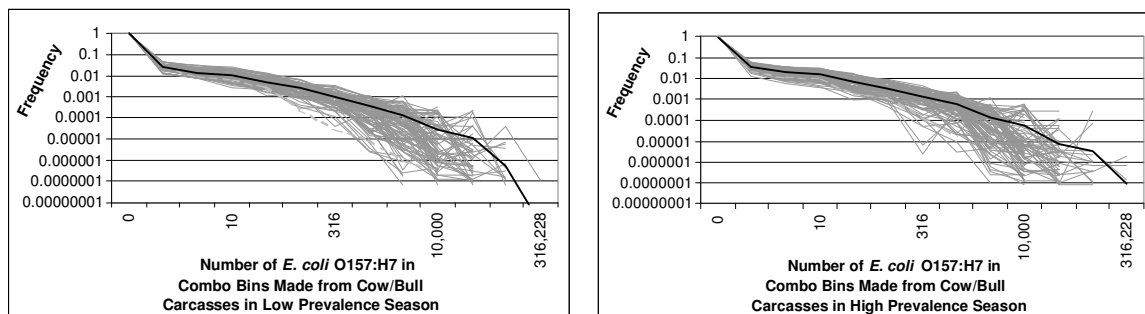


**Figure A3.1.** Comparison of seasonal distributions for number of *E. coli* O157:H7 in combo bins constructed from the slaughter of breeding (cow/bull) cattle. Dark lines are the mean distributions for each season.
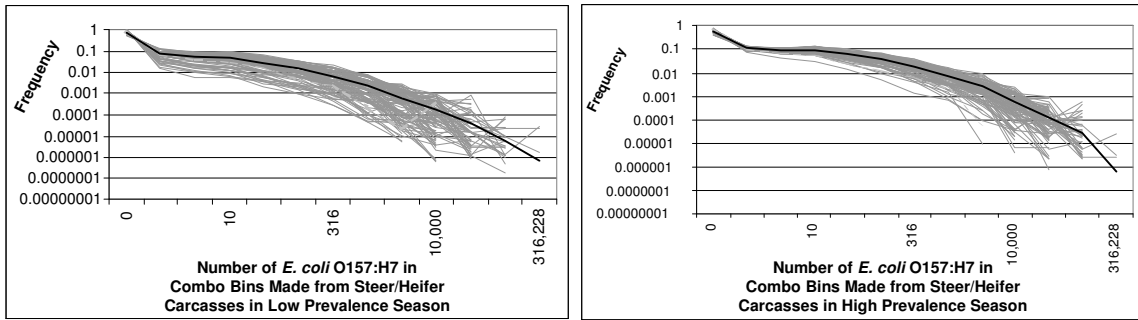
**Figure A3.2.** Comparison of seasonal distributions for number of *E. coli* O157:H7 in combo bins constructed from the slaughter of feedlot (steer/heifer) cattle. Dark lines are the mean distributions for each season.
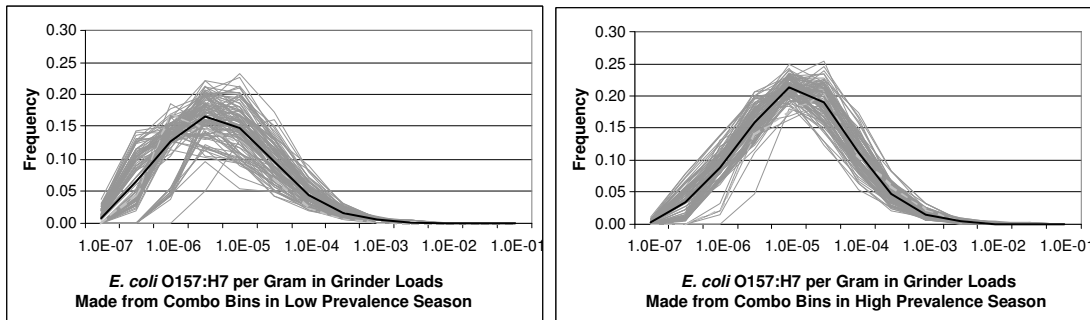


**Figure A3.3.** Frequency of ground beef contamination in contaminated grinder loads made from 2000-pound combo bins in low and high prevalence seasons. Grinder loads that are not contaminated are not shown in this chart. The mean grinder load distribution is represented by the dark line.

