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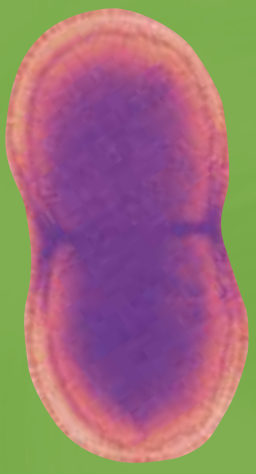
E. coli O157:H7 in beefburgers produced in the Republic of Ireland: A quantitative microbial risk assessment



E. coli O157:H7 in beefburgers produced in the Republic of Ireland: A quantitative microbial risk assessment

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1. SUMMARY

A quantitative microbial risk assessment (QMRA) model was developed for *E. coli* O157:H7 in beefburgers produced in the Republic of Ireland.
The risk assessment model was broken into three modules:

Module 1) slaughter process culminating in the production of boxed beef trimmings
Module 2) mincing of beef, beefburger formation and retail distribution
Module 3) domestic storage, cooking and consumption

Data inputs and assumptions

Initial data inputs to the model on the prevalence and concentration of *E. coli* O157:H7 were based on microbiological surveys on the pathogen in faeces (McEvoy *et al.,* 2003) and hide (O'Brien *et al.,* 2005a) of animals presented for slaughter at Irish beef abattoirs. The model outputs for prevalence and numbers of *E. coli* O157:H7 at the end of module 1 and 2 were validated using microbiological surveillance data for *E. coli* O157:H7 on beef trimmings (Carney *et al.,* 2006) and in beef mince / burgers at retail (Cagney *et al.,* 2004) in the Republic of Ireland.

The model assumed that contaminated hide and rumen contents were the vectors for cross contamination to carcasses and a cross contamination factor was created based on Irish surveillance data for the pathogen on bovine hide (O'Brien *et al.,* 2005a) in rumen contents (McEvoy *et al.,* 2003) and on beef carcasses (Mc Evoy *et al.,* 2003; Carney *et al.,* 2006). The changes in *E. coli* O157:H7 numbers on contaminated carcasses during carcass dressing operations including trimming of visibly dirty parts of carcass; carcass washing, evisceration and chilling were estimated based on research studies in the literature on the impact of these operations on pathogen numbers (Gill *et al.,* 1996; McEvoy *et al.,* 2003, McEvoy *et al.,* 2004). The potential increase in numbers of *E. coli* O157:H7 in the boning hall was assumed to be minimal (McEvoy *et al.,* 2004). A factor for estimating the transfer of contamination from carcass to trim was set in the model taking account of the surface area of carcass which was contaminated, the surface area of the trim, the weight of the trim and the number of trim in a box (27 kg). It was assumed that the beef trimmings were minced into 100g beefburger patties.
Input data for the retail/domestic part of the model was based on two main sources. Information on typical consumer storage and cooking practices in the domestic environment was derived from a questionnaire survey of consumers conducted by the Market Research Bureau of Ireland (MRBI) (Mahon *et al.,* 2003). Data on storage temperatures at retail and in domestic refrigerators were also gathered from temperature studies in both environments (Kennedy *et al.,* 2005; Carney *et al.,* personal communication) that found that temperatures ranged between 7 and 16°C. Based on the survey of consumer cooking practices, the model was based on the premise that 87% of consumers prepare hamburgers well done, 12% medium and 1% cooked them rare. A temperature distribution was set for the cooking temperature based on the assumption that beefburgers are cooked to mean temperatures of 68.3°C (well done), 62.7°C (medium) or 54.4°C (rare) (Jackson *et al.,* 1996). Consumption figures for beefburgers were derived from an Irish Food Consumption Survey carried out by the Irish Universities Nutrition Alliance (www.iuna.net) (Mahon *et al.,* 2003) and the serving size for a beefburger was set at a mean of 100g.

Model Outputs

The risk model was created in Excel with the add-on package @Risk™ (Pallisade Corporation, New York, USA). The output of module 1 indicated a mean simulated prevalence of *E. coli* O157:H7 on beef trimmings of 2.40 % and a mean count of $\log_{10}$ -2.69 CFU g^{-1}. This output was validated against a microbiological survey of *E. coli* O157:H7 on beef trimmings in Irish abattoir which indicated a prevalence of 2.36 % and counts of $\log_{10}$ 0.7 CFU g^{-1} to $\log_{10}$ 1.61 g^{-1} which indicates that the model simulated values and the survey results were similar.
The output of module 2 indicated a mean simulated prevalence in fresh beefburgers of 2.9% and 2.2% in frozen burgers while the mean simulated counts in fresh and frozen burgers were $\log_{10}$ 1.96 CFU g^{-1} and $\log_{10}$ -0.22 CFU

g^{-1} respectively. These predicted values were compared with microbiological survey data on prevalence and numbers of *E. coli* O157:H7 on these products on retail sale in the Republic of Ireland and shown to be similar (prevalence 2.8%; counts $\log_{10}$ 0.51 – $\log_{10}$ 4.03 CFU g^{-1}).

The dose response used was based on the model of Powell *et al.* (2000). The probability of illness caused by exposure to *E. coli* O157:H7 in fresh beefburgers was reported for an "average" individual. Overall, the model predicted that the risk of human illness from the consumption of a serving of minced beef and beefburgers was –5.94 log (1.1×10^{-6}). This is approximately 1 illness per one million burgers consumed.

Analysis of the risk model (by rank order correlation sensitivity analysis) indicated the following:

- The initial prevalence and numbers of *E. coli* O157:H7 on the bovine hide (correlation coefficient 0.62) had the greatest impact on overall probability of illness from *E. coli* O157:H7. Cross contamination at hide removal impacted on predicted risk (correlation coefficient 0.25).
- The impact of consumer practices on risk (calculated) from *E. coli* O157:H7 was examined. A sensitivity analysis revealed that one of the most important factors was the cooking preference (correlation coefficient –0.57). The higher the internal cooking temperature, the less the risk. "Well done" cooked burgers (mean internal temperature 68.3°C ± 2°C) virtually eliminated any probability of infection; "medium" cooked burgers (mean internal temp 62.7°C ± 2°C) also greatly reduced the probability of infection. Burgers cooked "rare" (mean internal temp 54.4°C± 2°C) constituted a significant risk to the consumer.
- Temperature abuse during retail storage temperature, during transport home and during home storage was deemed a significant parameter influencing model predictions (correlation coefficient 0.48). It is concluded that consumers can play a large role in reducing risk from *E. coli* O157:H7 in minced beef by keeping products properly refrigerated and cooking burgers to a "well done" state.
- The prevalence / contamination levels of *E. coli* O157:H7 (and calculated risk) in fresh chilled beefburgers and in frozen burgers were compared. Fresh burgers had a greater predicted prevalence (mean of 2.9% versus 2.2% for frozen burgers) and higher mean counts ($\log_{10}$1.96 CFU g^{-1} versus $\log_{10}$–0.22 CFU g^{-1} for frozen burgers). This was mainly due to the higher probability for temperature abuse of fresh burgers during retail display, transport and home storage.
- The difference in prevalence / contamination levels of *E. coli* O157:H7 (and calculated risk) in a beefburger made from 100% beef (meat) was compared with a burger made with added ingredients. As added ingredients were not identified as a significant risk factor in the contamination of beefburgers, they did not contribute directly to the contamination level. However, because of the reduced beef incorporated into burgers with added ingredients, a dilution effect was observed. The model indicated a reduction in prevalence of approximately 0.4% and a reduction in counts of approximately $\log_{10}$ 0.3CFU g^{-1} on contaminated beefburgers with added ingredients, resulting in a reduction in exposure and hence risk.
- The prevalence / contamination levels of *E. coli* O157:H7 (and calculated risk) from *E. coli* O157:H7 in beef mince purchased from a butcher shop was compared with product purchased from supermarket and the prevalence and count level was found to be virtually the same in both types of establishment with no difference in predicted risk.

2. INTRODUCTION TO QUANTITATIVE MICROBIAL RISK ASSESSMENT

2.1 Risk Analysis

Risk analysis is a valuable tool in the management of microbial food safety issues and a systematic approach for the regulatory authorities and the food industry to control the risk posed by a pathogen in a particular food commodity. Risk analysis, as defined by Codex Alimentarius Commission (Codex, 1999) consists of three elements: risk assessment, risk management and risk communication. Risk assessment is the part of the process in which the hazards are identified and the risk posed by that particular hazard (i.e. pathogen) is calculated. Apart from an end point calculation of risk, the risk assessment model can be used to develop risk based management options like identifying the critical control points and setting quantitative critical limits as part of HACCP (Hazard Analysis Critical Control Point) systems.

The principles of risk assessment and the fours stages involved (hazard identification, exposure assessment, hazard characterisation and risk characterisation) are outlined by the Codex Alimentarius Commission. Each of the stages is summarised below.

2.2 Hazard identification

A hazard can be defined as an agent with the potential to have an adverse effect on public health and may pose a short term, chronic, or fatal risk to a person. The identification of a microbial hazard associated with a particular food is generally based on information generated from routine microbial analysis of the commodity or from an epidemiological linkage of a particular pathogen with a case of food borne infection. Microbial pathogens may be present on raw food or may be introduced during processing, distribution, storage or final preparation by cross contamination. In particular, this may occur as a result of mincing, chopping or blending to homogenise foods or from cross contamination in the retail, food service or domestic environment.

2.3 Exposure assessment

Exposure assessment is a quantitative estimation of the amount of a contaminant in a typical serving of food. A microbial quantitative risk assessment can be based on the number of microorganisms at the time of consumption, or as close to this stage as is scientifically possible and practical. However, the final estimation of the numbers and prevalence of a pathogen in the food to be consumed is generally based on an accumulation of data on the prevalence and numbers of pathogen at key points in the food chain from the raw material though processing, retail distribution and domestic preparation. This provides information on the changes in pathogen numbers along the product chain and is a valuable tool in managing risk. The accuracy of an exposure assessment is highly dependent on the quality of the microbial data that is used in generating the assessment. In an ideal situation, data on prevalence, numbers, and virulence characterisation of the pathogen present are derived for the actual process, thus giving an exposure assessment with limited associated error. However, time restraints and lack of resources often rule this out as a viable option and so the data on the prevalence, numbers and types of microorganisms in a food are inferred from existing information sources. Sources used include the scientific literature, technical publications and conference abstracts, surveillance databases from national or regional public health bodies, industry surveillance testing and expert opinion where no data is available. Numerous problems arise in accessing data from these sources. Outside the scientific literature, available data is generally difficult to source. In particular, data for a specific raw product, ingredient or specific region / country can be difficult to obtain. Different microbiological methods may be used to obtain the results, the associated error may not be recorded or the method employed may not be mentioned. A further challenge is the lack of quantitative microbial data available for food borne pathogens.

In conducting an exposure assessment along a particular food chain, data is needed on how particular processes or stages along the food chain impact on microbial numbers i.e. increase or decrease microbial counts. It is often not practical to conduct challenge tests and so the alternative is to use predictive microbial models. Predictive

microbiology has developed as a science and is used as a means of predicting product self-life and to assist in factoring food safety into a product design. The models developed vary in sophistication from primary level models, which essentially collate pathogen numbers with time, to secondary models that recognise that the growth / survival of microorganisms is not merely a function of time but is significantly effected by their surrounding environmental parameters including temperature, pH, a_w, sodium chloride etc. The next level of model is based on tertiary equations which are computer software based and combine or build on elements of first and second order models into an easy-to-use system to predict the growth of particular pathogens under a particular set of conditions.

The overall exposure assessment relates the amount of contaminant in a designated amount of food with the amount of food typically consumed in a single serving. Information on food consumed is typically procured from food consumption databases developed for nutritional purposes.

The exposure assessment model can be 'deterministic', derived using single data points along the food chain. However, this approach may result in outlier values being ignored and thus potentially underestimates or overestimates the predicted risk, but is generally overly conservative. A more common approach is to use a probabilistic or stochastic analysis, which uses all available data in a data distribution at each step as opposed to a single value. Thus, at each sample point, account is taken not only of the values which fall in the mean range but also of the outlier values, thus giving a more accurate data picture. The distribution chosen should provide a good fit for the given data set and the analyses of all the distributions is generally conducted using a developed user-friendly software system (@ Risk™, Palisade, NY, USA) which facilitates a Monte Carlo analysis. In this process, a single data point is chosen at random from each data distribution and used to calculate an outcome. The process is repeated several thousand times (multiple iterations) with a new random data point from each distribution chosen each time and with the final output being based on all the iterations. Error in the prediction may be related to variability (a natural error related to randomness that cannot be altered by additional data or physical measurements) or uncertainty (due to a lack of data in an area where more research or more data can reduce the associated error). All risk models will have an error associated with their risk prediction and second order models can be used to separate out the part of the error which is associated with uncertainty, as opposed to variability. It is clear that the accuracy of the input data for the exposure assessment will influence the final output distribution and associated uncertainty. This highlights the need for accurate prevalence and count data on the microorganism in question and the importance of data on the impact that various process stages have on the microorganism.

