Human infection with *Campylobacter* spp. from chicken consumption: a quantitative risk assessment

Emma Hartnett

Ph.D Thesis

Department of Statistics and Modelling Science
University of Strathclyde
Glasgow, UK

2001

Abstract

Campylobacter is the commonest cause of human acute bacterial-enteritis in the developed world (ACMSF, 1993). Over the last ten years Great Britain has experienced an increase in the number of reported cases of campylobacter associated illness over the last ten years. There are numerous under reporting issues associated with campylobacter-related illness, and as such the actual number of cases that occur each year is unknown and the magnitude of the public health risk posed by this organism can only be hypothesised. Infection with campylobacter has been linked in epidemiological studies with the consumption of poultry, in particular chicken meat. A quantitative risk assessment (QRA) model has been produced to investigate this issue. Through the use of appropriate modelling techniques and collected data the QRA model assesses the risk of human infection with campylobacter consequent upon the consumption of a chicken meal. The model describes each of the stages of the chicken supply chain and the mechanisms by which the chicken/chicken product becomes contaminated was investigated thus allowing the identification of mitigation strategies, which can reduce such contamination. Model results estimate that the risk of infection with campylobacter associated with the consumption of a single serving of chicken has a mean value ranging from 0.040 to 0.070 with a 95^{th} percentile ranging from 0.098 to 0.160. These results have been used as a benchmark to which the impact of mitigation strategies are compared. Results clearly show that a reduction in the national flock prevalence, combined with a reduction in the within flock prevalence of positive flocks can have a dramatic impact upon the risk of infection. Further, freezing of chicken meat prior to consumption also considerably reduces the estimates risk.

Acknowledgements

I thank my supervisors Dr. Louise Kelly and Prof. George Gettinby for their continued support and guidance throughout the course of the project. I also thank Dr. Marion Wooldridge and Prof. Diane Newell for invaluable input during the development of this work.

I thank my friends for their constant encouragement. Finally I thank my family, specifically my parents and brother, for their support, both emotionally and financially, and their continuing belief in me. I dedicate this work to them.

This work was funded by the Veterinary Laboratories Agency, UK.

Thesis contents

Chapter 1

Introduction and thesis outline

Hartnett E., Kelly, L., Wooldridge, M., In Press(a). A quantitative risk assessment for campylobacter in broilers: work in progress. *Biodeterioration and degradation.*

1.1 Food Poisoning

Illness caused by the consumption of food contaminated with infectious and toxigenic micro-organisms is a major cause of suffering and death throughout the world (Allen & Kaferstein, 1983). Food-borne diseases have a major public health impact and in the year 2000 alone, there were 86,616 statutory notifications of food poisoning in England and Wales (CDSC, personal communication).

Food poisonings most commonly cause gastro-enteritis, a self-limiting illness which is only considered life threatening when the elderly, infantile or immuno-compromised are affected. However, with several food-associated pathogens there can be chronic sequelae or disability after infection; for example infection with *Listeria* species can result in miscarriage and meningitis.

Food-borne illness is a huge public concern. Food associated disease outbreaks capture the public's attention as every consumer is potentially at risk and consumers do not feel they can control these risks (Altekruse *et al.*, 1998). Such attention can have a large economic impact. For example, in the 1980s when the potential risks associated with bovine spongiform encephalopathies and beef were first publicised, the sale of beef products dropped. Each year millions of pounds are spent on scientific research into the understanding and control of food-borne pathogens. Further, the education of the public to prevent infection is a top priority. In line with this, in Great Britain (GB) April 1st 2000 saw the introduction of the Food Standards Agency (FSA), whose aim is to not only fund research but has boldly stated that it will achieve a 20 % drop in the number of reported cases by the year 2006. The FSA will also inform the public through openness of all results and decision-making processes.

Despite these research and education efforts, food poisoning is currently far from under control. Each year shows a steady increase in the number of laboratory confirmed cases. For example in the period from 1990 to 2000 the incidence of food poisoning in England and Wales increased from 52,145 to 86,616 laboratory

confirmed, and reported cases. Thus, further efforts are still required to aid in the discovery of a solution to the situation posed by this problem.

The epidemiology of food-borne disease is rapidly changing. New pathogens are constantly emerging, with many well-recognised pathogens increasing in prevalence or being associated with new food products. In the period from 1950 to 1995 the number of recognised types of food-borne illness-causing organisms trebled (Baird-Parker, 1994). This however may be misleading, as reports of the emergence of new pathogens may well be the recognition of those already present for decades. For example, *Campylobacter jejuni* was only identified as a human pathogen in the 1970s (Griffiths & Park, 1990) however, identification of the organism may have coincided with scientific developments enabling growth and culture of the organism.

Human demographics have had a major role to play in the changing face of food-borne illness (Altekruse *et al.*, 1998). Moreover, increasing portions of the population have a heightened susceptibility to food borne disease. The percentage of people with immune impairments is currently increasing due to factors such as HIV, age or chronic illness. In such groups of people, food-borne diseases are more likely to be severe, and recurrent infection and death becomes more likely. Further, surveillance of food-borne disease is not free from problems. Under-reporting, either by the sufferers or the clinical staff involved in the reporting system, result in surveillance data being hard to interpret and hence the true extent of the problem is difficult to establish. In 1990, the World Health Organisation (WHO), European Region, held a number of consultations on the reporting of national food-borne statistics in an attempt to improve the reporting so that more cases can be identified together with source and cause of illness (Baird-Parker, 1994). However, improvements in reporting have been slow to emerge. In 1992 The Public Health Laboratory Service (PHLS) began a survey of infectious intestinal diseases in GB. The outcome of this study was that for every 136 cases of infectious intestinal disease in the community only one will be reported to the PHLS.

All of these factors make the control of food-borne illness a challenge and new

approaches, which assist in the understanding of the epidemiology of disease and methods of control, are constantly being investigated.

1.2 Campylobacter Food Poisoning

Campylobacter is the commonest cause of human acute bacterial-enteritis in the developed world (ACMSF, 1993). Over the last ten years GB has experienced an increase in the number of reported cases of *Campylobacter* associated illness. In 1988 the total number of laboratory reports to the Communicable Disease Surveillance Centre (CDSC) of confirmed *Campylobacter* faecal isolates was approximately 28 thousand, this was seen to rise to 58 thousand in 1997 (CDSC, 1997). Whether this is a true reflection of the *Campylobacter* status is questionable. In particular both the standard of isolation techniques and awareness of the public health risk have both resulted in increased surveillance of the pathogen making campylobacter associated enteritis a notifiable disease. Thus there is increasing awareness regarding the severe nature of the problem that *Campylobacter* species pose to human health.

The genus *Campylobacter* is a family of bacteria that displays spiral morphology with flagella at one or both ends. They are Gram-negative and can be seen to utilise a corkscrew motility. They are microaerophillic, requiring specific oxygen and carbon dioxide concentrations for growth. Despite oxygen being a pre-requisite for growth, the concentration found in air is toxic to these bacteria (Griffiths & Park, 1990). They are sensitive to desiccation - especially at room temperature (Doyle & Roman, 1982) - and to heat, and do not usually survive pasteurisation procedures (Griffiths & Park, 1990). Campylobacters are also sensitive to freezing, but, after several weeks of storage, viable cells can be recovered from the surface of frozen meats, especially poultry (Simmons & Gibbs, 1979).

The disease spectrum from campylobacter is wide and varied. The commonest outcome of infection is diarrhoeal disease referred to as campylobacter enteritis. The initial symptoms are a fever of 40°C or higher with nausea and abdominal

cramps. This may lead to confusion and delirium. Profuse watery diarrhoea follows, which may contain blood. The duration of infection is normally between 2 and 7 days and the condition is usually self-limiting, not requiring treatment. If treatment is given then erythromycin is typically the antibiotic of choice. The severe nature of the abdominal cramps has led to campylobacter enteritis being mistaken for acute appendicitis (Hay & Ganguli, 1980).

As well as gastro-enteritis, campylobacter species have been associated with other illnesses such as urinary infections (Davies & Penfold, 1979), meningism (Wright, 1979), and the bacterium was also isolated in pure culture from the bile of several individuals with acute cholecystitis (Darling *et al.*, 1979). Further, severe sequelae have been associated with prior campylobacter infection, namely reactive arthritis (Ebright & Ryan, 1984), Reiter's syndrome (Jhonsen *et al.*, 1983) and Guillian-Barré syndrome (Kaldor & Speed, 1984) a neurological disorder reported to occur once in every thousand cases of campylobacter related illness.

Campylobacter is a ubiquitous organism. The sources of campylobacter infection have been extensively investigated; they are now described as common bacteria in the intestines of most animals over a world-wide distribution. As a result of this widespread distribution they are often recovered from carcasses, raw meat, and offal (Simmons & Gibbs, 1979; Skirrow, 1982; Hood *et al.*, 1988; Fricker & Park, 1989). Campylobacters are also isolated from raw milk (Hutchinson *et al*, 1985), milk products (Hudson, 1999), contaminated water (Mawer, 1988; Vogt *et al.*, 1982), sewage (Arimi *et al.*, 1988), and research into other reservoirs is ongoing. Each of these reservoirs has a role to play in the epidemiology of campylobacter human infection. Campylobacter-related food-borne illness has an unusual epidemiology in that the majority of cases are sporadic, with few outbreaks. In 1999 only 1% of laboratory confirmed cases were associated with an outbreak (CDSC, 1999). Most campylobacter infections have a zoonotic cause and have been associated with the consumption of contaminated food, water or animal contact (Altekruse *et al.*, 1994). Outbreaks have been attributed to water (Ano *et al.*, 1989; Broczyk *et al.*, 1987), raw milk (Hudson *et al.*, 1984) wild-birds (Stuart *et al.*, 1997) and tuna salad (Roels *et*

al., 1998). Interestingly poultry and poultry consumption are rarely associated with outbreaks. Despite this phenomenon, the consumption of poultry meat has been implicated as the major source in a number of case control studies performed in the US (Harris *et al.*, 1986; Deming *et al.*, 1987; Hopkins *et al.*, 1984). However, in other studies the domestic consumption of poultry meat was not a risk (Adak *et al.*, 1995).

The epidemiology of campylobacter infection in humans is poorly understood. The number of cases of campylobacter reported to CDSC shows a seasonal pattern with a peak in the 3rd quarter. This agrees with reports in the literature of a high incidence in the third quarter (Blaser *et al.*, 1982; Kist, 1982; Skirrow, 1982). Further, such seasonal patterns are seen in other European countries such as Sweden and Denmark. Seasonality occurs in the colonisation of broiler flocks in these countries, however there are discrepancies between the peaks of flock colonisation and human disease in all these countries (Shreeve *et al.*, 2000). These anomalies suggest that seasonality in human campylobacteriosis cannot be fully accounted for by increased poultry consumption levels or seasonal cooking regimes, for example barbecues. It seems likely that a further, as yet unidentified, source of human campylobacter infection exists that shows marked seasonal patterns.

Poultry are recognised as asymptomatic carriers of campylobacter. Campylobacters are frequently isolated from not only live poultry but also poultry products. (Doyle, 1984). Several studies suggest a link between poultry and human infection on examination of laboratory characteristics of strains from chickens and infected humans (Harris *et al.* 1986b; Bruce *et al.*, 1977; Shanker *et al.* 1982; Brouwer *et al.*, 1979; Pearson *et al.*, 1987; Hopkins & Scott, 1983; De Boer & Hahne, 1990), however given that there is debate over which molecular approaches are most appropriate for such investigations (Newell & Wassenaar, 2001) it is difficult to assess the validity of these findings. Similar investigations (Diane Newell, Personal communication) have found that of all the genotypes identified, only 23% were common to both the chicken and human population. This indicates that not all strains causing human illness originate from poultry, and not all strains found in

chicken are associated with human illness. However, the reasons why some chicken strains can be associated with human illness and not others are currently unknown.

Red meats and vegetables are rarely identified as being contaminated with campylobacters at retail (Kwiatek *et al.*, 1990) despite the meat-producing animals being heavily colonised with campylobacters (Giles Paiba, unpublished results).

The relative contribution of each of the potential food sources to the level of infection in the human population, is currently unknown. Some take the viewpoint that the evidence linking poultry consumption and campylobacter enteritis is presumptive, (Gill & Harris, 1982) but the epidemiological evidence suggests an important public health risk.

Campylobacter would appear not to be an essential part of the normal gut flora of poultry. Birds can be reared free of colonisation (Byrd *et al.*, 1998a; Engvall *et al.*, 1986). However the organism causes no apparent pathology in the growth of a colonised chicken (Stern *et al.*, 1990). This means that the only incentive to prevent colonisation is to increase the quality of the final product for human health.

1.3 The Poultry Industry

1.3.1 Economics of the Poultry Industry

The poultry industry is one of the most advanced sectors of agriculture in GB. At the retail level, poultry provides a very versatile product from whole birds and portions, fresh or frozen, cooked or part-cooked through to a wide range of poultry-containing products. Since the 1950s, the poultry sector has steadily increased in size and the output from this industry continues to grow, making poultry production fundamental to the British economy. In 1993 the output of the poultry industry was valued at £950 million, compared to an annual average of £585 million in the period 1981 to 1983 (ACMSF, 1996). Chicken meat has experienced great popularity with

UK consumers. From 1988 to 1997 chicken had the highest reported sales in the meat industry. In 1997, 1.13 million tonnes were sold through retail and catering outlets, and chicken represented 79% of the total retail poultry market and 38.6% of the retail primary meat market (BPMF market review).

1.3.2 Poultry production systems

Poultry production is a highly specialised area of agriculture, which is both complex and varied. The high level of inter-dependency in production results in each stage being influential to live bird welfare and product quality. Production begins with an elite breeding flock consisting of birds with advantageous genetic properties. This leads to the grandparent breeding stock, which are bred from a chosen line of the elite stock. From the grandparent stock comes the parent breeding stock which yield the fertile eggs that ultimately produce the table bird. Once fertile eggs are laid, they are transported to a hatchery, where they take 21 days to hatch. When the birds are 1 day old they are taken to a broiler-growing farm, where they remain until they reach slaughter weight (between 30 and 60 days old). Once the required weight is achieved the birds are transported to the slaughterhouse and killed. Post-slaughter, a series of processing steps follow which result in packaging and distribution of the meat to the retail or catering outlets. Each stage of the production chain will be described in greater detail in the appropriate chapter of this thesis.

Due to the integrated nature of production, contamination at any point in the process has potential to persist to the retailed product. Because of this, careful control measures are needed to ensure that exposure of the bird/carcass to any contaminant is kept to a minimum. With reference to microbial contamination this may occur via numerous pathways, e.g. infected parent flock, feed/water, staff, processing machinery, even vermin. Such sources need to be identified and minimised.

1.4 Food Safety and Control

In the past, safe food production was ensured by the application of several industry codes of practice and meat inspection regimes. The continued rise in incidents of food-associated disease indicates the limitations associated with traditional meat inspection and surveillance strategies often involving end product testing (Gill, 1999). As a step forward, Good Management Practice (GMP) was introduced, however this approach reflects general guidelines rather than an objective approach to the assessment of the risks associated with the food product in question. Therefore, this approach has been extended. For the design of microbiologically safe products a procedure called the Hazard Analysis Critical Control Point system (HACCP) is internationally promoted.

1.4.1 Hazard Analysis Critical Control Point System

The HACCP system enables the specific microbiological hazards associated with the production, manufacture, distribution, and use of a particular food to be identified in an objective, systematic comprehensive manner. Further, the precise means of controlling the identified hazard can be investigated. With reference to HACCP, a microbiological hazard is defined by Randell (1997):

> "The unacceptable contamination, growth and/or survival by micro-organisms of concern to safety or spoilage; and/or the unacceptable production or persistence in foods of products of microbial metabolism such as toxins"

The principles and procedures for applying the HACCP system are well documented (Notermans *et al.*, 1995; Gill, 1999; Buchanan, 1995). More recently these procedures have been improved by the use of risk assessment techniques. The best current forms of HACCP use multidisciplinary teams of experts applying a structured approach to hazard analysis, which considers the consequences of failure

to control a raw material, specific piece of equipment or operating practice or the potential for one or more significant microbiological hazards to occur. (Baird-Parker, 1994). HACCP systems applied to food safety require food industries to identify points in production where contamination may occur and target resources toward processes that may reduce or eliminate identified disease hazards. In these programs, industry takes lead responsibility for the control of food borne hazards and regulatory agencies maintain oversight.

There are seven steps to the application of the HACCP system

1. Identification of hazards and assessment of their severity and risk of occurrence in any given situation.
2. Determination of critical control points (CCPs) at which hazards can be controlled.
3. Establishment of limits and tolerances that indicate when an operation is being controlled at a CCP.
4. Development and use of monitoring procedures to ensure that each CCP is being controlled.
5. Identification of any corrective action needed when a CCP is not under control.
6. Verification of controls to ensure that the HACCP system is working.
7. Keeping records, including those of any corrective action.

Essentially, HACCP is a risk management tool which aims to improve the quality of food products. However, it may be useful to be able to predict the effectiveness of controls implemented under HACCP system. A tool available to make such predictions is quantitative risk assessment.

1.5 Risk Assessment

Risk assessment represents an evaluation of the likelihood and severity of a known or potential hazard (Hathaway, 1997). When constructed in a quantitative manner the outcome is a mathematical statement, which describes the chance of an adverse

outcome from exposure to a risk at some defined level (Covello & Merkhofer, 1993). Risk assessment is one step in an overall process termed risk analysis, the overall aim of which is to manage risks. Risk analysis consists of four stages, of which risk assessment is the second; the others are hazard identification, risk communication and risk management. A schematic representation of the four stages of risk analysis is shown in Figure 1.1.

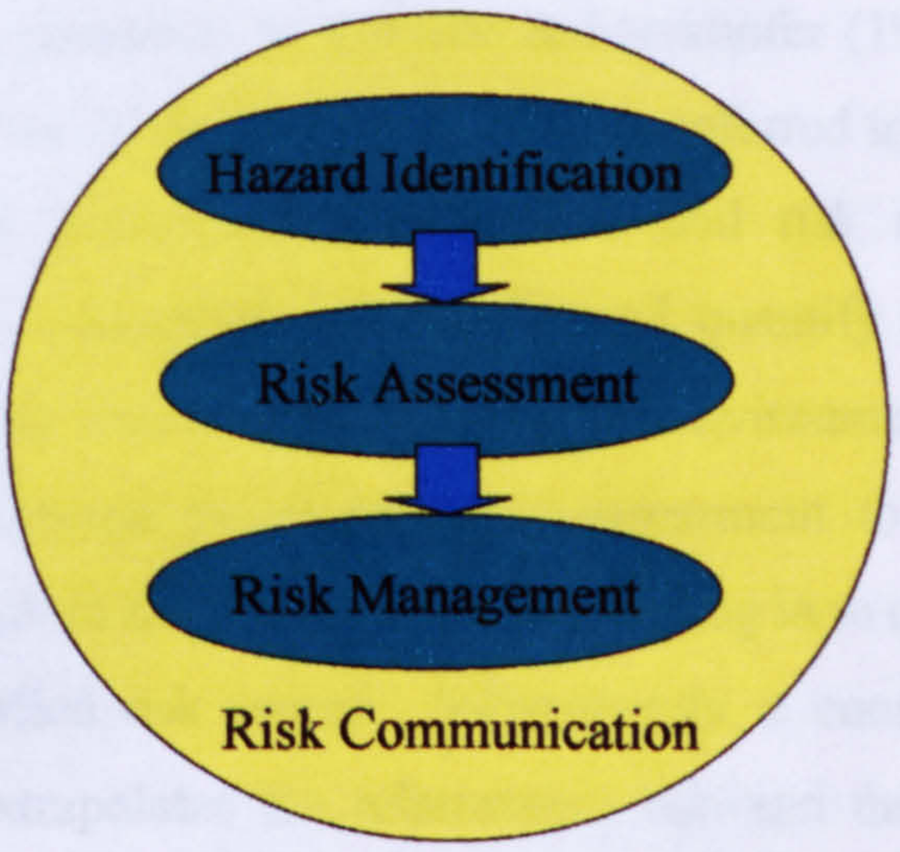


Figure 1.1: A representation of the four stages of risk analysis: hazard identification, risk assessment, risk management and risk communication.

Before a risk assessment can be carried out a hazard identification is undertaken which aims to identify factors that pose a risk, that is, result in an undesirable outcome to a given population. Once a hazard has been identified a risk assessment can be developed. This examines the conditions and extent of release of the hazard and all possible outcomes with their associated likelihoods. In particular, risk assessment provides a structured method for evaluating risks and allows the collection and analysis of available information in a logical fashion. Therefore such assessment will highlight areas of research need and provide managers with information on the identity and characterisation of risks so that control measures may be considered. These two stages feed into, and indeed are integral to, risk management which examines the feasibility of various mitigation strategies applied to the situation in question. Throughout each stage, risk communication is carried out. This involves an open exchange of information between all interested parties

and underpins the whole process of risk analysis ensuring appropriate communication of the risks and potential controls as defined by hazard identification, risk assessment and risk management. Ultimately, recommendations will be made which provide some risk reduction.

Following the framework adopted by the Office International des Epizooties (OIE), which was originally presented by Covello & Merkhofer (1993), a risk assessment can be divided into four distinct, interrelated steps referred to as release assessment, exposure assessment, consequence assessment and risk estimation. Initially, a release assessment is undertaken to describe and quantify the potential of a risk source to introduce the identified hazard into the environment of the population in question. This is followed by an exposure assessment to examine the relevant conditions which result in the study population coming in to contact with the defined hazard via the identified risk source. Subsequently, a consequence assessment is undertaken which extrapolates the relationship between the study population and exposure to the hazard at the levels previously defined. The process is completed by the integration of these three steps to attain an overall risk estimate. The efficiency of the model is increased in an iterative nature using sensitivity analysis techniques to identify key model parameters. Further, the effect of changes in the identified areas can be examined and the economic value of such strategies evaluated. This is advantageous, as the implementation of experimental strategies in practice is time-consuming and expensive in both human and monetary terms.

In addition to the system adopted by the OIE is the risk assessment framework implemented by the Codex Alimentarius Commission (CAC). This is based on a model presented by the USA National Academy of Science (NAS-NRC). Under this system the four components of risk assessment are defined as hazard identification, hazard characterisation, exposure assessment and risk characterisation. Here, hazard identification identifies the all potential hazards, hazard characterisation provides an estimate of the severity and duration of adverse effects due to the presence of the hazard, exposure assessment estimates the level of exposure to the hazard, and risk characterisation results in an estimate of the potential for adverse effects from the

particular hazard in a particular population.

Comparing the OIE and CAC risk assessment frameworks:

* Exposure assessment corresponds to the accumulation of the release assessment and exposure assessment as defined by OIE;
* Hazard characterisation corresponds to consequence assessment;
* Risk characterisation is equivalent to risk estimation;
* Finally, hazard identification is incorporated into the risk assessment process, where as this is a distinct element of risk analysis in the OIE code.

The framework adopted by OIE is in relation to international trade, however, the principles are relevant to many risk assessment issues. The emphasis in the CAC system is specifically on microbiological food safety risk assessments. As such, the system may be appropriate when considering other hazards. As such, the differences in the two systems are a result to the development of the systems in different areas and hence, in response to different requirements. Therefore, the system used is problem specific, the system adopted being that which best represents the situation posed for a specific hazard and a specific population.

Quantitative risk assessment (QRA) has been used for several years in areas such as finance, engineering, and the nuclear and chemical industries. In contrast, its use in the veterinary sphere, and in particular the area of food safety, is relatively recent. Buchanan & Whiting (1996) have postulated two reasons for the limited number of food related risk assessments. Firstly, lack of knowledge concerning dose-response relationships for many micro-organisms and secondly, difficulties in estimating the numbers of organisms ingested by humans.

Although such problems are widely recognised, risk managers and policy makers are now beginning to appreciate the benefits of undertaking good quality, transparent risk assessments for food related issues. Moreover, the Sanitary and Phyto-Sanitary (SPS) Agreement of the World Trade Organisation, allows countries to give priority to food safety over international trade only if a scientific basis for this priority can be

demonstrated by means of defendable assessments (Wooldridge, 1996). This means that use of this technique is essential to ensure safe food at both national and international levels. Consequently, QRA models for several micro-organisms in a variety of food products have appeared in the literature over the past two or three years. For example, Whiting & Buchanan (1997) have considered *Salmonella enteritidis* in pasteurised liquid eggs, Cassin *et al.* (1998) have modelled *Escherichia coli* O157:H7 in ground beef hamburgers and Nauta & Heuvelink (1998) have presented a model framework for *E. coli* O157:H7 in beef and beef products.

As well as the work mentioned above, there are several risk assessments currently being developed. These include two risk assessments which consider campylobacter in broilers. First, work by Fazil *et al.*, (Unpublished) estimates the risk of human infection as a result of the consumption of chicken in Canada. This model begins with the depopulation of the birds and transport to slaughter. The model then considers the stages of processing and cooking to provide an estimate of risk. This work specifically considers the consumption of an average, whole chicken by an average individual. Hence the resulting estimate of risk is a measure of the average risk for the population of Canada. Secondly, work by Christensen and colleagues estimates of the risk of campylobacter infection in Denmark (Christensen *et al.*, Unpublished). This model begins at the point of slaughter and considers the slaughter process and the preparation of a chicken meal. This model considers the consumption of a random chicken meal by a random member of the population of Denmark.

Risk assessment of microbiological hazards in foods has been identified as a priority area of work for the Codex Alimentarius Commission (CAC). In 1999, the Food and Agriculture Organisation (FAO) of the United Nations and WHO convened an expert consultation in Geneva, addressing the issue of microbiological hazards in foods. The consultation developed an international strategy and identified mechanisms required to support risk assessment of microbiological hazards in foods. As a result of that consultation, and in response to the Codex Committee on Food Hygiene (CCFH), FAO and WHO have jointly initiated a series of risk assessments

with the objective of providing expert advice on risk assessment methodologies to their member countries and CAC. The risk assessment modelling is undertaken as a two year project. In the first year hazard identification, exposure assessment, and hazard characterisation are undertaken. This work is then critiqued by a panel of WHO/FAO appointed experts at an expert consultation meeting. The result of this meeting is a report describing the state of the work, the experts opinion and suggestions which will enhance the usefulness of the work. In the second year risk characterisation is carried out. At the end of which a second expert consultation is held, the work critiqued and finally presented to CCFH. To date, this process has been completed for *Salmonella enteritidis* in eggs and broilers and *Listeria monocytogenes* in ready to eat foods (WHO, 2001). Further, two risk assessment projects are currently being developed as part of the WHO/FAO initiative. This work includes a risk assessment for *Campylobacter* species in broilers which brings together campylobacter risk assessment work from Canada, Denmark and the work presented in this thesis. The result will be a full farm-to-fork model assessing the risk to humans of campylobacter related illness as a result of the consumption of chicken. This project is in the second year and the report detailing hazard identification, exposure assessment, and hazard characterisation (Hartnett *et al.*, 2001b) and the report from the first expert consultation (WHO, 2001) are available

1.6 Focus of the thesis

Food poisoning, and particularly campylobacter-associated illness, poses a major public health risk. Further, despite continuing scientific research into the causes and prevention of such illness, the problem on the whole is escalating with a continual increase in the number of cases of food poisoning each year. The extent that each food source contributes to the human health burden is unknown. However, epidemiological investigations suggest that poultry and poultry products have an important role to play in the epidemiology of campylobacteriosis and hence control of the contamination of poultry products with campylobacter may reduce the risk of infection. Currently, there is a focus upon the use of HACCP principles to manage

microbial contamination in food production. However, prior to the implementation of any identified management actions it is desirable to be able to predict the impact any action will have upon both food contamination levels and public health. Such predictions can be made through the use of QRA. The application of QRA modelling in the food safety sphere is increasing in popularity with a number of models currently available in the literature. It provides a structured approach to the investigation and quantification of risk in a population.

Within this thesis a QRA model is developed. This model investigates and quantifies the risk of human infection with campylobacter as a result of the consumption of poultry and poultry products, specifically chicken meat, thus quantifying the contribution from chicken to the human disease burden.

The initial model framework for this risk assessment is illustrated in Figure 1.2. This framework is modular in nature whereby each module describes a distinct stage of the poultry production supply chain. All modules will be considered separately, using appropriate modelling techniques and collected data. Outputs from any one module will serve as inputs to the next module and the final module will generate estimated values of risk. In line with other QRA models for microbial risks (see for example Cassin *et al.*, 1998) this model is stochastic in nature and as such accounts for uncertainty and variability by appropriate uncertainty or probability distributions via Monte-Carlo simulation.

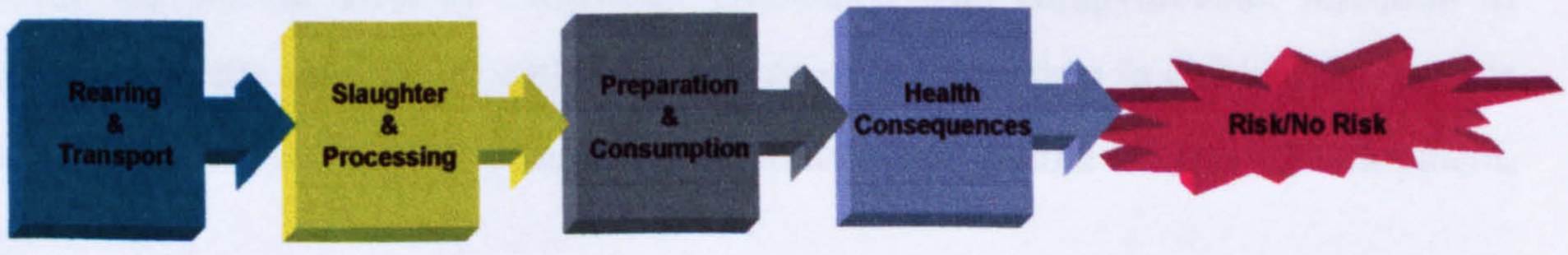


Figure 1.2: Model framework for the risk assessment investigating campylobacter infection in humans as a result of the consumption of poultry.

The modules illustrated in Figure 1.2 correspond to the fundamental steps of any risk assessment described by Covhello & Merkhofer (1993); that is release assessment, exposure assessment, and consequence assessment as described above. In the situation illustrated in Figure 1.2 it can be seen that the first two modules Rearing & Transport and Slaughter & Processing represent the release assessment, Preparation and Consumption module corresponds to an exposure assessment and the Health Consequences module corresponds to a consequence assessment.

This work will supplement current scientific research into the contamination of poultry at retail with campylobacter in that all stages in the poultry supply chain will be represented within the model framework. At each stage, the mechanisms by which the chicken/chicken product becomes contaminated will be investigated thus allowing the identification of mitigation strategies, which can reduce such contamination. Further the model development process will identify areas in which there is current data deficiency hence providing direction to future research efforts. In addition, the model development process will result in a better overall understanding of the infection pathway and provide structure to a complex problem, enhancing the current knowledge base with regards to this important public health risk. These are intrinsic characteristics for risk assessments that investigate problems of this type (Comer *et al.*, 1998).

1.6.1 Model Assumptions

Given the current level of knowledge associated with campylobacter infection in humans and the association with the presence of the organism in chicken flocks, it is necessary to make two general assumptions to enable model development, specifically:

* As discussed in this chapter, there are several strains of campylobacter species, and within each species there are numerous strains. However, it is currently unknown which strains are pathogenic to humans. As such an assumption is made that all strains present in chickens have the potential to cause disease in

humans.

* In addition to the lack of knowledge regarding the pathogenicity of different species and strains, the survival characteristics of different species and strains are also unknown. Therefore, it is assumed that all strains display the same characteristics.

As such the model makes use of all data available and does not discriminate between data for specific strains. However, should adequate data become available, the model presented in this thesis can be modified and hence these assumptions may no longer apply.

1.6.2 Thesis outline

The thesis is structured in a modular form whereby a number of chapters will describe each module shown in Figure 1.2.

In Chapter 2 risk assessment modelling techniques that are implemented throughout the thesis are described in detail and discussed. The focus of this chapter is on stochastic techniques and the manner in which uncertainty and variability can be dealt with when developing a risk assessment model.

Chapters 3 and 4 consider the module of the poultry supply chain, that is Rearing and Transport. In Chapter 3 a flock of chickens within a poultry shed is considered. The flock is exposed to campylobacter and a model is developed which describes the transmission dynamics within the flock on the farm. Further levels of infection and external contamination with campylobacter are considered and control measures on the farm are investigated. Following on from this, the transport of the flock to the slaughter facility is considered in Chapter 4, and a model is presented which predicts the within-flock cross contamination during transport. Overall, the models described in Chapters 3 and 4 provide estimates for the prevalence of colonised birds at the point of slaughter. Further the level of colonisation and external

contamination is also predicted. The models presented here provide a methodology for describing the dynamics of campylobacter within a chicken flock and hence fully describe the mechanisms which result in the entry of colonised/contaminated chickens in the slaughter facility.

In Chapters 5 and 6 consideration is given to the second module, Slaughter and Processing. Chapter 5 consists of a qualitative assessment of all the stages of poultry processing is given along with discussions on the potential impact of each stage upon the contamination levels of carcasses. The outcome is the identification of the key stages of processing which impact both the likelihood and magnitude of the contamination of retail products with campylobacter. Consequently, Chapter 6 presents a stochastic simulation model which quantifies the impact of the stages of processing identified in Chapter 5, thus estimating the probability of a random product being contaminated with campylobacter at the end of processing and the associated levels of contamination.

Chapter 7 investigates the Preparation and Consumption module. In this chapter, the storage of the product is first dealt with, followed by an investigation into the manner in which individuals prepare a chicken meal and subsequent cooking of the chicken product. These are combined to enable estimation of the probability of exposure to campylobacter from a single serving of a chicken meal and the likely number of campylobacter that will be ingested.

Following on from estimates of exposure, it is necessary to consider the subsequent health impact this may have upon a given individual. This is dealt with in Chapter 8. A full discussion about the way in which health outcomes are predicted by the use of dose-response modelling is presented followed by the derivation of a dose-response model for campylobacter. The outcome being an estimate of the probability that a random individual becomes infected with campylobacter following the consumption of a chicken meal.

In Chapter 9 the manner in which the models presented in Chapters 3 through to 8

are integrated is discussed, and the overall model results presented. This is followed in Chapter 10 by an investigation into ways in which the predicted risk of infection can be reduced. More specifically, mitigation strategies which have the potential to reduce the risk are implemented in the model and the effect quantified. Finally, The thesis concludes with Chapter 11 which consists of an overall discussion and summary of the work presented including a comparison of the work presented in this thesis with other risk assessment models developed for campylobacter.

In summary, described in this thesis is a stochastic risk assessment model, which provides and estimate of the risk of human infection with campylobacter, along with a measure of the degree of certainty associated with this estimate. Further, points in the supply chain which may reduce this risk will be identified, with a quantitative measure of the impact such mitigation strategies may have upon current estimates of risk.

Chapter 2

Techniques used in stochastic quantitative risk assessment modelling

2.1 Introduction

In recent years, the use of quantitative risk assessment (QRA) modelling by policy and decision-makers has increased substantially. This increase is apparent in many fields. In line with the expanding areas of application, the techniques and methodologies used are advancing at a rapid pace. In particular, the use of stochastic modelling techniques is now common place.

Within a stochastic model framework each input parameter is described by a probability distribution. This yields an output defined by a probability distribution describing the set of plausible risk estimates. Exact solutions describing the risk distribution are often analytically intractable, therefore risk analysts often employ numerical techniques such as Monte-Carlo simulation to estimate the final set of plausible risk estimates.

The proliferation of powerful personal computers and the availability of Monte-Carlo simulation software (e.g. @RISK) have made Monte Carlo simulation easy to implement. With trends moving toward more stochastic methodologies it is necessary to give consideration to what the input distributions represent. Generally, distributions either describe the variability in the value that a parameter can take or they describe the uncertainty in the true value of a parameter. In the risk assessment framework, variability represents the heterogeneity or diversity of a parameter. It describes the inherent randomness in the system under consideration and as such is irreducible by further measurement or study. In contrast uncertainty describes the level of ignorance regarding the particular parameter, and can be lessened by further investigations. As such, it is a function of the assessor as it represents the assessor's state of knowledge of the system in question. Both characteristics are inherent in the systems being described by QRA models, and are thus components of any data set. Further, unless there is perfect knowledge about a system, the true extent of the variability in a parameter may also be uncertain providing further complications

when interpreting QRA model systems. Given these complexities, a number of techniques are available to incorporate both features in the most appropriate way.

2.2 Variability and uncertainty in risk assessment models

When performing risk assessments using probabilistic methods it is necessary to be able to distinguish, analyse and visualise both the variability and uncertainty in any model parameter. Although these requirements have been considered in environmental risk assessments (See for example Cohen *et al.*, 1996; Bogen & Spear, 1987; Frey, 1998) the application of such techniques in the food safety and veterinary sphere has, thus far, been neglected, with variability and uncertainty normally remaining undistinguished in model frameworks. However, it has been demonstrated that the failure to appropriately deal with these characteristics can result in erroneous model results with risks potentially being underestimated (Nauta, 2000). Further, when uncertainty and variability are indistinguishable in a model this can complicate the decision making process as interpretation of the results is more complex. In particular, because uncertainty and variability describe very different aspects of the system, they can not be deemed equal and a model or distribution, which mixes both uncertainty and variability, will not provide clear characterisation of either uncertainty or variability (Brattin, 1996).

The technique of separating uncertainty and variability within a model is called second-order modelling. The application of second-order modelling can be mathematically complex and computer intensive. As a result of this complexity, there is currently much debate surrounding the merits of undertaking explicit separation. In particular, there is concern that the extra modelling effort required might not yield increased level of knowledge or precision in model results. In general, whether or not second-order modelling should be utilised, will be a problem specific decision which is likely to be influenced by the availability of resources.

When uncertainty and variability are treated separately within a QRA model, the model is structured around the variability of the problem. The uncertainty associated with the model parameters is then over-laid. There are two ways in which this can be dealt with in a model. The first method is to calculate the variability first and then to simulate the uncertainty. This involves analytically calculating the associated probability of each possible variable outcome from the model and adding uncertainty distributions to parameters. The model is then simulated to evaluate the effect of the uncertainty distributions on the variable results. This method quickly becomes intractable as models become increasingly complex. As an alternative, it may be more appropriate to simulate both the variability and uncertainty. Here the variable process is represented by use of multiple runs of the model. Upon each run, any parameters which have associated uncertainty are given a value from the previously defined uncertainty distribution for that parameter.

There is no rule as to which of these two approaches should be employed in any given situation, however Vose (2000) recommends that, when possible, the variability should be explicitly calculated and the uncertainty simulated. None the less, both methods described are asymptotically equivalent thus given sufficient simulation time, the method employed should not impact on the result.

2.2.1 Defining distributions of variability

There are several techniques available which look at how to interpret observed data for a variable in order to derive a distribution that realistically models its true variability.

When defining a parametric variability distribution, the properties of the variable in question should match those of the distribution chosen to model that variable. For example, whether the parameter is discrete or continuous, and does the theoretical range of the variable match that of the fitted distribution. There are several

theoretical distributions which comply with the mathematics of many variables. The binomial distribution describes trials with only two possible outcomes and as such has numerous applications when describing variability in data sets. If a distribution can be found with the same mathematical basis as the variable being modelled, it only remains to find the appropriate parameters to define the distribution. There are several techniques available which allow determination of the distribution parameters which best fit the data. Examples of such techniques are maximum likelihood methods, goodness-of-fit statistics and optimisation. There is extensive literature on the methods available see for example Schervish (1995).

Several distributions are known to fit certain types of data however this is based purely upon the shape of the distribution rather than any underlying process. For example the normal distribution is used to describe the distribution of several measures which are associated with nature such as weights and heights of individuals, yet there is no obvious mathematical rationale behind this, rather use is based upon observation of the data.

It is often the case that the observed data do not have an associated theoretical distribution or there are insufficient number of observations to determine whether or not the form of the variability matches a theoretical distribution. In this case an empirical distribution may be used. This relies solely upon the observed data to define the variability distribution and therefore makes no associated parametric assumptions. If the data are continuous a cumulative frequency plot of the data points can be used to define the variability. Given a data set of sample size n, x_i $i = 1,...,n$ ranked in ascending order, the associated cumulative probability of any given data point, $F(x_i)$ is given by the rank of the data point in the overall data set. More specifically

$$F(x_i) = \frac{i}{n+1}$$

If the data are discrete then the cumulative distribution function is given by

$$F(x_i) = \sum_{j=1}^{i} P(x_j)$$

where $P(X = x_i) = P(x_i)$.

2.2.2 Defining Uncertainty

In order to simulate both the variability and uncertainty present in the system, the uncertainty associated with any parameters must be defined. There are three general techniques used to quantify the uncertainty about a model parameter: Classical Statistics, Bayesian Inference, and Bootstrapping (Vose 2000). Each of these methods will now be briefly described, with reference to the treatment of a random sample of n data points $X = \{x_1,...,x_n\}$ from a cumulative variability distribution $F(x)$. In particular, each method is outlined with respect to the way in which the uncertainty associated with a statistical parameter θ of the parent distribution $F(x)$ is determined.

Classical statistics

There are several exact techniques available to characterise uncertainty when assumptions can be made about the parent distribution. Such assumptions often involve the parent distribution following either a binomial or normal model. Given that the normal distribution is a close approximation to several distributions under certain conditions, these exact techniques have a wide range of application. There is extensive literature available on the methods for estimating the uncertainty associated with a parameter, see for example Schervish (1995). For example in a situation whereby the mean and standard deviation of the parent distribution is unknown, the distribution of the mean of the population based upon the sample, $\bar{x}$, is commonly calculated from a student-t distribution as follows

$$\bar{x} = t(n-1)\left(\frac{\hat{s}}{\sqrt{n}}\right) + \bar{X}$$

and $t(n-1)$ is a student-t distribution with $n-1$ degrees of freedom. In the above equation $\bar{X}$ is the mean of the sample, $\hat{s}$ is an estimate of the true standard deviation based upon the data set calculated from

$$\hat{s}^2 = \frac{\sum_{j=1}^{n}(x_j - \bar{x})^2}{(n-1)}$$

The t is randomly sampled from a student-t distribution and this results in a range of $\bar{x}$ values according to each $\hat{s}$ and $\bar{X}$. The above method assumes a normally distributed population. It is therefore important to consider the relevance of the parametric assumption accompanying many of these techniques, as when they are not obeyed the degree of error introduced is difficult to quantify.

The Bootstrap

Bootstrap simulation was introduced by Efron in 1979 (Davison & Hinkley, 1997) for the purpose of estimating confidence intervals for a statistic using numerical methods. A key advantage of bootstrap simulation is that it can provide estimates of confidence intervals in situations for which analytical solutions may not exist. Hence, for example, when a parametric distribution such as the normal distribution cannot be assumed.

Consider a sample of n independently identically distributed random variables X_1, X_2, ..., X_n and a real-valued estimator $\theta(X_1, X_2, ..., X_n)$, denoted $\hat{\theta}$, of the distribution parameter θ. The bootstrap is a procedure that enables the assessment of the accuracy of $\hat{\theta}$, defined in terms of an empirical, cumulative distribution function F_n. The empirical distribution function is the maximum likelihood

estimator of the distribution for the observations when no parametric assumptions are made.

As defined by Efron *et al.*, (1994), bootstrap simulation is based on drawing multiple samples of size n, with replacement, from the empirical distribution F_n. This approach is referred to as re-sampling where the empirical distribution is described by an actual data set $x=(x_1,x_2,...,x_n)$. A random sample of size n from the original data-set is denoted by $x^*=(x_1^*,x_2^*,...,x_n^*)$ where the asterisks indicate that this is not the original data-set, rather a re-sampled version. As such the re-sampled data describe an empirical distribution, that is $\hat{F}\rightarrow(x_1^*,x_2^*,...,x_n^*)$. Note that since the sampling is done with replacement, it is possible to have repeated values within any given bootstrap sample.

It is possible to calculate any statistic based upon the bootstrap samples. This is referred to as the bootstrap replication of a statistic. More specifically $\hat{\theta}^*=s(X^*)$. Here $s(X^*)$ is some statistical estimator applied to a bootstrap replication of the original data-set. To quantify the uncertainty in any given statistic, for example the mean, multiple Bootstrap samples may be generated yielding multiple estimates of the statistic of interest. These estimates can be use to construct an uncertainty distribution to describe the parameter.

An alternative to the re-sampling procedure described above is the parametric bootstrap, in which the parent distribution is estimated using a parametric rather than an empirical distribution as the basis for re-sampling, for example it may be assumed that the data-set was drawn from a normal distribution. However it is often difficult to make such assumptions. Full discussion on parametric sampling is provided by Efron *et al.*, (1994).

Bayesian Inference

Bayesian inference is a technique based on Bayes' theorem (Lee, 1997) which focuses on using data to improve an initial estimate of a parameter. In brief, this technique involves estimation of a distribution for a parameter value θ, given that we have observed X. This distribution is denoted $f(\theta|X)$ and is called the posterior distribution. According to the Bayesian framework, estimation of $f(\theta|X)$ is based upon the accumulation of the prior distribution, denoted $\pi(\theta)$, and the likelihood function, denoted $l(X|\theta)$. The prior distribution is described as the density function for θ, before X was observed. It is not a probability distribution, but an uncertainty distribution describing the level of knowledge associated with θ prior to acquiring X. The likelihood function, $l(X|\theta)$, is the probability of X occurring for a selected value of θ. The posterior distribution is proportional to the product of the likelihood function and the prior distribution, that is

$$f(\theta|X) \propto \pi(\theta) l(X|\theta) \tag{2.1}$$

The result of equation (2.1) is a curve, which describes the shape of the posterior distribution, it is not the density function associated with the posterior distribution. The density function describing the posterior distribution is the result of equation (2.1), normalised such that the area under the curve equals unity, as shown in equation (2.2).

$$f(\theta|X) = \frac{\pi(\theta) l(X|\theta)}{\int_{\forall\theta} \pi(\theta) l(X|\theta) d\theta} \tag{2.2}$$

Fundamentally, Bayesian Inference takes an initial idea of what the posterior distribution looks like and then updates it with new information that has been acquired. Multiple data sets can be used as updates, for example, groups of animals from a herd are tested for a particular disease on three separate occasions. These

three data sets can be used as updates to provide an uncertainty distribution for the within herd prevalence of the disease.

The form of the likelihood function can provide information about the level of knowledge obtained from X. For example, if the shape of the likelihood function is similar to that of the prior distribution then the likelihood distribution embodies very little further knowledge regarding $f(\theta|X)$. In contrast if the shape is very different then a lot of knowledge has been obtained from X.

In summary, Bayesian Inference considers a data set X, and results in the formulation of an uncertainty distribution about a parameter θ, given X was observed. The methodology allows multiple data sets from one population to be combined in such a manner that each data set redefines the uncertainty distribution.