**Risk estimate**

The annual United States of America population risk estimate was nearly 1 illness in each 1 million ($9.6 \times 10^{-7}$) servings of ground beef consumed. When seasonality was considered, there was a 3× higher risk in June–September, namely 1 in every 600 000 servings ($1.7 \times 10^{-6}$) versus 1 in 1.6 million servings ($6.0 \times 10^{-7}$) during October–May. For children aged 0–5 years, the risk was estimated to be 2.5 times higher than for older consumers; although fewer exposures are expected, that they are at greater susceptibility was accounted for by using the upper bound of the dose-response curve.

**Key findings**

Various scenarios were modelled, and indicated that the likelihood of finding *E. coli* O157:H7 through testing of manufacturing trim was substantively higher, by approximately a 5-fold difference, than through testing of ground beef alone. Importance analysis for the occurrence and extent of *E. coli* O157:H7 contamination in beef trim and subsequent grinder loads ranked the following factors as the most important predictors: (i) feedlot and within-feedlot prevalence; (ii) probability of carcass contamination following de-hiding; (iii) amount of carcass contaminated; (iv) effectiveness of decontamination procedures; and (iv) carcass chilling. The effect of these factors on the occurrence and extent of contamination varied by season and type of cattle (feedlot herd or breeding herd).

Occurrence and extent of *E. coli* O157:H7 contamination in cooked ground beef was in addition influenced by (i) the proportion of ground beef that is frozen; (ii) the maximum population density of *E. coli* O157:H7 in ground beef; (iii) storage temperatures; and (iv) cooking. The United States of America population risk is influenced more by number of contaminated servings than number of *E. coli* O157:H7 per serving.

## A3.4. Risk assessment of Shiga-toxin producing Escherichia coli *O157 in steak tartare in the Netherlands (Nauta et al, 2001).*

**Model description**

Risk was estimated for the consumption of steak tartare patties, a lean (<10% fat) ground beef product typically eaten raw or partially raw in The Netherlands. The model considered three routes of exposure that encompass different slaughter practices and subsequent processing (industrial and traditional, differentiating size of slaughter operation and traditional butcher versus industrial preparation of product); three preparation styles of tartare patties (raw, medium and well done); and three age classes of consumers: 1–4 years, 5–14 years, and 15+ years of age.

**Initial data inputs**

Overall average animal-level prevalence of *E. coli* O157:H7 in Dutch cattle used for tartare was estimated to be 1%. (On the farm: 0–19 % and 0–25% for dairy cattle and veal calves, respectively; at the animal level: 0–9% and 0–61% for negative- and positive-tested farms for dairy cattle; for veal bulls, the animal level varied from 10 to 36% for farms found positive. Prevalences at herd and animal level at slaughterhouses also considered). Concentration of pathogen in faeces of shedding animals was based on studies of Zhao et al. (1995). Tartare was consumed raw 2.6% of the time and prepared (medium or well done) by remainder of consumers.

**Key assumptions**

Expert elicitation was used to estimate several parameters, including extent of faecal contamination of carcasses and percentage of patties thoroughly heated (20%). Given typical storage times and temperatures at retail and in the home, growth of pathogen in steak tartare not expected to be an important factor. The dose-response model was based on data from a 1996 outbreak in Japan and resulted in a dose-response similar to that for feed trials for *Shigella.* The model derived for this study predicts the highest probability of illness per single cell ingested compared with alternative models (Table A3.1).

**Table A3.1.** A comparison of dose-response models for STEC O157:H7 (adapted from Nauta et al., 2001)

| Model | Bacterial species | Probability of illness per single cell * | Reference |
|---|---|---|---|
| Exponential | *E. coli* O157:H7 | 0.005 | Nauta et al., 2001 |
| Beta-Poisson | Shigella and *S. flexneri* | 0.001 | Cassin et al., 1998 |
| Beta-Poisson | *S. dysenteriae* and enteropathogenic *E. coli* | 0.00003 | Powell et al., 2000; USDA-FSIS, 2001; Ebel et al., 2004; Teagasc, 2006 |

NOTES: * Probabilities given for the beta-Poisson model are mean values of the underlying beta distribution.