2.4 Hazard Characterisation

Hazard characterisation relates exposure to a hazard with a probable public health outcome (e.g. illness/death). A dose-response relationship can be used to estimate the exposure level (number) of microorganisms that will make a person ill or which may be fatal. The data used in generating dose response models are derived from a variety of sources including human clinical trials, epidemiological studies based on food poisoning outbreaks, animal clinical trials, in vitro studies using cell lines, biomarkers or expert opinion. The logarithm number of microorganisms ingested is plotted against the percentage of people that become ill to generate the dose response. Epidemiological data on clinical illness can also be taken into account, including the number of people affected in outbreaks, the profile of the population sickened (age, health status etc) and the severity of illness experienced (home recovery, hospitalisation, fatalities).

2.5 Risk Characterisation

The final stage in the process links the exposure assessment model with the hazard characterisation to give an estimate of the probability of an adverse health effect or risk to a population as a consequence of exposure to the hazard. In general, the risk characterisation sets out to estimate a number of factors which may include the expected risk of infection in an individual or the risk of illness in a population. These may be based on a prediction of illness per typical serving or calculated as an annual risk of illness. The risk estimates may be broken down into age categories, immune status etc. to identify groups which may be at higher risk from exposure to the contaminant.

As described for the exposure assessment model, the risk characterisation model is developed using commercial software such as @Risk™. The error associated with overall risk prediction can be separated to represent the contribution from uncertainty and variability. Other add-on software programmes for Excel including Crystal Ball (Decisioneering Inc., Denver USA) allow problem solving and more complicated risk estimation.

Apart from an overall estimate of risk and associated error, the risk model can be a valuable tool that can be used to determine the most important risk factors. This can be achieved by means of a sensitivity analysis. This process involves the determination of the effect of change in the input data (at different points along the food chain) on the overall risk estimate. Risk ranking is an approach used in exposure assessment to rank different categories / stages in the chain in order of potential risk from a particular microorganism, thus creating a sequential ordering system of risk posed to each product. Equally, risk ranking could be used to assess which of the factors along the chain contribute most to risk. Scenario analysis can be used to predict the expected reduction in risk which could be achieved by introducing a particular risk reduction option. It can also be used to direct the deployment of resources in a strategic manner and is a most useful tool from a risk management perspective.

3. OBJECTIVES AND SCOPE OF THE QUANTITATIVE RISK ASSESSMENT

Recognising the public health problem related to *E. coli* O157:H7 and the potential role of beef in its transmission, The Department of Agriculture and Food through the Food Institutional Research Measure (FIRM) funded a research programme to be conducted by Ashtown Food Research Centre, Teagasc and Biosystems Engineering Unit, University College Dublin, to develop a quantitative risk assessment model for *E. coli* O157:H7 in beefburgers produced in the Republic of Ireland. The risk assessment covered *E. coli* O157:H7 in the slaughter process culminating in the production of boxed beef trimmings; mincing of beef and burger formation, retail distribution; domestic preparation and cooking; consumption and predicted illness.

3.1 Risk Management Questions

Alongside the scientific team, a risk management forum was convened representing the key stakeholders including beef slaughter and processing sector, retail sector, public health, regulatory authorities and the food safety agencies (Food Safety Authority of Ireland, *safe*food, the Food Safety Promotion Board). At the outset of the programme, the risk managers set out the questions which they wished the scientific risk assessment to answer. These were as follows:

- Is there a difference in prevalence / contamination levels of *E. coli* O157:H7 (and calculated risk) in frozen burgers versus fresh chilled beefburgers ?
- Is there a difference in prevalence / contamination levels of *E. coli* O157:H7 (and calculated risk) from a beefburger made from 100% beef (meat) versus a burger made with added ingredients?
- Is there a difference in prevalence / contamination levels of *E. coli* O157:H7 (and calculated risk) from *E. coli* O157:H7 from beef mince purchased from a butcher shop versus a supermarket?

- What is the probability of a case of *E. coli* O157:H7 from a single serving of minced beef and what is the annual probability of infection?
- What is the probability of a case of *E. coli* O157:H7 infection in different risk groups (young children, healthy adults) from a serving of minced beef?
- What is the probability of *E.coli* O157:H7 illness from a minced beef meal prepared and consumed in the home versus outside the home?
- What impact do consumer practices have on the risk (calculated) from *E. coli* O157:H7?

4. QUANTITATIVE MICROBIAL RISK ASSESSMENT FOR *E. coli* O157:H7 IN BEEFBURGERS PRODUCED IN IRELAND

4. 1 Hazard identification

Escherichia coli

Escherichia coli O157:H7 is a member of the Enterhaemorrhagic group of *E. coli* (EHEC). Within this group of pathogens, *Escherichia coli* O157:H7 is the most notorious and was first implicated in infectious disease in the early 1980s (Riley *et al.,* 1982). The symptoms of infection from this group of organisms includes bloody diarrhoea and severe abdominal pain. Haemolytic uraemic syndrome (HUS), a cause of acute renal failure, may be a complication of the illness and neurological problems in the form of thrombotic thrombocytopaenic purpura (TTP) may also occur. Immuno-compromised patients including young children and the elderly are at a particular risk of developing HUS (Coia, 1998). Pathogenicity is related to the ability of the organism to adhere to and colonise the human large intestinal epithelial tissue, forming attachment and effacing lesions (encoded by eae gene) and the production of verocytotoxins (vt1, vt2). Between 2000-2004, clinical cases of *E. coli* O157:H7 in Ireland ranged from 1.0 to 2.2 cases per 100,000 (Table 1).

Table 1. Number of cases of confirmed *E. coli* O157 and crude incidence rate in the Republic of Ireland, 2000-2004 (source: HPSC annual reports www.hpsc.ie)

Year	Numbers of confirmed cases (number confirmed cases including non-residents)	Crude incidence rate per 100,000 population
2000	37(42)	1.0
2001	50 (52)	1.4
2002	68 (70)	1.7
2003	88	2.2
2004	52	1.3

E. coli O157:H7 in beef

While multiple sources and routes of transmission for *E. coli* O157:H7 are now recognised, beef and beef products remain an important vector for the pathogen and continue to be linked to outbreaks across the developed world (Table 2). Analyses of sporadic cases of *E.coli* O157:H7 infection have identified under-cooked beef as an important risk factor (Kassenbourg *et al.,* 2004).

The reported prevalence of *E. coli* O157:H7 in cattle faeces varies widely depending on location and study (Table 3). The typical pattern of shedding in a herd is sporadic with epidemic periods of shedding interspersed with periods of non-shedding. In addition, it is usual that only a small number of animals in the herd are shedders. These epidemics occur mainly during warm weather, suggesting that environmental proliferation may play an important role in the epidemiology of *E. coli* O157:H7 (Hancock *et al.,* 1998). Cattle are generally asymptomatic carriers of *E. coli* O157 with illness only reported in young calves (Dean Nystrom *et al.,* 1997). Studies have shown that within a herd there can be a small number of persistent high shedders or super shedders and the reported concentrations of the pathogen in naturally infected cattle is between 6.7×10^5 and 1.6×10^6 CFU g^{-1} (Matthews *et al.,* 2006).

E. coli O157:H7 can potentially be deposited on the surface of beef carcasses during the slaughtering process as a result of cross contamination from the bovine hide or gut contents. The hide is now generally accepted as an important vector of faecal contamination and therefore VTEC into the abattoir. Bell (1997) reported high contamination on sites associated with opening cuts and/or subject to hide contact during hide removal. Elder *et al.* (2000) reported that faecal and hide prevalence were significantly correlated with carcass contamination.

Carcass dressing operations which may reduce the number of *E. coli* O157:H7 include trimming of visibly dirty areas of carcasses; carcass washing (hot water); steam pasteurisation or treatment with decontaminants (organic acids). Carcass chilling is not likely to have any significant effect on *E. coli* prevalence or counts (McEvoy *et al.,* 2004). When the carcass is boned and trimmed into smaller cuts, the concentration of *E. coli* O157:H7 should not increase if the chill conditions are well-controlled but cross contamination may occur to other cuts and surfaces with distribution of the pathogen throughout the ground meat. The survival characteristics of *E. coli* O157:H7 are generally similar to most other *E. coli* strains. Storage temperature, pH, water activity and salt content are the most important factors in relation to the survival and or growth of the pathogen in the food environment. The pathogen survives at food freezing temperatures ($^{-1}$8°C). *E. coli* O157:H7 strains also show acid tolerance at the extreme range for *E. coli,* are capable of surviving at a pH of 2.5 (Waterman & Small, 1996) and as such may pose problems in ready-to-eat low pH fermented meats.

During distribution and storage, retail display etc., failure to maintain chill temperatures may allow growth of the pathogen. Improper handling of unpackaged meat or leakage from wrapped packages may also lead to cross contamination. Studies on beef and beef products in a range of countries at the retail stage have shown *E. coli* O157:H7 to be present in 0.43 to 5.22 % beef/beef products (Table 4). Epidemiologic evidence in outbreaks of *E. coli* O157:H7 attributed to beef, continue to be associated with consumers/service sectors who do not understand the risks of handling raw meat and have inadequate hygiene handling practices and undercook meat. A 1996 US survey indicated that 19.7% of the population consumed pink (undercooked) hamburgers at some time during the previous 12 months (CDC, 1998).

To strategically manage the risk posed by *E. coli* O157:H7 in ground/minced beef, a number of QMRA have been developed for *E. coli* O157:H7 in USA, Canada, Australia and The Netherlands (Cassin *et al.,* 1998; Lammerding *et al.,* 1999; Ebel *et al.,* 2004; Nauta *et al.,* 2001).

Table 2. Summary of selected outbreaks of *E. coli* O157:H7 attributed to contaminated beef

Location	Year	Beef product and mode of transmission	Number of Cases	Number of HUS (deaths)	Reference
France	2005	Frozen branded beefburgers at retail outlets	26	13 (0)	Anon., 2005
Japan	2005	Beef offal served in a restaurant	7	0 (0)	Maruzumi *et al.*, 2005
USA	2004	Beef tacos at fast-food restaurant	13	3 (0)	Jay *et al.*, 2004
USA	2003	Frozen steak : blade tenderised and injected with marinade	12	1 (0)	Laine *et al.*, 2005
Japan	2002	Grilled beef	28	0 (0)	Tsuji *et al.*, 2002
USA	2002	Ground beef at retail outlets (multi-state)	28	5 (0)	Anon., 2000
USA	1995	Roast beef at catered banquet	61	0 (0)	Rodrigue *et al.*, 1995
USA	1992-93	Undercooked beefburgers in fast-food outlets	501	45 (3)	Bell *et al.*, 1994

Table 3. Summary of studies on prevalence of *E. coli* O157:H7 in cattle faeces

Country	Sample Year	Type of Cattle	Number positive (%)	Reference
Spain	1993 - 1994	Beef / dairy cattle on farms	1/686 (0.1%)	Blanco *et al.*, 1997
Spain	1998 - 1999	Feedlot cattle	55/471 (12%)	Blanco *et al.*, 2000
Ireland	1998 - 1999	Faeces of slaughtered cattle	6/250 (2.4%)	McEvoy *et al.*, 2003
England/Wales	1999	Dairy, suckler and fattening herds	219/4,663 (4.7%)	Paiba *et al.*, 2003
Netherlands	1996	Dairy cattle on farms	75/1,152 (7%)	Heuvelink *et al.*, 1998
Norway	1995	Heifers and milking cows	6/1,970 (0.3%)	Vold *et al.*, 1998
Slovenia	1996 - 1997	Slaughtered cattle	2/250 (0.8%)	Andlovic and Marinsek, 1997
USA	2002	Cull dairy cattle	21/1,026 (2.1%)	Dodson and LeJeune, 2005
USA	2001	Feedlot cattle	1087/10,662 (10.2%)	Sargeant *et al.*, 2003
Canada	1992 - 1993	Dairy cattle	12/1,478 (0.8%)	Wilson *et al.*, 1996
Switzerland	2005	Dairy cattle	23/500 (4.6%)	Kuhnert *et al.*, 2005
Japan	1992 - 1994	Slaughtered cattle	7/387(2%)	Miyao *et al.*, 1998

Table 4. Prevalence of *E. coli* O157:H7 in beef mince /beef products

Country	Sample year(s)	Beef type	Number positive (%)	Reference
Ireland	2001-02	Beef mince and burgers	43/1,500 (2.8%)	Cagney *et al.,* 2004
Italy	2000-01	Minced beef	4/931 (0.43%)	Conedera *et al.,* 2004
U.K.	1996-97	Beef products	36/3,126 (1.1%)	Chapman *et al.,* 2000
Switzerland	2000	Beef mince	5/211 (2.3%)	Fantelli & Stephan, 2001
Argentina	2000	Beef mince	6/160 (3.8%)	Chinen *et al.,* 2001
USA	2000-03	Ground beef	189/26521 (0.71%)	Naugle *et al.,* (2005)
Botswana	2002-03	Beef cubes, minced beef, beef sausages	7/134 (5.22%) 5/133 (3.76%) 3/133 (2.26%)	Magwira *et al.,* 2005

4.2 Exposure assessment

The exposure assessment model was broken into three modules covering
module 1) slaughter process culminating in the production of boxed beef trimmings
module 2) mincing of beef, beefburger formation and retail distribution
module 3) domestic storage, cooking and consumption.

In order to validate the exposure assessment model, key outputs at end of module 1 (prevalence and concentration of *E.coli* O157: H7 in beef trimmings) and end of module 2 (prevalence and concentration of *E.coli* O157: H7 beef products at retail) were microbiologically examined to determine the prevalence and concentration of the pathogen and compared against the model outputs.