2.3 Propagating Variability and Uncertainty

Once the parameters of a QRA model have been fully quantified, there are several frameworks available in the literature that enable the propagation of variability and uncertainty within the simulation model.

An analytic approach for calculating risk that distinguishes between uncertainty and variability was presented by Bogen and Spear (1987). In their scheme, variability represents differences in risk between individuals whereas uncertainty is represented as the distribution of possible population risk values. This is calculated by summing individual risks present as a number of probabilistic descriptors of risk to different individuals in the population. These include a randomly selected individual, a mean risk individual, a 95th percentile individual, and a maximum risk individual. Each individual represents a different sample from the variability in the population distribution. For each of these defined individuals, an estimate of uncertainty in risk

is given this technique is parametric whereby the exact probability mass function of risk is calculated by combining distributions using Bayes' theorem.

This method becomes complex as models become larger and rapidly becomes intractable. Frey & Rhodes (1998) present a simulation based approach which avoids the need for analytic solutions hence simplifying the evaluation of larger models. The method is based upon two-dimensional probabilistic simulation. This can use a variety of sampling techniques such as Monte-Carlo and Latin Hypercube.

2.3.1 Two-stage Monte-Carlo simulation

In two-stage Monte-Carlo a two-stage simulation is carried out. In brief, the simulation consists of an "inner loop" (first stage) and an "outer loop" (second stage). The inner loop of the simulation resembles a traditional simulation. On each run of the model, samples are randomly drawn from probability distributions for quantities that vary from individual to individual. Based on these values, a risk estimate is calculated and the process is repeated. By repeating this process many times, the inner loop of the simulation creates a data set of risk values representing the range and distribution of risks for a hypothetical population.

Adding an outer loop to this traditional simulation, involves running the traditional simulation (inner loop) repeatedly, using different values for uncertain parameters each time. This produces multiple sets of simulation output, each of which characterises risk variability in the population assuming a different set of values for the uncertain parameters. Differences between the variability distributions generated reflect the impact of uncertainty on the characterisation of risk variability.

Using two-stage Monte-Carlo as a basis, Frey & Rhodes (1996) proposed a two-dimensional scheme that evaluates the risks associated with each combination of uncertain and variable quantities. This scheme is summarised as follows. The first step is to segregate the model inputs into variable and uncertain components. Following this frequency distributions are specified for all variable quantities and

for all uncertain quantities probability distributions are specified. For model inputs that have only variability or uncertainty, only a one-dimensional probabilistic characterisation in the variability or uncertainty dimension is required. For inputs that are both variable and uncertain a two-dimensional characterisation is required.

The scheme proposed by Frey & Rhodes (1996) is summarised in Figure 2.1. Assume for a given model there are M variable parameters and N uncertain parameters. On each run of the model all variable and uncertain distributions are sampled. Each of the M variable quantities are simulated with a sample size of m and each of the N uncertain dimensions of the model are simulated with a sample size of n. Thus the sample size for the two-dimensional simulation is the product of m and n. Clearly this can impose severe computational burden, depending on the required sample size.

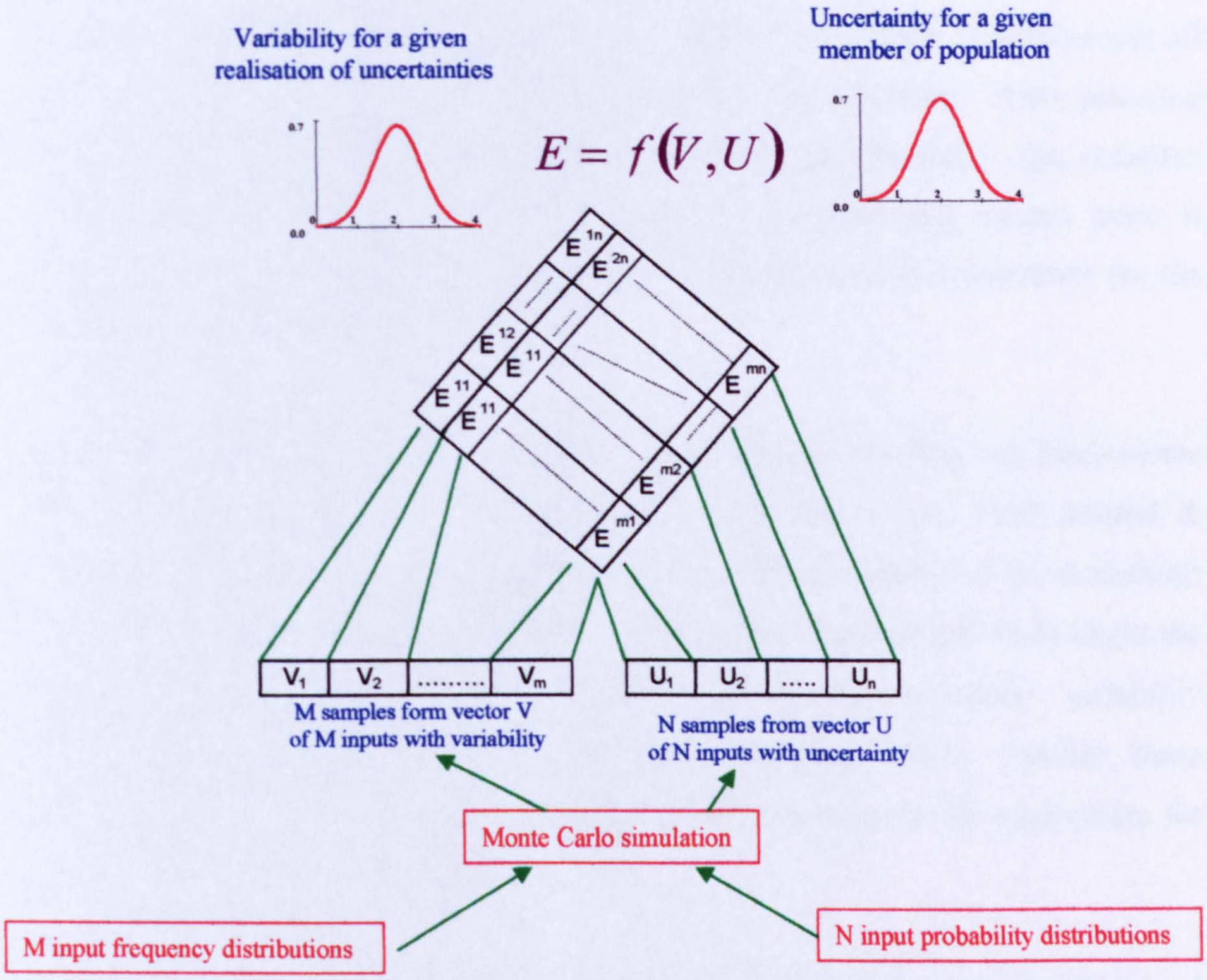


Figure 2.1: Schematic representation of the matrix for a two-dimensional Monte-Carlo simulation (After Frey & Rhodes, 1996).

The model is repetitively evaluated for each combination of samples from the variable and uncertain parameters. This is represented in Figure 1 by the matrix of values $E_{i,j}$ where i is an index from 1 to m of the sample values for the vector of variable quantities and j is an index from 1 to n of the sample values for the vector of uncertain quantities. Any column of the matrix represents the frequency

distribution for the variability in calculated values for a given realisation of uncertainties for each individual. Any row of the matrix represents the probability distribution for uncertainty in exposure level for a given member of the population.

The method presented here randomly selects all sets of variable quantities and all sets of uncertain quantities prior to calculating any risk estimates. After selecting these values it computes the risk estimates for each combination. For complex simulations this approach can impose a severe computational burden since it requires storage of all randomly generated variable and uncertain quantities for the duration of the simulation.

Similar two-dimensional methods appear in the literature regarding risk assessments in the environmental sphere (see for example Macintosh *et al.*, 1994; Helton & Shiver, 1996; Hoffman & Hammonds, 1994). A common feature of these methods is that they all have parametric elements. That is assumptions are made about the form of the distributions used to describe the uncertainty and/or variability associated with a model parameter (e.g. Bogen & Spear, 1987). Further, these techniques are highly computer intensive and therefore may not be appropriate for complex models (e.g. Frey & Rhodes, 1998).

An alternative method (Vose, 2000), which avoids both parametric assumptions, and the sampling issues associated with complex models is also available. This method involves the generation of non-parametric second-order random variables and is summarised as follows.

Consider a small data set drawn from a parent distribution that represents the variability in a particular model parameter. The data set consists of n data values $x_i,\ i=1,...,n$ each of which was randomly drawn from a distribution, $F(x)$. However, the form of this distribution is unknown and limited data makes parametric assumptions regarding the parent distribution $F(x)$ undesirable. A non-parametric approach is appropriate in this situation. The theory is as follows.

Given the data set $\{x_i\}$ each value x_i maps as a Uniform (0,1) distribution onto the cumulative probability distribution of that from which $\{x_i\}$ were drawn, $F(x)$. The data are ranked in ascending order such that $x_i < x_{i+1}$. As such, given n values were drawn from the distribution, the value x_i ranks ith. Therefore, there are $n-i$ values in the sample which are greater than x_i and $i-1$ values less than x_i. Such information enables the posterior marginal distribution to be determined, denoted P_i, for the cumulative probability associated with x_i, to be determined.

First, assume the prior distribution for the cumulative probability P_i takes the form of Uniform(0,1). Given the rank of a data point x_i, each other data point can then be considered as an independent Bernoulli trial as there are only two possible outcomes: the data point is either greater than or less than x_i. As such the probability density function for P_i given x_i can be described using the binomial theorem as follows

$$f(P_i|x_i; i=1,n) \propto (P_i)^{i-1}(1-P_i)^{n-i}$$

Here, a value less than x_i is be considered a "success" and a value greater than x_i a "failure". This is equivalent to a Beta distribution with parameters Beta$(i, n-i+1)$, and therefore

$$P_i = Beta(i, n-i+1)$$

Given that the data are ranked, $P_{i+1} > P_i$ and hence the Beta distributions describing P_i are not independent. As such it is necessary to determine the conditional distribution $f(P_{i+1}|P_i)$.

Consider two values, x_i and x_j drawn from the parent distribution $F(x)$, where $x_j > x_i$. The joint distribution for the associated cumulative probabilities for x_i and x_j, that is $f(P_i, P_j)$ is proportional to the probability that the cumulative probability of any given data point is in the range between P_i and P_j. More specifically

$$f(P_i, P_j) \propto P_i^{i-1}(P_j - P_i)^{j-i-1}(1-P_j)^{n-j}$$

Let $x_j = x_{i+1}$, this then becomes

$$f(P_i, P_{i+1}) \propto P_i^{i-1}(P_{i+1} - P_i)^{i+1-i-1}(1-P_{i+1})^{n-i-1}$$

which simplifies to give

$$f(P_i, P_{i+1}) \propto P_i^{i-1}(1-P_{i+1})^{n-i-1}$$

The conditional probability $f(P_{i+1}|P_i)$ is thus given by

$$f(P_{i+1}|P_i) = \frac{f(P_i, P_{i+1})}{f(P_i)} = k\frac{(1-P_{i+1})^{n-i-1}}{(1-P_i)^{n-i}}$$

where k is the constant of proportionality. The corresponding cumulative distribution function is given by evaluating the area under the density curve for the range P_i to P_{i+1}. More specifically

$$F(P_{i+1}|P_i) = \int_{P_i}^{P_{i+1}} k\frac{(1-y)^{n-i-1}}{(1-P_i)^{n-1}}dy = \frac{k}{(n-i)}\left[1-\left(\frac{1-P_{i+1}}{1-P_i}\right)\right]^{n-i} \qquad (2.3)$$

Given that the distribution $f(P_{i+1}|P_i)$ has a probability density of one when $P_{i+1}=1$ then $F(P_{i+1}|P_i)$ must also be equal to one, this then gives $k=n-i$ and, when substituted into equation (2.3), the result is equation (2.4)

$$F(P_{i+1}|P_i)=1-\left(\frac{1-P_{i+1}}{1-P_i}\right)^{n-i} \tag{2.4}$$

where this is Uniform(0,1) distributed as it is the cumulative distribution function.

Using this theoretical result, it is possible to generate a non-parametric second-order distribution for a given random variable for which $x_i \;\; i=1,...,n$ are samples from the parent distribution. In particular, given that $P_i=\text{Beta}(i,n-i+1)$, and the data are ranked in ascending order, the cumulative probability for the first data point, x_1, is $P_1=\text{Beta}(1,n)$. It then follows that the probability for the second data point can be determined from equation (2.4). Remembering that $F(P_{i+1}|P_i)=\text{U}(0,1)$, each of the uniform distributions are independent of each other, thus for clarity writing $\text{U}(0,1)=U_{i+1}$ and rearranging equation (2.4) yields

$$P_{i+1}=1-\sqrt[n-i]{U_{i+1}}(1-P_i)$$

Given that $1-\text{U}(0,1)=\text{U}(0,1)$ this can be re-written and hence the result is equation (2.5), the cumulative probability for P_{i+1}.

$$P_{i+1}=1-\sqrt[n-i]{1-U_{i+1}}(1-P_i) \tag{2.5}$$

Thus, given a sample data set, it is possible, by use of equation (2.5), to generate a second-order, non-parametric random variable.

When equation (2.5) is implemented within a two-stage Monte-Carlo simulation the result is a non-parametric, second-order distribution which describes not only the variability in the given parameter, but will also reflect the uncertainty which exists with respect to the true form of the variability. This uncertainty arises as a result of sampling and thus is a function of the sample size. On any given run of the model, a variability curve is defined from the uncertainty space associated with the parameter. Upon that run of the model one realisation of the variability in the parameter will be sampled. Multiple runs of the model then reflect multiple realisations of the variability in the parameter. As expected, when the sample size increases, the uncertainty space from which the variability distributions are defined decreases.

2.4 Regression analysis

A useful technique, which describes dependencies between variable parameters, is regression analysis. Regression analysis studies the effects of explanatory variables on a response variable. The simple linear regression model is given by

$$y_j = \beta_0 + \beta_1 x_j + \varepsilon_j \qquad j = 1,\ldots,n$$

where x_j is the independent variable, y_j is the dependent variable, ε_j is the error term, that is the difference between the observed value and that predicted by the model, β_1 is the regression slope, and β_0 is the y-axis intercept. The simplest analysis of data under the regression model is by the method of least squares. This method assumes that all individual y_j values are independent; for each value of x_j there is an infinite possible number of values of y_j which are normally distributed; the distribution of y_j given x_j has equal standard deviation for values of x_j and is centred about the regression line; and the means of this distribution at each value of x_j can be connected by a straight line. As such the least squares estimates for β are

$$\hat{\beta}_1 = \frac{\sum_{j=1}^{n}(x_j - \bar{x})(y_j - \bar{y})}{SS_x}, \quad \beta_0 = y - \hat{\beta}_1 \bar{x}$$

where $\bar{x} = n^{-1}\sum x_j$ and $SS_x = \sum_{j=1}^{n}(x_j - \bar{x})^2$.

The amount of variation in the dependent variable that is explained by the independent variable is given by the coefficient of determination, denoted R^2. This is given by

$$R^2 = \frac{\sum_{j=1}^{n}(\hat{y}_j - \bar{y}_j)^2}{\sum_{j=1}^{n}(\hat{y}_j - \bar{y})^2 + (y_j - \hat{y}_j)^2}$$

where $\hat{y}_j$ are the predicted values at each x_j. Therefore the ratio R^2 can be described as the fraction of the variation in the data explained by the model. This provides a quantitative measure of the linear relationship between the dependent and independent variables and ranges from –1 to +1. If the data are almost completely random, then none of the variance in the data is explained by the model. In this case $\sum_{j=1}^{n}(\hat{y}_j - \bar{y}_j)^2 \approx 0$ and hence $R^2 = 0$. In contrast, if the data lies very nearly on the regression line then $\sum_{j=1}^{n}(y_j - \hat{y}_j)^2 \approx 0$ and $R^2 = 1$. It should be noted that this is a model of variability. It is possible to include the uncertainty associated with parameter estimates.

2.4.1 Incorporating uncertainty into least squares regression models

Implementation of the least squares regression model, described in section 2.21 assumes that the relationship is correct and that the parameters are known. It essentially models the variability in a given parameter (dependent variable) with

respect to another parameter (independent variable). However, when only a small sample size is available it may be desirable to incorporate a measure of the associated uncertainty into the regression model. There are two methods available to incorporate uncertainty into a least squares regression model. These are classical statistical methods and the Bootstrap.

Classical statistics

When the assumption can be made that the model is correct, classical statistics provides a method to determine the best fitting values for β_0 and β_1. Further, it provides an exact distribution of the uncertainty for the estimate $y_j = \beta_0 + \beta_i x_j + \varepsilon_j$ at some value x_j. This distribution is given by

$$\hat{y}_j = t(n-2)s\sqrt{\frac{1}{n} + \frac{(x_j - \bar{x})^2}{\sum_{j=1}^{n}(x_j - \bar{x})^2}}$$

where $t(n-2)$ is a student-t distribution with $(n-2)$ degrees of freedom, and s is the standard deviation of the raw residuals $e_j = y_i - \beta_0 + \beta_1 x_j$, where

$$s = \sum_{j=1}^{n} \frac{e_j}{n-1}^2$$

This produces a relationship where the uncertainty is at its minimum in the middle of the data set. This is because the further away predictions are made form the actual data set the uncertainty increase with regards to the true nature of the relationship between the variables. The result of the application of this approach is that for any given value x_j there is a normal distribution describing the possible true value of y_j. However, when parametric assumptions are not desirable, this approach may be inappropriate.

Bootstrap

The bootstrap can be used to determine the uncertainty distribution associated with a regression analysis. There are two possible approaches: a parametric approach and a non-parametric approach. The use of either depends on the assumptions concerning the way in which the data arises. The first possibility, the basis for the parametric approach, is that the pairs are randomly sampled from a bivariate distribution F for (X,Y) and hence the regression coefficients can be thought of as parameters of a bivariate normal distribution. This is often referred to as type A data. In this situation the linear regression refers to linearity of the conditional mean of Y given $X = x$, that is

$$E(Y|X = x) = \mu_y + \gamma(x - \mu_x)$$

where

$$\gamma = \frac{\text{var}(X)}{\text{cov}(X,Y)}.$$

This corresponds to $\beta_0 = \mu_y - \gamma\mu_x$. The non-parametric bootstrap can then be used to resample from the paired observations $\{x_j, y_j\}$ and, at each Bootstrap replicate, calculate the regression coefficients.

In contrast to the above description, for any value of x_j the response y_j is sampled from a distribution $F_x(y)$ where the mean is $\mu(x)$ and the standard deviation is $\sigma^2(x)$ such that $\mu(x) = \beta_0 + \beta_1 x$. This is often referred to as type B data. It can be seen that $\beta_0 = \mu(0)$, and $\beta_1 = \frac{\sum(x_j - \bar{x})\mu(x_j)}{SS_x}$. Therefore $F_x(y) \equiv G\{y - \mu(x)\}$ where G is the distribution of random error.

For type B data the independent variables are fixed since they were predetermined. Therefore, assuming that the variations around the regression line are homoscedastic and the straight-line relationship is correct, the only random variable involved is that

producing the variations about the line, that is the residuals, e_j. The raw residuals estimate the random errors ε_j since

$$e_j = \varepsilon_j - \sum_{k=1}^{n} h_j \varepsilon_k$$

where

$$h_j = n^{-1} + \frac{(x_j - \bar{x})^2}{SS_x}$$

The values h_j are referred to as leverages.

For parametric re-sampling, G is estimated according to the assumed form of the error distribution. However, if it is known that the residuals are normally distributed then the classical statistics approach is the preferred choice as it provides an exact answer. For non-parametric re-sampling this method is extended. First a generalisation of the empirical distribution function is required and the raw residuals (r_j) are modified such that they are described by a non-parametric distribution and have constant variance, that is

$$r_j = \frac{y_j - \hat{\mu}_j}{(1 - h_j)^{\frac{1}{2}}}$$

where r_j are the modified residuals and $\hat{\mu}_j = \hat{\beta}_0 + \hat{\beta}_1 x_j$.

The next stage is to estimate G by use of the empirical distribution function of $r_j - \bar{r}$, where $\bar{r}$ is the average of r_j. It is assumed that the re-sampled model has the same design as the data, that is x_j^* it then specifies the conditional distribution of Y_j^* given x_j^* through the estimated version of the simple linear regression model, that is

$$Y_j^* = \hat{\mu}_j + \varepsilon_j^* \qquad j = 1,\ldots,n$$

with $\hat{\mu}_j = \hat{\beta}_0 + \hat{\beta}_1 x_j^*$ and ε_j^* randomly sampled from $\hat{G}$.

The steps to adding uncertainty to a linear regression model using a non-parametric Bootstrap can be summarised by the following stages:

- Observe data set x_j
- Assume that the bootstrap samples of the data follow the same model as the data such that $x_j^* \equiv x_j$
- Estimate the raw residuals e_j and modify according to yield r_j
- Estimate G from the empirical distribution function of $r_j - \bar{r} \Rightarrow \hat{G}$
- Finally $Y_j^* \left| x_j^* \right. = \hat{\mu}_j + \varepsilon_j^*$

Thus far the analysis of a linear model has been discussed. However, several biological situations are known not to have a linear relationship. An extension of the bootstrap technique described above to non-linear models is fully described by Davison & Hinkley (1997) however there is a simpler approach available. The method of least squares provides a tool for modelling functions other than lines. Consider the regression equation $\hat{y} = \beta_0 + \beta_1 x$. This is a linear function, however it is also a linear combination of 1 and x. As such it is a linear function of the variables β_0 and β_1. Therefore any model that is linear in these parameters, but not necessarily in the independent variable can use the method of least squares.

There are many situations where the most appropriate model is not linear, but which can be transformed into a linear relation. For example exponential and power equations can be transformed into linear equations. Given the relationship $y = Ae^{rx}$ taking logarithms of both sides the result is $\ln y = \ln A + rx$ which is a linear equation in the variables x and $\ln y$. And similar operations can be performed

upon many model forms resulting in a linear relationship then the bootstrap technique described above can be implemented.

2.5 Conclusions

The level of mathematical modelling incorporated into QRA models has dramatically increased over recent years due to an increase in demand for such approaches to decision making. QRA models are commonly formulated in a stochastic manner and thus incorporate variability and uncertainty. This is achieved using probability distributions and as a result, consideration must be given to the manner in which such probability distributions are used. In general, a probability distribution will either describe the variability or uncertainty associated with a particular parameter. These two characteristics are separate and distinct and as such require separate treatment within a risk assessment framework.

The variability in a parameter may be described parametrically when the process that leads to the variability is described mathematically by a theoretical distribution. Further, several measures are traditionally represented by theoretical distributions. However, this is not based upon any mathematical rationale, rather similarity in the form of the distribution. Variability is described non-parametrically by use of the empirical distribution defined from the observed data. Regression analysis can be used to describe the variability in a given parameter, in relation to another parameter in the model.

When small sample sizes are involved there may be uncertainty about a descriptive statistic for the population. Three methods have been described in this chapter, which estimate uncertainty in a given parameter based upon a sample from a population. The methods described are classical statistical methods, Bayesian Inference and Bootstrapping.

Once uncertainty and variability distributions have been defined, two-dimensional Monte-Carlo simulation can be used to propagate the variability and uncertainty through the model. There are several frameworks available to implement two-stage Monte-Carlo however caution must be given to the necessity for parametric assumption and computer efficiency for complex models. A method, which avoids these issues, has been described in this chapter. This involves the generation of non-parametric, second-order random variables which, when implemented within a two-stage Monte-Carlo simulation, provide the variability distribution and, upon multiple runs of the model, represent the associated uncertainty in any variable parameter.

Two-dimensional Monte-Carlo can also be used to add uncertainty to a regression analysis. To incorporate uncertainty into a regression analysis classical statistics provide exact methods of calculating the uncertainty distribution. When a non-parametric approach is preferred a non-parametric bootstrap can be employed.

The separate treatment of variability and uncertainty within food safety risk assessments is a novel approach. It is likely that the extent of explicit separation, which is carried out in such models, will be a project specific issue dependent upon resources. However, such techniques may be especially useful in food safety QRA modelling where data sets are often small due to the complexity and costly nature of experimental sampling.

In this thesis, a two-dimensional, Monte-Carlo simulation model is presented. Essentially, the model described the variability inherent in the processes leading up to the infection of humans with campylobacter as a result of the consumption of chicken. Where appropriate, uncertainty is incorporated into the model using the method of Vose (2000). That is, second-order non-parametric distributions are derived to describe the uncertainty in any variable parameters. Further, regression analyses are incorporated into the model to describe any dependencies. Any associated uncertainty is generated through the use of a non-parametric bootstrap as described in this chapter. The result is a distribution of the risk of infection with

campylobacter form the consumption of chicken, along with a measure of the level of uncertainty associated with this risk.

Chapter 3

The occurrence of campylobacter in birds at slaughter

Hartnett, E., Kelly, L., Newell, D., Wooldridge, M., & Gettinby, G., (2001). The occurrence of campylobacter in broilers at the point of slaughter, a quantitative risk assessment. *Epidemiology and Infection* 127;195-206.

3.1 Introduction

Broiler poultry production is highly specialised and follows a defined structure (ACMSF, 1996). When the birds are 1 day old they are moved to a broiler-growing farm, where they remain until they reach slaughter weight at ages of between 30 and 60 days. At this point depopulation occurs; that is birds are removed from the house, and transported to the slaughter facility for processing to produce the sale product.

A typical grow out house contains litter on the floor and troughs which are filled with feed and water in lines. Several birds will feed from a single feed/water trough and the feed and water may even be circulated on belts throughout the house. While on the growing farm, despite the strict controls often in place by a given company, the intensive nature of production means that the birds are exposed to a variety of sources of campylobacter. These may include contaminated farm staff, insects and even persistent contamination of the house itself as a result of a previously positive flock.

3.1.1 Campylobacter colonisation of poultry

The sources of campylobacter colonisation of poultry flocks are still debatable. Bacterial infections in both humans and animals are often of maternal origin. This, coupled with the fact that salmonella species in poultry are transmitted via this route, make vertical transmission a candidate. Transmission via contaminated eggs has been documented (Doyle, 1984) but remains an area of controversy. Isolation from eggs has been demonstrated as a rare event. In particular, Shanker *et al*, (1986) obtained only two positive eggs from a sample of 187 eggs from a campylobacter positive breeder flock. The occurrence of the two positive samples has been attributed to faecal contamination of the egg shell. Moreover, campylobacters have poor survival rates in egg albumen (Jones *et al.*, 1991). Therefore it seems that

vertical transmission is an unlikely source of infection (Annan-Prah & Janc, 1988; Van De Giessen *et al.*, 1992).

Transmission from flock to flock, referred to as 'carry-over', also seems an unlikely occurrence due to the poor survival of campylobacters in the environment under ambient conditions (Kapperud *et al.*, 1993; Jacobs-Reitsma *et al.*, 1995; Kapperud *et al.*, 1993). Further sources such as feed (Humphrey *et al.*, 1993; Mead & Hinton 1989) and litter (Pokamunsky *et al.*, 1986; Clark & Bueschkens, 1988) are unlikely as campylobacters are fragile organisms with an intolerance to desiccation (Doyle & Roman, 1982). As such they are unlikely to survive well in feed or litter. Most evidence, serotyping and case-control studies (Evans, 1992; Stern *et al*, 1997; Jacobs-Reitsma *et al.*, 1995) suggest that the primary source of introduction into the flock is the external environment. As campylobacters are ubiquitous this hypothesis is intuitive.

Once the flock has been exposed to colonisation, the water and feed play an important role in the dissemination of colonisation throughout the flock. When colonisation is first detected in the birds the feed soon becomes culture positive (Genigeorgis *et al.*, 1986). However the organism is rarely found in the water of flocks which contain colonised birds. The absence of campylobacters in water samples at early stages of colonisation has been attributed to viable but non-culturable forms (VNC) (Rollins & Colwell, 1986). Such forms of campylobacter may be capable of resuscitation *in vivo* (Mead & Hinton, 1989). Further, the importance of water in the transmission of the organism through a flock has been demonstrated experimentally. Chlorination of the water supply was shown to slow the within flock transmission of the organism (Pearson *et al.*, 1993).

Farm-workers play an interesting role in the epidemiology of flock colonisation. Case-control studies have identified farm staff as a risk factor (Lindblom *et al.*, 1986; Evans, 1992) and external contamination of a flock by catchers has been demonstrated (Tom Humphreys, unpublished data).

The risk of campylobacter colonisation is strongly associated with age (Evans, 1996) with the probability of infection increasing with age. Survival analysis has indicated that a number of management factors acted as predictors of the age at which flocks became colonised but in a follow up study, intervention methods were only successful in delaying the survival time (Evans, 1996). An interesting feature in the epidemiology of flock infection is the presence of a lag period which occurs during the first 14 days in the house. During this period no birds can become colonised. This is consistently seen in commercial flocks (Lindblom *et al.*, 1986; Mead & Hinton 1989) but absent in laboratory experiments (Shanker *et al.* 1990). The reasons for this difference are unknown.

Seasonality of the colonisation of broilers, i.e. a higher contamination rate during warmer periods, has been reported in certain countries, (Kapperud *et al.*, 1993; Jacobs-Reitsma *et al.*, 1994; Newell *et al.*, 1998). This seasonality of colonisation has been further demonstrated within GB (Newell *et al*, 1998), but in other countries such as the USA and Canada (Quebec) no evidence of seasonal variation has been found (Gregory *et al.*, 1997).

To consider the extent of exposure of humans to contaminated chicken products and methods of control, estimation of the probability of a random chicken destined for human consumption being campylobacter positive at the point of slaughter is required to enable the 'farm to fork' pathway to evolve. This chapter considers the rearing part of the first module of the 'farm to fork' framework previously described. The model presented here estimates the probability of a random bird from within the British national flock being campylobacter positive at the time of slaughter, together with an estimation of the uncertainty in this probability.

3.2 Model Development

The aim of the rearing module is to estimate the probability that a random bird from the GB poultry flock will be campylobacter positive at the point of slaughter. This probability is defined as P_{pb} and can be estimated as shown in equation (1)

$$P_{pb} = P_{fp} * P_{wfp} \quad (1)$$

where P_{fp} is the flock prevalence, that is the proportion of the national flock that is positive, and P_{wfp} is the within-flock prevalence of a positive flock at the time of slaughter. A positive flock is defined as a flock that contains one or more birds colonised with campylobacter. Estimation of P_{fp} and P_{wfp} was undertaken as follows.

3.2.1 Estimating flock prevalence, P_{fp}

The frequent colonisation of poultry flocks with campylobacter is well documented (Byrd *et al.*, 1998; Gregory *et al.*, 1997) however little data exists on the prevalence of positive flocks within GB or, indeed, world-wide. Currently there are no national surveillance schemes in GB. Although some poultry production companies carry out routine monitoring, the asymptomatic nature of the colonisation means that this a low priority. Consequently this highlights an area of limited data.

Sample data obtained from two fully-integrated poultry companies, an epidemiological study (Evans, 1996) and a published source (Humphrey *et al.*, 1993) were used to obtain an estimate of P_{fp}. More specifically, individual estimates of flock prevalence were derived for each source using beta distributions as follows

$$P1_{fp} = Beta(r_1 + 1, s_1 - r_1 + 1)$$
$$P2_{fp} = Beta(r_2 + 1, s_2 - r_2 + 1)$$
$$P3_{fp} = Beta(r_3 + 1, s_3 - r_3 + 1)$$
$$P4_{fp} = Beta(r_4 + 1, s_4 - r_4 + 1)$$

where $P1_{fp}$ and $P2_{fp}$ are estimates of flock prevalence derived from data from the two leading GB poultry producers which together account for 35% of national chicken production; $P3_{fp}$ is an estimate of flock prevalence based on the epidemiological

study (Evans, 1996) which involved 5 separate poultry producers, together responsible for 50% of the national flock, and $P4_{fp}$ estimates flock prevalence from a published study (Humphrey *et al.*, 1993). In each case, r denotes the number of positive flocks and s the number of flocks sampled. The beta distribution is used to characterise the uncertainty in the sample data and assumes a random sample and that the sample size is smaller than the total population. It also assumes that each positive flock is equally likely to be detected (Vose, 2000). The values corresponding to each r and s cannot be disclosed due to data confidentiality, however the resulting beta distributions, $P1_{fp}$, $P2_{fp}$, $P3_{fp}$, and $P4_{fp}$ are shown in 3. 1.

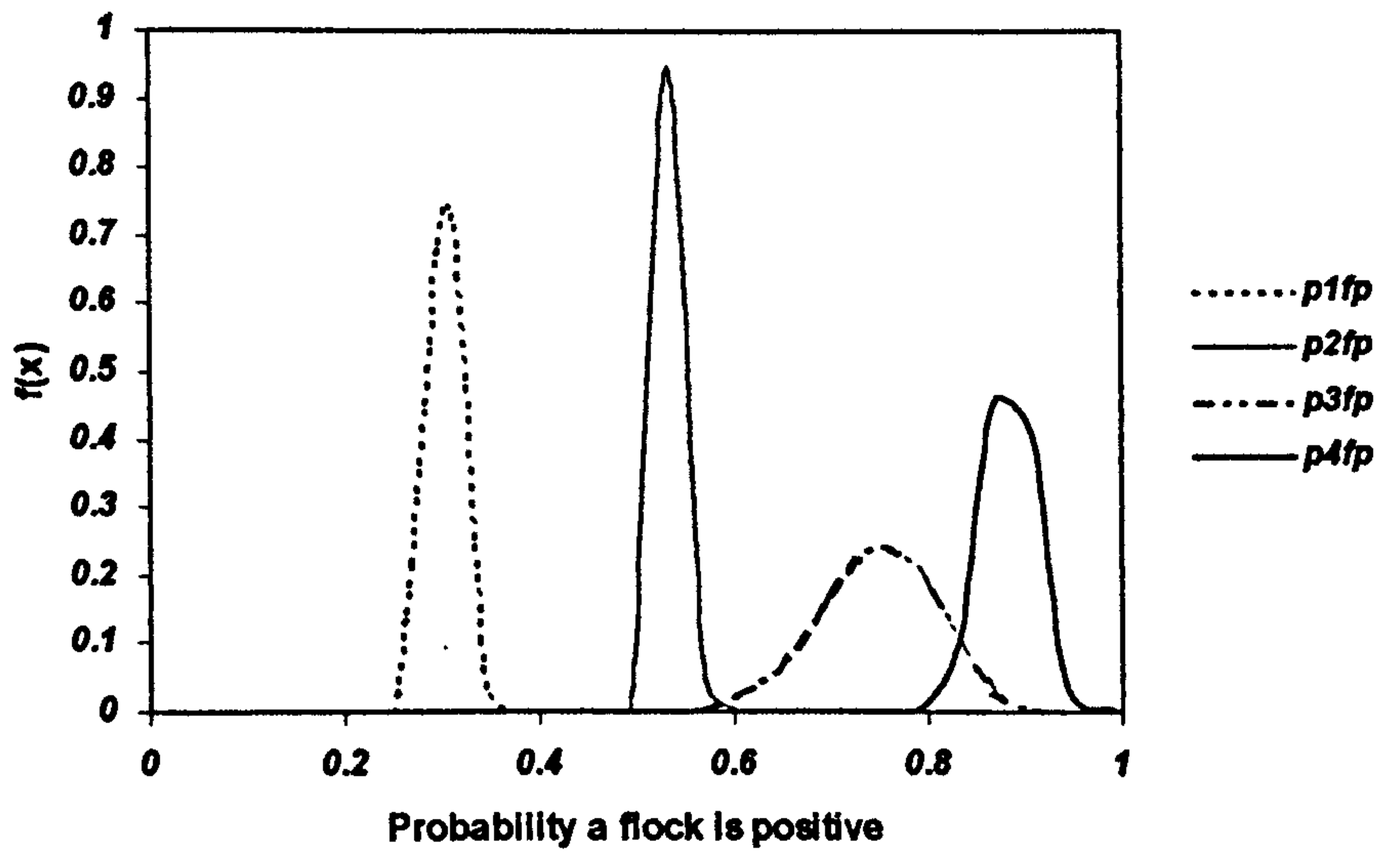


Figure 3.1: Graph to show the uncertainty distributions for the probability that a flock is positive for each of the data sources $P1_{fp}$, $P2_{fp}$, $P3_{fp}$, and $P4_{fp}$.

The prevalence of positive flocks based on each source, $P1_{fp}$, $P2_{fp}$, $P3_{fp}$, and $P4_{fp,}$ are weighted according to market share to give the overall flock prevalence, that is

$$P_{fp} = (P1_{fp}w1) + (P2_{fp}w2) + (P3_{fp}w3) + (P4_{fp}w4)$$

where *w*1, *w*2, *w*3 and *w*4 are the associated weights. The values for *w*1, *w*2, *w*3 and *w*4 are based on the companies market share using denominator data derived from MAFF statistics department (2000).

3.2.2 Estimating within-flock prevalence, P_{wfp}

Within-flock prevalence (WFP) is a measure based on the number of birds expected to be colonised with campylobacter within a positive flock. The WFP is directly related to the rate of transmission and is therefore a time dependent phenomenon for a positive flock. It has been reported that the within-flock transmission of campylobacter is rapid and that once campylobacter has been detected the WFP reaches 100% within seven days (Shanker *et al.*, 1990; Jacobs-Reitsma *et al.*, 1995), even in houses where bird movement is restricted (Shreeve *et al*, 2000). However the precise dynamics of campylobacter transmission in poultry flocks is poorly understood.

Mathematical models have been used previously to investigate the pattern of disease epidemics (Bailey, 1975; Fukuda *et al.*, 1984) in both human and animal populations. Here, a mathematical approach has been adopted to describe the transmission of campylobacter within a flock.

As discussed previously, poultry production is highly specialised and follows a defined structure (ACMSF, 1996). Briefly, when the birds are 1 day old they are taken to a broiler-growing farm, where they remain until they reach slaughter weight at ages between 30 and 60 days to become 'table birds'. At this point depopulation occurs; that is birds are removed from the house, and transported to the slaughter facility for processing to produce the sale product.

Upon arrival at the growing farms the birds are placed in a house where they form spatial clusters. This clustering effect is likely to be due to social factors. The display of social behaviour is common to fowl and has been well documented (McBride & Foenander, 1962; Collias *et al.*, 1966; McBride *et al.*, 1969; Wood-

Gush *et al.*, 1978; Tribe, 1980; Pamment *et al.*, 1983) and experimental work suggests a similar social behaviour is displayed by birds in the commercial rearing environment (Preston & Murphy,1989). The area explored by a given bird diminishes with age (Preston & Murphy, 1989) thus enhancing the clustering effect. This reduction can be attributed to the increase in size of birds in a fixed environment.

The transmission of campylobacter in a flock is believed to begin with a single bird becoming colonised. The mechanism by which a single bird becomes colonised and the time at which this occurs is unknown. As discussed previously case-control studies have demonstrated several reservoirs to which a flock may be exposed. These include wild birds, rodents, and cross-contamination from the environment via farm workers (Annan-Prah & Janc, 1988; Engvall *et al.*, 1986).

Following colonisation of the first bird within the flock, it is likely that transmission will initially be confined to the cluster in which this bird resides. During this process campylobacters are excreted in the faeces of positive birds. As broilers are coprophagic this leads to ingestion of the organisms by other birds in the flock and hence bird to bird transmission. As well as bird to bird transmission, excretion of the organism results in the contamination of the feed and water. In a short time period (4 days) a threshold will be reached where the contamination level of feed and water is sufficient to cause extensive colonisation in birds as a result of the ingestion of these products. This allows for dissemination of campylobacters throughout the whole flock until all birds are colonised.

Given this description of transmission, it is appropriate to model the time dependent process of flock colonisation in two stages. The first stage is the initial transmission within the cluster containing the first bird that is colonised, and the second stage is the transmission throughout the remainder of the flock.

Within this model it is assumed that the first bird becomes colonised at a time $t = t_{ex}$. This time is defined as the age at first successful exposure of a bird in the

flock, that is, exposure which leads to colonisation with campylobacter. This time is set to zero, that is $t_{ex} = t_0$. Stage 1 is described by a modified chain binomial model until a threshold time is reached. Experimental studies have shown that, following colonisation of the first bird, campylobacters can be detected in the feed, water and litter after 3 days (Shanker *et al.*, 1990). It is therefore assumed that the levels of contamination become sufficient to allow widespread dissemination of the organism throughout the flock 4 days following colonisation of the first bird. Thus a model for simple epidemic spread can be used to represent the second stage of the colonisation process. Thereafter transmission continues until either all birds become colonised or depopulation occurs at time t_A. Each of these stages can be described by the following models.

Stage 1: Chain Binomial

In 1982 Reed & Frost developed chain-binomial models of epidemic spread (Jacquez, 1987). Although this work was not published, the theory was popularised by Bailey (Bailey, 1975) and these models have frequently appeared in the literature, for example to study HIV epidemics (Ng & Orav, 1990). The initial transmission of campylobacter within a flock is described using such a model (Bailey, 1975; Jacquez, 1987). Such a model is deemed appropriate when the data available for parameter estimation are measured in discrete time (Bailey, 1975) as in the occurrence of colonised birds within the cluster containing the first positive bird.

In the situation presented here the basic chain binomial model describes the colonisation of a random susceptible bird which becomes colonised after a fixed constant time. The colonised bird is then removed from the susceptible population. New cases occur within the cluster in distinct groups at each time point, as described by the recurrence equation (3.2)

$$I_c(t+1) = I_c(t) + NI_c(t+1) \qquad (3.2)$$

where $I_c(t)$ is the number of colonised birds in the cluster at t, and $NI_c(t+1)$ is the number of newly colonised birds in the period $(t, t+1]$ where $(t, t+1]$ is defined as

one day. The number of newly colonised birds at each time point will follow a binomial distribution which depends upon the probability that any susceptible bird in the cluster becomes infected in time $(t, t+1]$, that is $p(t)$. Following on from this, the binomial likelihood for $NI_c(t+1)$ can be written as:

$$P\left[NI_c(t+1)=x_{t+1}, NI_c(t)=x_t, \ldots, NI_c(1)=x_1 \middle| I_c(0)=x_0\right]=\prod_i P\left[NI_c(i)=x_i \middle| H(i-1)\right]$$

where this binomial likelihood is given by the binomial probabilities dependent on $p(t)$, the probability that a susceptible bird becomes colonised in the period $(t, t+1]$, and $H(t)$ can be described as the history of the epidemic up to that point. More specifically

$$P\left[NI_c(t+1)=x_{t+1} \middle| H(t)\right]=\binom{S_c(t)}{x_{t+1}} p(t)^{x_{t+1}}\left[1-p(t)\right]^{S_c(t)-x_{t+1}}$$

$$H(t)=\left\{NI_c(t)=x_t, NI_c(t-1)=x_{t-1}, \ldots, NI_c(1)=x_1, I(0)=x_0\right\}$$

where $S_c(t)$ is the number of susceptible birds in the cluster at time t.

When considering transmission of campylobacter within a flock, the probability that a bird becomes colonised is dependent upon the transmission rate, the social need to make contact with other birds, and the probability of contact with a colonised bird. The generic form of the chain binomial model assumes a randomly mixing population, that is, a given bird would be equally likely to make a contact with every infected bird (Jacquez, 1987). In reality, commercial flocks can be many thousands in size, hence random mixing is not a reasonable assumption. However, by assuming a bird moves around a limited number of birds, defined as a cluster, and by considering the number of birds a given bird comes into contact with, and the number of times contact is made, we are able to model the spread of infection in a small neighbourhood. The basic chain binomial model described above is then modified to include these factors. Such a modified chain binomial model has been used previously by Ng & Orav (1990) to describe the transmission of HIV within a male community. Within this work the number of sexual partners an individual had

and the number of times sexual contact was made were considered. Within the present problem each sexual partner is analogous to the number of birds a given bird makes contact with, and each sexual contact is analogous to the number of times contact is made with each bird. Use of the modified model requires several assumptions (Ng & Orav, 1990):

(i). The total cluster size remains constant i.e. $S_c(t)+I_c(t)=n_c$ for all values of t where n_c is the total cluster size;

(ii). A bird, which becomes colonised at time t, cannot transmit the organism to another bird until time $t+1$, this allows for a fixed latent period of one day;

(iii). Birds within the cluster act independently; and

(iv). Each non-colonised bird has the same probability of being colonised at time t

Let b equal the probability of transmission given a single contact of a susceptible bird with a colonised bird, A equal the number of birds a given bird comes into contact with in one day, that is $(t, t+1]$ and R equal the number of times the bird is contacted by each of the A contacts in $(t, t+1]$. The parameters A and R are random variables which have probability density functions given by

$$P(A=a)=f(a)$$
$$P(R=r)=g(r)$$

Within the model probability generating functions are used for A and R as they are easier to manipulate (Jacquez, 1987). The associated probability generating functions are given by

$$\Phi_A(s)=E(s^A)=\sum_{a=0}^{\infty} f(a)s^a \qquad 0\le s\le 1$$
$$\Phi_R(s)=E(s^R)=\sum_{r=0}^{\infty} g(r)s^r$$

From the work of Ng and Orav (1990) assuming independence of individual birds, the probability that a susceptible bird becomes colonised in the period $(t, t+1]$, $p(t)$, is derived as follows:

$$P(\text{no transmission occurs} \mid \text{contact with one colonised bird}) = (1-b)$$

$$P(\text{no transmission occurs} \mid R \text{ contacts with one colonised bird}) = \Phi_R(1-b)$$

$$P(\text{no transmission occurs} \mid R \text{ contacts with a random bird in cluster})$$

$$= 1-\left\{\left[\frac{I_c(t)}{n_c(t)}\right]\left[1-\Phi_R(1-b)\right]\right\}$$

Therefore, the probability that a susceptible bird becomes colonised in the period $(t, t+1]$, $p(t)$, is given by equation (3.3)

$$p(t)=1-\Phi_A\left[1-\left\{\left[\frac{I_c(t)}{n_c(t)}\right]\left[1-\Phi_R(1-b)\right]\right\}\right] \qquad (3.3)$$

This can be written equivalently without the use of generating functions:

$$p(t)=1-\sum_a f(a)\left\{1-\frac{I_c(t)}{n_c(t)}\left[1-\sum_r g(r)(1-b)^r\right]\right\}^a$$

It is assumed that the variable A, the number of contacts a bird makes with an individual in one day follows a binomial distribution i.e. Binomial(n_c,P_c) where P_c is the probability that contact is made with another bird. Also it is assumed that the variable R, that is the number of times that a bird makes contact with a given bird follows a Poisson distribution, i.e. Poisson(y), where y is the mean number of times contact is made with each bird. In this way the number of contacts is limited to be equal to or less than the cluster size, but the number of times contact is made is theoretically unbounded.