**Intermediate outputs**

For industrial ground beef (i.e. large volumes processed), the model predicted that the prevalence of contaminated batches is higher than that prepared in traditional facilities, where meat of only one or a few carcasses is used per batch. However, at the level of raw steak tartare patties (unit sizes), prevalences are almost equal. Pathogen numbers in contaminated product were smaller in industrially produced patties, a result of diluting contamination throughout large volumes of uncontaminated meat. Overall, the exposure model predicts 0.3% of raw steak tartare patties are contaminated; about 64% of positive patties contain only 1 cfu, and only 7% contain more than 10 cfu. Given the model assumption that 1 cfu per 100 g is detected, the results underestimate national Netherlands survey data for the product, which indicated 1.2% of raw patties positive (1/82 samples), noting that the realistic sensitivity of testing is closer to 10 cfu/100 g.

**Risk estimation**

The predicted number of illnesses associated with the consumption of steak tartare in The Netherlands is 1284 cases per year, an incidence rate of 8 per 100 000 persons. By comparison, the incidence rate of STEC O157 illnesses from all sources, based on epidemiological data, is estimated to be 13 cases per 100 000. The authors suggest that the model prediction may be overestimating the number of illnesses associated with the product, possibly driven by the dose-response model used, which predicts a higher probability of illness than models used by other authors. Using an alternative dose-response model (Powell et al., 2000), only 17 cases per year were predicted from the consumption of steak tartare.

**Key findings**

Scenario analysis was used to identify the important parameters of the model and model assumptions. Effects on the risk estimate of uncertainty in farm prevalence and concentration of the pathogen in faeces were large, as were effects of growth and inactivation on carcasses. Based on the model and expertise, the factors that are most likely to decrease the risk

associated with the product are: lowering prevalence and concentration of *E. coli* O157 in cattle, improved hygiene at slaughter, or increased frequency of industrial processing. Product control by monitoring at retail did not appear to be practical given low prevalence and concentrations, and growth during storage unlikely. Intervention at the consumer level, using an information campaign to influence preparation practices, was not part of the risk assessment model. However, this aspect is addressed through professional opinions from communication experts on potential effectiveness of an information campaign. It was concluded that this strategy would probably not be favourable to risk reduction for the product.

## A3.5. E. coli *O157:H7 in beef burgers produced in the Republic of Ireland: A quantitative microbial risk assessment (Teagasc, 2006).*

### Model description

The model considered slaughter and the potential contamination of carcasses in the abattoir with *E. coli* O157:H7, taking into account the impact various slaughtering processes may have on the distribution of the bacteria; preparation of beef burgers, focusing on processing of beef trimmings (from one or more 27.5-kg boxes of beef trimmings) into beef burgers; retail handling practices and their effects on bacterial numbers; and finally domestic storage, preparation and cooking were considered. In the model developed, variability and uncertainty in the input parameters were incorporated by the construction of a second-order model by means of probabilistic distributions.

### Data inputs

Data inputs on the prevalence and concentration of *E. coli* O157:H7 in the Irish beef chain were based on a number of microbiological surveys on the pathogen at key points in the chain, including bovine faeces (McEvoy et al., 2003), bovine hide (O'Brien et al., 2005), carcass and beef trimmings (Carney et al., 2006) and beef burgers and minced beef at retail (Cagney et al., 2004).

Data shown in Table A3.2 include results for bovine hide, used as starting input data set for the model. The additional data shown were collected to validate the results of the risk model outputs.