4.2.1 Data inputs

4.2.1.1 Microbiological Data

It was recognised that there were large gaps in the data on *E. coli* O157 in the Irish beef chain which would be essential to develop the model (data on numbers/ prevalence of *E. coli* O157:H7 on hides of animals presented for slaughter in Irish abattoirs) and to validate the outputs of the model (data on numbers/ prevalence of *E. coli* O157:H7 on beef trimmings, and on beef products at retail in the Republic of Ireland) for Irish-produced minced beef. Substantial research was conducted to fill in data gaps as outlined below.

4.2.1.1.1 Microbiological methods (O'Brien et al., 2005b)

It is well recognised that some of the error in a microbiological risk assessment is related to the microbiological data on which it is based. Equally, the microbiological data is only as good as the method used to generate the data. It is important in risk assessment to be able to attribute the error related to the methods used in generating the data. In this study, experiments were carried out to assess the detection limits for cultural methods used to enumerate

and recover *E. coli* O157:H7 from beef mince and bovine hide (O'Brien *et al.,* 2005b). Minced beef and bovine hide were inoculated with varying concentrations (log_{10}1.58-2.58 CFU g^{-1} and log_{10} 2.20-4.49 CFU 100 cm^2 respectively) of *E. coli* O157:H7 and recovered using an enumeration (direct plate method) or presence /absence method (enrichment / immunomagnetic separation) and then plated onto Sorbitol McConkey Agar (SMAC) or SMAC-Cefixime Tellurite (CT) in both cases. The direct plate method detected the pathogen consistently from minced beef samples with an average recovery of 69.2 - 91.2 %. From faecal material on the bovine hide, the recovery of the pathogen ranged from 1.80 – 64.5 % with fresh faeces, depending on the inoculum, while from dried faeces on hide, the results ranged from no recovery to 25 %. Enrichment/ immunomagnetic separation (IMS) recovered *E. coli* O157:H7 at all inoculum levels tested in beef mince, while the pathogen was only detected consistently at an average inoculum level of log_{10} 2.20 CFU 100 cm^2 from fresh faeces and log_{10} 4.49 cfu 100 cm^2 from dried faeces on bovine hide. These errors (underestimation of counts) associated with the method were included in the developed model.

Microbiological input data (O'Brien et al., 2005a; Carney et al., 2006; Cagney et al., 2004)
Bovine hides samples (n = 1500) were collected over a 17 month period (30 samples per week) by sponge swabbing areas of approximately 122 cm^2 of the bovine rump of slaughtered cattle at an early stage of carcass processing (first legging). Sponge samples (n= 1500) were stomached in Buffered Peptone Water supplemented with novobiocin and either directly plated on SMAC-CT agar or enriched for 24 h, extracted by immunomagnetic separation (IMS), and plated onto SMAC-CT. Overall, *E. coli* O157 was recovered from 109 samples (7.3 %) at concentrations ranging from <log_{10} 0.13 to log_{10}4.24 CFU 100 cm^2 (Figure 1). PCR analysis revealed a wide diversity in genetic profiles among recovered isolates of *Escherichia coli* O157 (Table 5)

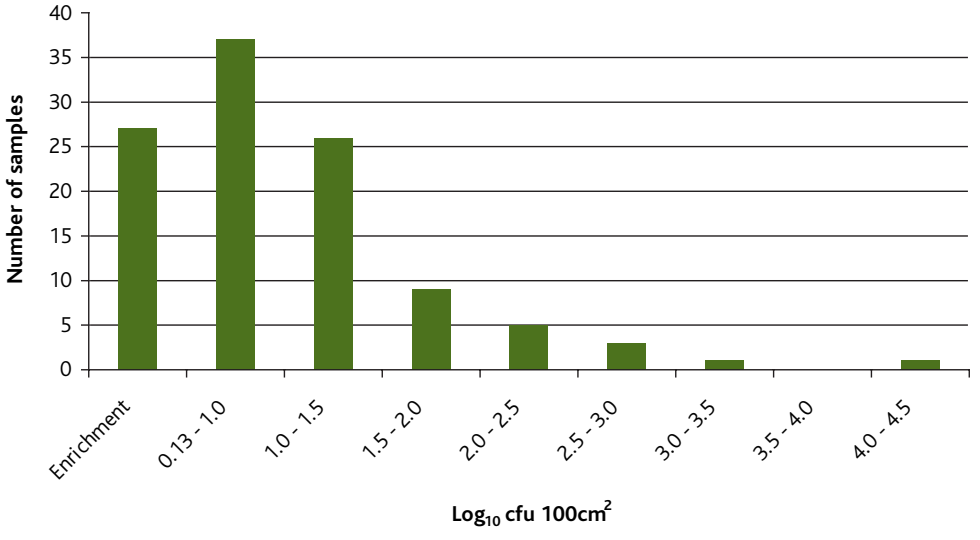


Figure 1. *E. coli* O157 counts (log_{10} cfu 100 cm^2) on bovine hide (n = 109)

Note: Enrichment indicates the organism was present but numbers were below the detection limit of the enumeration method

Table 5. Virulence profile for *E. coli* O157 isolates from bovine hide (n=109)

		Virulence Genes					
No. isolates	**(%) total isolates**	***hlyA***	***eaeA***	***vt1***	***vt2***	***rfbO157***	***fliCh7***
62	56.9	+	+	-	+	+	+
29	26.6	+	+	-	+	+	-
9	8.3	-	-	-	-	+	+
5	4.6	+	+	+	+	+	+
2	1.8	+	+	-	-	+	+
1	0.9	+	+	+	+	+	-
1	0.9	-	-	+	-	+	-

+ = Positive - = Negative

Microbiological surveillance data used to validate the model (Carney et al., 2006, Cagney et al., 2004)
The prevalence and number of *E. coli* O157 was assessed on samples of beef trimmings (fragments of beef derived during carcass deboning) (n=1351), beef carcasses (n= 132) and bovine head meat (n=132) in a beef slaughter plant in Ireland over an 18 month period (2002 to 2003) (Carney *et al.,* 2006). Samples were examined for the presence of *E. coli* O157:H7/H- by direct plating on SMAC-CT and by enrichment / immunomagnetic separation (IMS) with plating of recovered immunobeads onto SMAC-CT agar. Presumptive *E. coli* O157:H7/H- isolates were confirmed by PCR targeting a range genes i.e. *vt1, vt2, eaeA, hlyA* and the O-antigen encoding region of the pO157 gene. *E. coli* O157:H7/H- was recovered from 2.36 % (32/1351) of beef trimming samples at concentrations ranging from $<\log_{10}$ 0.70 to $\log_{10}$ 1.61 CFU g^{-1}. The virulence profile is shown in Table 6.

E. coli O157:H7/H- was recovered from 3.0 % (4/132) of carcass samples, at concentrations ranging from $<\log_{10}$ 0.70 to $\log_{10}$ 1.41 CFU g^{-1}. All of the carcass isolates contained the *eaeA, hylA* and *fliCh7* genes. One isolate contained both the *vt1* and *vt2* genes, 2 contained the *vt1* gene only and 1 contained the *vt2* gene only.

E. coli O157:H7/H- was recovered from 3.0 % (3/100) of head meat samples, at concentrations of $\log_{10}$ 0.7 to $\log_{10}$ 1.0 cfu g^{-1}. All of the head meat isolates contained the *eaeA, hylA, fliCh7* and *vt2* genes. No head meat isolates contained the *vt1* gene.

At study on prevalence and numbers of *E. coli* O157: H7 in minced beef (unpackaged or packaged) and beefburgers (frozen, fresh and unpackaged or packaged) at retail was carried out over a period of 12 months in the Republic of Ireland funded by the Food Safety Authority of Ireland (Cagney *et al.,* 2004). A total of 1533 products were tested with approximately 15 products collected from each of the 26 counties every 3 months. Beef mince and burgers were collected from both supermarkets and butcher shop outlets. A standard analysis was conducted by sample enrichment, IMS extraction and plating onto SMAC agar with confirmation by PCR. The results showed that 43 retail

Table 6. Virulence profile of *E. coli* O157 isolates recovered from beef trimming 90 vl* (n=14) or 70 vl (n=18), carcasses (n= 4) and head meat (n=3)

		Virulence Genes				
Source	**No. of Isolates**	***hlyA***	***eaeA***	***vt1***	***vt2***	***H7***
Trimming	7	+	+	+	-	+
Trimming	5	+	+	-	+	+
Trimming	1	+	+	-	+	-
Trimming	1	-	-	-	-	+
Trimming	9	+	+	-	+	+
Trimming	6	+	+	+	-	+
Trimming	2	+	+	+	+	+
Trimming	1	+	+	+	-	-
Carcass	1	+	+	+	+	+
Carcass	2	+	+	+	-	+ _
Carcass	1	+	+	-	+	+
Head meat	3	+	+	-	+	+

*vl = visual leanness, + = Positive, - = Negative

beef products (2.8 %) contained *E.coli* O157:H7. The number of *E.coli* O157: H7 in 21 of these samples ranged from $\log_{10}$0.51 to $\log_{10}$ 4.03 CFU g^{-1} (i.e. 3 to 10,700 bacteria per gram) while in the remaining 22, the pathogen was detectable by enrichment only. There was a seasonal effect observed with 33 of 43 positive samples detected in January (n = 8), April /May (n = 20) and August (n = 5) and the remaining 10 positive samples detected over the other 8 months. Of the beef products testing positive, 32 were purchased from supermarkets and 11 from butcher shops. *E.coli* O157: H7 was recovered from 2.8% (13 / 457) fresh packaged mince and from 1.88 % (3 / 160) of fresh unpackaged burgers purchased from butcher shops. Of the 43 isolates recovered, 41 contained the virulence genes, *vt1, vt2, eaeA* and *hlyA* genes while the remaining 2 isolates contained only one of the *vt* producing genes (*vt 1*or *vt2*).

The overall microbiological results used as input and to validate the model are summarised in Table 7 below.

Table 7. Summary of prevalence and numbers of *E. coli* O157:H7 at various sample points along the beef chain in Ireland used to develop (hide, carcass, faeces, rumen contents) or validate (trimmings, retail mince/burgers) the QMRA model

Sample type	Sample number	Number positive (%)	Numbers present (log_{10} CFU)	Reference
Bovine faeces	250	6 (2.4%)	-	McEvoy *et al.,* 2003
Rumen contents	250	2 (0.8)	-	McEvoy *et al.,* 2003
Bovine hide	1500	109 (7.3)	0.13 - 4.24 /100 cm^2	O'Brien *et al.,* 2005
Beef carcasses	250	8 (3.2)	-	McEvoy *et al.,* 2003
Beef carcasses	132	4 (3.0)	0.70 - 1.41 g^{-1}	Carney *et al.,* 2006
Head meat	100	3 (3.0)	0.70 -1.00 g^{-1}	O'Brien *et al.,* 2005
Beef trimmings	1351	32 (2.36)	0.70 - 1.61 g^{-1}	O'Brien *et al.,* 2005
Retail beef mince/burgers	1533	43 (2.8)	0.52 – 4.03 g^{-1}	Cagney *et al.,* 2004

4.2.1.2 Consumer handling and cooking practices

One of the most difficult parts of the exposure assessment is the final phase of the chain, from consumer purchase of beef at retail, through domestic storage and preparation. This part of the chain is not regulated and it is difficult to conduct microbiological sampling or to predict the fate of the pathogen as exact storage and preparation methods are unknown and may be highly variable. In order to get an estimate and make assumptions about the fate of *E.coli* O157:H7 in this part of the beef chain in Ireland, the Market Research Bureau Ireland (MRBI) was commissioned to conduct a specially-designed questionnaire (multiple-choice questions) which was administered by telephone survey to 500 people (covered gender, age and socio – economic diversity) to beef consumers who were the main purchaser of beef in the household.

The study generated on consumer habits regarding the purchase and preparation of beef mince is complied in a report (Mahon *et al.,* 2003). To summarise, a high percentage of consumers (59 %) purchased their beef early during their shopping trip and the majority of respondents (83%) did not use cooler bags for their chilled or frozen products. The majority of respondents (97%) returned home within two hours of shopping and refrigerated or froze the meat immediately. Approximately 44% of consumers stored their mince beef or burgers on a middle or high shelf in the fridge, or uncovered, thus contributing to the risk of cross contamination from meat drip to ready-to-eat foods on a lower shelf or from contact with adjacent food. The majority (96%) consumed fresh mince beef within two days of purchase.

Regarding the handling and preparation of food, the majority of respondents (58%) thawed their meat at room temperature as opposed to the refrigerator or microwave. A significantly higher proportion of females (94%) cooked their burgers more thoroughly than males (67%). 87% of consumers prepare hamburgers well done, 12% medium and 1% cooked them rare. Although differences were noted between food handling practices across gender and the different age groups, differences were not statistically significant.

4.2.1.4. Beef consumption patterns

Data on consumption of beef in Ireland was collated from the Survey of Lifestyle, Attitudes and Nutrition (S.L.A.N) database (National University of Ireland, Galway) and the Irish Universities Nutritional Alliance database (University College Cork) (www.iuna.net). The latter database was set up based on a nutritional survey conducted between 1997 and 1999 to provide up-to-date information on habitual food and drink consumption in Irish adults. The executive summary and the complete survey report are available on the website www.iuna.net.