The generating functions for the number of contacts made, Φ_A and the number of times contact is made with each bird, Φ_R are therefore given by

$$\Phi_A = \left(1 - P_c + P_c s\right)^{n_c}$$

$$\Phi_R = e^{(-y(1-s))}$$

Thus substituting these generating functions into equation (3.3), the probability that a non-colonised bird becomes colonised in one day, that is *p(t),* is given by:

$$p(t) = 1 - \left[1 - P_c\left(\frac{I_c(t)}{n_c(t)}\right)\left(\frac{1-\exp^{-yb}}{1-\exp^{-y}}\right)\right]^{n_c}$$

The mean number of newly colonised birds is then given by:

$$NI_c(t+1) = p(t)S_c(t)$$

The way in which *p(t)* and the resultant prevalence of positive birds within a given cluster varies over time is illustrated in Figure 3.2. In this illustration the mean number of times contact is made with another bird (y) is 5 contacts per bird per day, n_c the number of possible contacts (n_c) is 109 contacts per day, where 109 is the mean value of the distribution, and the probability that contact is made (P_c) is 0.07. It can be seen that as the prevalence within a cluster increases so does *p(t)*. Further, the probability that a bird becomes colonised is greater than the prevalence of positive birds in the cluster. This is due to the occurrence of multiple contacts per bird with another bird.

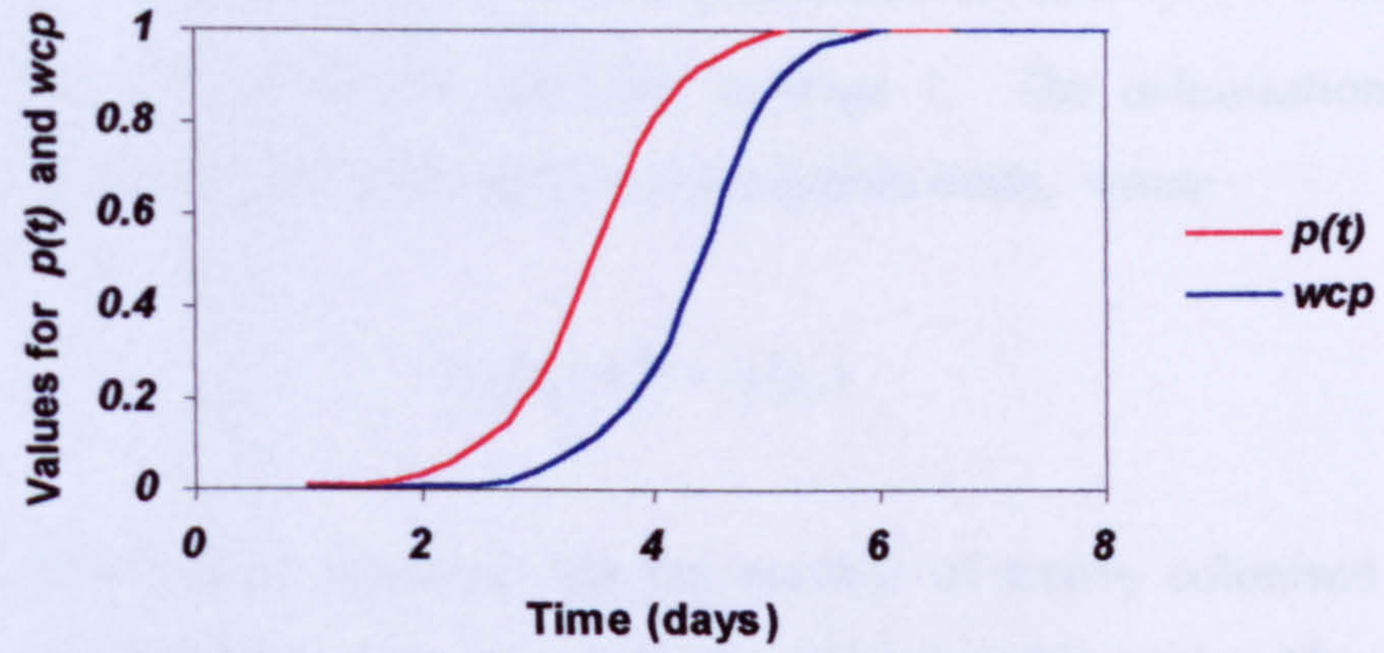


Figure 3.2: Graph to show the way in which the probability that a non-colonised bird will become colonised, $p(t)$, and the resultant prevalence of campylobacter positive birds within a cluster, *wcp*, for a given cluster vary over time. Here the mean number of times contact is made with each bird is 5 contacts per bird per day, the number of possible contacts is 109 contacts per day and the probability that contact is made is 0.07.

Stage 2: Epidemic spread

As previously discussed during the process of campylobacter colonisation within a flock, a threshold time is reached when the water and feed become contaminated. This threshold normally occurs 4 days after the first bird in the cluster becomes colonised and colonisation rapidly spreads throughout the remainder of the flock. Thereafter, stage 2 begins at time $t=t_5$. In the second stage it is assumed that the number of newly colonised birds at any time point is dependent upon the initial number of colonised birds, that is, the number of birds colonised within the cluster, at the time when stage 2 begins ($I_c(t_4)$) and the transmission rate. Under this

assumption, the colonisation process in stage 2 can be represented by a simple epidemic model.

It is assumed that in stage two, n is the total population size and $I_c(t_4)$ is the number of colonised birds in the cluster modelled in stage 1. The colonisation process begins with $I_c(t_4)$ colonised birds and $S_B(t_4)$ susceptible birds, where

$$S_B(t_4) = n - I_c(t_4)$$

In any time period, it is assumed that the number of newly colonised birds is proportional to both the numbers of colonised and susceptible birds. Therefore the process can be described by the differential equation (3.4)

$$\frac{dS_B}{dt'} = -b_B S_B(t')[n - S_B(t')] \qquad (3.4)$$

where $S_B(t)$ is the number of susceptible birds, b_B is the biological transmission and t' is equal to $(t-4)$ where the value 4 is the time in days until the second stage begins. By incorporating t' into the differential equation the result is a small lag in the overall epidemic curve at the point when the change occurs from the first to the second stages of the model. This is biologically consistent as the organism changes mode of transmission, from bird to bird to environmental transmission via feed and water. The transmission probability, b_B is assumed to be proportional to the transmission probability b. This assumption is made because in the second stage, transmission occurs both directly and indirectly from bird to bird. In the indirect case, colonised birds contaminate feed and water which then leads to exposure and subsequent colonisation of susceptible birds. Thus the probability of transmission in stage 2 is related to the probability of transmission in stage 1. The constant of proportionality is calculated as $\frac{1}{10n}$.

Solving (3.4) for the number of susceptibles gives equation (3.5) (Bailey, 1975)

$$S_B(t') = \frac{S_B(t_4)n}{S_B(t_4) + I_c(t_4)\exp^{[nb_B t']}} \qquad (3.5)$$

After completion of the first and second stages the total number of colonised birds within a flock *I(t)* is given by

$$I(t) = n - S_B(t)$$

Therefore the within-flock prevalence at time *t* since the time of exposure can be calculated directly from equation (3.6):

$$P_{wfp}(t) = \frac{I(t)}{n} \qquad (3.6)$$

A schematic representation of the overall model used to estimate the within-flock prevalence of a positive flock at slaughter, P_{wfp}, is shown in Figure 3.3.

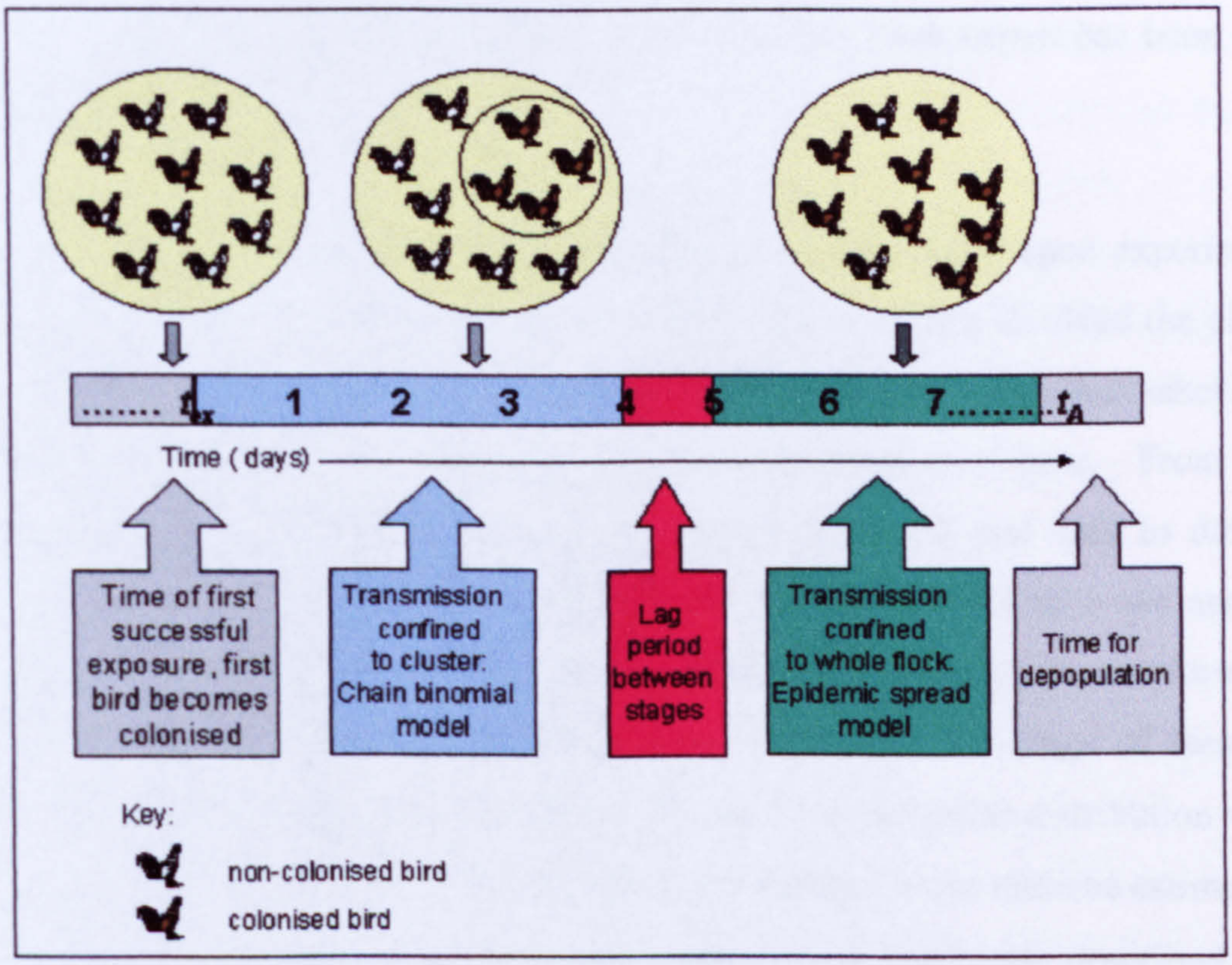


Figure 3.3: Schematic representation of the model to estimate the within flock prevalence of a campylobacter positive flock at slaughter, P_{wfp}.

3.3 Parameter Estimation and Simulation

The parameters and their estimated distributions are listed in Table 3.1. There is extensive published work on campylobacter, however the number of studies that investigate the dynamics of within flock transmission of this organism is limited. As a result, values for A, R, and n_c are based upon expert opinion. Experts, including a veterinary epidemiologist, an avian ecologist and a broiler farm manager, selected for their experience with broiler flocks, were asked to provide estimates for minimum, most likely and maximum values for A, R, and n_c. These estimates have been used to define triangular distributions and opinions are combined within a discrete distribution as described in Vose (2000). More specifically, by using Discrete($\{E_1,E_2,\ldots,E_n\},\{w_{E1},w_{E2},\ldots,w_{En}\}$) where E_1, $E_2,\ldots,E_n$ are n individual experts opinions, defined by the associated triangular distributions, and w_{E1},

$w_{E2},\ldots,w_{En}$ are the associated weights of each opinion. Each expert has been given equal weighting.

The biological transmission rate for campylobacter, b, is based upon experimental studies (Stuart *et al.*, 1997; Shanker *et al.*, 1990). These studies involved the placing of a colonised bird in a group of un-colonised birds. Samples were then taken daily to measure the change in the number of colonised birds over time. From these studies two values for the transmission rate were estimated and used to define a uniform distribution; that is, all values between the two values of b are assumed equally likely to be the estimated value for a given flock. Ideally, more information is required, for example the most likely value of b within the range of these two values. If this information were available the use of a triangular distribution would allow values within the range to be weighted, providing a more realistic estimate for this parameter. The value of b_B is proportional to b as previously described. The proportionality factor is equal to $\frac{1}{10n}$. Due to the absence of data, experts in the area of the colonisation of chickens with campylobacter agreed this factor by inspection of the resulting epidemic curve.

Table 3.1: Probability distributions and associated parameter values used in the model to estimate the probability distribution for a random bird selected from the UK chicken flock being campylobacter positive at the point of slaughter

Parameter	Symbol	Probability Representation
Experimental Data		
Transmission rate per day	b	Uniform(0.1,0.3)
Expert Opinion		
Number of contacts a bird makes with other birds in one day	A	RiskDiscrete$(\{\alpha,\beta,\gamma\},\{P_\alpha,P_\beta,P_\gamma\})$ Where: α~Triang(12,100,500)* β~Triang(30,50,120)* γ~Triang(20,45,100)*
Number of times a bird comes into contact with a given bird in one day	A	RiskDiscrete$(\{\alpha,\beta\},\{P_\alpha,P_\beta\})$ Where: α~Triang(3,5,6)* β~Triang(2,6,8)*
Size of Cluster	n_c	RiskDiscrete$(\{\alpha,\beta\},\{P_\alpha,P_\beta\})$ Where: α~Triang(n/12,n/10,n/8)* β~Triang(100,300,1000)*
Industrial Data		
Flock size	n	Triang(7800,30750,41596)
Age at depopulation in days	t_A	Triang(28,42,64)
Age at first exposure to campylobacter in days	t_{ex}	Uniform(14,t_A)

* These parameters are represented by Triangular distributions based on expert estimates

The age at first successful exposure, t_{ex}, is an unknown parameter in the model. Several studies have shown that campylobacters are rarely isolated from commercial flocks under three weeks of age. One explanation of this is that the colonisation process probably begins with a single bird and it is possible that it takes time before positive birds are detectable in large commercial flocks. It is assumed that the time until the number of birds colonised is large enough to allow detection, after exposure to campylobacters, is one week. Therefore, the time of exposure, t_{ex}, is assumed to be a uniform random variable between fourteen days and the age at depopulation.

Finally, distributions for flock size (n), and time of depopulation (t_A) are derived directly from data involving several industrial sources that together are representative of approximately 50% of the national flock.

The simulation model was developed in the software package @RISK. To run the model, simulations were carried out in two parts. An initial assumption is made that prior to the time at first successful exposure to campylobacter and appearance of the first positive bird, t_{ex}, the within flock prevalence for a given flock is zero.

In the first part of the simulation the two stage model to calculate the within flock prevalence, P_{wfp}, is run. More specifically, values for t_{ex}, t_A, are randomly selected from the associated distributions (shown in Table 1) and used to generate the time to run the within-flock prevalence model. This is a result of the time of first successful exposure and the age at slaughter for a given flock, and is given by $t_{run} = t_A - t_{ex}$, where t_A is the age at slaughter, t_{ex} is the time of first successful exposure and t_{run} is the time for which the within-flock prevalence model is run. Values for n, b, R, A are then selected from the associated distributions and P_{wfp} estimated. This process is repeated 4,000 times. The result is a variability distribution for P_{wfp}. Therefore, for a random flock the within flock prevalence is the sum of each within flock prevalence weighted by the frequency of occurrence of that prevalence. More specifically

$$P_{wfp} = \sum_{i=1}^{i=4000} P_{wfp_i} f\left(P_{wfp_i}\right)$$

This is the equivalent of taking the mean of the distribution. Following on from this, for the second part of the simulation 1000 values for the flock prevalence, P_{fp}, are randomly selected from the distribution and P_{pb} is then calculated as shown in equation (3.1). The result is P_{pb} and the associated uncertainty distribution.

The number of iterations for the first simulation, that is to calculate P_{wfp}, was chosen according to when the model output mean was considered stable (Vose, 2000) that is when it varied less than 1% from the mean output at 5000 iterations. The variation from the mean at 10,000 iterations for a given number of iterations is shown in Figure 3.4. It can be seen that the model output stabilises at 4,000 iterations. The number of iterations for the second stage, calculation of P_{pb} was chosen to allow adequate selection of the range of values from the distribution for the flock prevalence, P_{fp}. Values above those selected did not result in any notable differences to output estimates for the model.

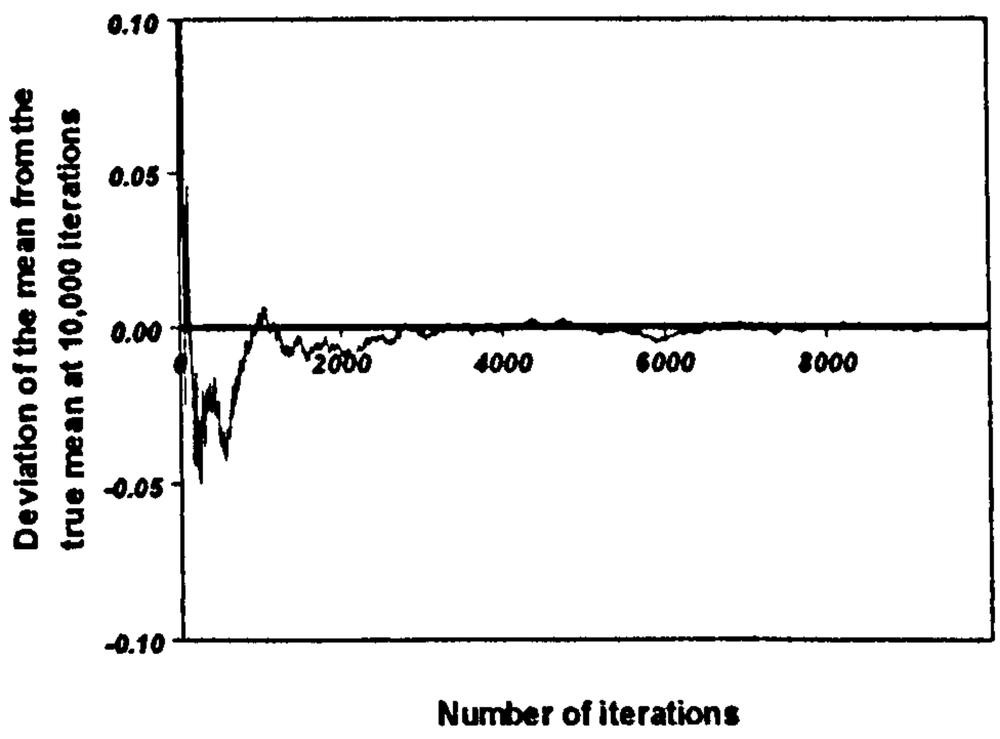


Figure 3.4: Graph to show the variation in the running mean of the probability that a bird will be campylobacter positive at slaughter, P_{pb}, from the mean of this probability for numbers of iterations up to 10,000.

3.4 Results

The density and cumulative uncertainty plots for the probability that a random bird is campylobacter positive, P_{pb}, using Latin Hypercube sampling, is shown in Figure 3.5. From the model results, although 25% of randomly selected birds from the national flock will have a probability of less than 0.52 of being campylobacter positive, the most probable value that a bird is positive is 0.53. Moreover, 25% of randomly selected birds from the national flock will have a probability in excess of 0.54 of being campylobacter-positive.

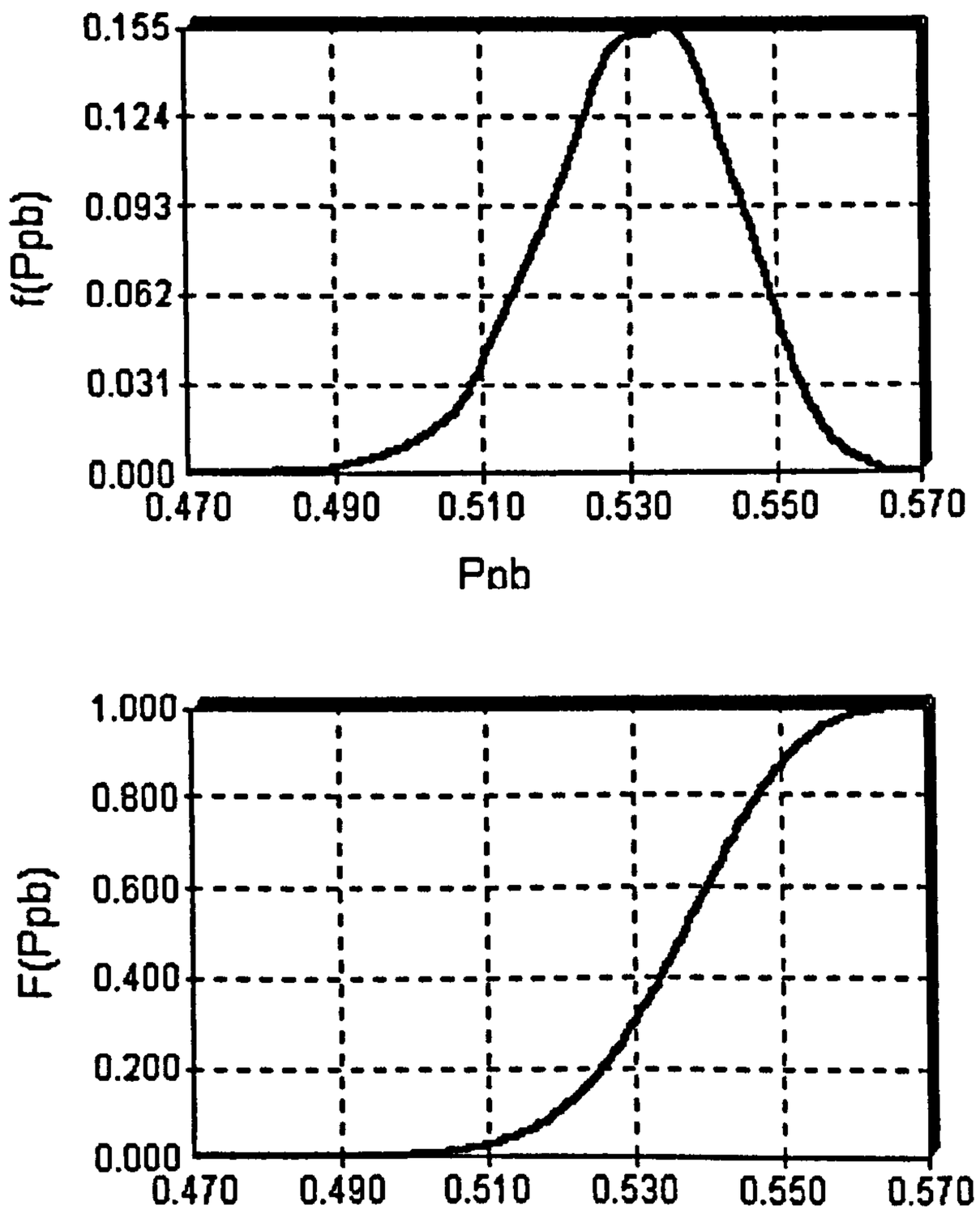


Figure 3.5: Density and cumulative plots for the probability a random bird selected at slaughter from the GB chicken flock is campylobacter positive.

3.5 Identification of Risk Control Points

The sensitivity of the probable within-flock prevalence at slaughter, P_{wfp}, to controllable variables was examined. More specifically the sensitivity analysis considered the relationship of P_{wfp} and the age at first successful exposure (t_{ex}), the transmission rate (b), cluster size (n_c), number of contacts made in one day (A), and the time of depopulation (t_A). For a given variable, t_{ex}, b, n_c, A, and t_A, the value was varied across the minimum and maximum values of the distribution while all other parameters remained as described by their associated distributions (Table 3.1). The relationship between the variables and P_{wfp} was determined upon the basis of a scatter plot matrix.

Scatter plot matrices allow the illustration of the interrelationships between several variables. As an illustration of how to interpret the information on a scatter plot matrix consider the situation where there are n variables for which an illustration of the relationship between them is required. The matrix consists of n^2 sectors. For any given variable $i\,(i=1,\ldots,n)$ the relationship between the variable and another variable j $(j=1,\ldots,n)$ is shown in the quadrant (i,j). As an illustration consider the matrix shown in Figure 3.6. Here we have 3 variables α, β, and γ and the matrix is divided into 9 areas (3^2). Therefore, from Figure 3.6 it can be seen that the relationship between α and β is shown in sector (1,2), and the relationship between α and γ is shown in sector (1,3).

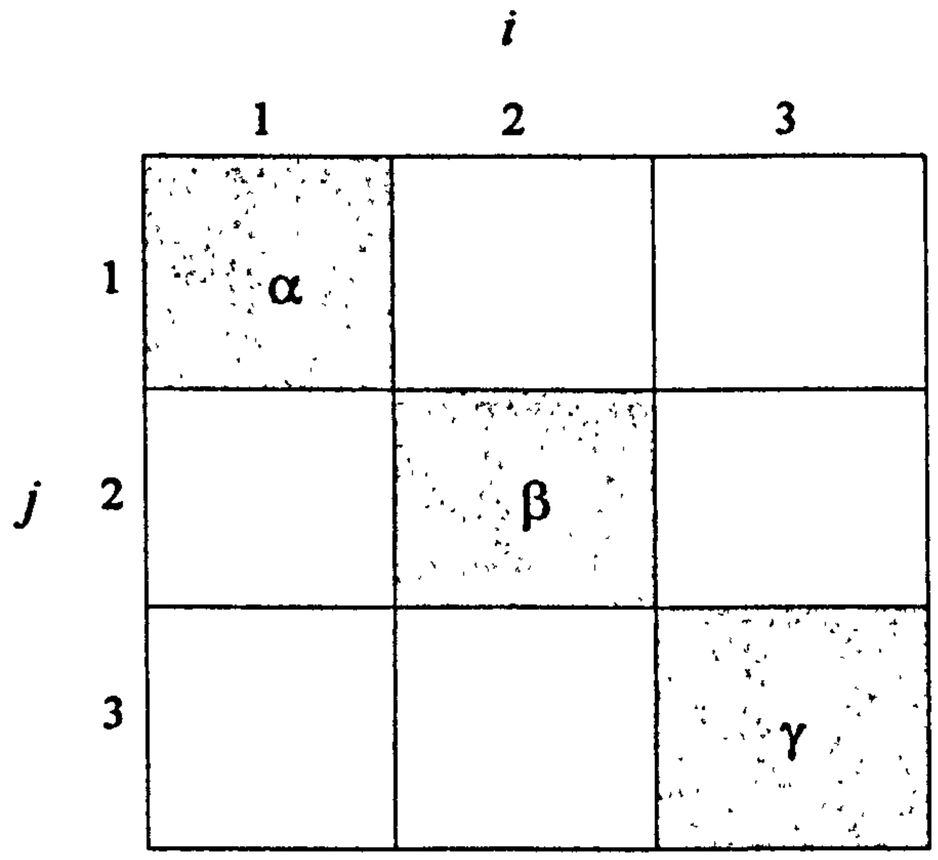


Figure 3.6: Illustration of how to interpret a scatter-plot matrix

The scatter plot matrix for P_{wfp}, t_{ex}, b, n_c, A, and t_A is shown in Figure 3.7. Examination of the matrix (Figure 3.7) shows, as required, no apparent interrelationship between variables b, N, and t_{run}. However it can be seen that P_{wfp} is sensitive to t_{run}, the time which the organism has to disseminate through the flock. Further, Figure 3.5 shows no distinct correlation between the transmission rate and flock size with P_{wfp} as P_{wfp} can still take any value between zero and one as the other variables adopt values from their allowable ranges determined by the associated distributions.

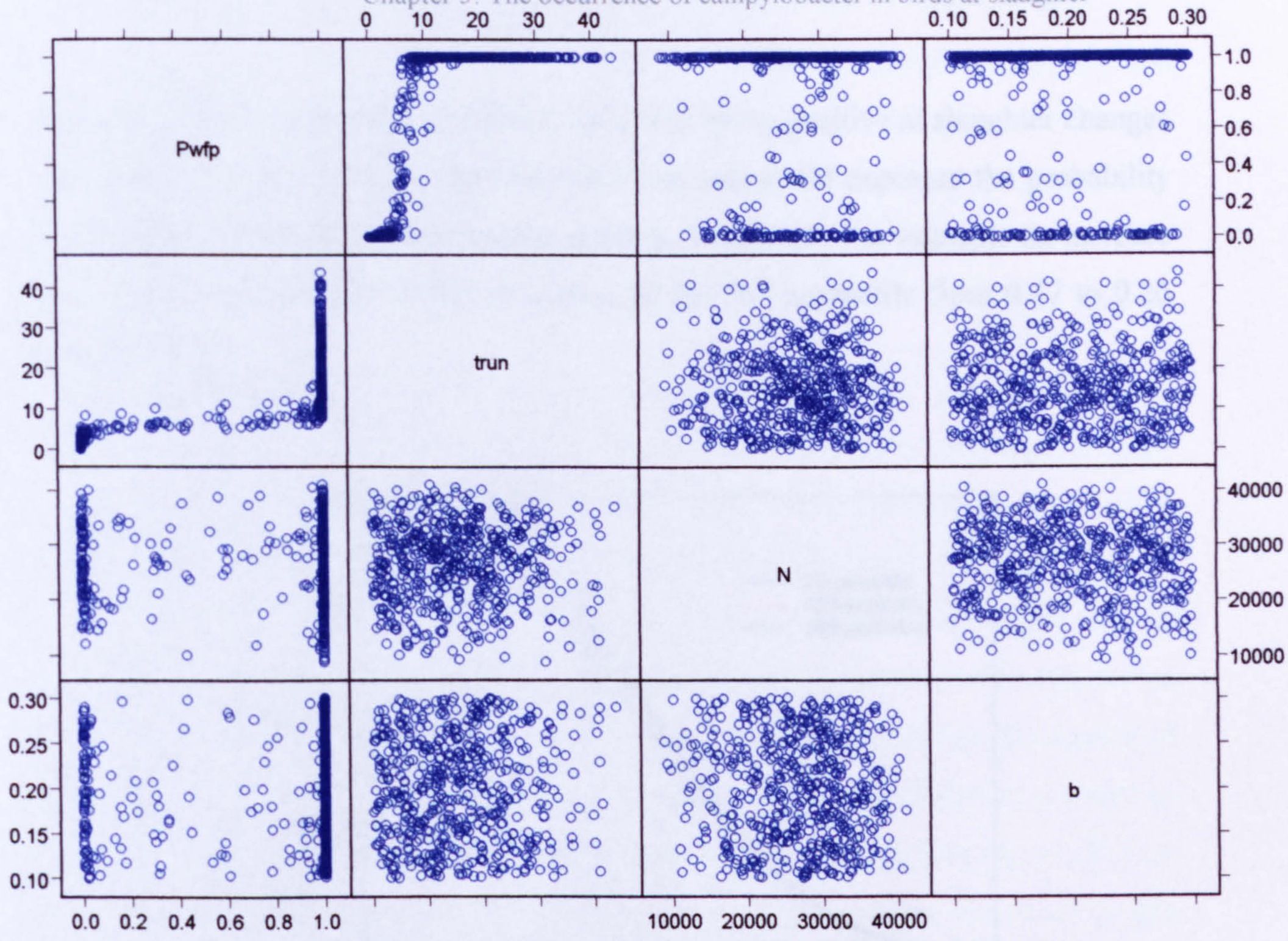


Figure 3.7: Scatter plot matrix showing the relationship between the variables P_{wfp} t_{run}, N, and b.

From equation 3.1 it can be seen that as P_{wfp} increases so does P_{pb}, the probability that a bird is campylobacter positive. Given the relationship between t_{run} and t_{ex}, the way in which P_{pb} varies with t_{ex} is shown in Figure 3.8. From this it can be seen that as the age at first successful exposure (t_{ex}) increases the probability that a bird is campylobacter-positive can be reduced. This is a reflection of the time at which depopulation occurs. As the time till first successful exposure increases the number of days until depopulation decreases thus reducing the chance of flock colonisation resulting in a diminished within flock prevalence. Similarly a reduction in the time to depopulation, t_A, also results in a reduced P_{pb}.

The model indicates that the probability of a bird being positive at slaughter changes over time. Thus, by delaying the time until first successful exposure the probability of a random bird being campylobacter positive is reduced. For example an increase in t_{ex} from 30 to 38 days results in a drop in the 50th percentile from 0.57 to 0.26 (Figure 3.8).

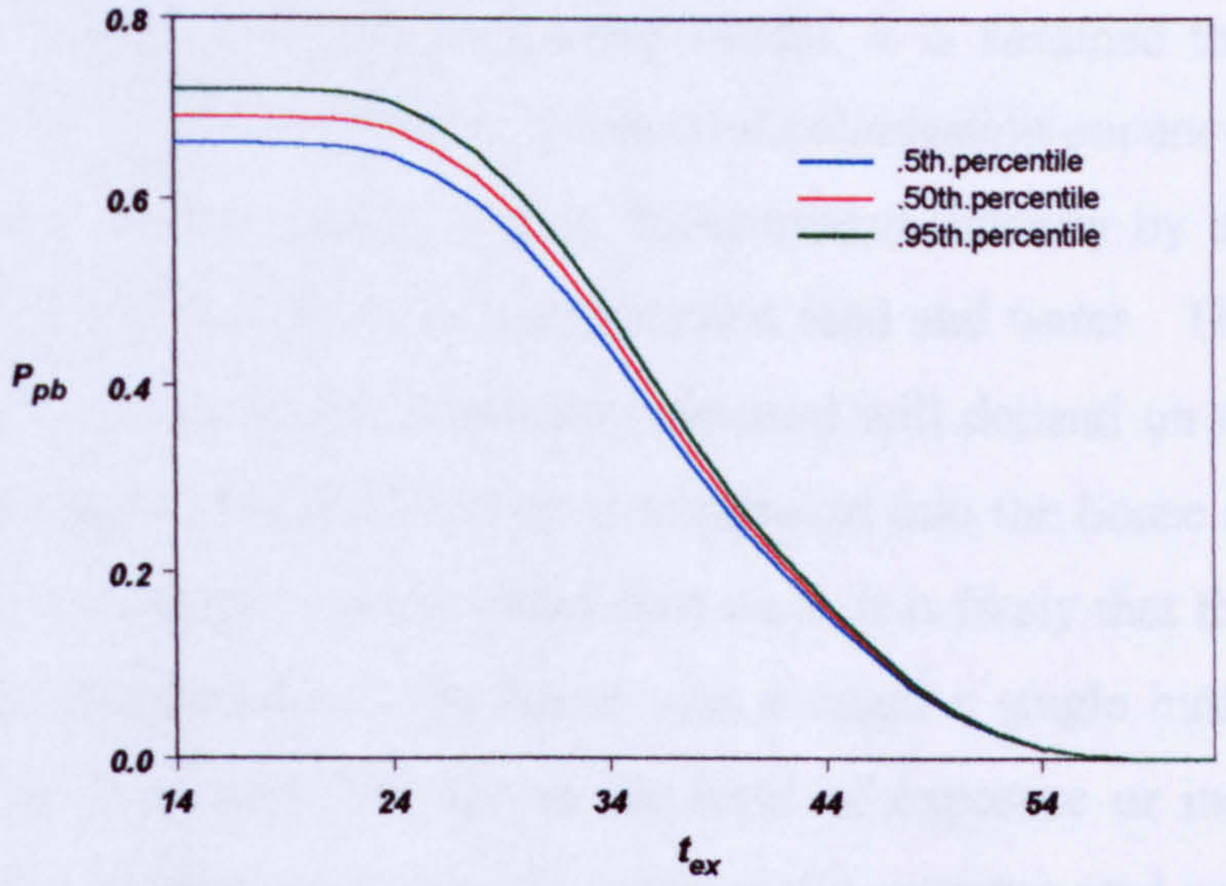


Figure 3.8: The sensitivity of the probability that a random bird is campylobacter positive (P_{pb}) to the time of first exposure (t_{ex})

This reduction suggests that this is a potential critical control point. This is an intuitive result as delaying the time at which a flock becomes positive will reduce the number of birds which become colonised before the flock is removed for slaughter. Previous reports indicate that the implementation of strict biosecurity measures, such as boot dips and sanitary barriers (Evans, 1996) can delay the time until first successful exposure. However, the successful application of such measures in the day-to-day workings of a poultry farm is difficult, with compliance by staff difficult to monitor (Shreeve *et al.*, 2000). Thus delaying the time until first successful exposure may prove an impractical strategy. An alternative is to reduce the age at depopulation that also reduces the probability a random bird is positive.

Although this is also a potential critical control point, given market demands on size and weight of table birds at sale, this is not a feasible option.

3.6 Possible model modifications

The assumptions on which the model that represents the process of flock colonisation, that is estimation of P_{wfp}, are based are important in the interpretation of the generated results. Within the current model, it is assumed that a flock is initially comprised of birds in clusters. Successful colonisation occurs from a single bird in one cluster. The organism is then disseminated, initially by direct contact with the colonised bird and then via contaminated feed and water. The validity of the assumption that a single bird becomes colonised will depend on the source of infection. For example if campylobacter is introduced into the house as a result of farm staff with, for example, contaminated foot wear, it is likely that there will be a point source of contamination in the house. As a result a single bird near to this point will become colonised first due to the level of exposure or individual bird characteristics such as immune status. In contrast if a contaminated water supply is the source of flock infection, the situation is somewhat different. This can be described as follows.

Campylobacters are frequently isolated from water sources and contaminated water has been associated with human outbreaks of campylobacteriosis (Vogt *et al.*, 1982). If a flock is exposed to contaminated water multiple colonised birds will initiate the colonisation process. Homogeneous mixing could be expected as the water is circulated through the house. This could be described by use of the differential equation for epidemic spread, that is equation (3.4), and disregarding the chain binomial model.

In addition it is debatable whether vertical transmission of campylobacters can occur (Cox *et al.*, 1999; Jacobs-Reitsma, 1997). Certainly if vertical transmission does occur it is likely to be an infrequent event with only up to 10 out of 1000 birds being

colonised via this route. Such an occurrence would result in multiple colonised birds and multiple initial clusters containing colonised birds. This can be modelled by use of multiple chain binomial models (Ng & Orav, 1990).

It can therefore be seen that such modifications could impact on the current model results. To investigate the impact of such modifications three situations are considered and the results presented for comparison.

3.6.1 Source of infection is contaminated feed, litter and/or water

When the source of campylobacter that a flock is exposed to is contaminated feed and/or water it can be assumed that the whole flock will be exposed. In this situation there will be random appearance of colonised birds beginning from the time that the feed and/or water enters the house. This is the situation presented above in stage two of the model and therefore the colonisation process can be described by use of the following equation

$$\frac{dS_B}{bt} = -b_B S_B(t)[N - S_B(t)]$$

which when solved for the number of susceptibles yields

$$S_B(t) = \frac{S_B(t_4)N}{S_B(t_4) + I_c(t_4)\exp^{[Nb_B t]}}$$

It can be seen that this is analogous to equation 5 except the equation is differentiated with respect to t as opposed to t'. This is because here the differential equation is used in isolation, there is no first stage of transmission to consider.

3.6.2 Source of infection is via vertical transmission

It is currently debatable whether or not vertical transmission occurs. However if this mode of transmission is possible it has implications regarding the model described in this chapter. Initially there will be a number of birds that are colonised and as such begin the infection process. Each of these birds will then initiate colonisation of its social cluster and consequently colonisation of the whole flock.

Consider a flock that initially has i birds which become colonised at some time as a result of vertical transmission. This results in i clusters initiating the colonisation process of the flock. Therefore, an assumption is made that each cluster begins with one colonised bird. Assuming that each cluster acts independently, the probability that a bird will become colonised in one day in cluster i, that is $P(t)_i$, is given by

$$p(t)_i = 1-\left[1-P_i\left(\frac{I_i(t)}{n_i(t)}\right)\left(\frac{1-\exp^{-y_i b}}{1-\exp^{-y_i}}\right)\right]^{n_i}$$

where $I_i(t)$ is the number of colonised birds in cluster i, n_i is the total number of birds in cluster i, P_i is the probability that contact is made with another bird in cluster i, and y_i is the mean number of times contact is made with each bird in cluster i. The number of colonised birds at time t is then given by

$$NI_c(t) = \sum_{i=1}^{n} p(t)_i S_i(t)$$

where $NI_c(t)$ is the total number of newly colonised birds to appear in t in all the clusters in the flock, and S_i is the number of susceptibles in cluster i. Following on from this stage 2 therefore begins with $NI_c(t_4)$ colonised birds. Stage 2 is then as described in section 2.

For comparison the uncertainty distribution for the probability that a bird will be campylobacter positive if selected at random from the GB broiler flock at slaughter assuming the current model framework, assuming flock colonisation results form vertical transmission and assuming that flock colonisation results from contaminated feed and litter is shown in Figure 3.9. Further, summary statistics for each of these situations is shown in Table 3.2.

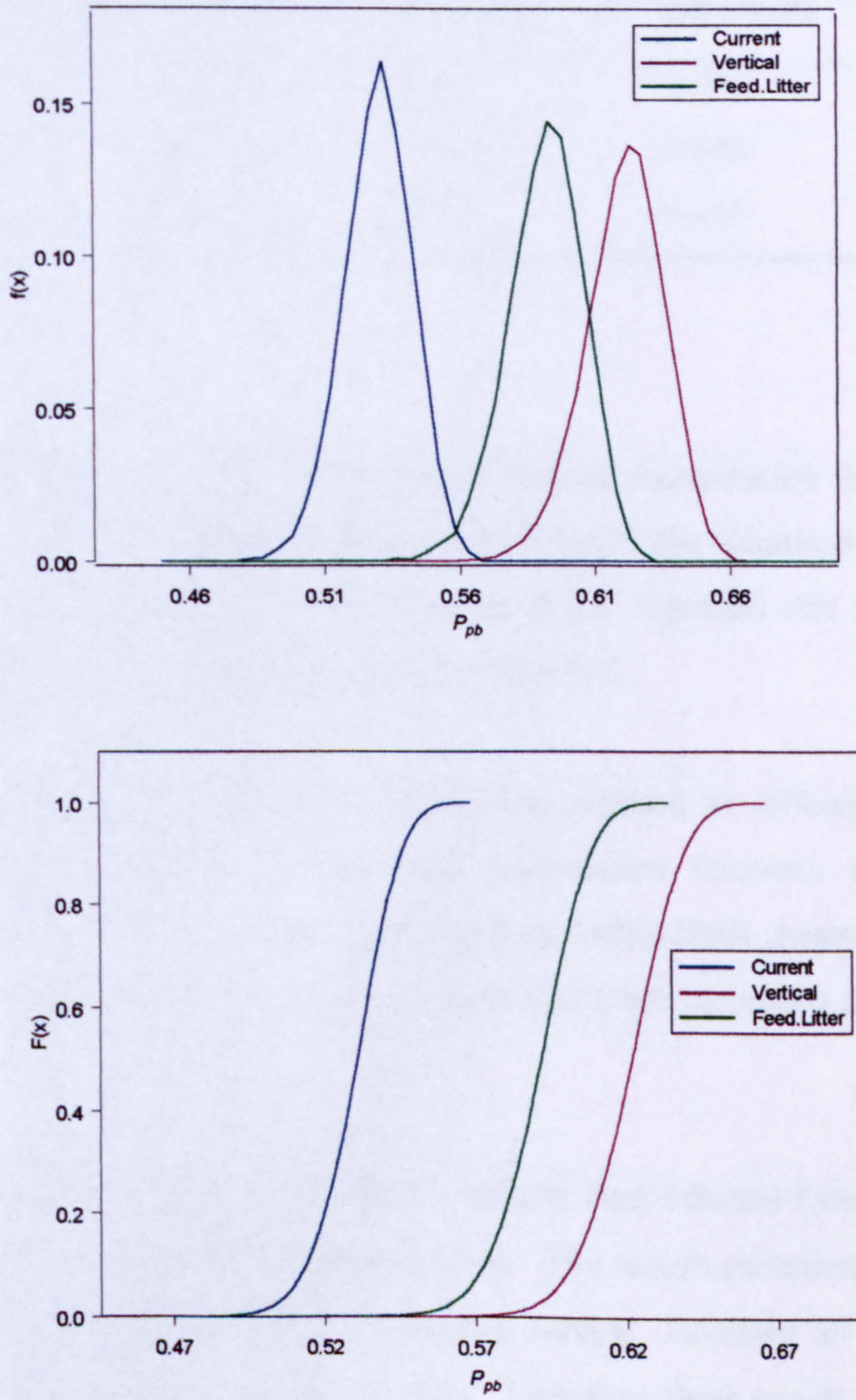


Figure 3.9: Density and cumulative plots for the uncertainty distribution for the probability that a bird is positive at slaughter under the current model assumptions,

Flock colonisation due to vertical transmission and flock colonisation as a result of contaminated feed and water

Table 3.2: Summary statistics for the probability that a bird is colonised at slaughter in GB under current model assumptions, Flock colonisation due to vertical transmission and flock colonisation as a result of contaminated feed and water.

Modification	5th Percentile	50th Percentile	95th Percentile	Mean P_{wfp}
Current	0.509	0.531	0.55	0.764
Vertical	0.596	0.622	0.643	0.895
Feed/litter	0.567	0.592	0.613	0.852

It can be seen that if flocks are colonised via vertical transmission the probability that a bird is positive at slaughter is greater than if flocks are colonised as a result of contaminated feed and litter or another source of the organism that results in the colonisation of the flock being initiated by a single bird.

At present the frequency with which flocks are exposed to different sources of campylobacter is unknown. Once such information becomes available the incorporation of source of organism and resulting within-flock dynamics weighted by frequency of occurrence may lead to a model that more accurately represents real life.

The model considers the probability that a random bird selected from the national slaughter flock will be campylobacter positive. The results presented here can be validated by the implementation of an abattoir survey. However all birds from a given flock will go through the same abattoir. Therefore, these results should not be interpreted as estimating the likely probability of a random bird being campylobacter positive within one abattoir, rather it estimates random selection from the whole national flock.