**Table A3.2.** Prevalence and numbers of *E. coli* O157:H7 at various sample points along the beef chain in Ireland.

| Sample type | Sample numbers | Number positive (%) | Numbers present ($log_{10}$ cfu) | Reference |
|---|---|---|---|---|
| Bovine hide | 1500 | 109 (7.3) | 0.13–4.24/100 cm$^2$ | O'Brien et al., 2005 |
| Beef carcasses | 132 | 4 (3.0) | 0.70–1.41/g | Carney et al., 2006 |
| Head meat | 100 | 3 (3.0) | 0.70–1.00/g | O'Brien et al., 2005 |
| Beef trimmings | 1351 | 32 (2.4) | 0.70–1.61/g | O'Brien et al., 2005 |
| Retail minced beef and burgers | 1533 | 43 (2.8) | 0.52–4.03/g | Cagney et al., 2004 |

Data for the retail and domestic part of the model was based on two main sources. Information on typical consumer handling practices in the domestic environment was derived

from a questionnaire survey of consumers conducted by the Market Research Bureau of Ireland (MRBI) (Mahon, Cowan and Henchion, 2003). Data on storage temperatures at retail and in domestic refrigerators was gathered from temperature studies in both environments (Kennedy et al., 2005; Duffy et al., 2005). Consumption data figures for minced beef was derived from an Irish Food Consumption Survey carried out by the Irish Universities Nutrition Alliance (Mahon, Cowan and Henchion, 2003).

**Key assumptions**

- The assumption is that the beef trimmings are boxed into 27 kg lots and trimmings from these boxes are minced into 100 g beef burger patties.

- The potential growth for *E. coli* O157:H7 in the beef burger (at retail, transport to home, and in domestic environment) was adapted from the model employed in the USDA-FSIS (2001) risk assessment model, using Irish data on storage times and temperatures at retail, transport and domestic stages. The calculation for the probability temperature abuse was carried out in a different manner to the FSIS model.

- The product is a 100 g fresh beef burger sold in either a supermarket or a butcher's shop.

- A temperature distribution was set for the cooking temperature based on the assumption that beef burgers are cooked to mean temperatures of 68.3°C (well done), 62.7°C (medium) or 54.4°C (rare). (Normal Distribution: standard deviation ±2°C). The log reduction as a result of cooking was then estimated. Based on a population survey, 87% of consumers prepare hamburgers well done, 12% medium and 1% cooked them rare.

The dose response used was chosen from the literature based on United States of America data (Powell et al., 2000). In this EPEC was chosen to represent the lower bound of an *E. coli* O157:H7 dose-response function, as has been done in previous studies, and *S. dysenteriae* was selected as an upper bound to the *E. coli* O157:H7 dose-response function. The dose-response analysis was performed using a beta-Poisson function.

**Risk estimate**

Transposing the exposure assessment data through a dose response model yielded an estimate of the probability of illness caused by exposure to *E. coli* O157:H7 in beef burgers. The probability reported is for an 'average' individual. It is acknowledged that this dose response relationship may be an underestimate for immuno-compromised individuals; however, to try to create one for individual risk groups was not possible given the lack of reliable data for a dose-response relationship in these categories. The simulated probability of illness from a contaminated serving of fresh beef was -5.94 log (i.e. $10^{-5.94}$ = approximately 1 chance in a million).

**Key findings**

The sensitivity of model inputs to model predictions was modelled by rank order correlation sensitivity analysis. The initial count on bovine hides and the initial hide prevalence were significant parameters, indicating the importance of minimizing contaminated hides entering the slaughter plant. Cross-contamination at hide removal was also a significant parameter, indicating where producers might focus efforts to reduce risk. Consumer behaviour in terms of cooking temperature and temperature abuse during transport and storage also plays an important part in dictating the final risk value, indicating the important role consumers have to play in ensuring their food is safe for consumption.

## *A3.6. Summary*

Each of the available risk assessments indicate the limitations of data and understanding in modelling the events that result in contamination of ground beef with *E. coli* O157:H7. Empirical survey data and in-depth investigation following episodes of illnesses associated with product substantiate MRA predictions that the pathogen occurs in ground beef at low levels and low prevalence most of the time, with sporadic occurrences of high levels leading to a contaminated lot. The risk assessments all important factors identified correlated with risk, from pre-harvest to consumer, some of which may be potential targets for risk mitigation and intervention, or are highly uncertain and require further research. However, beyond application of good hygienic practices, adoption of multiple interventions at slaughter, and strict temperature controls throughout the food chain, there are no practical risk management options available today that would entirely eliminate the pathogen from live animals, from carcasses, or in raw ground product, with the exception of irradiation. Irradiation can effectively reduce the number of pathogens and result in a microbiologically safe product that retains its raw appearance.