The purpose of the analysis in this study was to estimate minced meat and beefburger intakes in the adult population as a whole, for men and women of different ages, taking into account seasonality and location where beef consumed. Emphasis was placed on describing the quantity of minced meat/burgers consumed on average per eating occasion. The data is included in a consumption report (Mahon *et al.,* 2003).

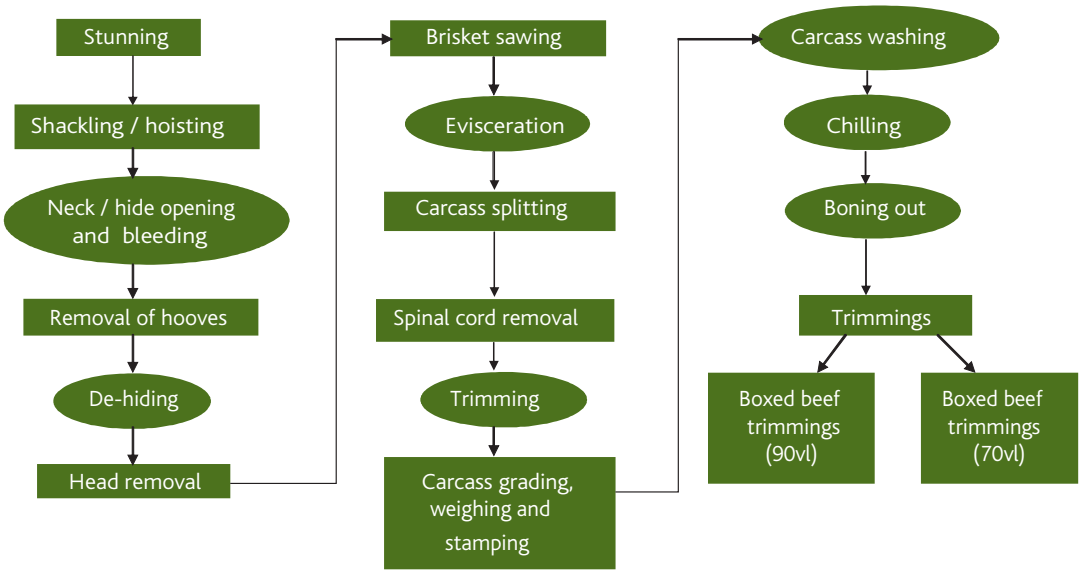
4.2.2 Exposure Assessment Model

The exposure assessment model was developed in a spreadsheet (Microsoft Excel 97) with the @Risk add-on package (Palisade Software, Newfield, N.Y.) and the simulation was performed using Latin Hypercube sampling. The exposure model developed was a second order model for both the production of beef trimmings and the production of retail beefburgers. Distributions were used to represent data rather than point estimates. Bayesian analysis was used to reduce the uncertainty around the predicted risk estimate. Variability (natural randomness in system e.g. number of animals slaughtered) and uncertainty (lack of knowledge e.g prevalence) were incorporated into the model.

4.2.2.1 Module 1: Slaughter module

The slaughter module simulated the potential contamination of carcasses in the abattoir with *E. coli* O157:H7, taking account of the impact various slaughtering processes may have on the distribution of the bacteria. A flow diagram of the slaughter process is shown in Figure 2 and the initiating parameters for the model are shown in Table 8. The data inputs for the model included the prevalence and number of *E.coli* O157:H7 on the hide and in the rumen contents of animals presented for slaughter as described above (O'Brien *et al.,* 2005a and McEvoy *et al.,* 2003, Table 7). The initial number of bacteria on animal hides was modelled by fitting a second-order continuous non-parametric distribution to a data set using methodology as detailed by Vose (2001). The distributions are shown in Figures 3, 4 and 5.

Figure 2: Flow diagram of slaughter module Note: (vl = visual lean meat)



The model assumed that contaminated hide and gut contents were the main vector for cross contamination to carcasses at the hide removal stage and at evisceration cross contamination factors from hide to carcass and from rumen contents to carcass were created (Figure 6, 7) based on Irish surveillance data for the pathogen on bovine hide (O'Brien *et al.,* 2005a) and on beef carcasses (McEvoy *et al.,* 2003; Carney *et al.,* 2006).

Operations during the slaughter process (Figure 2) may impact on the number of *E. coli* O157:H7 (CFU /cm^2) on contaminated carcasses. Trimming visibly dirty parts of carcass can significantly reduce bacterial counts on carcasses (Gill *et al.,* 1996). Carcass washing with potable water at a temperature of 35 - 40°C reportedly has no significant change on generic *E. coli* counts (McEvoy *et al.,* 2004) but there is also evidence that some washing procedures may redistribute bacteria to other parts of the carcass (McEvoy *et al.,* 2003; Bell, 1997). The overall reduction in counts as a result of these operations was modelled using a triangular distribution with a minimum reduction of zero and uncertainty about the mean was modelled using a uniform distribution (uniform distribution (0.3, 0.7) and uncertain maximum value (uniform distribution (0.8, 1.2).

Table 8. Initiating parameters distributions and inputs

Parameter	Symbol	Distribution/model	Category	Units
Animals slaughtered in a day	A_s	350	Fixed value	animals
Prevalence in the gut	P_g	beta(3,247)	Uncertainty	prevalence
Prevalence on the hide	P_h	beta(110,1390)	Uncertainty	prevalence
Test sensitivity	Tse	Derived by bayesian Inference (see text)	Uncertainty	factor
True Prevalence	P_{ht}	Ph/Tse		prevalence
Number of animals with *E. coli* in gut	A_g	binomial(A_s,P_g)	Variability	animals
Number of animals with *E. coli* on hide	A_h	binomial(A_s,P_{ht})	Uncertainty	animals
Transfer ratio between hide and carcass	TR	beta(4,32)/beta(110,1390)	Uncertainty	prevalence
Prob of infected carcass	P_c	1-(1-Pht × (TR/A_s))^A_s	Calculation	ratio
Number of contaminated carcasses	C_c	binomial(A_s,P_c)	Variability	animals
Total surface area of animal	TSA	32000	Fixed value	cm^2
Mass of a combi bin	M	2700	Fixed	g

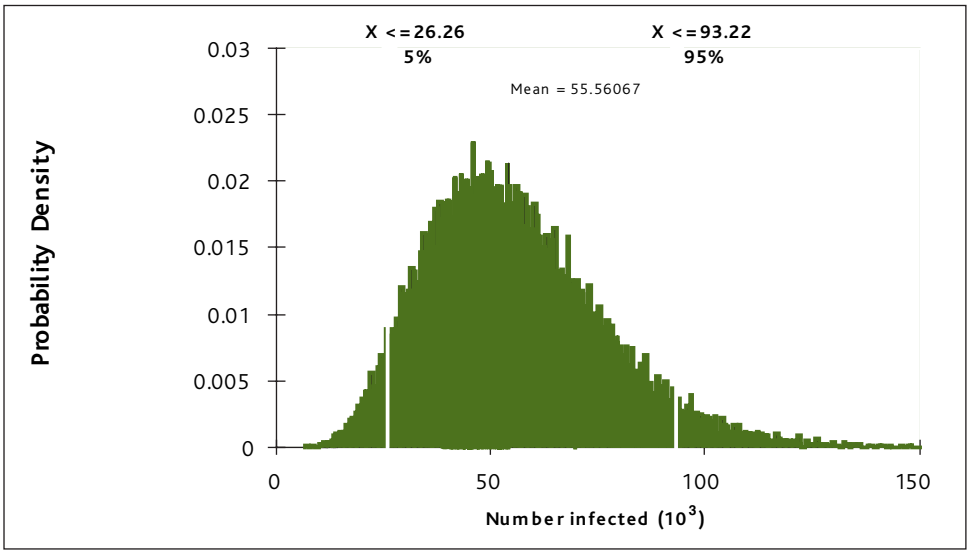


Figure 3. Distribution curve for number of animals with *E.coli* O157:H7 on hide (Distribution N_h~ Poisson(S,P_h)

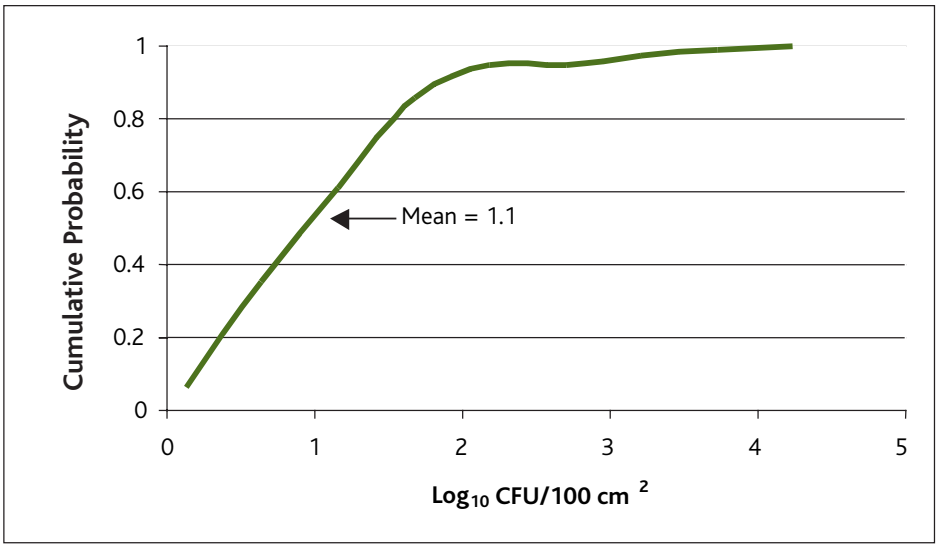


Figure 4: Cumulative distribution curve for counts of *E.coli* O157:H7 on hide (Distribution N_h~ Poisson(S,P_h)

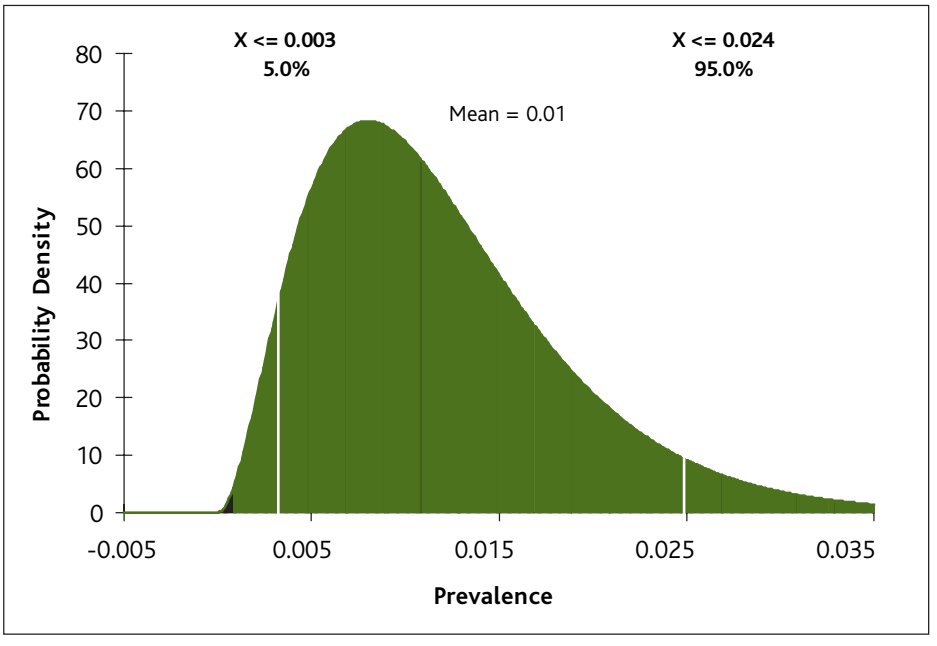


Figure 5. Distribution curve for prevalence (Pi) *E. coli* O157:H7 in rumen contents (n= 50 animals) (Distribution ~ Beta (2+1, 250-2+1)

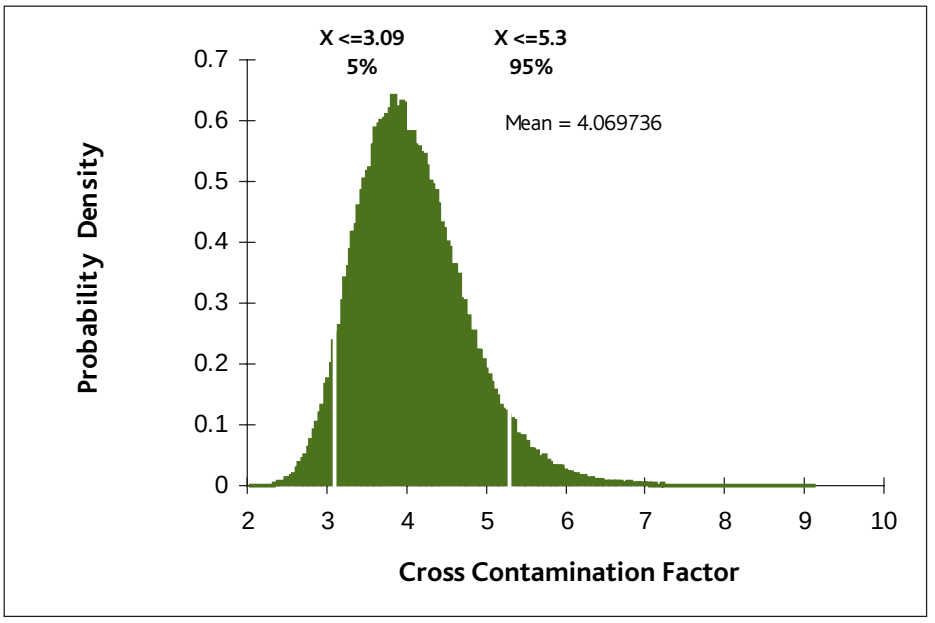


Figure 6. Distribution curve for *E .coli* O157:H7 cross contamination factor from hide to carcass (Distribution ~ Beta(148+1,341^{-1}48+1)/Beta(38+1,355-38+1)