3.7 Conclusions

The sources of colonisation for broiler flocks are currently unknown. Epidemiological investigations have implemented break down in biosecurity, contaminated water supplies and infected rodents and insects. However, for many flocks the source of campylobacter remains unknown. Despite this, once a flock has been exposed the entire flock can become colonised in as little as seven days. In this chapter a model is presented which describes the colonisation of a broiler flock with campylobacter over time. This model assumes that the colonisation process begins with a single bird. The colonisation of the flock is initiated by the colonisation of the birds which the first positive bird makes contact per day, that is the bird's social cluster. This process is represented by the use of a modified chain binomial model. It has been reported that four days following the initiation of flock colonisation the levels of campylobacter in the environment of the house is sufficient to result in the colonisation of a bird. At this point colonised birds appear at random throughout the flock. Here, a differential equation model, traditionally used to represent the epidemic spread of infectious diseases, is employed. This two-stage model is combined with an estimate of the prevalence of positive flocks in the national flock, the result being an uncertainty distribution for the probability that a bird will be campylobacter positive at the point of slaughter.

Model results indicate that 50% of randomly selected birds from the national flock will have a probability of between 0.52 and 0.54 of being campylobacter positive. The mean value for this probability is 0.53. Sensitivity analysis has indicated that the time of first successful exposure is a critical control point which can dramatically reduce this probability. However, given the current lack of knowledge regarding why flocks are exposed to campylobacter, this is a difficult factor to control. It may be that this, in conjunction with control points identified in later stages in the supply chain will result in a significant reduction in the level of human illness.

The basic model describes the colonisation of a flock, initiated by the colonisation of a single bird. However it may be that the colonisation process begins with multiple birds. In this chapter, modifications to the basic model are described. These are used to describe flock colonisation initiated as a result of vertical transmission and a situation where a large portion of the flock will be exposed such as contaminated feed, litter or water. Comparison of the probability a bird is positive at slaughter as estimated from the basic model and the modified versions show that when the source of colonisation is vertical transmission or contaminated feed, litter or water the probability that a bird will be positive at slaughter is increased. Currently, the frequency with which each of these model frameworks applies is unknown. However should the information become available, these could be weighted accordingly and potentially result in a more realistic estimate of the probability that a bird is positive for campylobacter at slaughter.

Chapter 4

The contamination of chickens on the farm, and during transport to the slaughter facility

4.1 Introduction

It is well recognised that the presence of pathogenic organisms in the gut of food-producing animals provides the potential to contaminate food products and hence result in exposure of the human population. As such, it is necessary to be able to quantify the level of campylobacter likely to be present in the gut of a colonised bird at the point of entry into the processing facility, that is, the point of slaughter. This will enable a full estimation of the risk posed to the population as a result of such colonisation. However, there is a further reservoir of organisms which may enter the supply chain, these are organisms which contaminate the exterior of the birds. Such organisms may also result in the contamination of food products, hence the levels of such contamination are also required to enable estimation of the risk to the human population of campylobacter infection from consumption of chicken meat/products.

When a bird becomes exposed to, and ingests a level of campylobacter, if the level is sufficiently high, the organisms will establish and reproduce within the gut of the bird. This process will continue until equilibrium is reached, that is, where the level of colonisation will be maintained and the bacterial population is stable. Once colonised, broiler birds remain colonised. This is referred to as maximal colonisation. The sufficient level to initiate this process, is currently unknown. The source of campylobacter on a farm is often undetermined during investigations into flock colonisation. Consequently, the levels of exposure in the farm setting are unlikely to be measured. Further, the viability of the organisms may be dependent upon the reservoir from which they originate, for example, organisms that originate from a contaminated puddle may be more efficient at colonising the gut than organisms from contaminated soil, or vice versa.

Once a bird is colonised with campylobacter it will excrete large numbers of campylobacters in faeces. Contact with the faeces of such bird is one mechanism by which the organisms spread throughout a flock, as discussed in Chapter 3. However, there is a second consequence of this excretion of organisms, namely the

contamination of the exterior of the birds. There are two important factors in the contamination of the exterior. Contamination that occurs while the birds are on the farm and contamination that occurs during the transportation to the slaughter facility. Together these contamination events lead to contaminated birds entering the slaughter facility. Such birds will be critical for the final level of contaminated products.

In this chapter, a model, which estimates the prevalence and levels of contamination with campylobacter at the point of birds entering the slaughter facility, is described. Estimation covers 3 related steps, the number of campylobacters within colonised birds, the level of contamination on the farm and finally the amount of cross-contamination during transport.

4.2 The occurrence of contamination

Given a bird is colonised, it is biologically consistent that such a bird will also be contaminated on its exterior due to, for example, contamination with the faeces that it excretes. Given this situation, a flock with a within flock prevalence of 1 will have all birds contaminated. In a flock which contains colonised birds but has a within-flock prevalence of less than 1, there is the opportunity for the birds which are not colonised to become contaminated on their exteriors. This can occur as a result of contact with either a colonised, and hence a contaminated bird, or contaminated faeces.

The probability that a non-colonised bird will become contaminated on its exterior can be expected to be related to the within-flock prevalence of the flock. This relationship can be derived by considering the within-flock transmission dynamics discussed in Chapter 3.

In brief, once a flock is exposed and a single bird is successfully colonised, transmission ensues amongst the bird with which the first colonised bird makes contact with on a daily basis, that is the birds social cluster. This continues until a threshold time is reached where the level of contamination in the feed, and water supply is sufficient to result in the colonisation of an exposed bird. From here onwards colonised birds appear randomly throughout the entire flock. This process continues until either all the birds become colonised or depopulation occurs and the birds are removed for slaughter.

Under the circumstances described above there are two stages of transmission. Contamination can also be defined in terms of these stages. The initial stage of within-flock transmission is transmission amongst the social cluster with which the first colonised bird interacts. Under such circumstances the probability that a random bird in the flock becomes contaminated on its exterior is assumed to be the probability that the bird is within the cluster containing the first positive bird. In this stage, the bird has the opportunity to come into contact with colonised and contaminated birds. Once transmission enters the second stage, colonised birds appear in a random fashion throughout the flock. It is now highly likely that a random bird will come into contact with either a contaminated bird or contaminated faeces. Therefore it is assumed that the probability that a bird is contaminated during this stage of transmission is equal to 1.

Once the birds in a given house have reached the desired slaughter weight the birds are caught, loaded onto a vehicle and transported to the slaughter facility. Commonly, the birds are loaded into baskets. The number of birds per basket is regulated by the Welfare of Animals Transport Act (Line *et al.*, 1997) and depends upon the weight of the birds. The baskets are grouped together in modules, each module containing three rows of four baskets. The modules are placed in the vehicle in two rows, stacked one on top of the other. In this chapter, two modules stacked on one another is referred to as a section. Each vehicle consists of between nine and eleven sections, depending upon the size of the vehicle. Each module has a solid metal floor, but the baskets are designed such that the floor of the basket allows any

excrement to pass away from the birds. One vehicle holds 5000 to 6000 birds, depending on the size of the vehicle and weight of the birds. Therefore, multiple vehicles are used for any given flock.

During transportation to the slaughter facility, the stress the process places on the birds results in changes in the consistency of the faeces. In particular, they become liquid in nature (Mulder, 1995). This causes contamination of the exterior of a large proportion of the birds in the transport vehicle, despite the metal sheeting separating the modules (Andrew Gibson, Personal Communication). More specifically, it is likely that in any given section of the vehicle, there will be contamination of the birds with faeces originating from the birds in rows above and also from the modules adjacent. However, in the current context, this contamination is only of interest if there are birds present which are excreting campylobacters. Therefore, the probability that a bird becomes contaminated during transport is a function of the number of rows that contain colonised birds and the location of these birds within the vehicle in relation to non-colonised birds.

When estimating the level of contamination on the exterior of a bird on arrival at the slaughter facility there are two distinct situations to consider. These are the transportation of a campylobacter positive flock and the resulting cross contamination that may occur within that flock, and the transportation of negative flocks. Within a campylobacter negative flock by definition there are no colonised birds, hence no birds are shedding the organism. Each of these situations will now be discussed in turn.

In this chapter, positive and negative flocks are considered separately, hence there are two distinct models. Each of these will now be described.

4.3 The contamination of positive flocks

Within a positive flock the level of cross-contamination that occurs during transport is governed by the location of the birds which are shedding the organisms within the vehicle. When a flock is caught and loaded onto the vehicle the lights in the house are dimmed such that bird movement is minimised. Therefore, the order in which the birds are loaded onto the vehicles is related to their location in the house. The birds nearest to the front of the house will be loaded on the first vehicle and the birds at the back of the house onto the last vehicle.

Initially, each flock is assigned a campylobacter status defined as θ_f where $\theta_f \in \{0,1\}$ such that $\theta_f = 1$ means that the flock is positive for campylobacter and $\theta_f = 0$ means that the flock is negative for campylobacter. The condition $\theta_f = 1$ occurs with probability P_{pf}, the probability that a random flock is campylobacter positive, therefore $\theta_f = 0$ occurs with probability $1-P_{pf}$.

It is assumed that the flock can be spatially represented within the house by an $a \times b$ lattice structure where A represents the horizontal distance, measured in number of birds, within the house and A represents the vertical distance, measured in number of birds. The total number of birds within the flock is $N = A \times B$. At a particular time t each bird in the flock is in a colonisation state defined as $c_x \in \{0,1\}$ where $x = (a,b)$, $a = 1,...,A$ and $b = 1,...,B$, such that $c_x = 1$ means that the bird at location x is colonised with campylobacter, and $c_x = 0$ means that the bird at location x is not colonised with campylobacter. For $t < t_{ex}$, $c_x = 0$ for all $x = (a,b)$. Once a bird is colonised, $c_x = 1$ it cannot change status as it remains colonised. Note that if $\theta_f = 0$ then $c_x = 0$ for all $x = (a,b)$ over all t.

At time $t = t_{ex}$, t is set to $t = 0$ and a random bird is located by selection of a random $a \times b$ location on the lattice. This bird is designated as the first bird to

become colonised within the flock, that is $c_x = 1$. The cluster to which this bird belongs is then marked on the lattice and each other bird in the flock is assigned a cluster status, defined as $cl_x \in \{0,1\}$, where $cl_x = 1$ if the bird is in the cluster, and $cl_x = 0$ if the bird is not in the cluster. For $t = 1,..,4$, where t is measured in days, a bird within the flock at any x location changes status with probability $p_c(t)$ given by

$$p_c(t) = \begin{cases} cl_x \times p(t) & c_x = 0 \\ 0 & c_x = 1 \end{cases}$$

where $p(t)$ is the probability that a susceptible bird in the cluster becomes colonised according to the chain binomial model, as described in Chapter 3.

Once $t > 4$, the number of birds that will change colonisation status is governed by the differential equation described in Chapter 3 (equation 3.4). Birds which become colonised are picked at random locations throughout the flock, sampling without replacement. Therefore, given a bird at location x the probability it will change status is given by

$$p_c(t) = \begin{cases} I_n(t)/N & c_x = 0 \\ 0 & c_x = 1 \end{cases}$$

Here $I_n(t)$ is the number of newly colonised birds at time t, $I_n(t) = I(t) - I(t-1)$ where $I(t)$ is calculated as described in Chapter 3. This process continues until $t = t_A$, the time for depopulation. Given a bird is colonised, the number of organisms in the gut of the bird is defined as Λ_g.

Consider the contamination of the exterior of a bird in position x at the point of depopulation. As described previously, the contamination status of a bird is

dependent upon both the cluster status of the bird and the stage of within-flock transmission. The contamination status of a bird in position x is defined as $CD_x \in \{0,1\}$ such that $CD_x = 0$ means that a bird in position x is not contaminated at depopulation, and $CD_x = 1$ means that this bird is contaminated at depopulation. This status is governed by the following condition

$$CD_x = \begin{cases} 0 & t \leq 4;\ cl_x = 0 \\ 1 & t \leq 4;\ cl_x = 1 \\ 1 & t > 4 \end{cases}$$

The result of the model described above is a co-ordinate for each bird in the flock and an associated colonisation status and contamination status at the point of depopulation. Given a bird is contaminated at depopulation, the level of contamination on the exterior is defined as Ω_d.

An illustration of the implementation of this model is shown in Figure 4.1. This demonstrates the use of the model in Excel with the model programmed using Visual Basic for Applications. The flock used to illustrate the model is assigned parameter estimates as shown in Table 4.1. This is not a representative flock, as a small flock size and cluster size are chosen for ease of illustration.

Table 4.1: Parameter values used to illustrate the spatial spread of campylobacter within a small flock represented by Figure 4.1.

Parameter description	Symbol	Parameter value
Flock size	n	8010
Cluster size	n_c	467
Number of contacts a bird makes in one day	A	109
Number of times a bird comes into contact with any given bird in one day	R	55
Probability of making contact with another bird	P_c	0.143
Biological transmission rate	b	0.2

In Figure 4.1 un-colonised birds are represented by yellow squares, un-colonised birds which are in the cluster containing the first positive bird are represented by blue squares and positive birds are represented by red squares. As presented in detail in Chapter 3, the transmission begins at $t = 0$ with a single bird becoming colonised. Following this, for $t < 5$ transmission is confined to the cluster, and the increase in the number of colonised birds within the cluster for this time can clearly be seen from Figure 4.1. In this particular case the whole cluster becomes colonised by $t = 3$. This is because as the flock is unusually small, the cluster size is also small and as such, less time is required for the birds in that cluster to become fully colonised. Once $t = 5$ transmission now involves the whole cluster and the random appearance of colonised birds throughout the flock, as predicted by the model, can be seen in Figure 4.1. This process continues until either the whole flock is colonised or the flock is depopulated

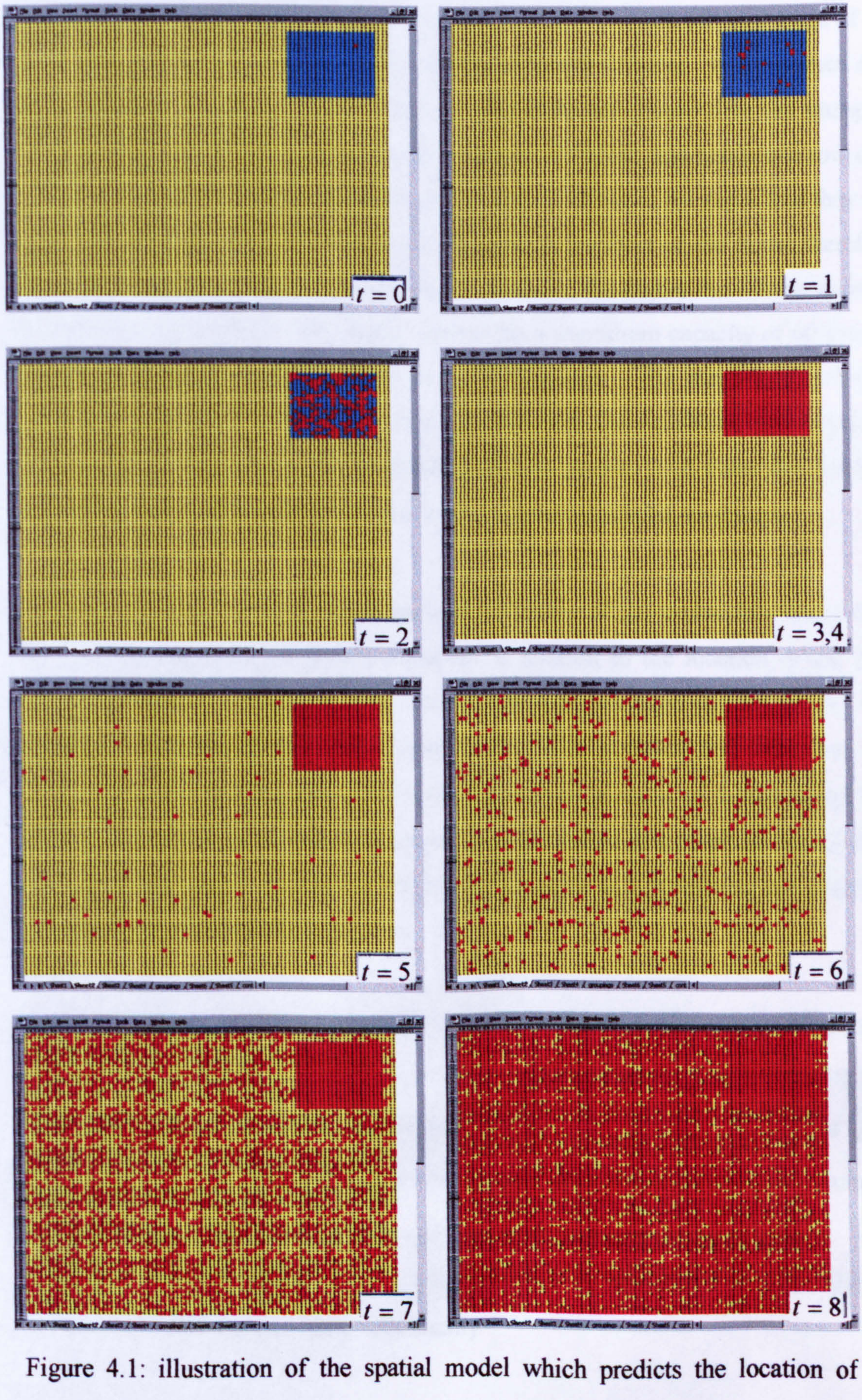


Figure 4.1: illustration of the spatial model which predicts the location of positive birds within a flock over time. Negative birds are represented by yellow squares, birds which are assigned to the cluster are identified by blue squares and positive birds are represented by red squares.

Having described the colonisation and contamination processes, the next stage of the model considers placing of the birds into the transport vehicles. It is assumed that for all flocks, a basket contains 25 birds. The birds are placed onto the transport vehicles, in groups of 100, as each basket contains 25 birds, and hence one row of a section will carry 100 birds. This placement is carried out in the order that they are in the house. One end of the house is allocated as the front of the house therefore the further away from the front of the house a bird is, the higher the vehicle number the bird will be transported in. Each vehicle has a maximum capacity of 60 groups of birds, assuming that all vehicles carry 10 sections. The number of vehicles required for any flock is therefore a multiple of 6,000. Once all the birds are placed into the transport vehicles the probability that a random bird from the flock is contaminated during transport, defined as P_{ct} can be calculated, as follows.

There are no data available which provide estimates of the probability that a bird will become contaminated during transport in relation to the location of the bird within the vehicle. Therefore, this contamination is predicted using a model that describes the biological situation. Specifically, it is assumed that there are two modes of contamination. First, that which occurs as a result of a bird being in a position below colonised birds and second, contamination as a result of the adjacent sections of the vehicle containing contaminated birds and generating the potential for horizontal spread of the organisms.

Consider a random bird in section i $(1 \le i \le 10)$, and row j $(1 \le j \le 6)$, where row $j = 1$ is the top row and $j = 6$ is the bottom row of the vehicle. The probability that transmission occurs vertically, that is from the birds in section i, rows 1 to j is defined as $C_T(d)_{i,j}$. This probability is dependent upon the distance, that is number of rows, between the selected bird and the nearest colonised birds above. It is assumed that the probability that a bird becomes contaminated is given by the reciprocal of this distance, more specifically

$$C_T(d)_{i,j} = \frac{1}{j - \max\{C_{x,j}.z\}} \qquad z = 1,..,i-1$$

Here, $C_{x,j}$ is the status of section $\{i,j\}$ where $C_{x,j} \in \{0,1\}$ such that $C_{x,j} = 1$ means that there is at least one colonised bird in section $\{i,j\}$. Therefore, that $C_{x,j} = 0$ means that there are no colonised birds in section $\{i,j\}$. Next, consider cross-contamination from the adjacent birds that is birds in row j, sections 1 to 10. The probability that transmission occurs horizontally, that is from the birds in row j, and all sections defined as $C_T(V)_{i,j}$. It is assumed that the probability that a given bird will become contaminated by this route is given by the product of the probability that birds in any one of the sections 1 to 10 is contaminated and the reciprocal of the distance between this section and the selected section. More specifically

$$C_T(V)_{i,j} = \left[\sum_{z=1}^{j-1} C_T(d)_{i,z}.\frac{1}{j-z+1}\right] + \left[\sum_{z=j+1}^{10} C_T(d)_{i,z}.\frac{1}{z-j+1}\right]$$

Therefore, the probability that a random bird located in section i, row j will become contaminated during transport, that is $P(CT)_{i,j}$ is given by

$$P(CT)_{i,j} = C_T(V)_{i,j} + C_T(d)_{i,j} - C_T(V)_{i,j}.C_T(d)_{i,j}$$

Hence, on arrival at the slaughter facility, each bird has an associated status for the occurrence of contamination during transport, defined as $CT_{i,j} \in \{0,1\}$ such that $CT_{i,j} = 1$ means that the bird located within a vehicle in position (i,j) became contaminated externally during transport, and $CT_{i,j} = 0$ means that this bird did not become contaminated during transport. The condition $CT_{i,j} = 1$ occurs with probability $P(CT)_{i,j}$, therefore $CT_{i,j} = 0$ with probability $1 - P(CT)_{i,j}$.

Given a bird which has become contaminated during transport the level of contamination on the birds exterior is defined as Ω_t.

4.3.1 Level of contamination at slaughter

The level of external contamination that is present upon a bird on arrival at the slaughter facility has been observed to be significantly different ($P<0.05$) than that which is present before the flock are transported (Stern *et al.*, 1995), thus suggesting that transportation allows cross contamination within the flock. However, as previously described in section 4.3, the probability that a random bird will become contaminated during transport is a function of the location of that bird in relation to the location of the colonised birds in the flock within the transport vehicles. As such the level of contamination on the exterior of a bird is governed by the probability that the bird became contaminated during transport. As described previously, when the birds are placed in the transport vehicle each bird has a contamination status at the point of depopulation, (CD), and a status for the occurrence of contamination during transport, (CT). The level of contamination at the point of slaughter, that is Ω_s is governed by these two factors, as described by equation (4.1).

$$\eta_{ext} = \begin{cases} 0 & CD = 0; CT = 0 \\ \Omega_d & CD = 1; CT = 0 \\ \Omega_t & CT = 1 \end{cases} \qquad (4.1)$$

Here, Ω_d is the level of contamination on the exterior of a bird at depopulation, Ω_t is the level of contamination on a bird after transport, and η_{ext} is the level of contamination on a random bird from positive flocks at the point of slaughter.

4.4 The contamination of negative flocks

Consider a negative flock, that is a flock which has not been exposed to campylobacter at a level sufficient to result in the colonisation of any birds in the flock. Given that birds within a negative flock by definition contain no colonised birds, there are therefore no birds shedding the organisms during transport. As such the above model description does not apply.

Given the absence of colonised birds, it could be assumed that within such a flock there is no opportunity for bird to become contaminated on their exteriors. In reality, this is not the case. Experimental data suggests that there are at least two occasions when birds in negative flocks may become contaminated (Tom Humphreys, personal communication). First, it has been hypothesised that when the birds are caught the catchers hands may be contaminated with organisms as a result of previously catching a positive, and hence contaminated, flock. Second, it has been reported in the literature that the baskets within which the birds are transported may be contaminated with campylobacters. The baskets are routinely cleaned once the birds are removed at the slaughter facility however such cleaning may be inadequate to remove all the organisms present.

4.4.1 Level of contamination at slaughter

There are no data available that enable the estimation of either the probability that a negative flock will become contaminated or the extent of such contamination. However, given the two opportunities for contamination described above, it is intuitive that the probability that a flock will become contaminated is dependent upon either the catchers or the crates coming into contact with a positive flock at some point previous to contact with the negative flock. Therefore an assumption is made that the probability that a bird from negative flock will come into contact with a contaminated crate or catcher is equal to the national flock prevalence, that is P_{pf}.

Here the flock prevalence is as described in Chapter 3. Given contact with a contaminated catcher or crate, the probability that a bird will become contaminated is assumed to follow a Uniform(0,1) distribution. Therefore, the probability that a bird from a negative flock will become contaminated on its exterior, defined as $P(Cnf)$ is given by $P(Cnf) = P_{pf}\mathrm{U}(0,1)$.

Given that birds from negative flocks can become contaminated, the extent of this contamination is related to the level of contamination in the positive flocks. Consider contamination by catchers hands. Experimental focussing on the cross-contamination of organisms from surfaces to hands and hands to surfaces suggests transfer rate of 10% (Zhao *et al.*, 1998). That is given one contact with a contaminated surface approximately 10% of the organisms will be transferred. For a bird to become contaminated via catcher's hands two events must occur, first the catchers hands must come into contact with a contaminated bird and hence become contaminated. Second the contaminated hands must transfer the organisms to a previously uncontaminated bird. Therefore an assumption is made that the level of contamination that a random bird in a negative flock receives is 1% of the contamination on the exterior of a random positive bird, that is a colonised bird.

The same assumption is made regarding contamination via crates as the birds must contaminate the crates, and then the contaminated crates must come into contact with the exterior of a bird from a negative flock. As such, there are two points of contact and therefore it is assumed that the transfer rate is 1% of the level of exterior contamination on a random bird from a random positive flock.

The overall model can then be summarised as follows. The probability that a bird will be contaminated at the point of slaughter, defined as P_{cs} is given by equation (4.3)

$$P_{cs} = \begin{cases} P(CT)_{i,j} & \theta_f = 1 \\ P(Cnf) & \theta_f = 0 \end{cases} \qquad (4.3)$$

The contamination level on the exterior of a bird selected at random from the national flock at the point of slaughter, defined as η_{ext}, is given by equation (4.4).

$$\eta_{ext} = \begin{cases} \Omega, & \theta_f = 1 \\ 0.01\Omega, & \theta_f = 0 \end{cases} \tag{4.4}$$

4.5 Parameter estimation

4.5.1 Colonisation level

The level of colonisation within the caeca of several birds within random flocks is reported by Stern *et al.*, (1995). This data set recorded levels of colonisation before and after the birds had been transported and suggests that there is no significant difference in colonisation levels at these two time points. There are several other reports in the literature which give an indication of colonisation levels in positive birds (Jacobs-Reitsma *et al.*, 1994; Stern, 1988; Aho & Hirn, 1988; Atabay & Correy, 1997; Berndston *et al.*, 1996). However, such studies commonly report only the mean value, or maximum colonisation observed. Data of this type does not enable the definition of a variability distribution to describe colonisation levels in random birds. Given this, only the data set from Stern *et al.*, (1995) is utilised to define the variability distribution describing the colonisation level at slaughter, that is Λ_s. The data set reported by Stern *et al.*, (1995) is shown in Table 4.2. From this table it can be seen that this data consists of only 9 samples. As such, there is uncertainty associated with the form and extent of the variability distribution. Further, the data points reported are means of several samples. There are numerous combinations of colonisation levels that could have lead to the reported mean for any given farm. Therefore, this data is used to define a non-parametric, second-order distribution as described in Chapter 2. The mean of this second-order distribution will be the reported data set and all possible ways by which the reported data could

have occurred is accounted for by the two-dimensional nature of the distribution. By comparison with reports of colonisation levels in the literature, it can be seen that this data set is consistent with other findings (Jacobs-Reitsma *et al.*, 1994; Stern, 1988; Aho & Him, 1988; Atabay & Correy, 1997; Berndston *et al.*, 1996).

Table 4.2: The number of campylobacters colonising the caeca of broilers at slaughter from Stern *et al.*, (1995).

Farm number	Mean Log cfu/gram ceacal contents post transport per farm
1	7.08
2	5.74
3	5.11
4	7.00
5	5.40
6	6.38
7	7.28
8	6.28
9	4.11

4.5.2 Contamination level

There is little information in the published literature with regards to the level of contamination on the exterior of birds either before or after transport. An investigation by Stern *et al.* (1995) recorded measurements of external contamination with campylobacters both before and after transport. This data set is shown in Table 4.2. The data consist of mean counts taken from 10 farms which were under experimental control (numbered 1 to 10) and duplicate samples taken from 5 farms, randomly chosen, not under experimental control (numbered 11 to 15 denoted by *). Given that the cross-contamination that occurs during transport is not controlled on the farms classed as under experimental control it is appropriate to

pool the two data sets. It can be seen from Table 4.3 that on 7 occasions no campylobacters were retrieved from the birds prior to transport, yet a high level of contamination was recorded post-transport, further strengthening the importance of cross-contamination during this stage.

Table 4.3: The number of campylobacters (mean log cfu per carcass) contaminating the exterior of broilers prior and post transport taken from Stern *et al.* (1995).

Farm Number	Mean Log cfu/carcass prior to transport	Mean Log cfu/carcass post-transport
1	ND	7.53
2	ND	ND
3	ND	7.05
4	6.16	7.48
5	6.09	8.18
6	6.38	8.66
7	5.97	7.34
8	5.81	7.34
9	6.23	7.75
10	ND	6.82
11*	2.4	5.8
11*	4.3	6
12*	2.65	5.53
12*	ND	4.93
13*	6.23	9.62
13*	6.15	ND
14*	2.37	6.61
14*	ND	6.36
15*	ND	ND
15*	2.88	6.67

As with colonisation levels, this data set is based on a small sample size hence there is uncertainty with regards to the extent of the variability in the population. Therefore, as previously, second-order non-parametric distributions for the number of organisms contaminating the exterior of a bird before and after transport were derived from the data shown in Table 3. These before and after distributions are then correlated to account for the fact that the level of contamination after transport depends on the level of contamination before transport. Using Spearman's rank order the correlation coefficient, ρ, is given by

$$\rho = 1 - \left(\frac{6\Sigma(\Delta R)^2}{n(n^2 - 1)} \right)$$

where ΔR is the difference in rank of the data in a data pair and n is the number of data pairs. The data shown in Table 4.3 results in $\rho = 0.895$. This indicates that the number of organisms before and after transport that contaminate the exterior of are bird as positively correlated, as expected.

Note that the data points reported in Table 4.2 and Table 4.3 are means of several samples for a given broiler farm. There are numerous combinations of colonisation levels that could have lead to the reported mean for any given farm. Therefore it is appropriate to use non-parametric second-order distributions to describe the variability present. The mean of these second-order distributions will correspond to the reported data sets. As such all possible ways by which the reported data could have occurred is accounted for by the two-dimensional nature of the distributions.

The model contains several parameters, each of which is described by an appropriate variability distribution. The variability distributions have associated uncertainty with respect to the true variability. As such the model can be run with different combinations of variability distributions with each combination representing one possible realisation of the contamination of a flock during rearing and transport. Of course if there was no associated uncertainty then there would be only one possible

combination. These different realisations are mimicked by simulating the model a number of different times and the result is multiple distributions describing the variability in the number of campylobacters colonising the intestines and contaminating the exterior, and multiple estimates of the prevalence of contaminated birds at the point of slaughter. This allows the quantification of the level of uncertainty with respect to the outputs of the model the number of organisms colonising the intestinal tract (Λ_s), the number contaminating the exterior of a bird at the point of slaughter (η_{ext}), and the probability that a bird is contaminated at slaughter, that is $P(CT)$.

The model is run for 50 simulations. Upon each simulation a variability distribution is selected from the associated uncertainty space for each second-order random variable and kept constant for any given simulation. Within each simulation the selected distributions are sampled 5000 times and a value for Λ_s is sampled and η_{ext} is calculated as shown in equation (4.4). At the end of each simulation $P(CT)$ is given as shown in equation (4.5). Multiple simulations result in the uncertainty distribution for $P(CT)$. The number of samples taken within a simulation was chosen according to when the running mean of $P(CT)$ no longer deviates ±1% from the 'true' mean, defined as the mean of $P(CT)$ at 8000 iterations. The deviation of the running mean from the 'true' mean is illustrated in Figure 4.2. It can be seen that 2000 samples is adequate to fulfil the criteria. The number of simulations was chosen to ensure adequate selection of the variability from the uncertainty interval for each of the second-order random variables.

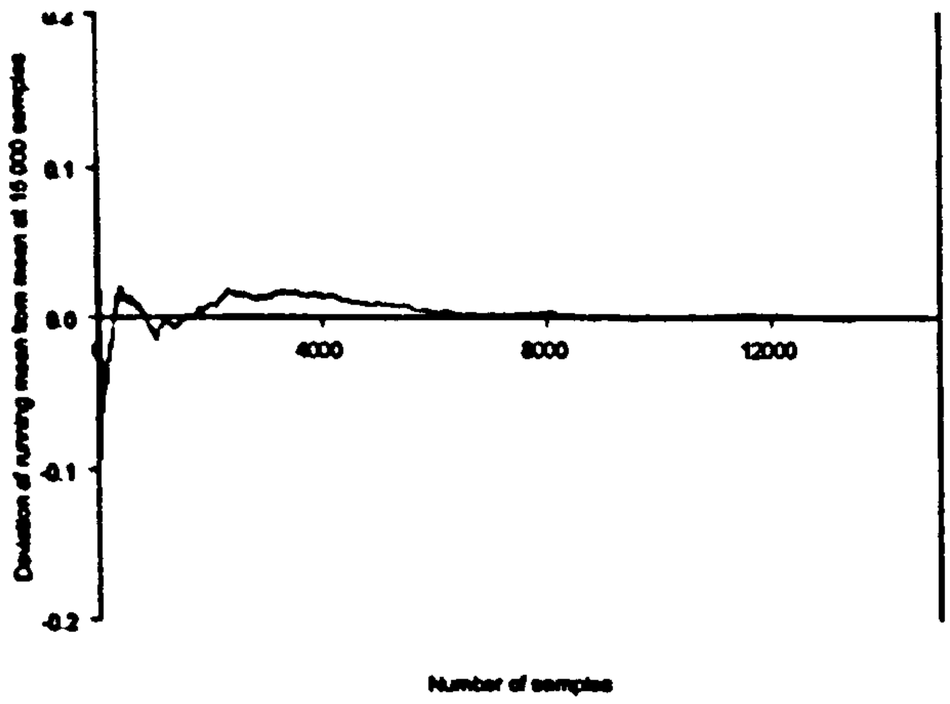


Figure 4.2: Deviation of the running mean from the true mean defined as the mean at 15 000 samples from the distribution for the probability a bird is contaminated on arrival at slaughter.

4.6 Results

The density graph for the number of organisms which colonise the ceaca of a positive bird are shown in Figure 4.3. The mean number of organisms has an uncertainty range from 5.86 to 6.80, further the 5th percentile ranges from 3.7 to 5.5 and the 95th percentile from 7.2 to 8.6. This indicates that, on average, a bird will be colonised at a level of 3.7 to 5.5 log cfu per gram of ceacal contents. However, 95 times out of 100 this level will be up to 7.2 to 8.6 log cfu per gram of ceacal contents.

The uncertainty distribution for the probability that a bird is contaminated on arrival at the slaughter facility is shown in Figure 4.4. Essentially, this is the accumulation of the distributions for the probability that a bird is contaminated on the farm and the probability that a bird is contaminated during transport, as described by equation (4.1). The mean value for the probability that a bird will be contaminated on the

farm is 0.6. Further, the probability that a bird will become contaminated during transportation to the slaughter facility has a mean value of 0.29, as estimated from the transport section of the model described above. The resulting probability that a bird will be contaminated at slaughter has a mean value of 0.83. That is 83 out of 100 birds will have 1 or more campylobacters in the exterior at the point of slaughter. This probability ranges from 0.80 to 0.85 as shown in Figure 4.4.

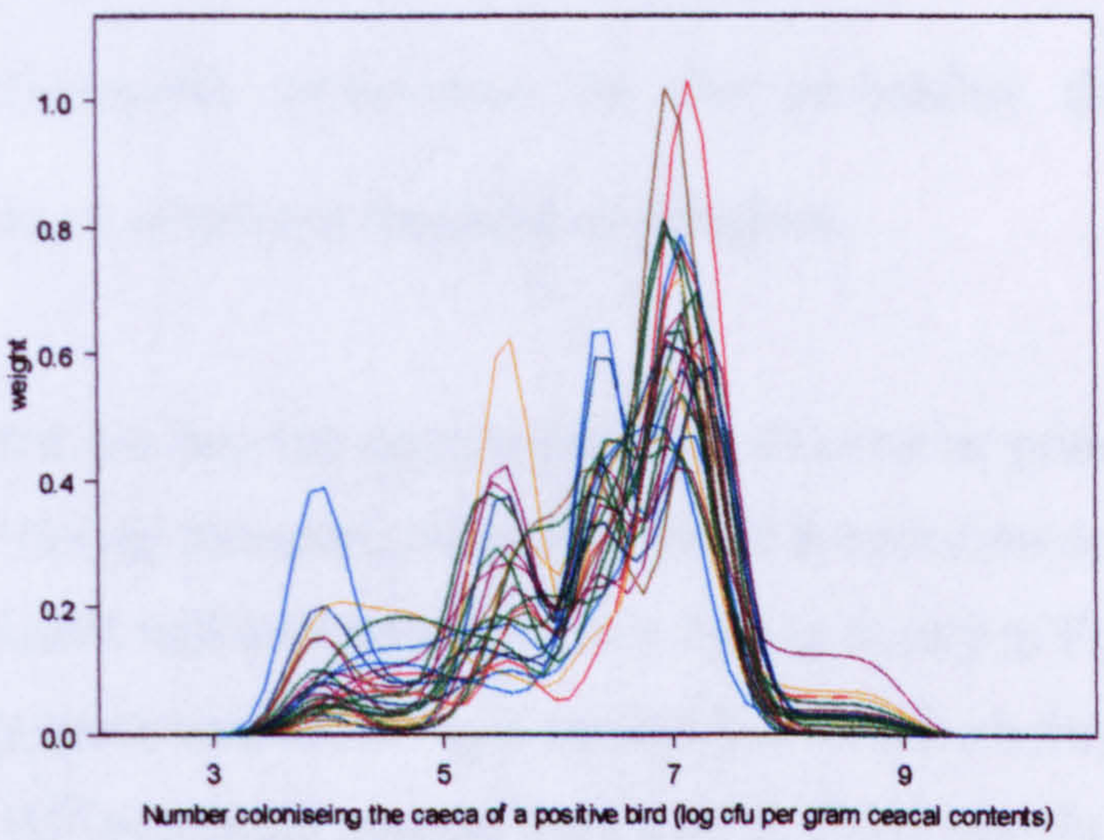


Figure 4.3: Second-order distribution for the number of organisms colonising the caeca of a positive bird. This distribution reflects the level of uncertainty associated with this model parameter.

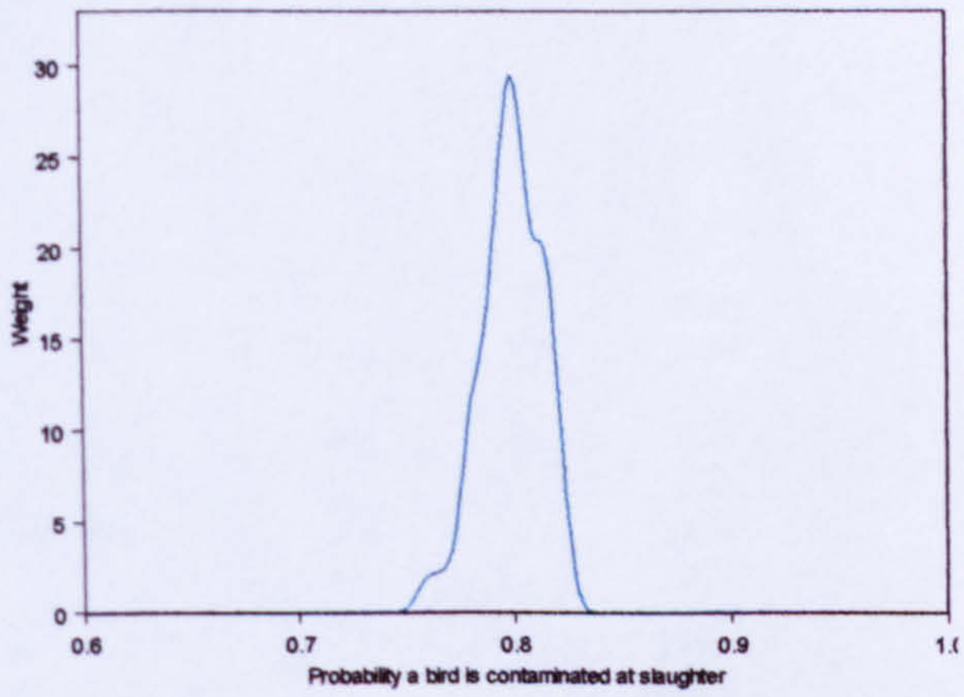


Figure 4.4: Uncertainty distribution for the probability that a bird will be contaminated on its exterior at the point of slaughter.

Given that a bird has become contaminated on its exterior prior to slaughter, either on the farm or during transport, the second-order distribution describing the number of organisms which will contaminate such a bird is shown in Figure 4.5. The mean number of organisms contaminating a carcass has an uncertainty range from 6.08 to 7.23, with the 95th percentile ranging from 8.33 to 10.62 log cfu per carcass. That is 95 times out of 100 a bird will be contaminated with up to 8.33 to 10.62 log cfu per carcass.

From Figure 4.5 it can be seen that there are two distinct peaks in the distribution for the number of organisms contaminating the exterior of a carcass at slaughter. This is a direct result of the two populations which are contaminated. More specifically there are birds which are contaminated as a result of being from a positive flock, and there are birds which are from negative flocks but have become contaminated by some other mechanism prior to slaughter. The two peaks are in fact related by the model assumption that the level of contamination on a bird from a negative flock is 1% of the contamination that would be observed on a random bird originating from a positive flock. However, given that there is no information currently available

about the contamination of bird from negative flocks, the validity of this assumption is unknown.

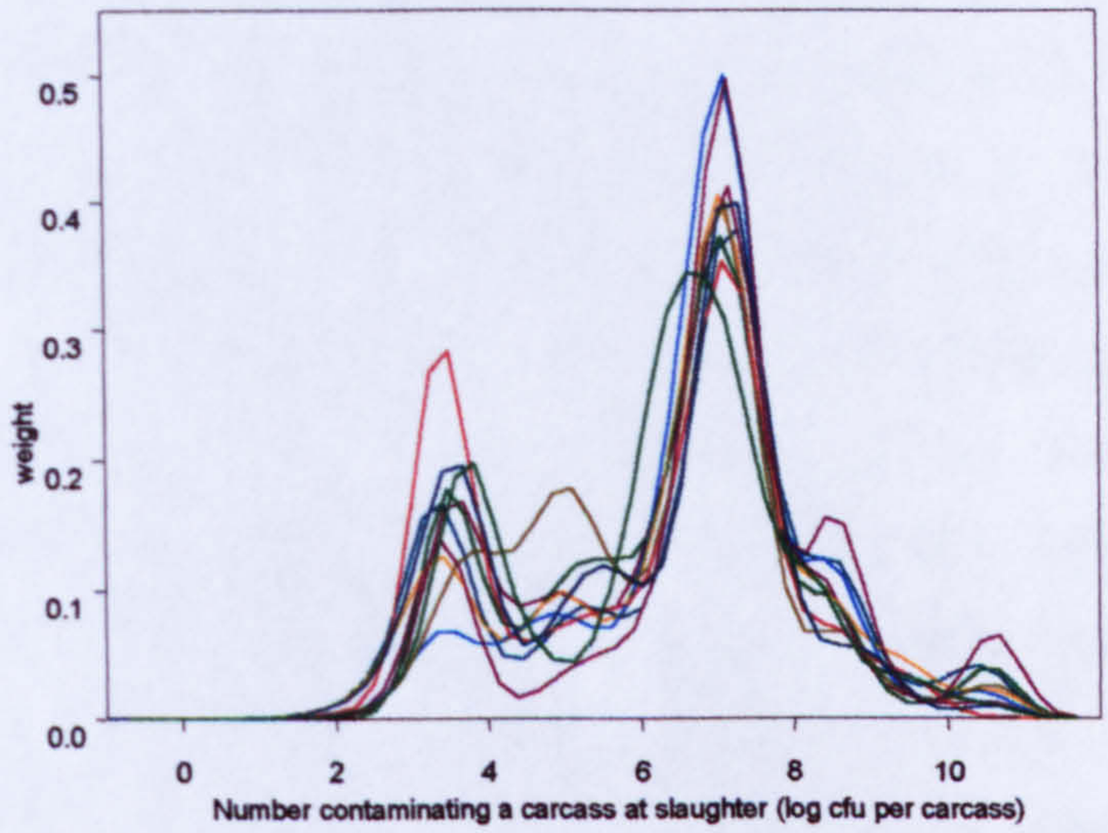


Figure 4.5: Second-order distribution describing the level of contamination on a contaminated bird at slaughter, log cfu per carcass.

4.7 Investigation of risk control points

Positive birds, when slaughtered, carry large numbers of organisms in their caeca with model results indicating that 95 percent of the time this can be as high as 7.2 to 8.6 log cfu per gram of ceacal contents. Thus contamination of the exterior of birds provides a massive reservoir of organisms which have the potential to persist through to any processed chicken product. There are a number of ways that could be considered to reduce these.

As discussed in Chapter 3, the sources via which flocks become colonised with campylobacter are wide and varied with a number of potential sources for any given flock such as the external environment following a breakdown in bio-security or

contaminated feed, water or litter. This makes preventing the exposure of flocks to campylobacter a difficult task. Consequently, research is now often directed to methods of preventing a bird becoming colonised given it is exposed to high levels of the organisms. Such methods include vaccination and competitive exclusion.

The use of vaccines to significantly reduce the colonisation of chicken flocks with food poisoning organisms such as *Salmonella typhimurium* DT104 has had great success, with numerous flock infections now prevented due to the routine use of the vaccine. However, such a solution for campylobacter is, thus far, elusive with vaccines in experimental trials having limited success, only succeeding in lowering the levels of colonisation rather than preventing colonisation (Diane Newell, personal communication). A similar situation is also currently apparent for competitive excluders, where one strain of campylobacter which is assumed to be non-pathogenic to humans is used to colonise the birds and hence prevent the colonisation of the bird with any pathogenic campylobacters. Research in this area is continually growing, however it is questionable whether such a strategy is feasible given public perception of an assumed 'safe' strain of a known food poisoning organism. However, with further research this may provide a method by which birds are able to enter the slaughter facility without the presence of campylobacters in the cut of any birds.