## *A3.7. References*

Cagney, C., Crowley, H., Duffy, G., Sheridan, J.J., O' Brien, S., Carney, E., Anderson, W.A, McDowell, D.A., Blair, I.S. & Bishop, R.H. 2004. Prevalence and numbers of *Escherichia coli* O157: H7 in minced beef and beef burgers from butcher shops and supermarkets in the Republic of Ireland. *Food Microbiology,* 21(2): 203–212.

Carney, E., O'Brien, S.B., Sheridan, J.J., McDowell, D.A., Blair, I.S. & Duffy, G. 2006. Prevalence and level of *Escherichia coli* O157 on beef trimmings, carcasses and head meat at a beef slaughter plant. *Food Microbiology,.* 23(1): 52–59.

Cassin, M.H., Lammerding, A.M., Todd, E.C.D., Ross, W. & McColl, R.S. 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *International Journal of Food Microbiology,* 41: 21–44.

Duffy, G., Butler, F., Cummins, E., O'Brien, S., Nally, P., Carney, E., Henchion, M., Mahon, D. & Cowan, C. 2005. *E. coli* O157:H7 in minced beef produced in Ireland: A quantitative risk assessment. Draft report. The National Food Centre, Teagasc, Ashtown, Dublin

Ebel, E., Schlosser, W., Kause, J., Orloski, K., Roberts, T., Narrod, C., Malcolm, S., Coleman, M. & Powell, M. 2004. Draft risk assessment of the public health impact of *Escherichia coli* O157:H7 in ground beef. *Journal of Food Protection*, 67(9): 1991–1999.

Kennedy, J., Jackson, V., Blair, I.S., McDowell, D.A., Cowan, C. & Bolton, D.J. 2005. Consumer food safety knowledge and the microbiological and temperature status of their refrigerators. *Journal of Food Protection*, 68: 1421–1430.

Mahon, D., Cowan, C. & Henchion, M. 2003. Report on mince beef and beef burger consumption and handling practices of Irish consumers. Food Marketing Group, The National Food Centre, Teagasc, Ireland.

McEvoy, J.M., Doherty, A.M., Sheridan, J.J., Thomson-Carter, F.M., Garvey, P., McGuire, L., Blair, I.S. & McDowell, D.A. 2003. The prevalence and spread of *Escherichia coli* O157:H7 at a commercial beef abattoir. *Journal of Applied Microbiology,* 95: 256–266.

Nauta, M.J., Evers, E.G., Takumi, K. & Havelaar, A.H. 2001. Risk assessment of Shiga-like producing *Escherichia coli* O157 in steak tartare in the Netherlands. RIVM report 257851003. RIVM, Bilthoven, The Netherlands.

O'Brien, S.B., Duffy, G., Carney, E., Sheridan, J.J., McDowell, D.A. & Blair, I.S. 2005. Prevalence and numbers of *Escherichia coli* O157 on bovine hides at a beef slaughter plant. *Journal of Food Protection,* 68(4): 660–665.

Powell, M.R., Ebel, E., Schlosser, W., Walderhaug, M. & Kause, J. 2000. Dose response envelope for *E. coli* O157:H7. *Quantitative Microbiology,* 2: 141–163.

Teagasc [Agriculture and Food Development Authority of Ireland]. 2006. *E. coli* O157:H7 in beef burgers produced in the Republic of Ireland: A quantitative microbial risk assessment. Ashtown Food Research Centre, Dublin, Ireland.

USDA-FSIS [U.S. Department of Agriculture, Food Safety and Inspection Service]. 2001. Draft risk assessment of the public health impact of *Escherichia coli* O157:H7 in ground beef. USDA, Washington, DC, USA. Available at http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/00-023N/00-023NReport.pdf (Accessed 2011-05-27)

Zhao, T., Doyle, M.P., Shere, J. & Garber, L. 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Applied and Environmental Microbiology,* 61(4): 1290–1293.