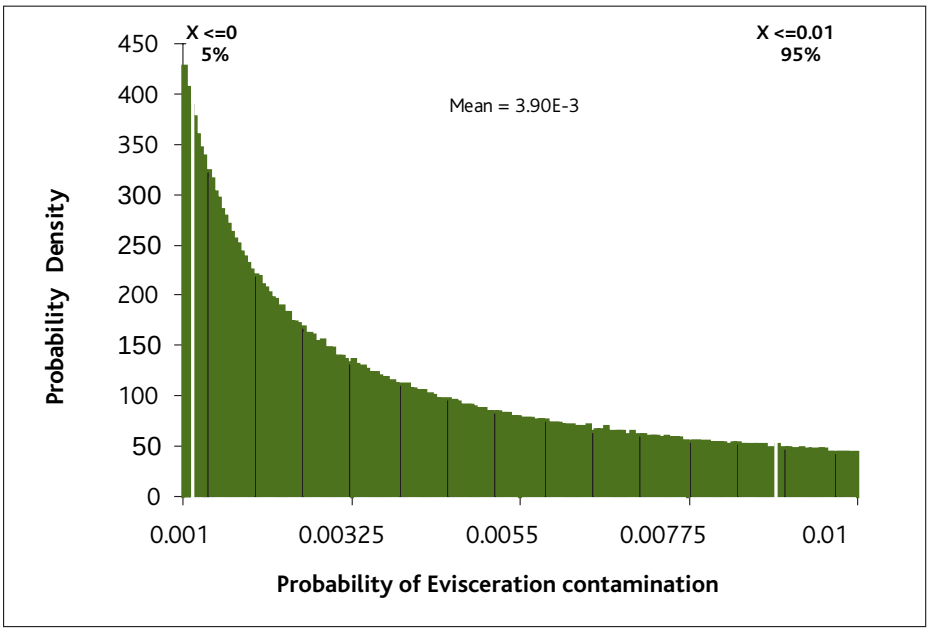


Figure 7. Distribution curve for *E. coli* O157:H7 cross contamination factor from gut contents to carcass during evisceration (Distribution curve 10^-Uniform(2,3)

Chilling can have an impact on bacterial numbers and McEvoy *et al.* (2003) reported a reduction in prevalence on carcasses after chilling for 24 hours. Similarly Gill *et al.* (1996) reported a reduction in coliforms and *E. coli* on carcasses following cooling processes of between 0.5 $\log_{10}$ units and 2 $\log_{10}$ units. Sheridan (2000) noted that carcass contamination may increase, decrease or remain unchanged following chilling and depended on parameters such as temperature, air speed and relative humidity. In the model, growth or decline is assumed to occur only on carcasses that are contaminated on entering the chiller. The change in counts on contaminated carcasses during chilling was modelled in this study using a normal distribution with an uncertain mean ranging from -0.5 to 0.5 $\log_{10}$ and a standard deviation of 1 (Figure 8).

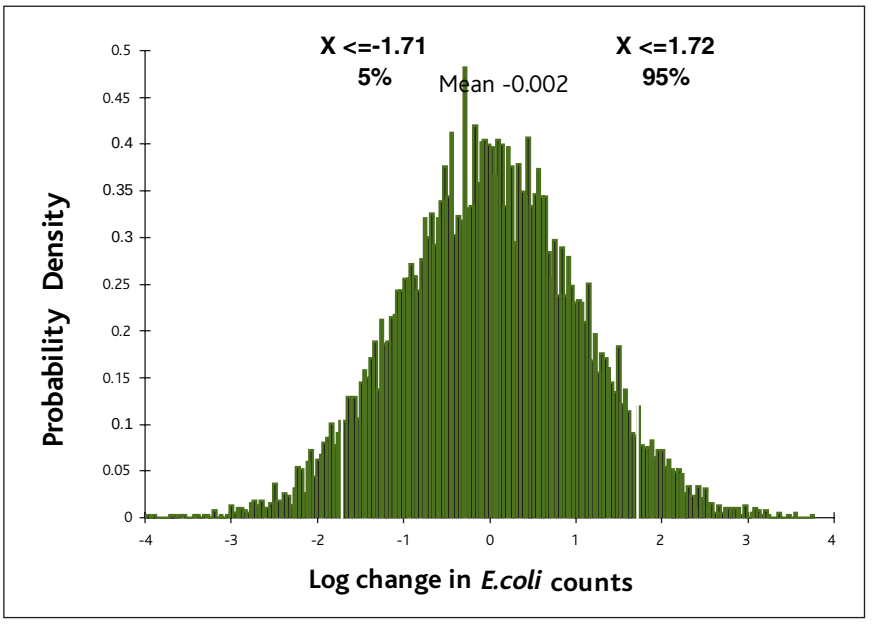


Figure 8. Distribution curve for reduction in *E.coli* O157:H7 from chilling Normal(Uniform(-0.5, 0.5),1)

The potential increase in numbers of *E. coli* O157:H7 during boning was assumed to minimal. McEvoy *et al.* (2004) reported a mean increase of approximately 0.33 $\log_{10}$ during this operation. Growth was modelled using a triangular distribution with a minimum of 0 logs of growth, most likely value of 0.33 and a maximum growth of 2 logs of growth, in line with published literature. A summary of model inputs and distributions for carcass simulations is shown in Table 9.

Table 9. Model inputs and distributions for carcass simulation

Parameter	Symbol	Distribution/model	Category	Units
Flag for Carcass infected	F_c	binomial(1,P_c)	Variability	FLAG
Flag for Infected gut	F_g	binomial(1,P_g)	Variability	FLAG
Initial number on hide	I_h	Second order continuous non parametric distribuion fitted to data	Variability and Uncertainty (see text)	$\log_{10}$ cfu/100 cm^2
Factor increase for test se	F_i	Uniform(0.5,1.5)	Uncertainty	
True number on hide	I_{ht}	LOG(10^(I_h+F_i)/100)	Calculation	$\log_{10}$ cfu/cm^2
log factor for decrease from hide to carcass.	R	Cumulative distribution fitted to data	Variability	factor
Initial number introduced during dehiding (on carcass)	I_c	I_{ht}-R	Variability	$\log_{10}$ cfu/cm^2
Total contaminated surface area	A	10^(Triang(LOG(30), LOG(300), LOG(3000)))	Variability	cm^2
Total organisms on contam carcass at dehiding	$B_{c,h}$	LOG((10^I_c) × A)	Variability	$\log_{10}$ CFU/carcass
Most likely reduction due to decontam	$D1_{mm}$	Uniform(0.3, 0.7)	Uncertainty	logs
Maximium reduction due to decontam	$D1_{max}$	Uniform(0.8, 1.2)	Uncertainty	logs
Reduction due to decontamination	D1	Triang(0,$D1_{mm}$,$D1_{max}$)	Variability	logs
Probability of contam at evis	E	10^-(Uniform(2,3))	Uncertainty	probability
Cut at evis flag	E_f	Binomial(1,E)	Variability	FLAG
Total organisms on contam carcass due to evis	$B_{c,e}$	LOG((10^I_c) × A)	Calculation	$\log_{10}$CFU/carcass
Most likely decontamination	$D2_{mm}$	Uniform(0.3, 0.7)	Uncertainty	logs
Most likely max decontamination	$D2_{max}$	Uniform(0.8, 1.2)	Uncertainty	logs
Decontamination (pasteurise)	D2	Triang(0,$D2_{mm}$,$D2_{max}$)	Variability	logs
Change in numbers during Chilling mean	CR_m	Uniform(-0.5, 0.5)	Uncertainty	logs
Stdev of numbers during chill	CR_{stdev}	1	fixed value	logs
Change in numbers during Chilling	CR	Normal(CR_m, CR_{stdev})	Variability	logs
Change in numbers in boning hall (growth)	G	Triang(0, 0.33, 2)	Variability	logs
Number of organisms per carcass after boning out	N_d	LOG(10^$B_{c,h}$ × 10^-D_1 × 10^-D_2 × 10^CR × 10^G)	Calculation	Log number of *E.coli* organisms
Number of organisms per carcass after boning out	N_e	LOG(10^$_{Bc,e}$ × 10^-D_1 × 10^-D_2 × 10^GR × 10^G)	Calculation	Log number of *E.coli* organisms
Number of organisms per carcass after boning out (contam at evis	N_f	LOG(((10^$B_{c,h}$ × 10^-D_1)+10^$B_{c,e}$) × 10^-D_2 × 10^CR × 10^G)	Calculation	Log number of *E.coli* organisms
Density on carcass (hide only)	n_d	LOG((10^N_d)/A)	Calculation	log organisms/cm^2
Density on carcass (evis only)	n_e	LOG((10^N_e)/A)	Calculation	log organisms/cm^2
Density on carcass (both)	n_b	LOG((10^N_f)/A)	Calculation	log organisms/cm^2

Beef trimmings

A factor for estimating the transfer of contamination from carcass to trim was set in the model, taking account of the surface area of carcass which was contaminated, the surface area of the trim, the weight of the trim and the number of trim in a box (27 kg). The data inputs for modelling the prevalence and number of *E. coli* O157:H7 are shown in Table 10.

Table 10. Summary of inputs for beef trimmings simulation

Parameter	Symbol	Distribution/model	Category	Units
Mean Mass of trimming (70VL)	M_m	Bootstrap on data set (see text)	Uncertainty	g
Standard deviation for mass trimming	M_{stdev}	Bootstrap on data set (see text)	Uncertainty	g
Mass of trimming (70VL)	$M_{trim,i}$	Normal(M_m, M_{stdev}, Truncate(2000,)	Variability	g
Total scrap wt, Mass of trim a carcass contributes to a box	$M_{c,a}$	$\sum_{i=1}^{N_{tc}} M_{trim,i}$		g
Cumulative mass in box	Cm	$\sum_{a=1}^{x} M_{ca}$		g
Number of trimming per carcass(70VL)	Nc	Triang(5, 6,7)	Variability	trimmings
Number of trimmings a carcass contributes to a box	Ntc	Uniform(4,Nc)	Variability	trimmings
Surface area of trim	$Sa_{trim,i}$	Uniform(0.1, 0.5)	Uncertainty	cm^2/g
Total cm^2 placed in a box by animal	$A_{trim,i}$	$M_{trim,i} \times Sa_{trim,i}$		cm^2
Expected number of contaminated cm^2 per trimming	$A_{contam,i}$	$Poisson\left(A_{trim,i} \times \left[\frac{A - \sum_{x=0}^{i} A_{contam,x}}{TSA - \sum_{x=0}^{i} A_{trim,x}}\right]\right)$	Variability	cm^2
Expected number of contaminated cm^2 a carcass contributes to a box	$Acc_{,a}$	$\sum_{i=o}^{x} A_{contam,i}$	Calculation	cm^2
E. coli numbers, hide only	$C_{trim,i}$	Poisson($10^{nd} \times A_{contam,i}$)	Variability	CFU
E. coli numbers, gut only	$C_{trim,i}$	Poisson($10^{ne} \times A_{contam,i}$)	Variability	CFU
E.coli numbers, both	$C_{trim,i}$	Poisson($10^{nf} \times A_{contam,i}$)	Variability	CFU
Infected trimmings	It	count if (C_{trim} >0)	Calculation	trimmings
Total *E.coli* in combi		$\sum_{a=1}^{x} \sum_{i=1}^{Nt} C_{trim,a,i}$	Calculation	CFU
Mean trimmings per carcass	TC_m	From Nc distribution		trimmings
Standard deviation of trimmings per carcass	TC_{stdev}	From Nc distribution		trimmings
Total trimmings produced	T_p	Normal($A_s \times TC_m$,SQRT(A_s) × TC_{stdev})		trimmings
Probability that a contaminated carcass will produce a contaminated trim	P_c	Precedure in model, see text.		probability
Total infected trimmings	T_{ip}	binomial(Normal($C_c \times TC_m$,SQRT(C_c) × TC_{stdev}), P_c)		trimmings

Model outputs for slaughter module

The model indicated a mean simulated prevalence of *E. coli* O157:H7 on beef trimmings of 2.40 % and mean counts of -2.69 log_{10}CFU g^{-1} (distribution, Figure 9). This output was compared against a microbiological survey of the *E. coli* O157:H7 on beef trim in Irish abattoir which indicated prevalence (2.36 %) and numbers (0.7 - 1.61 log_{10} CFU g^{-1} (Figure 10). The mean values for the simulation and the survey are similar, although the simulated distribution is considerably wider, highlighting the uncertainty in the input parameters.