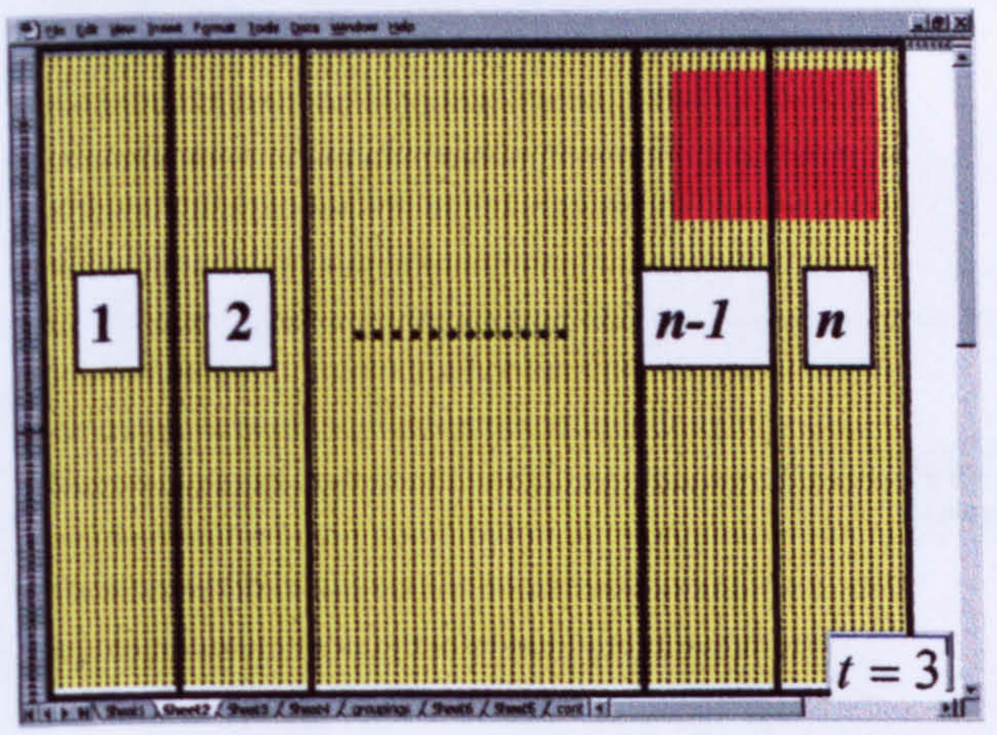
Although not explicitly modelled here, any measure which reduces either the probability of exposure of flocks to sources of campylobacter, or prevents the process of colonisation once exposure has occurred are likely to have an impact upon model results, specifically the probability that a flock is positive at slaughter, P_{fp} and the colonisation levels at slaughter, Λ_s.

The model presented in section 4.3 demonstrated that the transportation of flocks from the slaughter facility is an important stage in predicting the microbiological profile of the exterior of a carcass at the point of slaughter. Further, the stage of within flock transmission is also an important factor. Consider the transmission dynamics and the two stages of transmission which occur during the colonisation of

a flock as described in 4.2. The time following exposure to campylobacter which this flock is depopulated is crucial in predicting the extent of cross-contamination which may occur during transportation to the slaughter facility.

To illustrate this, consider the colonisation of the flock as shown in Figure 4.1. When depopulated this flock will be divided into n vehicles. The division of this flock into n vehicles for $t=3$ days and $t=7$ days is shown in Figure 4.6. It can be seen that if this flock is depopulated at $t=3$ only 2 vehicles will contain colonised birds. Therefore there is the potential for birds to arrive at the slaughter facility without any effect from transportation. However, if this flock is depopulated at, for example, $t=7$, then as can be seen from Figure 4.6 all the vehicles will contain positive birds and hence it is likely that all the birds will be further contaminated by transportation, increasing the microbial loads on the exterior of these birds.

As identified in Chapter 3, the time of exposure to campylobacter is a potential risk control point. By delaying the time since exposure to campylobacter, the earlier the flock will be in the process of flock colonisation and hence providing the opportunity to minimise the effect of cross-contamination during transportation as only a limited number of vehicles will contain positive birds.



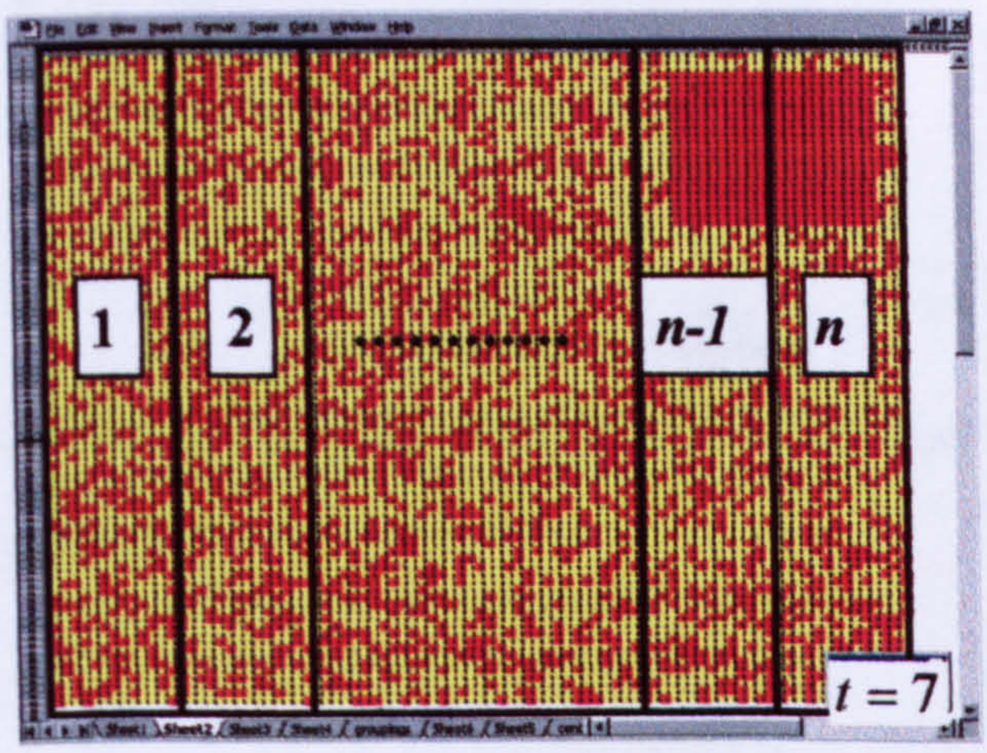


Figure 4.6 : Illustration of the placement of the flock loaded into vehicles 1 to n at $t = 3$ days and $t = 7$ days since the flock was exposed to campylobacter. Depicted is the importance of the stage of flock colonisation at loading in relation to the number of vehicles that will contain colonised birds.

The occurrence of cross contamination during transport is reported to contribute to the probability that a bird is contaminated at slaughter. To illustrate the impact this cross contamination has upon the probability of contamination at slaughter Figure 4.7 shows density plots of the probability that a random bird is contaminated on the

farm, and the probability that a bird is contaminated at slaughter. From this figure it is clearly illustrated that the stages between the birds located in the shed on the farm, and the point of slaughter contribute to the probability that a random bird is contaminated at slaughter.

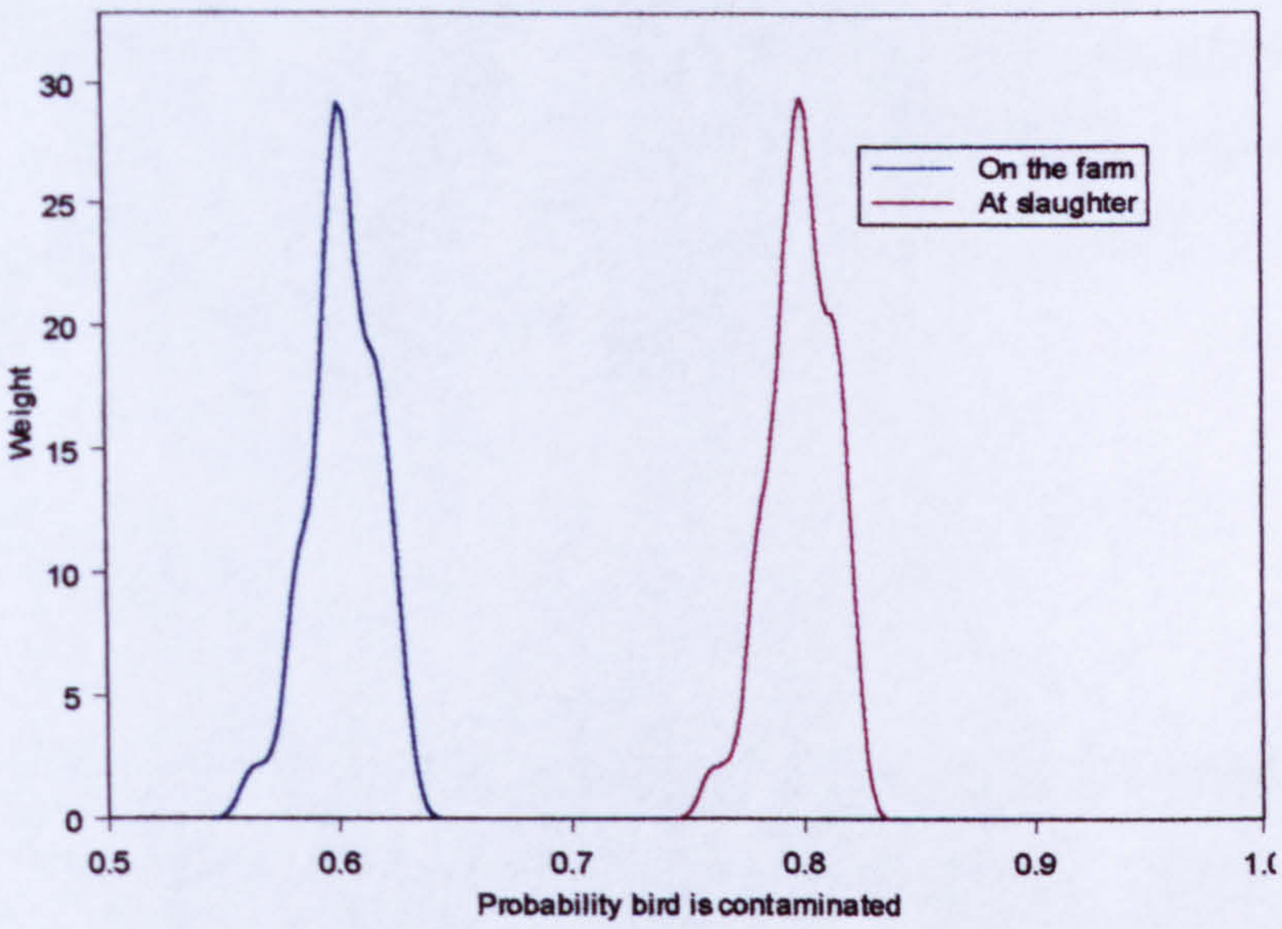


Figure 4.7: Uncertainty distributions for the probability that a bird is contaminated on the farm, and at slaughter.

A further potential control point is to minimise the levels of cross-contamination that occur during transportation. This could be achieved by preventing the passage of excrement from one basket to another. However, during the transportation to the slaughter facility the welfare of the birds is a top priority and as such it is unlikely that changes are feasible to the current structure of the process.

It has been presented that birds from negative flocks frequently arrive at the slaughter facility with external contamination. This is an area of data deficiency with very little currently known regarding the frequency and levels of contamination of the exteriors of birds form negative flocks. Such contamination provides a further route by which chicken products may become contaminated with campylobacters. This population of contaminated birds could be removed by adequate crate cleaning

and improved hygiene during the catching of the flocks. Due to the current lack of knowledge associated with this aspect of the model the full extent of the removal of this population is unknown.

In summary, currently there are limited avenues available to reduce the levels of colonisation and the levels of contamination on the exterior of a bird at the point of slaughter. One possibility is to delay the time of exposure and therefore minimise the extent of cross-contamination that occurs during the transportation of a positive flock. This control point was also identified as a factor in reducing the probability that a bird is colonised at slaughter in Chapter 3.

4.8 Conclusions

Once exposed to campylobacters, broilers may become colonised. Often this colonisation is to high levels with recorded measurements of 9 log cfu per gram of ceacal contents common in the literature (Stern *et al.*, 1995; Line *et al.*, 1997; Jacobs-Reitsma *et al.*, 1994). Once colonised, this level is maintained until slaughter. There are several research avenues focussed upon preventing the colonisation of broiler flocks. However, methods such as vaccination and competitive exclusion have, thus far, had limited success but research in these areas is currently on going.

A consequence of the colonisation of a flock is the external contamination of the birds in that flock. This occurs either by self-contamination for a bird which is colonised and hence likely to become contaminated as a result of the excretion of campylobacters in the bird's faeces or by contact with faeces containing campylobacter but the bird need not necessarily be colonised. This contamination is then magnified during the transportation of the flock to the slaughter facility. This is a result of the dispersal of contaminated faeces throughout the vehicle.

In this chapter a model has been presented which predicts the level of contamination on the exterior of a bird on arrival at the slaughter facility. This model utilises the spatial location of colonised birds within a flock at depopulation and considers the placement of the birds into the transport vehicles predicting the impact they will have on any random bird in the flock. This model demonstrates that the time since exposure of a flock to campylobacter is important when quantifying the level of cross-contamination likely to occur during transportation. More specifically, if a flock reaches the second stage of transmission then it is likely that the probability that a bird will be contaminated during transport is equal to 1.

The contamination of the exterior of birds is not unique to positive flocks. Experimental studies suggest that birds from negative flocks can become contaminated on their exteriors at some point prior to slaughter. However, the frequency and extent of such contamination is currently unknown, as such this is an area of data deficiency. An assumption is made that a negative bird can become contaminated with 1% of the contamination on a random positive bird. However, in the absence of data the impact this has upon the probability and levels of contamination of the exterior of a random bird at slaughter, predicted by the model and the validity of these predictions are unknown.

Chapter 5

The slaughter and processing of chicken: a review

5.1. Introduction

The processing of chicken meat consists of a highly controlled sequence of events, beginning with the slaughtering process through to transport of the final sale product. Welfare of the live birds and carcass quality are top priorities and so these two factors govern the way in which processing procedures are carried out. If human pathogens are present in the intestinal tract of chickens, forming part of the faecal micro-flora, the potential is there for contamination of carcasses during slaughter and processing. The extent of this will depend on the prevalence of the organisms in and on the birds as well as the hygienic standards employed during processing. Such contamination can be described in two ways (Gill, 1999), first vertical contamination resulting from colonisation of the live bird, secondly horizontal or cross contamination which results from a source other than the bird/carcass, for example, the processing equipment or another bird/carcass. Horizontal contamination may augment vertical contamination and is especially important when considering uncontaminated carcasses that are being processed alongside contaminated carcasses.

Under the implementation of Hazard Analysis Critical Control Point (HACCP) systems in poultry processing, for each operation which affects the safety of the product, critical control points are identified and can be controlled to contain or eliminate an identified hazard (Gill, 1999). The identification of such control points within chicken processing requires an understanding of the process itself and the behaviour of the microbial hazard within that environment. There are four main points for consideration when investigating organisms in a processing environment. These are the temperature range for growth, the range of water activity over which the bacteria can grow, nutritional requirements, and resistance to heat and other stressing environmental factors. These factors will determine the behaviour of a given organism within this environment.

For most bacterial species found in the food chain, for example salmonella and staphylococcus, these characteristics facilitate amplification through processing as a

result of growth and establishment within the environment. Consequently their total elimination from the food chain may only be possible when the bacteria are eliminated from the livestock. Any intervention strategies at later stages of the food chain may reduce the magnitude of the problem and should certainly be taken for that reason, but the main problem should be attacked at the origin. This is not the case for campylobacters as they are thermophillic and strictly microaerophillic, having an optimal growth temperature of 42°C with a permissible growth range of 32-35°C and an optimum oxygen concentration of as little as 5% along with 10% carbon dioxide (ICMSF, 1996). Unless these conditions are met, they are not able to propagate in the processing environment or on the raw, processed product. The pattern of contamination is therefore different from many other bacteria the main problem being horizontal contamination. Therefore, preventive measures in processing may be very effective and have the potential of eliminating the organism from the food chain.

In this chapter, a qualitative assessment of each of the stages of processing is carried out. In particular, consideration is given to the impact each of the stages of processing has upon the prevalence and extent of carcasses contaminated with campylobacters. Thus the aim is to identify the key stages of processing which influence the probability and magnitude of contaminated products such that a quantitative assessment can then be developed.

5.2. The Stages of Chicken Processing

The processing of chicken consists of nine main stages beginning with the slaughter of the birds through to final grading and packaging of the sale product which is then transported to the retail outlet for distribution. The overall process is illustrated in figure 5.1. Each of these nine stages will now be described.

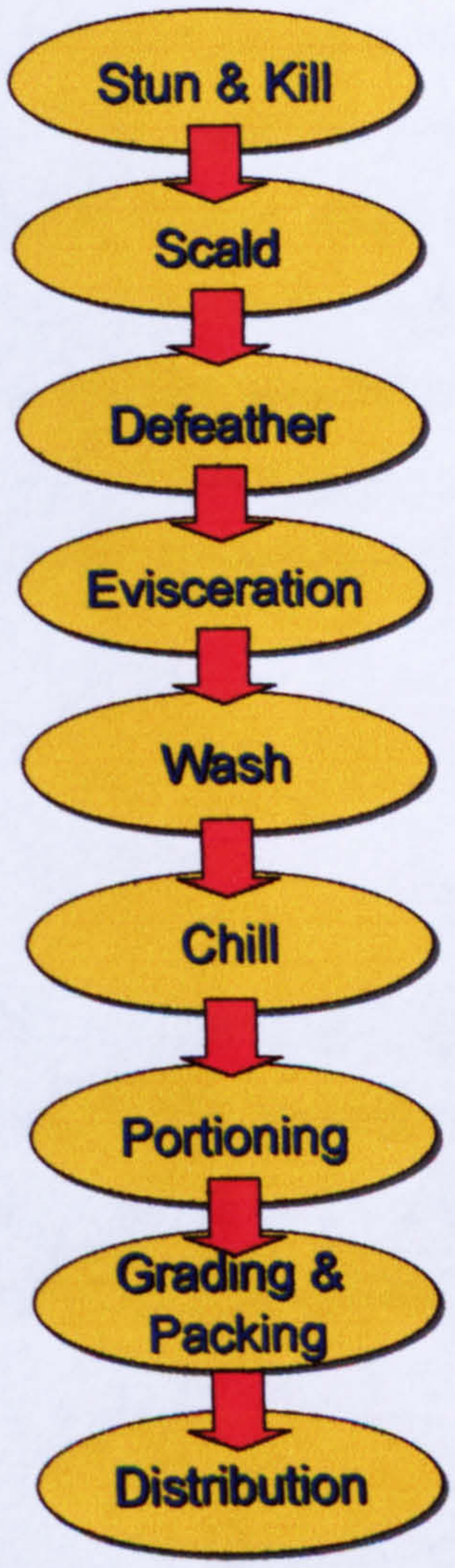


Figure 5.1: Diagram illustrating the ten main stages of the processing of chicken from stunning to retail and distribution.

5.2.1 Stun and Kill

The first stage involves the stun and subsequent kill of the live birds. Upon arrival at the slaughter facility, the birds are removed from their crates and put onto the killing line, where they are hung upside down by their feet in shackles. It is a legal requirement that there is physical separation between this and the rest of the slaughterhouse to prevent the spread of the dust and dirt generated by this process. From here, a conveyor moves the birds towards the stunning equipment. Commonly

electrical water-bath stunning is used however other methods are available such as gassing. After stunning, the birds are bled for up to two minutes before processing begins. Due to the high intensity of the slaughter process, birds are hung in close proximity and will be in contact with each other, as well as with machinery, throughout stun and kill. Despite this, these stages have few microbiological implications; although electrical, water-bath stunning may lead to both inhalation of contaminated water by the birds and microbial contamination of the carcass tissues (Lillard, 1973). Consequently, the effect of stun and kill is assumed to be negligible.

5.2.2 Scald

Once birds have been slaughtered the carcasses are immersed in a scald tank. This process loosens the feathers and facilitates plucking. As birds enter the scald tank there may be involuntary defecation, leading to accumulation of faecal matter in the tank. In the case of birds colonised with campylobacter this results in contamination of the scald water.

The process of scalding depends upon whether the carcass is destined for fresh or frozen sale. Carcasses used for fresh products undergo soft scald where the water is at a temperature of 50-52°C for up to 3.5 minutes, those used for frozen products undergo hard scald, and in this case the water is at 56-58°C for 2-2.5 minutes. The different scalding methods are used as soft scalding avoids damage to the cuticle and hence prevents skin discolouration, an undesirable quality in fresh sale chickens but not a large concern for frozen products. The slaughtering and scalding processes interact in influencing microbial contamination of the internal organs. If the birds are not given long enough to stop breathing or gasping before scalding, there is a danger that they will inhale the scald water. This may result in the trachea, oesophagus, lungs, crop, gizzard and air sacs becoming contaminated with scald water during the scalding procedure (Lillard, 1973). This would not assume any importance if the contamination was restricted to the inedible offal, but Lillard (1973) showed that low

level internal contamination could occur. Further, scalding may lead to external contamination if an uncontaminated carcass is passed through contaminated scald water.

5.2.3 De-feathering

De-feathering is a mechanical process, which occurs immediately after scalding. This process is carried out by a series of in-line plucking machines that comprise of banks of counter rotating stainless steel domes, or discs, with mounted rubber fingers. These machines incorporate continuous water sprays that flush out the removed feathers. Any remaining feathers are removed by hand. These machines are major sites of potential cross-contamination in primary processing. Rubber fingers can scour the carcass and can also harbour contamination, following contact with a contaminated carcass, in the 'cobweb' of tiny cracks that form when the rubber becomes brittle. This has the potential to result in contamination of a previously uncontaminated carcass. However, such contamination will be low-level. In contrast, significant contamination results due to the spinning action of the plucker heads. In particular, this action results in the formation of aerosols, which spread contamination (Hinton *et al.*, 1996). The process of defeathering has been demonstrated to generally increase the number of carcasses contaminated with organisms (Oosterom *et al.*, 1983a; Oosterom *et al.*, 1983b ; Izat *et al.*, 1988). This is due to re-distribution of the organisms and therefore has a large impact on previously 'clean' carcasses. Redistribution is due to the aerosol spray and contamination of machinery.

5.2.4 Evisceration

Following plucking the head and feet are removed and the birds are eviscerated, that is the internal organs are removed. In some plants, carcasses are detached from the hanging hooks and transferred to the evisceration area to be re-hung. This handling

activity provides the opportunity for organisms to spread. For the majority of production, evisceration is carried out mechanically, but manual evisceration is still practised. On automated lines, a cut is made around the vent of the carcass, a spoon-shaped device is inserted into the opening and the viscera are withdrawn. The intestines etc. remain attached for inspection, hanging over the back of the carcass connected by their natural tissues, and gross contamination of the carcass may result if they are damaged. This is not an uncommon occurrence because the machinery used is not able to allow for natural variation in the size of the carcasses being processed (European Union, 1997). It has been shown that even when the viscera remain in-tact the levels of enteric bacteria, including campylobacter, increase on the exterior of the carcass (Oosterom *et al.*, 1983; Izat *et al.*, 1988). If a carcass originates from a classified negative bird then damage to the viscera can be ignored. For birds colonised with campylobacters gross contamination may result if damage occurs to the viscera during this process.

Partial evisceration is practised in GB. The intestines are removed but the remaining viscera are left inside the carcass. Delayed evisceration is also permissible, where un-eviscerated birds are held for up to 15 days under refrigeration at no more than 4°C. With regards to campylobacter this will have few microbiological implications. Due to the thermophillic nature of the organism it can be assumed no growth will occur. Despite these methods being employed only complete evisceration is considered within this model as the frequency and microbial implications of partial and delayed evisceration are currently unknown.

5.2.5 Washing

After post-mortem inspection the viscera are separated into edible and inedible offal. The eviscerated carcass is spray washed internally and externally. It is EU regulation that following evisceration there is a carcass wash. The mandatory use of inside-outside carcass washes removes visible faecal contamination, but does not eliminate

bacteria attached to the surface. Attachment is a time-dependent process; therefore washing the carcasses at different stages may remove bacteria before they become attached to the carcass. It has been demonstrated (Cudjoe *et al.*, 1991) that the washing procedure typically reduces the numbers of campylobacter on a carcass by 90% percent.

5.2.6 Chilling

The poultry meat hygiene regulations (European Union, 1997) require that poultry meat be chilled to 4°C or less as soon as possible after evisceration. Within the EU, three types of chilling process are used. These are air-chill, water immersion and spray chilling. All three methods may lead to cross-contamination, however, the problem is greatest in systems that use water. Air chillers are generally used where carcasses are for fresh sale and methods employing water are mainly used for frozen products. With regards to campylobacter, despite the potential for cross-contamination to occur, water chilling reduces the levels of contamination on a carcass as they move through a counter-flow current (Laisney *et al.*, 1991). Further, it has been demonstrated that the addition of chlorine to the chill water prevents the cross contamination of organisms which have been washed-off into the water. Despite campylobacters being able to survive levels of chlorine likely to be present in poultry processing water, the chemical hinders the attachment of the organisms to a carcass. Air chilling has been shown to have no effect on the levels of campylobacter due to their ability to survive under these conditions. (Cudjoe *et al.*, 1991). Spray chillers are rarely employed as they require large volumes of water but this technique avoids cross contamination.

5.2.7 Portioning

There is a growing trend towards production of poultry meat for the retail and catering sectors as portions rather than whole carcasses. In 1998, 41% of all chicken sold in GB was portioned and amounted to approximately 148 thousand tonnes (BPMF, 1998). The jointing of the carcass is increasingly being carried out using mechanical or semi-mechanical methods which allow faster line speeds and higher through puts required by the industry. However, the increased contact with machinery and/or human hands and tools during portioning could result in higher numbers of pathogens and spoilage organisms contaminating the product due to cross-contamination. This occurs as a result of either redistribution of the organisms contaminating the carcasses which have been processes that day or carry-over of contamination from the day before that has persisted through cleaning procedures. A variety of cuts are marketed, and the principal ones have been defined by a working party on standardisation of perishable produce: (ACMSF, 1996) these are

(i) Half: half the carcass obtained by a longitudinal cut in plane through the sternum and the backbone;

(ii) Quarter: a half divided by a transversal cut, by which the leg and breast quarters are obtained;

(iii) Breast: sternum and the ribs distributed on both sides of it, together with the surrounding musculature;

(iv) Leg: femur, tibia, and fibula, together with the surrounding musculature;

(v) Thigh : femur together with the surrounding musculature;

(vi) Drumstick: tibia, and fibula together with the surrounding musculature.

Few reports have been found in the scientific literature regarding contamination of poultry meat during portioning but automatic portioning equipment is likely to be a potential source of contamination (Gill, 1999). In addition, other surfaces with which the portions come into contact, such as conveyor belts, cutting boards, and packaging

material, may add to the microbial load of the final product. Hands and clothing of factory personnel and utensils such as knives are also likely to contribute to microbiological contamination. The degree of microbial contamination on cut portions reflects their degree and duration of exposure to the processing environment.

5.2.8 Carcass de-boning and mechanically recovered meat

The growth in the sale of further-processed poultry has placed a heavy demand on the production of de-boned poultry meat. Mechanically recovered meat of good quality has found a ready market and is widely used in a variety of white and red meat products such as frankfurters, sausages and burgers. Mechanically recovered meat can be held chilled at 2°C for use within 48 h or frozen in shallow layers in a plate freezer.

Due to the absence of data with regards to the microbial implications of carcass de-boning and mechanical recovery any effect is assumed to be negligible, however this assumption can easily be modified should such information become available.

5.2.9 Grading and Packaging, and Distribution

Once carcasses have been portioned they are weighed and graded. This can result in the cross-contamination of organisms from the equipment to the carcasses. The carcasses are then packed. Packing is governed by the scald and chill system used. If carcasses are water chilled they may be trussed with pre-packed giblets inserted into the body cavity and then packed in a polythene bag. Air chilled carcasses are usually packed without giblets on polystyrene trays and wrapped in cling film. Alternatively

they may be bulk packed. Again there is potential for cross-contamination here. Despite the opportunities for contamination there is no information in the literature on the effect of grading and packaging on contamination levels. Therefore it is assumed that grading and packing have no effect on carcass contamination levels. The packaged carcasses are then distributed appropriately.

5.3 Conclusions

The processing of chicken meat is a highly structured event and provides several opportunities for the contamination and cross-contamination of chicken products during the process. The stages of processing have been summarised in this chapter as stun and kill, scald, de-feather, evisceration, wash, chill, portioning, grading and packaging and distribution. Each of these processes contributes in a unique manner to the microbiological profile of the finished product. However, certain processes, namely de-feathering and evisceration are well recognised as important with regards to the impact the stages have upon the contamination level of a finished product.

Given the nature of chicken process, that is a high through put process with a great deal of structure, control measures which are able to reduce and even eliminate the microbial contamination on a finished product may be difficult to identify and costly to implement. As discussed in Chapter 1, the HACCP system provides the means to control a hazard once mitigation strategies have been identified. However, it is desirable to have a measure of the likely impact of each stage of processing upon the final contamination levels to help better understand the process by which contaminated chicken products enter the retail market. Further, the relative impact any given mitigation strategy would have upon contamination levels may also be invaluable prior to the undertaking of control measure. Both of these requirements can be approached by the use of mathematical modelling and risk assessment.

From the qualitative assessment of each of the stages of processing presented in this chapter, it can be seen that the most significant stages when considering the contamination of carcasses with campylobacters are scald, defeathering, evisceration, wash and chill. Therefore, the next stage is to quantify the impact each of these has upon prevalence and magnitude of carcass contamination. This is approached in the next chapter.

Chapter 6

Simulation model describing the slaughter and processing of chicken

6.1 Introduction

The processing of poultry is a sequential process that provides a number of opportunities for contamination of a carcass with food poisoning organisms such as *Campylobacter* spp. A qualitative assessment of each of the stages of processing has been presented in Chapter 5. From this assessment, the key stages regarding campylobacter contamination have been identified as scald, de-feathering, evisceration, washing, and chilling.

However, given that chicken processing is highly controlled by governing bodies such as the European Union (EU), this presents the opportunity for the application of mitigation strategies, which have the ability to reduce current contamination levels. To be able to consider control of potential contamination the process and the factors contributing to contamination must be understood. In this chapter a simulation model is presented which describes the processing of chickens in a random plant within Great Britain (GB). Stochastic in nature, the model mimics the uncertainty and variability present in such an intensive but highly regulated process.

6.2 The slaughter and processing model

6.2.1 Model overview

The model considers the stages of processing which may have an impact upon the level of campylobacter contaminating a carcass. As detailed in Chapter 5, these stages are scald, de-feathering, evisceration, washing, and chilling.

In the first instance, the simulation model considers a group of 100 birds from a random flock at the point of slaughter in a randomly selected processing plant in

GB. Based on the outputs from the models describing the rearing and transport stages of broiler production, that is the models described in Chapter 3 and Chapter 4, each bird in this group is assigned a history. More specifically the group is assigned a flock status and each bird within the group is assigned a campylobacter status, a level of contamination and a level of colonisation.

The status of the flock the group originates from is defined as θ_f where $\theta_f \in \{0,1\}$ such that $\theta_f = 1$ means that the flock is positive for campylobacter and $\theta_f = 0$ means that the flock is negative for campylobacter. The condition $\theta_f = 1$ occurs with probability P_{pf}, the probability that a random flock is campylobacter positive, therefore $\theta_f = 0$ occurs with probability $1-P_{pf}$. Further, the colonisation status of a bird in the group is defined as C_x, where $C_x \in \{0,1\}$ such that $C_x = 1$ means that the bird is colonised with campylobacter, and $C_x = 0$ means that the bird is not colonised with campylobacter. The subscript x indicates the location of the bird in the rearing house at de-population, as determined in Chapter 4, where $x = (i, j)$, such that $i = 1,...,a$ and $j = 1,...,b$ where a is the number of birds horizontally, and b the number of birds vertically represented on a lattice within the house. The condition $C_x = 1$ occurs with probability P_{wfp}, the probable within-flock prevalence of a positive flock, therefore $C_x = 0$ occurs with probability $1 - P_{wfp}$. If $C_x = 1$ the bird is colonised with Λ_s organisms. It follows that if $\theta_f = 1$, then $C_x \in \{0,1\}$, however if $\theta_f = 0$ then $C_x = 0$. Next the contamination status is considered. This is θ_c where $\theta_c \in \{0,1\}$ so that θ_c=1 means that a given bird has organisms contaminating the exterior and θ_c=0 means that the bird has no external contamination with C*ampylobacter* spp. The condition $\theta_c = 1$ occurs with probability P_{cs}, the probability that a bird is contaminated at slaughter. Therefore, the condition $\theta_c = 0$ occurs with probability $1 - P_{cs}$. If $\theta_c = 1$ a given bird is assigned η_{ext} contaminating organisms. The variables P_{pf}, P_{wfp}, P_{cs}, C_x, Λ_s and η_{ext} are generated from the model described in Chapter 3 and Chapter 4.

Once carcass history has been designated the position of the flock in the flocks to be processed that day is allocated. On any given day, 4 to 6 flocks may be dealt with in a processing facility (Andrew Gibson, Quality Control Manager, Premier poultry, *Personal Communication*). For the purposes of this model, it is assumed that a plant processes five flocks. The position of the selected flock in the processing day is given by θ_p, where θ_p is a uniform random integer variable between 1 and 5. Here, $\theta_p = 1$ means that the flock is the first in the day to be processed, $\theta_p = 2$ means that the flock is the second to be, and so on until $\theta_p = 5$, the fifth flock to be processed.

Following characterisation of history and flock position, the product type of the group of carcasses at the point of sale is determined. Product types are defined as (i) fresh and whole, (ii) fresh and portioned, (iii) frozen and whole, and (iv) frozen and portioned. Hence, at the point of sale, a random carcass is product type θ_s, where $\theta_s \in \{\alpha, \beta\}$ such that α represents a fresh product, and β a frozen product. Further, the product is in state θ_{por}, where $\theta_{por} \in \{0,1\}$ such that $\theta_{por} = 0$ means that the product is sold whole, and $\theta_{por} = 1$ means that the product is sold portioned. The probabilities of each of these product types occurring are derived from market shares of fresh and whole, fresh and portioned, frozen and whole, and frozen and portioned products provided by BPMF (1997).

Given characterisation of history, flock position and product type the model follows the group of carcasses through the first stages of processing, that is stun and kill, scald and de-feathering. Subsequent to these steps a random bird is selected from the group and followed through the remaining stages of processing, that is evisceration, wash and chill.

The model estimates the stochastic effect of each of the processing stages on the contamination levels on the carcass(es). Multiple runs of the model reflect the processing of multiple birds from multiple flocks and hence a probability

distribution for the number of campylobacters contaminating a product and the probability that a product is contaminated at the point of sale are generated. Derivation and estimation of these distributions is now described.

6.2.2 Level of Contamination

The model considers what happens during all stages of processing. From the qualitative assessment of chicken processing presented in Chapter 5, it can be seen that scalding results in a proportion of organisms to be washed off the carcass. De-feathering causes both a proportion to be washed off /removed with the feathers, and a number of organisms to be added from cross-contamination. Evisceration allows a number of organisms to contaminate a carcass from both cross- and vertical-contamination but may also result in a proportional reduction. During washing a proportion of organisms will be washed off. Finally chilling results in either no effect (air chilling) or a proportional wash-off (water-chilling). The final number of organisms that are on any carcass is a result of the effect of all stages of processing. Hence, it is necessary to estimate the changes afforded by each of the processing stages. The cumulative effect of these changes results in the number of organism contaminating a random carcass. This effect is quantified in equation (6.1) where the contamination level on a selected carcass *i*, defined as η_{p_i} is given by

$$\eta_{p_i} = \tau_i \nu_i (\mu_i \eta_{ext_i} + \varphi_i + \xi_i) \qquad (6.1)$$

where η_{ext_i} is the number of campylobacter contaminating bird *i* at the point of slaughter that is the level of contamination on entry into the processing plant, μ_i is the proportion remaining after scalding, φ_i is the change in numbers due to de-feathering, ξ_i is the change in numbers due to evisceration, ν_i is the proportion remaining after washing and τ_i is the proportion remaining on a carcass after chilling. The distribution for each of these parameters is estimated by use of

available sample data which measures the levels of contamination on a carcass before and then after a given process. There are several methods available to make such measurements, for example, counting levels of contamination on the neck skin, estimating levels by swabbing a particular section of the carcass of a fixed size, or enumerating the contamination on the whole carcass via a carcass rinse. Given some of the parameters of equation (6.1) are measures of proportion change in organisms it is assumed that on any given carcass the measured proportion reduction on one site of the carcass will be consistent across the whole carcass. Therefore, all data that measures levels of contamination before and after sampling in a consistent manner can be utilised to estimate model parameters. As such, throughout this chapter measures of contamination on a carcass in a data set are referred to as mean log cfu per unit as each study will have used a different sampling strategy and hence measured a different unit.

Due to the use of sample data, there is associated uncertainty with regards to the true distribution of the variability in these parameters. This is dealt with by the use of second-order non-parametric distributions as previously discussed in Chapter 2. Each of these variables is estimated as follows.

Estimating the effect of scald

The probable proportion of organisms remaining after the scalding process, that is μ_i, is dependent upon whether a carcass undergoes hard or soft scald, this is governed by product type, θ_s under the following condition:

$$\mu_i = \begin{cases} SS & \theta_s = \alpha \\ HS & \theta_s = \beta \end{cases}$$

Here *SS* and *HS* are distributions describing the variability in the proportion of organisms remaining after the processes of soft and hard scald respectively. Sample data consisting of the mean microbial counts of *n* carcasses selected at random before and after scalding were used to estimate the distribution for the variables *HS* and *SS*. In particular, for each scald type, proportions remaining were calculated for each data point. The data points and calculated proportions are given in Table 6.1. The variability distributions were then derived as follows.

For soft scald, the calculated proportions were used to derive a non-parametric second-order distribution (see Chapter 2) for the variability in the proportion remaining. Currently, it is assumed that scalding can only decrease the contamination levels. On one observation an increase was recorded. Given that all other data points are reductions and the process itself is one of washing-off, this point is omitted from the distribution. However, should more data become available which documents increases in contamination levels and hence provides more information about the process resulting in an increase in contamination as a result of scalding, this can be incorporated into the model.

For hard scald, there are only three data points available. Therefore, the variability in the effect of this process on the contamination level of a carcass is assumed to be a uniform random variable between zero and 10% above the maximum value observed for proportion remaining (Table 6.1). The maximum proportion remaining observed is 0.16 therefore this translates to a Uniform(0,0.0176). Further, due to limited data an assumption is made that there is no associated uncertainty with this maximum value. This assumption can be modified should more information become available.

Table 6.1: Measured mean log cfu campylobacter on a carcass before and after soft and hard scald and the calculated proportion remaining as a result of the scalding process.

Number of carcasses sampled	Scald type	Mean log cfu per unit before scald	Mean log cfu per unit after scald	Proportion of organisms remaining post scald	Reference
8	Soft	3.99	1.37	0.002	Oosterom *et al.*, 1983
8	Soft	3.30	1.68	0.020	Oosterom *et al.*, 1983
8	Soft	2.18	2.40	1.660	Oosterom *et al.*, 1983
8	Soft	3.74	<1.26	0.003	Izat *et al.*, 1988
8	Soft	3.56	1.26	0.005	Izat *et al.*, 1988
8	Soft	3.03	1.19	0.014	Izat *et al.*, 1988
5	Soft	2.9.0	1.00	0.012	Berrang *et al.*, 2000
5	Soft	5.00	2.00	0.001	Berrang *et al.*, 2000
5	Soft	5.00	1.70	0.001	Berrang *et al.*, 2000
5	Soft	3.10	2.40	0.199	Berrang *et al.*, 2000
5	Soft	5.80	2.40	0.0003	Berrang *et al.*, 2000
5	Soft	4.60	1.50	0.001	Berrang *et al.*, 2000
8	Hard	2.39	0.61	0.016	Oosterom *et al.*, 1983
8	Hard	3.42	1.25	0.007	Oosterom *et al.*, 1983
8	Hard	3.44	1.26	0.007	Oosterom *et al.*, 1983

Estimating the effect of de-feathering

The change in contamination due to de-feathering, defined as φ_i, is estimated by considering the cross-contamination effects of de-feathering. Experimental work based on the use of a 'seeder' carcass artificially contaminated with a marker organism has demonstrated that contamination with the marker can be detected as far as 200 carcasses away from the 'seeder' carcass after the de-feathering procedure (Hinton *et al.*, 1996). Further, the level of contamination was shown to be an inverse function of the number of birds between the nearest contaminated carcass and any given carcass.

As discussed in Chapter 4, when carcasses originate from a positive flock, the nearest positive carcass will most likely be the one next to it as a high proportion of the birds will be contaminated. In contrast, consider a group of carcasses that come from a flock previously classified as campylobacter negative. Within such a group there will be a proportion of carcasses that are contaminated. This can be accounted for in two main ways, first the crates in which the birds are transported are cleaned between flocks. However this process has been demonstrated as ineffective at removing the campylobacter contamination resulting from the transport of a positive flock. Secondly, when the birds are caught, the hands of the catchers can cause contamination (T. J. Humphreys, *Pers. Comm.*). In this situation, only low level contamination may occur and the nearest contaminated carcass may be several carcasses away. However, it is important to consider the effect of contamination caused by de-feathering in negative flocks as such contamination may persist to the final sale product.

From the above description, it is apparent that the effect of de-feathering on any given carcass is dependent upon the place of the carcass in the de-feathering line with respect to any contaminated carcasses in the line. If there are no contaminated carcasses preceding a selected carcass then the numbers contaminating the carcass,

if there are any, decrease due to the removal of feathers. It has been demonstrated that the de-feathering process can reduce numbers by 1000-fold (Hinton *et al.*, 1996) but there is no indication of the variability surrounding this decrease for different carcasses or indeed no suggestion of the uncertainty surrounding this point value. If there are contaminated carcasses in front of a given carcass, the numbers on the selected carcass may increase due to the aerosol spread and machinery contamination.

As previously mentioned, the increase in contamination is related to the number of carcasses between a selected carcass and the nearest contaminated carcass. Therefore, the model simulates the sequential de-feathering of the group of 100 birds and estimates the random effect of the de-feathering process on all 100 birds with respect to each de-feathering event within the group. This is shown schematically in Figure 6.1.

Figure 6.1 illustrates the 100 carcasses at the 100 different positions in the de-feathering line. Consider a random carcass, the position of the selected carcass is given by i (i=1,...,100) and the position of the carcass being de-feathered at the selected step is given by j (j=1,...,100). Thus when $i = j$ a selected carcass is being de-feathered. It therefore follows that for a selected carcass if $i < j$, the carcass is still to be de-feathered and for $i > j$ the carcass has been de-feathered. It can be seen that if a carcass being de-feathered is uncontaminated, $\theta_c = 0$, the contamination status of the birds behind that carcass does not change. However if a bird is contaminated, the result is a reduction on the level of contamination on the carcass being de-feathered and an increase on the carcasses following due to cross-contamination. This is illustrated by un-contaminated carcasses becoming contaminated. For example, consider the carcass in position $i = 3$ in Figure 6.1. It can be seen that when $j = 2$ the carcass in position $i = 3$ is uncontaminated, however in the next de-feathering step, that is $j = 3$, the carcass in position $i = 3$ has become contaminated as a result of the de-feathering of a contaminated carcass.

The extent of cross-contamination is related to the number of shackles, that is the number of birds, away a given carcass is from the carcass being de-feathered. Sample data (Hinton *et al.*, 1996) was used to estimate the effect of de-feathering on a series of carcasses. These data are shown in Table 6.2 and can be summarised as follows.

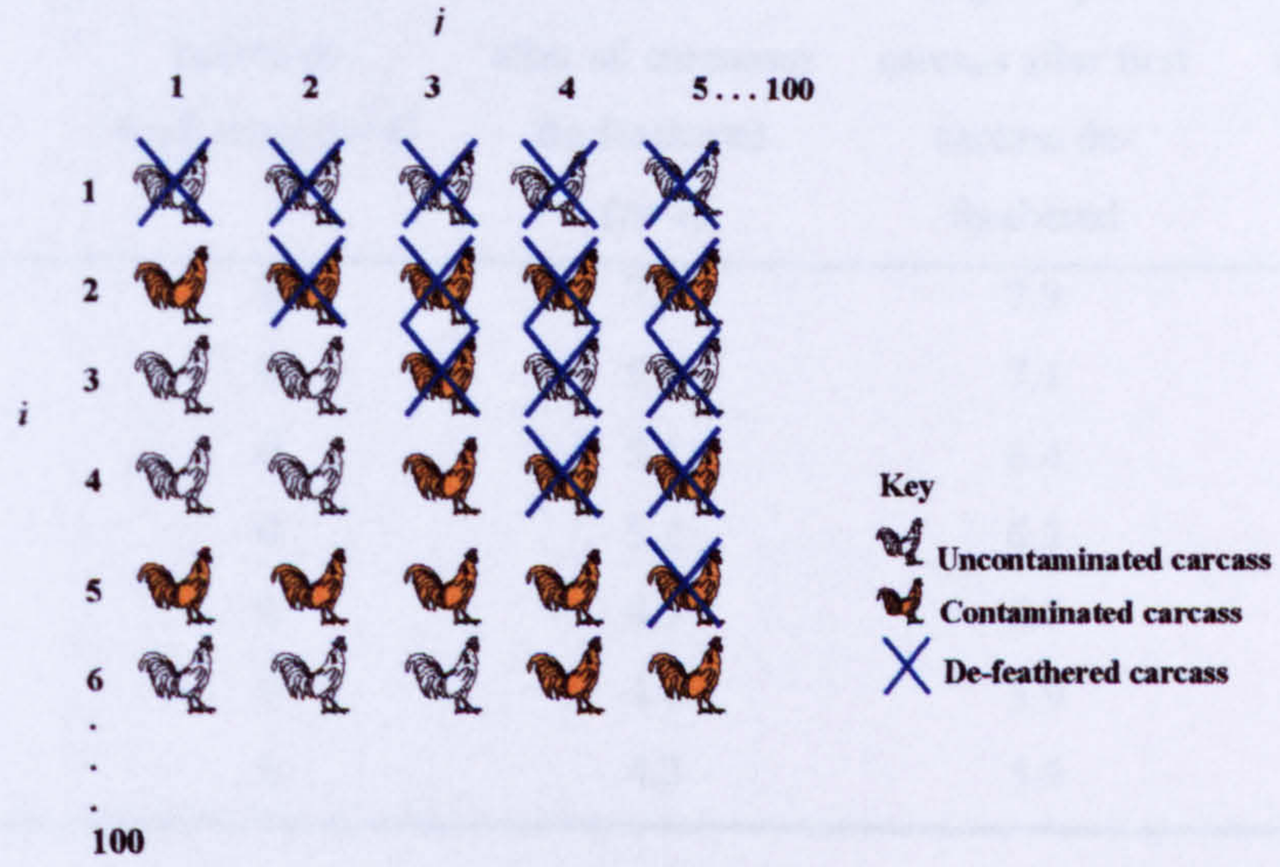


Figure 6.1: Schematic representation of the effect of position in the de-feathering process on cross-contamination of carcasses

Table 6.2: Experimental data showing the spread of organisms from a contaminated seeder carcass to subsequent uncontaminated carcasses (after Hinton *et al.*, 1996)

Carcass Number	Mean log cfu per carcass before de-feathering (n=4)	Mean log cfu per carcass after all carcasses de-feathered (n=4)	Calculated mean log cfu per carcass de-feathered	Proportion of seeder contamination received
'Seeder'	9	7.9	7.9	N/A
1	0	5.9	7.1	0.0125
2	0	5.3	6.4	0.0025
3	0	5.2	6.3	0.0029
4	0	4.5	5.7	0.0005
5	0	4.8	5.9	0.0008
6	0	4.3	5.5	0.0003

A seeder carcass was artificially contaminated with 9 log cfu of a marker organism. A further six carcasses were then set in the shackle line proceeding the seeder bird. These six carcasses were known to be uncontaminated with respect to the marker organism. Microbial counts were then taken after all six birds had been through the de-feathering process. Using this data (Table 6.2) the effect of de-feathering the seeder carcass on the six proceeding carcasses was estimated. In particular, taking this information and making two assumptions,

(i) the effect of the process on the carcass being de-feathered does not vary from carcass to carcass,

(ii) only the seeder carcass contributes to the contamination of the following carcasses;

the proportion of contamination a carcass receives from the carcass being de-feathered, given the distance between them, is estimated. A regression model was fitted to the experimental data using least squares to quantify the relationship between the proportion of seeder contamination received by a carcass and shackle position in relation to the seeder carcass. The predicted points and data points are plotted in Figure 6.1. The regression equation is $y = 0.0114(i-j)^{-1.8679}$ where y is the proportion of contamination a selected carcass receives from the carcass being de-feathered and (i-j) is the shackle position of the selected carcass, and the R^2 value is 0.91.

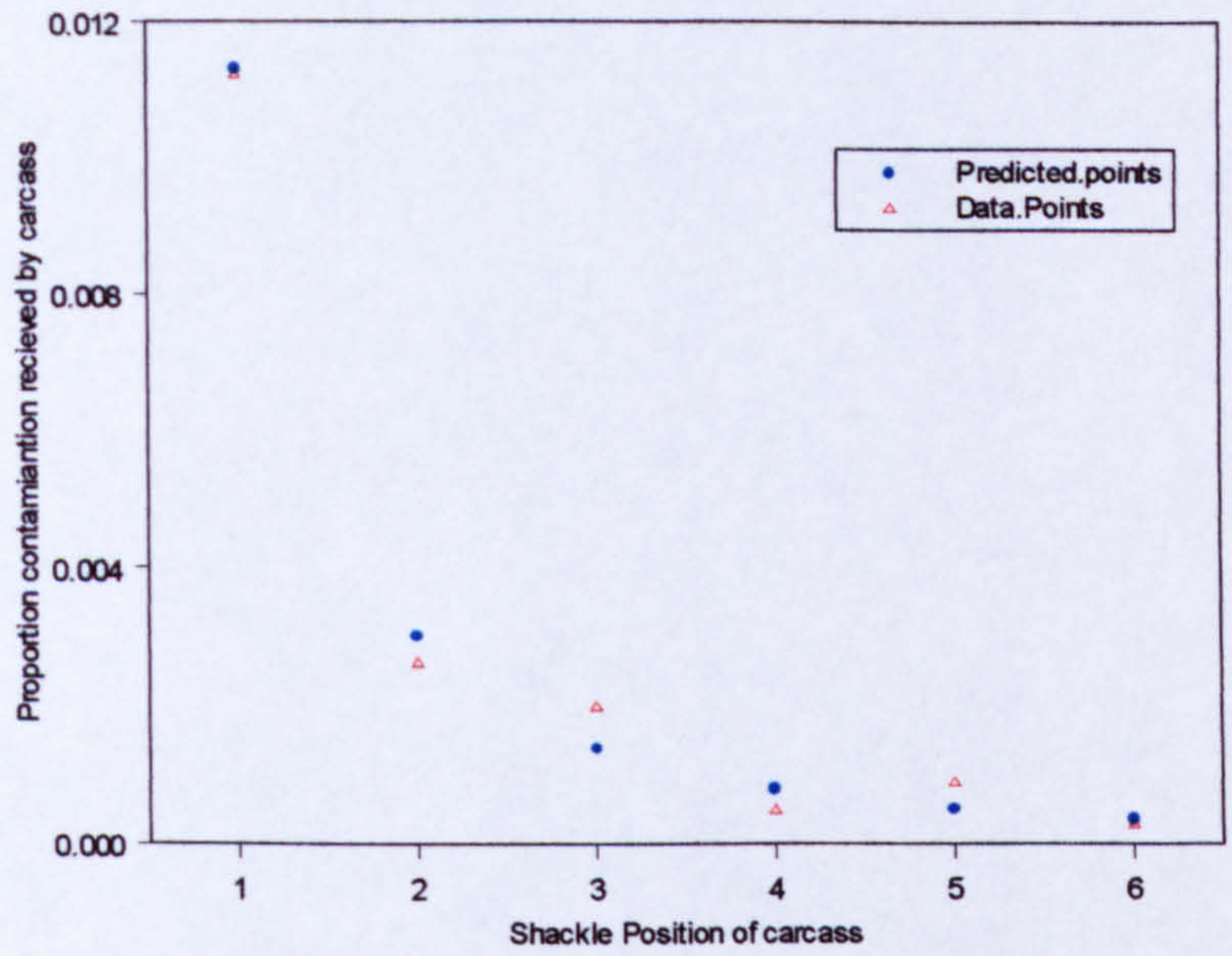


Figure 6.2: Graph showing the experimental data in comparison with the corresponding points predicted from the regression equation $y = 0.0113(i-j)^{-1.9067}$ to estimate the proportion of contamination received form a carcass being de-feathered given the number of shackles away a carcass is from the carcass undergoing the process, $(i-j)$. Here i is the position of the selected carcass and j is the position of the carcass being de-feathered.

Uncertainty is incorporated into the regression model as described in Chapter 2. In brief, the regression line is linearised and the Bootstrap method is then employed to estimate the distribution of uncertainty associated with the residuals at each observation point, that is, the number of shackles between a given bird and the bird being de-feathered.

For a given carcass the change in contamination resulting from the de-feathering process is the sum of the number of organisms gained from the de-feathering of the preceding birds, minus the sum of the number of organisms lost to the birds proceeding the carcass as a result of de-feathering of the carcass, and the reduction that results from de-feathering due to organisms being lost via the removal of feathers and flushing action of the water. Therefore the change in contamination due to de-feathering for the carcass in position i in the group given the carcass being de-feathered is in position j, that is φ_i, is given by equation 6.2.

$$\varphi_i = \left(\sum_{j=1}^{j=i-1} \eta_{c,d(i-j)} \left(0.0113 (i-j)^{-1.9067} \right) \right) - \left(\sum_{j=i+1}^{j=100} \eta_{c,d_i} \left(0.0113 j^{-1.9067} \right) + r_i \right) \qquad (6.2)$$

Here r is the reduction in the level of contamination on the carcass being de-feathered as a result of the removal of feathers and washing action of the water, and $\eta_{c,d(i-j)}$ is the level of contamination on the carcass being de-feathered which is given by $\mu_{(i-j)}\eta_{ext(i-j)}$ and η_{c,d_i} is the level of contamination on carcass i at the point of de-feathering given by $\mu_i \eta_{ext_i}$. To illustrate how Equation 6.2 works consider a group of 10 carcasses to be de-feathered. Table 6.3 shows how φ_i is calculated for the carcass which is fifth in this group, that is $i = 5$.

Table 6.3: Illustration of the calculation of the variable φ_i, the change in contamination levels as a result of de-feathering, carried out by use of equation 6.2. Assume number on carcass $i=5$: 2000 cfu, and the reduction is $r_{i=5}=1200$, de-feathering results in an increase of contamination of 6607cfu.

j	Number on carcass $i=j$	Number carcass $i=5$ gains from preceding carcasses	Number carcass $i=5$ loses to following carcasses
1	100000	804	0
2	31623	440	0
3	125893	3794	0
4	251	28	0
5	N/A	N/A	N/A
6	0	0	226
7	0	0	60
8	0	0	28
9	0	0	16
10	0	0	11
	Total cfu carcass $i=5$ gained		5066
	Total cfu carcass $i=5$ lost		1541
	$\phi_{i=5}$		6607

Estimating the effect of evisceration

The probable change in numbers due to evisceration, ξ_i, is dependent upon the colonisation status of the bird, θ_b, and the probability that cross-contamination occurs during evisceration.

In previous processing steps where cross-contamination has been considered, the focus has been upon the redistribution of contaminating organisms within a given flock. During evisceration, the potential for cross-contamination between flocks is introduced. This contamination could result from a flock processed earlier in the day or even on a previous day, where contamination has persisted despite cleaning procedures.

Sample data measuring the level of campylobacter contamination on carcasses before and after the evisceration procedure are shown in Table 6.4.

Inspection of this data indicates that evisceration has mixed effects on the level of carcass contamination. Increases, decreases and no change in contaminating load are recorded in the data set. The carcasses considered in this data were reported as 'remaining intact' during evisceration, that is no damage occurred to the viscera during the procedure and as such an increase in contaminating load is a result of horizontal contamination rather than vertical-contamination. However, damage to the viscera is not an uncommon occurrence and provides the opportunity for gross contamination of the carcass exterior. Within the model, both horizontal and vertical contamination are considered.

Let, C_c be the change in contamination due to horizontal-contamination from, for example, workers and machinery and C_d the increase in contamination due to damage to the viscera. The variable C_c is dependent upon the probability that change to the contamination levels (either increase or decrease) occurs, this is

defined as κ. The variable $\kappa \in \{0,1\}$ such that $\kappa = 1$, means that, not considering damage to the viscera, a change, either increase or decrease, in contamination level on a carcass has occurred during evisceration. In contrast, $\kappa = 0$ means that evisceration, in the absence of damage, has no effect on contamination levels. The condition $\kappa = 1$ occurs with probability P_κ therefore $\kappa = 0$ occurs with probability $1 - P_\kappa$. The probability that $\kappa = 1$, that is P_κ is estimated from the experimental data shown in Table 6.3. It can be seen in this data set that no change in contamination level on a carcass after evisceration was recorded twice out of 18 observations. Using this information a beta distribution is used as previously described in Chapter 3 to describe the uncertainty surrounding the true value of P_κ more specifically $P_\kappa \sim \text{Beta}(3,17)$.

If $\kappa = 1$ the effect, either increase or decrease in contamination, must be determined. The effect of evisceration given that a change in contamination results is defined as Ev. The variable $Ev \in \{\varsigma, \varphi\}$ such that $Ev = \varsigma$ means that disregarding damage, evisceration results in an increase in contamination of d_ς organisms, $Ev = \varphi$ means that, not considering damage, evisceration results in a decrease in contamination with the proportion of organisms remaining given by d_φ. The condition $Ev = \varsigma$ occurs with probability P_ς, therefore $Ev = \varphi$ occurs with probability $1 - P_\varsigma$. The probability P_ς. is estimated based on the data shown in Table 6.3. An increase in contamination was seen on 14 out of 18 observations where there was a change in contaminating load as a result of evisceration. Therefore, using the beta distribution to quantify the associated uncertainty, $P_\varsigma \sim \text{Beta}(15,5)$. Due to the small sample size the variability in the number of campylobacters added to the carcass, d_ς, and proportion of organisms remaining following a reduction in contamination, that is d_φ, are described by non-parametric second-order distributions. The distributions are estimated, as previously described in Chapter 2, using the sample data shown in Table 6.4.

Table 6.4: Sample data measuring the levels of campylobacter contamination on a carcass before and after evisceration.

Number of carcasses sampled	Log cfu per unit before evisceration	Log cfu per unit after evisceration	Change observed	Change in contamination level	Reference
				Increase log cfu per carcass	
8	1.99	2.44	Inc	1.979019	Oosterom *et al.*, 1983
8	1.07	2.58	Inc	0.962321	Oosterom *et al.*, 1983
8	2.09	2.62	Inc	2.080651	Oosterom *et al.*, 1983
8	2.18	2.5	Inc	2.172767	Oosterom *et al.*, 1983
8	2.37	3.12	Inc	2.364181	Izat *et al.*, 1988
8	2.82	3.49	Inc	2.8177	Izat *et al.*, 1988
8	2.82	3.49	Inc	2.8177	Izat *et al.*, 1988
5	3.2	3.2	None	0	Berrang *et al.*, 2000
5	3.7	3.7	None	0	Berrang *et al.*, 2000
				Proportion of organisms remaining post evisceration	
5	4.5	3.7	Dec	0.158489	Berrang *et al.*, 2000
5	3.1	2.53	Dec	0.269153	Berrang *et al.*, 2000
5	4.1	4	Dec	0.794328	Berrang *et al.*, 2000
5	<3	1.6	Dec	0.039811	Berrang *et al.*, 2000
11	5.75	5.7	Dec	0.891251	Abu-Ruwaida 1994
8	3.68	3.49	Dec	0.645654	Izat *et al.*, 1988
8	2.46	2.24	Dec	0.60256	Oosterom *et al.*, 1983
8	2.85	2.6	Dec	0.562341	Oosterom *et al.*, 1983

inc = increase dec = decrease none = no change

If it is decided that an increase in contamination occurs, that is $Ev = \varsigma$ then it is necessary to consider the position of the flock in the facility processing day, given by θ_p where $\theta_p \sim \mathrm{U}(1,5)$ as defined earlier in section 2.2.1. The increase in contamination due to evisceration is not only due to re-distribution of organisms from within a given flock, but also cross-contamination from other flocks. This cross-contamination may be from positive/contaminated flocks processed that day or carry over from a previous day on which positive/contaminated flocks were dealt with. Given this, it can be seen that there are three possible scenarios:

(i). A positive flock has preceded the selected flock resulting in contamination of the machinery and hence cross-contamination of the selected carcass;

(ii). No positive flocks have been processed so far that day in the selected facility. However, the national flock prevalence is not zero and therefore positive flocks have been processed on previous days. As such it is likely there is low-level contamination of equipment in the plant which may be due to persistence of the organisms through cleaning procedures;

(iii). There are no positive flocks in the national flock, that is $P_{pf} = 0$. As such contamination of the plant that could occur from, for example animal reservoirs contaminating workers shoes and clothing, can be assumed to be negligible.

In the first scenario the distribution d_ς is selected. In the second scenario d_ς is truncated at the 20th percentile and all values below this percentile are assumed to be equally likely. More specifically, this becomes $\mathrm{Uniform}\left(0, F\left(d_\varsigma < 0.2\right)\right)$. There is currently no data available which gives level of contamination in processing plants where no positive flocks have been processed. Therefore, it is assumed that truncation at the 20th percentile provides an appropriate distribution for the residual contamination. However, should such data become available, this assumption can be modified. In the final scenario $d_\varsigma = 0$ as it is assumed there is no contamination of equipment or workers.

It can be seen that the variable C_c, the change in contamination due to horizontal contamination, is dependent upon κ, the change in contamination levels during evisceration. If $\kappa = 1$, that is evisceration has resulted in either an increase or decrease in contamination, C_c is given under the following condition

$$C_c = \begin{cases} d_\zeta & Ev = \zeta \\ \eta_{ce}.d_\varphi & Ev = \varphi \end{cases}$$

Here η_{ce} is the number of campylobacters contaminating a carcass at the start of evisceration which is given by $\mu_i \eta_{exti} + \varphi_i$. If $\kappa = 0$ then, disregarding damage, no change in contamination levels has occurred during evisceration and, therefore, $C_c = 1$. The result of the above condition is that C_c will always be a number of organisms, even when a proportion decrease occurs.

Let C_d be the increase in contamination as a result of damage to the innards during the evisceration procedure. The variable C_d is assumed to be a uniform random variable with a possible minimum value of zero and a maximum value of η_{caeca}, that is the number of campylobacters colonising 1 gram of the ceacal contents of a bird. This assumption is due to the lack of data with respect to the level of contamination that will occur given a carcass is colonised and the viscera are damaged. Should such data become available, this assumption can be modified as appropriate. The probability that damage occurred to the innards is defined as Da. Here $Da \in \{0,1\}$ such that $Da = 0$, with probability P_{Da} where P_{Da} is a uniform random variable between zero and one, means that damage occurred to the viscera during the evisceration process; $Da = 1$, with a probability 1-P_{Da}, means that damage did not occur during evisceration.

Considering both vertical and horizontal contamination during evisceration the overall change in contamination can be quantified. If damage occurs during the process then the change in contamination is the sum of the increase in numbers

resulting from contamination due to damage, C_d, and the change in numbers presented by the overall process, that is C_c. If, however, damage does not occur then $C_d = 0$ and the change in contamination is simply that afforded by the overall process, C_c. More specifically

$$\xi_i = \begin{cases} C_c + C_d & Da = 1 \\ C_c & Da = 0 \end{cases}$$

It can therefore be seen that the change in contamination as a result of evisceration, that is ξ_i, can be summarised by the following statement

$$\xi_i = \begin{cases} C_c + C_d & Da = 1\ \kappa = 1 \\ C_c & Da = 0\ \kappa = 1 \\ C_d & Da = 1\ \kappa = 0 \\ 0 & Da = 0\ \kappa = 0 \end{cases}$$

Estimating the effect of washing

Washing reduces the level of contamination on a carcass. This can be seen in the data shown in Table 6.5. This table consists of measurements of the level of carcass contamination taken before and after the washing process. Given the data in Table 6.5, the proportion of organisms remaining after a wash of the carcass, defined as v_i, is estimated. A distribution describing the variability in the remaining proportion is then estimated. As the sample data set is small there is associated uncertainty with regards to the true variability of this parameter. Therefore v_i, the proportion remaining after washing, is described by a second-order non-parametric distribution estimated using methods previously described (Chapter 2).

Table 6.5: Sample data showing measures of campylobacter before and after carcass washes.

Number of carcasses sampled	Mean log cfu before washing	Mean log cfu after washing	Proportion of organisms remaining	Reference
5	1.60	1.00	0.2511	Berrang *et al.*, 2000
5	3.20	2.10	0.0794	Berrang *et al.*, 2000
5	3.70	3.30	0.3981	Berrang *et al.*, 2000
5	2.53	2.00	0.2951	Berrang *et al.*, 2000
5	4.00	1.60	0.0039	Berrang *et al.*, 2000
5	3.70	2.70	0.1000	Berrang *et al.*, 2000
8	2.83	1.71	0.0758	Berrang *et al.*, 2000
8	2.94	2.39	0.2818	Berrang *et al.*, 2000
8	3.50	3.04	0.3467	Berrang *et al.*, 2000
11	5.70	5.10	0.2511	Berrang *et al.*, 2000

Estimating the effect of chilling

Within the EC, only air chilling is used for carcasses to be sold as fresh products. Air chilling is assumed to have no effect on the organism levels on the carcass (Cudjoe *et al.*, 1991). However, if a carcass is to be sold as frozen products, it is assumed that water chilling is used. This has been shown to have an impact on contamination levels. Further, when water chilling is used chlorine may be used as an additive. This affects the carcass contamination as can be seen in Table 6.6. Here carcass contamination was measured before and after chilling with water. Procedures both with and without chlorine are included in this data set.

Table 6.6: Data measuring the levels of campylobacter contamination on a carcass before and after water chilling with and without chlorine added to the chill water.

Number of carcasses sampled	Chlorine added	Mean log cfu before chilling	Mean log cfu after chilling	Proportion remaining	Reference
8	-	1.71	1.43	0.52	Izat *et al.*, 1988
8	-	2.39	1.85	0.29	Izat *et al.*, 1988
8	-	3.04	1.18	0.01	Izat *et al.*, 1988
15	-	2.92	1.74	0.07	Wempe *et al.*, 1983
15	-	2.62	1.38	0.06	Wempe *et al.*, 1983
15	-	3.32	2.33	0.10	Wempe *et al.*, 1983
15	-	2.50	1.76	0.18	Wempe *et al.*, 1983
5	+	2.10	1.20	0.13	Berrang *et al.*, 2000
5	+	3.30	1.10	0.01	Berrang *et al.*, 2000
5	+	2.00	0.90	0.08	Berrang *et al.*, 2000
5	+	1.60	3.20	N/A	Berrang *et al.*, 2000
5	+	2.70	1.10	0.03	Berrang *et al.*, 2000
90	+	5.35	3.86	0.03	Cason, 1997

The frequency with which chlorine is used, in GB is currently unknown. This frequency is defined as P_{Cl} and is assumed to be a uniform random variable with a minimum value of zero and a maximum of one. Based on this, the use of chlorine in the chilling of a given carcass is defined as Cl where $Cl \in \{0,1\}$ such that $Cl = 1$ means that chlorine is used as an additive to the chill water; this occurs with probability P_{Cl}. Further, $Cl = 0$ means that chlorine was not used as an additive to the chill water. This condition therefore occurs with probability $1 - P_{Cl}$.

It can therefore be seen that the probable reduction achieved by chilling the carcass is dependent upon the status of the product, either fresh or frozen. If the product is to be sold as fresh, $\theta_s = \alpha$, there is assumed to be no change in contamination levels and $\tau_i = 1$. In contrast, if the product is to be sold as frozen, $\theta_s = \beta$, water chilling will be used and this may have an impact on microbial levels on the carcass. This impact depends on the use of chlorine in the water. More specifically the proportion of organisms remaining following chilling is given under the following condition

$$\tau_i = \begin{cases} 1 & \theta_s = \alpha \\ \tau_{cl} & \theta_s = \beta; \quad Cl = 1 \\ \tau_{ncl} & \theta_s = \beta; \quad Cl = 0 \end{cases}$$

Here τ_{cl} is the proportion of carcass contamination remaining following water chill without chlorine, and τ_{ncl} is proportion of contamination remaining after a water chill which has chlorine added to the water. The variables τ_{cl} and are τ_{ncl} estimated, first by calculating the reduction in contamination in the samples shown in Table 6.6 with and without chlorine being added to the water. These data are then combined to give a second-order non-parametric distribution using previously described methods.

6.2.3 Summary of model

In summary, a description of a simulation model, which predicts the number of campylobacter that will contaminate a carcass post-processing in a random plant in GB has been provided. This model looks at the major stages of processing and involves several parameters, the outcome of this model is a processed carcass with an associated level of campylobacter contamination. To illustrate how the inputs fit together Figure 6.3 is a schematic summary representation of the model illustrating where the various variables described above are inputs into the overall model.

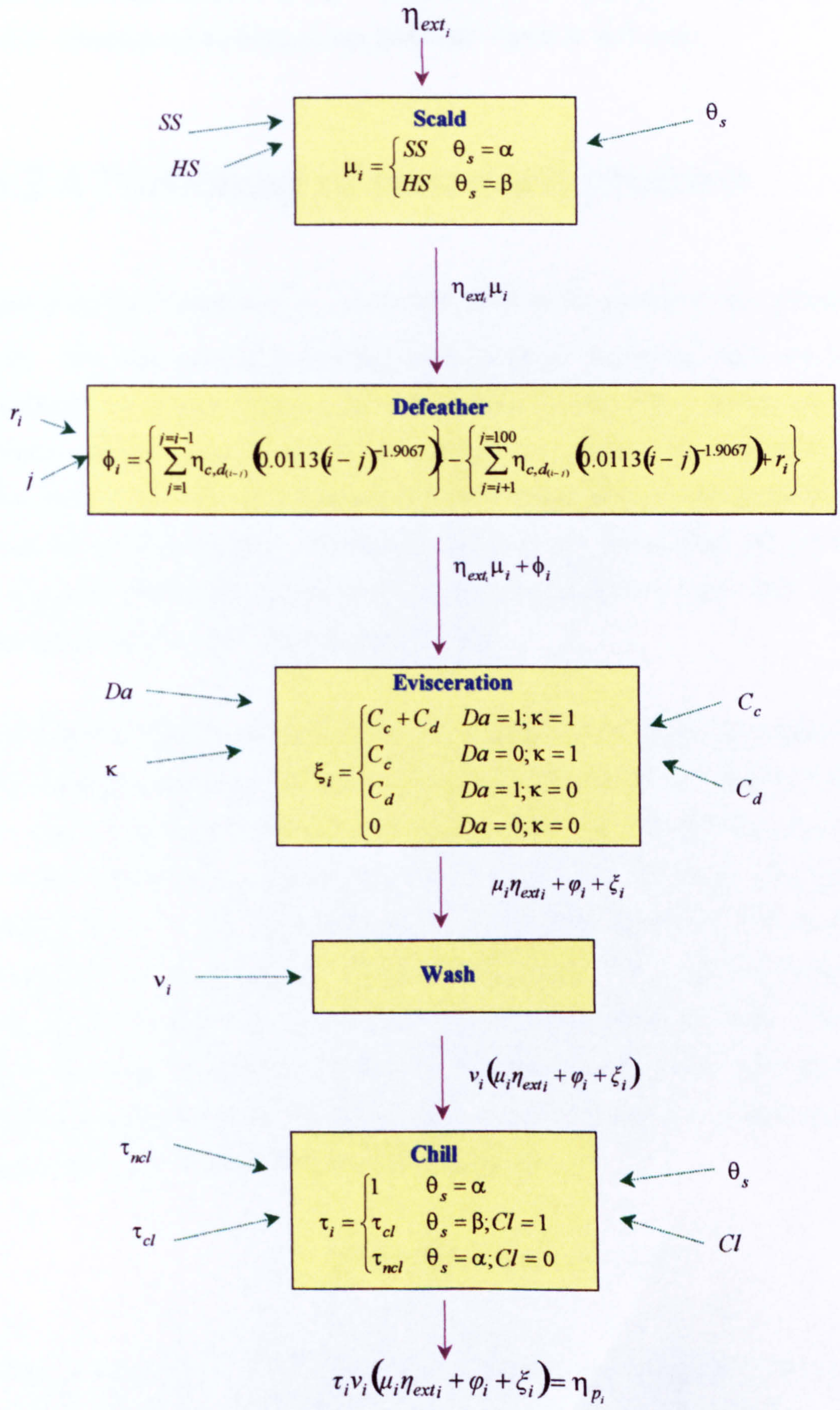


Figure 6.4: Summary of the simulation model to describe the processing of a

chicken in a random plant in GB. For details of the mathematical terms see section 6.2.2. Directed arrows indicate key parameter inputs at each stage.

6.2.4 Portioning of processed products

Following the estimation of η_{pl} as described above, the product is then prepared for sale. This may involve portioning of the product. Currently, there are no data available which give changes in contamination which occur during processing. Further reports have recorded no increase in overall contamination during portioning. Therefore it is assumed that portioning does not change the microbial load on a whole carcass. However, it is necessary to estimate the number of campylobacters which are present on a portion of chicken resulting from a carcass, for which the level of contamination is known.

An approach which could be adopted is to assume that the organisms contaminating the processed carcass are uniformly distributed over the carcass, however, there may be some clustering of organisms. This clustering could result from areas of the carcass being more exposed to contamination and therefore carrying more organisms, or a result of an area of the carcass being protected from removal of organisms by, for example, washing of the carcass. This could be because the organisms are under a wing, or embedded in the skin or feather follicles. Therefore, it is necessary to allow for variability between the extent of contamination on portions originating from the same carcass. A method available to deal with such a situation is presented by Nauta (2001) and is given by

$$\eta_{por} \sim \text{Binomial}\left(\eta_{pl}, \text{Beta}\left(b, b(w-1)\right)\right)$$

Here, the parameter b is a measure of the degree of clustering, that is the variability in the number of organisms that contaminate each portion, of weight w. This is a

relative value which is an integer >1. The greater the value of b the lesser the extent of the variability. The result is a variability distribution for the number of organisms which contaminate a portion of weight w. The weight of a carcass, in grams, is described by a triangular distribution, more specifically Triang(110,1500,2500) (Andrew Gibson, premier Poultry; Personal Communication). The weight of meat which is edible on a carcass is 30% of the weight of the carcass, and finally, the size of the portion, that is w, is therefore assumed to be one quarter the weight of edible meat on the carcass.

The value that the parameter b should take is unknown. Nauta *et al.*, (2001) carried out an expert consultation to determine the value of b for clustering of *Escherichia coli* on raw beef carcasses. The mean value determined was 4. This value is assumed to be appropriate for the clustering of campylobacter on chicken.

Therefore, the number contaminating a product at the end of processing, that is η_p, is given by the following condition

$$\eta_p = \begin{cases} \eta_{pi} & \theta_{por} = 0 \\ \eta_{por} & \theta_{por} = 1 \end{cases}$$

6.2.5 Estimating the prevalence of contaminated products

The model described above initially follows a group of 100 birds from a randomly selected flock and subsequently a random bird from within this group through the processing plant. At the end of each of the processing stages modelled the number of contaminating organisms on the carcass is calculated. This calculation mimics the changes in numbers as a result of the particular step.

At the end of processing, a carcass can be defined as contaminated if it carries at least one organism. By means of a conditional statement, the model can state whether a selected product is contaminated or not. The conditional statement is

$$\psi = \begin{cases} 1 \text{ if } \eta_p \rangle 1 \\ 0 \text{ if } \eta_p \langle 1 \end{cases}$$

where ψ_i is whether or not the product is contaminated at retail. Within a given simulation, distributions are sampled n times and each time the result is either a contaminated or uncontaminated product. Multiple samplings of the distributions represent the production of multiple products. Therefore, running the model allowing for n samplings of each distribution the probability that a product is contaminated can be calculated by use of Equation 6.3.

$$P_{pp} = \frac{\sum_{i=1}^{i=n} \psi_i}{n} \qquad (6.3)$$

where P_{pp} is the probability that a product is contaminated based upon n samplings within a simulation of the model.

6.3 Running the model

The model contains several parameters, each of which is described by an appropriate variability distribution. The variability distributions have associated uncertainty with respect to the true variability. As such the model can be run with different combinations of variability distributions with each combination representing one possible realisation of the processing of chicken. Of course, if there was no associated uncertainty there would be only one possible combination. These different realisations are mimicked by simulating the model a number of different times and the result is multiple distributions describing the variability in the number of campylobacters contaminating a product and multiple estimates of the prevalence of contaminated products at the end of processing. This allows the quantification of the level of uncertainty with respect to the outputs of the model η_p, the number contaminating a product at the end of processing, and P_{pp}, the probability that a product is contaminated at the end of processing.

The model is run for 50 simulations. Upon each simulation a variability distribution is selected from the associated uncertainty space for each second-order random variable and kept constant for any given simulation. Within each simulation the selected distributions are sampled 5000 times and η_p calculated as shown in equation (6.1). At the end of each simulation P_{pp} is then given as shown in equation (6.3). Multiple simulations result in the uncertainty distribution for P_{pp}. The number of samples taken within a simulation was chosen according to when the running mean of η_p no longer deviates ±1% from the 'true' mean, defined as the mean of η_p at 5000 iterations. The deviation of the running mean from the 'true' mean is illustrated in Figure 6.4. It can be seen that 5000 samples are adequate to fulfil the criteria. The number of simulations was chosen to ensure adequate selection of the variability from the uncertainty interval for each of the second-order random variables.

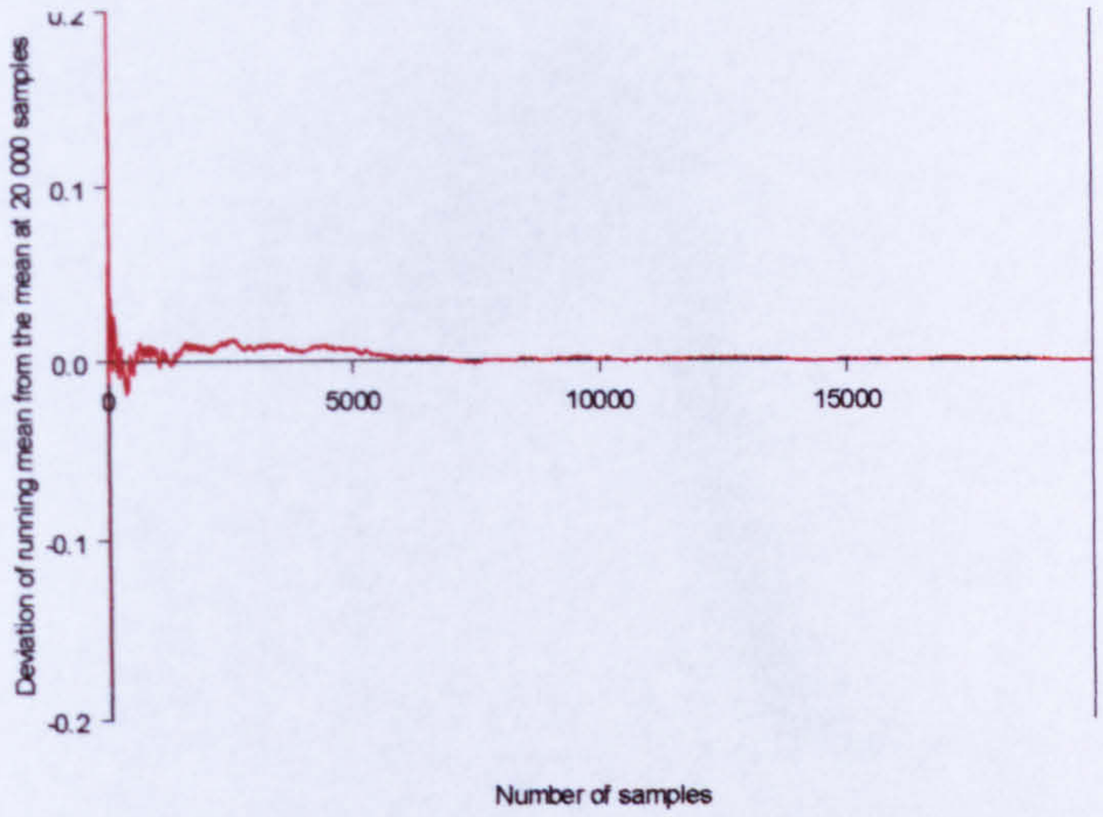


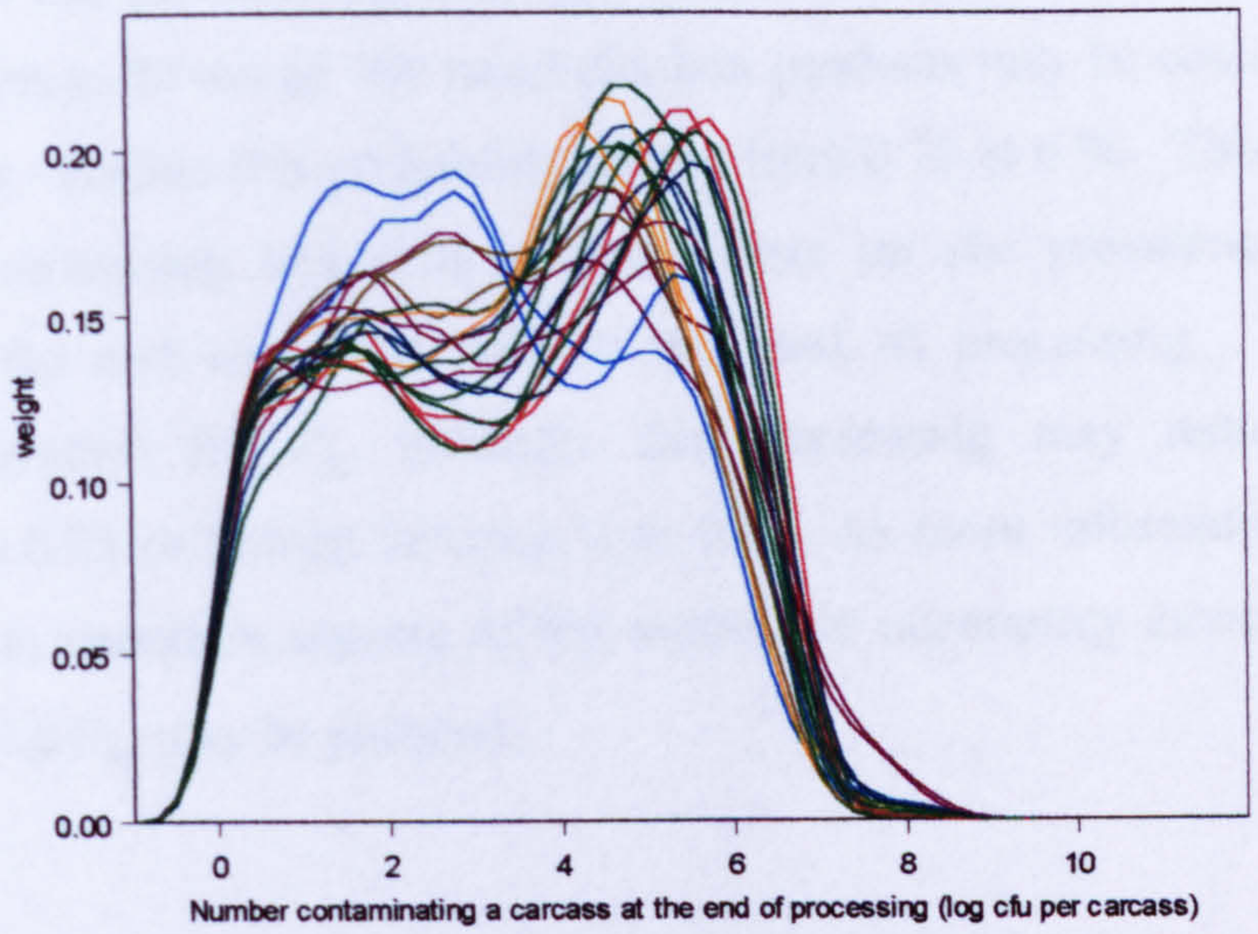
Figure 6.4: Graph to show the deviation of the mean number organisms contaminating a product, η_p, from the 'true' mean of η_p defined as the mean at 10,000 distribution samples (iterations).

6.4 Model results

The density and cumulative distributions for the probable number of campylobacters that will contaminate a random chicken product at the end of processing, η_p, based on 50 simulations made up of 5000 distribution samples are shown in figure 6.5.

In this figure, each individual line represents one possible variability distribution for the number of campylobacters contaminating a product, η_p. The multiple curves and their locality, resulting from multiple simulations of the model, indicate the degree of uncertainty surrounding this variability. Considering this, the mean

number of organisms on a random product ranges from 5.43 to 6.28 log cfu per product.



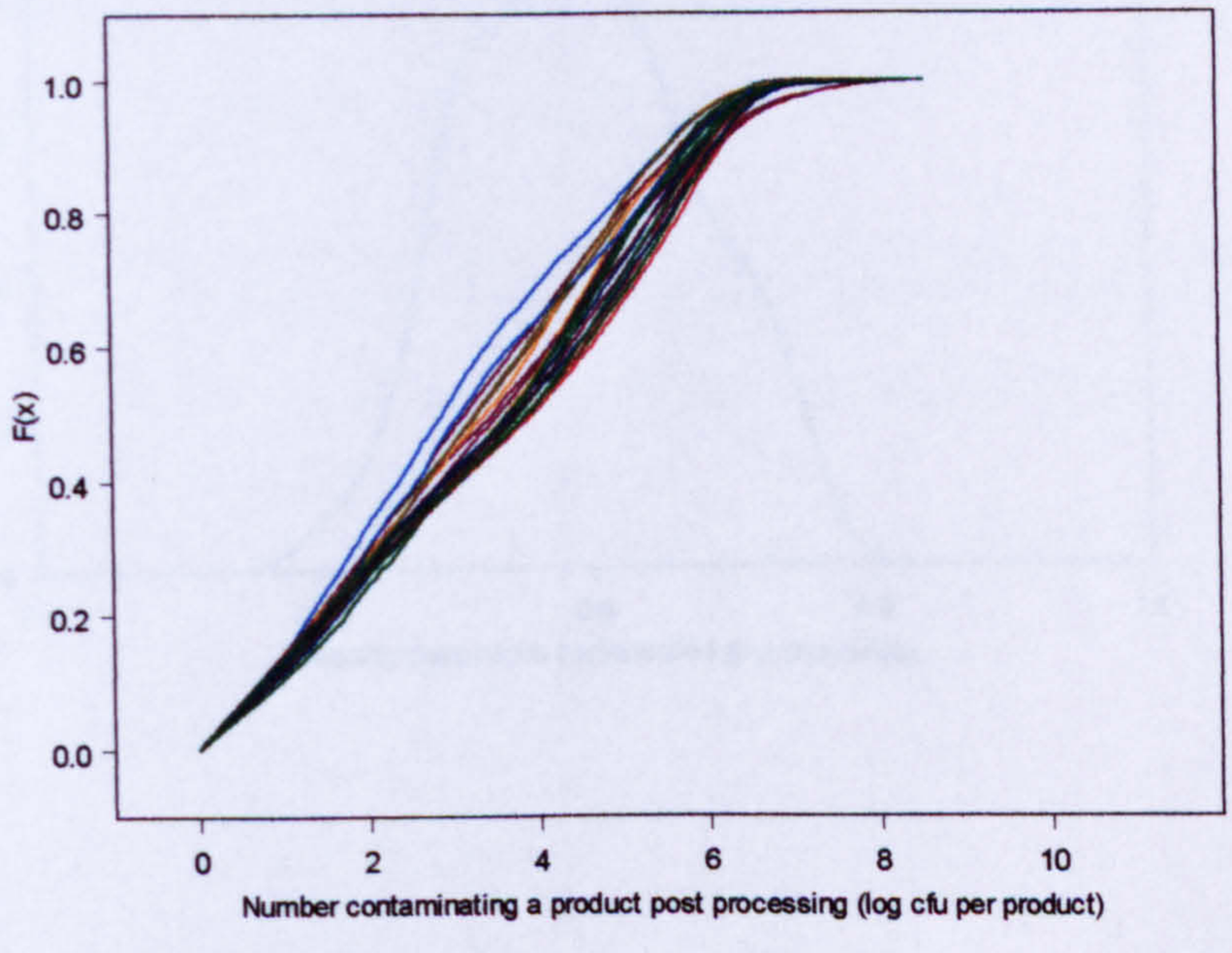


Figure 6.5: Second-order density and cumulative plots describing the number of campylobacter contaminating a product, η_p estimated using the model, and illustrating the uncertainty that arises due to the variability in the combined process.

Based on the distributions for η_p, the probability that a product is contaminated with campylobacter at the point of sale that is $P_{pp,}$ is also uncertain, each simulation resulting in a possible value for this probability. The resulting density plot for P_{pp} is shown in Figure 6.6. This figure shows the uncertainty in the true value of P_{pp}. The mean value for this probability, based upon 50 simulations of 5000 iterations is 0.8, That is, on average 80 out of 100 retail chicken products may be contaminated with campylobacter. Further this probability ranges from 0.73 to 0.90. This suggests that on average, processing has little overall effect on the prevalence of positive carcasses at the end compared with at the start of processing. However, the uncertainty interval for P_{pp} indicates that processing may reduce the mean prevalence to 0.73 or indeed increase it to 0.90. As more information is gathered with respect to uncertain aspects of the model the uncertainty associated with the outputs η_p, and P_{pp} may be reduced.

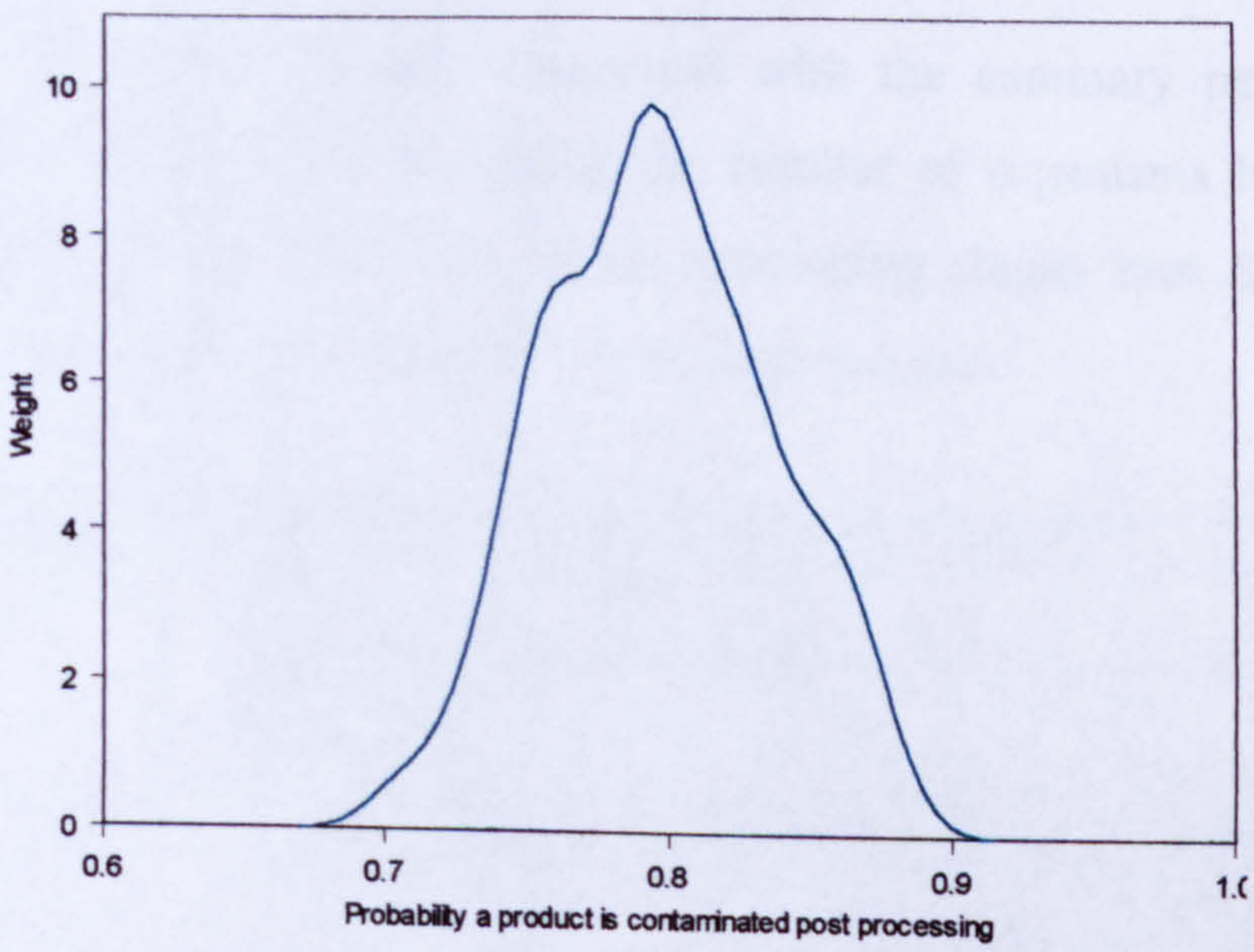


Figure 6.6: Uncertainty plot for the probability that a product is contaminated post processing, P_{pp}

6.5 Sensitivity of the processing stages on model results

The outputs of this model, the probable number of organisms contaminating a random retail product, and hence the probability that a retail product is contaminated are the result of the integration of all stages of processing. Figure 6.7 illustrates the individual effects of the processing stages, specifically on the number of organisms on a carcass at the end of the respective step. The figure shows the second-order distributions for the number of organisms on a carcass (Log cfu per carcass) as predicted by the model following stun & kill, scald, de-feather, evisceration, wash and chill. Table 6.7 shows the summary statistics for the number of organisms per carcass after each of the stages.