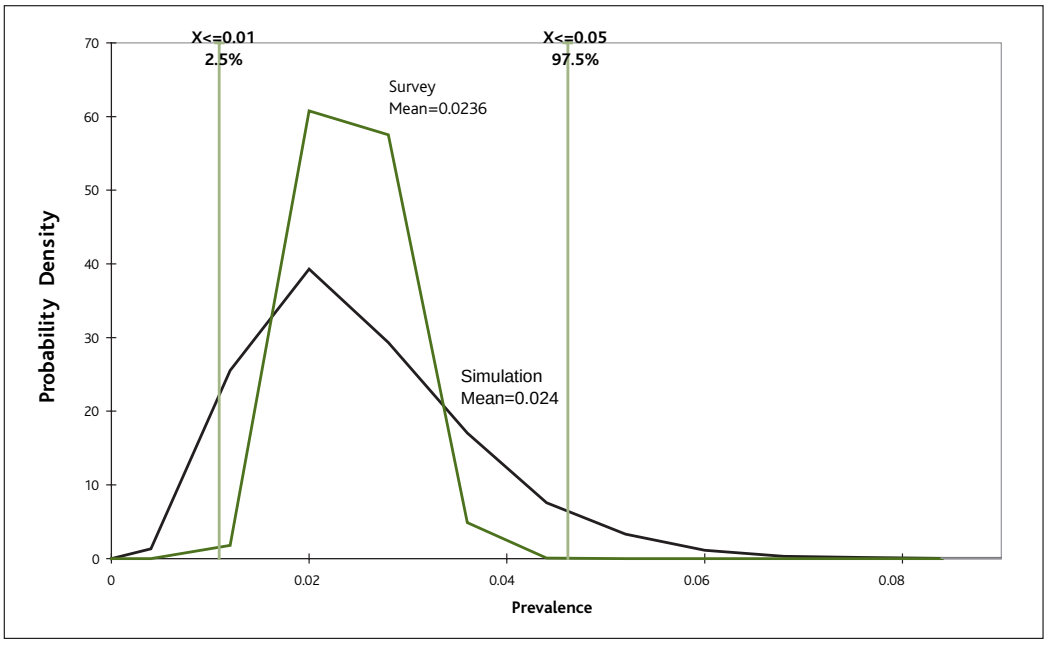


Figure 9: Model (simulation) results for the prevalence of *E. coli* O157:H7 in beef trimmings compared to microbiological survey results (including uncertainty analysis)

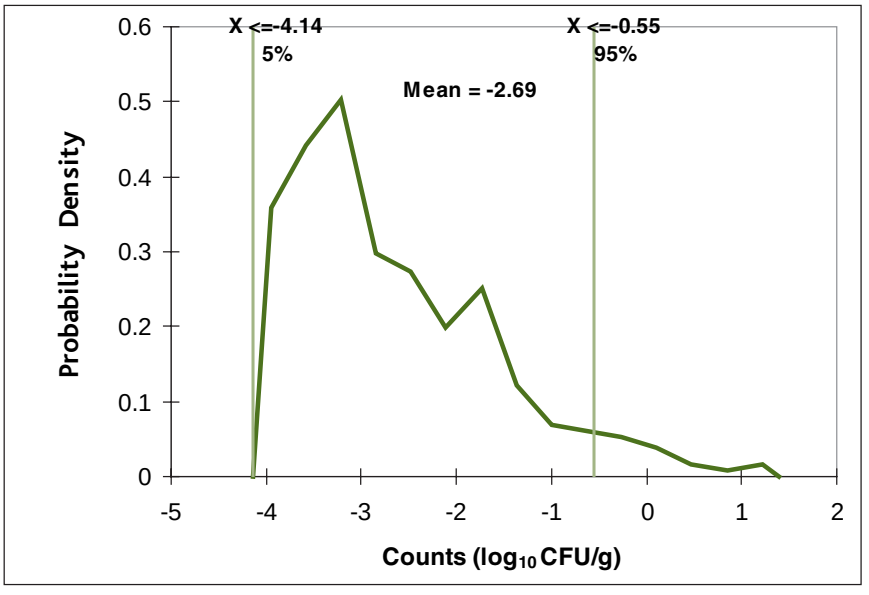


Figure 10: Distribution curve of predicted numbers (log_{10} CFU g^{-1}) of *E.coli* O157:H7 in beef trimmings.

The simulated results indicate that many of the trimmings may have very low bacterial counts with 95th percentile < Log_{10} -0.55 CFU g^{-1} (Figure 10). This is supported by the fact that very few of the *E. coli* O157:H7 positive trim samples could be enumerated using direct plate techniques. Of the ones that were enumerated, counts were between 0.05 - 0.65 log_{10} CFU/g which is within the range indicated by the model.

To determine the uncertainty parameters responsible for the wide spread of the probability distributions for both prevalence and counts, a sensitivity analysis was performed. A sensitivity analysis is a systematic evaluation of model inputs and assumptions. The parameters were ranked in accordance with the magnitude of their effect on model predictions. The sensitivity of the prevalence and counts of *E. coli* O157:H7 on contaminated trimmings to input values was measured by rank correlation

The input having greatest impact on *E. coli* prevalence (Figure 11) was the test sensitivity, followed closely by the hide carcass transfer factor and the initial hide prevalence. The analysis reveals that additional efforts are also needed to understand the processes involved in the initial transfer of *E. coli* to the carcass and to reduce or limit such a transfer. Hide prevalence was significantly correlated with carcass contamination, indicating a role for control of *E. coli* O157:H7 in live cattle

The initial count on the animal hide was the parameter having the greatest impact on count predictions in the model, highlighting the need to investigate the uncertainty about this parameter. The contaminated surface area and the transfer of the pathogen from hide to carcass also had an impact on model predictions, highlighting the requirement to better understand the dynamics of microbial transfer from hide to carcass. Other input parameters in the model had a lesser effect on model predictions.

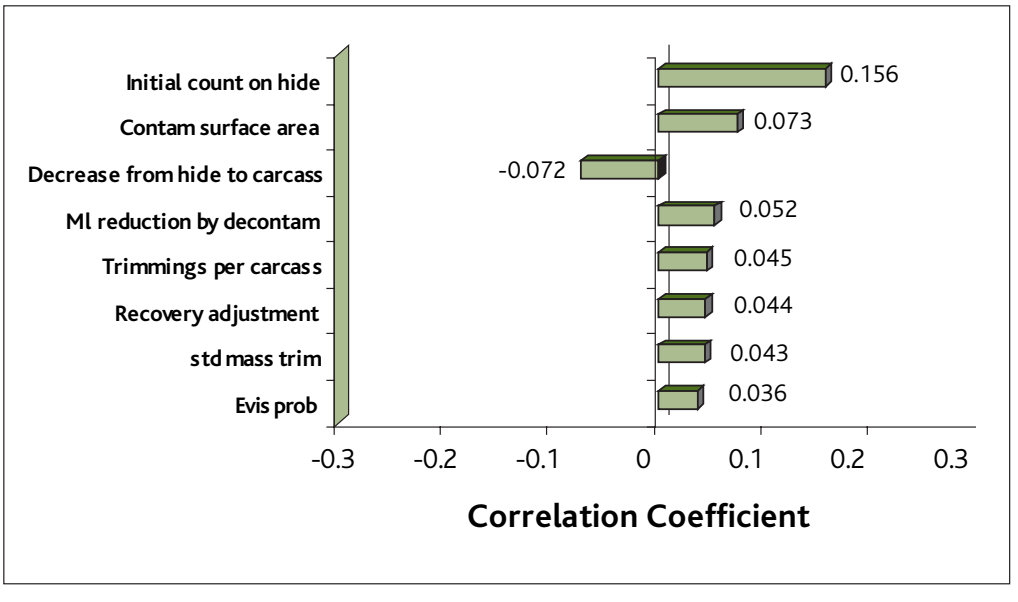


Figure 11: Sensitivity analysis on factors (uncertainty input parameters) affecting the counts of *E. coli* O157:H7 on contaminated beef trimmings

4.2.2.2 Module 2: Minced beef and burger preparation to retail

This module focused on the processing of beef trimmings coming from one or more 27.5 kg boxes of beef trimmings into products i.e. beefburgers (100g) to be sold at butchers shops or supermarkets. Figure 12 shows a flow diagram of the module.

Data inputs

The input data on prevalence and counts for *E. coli* O157:H7 in beef trimmings was the simulated output from module 1 as described above i.e 2.40 % (95th percentile range, 95% - 5.1%) and mean counts of -2.69 $\log_{10}$CFU g^{-1}). The probability of each trimming contributing to a batch of minced beef and the number of *E. coli* O157:H7 it contributes to the batch if contaminated was modelled.

The potential growth of *E. coli* O157:H7 during retail storage was modelled. Research has shown that *E. coli* O157:H7 can grow at temperatures of 7.2°C or higher (Palumbo *et al.,* 1995) so if temperature abuse of minced beef occurs during storage, *E. coli* O157:H7 growth can potentially occur. Gompertz microbial growth equations (Marks *et al.,* 1998) were used to predict the amount of *E. coli* O157:H7 growth that would occur over a given time at a given temperature in minced beef that suffered temperature abuse during retail sale. The model to describe the potential growth for *E. coli* O157:H7 in beefburger was adapted from the model employed in the FSIS / USDA risk assessment model (Ebel *et al.,* 2004) but was adapted to represent the results of the survey conducted in Ireland on typical storage times and temperatures at retail (range 7 to 16°C) (Carney, personal communication).

The effect of freezing on *E. coli* O157:H7 numbers was modelled using a distribution that represented a decline in numbers of between 0 $\log_{10}$ CFU g^{-1} and 3 $\log_{10}$ CFU g^{-1} based on literature data (USDA-FSIS, 2004). Using relevant distributions the model was used to estimate the prevalence and counts of *E. coli* O157:H7 in a 100 gram serving of fresh minced beef which represented the upper end of consumption size and exposure.

Model outputs

The distribution for the prevalence of *E. coli* O157:H7 in raw fresh and frozen burgers is shown in Figure 13. The mean simulated prevalence generated by the model in fresh burgers was 2.9%. In frozen beefburgers, the simulated

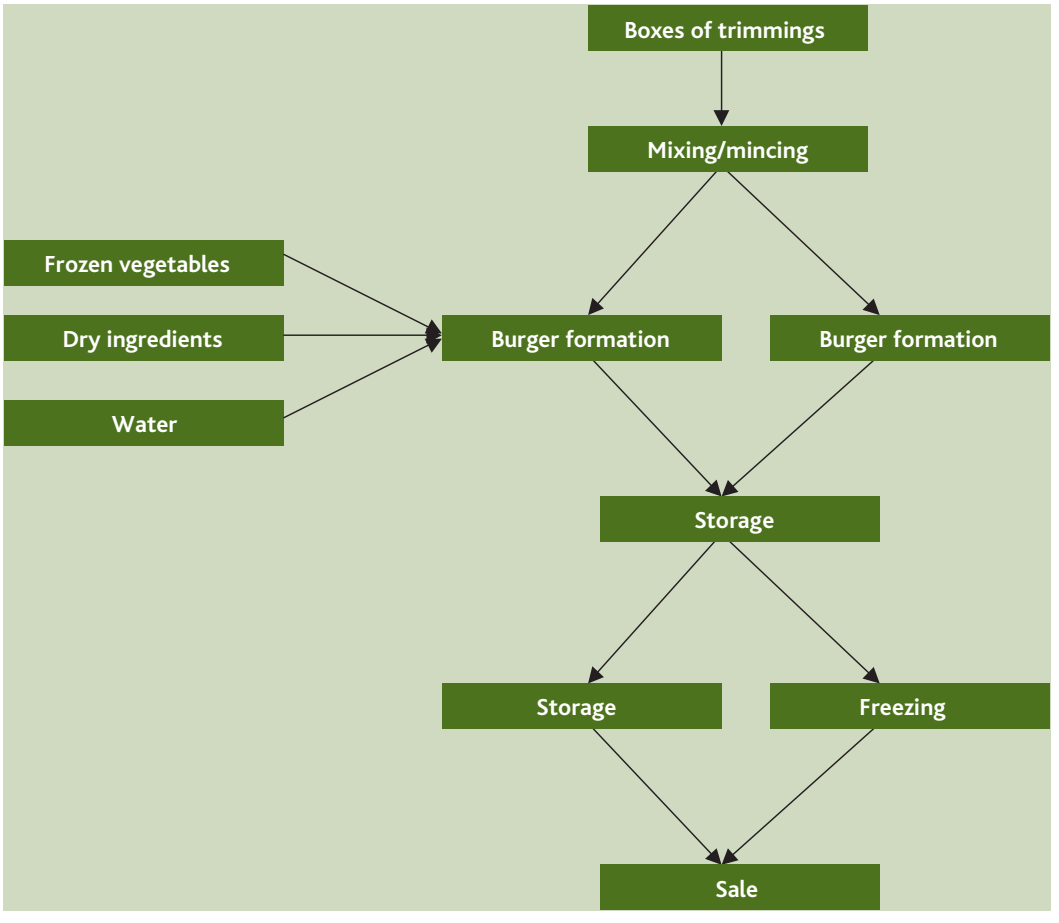


Figure 12. Flow diagram from beefburger preparation through retail distribution

distribution for prevalence had a mean of 2.2%. This is in good agreement with a survey of fresh beef on retail sale, that found 2.8% of samples were contaminated with *E. coli* O157:H7 (n=1533, 90% confidence interval of 2.2% to 3.5%)(Cagney et al., 2004). It is noticeable that the simulated prevalences have wider distributions than the survey indicating that uncertainties still remain in the model. Alternatively the result could reflect deficiencies in the results of the snap shot survey at retail (i.e. necessarily limited to set number of sample, distribution etc.).