Table 6.7: Uncertainty ranges associated with the summary percentiles for the second-order distributions describing the number of organisms likely to be on a contaminated carcass after each of the processing stages stun & kill, scald, de-feather, evisceration, wash and chill, log cfu per carcass.

Processing stage	Uncertainty range associated with the summary percentiles of number of campylobacter contaminating a carcass (log cfu per carcass)		
	5th percentile	*50th percentile*	*95th percentile*
Stun & kill	3.07 – 3.72	6.08 – 7.23	8.33 – 10.62
Scald	0.40 – 1.84	3.64 – 5.27	6.79 - 8.06
De-feather	0.22 – 0.89	1.96 – 3.10	4.67 – 5.79
Evisceration	0.57 – 1.04	3.94 – 5.30	7.00 – 7.95
Wash	0.39 – 0.61	3.33 – 4.77	6.22 – 7.10
Chill	0.35 – 0.53	3.07 – 4.23	6.10 – 6.91
Post processing	6.34 – 0.47	2.91 – 3.98	5.96 – 6 65

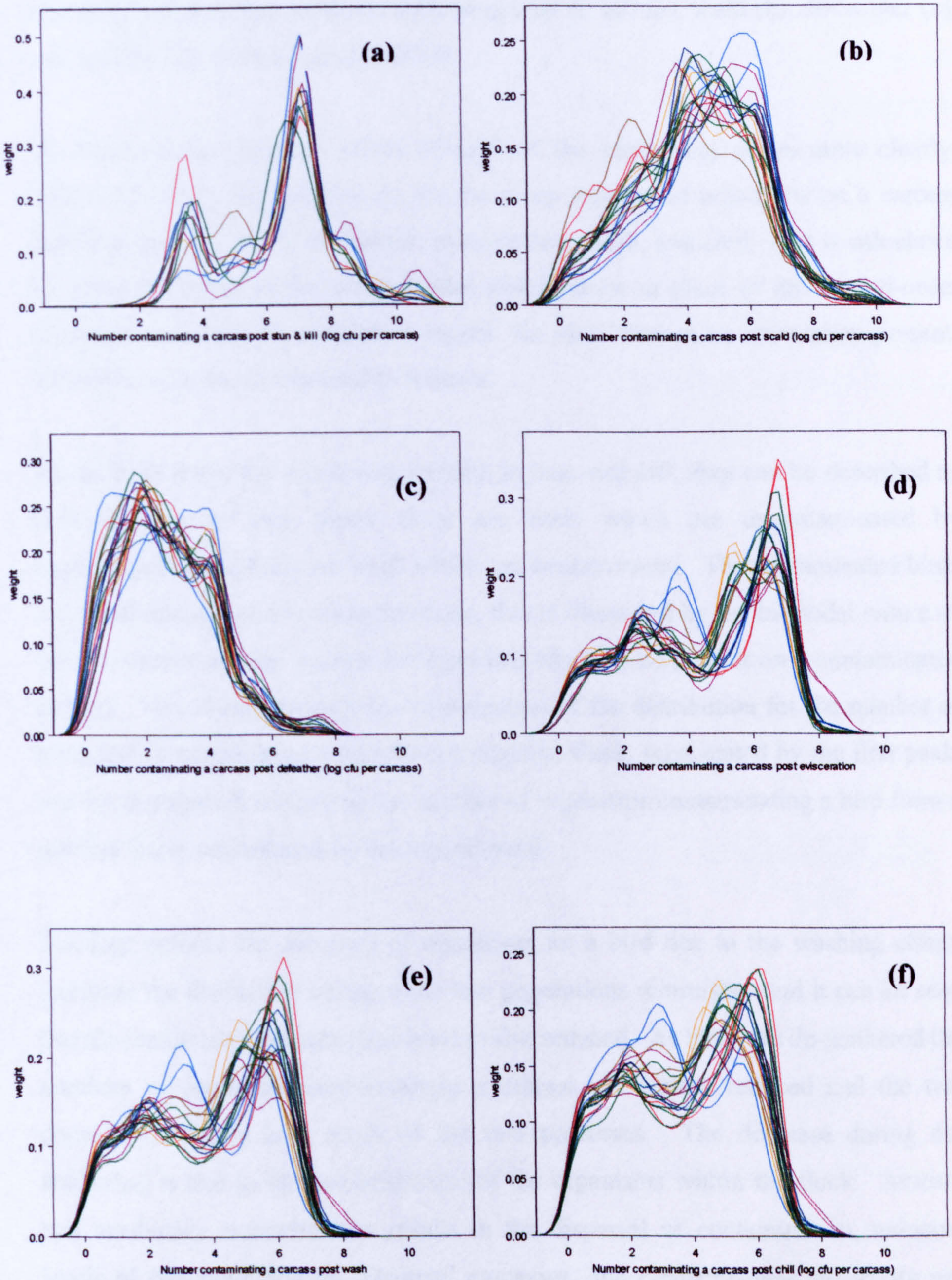


Figure 6.7: Second-order distributions for the number of campylobacter (log cfu per

carcass) contaminating a carcass following stun & kill (a), scald (b), de-feather (c), evisceration (d), wash (e), and chill (f).

To illustrate the predicted effect of each of the processing stages more clearly, Figure 6.8 shows the distribution for the mean number of organisms on a carcass post stun and kill, scald, de-feather, evisceration, wash, and chill. This is calculated by using the mean of the second-order distributions in place of the second-order distributions within the simulation model. As such there is no uncertainty present. The effects can be summarised as follows.

As the birds enter the processing facility, at stun and kill, they can be described as being in one of two states; there are birds which are uncontaminated by campylobacter and there are birds which are contaminated. The contaminated birds are the accumulation of two populations, this is illustrated by the bi-modal nature of the distribution for the number of organisms likely to be present on a contaminated carcass. This is explained by the combination of the distribution for the number of organisms contaminating a bird from a negative flock, represented by the first peak, and the distribution describing the number of organisms contaminating a bird from a positive flock, represented by the second peak.

Scalding reduces the numbers of organisms on a bird due to the washing effect, therefore the distinction between the two populations diminishes and it can be seen that the maximum contamination level is also reduced. As birds are de-feathered the numbers of organisms contaminating a carcass are further reduced and the two populations merge as a result of the two processes. The decrease during de-feathering is due to the re-distribution of the organisms within the flock. Aerosol and machinery contamination results in the dispersal of contamination, reducing levels of contamination on 'positive' carcasses, and contaminating previously un-contaminated carcasses. However, during evisceration there is an increase in the maximum numbers of organisms that contaminate a carcass and the two populations re-emerge. This is a result of the process of evisceration. Here there is the possibility of cross contamination from the machinery and also the potential for

damage to the viscera resulting in further contamination of the exterior. The populations on the exterior are then reduced by washing and then further still reduced by chilling. The final two populations are evident in the second-order distribution for the number contaminating a carcass post process (Figure 6.5)

The prevalence of contaminated carcasses, P_{pp}, at the end of processing is also the result of all stages in the procedure. Each of the stages, scald, de-feather, evisceration, wash and chill, have distinct effects on P_{pp}. These effects are illustrated in Figure 6.9. This figure shows the uncertainty distribution for the probability a carcass is contaminated following scald, de-feather, evisceration, wash and chill and the associated mean value and uncertainty interval for this probability is given in Table 6.8.

Table 6.8: Mean probability that a carcass is contaminated after each processing stage along with the maximum and minimum values of this probability based upon the simulation model described in Section 6.1.1.

Processing stage	Mean	Simulation minimum	Simulation maximum
Scald	0.83	0.80	0.85
De-feather	0.95	0.89	1
Evisceration	0.94	0.91	0.99
Wash	0.88	0.82	0.96
Chill	0.83	0.78	0.93
Final product	0.80	0.73	0.90

Initially, based on the results from the model describing the rearing and transport of the birds (see Chapters 3 and 4), a carcass has a mean probability of 0.85 of being contaminated when entering the processing facility, this is not altered by stun and kill due to the assumption any effect here on contamination is negligible. The

process of scalding results in a reduction of this value to 0.83 due to the 'washing-off' effect of this process. De-feathering increases this probability to a value of 0.95. This is in contrast to the decrease in numbers per carcass observed during de-feathering (Table 6.7) and is explained by aerosol and machinery contamination of previously uncontaminated carcasses, that is redistribution of the organisms. The removal of the innards during evisceration results in a slight decrease in the mean prevalence of contaminated carcasses with a decrease in the prevalence to 0.94.

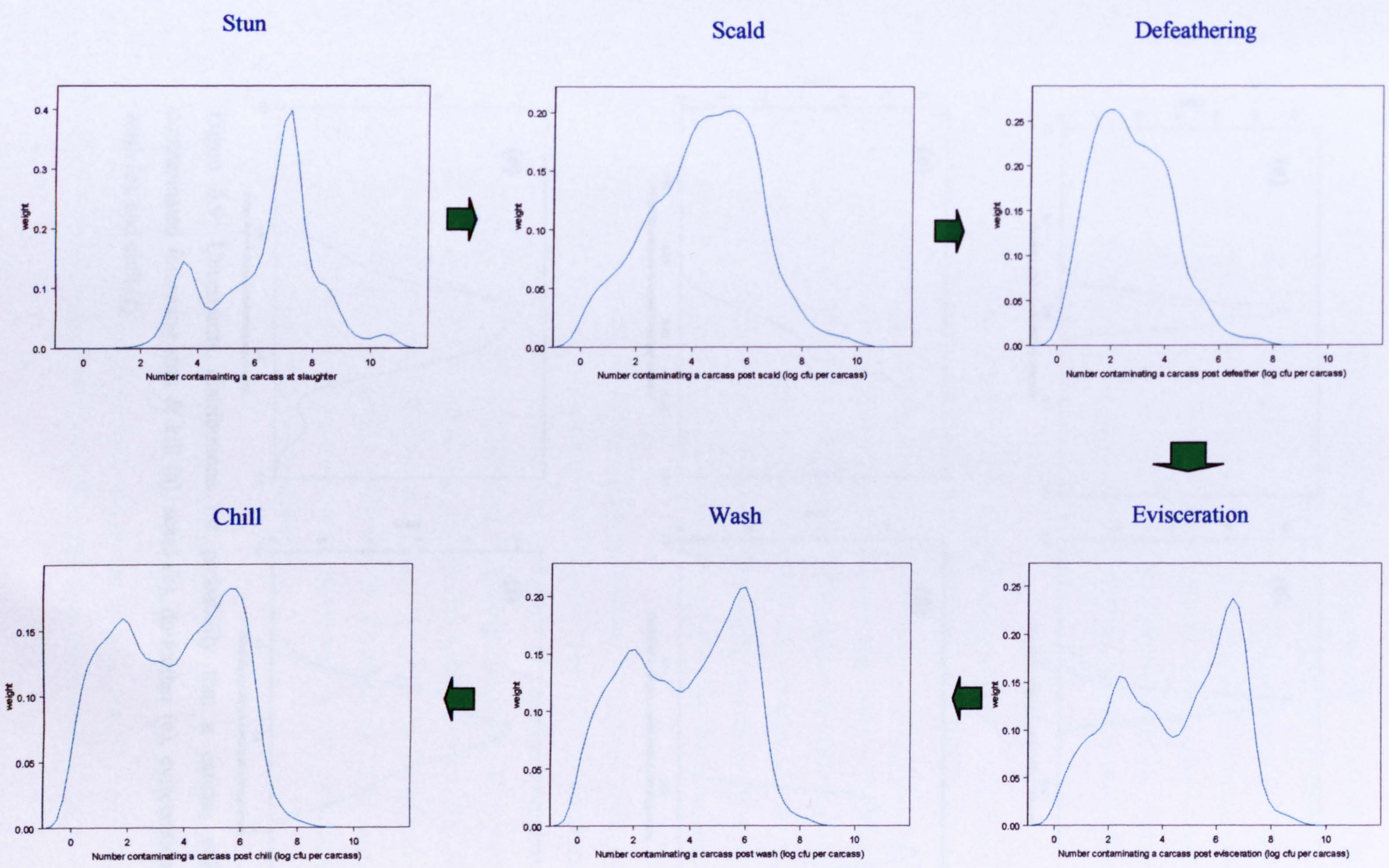
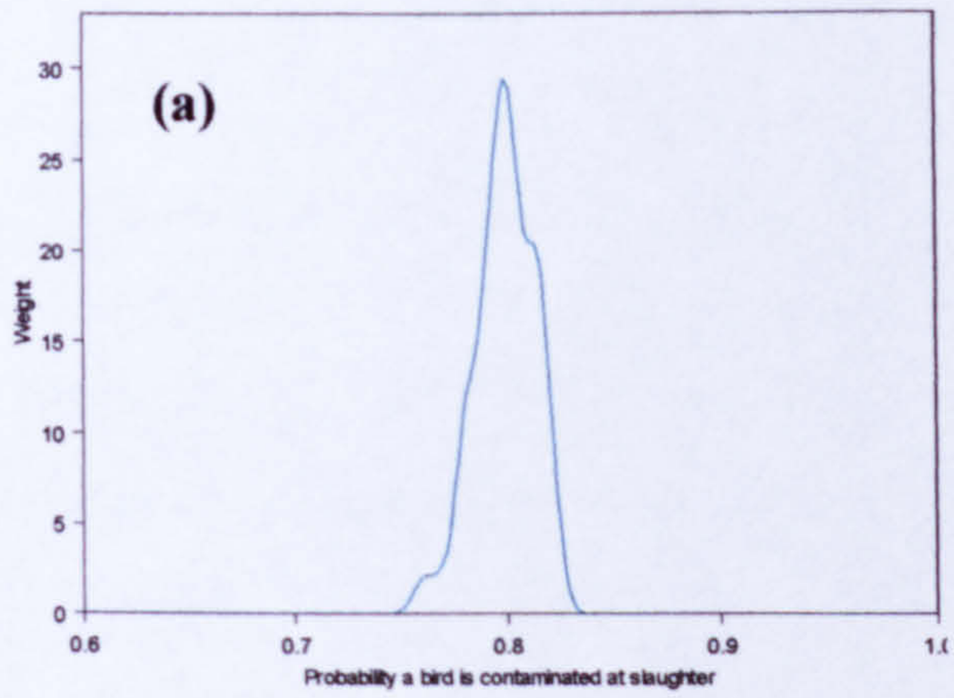
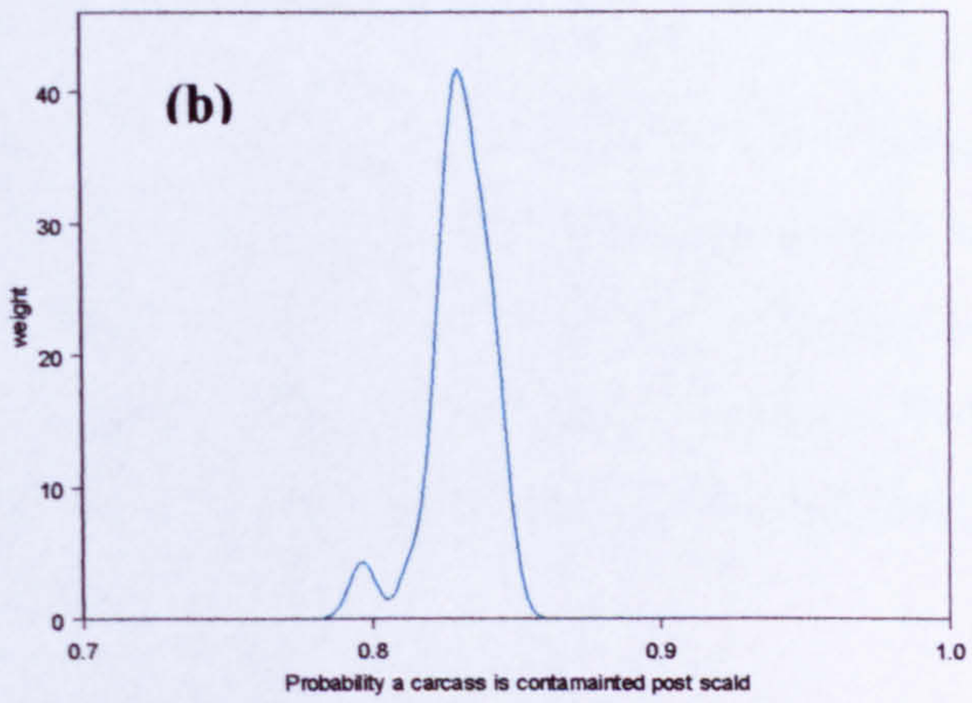
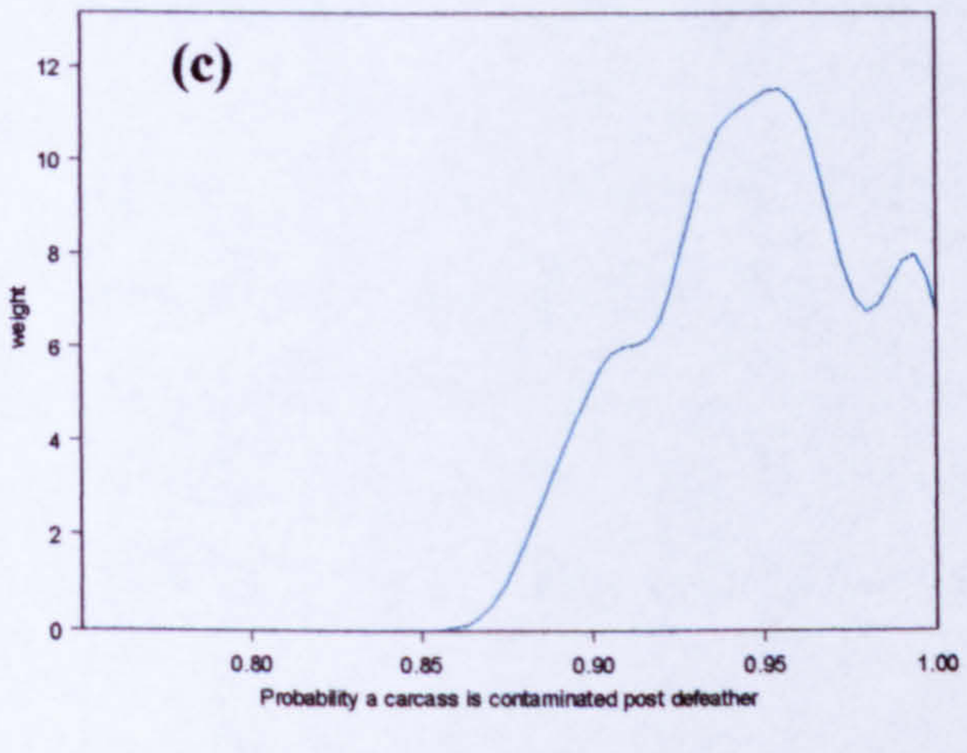
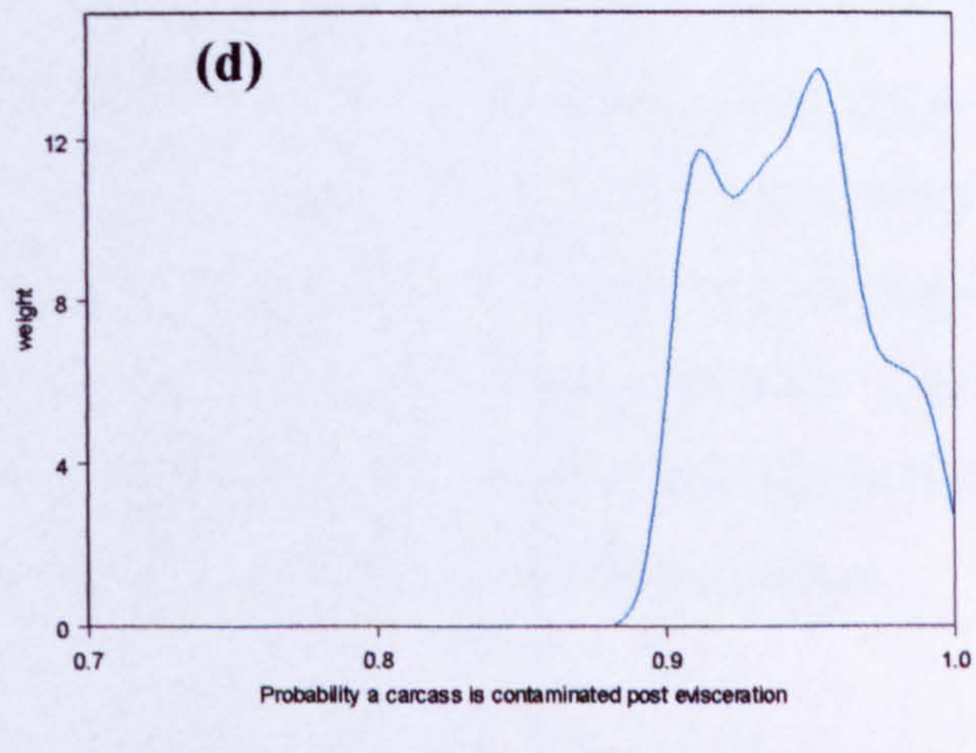


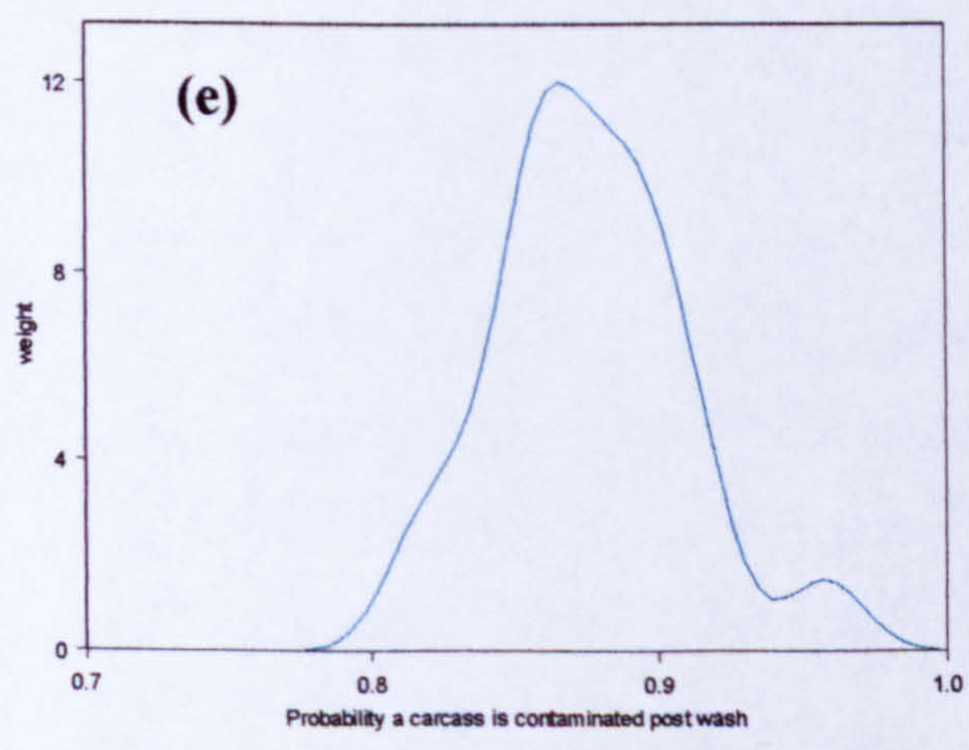
Figure 6.8: Graphs showing the change in the distribution for the number of organisms contaminating a carcass after each processing stage











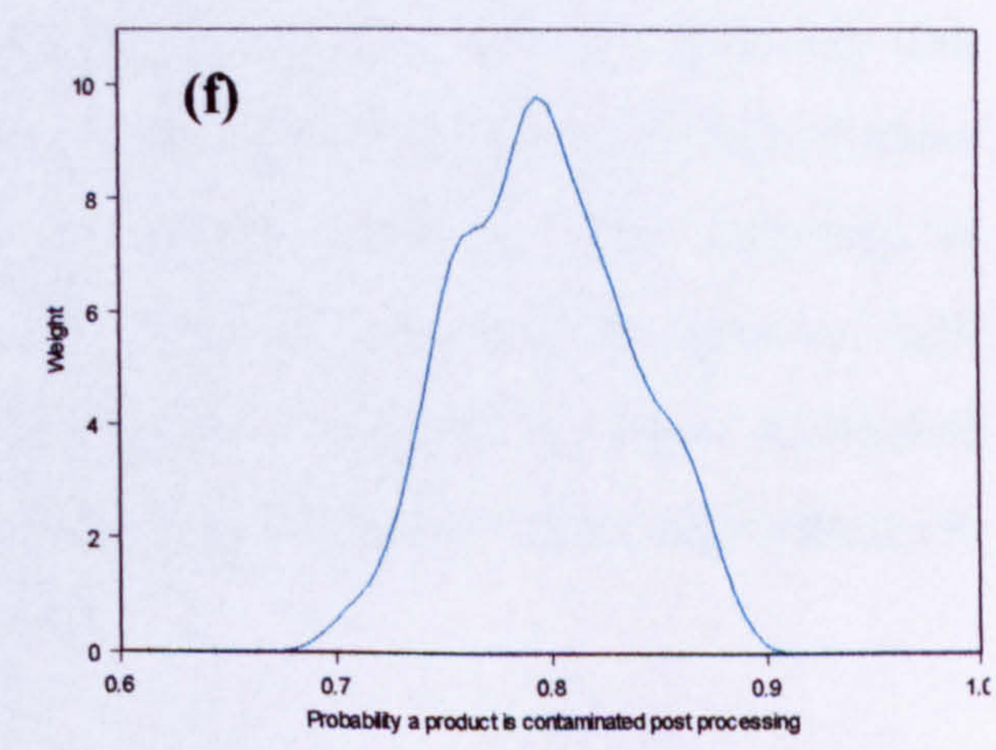


Figure 6.9: Uncertainty distributions for probability that a carcass will be contaminated following stun & kill (a), scald (b), de-feather (c), evisceration (d), wash (e), and chill (f).

Although the mean probability of contamination decreases after evisceration, there is an increase in the minimum of the uncertainty interval for this probability from a value of 0.89 post de-feathering to a value of 0.91, consequent upon the occurrence of cross-contamination and damage to the viscera. Next the washing stage causes a reduction to 0.88 and finally chilling further decreases the probability a carcass is contaminated to 0.83 as a result of the washing effect of the water. Overall, the important stages here are de-feathering and evisceration, both resulting in a possible increase in the prevalence of positive carcasses.

Further examination of Table 6.8 shows that processing can have mixed effects. The uncertainty in the probability a carcass is contaminated at the start of processing ranging from 0.8 to 0.85 compared to a range of 0.73 to 0.9 post processing (post-chill). It can be seen that de-feathering is the key stage here, as the uncertainty intervals for scald and de-feathering overlap. This suggests de-feathering, although on average results in an increase in prevalence may also decrease prevalence. This is likely if carcasses only have low-level contamination post-scald as the removal of feathers and the washing effect of the water has the potential to 'clean' carcasses.

Examination of Figure 6.8 also shows that evisceration has a major impact on the extent of contamination on a carcass. Currently this section of the model has two main parameters, the probability that damage occurs, *Da*, and the probability that cross contamination occurs, *Xc*. Therefore, to investigate the effect of each of these one of the parameters *Da* and *Xc* was set to zero while the other remained as described by the associated distribution. Figure 6.10 compares the effect of both cross contamination and damage remaining as described by there associated distributions, setting *Xc* to zero and allowing only damage to occur, and setting *Da* to zero allowing only for cross-contamination.

From Figure 6.10, when no damage to the viscera is allowed the prevalence of positive carcasses is reduced due to the mixed effects produced by cross-contamination previously described, and it can be seen from the corresponding distribution that it is more likely a carcass will have lower levels of contamination

than in the current situation. In contrast, when only damage is possible and there is no cross-contamination this results in no change in the prevalence of carcasses, as expected, as there is no cross-contamination effect. Further the associated distribution for the number of organisms contaminating a carcass is similar to that seen in the current model. Hence cross contamination has the greatest influence on the prevalence of contaminated carcasses following evisceration and damage to the innards has the greatest influence on the predicted number of organisms contaminating a carcass following evisceration.

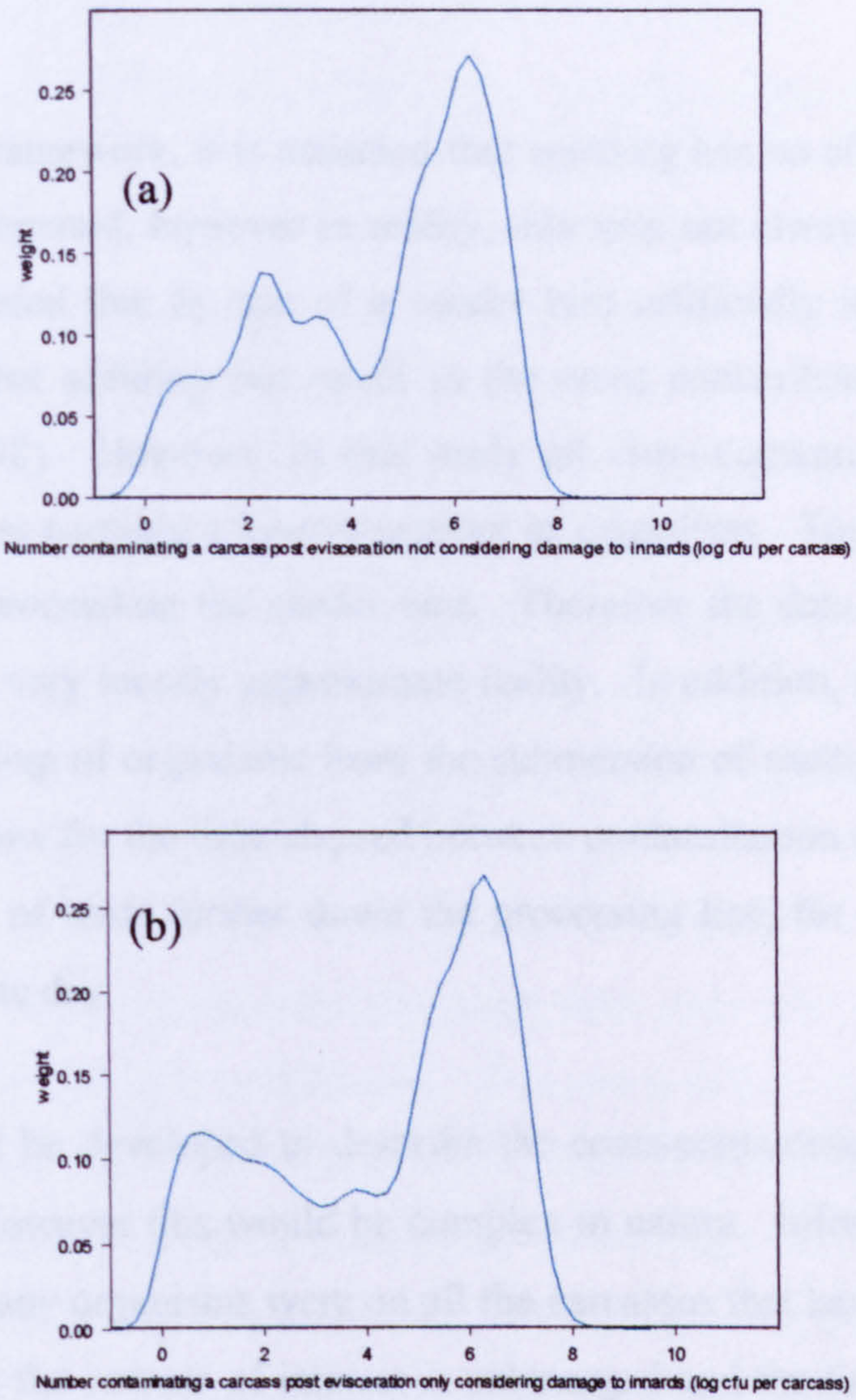


Figure 6.10: Density distributions comparing the number of organisms contaminating a carcass when only cross-contamination can occur during evisceration (a), and only damage to the innards can occur during evisceration (b).

6.6 Discussion

Within the model framework, it is assumed that scalding has no effect on carcasses which are uncontaminated, however in reality, this may not always be the case. It has been demonstrated that by use of a seeder bird artificially inoculated with a marker organism that scalding can result in the cross contamination of carcasses (Mulder *et al.*, 1978). However, in this study all cross-contamination originates from a single carcass carrying a known number of organisms. The carcasses tested are those directly proceeding the seeder bird. Therefore the data presented are of limited use as they very loosely approximate reality. In addition, the data does not allow for any build-up of organisms from the submersion of multiple contaminated birds nor does it allow for the time elapsed between contamination of the scald water and the immersion of birds further down the processing line, for example, a flock processed later in the day.

An approach could be developed to describe the cross-contamination of carcasses during scalding. However this would be complex in nature. Information would be required on how many organisms were on all the carcasses that had entered the tank up to the point that the carcass of interest is submerged and the time between these events. Further, information on the survival capabilities of campylobacter in the scald water accounting for changes in temperature and other properties such as $NaCl_2$ concentration with time, would be required. Competition factors associated with other micro-organisms in the scald water, would also need to be considered along with attachment properties to decide how many campylobacters successfully contaminate a given carcass. Several of these variables are currently unknown. Whether contamination of a carcass at this stage of processing would persist through the latter stages to the final product is questionable, but if so, the assumption made here may result in an under estimate of P_{pp}.

Overall, there are currently three unknown variables in this model, these being the probability that damage occurs to the viscera during evisceration *Da*, the probability

that chlorine is used during chilling procedures involving water, τ_c, and the effect of portioning on the prevalence and microbial load of a carcass(es). These unknowns may have implications for the model results, however obtaining quantitative data for them may be difficult.

(i). The probability that damage occurs during evisceration is assumed to be a uniform random variable between zero and one. In the field situation, this could be measured by observing an evisceration line and counting the number of times the viscera are impaired. However, there will be variability between processing facilities, production companies and possibly daily variability inherent in the probability of damage occurring making this a difficult variable to describe with certainty. Therefore, second-order modelling of any data acquired may be appropriate.

(ii). During the chilling procedure the frequency with which chlorine is used as an additive to the chill water is unknown. This is a sensitive issue. Chlorination of the water has beneficial effects on the level of microbial contamination. Mead and colleagues (1989) demonstrated that although there is no effect upon the organisms that remain attached to a carcass, those that are washed into the chill water are prevented from re-attaching due to the chlorine. This makes chlorination of chill water a frequent retailer demand in GB. In contrast, the EU directive (EU, 1997) stipulates that only potable water may be used throughout the processing of chickens so as to ensure no residues are present on the retail product. Given the sensitivity of this issue, information with relating to the frequency of chlorination use in GB is difficult to obtain.

(iii). It is assumed that portioning has no effect on the microbial load of a carcass. It has been demonstrated by sampling the same site before and after portioning that there was no significant change in the numbers of organisms present. However, at a facility with poor hygiene practices this may not be the case. If

machinery is inadequately cleaned or there is poor training of staff, portioning could potentially be a site of cross-contamination.

Additional information relating to these unknown variables could result in a model which better describes the true situation, hence resulting in a potentially more accurate estimate of the outputs of this model.

The model section which describes de-feathering considers 100 carcasses and the sequential removal of feathers and effect on proceeding carcasses. The 100^{th} bird is followed through the rest of the processing stages. The number of carcasses was chosen to allow the distribution of cross-contamination to be adequately represented. However, there is the possibility that during processing the random bird selected may be the less than the 100^{th} bird processed in the flock. Given that flocks are large (up to 42 thousand birds in size) the probability of the bird being in a position less than 100 in the slaughter line is low. As such it is unlikely that modification of the model describing de-feathering to allow for this possibility will have any significant impact on current estimates of P_{pp} and η_p.

The results of this model indicate that de-feathering and evisceration are major stages in determining not only the probability that a carcass will be contaminated but also the extent of this contamination. De-feathering, despite reducing the numbers contaminating a carcass, increases the prevalence of contaminated carcasses due to cross-contamination resulting from the water sprays. It has been demonstrated that placing aluminium sheets between carcasses during de-feathering can reduce the level of cross-contamination (Hinton *et al.*, 1996). During evisceration it was demonstrated that the greatest influence is from the occurrence of damage to the viscera. However cross-contamination is also of significance as when the probability of damage occurring to the viscera, *Da*, was set to zero and only cross-contamination was allowed to occur the prevalence of 'positive' carcasses still increased from the previous stage, de-feathering (Figure 6.9). Therefore, changes in the way birds are de-feathered and a reduction on the occurrence of damage, which

could be implemented by more frequent use of manual evisceration or machinery that can account for the variability in carcass dimensions and containment of the level of cross-contamination, perhaps achievable by better staff training and more frequent/rigorous hygiene practices may result in a reduction in the probability that a carcass is contaminated at the end of processing.

Currently, during evisceration, the possibility that carry-over occurs from the previous flock/previous day is accounted for by assigning a position to the selected flock in the processing day. One possible control measure is the slaughtering of classified negative flocks first in the day followed by the positive flocks. Given that negative flocks can be contaminated this will not eliminate contamination from such flocks but will reduce the extent of cross-contamination from other flocks for example during evisceration.

6.7 Conclusions

The processing of chicken meat is a crucial stage in predicting the microbiological profile of retail chicken products. Each stage of processing contributes in a unique manner to the final contamination level of a chicken product.

In this chapter a model has been developed which investigates five of the ten main stages of processing. These stages were selected upon the basis of importance with respect to the campylobacter contamination levels on chicken products. The stages are scald, de-feather, evisceration, wash and chill. The model takes the form of a simulation model which, at each stage of processing, using the appropriate mathematical formulation estimates the effect of the stage upon the contamination level of a carcass. Hence the result is an estimate of the number of campylobacter that will contaminate a processed product. This then enables an estimate of the probability that a random product will be contaminated post processing. The

products considered are both whole and portioned chicken carcasses that are to be sold as either fresh or frozen products.

The data available to parameterise the change that any one process may have upon the contamination level on a carcass mainly consist of small sample sizes, and are often reported simply as a mean value of a group of samples. As such there is a high level of uncertainty with respect to the form of the distribution describing the processing effects. As such second-order modelling is used as described in Chapter 2 enabling quantification of the level of uncertainty resulting from the small data sets.

In summary, the model described here simulated the slaughter and processing of a random bird selected from a random flock in GB. On average a carcass has a probability of 0.8 of being contaminated at the end of processing. This probability ranges from 0.73 to 0.90 suggesting that processing may have mixed effects on the prevalence of contaminated carcasses. The likely number of organisms contaminating a carcass has a mean uncertainty interval of 5.43 to 6.28 log cfu per carcass, with a 95th percentile uncertainty interval of 5.96 to 6.65 log cfu per carcass. It was demonstrated that de-feathering and evisceration are important in controlling the probability that a carcass is contaminated at the end of processing and the associated level of contamination. There are several measures that could be taken to reduce the probability that a carcass is contaminated and the microbial loads, such as adjustments of current processing practices. However without adequate staff training in good hygiene practices and adequate routine cleaning the effect of these costly adjustments may be limited. These stages should be areas of focus for future research with an aim to identify ways in which the increases in prevalence and contamination levels can be minimised.

Chapter 7

The preparation and cooking of a chicken product

7.1 Introduction

The majority of cases of food poisoning are associated with exposure to pathogenic organisms as a result of the preparation of meals in the home (Altekruse *et al.*, 1998). In a UK National Food Safety Report (Griffiths *et al.*, 1998) only 40% of respondents claimed to store food according to recommended practices and over half indicated they did not follow manufacturers instructions for preparing and cooking food.

The consumption of under-cooked food is well recognised as a risk. In addition, the direct or indirect cross-contamination of cooked products by raw products, combined with poor storage are also major concerns when food is prepared in the home (Worsfold & Griffith, 1997). Direct contamination of food products involves the passage of pathogens from a contaminated source directly to the food item. Indirect contamination occurs when pathogens are transferred from a source, via a vehicle, to the food product. The main vehicles for indirect contamination are hands, equipment, utensils, surfaces and kitchen cloths.

The full extent to which cross-contamination is responsible for causing food related illness in the UK is yet to be determined. Information on consumer food safety behaviour has been derived mainly from questionnaires and telephone surveys. However, the use of interviews and questionnaires has a number of limitations. The greatest obstacle being that it can be difficult to interpret and verify the responses with individuals often claiming a better standard of hygiene than is in practice. Of most use is data involving actual observation of individuals during the preparation of a meal. Such data sets are sparse but two have been presented by Worsfold and Grffith (1997) and Jay *et al.*, (1999). These data demonstrate that the occurrence of cross-contamination during preparation of food and the inadequate cooking of foods are not uncommon.

In this chapter a model is presented which estimates the likelihood and magnitude of exposure to *Campylobacter* spp. as a result of the preparation and consumption of a serving of chicken in the home. There are three main sections to the model, these are the storage of the chicken product prior to cooking, preparation of the meal which includes the chicken product, and the consumption of a serving of the prepared meal. These sections are inter-linked and hence all three are crucial to predicting exposure. The way in which these three sections are linked is illustrated in Figure 7.1. The accumulation of the sections shown in Figure 7.1 results in an estimate of the probability of exposure to campylobacter and, given exposure the number of campylobacter ingested. Throughout this chapter exposure is defined as the ingestion of one or more cfu of campylobacter.

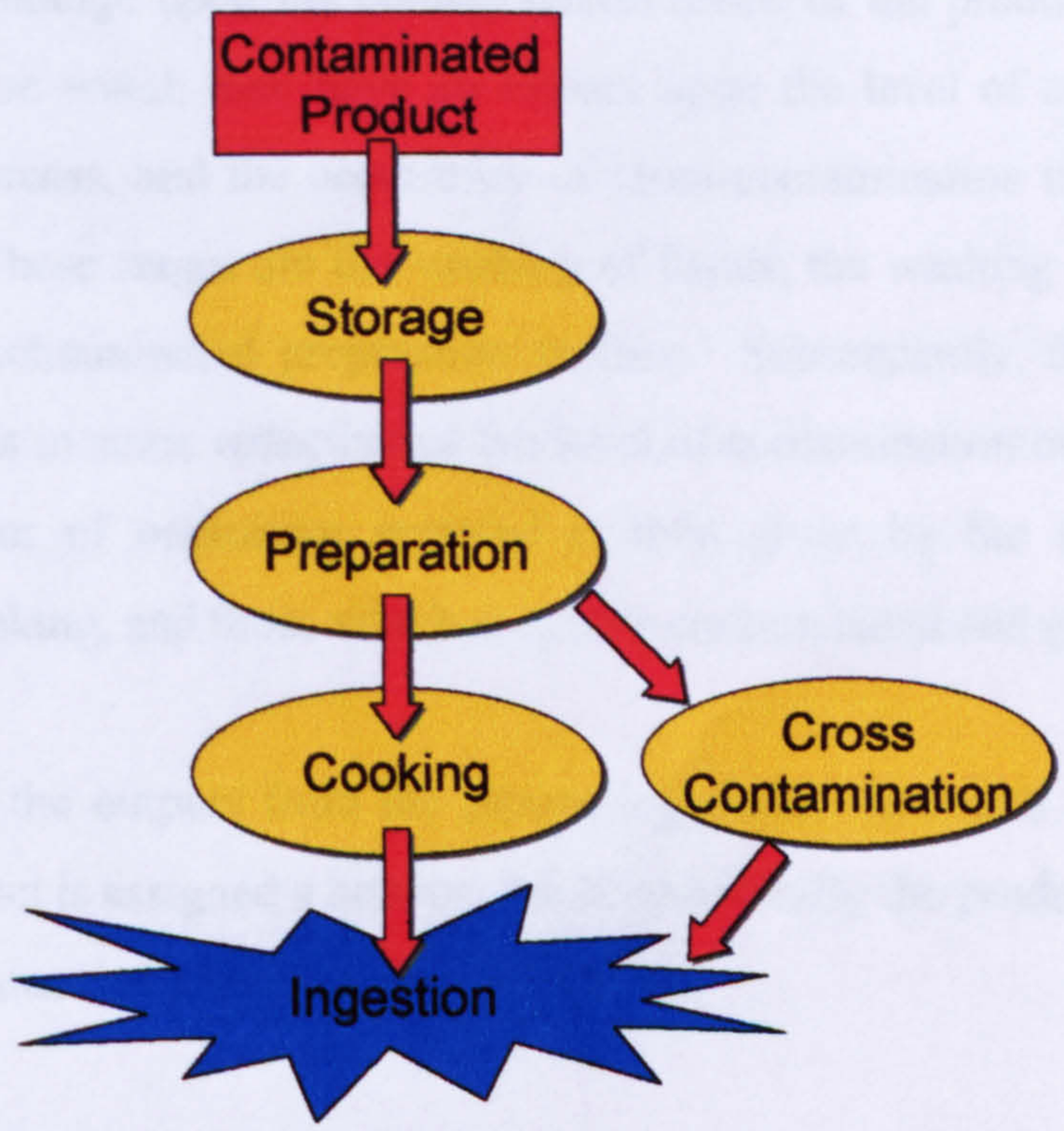


Figure 7.1: Illustration of the three sections of the model to estimate the likelihood and magnitude of exposure to campylobacter from the consumption of a chicken meal in the home.

7.2 Estimating the number of organisms ingested

As illustrated in Figure 7.1, in the first instance, the model considers storage of the product from the end of processing to preparation. More specifically, this is the period of time for which the product is subject to either refrigerated or frozen storage. In this way, the link is made between the end of processing and the preparation of the meal. The storage incorporates anytime which the product may have been stored at retail as well as storage in the home. The model then estimates the effect of such storage upon the contamination levels of the product. Next, the stages of preparation which may have an impact upon the level of campylobacter contaminating a carcass, and the occurrence of cross-contamination in the kitchen, are investigated. These stages are the washing of hands, the washing of vegetables and the use of a contaminated preparation surface. Subsequently, the product is cooked. This results in some reduction of the level of contamination on the product. Finally, the number of organisms ingested is then given by the sum of those remaining after cooking, and those which are cross-contaminated and get ingested.

Initially, based on the outputs from the processing model described in Chapter 6, each chicken product is assigned a history. More specifically the product is assigned a contamination status and a level of contamination.

The contamination status is given by θ_c where $\theta_c \in \{0,1\}$ so that θ_c=1 means that a given product is contaminated, and θ_c=0 means that the product is not contaminated with campylobacter. The condition $\theta_c = 1$ occurs with probability P_{pp}, therefore $\theta_c = 0$ with probability $1-P_{pp}$ where P_{pp} is the probability that a product is contaminated at the end of processing. If θ_c=1 a given product is assigned η_p contaminating organisms where η_p is the number of organisms contaminating a

product at the end of processing. The variables P_{pp} and η_p are generated from the model described in Chapter 6.