The simulated counts of *E. coli* O157:H7 in fresh and frozen burgers is given in Figure 14. The simulated counts in frozen burgers (mean of -0.22 $\log_{10}$ CFU g^{-1}) are less than those for fresh burgers (mean of 1.96 $\log_{10}$ CFU g^{-1}), this is mainly due to the greater probability of temperature abuse of fresh burgers on retail display. The simulated counts compare favourably with results of a retail survey where counts varied from $\log_{10}$0.51– 4.03 CFU g^{-1}.

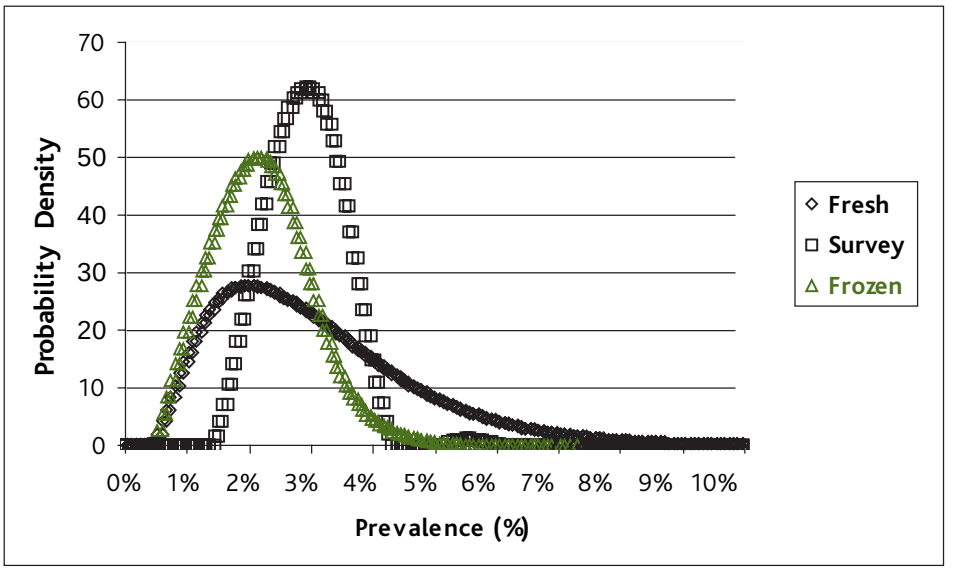


Figure 13. Distribution curves for prevalence of *E. coli* O157:H7 in contaminated raw beefburgers

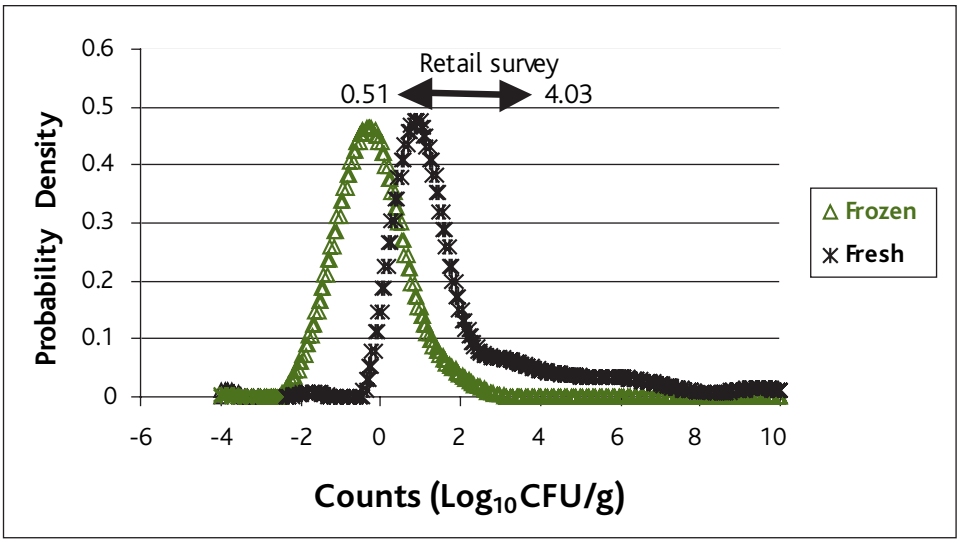


Figure 14 Distributions (simulated) for counts of *E. coli* O157:H7 in contaminated raw beefburgers

4.2.2.3 Module 3: Domestic storage and cooking

This model took account of domestic storage and cooking (Figure 15)

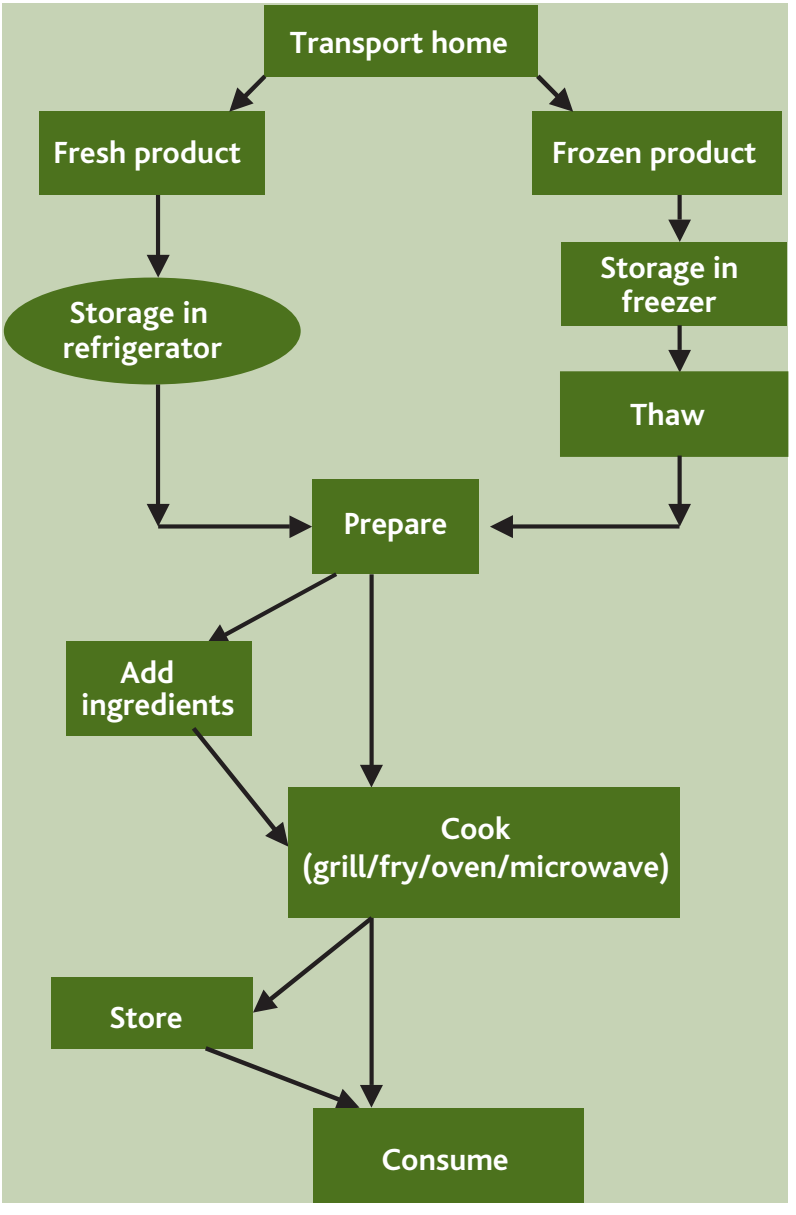


Figure 15. Flow diagram of module on domestic storage and cooking

Data inputs

The input data to this module on prevalence and numbers of *E. coli* O157:H7 were the outputs from module 2. The assumed temperature range (7 to 22°C) during transport of product from retail outlets and at home was based on data derived from the survey of consumer habits (Mahon *et al.*, 2003) and during domestic storage were based on the temperature studies in domestic refrigerators in Ireland (Kennedy *et al.*, 2005)

Cooking practices in Irish homes were simulated by taking three cooking categories (well done, medium and rare) and applying an inactivation model (Jackson *et al.,* 1996) to simulate the effect of cooking on *E. coli* O157:H7 counts. The temperature distribution was set for the cooking temperature based on the assumption that beefburgers are cooked with a mean temperature of either 68.3°C (well done), 62.7°C (medium), or 54.4°C (rare) (Jackson *et al.,* 1996) (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 (Mahon *et al.,* 2003; Kennedy *et al.,* 2005). An estimate of the prevalence and counts of *E. coli* O157:H7 after cooking was obtained.

Outputs

The simulated prevalence of *E. coli* O157:H7 in cooked fresh burgers is shown in Figure 16. The mean prevalence in cooked fresh burgers was 0.4%. The simulated counts of bacteria are given in Figure 17. The mean simulated counts remaining in the product was 0.153 $\log_{10}$ CFU/serving.

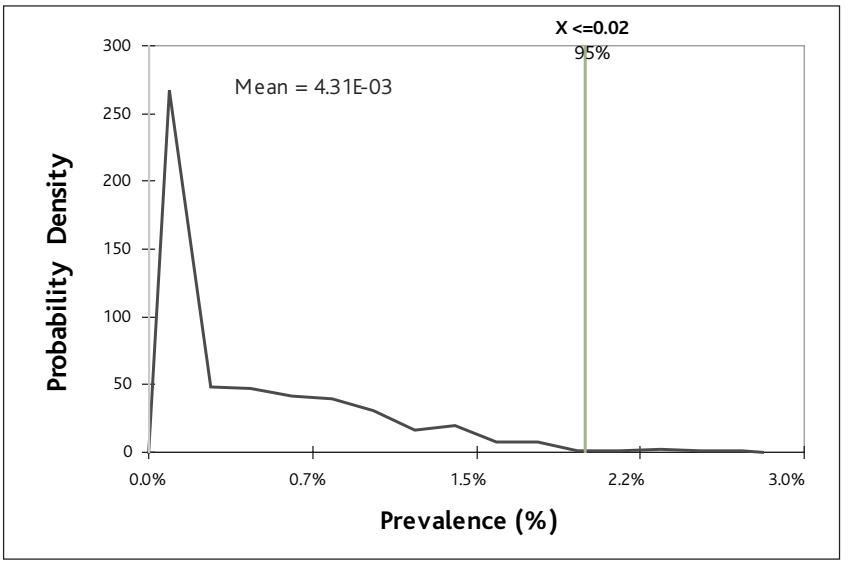


Figure 16: Distribution for prevalence of *E. coli* O157:H7 in cooked fresh burgers.

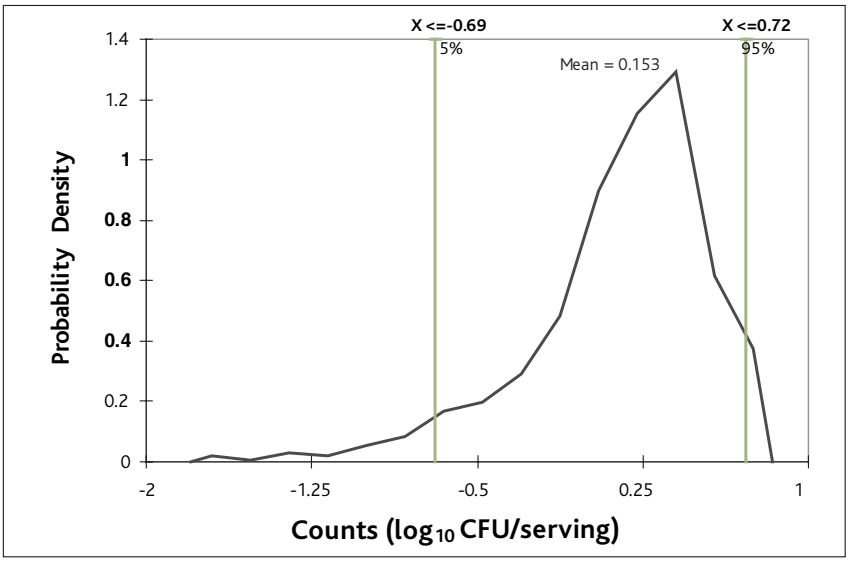


Figure 17. Distribution of *E. coli* O157:H7 counts per serving of beefburger (100g)

4.3. Hazard Characterisation

4.3.1 Dose response model

The objective of this study was to estimate the probability of *E. coli* O157:H7 infection resulting from a certain level of exposure. Human dose response trials have not and cannot be carried out with this highly pathogenic bacteria and so only estimates of the number of *E. coli* O157:H7 required to cause infection in humans are available. It was decided to use data available in the literature on infectious dose levels for humans infected with closely related bacteria - *Shigella dysenteriae* and Enteropathogenic *E. coli* (EPEC). EPEC was chosen to represent the lower bound of an *E. coli* O157:H7 dose-response function as has been done in previous studies based on the assumption that *E. coli* O157:H7 is unlikely to be less pathogenic than EPEC. *S. dysenteriae* was selected as an upper bound to the *E. coli* O157:H7 dose-response function based on the assumption that *E. coli* O157:H7 is unlikely to be more pathogenic than *Shigella dysenteriae* (Powell *et al.,* 2000). Information on the number of illnesses attributed to beef in Ireland was deemed to be too small to derive any statistical significance. The output of the dose-response model is an estimate of the number of people expected to fall ill for a given dose. The dose-response analysis was performed using a beta-poisson function.

The resulting dose-response model is given in Figure 18. The model predicts the average response for an administered dose given that organisms are randomly-distributed in the medium. The function assumes that a single organism is capable of inciting illness in an individual. The output of the dose-response model is an estimate of the probability of human illness given a specific dose. The dose response is combined with exposure predictions. Transposing the predicted exposure through the dose-response curve will result in an estimate of the number of people expected to become ill during a year as a result of a specific level of exposure. It is accepted that this dose-response relationship may be an underestimate for immune compromised individuals; however, to try to create one for individual risk groups would merely be a "shot in the dark" given the lack of reliable data for a dose-response relationship.

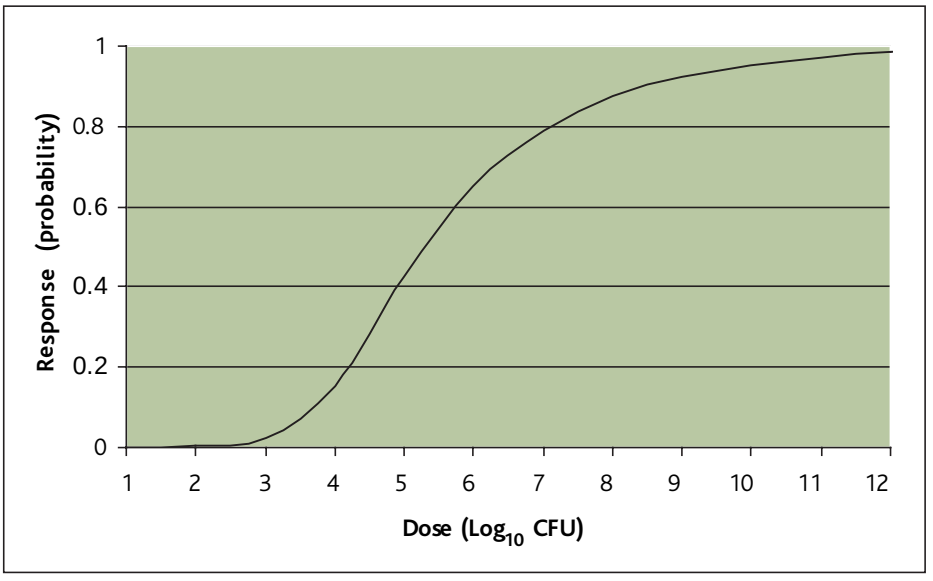


Figure 18: Dose-response model for *E. coli* O157:H7 (Powell et al., 2000)

4.4 Risk Characterisation

In the final phase, the estimates of human exposure to *E. coli* O157:H7 were modelled through the above dose response model. Transposing the exposure assessment data through a dose response model yields an estimate of the probability of illness caused by exposure to *E. coli* O157:H7 in beefburgers. The probability reported is for an "average" individual. The simulated probability of illness from a contaminated serving of fresh beef is given in Figure 19. The mean probability of illness was –5.94 log (i.e. $10^{-5.94}$ = approximately 1 chance in a million).

The sensitivity of model inputs to model predictions was modelled by rank order correlation sensitivity analysis. The results are presented in Figure 20. The initial count on bovine hides was the parameter having the most impact on predicted risk (correlation coefficient 0.62). Cross contamination at hide removal was also important (correlation coefficient 0.25) indicating where producers might focus efforts to reduce risk. Consumer behaviour in terms of cooking temperature (correlation coefficient -0.57) and temperature abuse (0.48) during transport and storage are very important part in dictating the final risk value, indicating the important role consumers have to play in ensuring their food is safe for consumption.

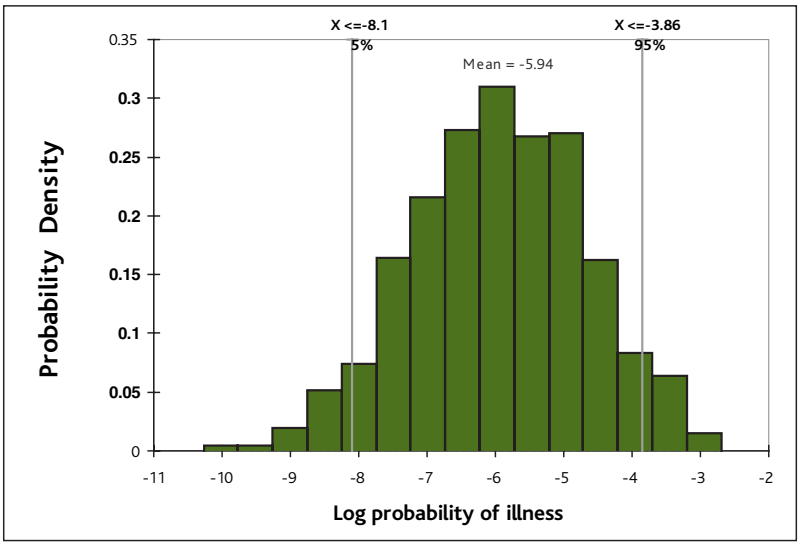


Figure 19: Probability of illness from a contaminated serving of fresh beef was mean log 1.1 x 10^{-6} (–5.94) which is approximately 1 burger in a million.

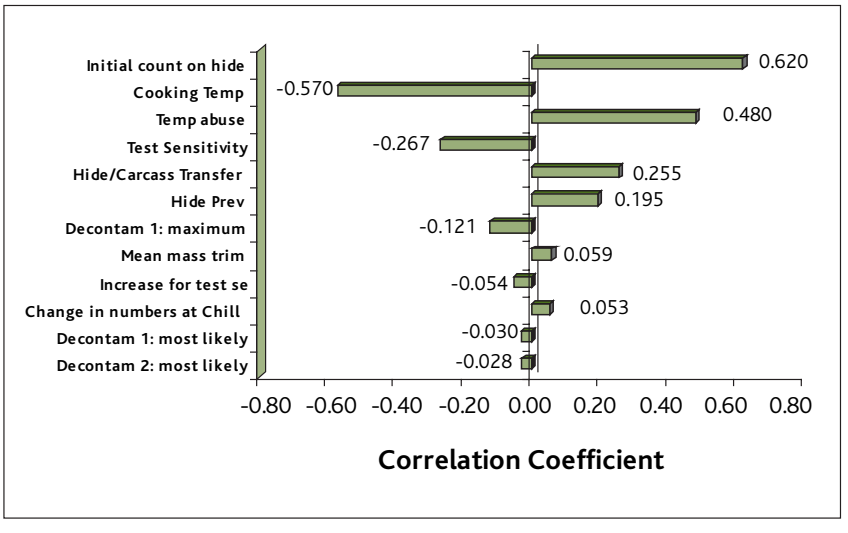


Figure 20: Sensitivity analysis between the probability of illness and the most important parameters.

5. ANSWERS TO RISK MANAGEMENT QUESTIONS

The programme has allowed the risk management questions outlined in section 3.1 to be answered

- Is there a difference in prevalence / contamination levels of *E. coli* O157:H7 (and calculated risk) in frozen burgers versus fresh chilled beefburgers ?

It was found that fresh burgers had a greater prevalence (mean of 2.9% versus 2.2% for frozen burgers) and higher counts (mean $\log_{10}$1.96 CFU g^{-1} versus $\log_{10}$ –0.22 CFU g^{-1}) than frozen burgers. This is mainly due to the higher probability of temperature abuse of the fresh burgers on retail display, during transport and home storage.

- Is there a difference in prevalence / contamination levels of *E. coli* O157:H7 (and calculated risk) from a beefburger made from 100% beef (meat) versus a burger made with added ingredients?

As added ingredients were not identified as a significant risk factor in the contamination of beefburgers, they did not contribute directly to the contamination level. However, due to the reduction in the amount of beef incorporated into burgers with added ingredients, a dilution effect was observed. The model indicated a reduction in prevalence of approximately 0.4% and a reduction in counts of approximately 0.3 $\log_{10}$ CFU g^{-1} on contaminated beefburgers with added ingredients, resulting in a reduction in exposure and hence risk. The use of head meat does not impact on the prevalence/contamination level in burgers and did not constitute any additional risk.

- Is there a difference in prevalence / contamination levels of *E. coli* O157:H7 (and calculated risk) from *E. coli* O157:H7 from mince beef purchased from a butcher shop versus a supermarket?

The prevalence and number of *E. coli* O157:H7 was similar in beef mince/ burgers purchased from butchers' shops and supermarket.

- What is the probability of a case of *E. coli* O157:H7 from a single serving of minced beef and what is the annual probability of infection?

The calculated mean probability of *E. coli* O157:H7 infection and ensuing illness from a serving of minced beef was found to be log 1.1 x 10^{-6} ($\log_{10}$ –5.94) (90th percentile range $\log_{10}$ –8.1 to $\log_{10}$–3.86) which is approximately 1 burger in a million.

- What is the probability of a case of *E. coli* O157:H7 infection in different risk groups (young children, healthy adults) from a serving of minced beef?

Data on dose-response relationships for individual risk groups was not available; indeed data on "conventional" dose-response for *E. coli* O157:H7 is sparse. As a result, it was decided to use the best available dose-response model from the literature without reference to risk groups. It is accepted that this dose-response relationship may be an underestimate for immune compromised individuals.

- What is the probability of *E. coli* O157:H7 illness from a minced beef meal prepared and consumed in the home versus outside the home?

With currently available data, it was only possible to predict the risk from *E. coli* O157:H7 in beefburgers consumed at home.

- What impact do consumer practices have on the risk (calculated) from *E. coli* O157:H7?

Consumer practices have a large impact on predicted risk of illness. In particular, a sensitivity analysis revealed that one of the most important impacting factors was the cooking preference (correlation coefficient –0.57). The higher the internal cooking temperature of the burger, the lower the risk. Burgers that are cooked "well done" (mean

internal temperature 68.3°C ± 2°C) virtually eliminated any probability of infection; burgers cooked "medium" (mean internal temp 62.7°C ± 2°C) also greatly reduced the probability of infection. Burgers cooked "rare" (mean internal temp 54.4°C± 2°C) constitute a significant risk to the consumer. Temperature abuse was also deemed a significant parameter influencing model predictions (correlation coefficient 0.48) including temperature abuse during transport and home storage. It is concluded that consumers can play a large role in reducing risk from *E. coli* O157:H7 in minced beef by keeping products properly refrigerated and cooking burgers to a "well done" state.

6. CONCLUSIONS

- While the predicted prevalence of *E. coli* O157:H7 remains low throughout the beef chain (2.51% to 2.9%), the predicted number of pathogens on contaminated samples ranged from $\log_{10}$ -0.22 to $\log_{10}$1.96 CFU g^{-1} is highly variable and sporadic samples with high numbers pose a particular risk to the consumer. Better temperature control (mean ≤ 7°C) during retail, and in particular during domestic transport and storage would reduce the likelihood of *E. coli* O157:H7 growth and thus the risk of servings with very high numbers which have a greater risk of predicted illness.

- The *E. coli* O157:H7 isolates recovered from retail beef in Ireland all possessed an array of virulence factors which would make them potentially pathogenic to humans indicating that beef is a possible vector of *E. coli* O157 infection for humans, either directly as a result of eating undercooked beef or from cross contamination in the retail or domestic environment.

- The calculated mean probability of *E. coli* O157:H7 infection and ensuing illness from a serving of minced beef was found to be log 1.1 x 10^{-6} ($\log_{10}$ –5.94) (90^{th} percentile range $\log_{10}$ –8.1 to $\log_{10}$–3.86) which is approximately 1 burger in a million.

- Further research is needed on the significance of the bovine hide as a source of contamination with *E. coli* O157:H7, in particular the transfer of contamination from the hide to the meat, the rate at which this happens, the amount of pathogen transferred and the operational procedures which contribute to it, including the effect of hide pulling equipment.

The application of quantitative risk assessment to microbial food borne pathogens is still a new and very dynamic field of research and advancements in the area continue at a fast pace. It is an approach to food safety management which has now been adopted by major national and international agencies and advancements are continuing from a number of avenues. New and better modelling techniques are now emerging both in terms of the models employed in exposure assessment to predict microbial growth / survival and the models used in exposure assessments and risk characterisations. As the field of quantitative microbial risk assessment develops, it will have better linkages with other management systems, including HACCP, economic cost benefit analysis, appropriate level of Public Health Protection (ALOPs) and Food Safety Objectives. With this multi-disciplinary approach, food safety will in the future be managed more strategically, leading to overall improvements in public health protection from microbial food contaminants.

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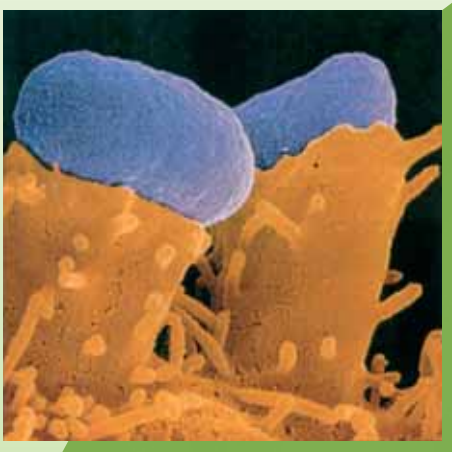

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