Next, the storage and subsequent preparation are considered. As defined in Chapter 6, at the point of sale, a random carcass is product type θ_s, where $\theta_s = \{\alpha_p, \alpha_w, \beta_p, \beta_w\}$ such that α_p represents a portioned fresh product, α_w represents a fresh whole product, that is a whole carcass, β_p a frozen portioned product and β_w a fresh whole product. Freezing includes only manufacturers freezing of the product. That is the product is purchased frozen. Currently, there is no information regarding the frequency with which a product will be purchased fresh and subsequently frozen in the home prior to consumption and hence this is not currently considered in the model. Finally a preparation status is assigned. This is defined as θ_{prep} where $\theta_{prep} \in \{0,1\}$ such that $\theta_{prep} = 1$ means that prior to consumption some preparation will be carried out and $\theta_{prep} = 0$ means that prior to consumption no preparation will occur, however the product will be handled in some way. The value of θ_{prep} is given by the following condition

$$\theta_{prep} = \begin{cases} 1 & \theta_s = \alpha_w, \beta_w \\ 0 & \theta_s = \alpha_p, \beta_p \end{cases}$$

The total number of organisms which contaminate a chicken meal, and are subsequently ingested is a function of the number of organisms which survive storage, and are ingested as a result of either cross contamination of or inadequate cooking of the meal. As such, the number of organisms ingested from a random serving of chicken, defined as η_{exp}, is given by equation (7.1).

$$\eta_{exp} = \eta_{pc} + \eta_{cc}^{*} \qquad (7.1)$$

Here, η_{pc} is the number of organisms surviving cooking and hence ingested, and η_{cc}^{*} is the number of organisms cross-contaminated during preparation that are ingested. Both η_{pc} and η_{cc}^{*} are a function of the number of organisms which survive storage of the chicken product, defined as η_{ps}.

From equation (7.1) it is necessary to first estimate the number of organisms which survive storage, and hence contaminate the product at the point of preparation. Following this, the effects of preparation and cooking can be quantified.

7.2.1 Estimating the effect of storage

The quantification of the effect of storage the number of organisms that will survive storage, that is η_{ps}, is dependent upon the manner in which campylobacters respond to temperature and other environmental factors to which they may be exposed during this process and the period of time of exposure.

Modelling the response of bacterial populations to environmental conditions

Describing the response of bacterial populations to environmental conditions is an extensive field of scientific research. Such descriptions often consider the growth of the bacterial population. Numerous models are available to described the response of a given organism to changes in the environment over time. These range in the level of both mathematical complexity and the extent to which the models describe the biological processes occurring. Such predictive models are grouped into one of three

classes, these are primary, secondary and tertiary models. Primary models describe the growth or inactivation curve observed over time when a population is exposed to a given temperature. Secondary models introduce another level of complexity in that they describe the kinetic parameters of primary models in terms of the environmental conditions such as pH and salt concentration. Tertiary models integrate all the information regarding the way in which the given organisms responds to all aspects of the environment and often utilise decision support systems to predict growth.

The simplest way to describe growth and decline is to assume first order kinetics. Bacterial growth is often described by sigmoidal curves. An example is the exponential model. Given a population of bacteria where growth is unrestricted the rate of increase in the population is proportional to the size of the population itself. This can be expressed as

$$\frac{dN}{dt} = \mu N$$

Where N is the size of the bacterial population, t is time and μ is some constant which describes the rate at which the population changes with t. When solved for N the result is

$$N = N_0 e^{\mu t}$$

On taking logarithms the result is equation (7.2). This is referred to as the exponential model describing bacterial growth. Here, μ is defined as the specific growth rate constant.

$$\ln N = \ln N_0 + \mu t \qquad (7.2)$$

The death of bacteria is usually most simply described by the Arrhenius equation, that is

$$\log_{10} N = \log_{10} N_0 - \left(\frac{kt}{2.303}\right)$$

where $k = Ae^{\frac{E_a}{RT}}$, A is the pre-exponential factor, E_a is the activation energy, R is the molar gas constant, and T is the absolute temperature. However, in most cases researchers do not quote such detailed properties for a given bacterial species and usually refer to the D-value. The D-value is the time required to observe a 1 log reduction in the population size, in minutes. As such, the Bigelow model (Nauta, 2001) can be adopted where the inactivation rate is a function of temperature. The result is equation (7.3).

$$\log N = \log N_0 - \frac{t}{D} \qquad (7.3)$$

Several sigmoidal functions have been used to empirically describe the growth and inactivation curves, for example the logistic, Gompertz, Richards and others (Zwietering *et al.*, 1990). However, models based on the life cycle have also been developed (Whiting & Cygnarowicz-Provost 1992). In general, models describing growth or decline are empirical models or analytical solutions of differential equations, describing the number of micro-organisms in time under constant environmental conditions.

Secondary models are commonly divided into three main categories. These are square root models, Arrehenius type models, and polynomial models. Ross and McMeekin (1994) have comprehensively described these models in a review paper on predictive microbiology. An example of a secondary growth model is the Gamma model, which is given by

$$N_t = N_0 e^{\mu_{max}(t-\lambda)}$$

Where

$$\mu_{act} = \mu_{opt}\gamma(T)\gamma(pH)\gamma(a_w)$$

$$\gamma(T) = \left(\frac{T - T_{\min}}{T_{opt} - T_{\min}}\right)^2$$

$$\gamma(pH) = \frac{(pH - pH_{\min})(pH_{\max} - pH)}{(pH_{opt} - pH_{\min})(pH_{\max} - pH_{opt})}$$

$$\gamma(a_w) = \frac{a_w - a_{w\min}}{1 - a_{w\min}}$$

This model describes the specific growth rate in terms of the environmental conditions. Specifically the temperature, T, pH and water activity a_w and the minimum, maximum and optimum growth requirements of these environmental characteristics. These three characteristics are organism specific and therefore require information regarding these characteristics for any given bacterial species. There are many other models available, these have been described in detail by McMeekin *et al.* (1993).

Crucial to using any growth or inactivation model is adequate data for a given bacterial species. The availability of such data can have an impact upon the model selected to describe a particular organism response to the environment. For example, the exponential model only requires the specific growth rate constant, however the gamma model requires parameters, which not only describe the response to temperature but also the way in which the species will respond to pH, water activity. However, in contrast to the exponential model the gamma model provides quantitative insight into the relevance of several environmental conditions for growth. Thus it provides a more detailed biological description of the processes occurring.

There are very few data available which describe the response of campylobacters to temperature, pH and other environmental factors. Of the data available, most studies

only include a limited number of temperature points at which the relationship has been determined, with very few studies looking at the combined effects of temperature, pH, water activity and others.

Data available to model the response of campylobacter populations

Comparison of available studies suggests that the food matrix has an important role to play in the survival of campylobacters in response to changes in temperature. This is highlighted by Bandres *et al* (1988) where a direct comparison of the survival of several entero-pathogens in a variety of foods is carried out. The results clearly show that the survival traits of a given species is dependent upon the food vehicle.

There is very little data in the literature that considers the response of campylobacter to temperature, when in chicken meat. Studies involve mediums from ground beef, brucella broth, milk, water melon, water, pork, turkey roll, red meats, oyster, fish, and a variety of cooked foods. Despite the assumed importance of chicken in the epidemiology of campylobacter food poisoning it is surprising that so few studies involve chicken meat as the response medium.

The data available show clear differences in the manner in which different strains of campylobacter respond to temperature. Doyle & Roman (1981) tested 3 separate strains for the effect of temperature on the generation times and 5 separate strain measuring the effect of temperature on the D-values. The results demonstrate the inter-strain variability present. These findings are supported by other researchers (Sorqvist, 1989; Waterman, 1982; Gill & Harris, 1982; Hanninen, 1981; Christopher *et al*, 1982)

It can be seen that modelling the change of bacterial populations in response to the environment is a well established field of scientific research. There is extensive

published literature available on the subject and an overall introduction to the area is provided by McMeekin *et al.* (1993).

Campylobacter are thermophillic in nature and therefore will not grow during storage. As such, during refrigeration and freezing, inactivation of campylobacter is the only consideration. Several studies demonstrate that campylobacter have high death rates during cold storage, especially during frozen storage. It has been demonstrated that only 4 weeks of frozen storage are necessary to render a previously contaminated product uncontaminated (Rosef *et al.*, 1984).

Survival during refrigeration and storage

To describe the effect of refrigeration, the Bigelow model is used to predict the size of the population after some time, t, of refrigerated storage. More specifically

$$\log_{10}(\eta_{ps}R) = \log_{10}(\eta_{pp}) - \left(\frac{t}{D}\right)$$

where $\eta_{ps}R$ is the number of organisms contaminating a product following refrigerated storage, η_{pp} is the number of organisms contaminating a product post-processing, and D is the D-value for campylobacter. The time for which the product is stored is based upon data from a consumer survey (MAFF, 1991). The range of temperatures at which a chicken product will be stored in the retail environment and the home is currently unknown. Therefore an assumption is made that refrigerated storage will be at 4 °C. As such the D-value is that measured for campylobacter at 4 °C, and is given by Koidis & Doyle (1983).

To describe the effect of frozen storage, a regression model was fitted to the experimental data of Aho & Hirn (1988) using least squares. This data set describes the relationship between the time of storage and the proportion of the initial

campylobacter population which survives such storage. The data are given in Table 7.1. Inspection of the data suggest that the proportion of the population which survives over time follows exponential decay, hence an exponential regression model is fitted to the data points to calculate the rate of exponential decline. The predicted regression line and the data points are plotted in Figure 7.2. The regression equation is $F = 1.2e^{-1.9802t}$ and the R^2 value is 0.91. Hence, from the rate of exponential decline, $F = e^{-1.9802t}$ where F is the proportion of the population which remains after storage for time t.

Table 7.1: The survival of campylobacter during frozen storage over time, after Aho & Hirn (1988) and calculated proportion of initial population remaining over time.

Time (weeks)	Population size (log cfu)	Proportion of initial population remaining
0	5.5	1.0000
1	4.7	0.1580
2	3.7	0.0168
3	3.7	0.0168
4	1.7	0.0002

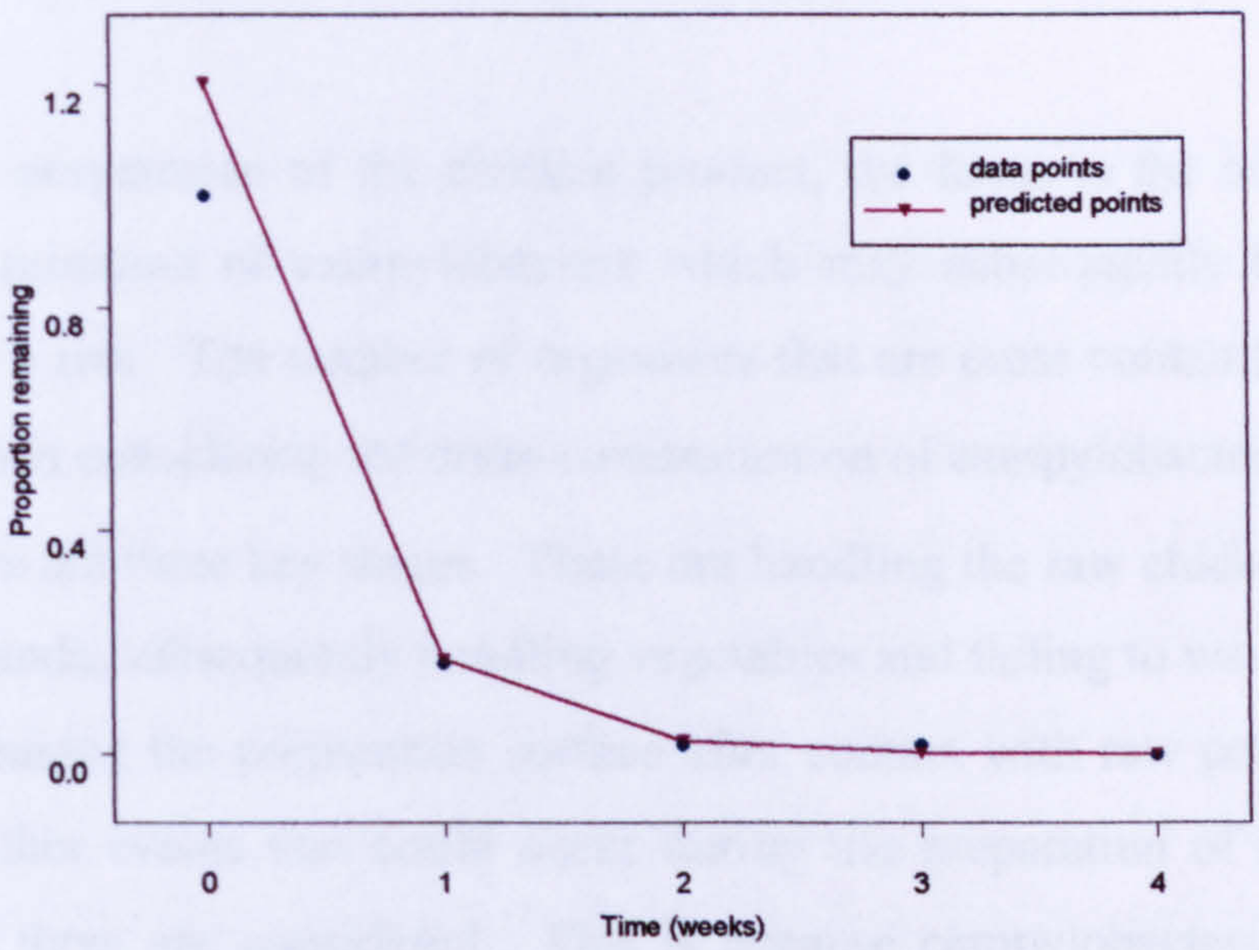


Figure 7.2: Plot of the data points and regression line quantifying the relationship between the proportion of the campylobacter population remaining with time during frozen storage

Therefore, the number of organisms which contaminate a product post storage is given by the following condition

$$\eta_{st} = \begin{cases} \eta_{ps}(R) & \theta_s = \alpha_w, \alpha_p \\ \eta_{pp} F & \theta_s = \beta_w, \beta_p \end{cases}$$

7.2.2 Preparation of the product

During the preparation of the chicken product, the focus is the occurrence of the cross-contamination of campylobacters which may subsequently be ingested and hence pose a risk. The number of organisms that are cross-contaminated is defined as η_{α}. When considering the cross-contamination of campylobacter in the domestic setting there are three key stages. These are handling the raw chicken and failing to wash the hands, subsequently handling vegetables and failing to wash the vegetables and not cleaning the preparation surface after contact with raw poultry. There are several further events that could occur during the preparation of a meal however only these three are considered. This is because campylobacters are unlikely to grow in the kitchen environment, as they are thermophiles. Hence, the effects of storage at room temperature, failure to cool cooked foods appropriately and several other events associated with an increased risk of a food related illness are assumed to be negligible when the organism of interest is campylobacter.

The order in which these stages are considered by the model is important. First the individual, if necessary, handles the product in some manner that organisms may contaminate the hands of the individual. If the individual then washes their hands it is assumed that they are adequately cleaned (Haas *et al.*, 1999) and the hands then pose no risk. However, if they fail to wash their hands then there is the opportunity for the organisms present on the hands to transfer to other items or food products. In this model, the transfer to vegetables from the hands is explicitly considered. However, as with the contamination of the hands, if the individual washes the vegetables prior to cooking/eating then there is assumed to be no risk via this pathway. Next the preparation of the chicken is considered. If the product is prepared in some way, that is $\theta_{prep} = 1$, it is assumed that some preparation surface is used. This could be a plate or a chopping board. If this is not cleaned after the

chicken has contaminated the surface then the organisms which now contaminate the surface may pose a risk.

It is not the aim of this model to incorporate all possible ways in which an individual may ingest organisms as a result of cross contamination. Rather, the three scenarios above are considered and then it is assumed that the organisms which are contaminating the hands, vegetables and/or the surfaces are ingested by some mechanism without explicitly specifying that mechanism. There are numerous pathways by which organisms contaminating the hands of an individual or a work surface can become ingested. There may be several indirect cross contamination events, which result in the contamination of a food, or the organisms may be ingested directly from the hands, for example, by licking the fingers. The pathways are numerous and highly variable between individuals with factors such as level of education, social status, gender, age and many more playing a role in the manner in which an individual behaves in the kitchen (Worsfold & Griffiths, 1997; Jay *et al.*, 1999; De Boer *et al.*, 1990).

For each product, a random individual prepares the meal. Dependent upon the preparation status of the product, θ_{prep}, each individual is assigned a profile of how they will prepare the meal and which of the cross-contamination events will occur. More specifically, each individual is assigned a status regarding whether or not the hands, vegetables or preparation surface are cleaned after direct or indirect contact with a chicken product. If $\theta_{prep} = 0$ then no preparation is required, hence contamination of the surface is not considered.

The hand status is defined as H_w where $H_w \in \{0,1\}$ such that $H_w = 1$ means that the individual failed to wash their hands after handling a chicken product and $H_w = 0$ means that the individual did wash their hands post handling. The probability that an individual will wash their hands after contact with a chicken product is defined as P_H. As such the condition $H_w = 0$ occurs with probability P_H, therefore $H_w = 1$

occurs with probability $1-P_H$. The vegetable status is defined as V_w where $V_w \in \{0,1\}$ such that $V_w = 1$ means that the individual failed to wash the vegetables after handling and $V_w = 0$ means that the individual did wash the vegetables. The probability that an individual will wash the vegetables is defined as P_V. As such the condition $V_w = 0$ occurs with probability P_V, therefore $V_w = 1$ occurs with probability $1-P_V$. Similarly, the preparation surface status is defined as S_w where $S_w \in \{0,1\}$ such that $S_w = 1$ means that the individual failed to wash the surface after preparation and $S_w = 0$ means that the individual did wash the surface after preparation of the product. The probability that an individual will wash the vegetables is defined as P_S. As such the condition $S_w = 0$ occurs with probability P_S, therefore $S_w = 1$ occurs with probability $1-P_S$. If $\theta_{prep} = 0$ then $S_w = 0$.

The probabilities P_H, P_V, and P_S are estimated using data from an observational study carried out in the UK. In this study, 108 individuals were asked to prepare a meal and the number of people which performed pre-defined action during preparation was recorded. The results for the number of people that washed their hands after handling raw chicken, failed to wash the vegetables and failed to clean the preparation after contact with the raw chicken are shown in Table 7.2 along with the resulting distributional assumptions for the probability of occurrence. In each case the probability of occurrence is defined by use of the beta distribution. In this way the uncertainty in the probability of occurrence of each action is described.

Table 7.2: the number of individuals who performed out a particular poor hygiene action during the preparation of a meal. Total sample size was 108 individuals (After Worsfold & Griffiths, 1997).

Action	Percent occurrence	Distributional assumption
Failure to wash hands after handling raw meat	58	Beta(63,46)
Failure to wash vegetables	41	Beta(44,65)
Failure to clean preparation surface for preparation of different products	60	Beta(65,44)

Once the preparation profile is assigned the levels of organisms contaminating the hands, vegetables and surface are estimated. In each case, that is transfer from product to hands, hands to vegetables and product to preparation surface, it is assumed that some proportion of the total number of organisms contaminating the product have the potential to be transferred. This is a result of the proportion of organisms that come into direct contact with the hands, vegetables, and preparation surface.

There are no data which provides information about the levels of contact which occur during preparation of a chicken meal. Therefore it is assumed that in each case, the proportion of product which contacts the hands and preparation surface, defined as pc, is uniformly distributed between ½ and ⅙ of the total surface area of the product, that is $pc \sim U(\frac{1}{2}, \frac{1}{6})$. It is likely that the organisms will not be uniformly distributed about the product, there may be some clustering in specific regions of the product. To address this issue, the number of organisms that are present on the contact surface and hence have the potential to transfer is defined as η^{*}_{con}. The asterisk denotes the stage of preparation being considered such that η^{H}_{con} indicates contact with hands and η^{S}_{con} indicates the contact with the preparation

surface is occurring. Estimation of parameters such as η^{*}_{con} which allow for microbial clustering has been defined by Nauta (2001) and is given by

$$\eta^{*}_{con} \sim \text{Binomial}\left(\eta^{*}, \text{Beta}\left(b, b\left(pc-1\right)\right)\right)$$

Here, the parameter b is a measure of the degree of clustering, that is the variability in the number of organisms that contaminate each proportion, of size pc, of the product. This is a relative value which is an integer >1. The greater the value of b the lesser the extent of the variability. The result is a variability distribution for the number of organisms which contaminate the proportion, pc, which contacts the hands or preparation surface.

The value that the parameter b should take is unknown. Nauta (2001) carried out an expert consultation to determine the value of b for clustering of *Escherichia coli* on raw beef carcasses. The mean value determined was 4. This value is assumed to be appropriate for the clustering of campylobacter on chicken.

Given the number of organisms which contact the surface and hence have the potential to transfer, the proportion of these that will transfer is given by γ_t. The rate of transfer for campylobacter is unknown and has not been the subject of experimental studies to date. However, transfer studies have been conducted for several other organisms, (Mackintosh & Hammonds, 1984) these data demonstrate that transfer rates vary between bacterial species. In these trials, transfer from the hands to a cloth, and vice versa was specifically considered. As such, these data cannot be used to calculate transfer probabilities as required by the model described here. Further data are presented by Zhao *et al.*, (1998). This study investigates the transfer of organisms from a chicken portion to a cutting board. Although this study does not use campylobacter, it is assume that the transfer rates are equivalent. Further, it is assumed that the transfer rate from product to chopping board also apply to transfer from product to hands, and hands to vegetables. To incorporate

variability in this transfer rate, it is assumed that the minimum proportion of organisms which transfer is 0, the maximum proportion of organisms which transfer is 0.15, and the most likely proportion is 0.1, the value reported by Zhao *et al.*, (1998). These are then incorporated in a triangular distribution, that is $\gamma_t = T(0,0.1,0.15)$. Should more data become available, this distribution can be updated in the model to better reflect the transfer of campylobacter.

The number of organisms which are cross contaminated during the preparation of the chicken meal and hence have the potential to be ingested, defined as η_{cc} is given by equation (7.4).

$$\eta_{cc} = \begin{cases} \left[H_w \eta_{ps} \gamma_t\right] + \left[V_w \gamma_t \left(H_w \eta_{con}^H \gamma_t\right)\right] + \left[S_w \left(\eta_{ps} - \eta_{con}^H\right) \eta_{con}^S \gamma_t\right] & \theta_{prep} = 1 \\ \left[H_w \eta_{ps} \gamma_t\right] + \left[V_w \gamma_t \left(H_w \eta_{con}^H \gamma_t\right)\right] & \theta_{prep} = 0 \end{cases} \tag{7.4}$$

The proportion of organisms which are cross contaminated that are ingested with the meal is unknown. This proportion is highly variable between individuals and different meals prepared by any given individual. Therefore, it is assumed the proportion of organisms which are cross contaminated that are ingested, defined as η_{cc}^* is uniformly distributed between zero and the total number cross-contaminated. This translates to $\eta_{cc}^* \sim U(0, \eta_{cc})$.

7.2.3 Cooking of the product

The number of organisms that survive the cooking process and are subsequently ingested is defined as η_{pc}. This number is a function of the starting population and survival characteristics of campylobacter. As previously described, the survival and growth characteristics of campylobacter are temperature dependent. However, in

contrast to storage, during the cooking of a product the temperature will be time dependent. As the product is to be cooked a measure of the temperature of the product throughout cooking is required to estimate the decline in the contaminating population. This can be achieved by modelling the rate at which heat passes through the product over time during cooking.

Modelling the transfer of heat during cooking

Given an object of homogeneous material, there will be an energy transfer rate within the object. This rate is defined as q and is governed by Fouriers' law, which results on the expression

$$q = -kA\frac{\delta T}{\delta x}$$

Where $\frac{\delta T}{\delta x}$ is the temperature gradient in the body, k is the thermal conductivity of the material and A is the area. If the temperature profile is linear then $\frac{\delta T}{\delta x}$ becomes

$$\frac{\Delta T}{\Delta x} = \frac{T_2 - T_1}{x_2 - x_1}$$

Such linearity exists in a homogeneous medium of fixed thermal conductivity during steady state heat transfer. Steady state transfer occurs when the temperature throughout the object is independent of time. However, if the temperature is time dependent, this is referred to as time varying transfer (Pitts & Sissom, 1998).

The conductive heat transfer rate at any point within a medium is related to the local temperature gradient by Fouriers law. From the temperature distribution the temperature gradient at any location can be derived and consequently the heat transfer rate.

Consider a cube which is subsequently heated. This results in an energy flux throughout the cube. This flux is a three-dimensional vector. The general conduction equation describing the rate of change of temperature with time is given by

$$\frac{\partial}{\partial x}\left(k\frac{\partial T}{\partial x}\right)+\frac{\partial}{\partial y}\left(k\frac{\partial T}{\partial y}\right)+\frac{\partial}{\partial z}\left(k\frac{\partial T}{\partial z}\right)+q'=\frac{1}{\alpha}\frac{\partial T}{\partial t}$$

Where q' is the rate of heat generation per unit volume. However, this is often equal to zero, thus given a constant thermal conductivity throughout the body, this becomes equation (7.5), which is known as the Fourier equation.

$$\frac{\partial^2 T}{\partial x^2}+\frac{\partial^2 T}{\partial y^2}+\frac{\partial^2 T}{\partial z^2}=\frac{1}{\alpha}\frac{\partial T}{\partial t}$$

(7.5)

When a body is in steady state heat conduction the left hand side of the above equation equates to zero. However, when considering the cooking of a chicken product the temperature is varying with time and hence Fouriers equation is applied.

To illustrate how such techniques can be applied to the problem of modelling the rate of heat transfer in a chicken predict, the situation is simplified such that the product can be represented by a rectangular slab of finite thickness. The flow of heat is assumed to flow in only one direction. This could be thought of as representing pan frying of a portion of chicken breast meat. However, in reality there may be flow of heat in the y and z directions within the product.

The temperature at any point within a product is dependent upon several factors. These include the temperature of the surrounding medium, the geometry of the

product, and the products' heat transfer coefficients. Initially, assume that the temperature only moves through the product in a single direction. The temperature, at any point x in the product at time t, denoted $T_{t,x}$, is then given by

$$T_{t,x} = T_m + \theta_{t,x}(T_0 - T_m)$$

where T_0 is the initial temperature of the product, T_m is the temperature of the heating medium, x is the distance of the sector from the centre of the product and $\theta_{t,x}$ is the rate of heat transfer at point x and time t.

Under the assumption heat transfer is uni-directional it can be assumed that the product is of very large dimensions in the y and z directions. As such, $\frac{\partial^2 T}{\partial y^2} = 0$, and $\frac{\partial^2 T}{\partial z^2} = 0$ and the Fourier equation can be simplified to yield equation (7.6), the one-dimensional conduction equation.

$$\frac{\partial^2 \theta}{\partial x^2} = \frac{1}{\alpha}\frac{\partial \theta}{\partial t} \qquad (7.6)$$

where θ is the rate of heat transfer.

The approach discussed above has been illustrated by Hartnett *et al.*, (2001). The authors assume a one dimensional slab model, described above, is appropriate. An assumption is made about the distribution of organisms at various depths below the surface and the number of organisms surviving at each depth as a function of temperature and time are then calculated using the model described above. The results are illustrated in Figure 7.3.

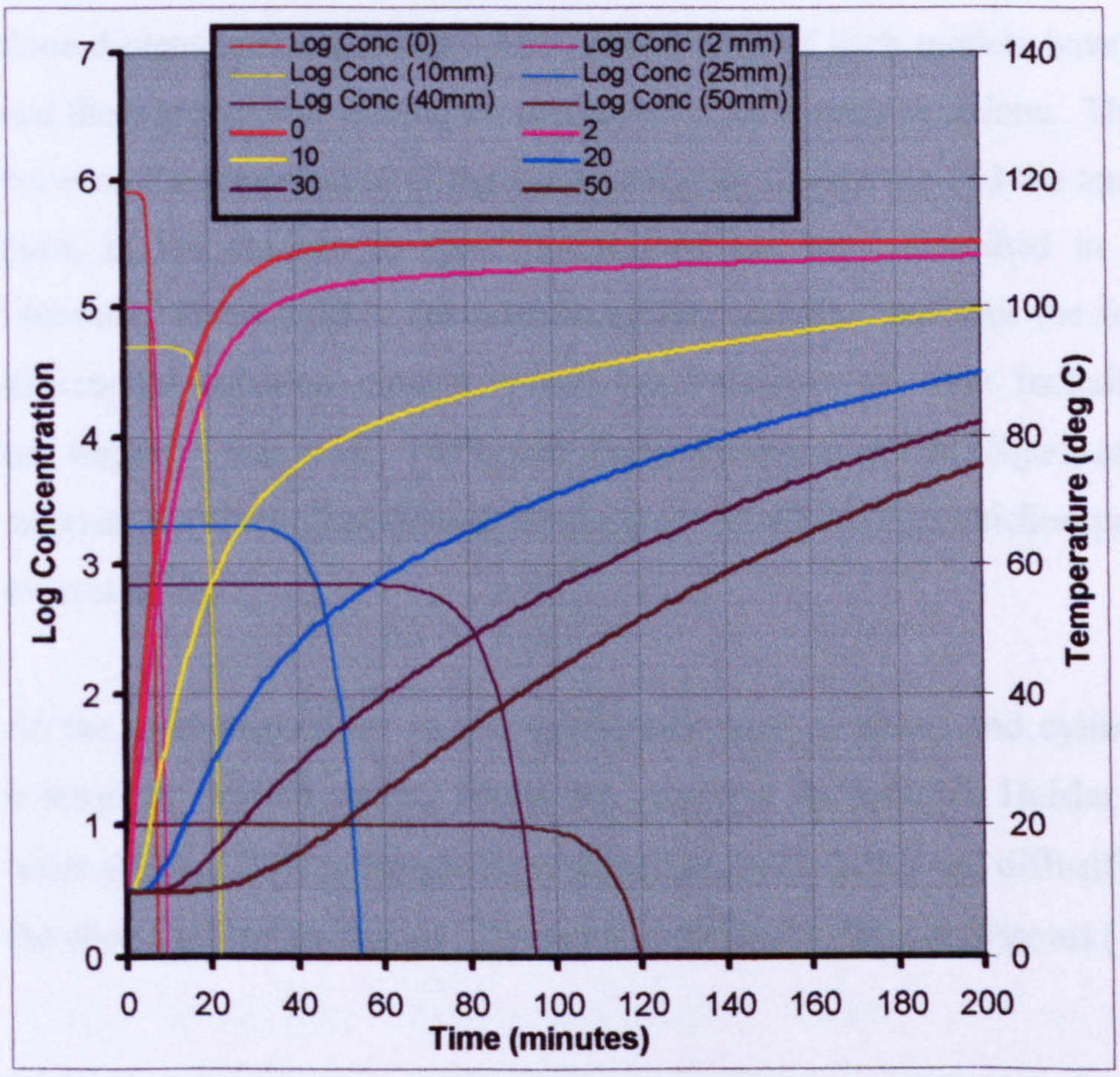


Figure 7.3: Graph showing the manner in which temperature at different depths increases and the bacterial population present at that depth decreases as heating proceeds, as described by the one dimensional heat transfer model for a slab of chicken of finite thickness.

The method presented above can be adjusted to model the transfer of heat in two- and three-dimensional systems. This involves solving the Fourier equation for the appropriate geometry as functions of x and y for two-dimensional and x, y, and z for three-dimensional situations. The mathematics of such models have been studied and there are several techniques presented to solve such equations. The relationship between the temperature of the surrounding air in an oven and the temperature of a point in the chicken in three dimensions has been described in the scientific literature. These models are mathematically complex and take the form of partial differential equation models solved in a number of ways including analytical techniques (Chen *et al.*, 1999) and finite element methods (Ngadi *et al.*, 1997) to estimate the three dimensional temperature distribution in chicken products during oven cooking.

As the mathematics for simple geometries such as cubes and cylinders has been extensively studied several charts are available for example Heisler charts, which relate the heat flow to the physical properties, such as thermal diffusifity, and size of the object subject to heating. These are presented in Pitts & Sissoms (1999).

To implement the one dimensional heat transfer model described above it is necessary to define the distribution of organisms throughout the product as a function of depth from the surface. The majority of the organisms will contaminate the surfaces of the product rather than the muscle as a result of the cross contamination, which occurred during processing. However, it is currently an area of scientific debate whether campylobacters penetrate the muscle and are therefore found at a variety of depths within a product (Tom Humphreys, personal communication). However, there is currently no scientific evidence of such an occurrence. As such it is not necessary to develop a complex heat transfer model predicting the temperature within the product during cooking.

7.2.4 Model description

In this chapter, the model chosen to describe the way in which a campylobacter population changes with temperature is driven by data availability. From the data for campylobacter FRI-CF8, campylobacter is a sensitive organism, with high death rates at temperatures above 50 °C. Cooking of chickens is likely to take the product to temperatures above this. As there is data available providing a thermal profile for the temperature of a chicken product over time during cooking, a simple empirical model can be developed to describe the change in temperature of the product over time. Given the lack of data available in the published literature which refers to the response of campylobacters to environmental conditions the simple exponential model is chosen to describe the way in which growth responds to the changes in temperature which may occur during cooking.

The temperature of the chicken product over time is estimated from experimental data. Kelly *et al.*, (2000) present temperature profiles for the internal and external temperature of chicken drumsticks when oven roasted. The data is shown in Table 7.4. Similar profiles are given by Bryan *et al.*, (1971), Bryan & McKinley (1974), Lyon *et al.*, (1975), Chang *et al.*, (1998). Inspection of the data suggest a logarithmic function is appropriate to describe the change in temperature of a chicken product as heating proceeds. Using the method of least squares, the regression model quantifying the relationship between the temperature of the product during cooking, $T(t)$, and time is given by $T(t) = 46.747\ln(t) - 94.05$ where $R^2 = 0.95$. The predicted regressions line and data points are plotted in Figure 7.4.

Table 7.5: Experimental data showing the internal temperature of a chicken drumstick during oven roasting.

Time (mins)	Internal temperature °C
0	14.9
5	14.9
10	13.7
15	28.8
20	43.2
25	56.2
30	68.6
35	78.0
40	85.8
45	83.7
50	93.3
55	94.9
60	82.1

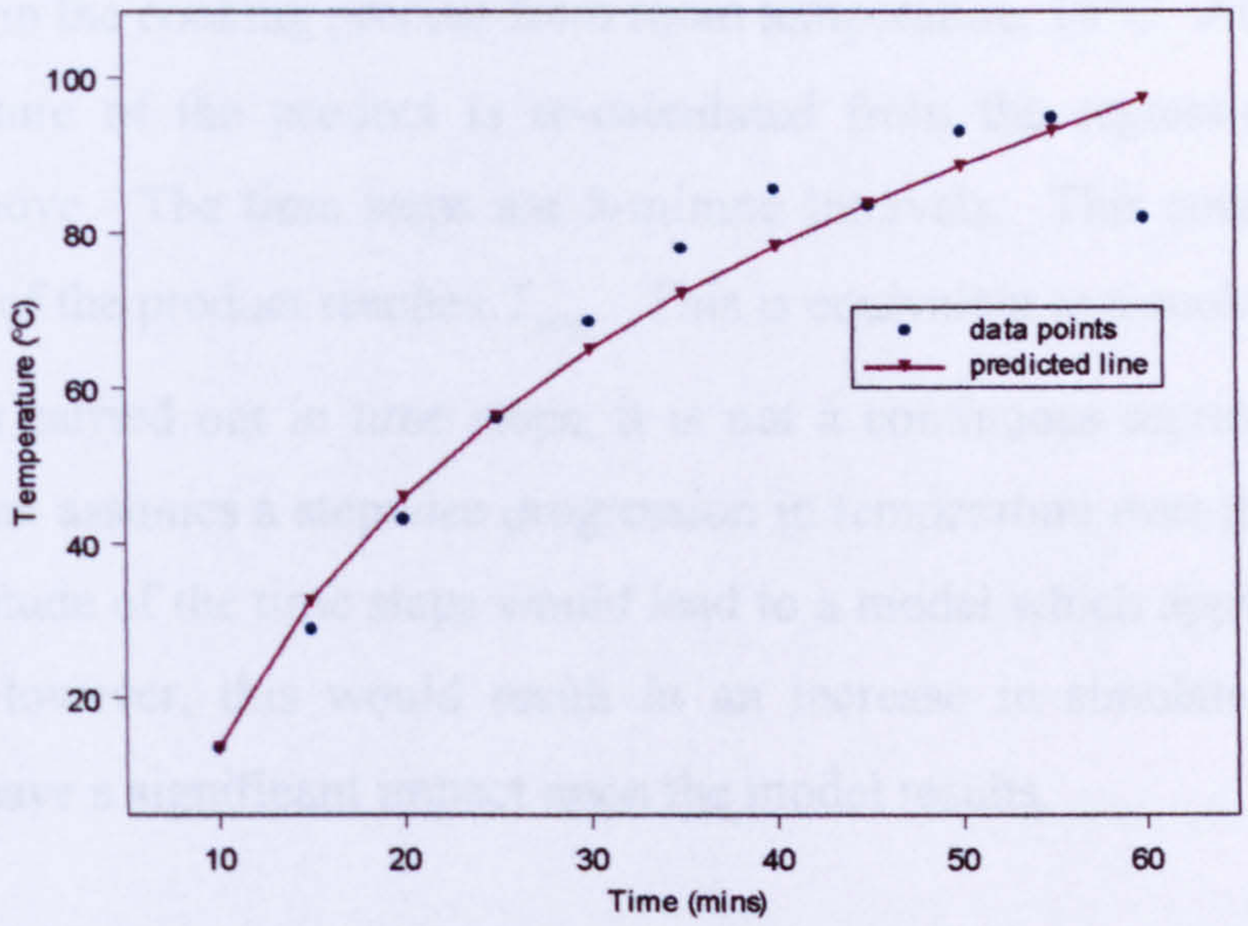


Figure 7.4: Graph showing the experimental data in comparison with the corresponding points predicted from the regression equation $T(t) = 46.747\ln(t) - 94.05$ to estimate the temperature of a chicken product during cooking.

The manner in which temperature changes with time during the cooking process is described by $T(t)$. The amount of growth or inactivation can be estimated by considering each time step and re-evaluating equation (7.6) for each time interval until cooking stops. This usually requires knowledge of how long individuals cook chicken meal, this is data which is currently unavailable. An alternative approach is possible, however, in the US, a large survey was conducted during which individuals were asked to measure the temperature of the chicken product immediately after they had cooked the item (Audits, 2001). This information enables both the temperature at which the product was cooked and the amount of time for which the item was cooked for to be calculated. The temperature to which a product is cooked is defined as T_{stop}.

The model begins at a starting temperature of the product. It is assumed that all products begin the cooking process from room temperature, 18°C. At each time step the temperature of the product is re-calculated from the regression relationship described above. The time steps are 5-minute intervals. This continues until the temperature of the product reaches T_{stop}. This is equivalent to a cooked product. As the model is carried out in time steps, it is not a continuous representation of the process, rather assumes a stepwise progression in temperature over time. Reduction in the magnitude of the time steps would lead to a model which approaches the true situation. However, this would result in an increase in simulation time and is unlikely to have a significant impact upon the model results.

Now that a model has been developed to describe the manner in which temperature of the product changes throughout the cooking process it is necessary to predict the response of the campylobacter populations to such temperatures. Thus, enabling predictions of the level of contamination on the cooked product to be made. The model chosen to describe the way in which a campylobacter population changes with temperature is driven by data availability. Given the lack of data available in the published literature which refers to the response of campylobacters to environmental conditions the simple exponential model is chosen to describe the way in which growth responds to the changes in temperature which may occur during cooking. From the data for campylobacter FRI-CF8, campylobacter is a sensitive organism, with high death rates at temperatures above 50 °C. Cooking of chickens is likely to take the product to temperatures above this.

The size of the bacterial population after exposure for a given amount of time, t at temperature T is given by equation (7.7).

$$N_t = \begin{cases} N_{(t-1)}e^{\mu t} & T < T_c \\ 10^{\log(N_{(t-1)}) - \left(\frac{t}{D}\right)} & T \Rightarrow T_c \end{cases} \qquad (7.7)$$

Here $N_{(t-1)}$ is the population size at the previous time step, prior to exposure to the temperature for time t, μ is the specific growth rate constant and D is the D-value, that is the time required for a 1 log reduction in the size of the bacterial population at a given temperature. The above is an accumulation of the exponential models for growth and inactivation. As such, the model describes the growth of campylobacters in the exponential phase. The parameter T_c is the temperature at which growth does not occur and the numbers of campylobacter begin to decline. The temperature at which growth ceases and inactivation begins is $T = 46°C$ (Doyle & Roman, 1981).

Given the variability that is evident between strains of campylobacter data is chosen upon the basis of the availability of measurements at a range of temperatures for the same strain. There is no such data available with measurements taken in chicken meat, therefore, data sets are combined to provide a response profile across temperatures for a given single strain. From the literature, a range of temperature measurements were available for only a single strain, that is *C. jejuni* FRI-CF8. This data will now be described.

For the survival during storage data from Koidis & Doyle, (1983) using *C. jejuni* FRI-CF8 is used. The measurements were taken in Brucella broth. Growth is parameterised using the data from Doyle & Roman (1981) covering temperatures from 25 to 47 °C using *C. jejuni* FRI-CF8. As growth is being considered, only those temperatures which are in the permissible growth range for campylobacters are used, that is 32 to 45 °C. Inactivation is measured using D-values presented by Doyle & Roman (1981) for using *C. jejuni* FRI-CF8 and Gill & Harris (1982) where a strain referred to as animal strain 6 was used. No data are available for temperatures corresponding to these reported by Gill & Harris (1982) for *C. jejuni* FRI-CF8, therefore an assumption is made that animal strain 6 displays the same survival characteristics as *C. jejuni* FRI-CF8.

To describe the rate of growth during cooking, the specific growth rate, μ, is related to temperature. To describe the effect of temperature, a regression model was fitted

to the experimental data using least squares to quantify the relationship between the specific growth rate and temperature of the product, using data presented by Blankenship & Craven (1982). The data are given in Table 7.3. Inspection of the data suggest a logistic model is appropriate. Therefore, a logistic regression model was fitted to the data and the predicted regression line and the data points are plotted in Figure 7.5. The regression equation is $\mu = 1.4943\ln(T) - 5.0885$ and the R^2 value is 0.95.

Table 7.4: Experimental data showing the doubling time, and hence the associated specific growth rate constant as a function of temperature (after Blankenship & Craven, 1982)

Temperature	Doubling time (t_d)	Specific growth rate ($\mu = \frac{\ln 2}{t_d}$)
32	11.1	0.06
35	2.96	0.23
37	2.16	0.32
42	1.24	0.56
45	1.28	0.54

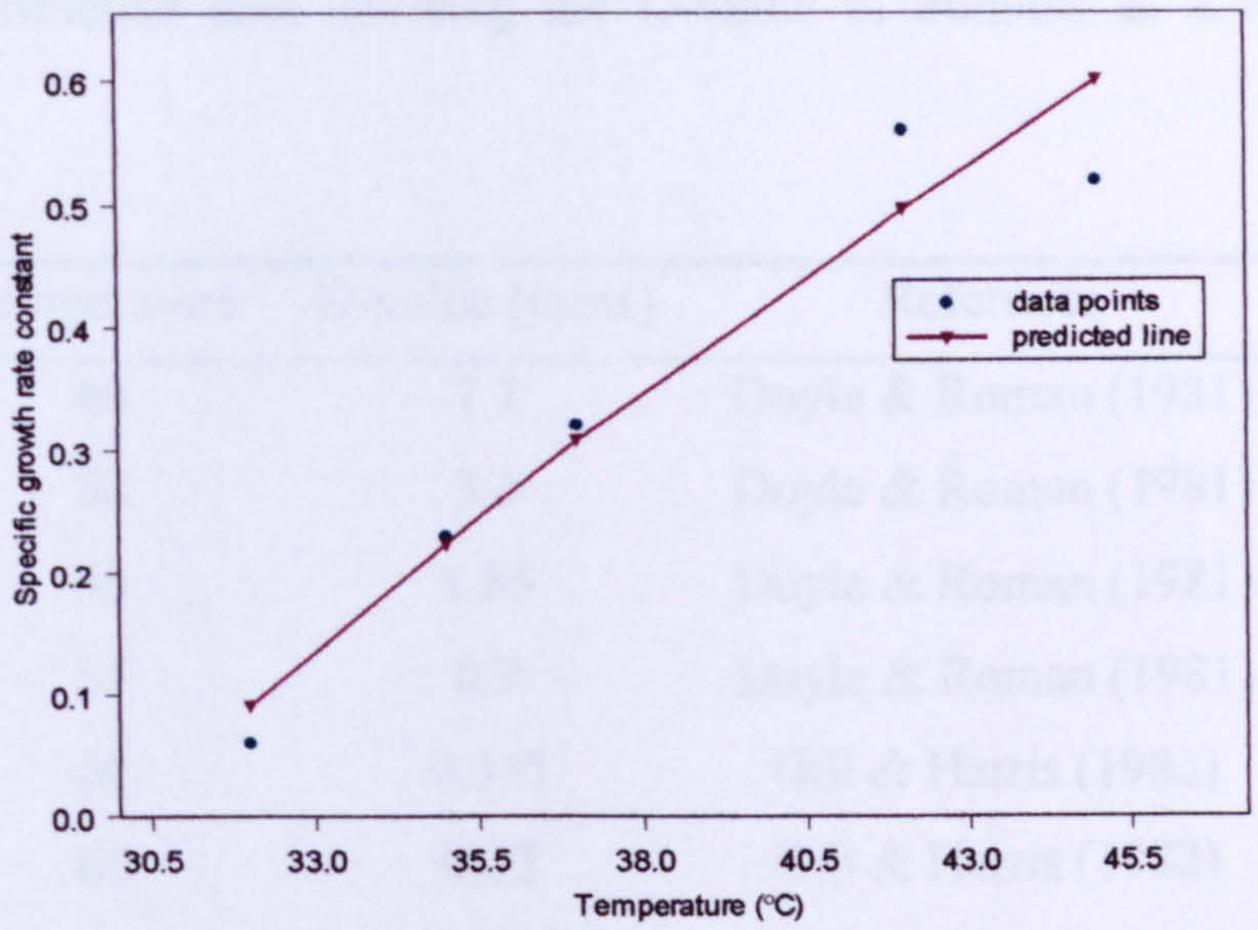


Figure 7.5: Graph showing the experimental data in comparison with the corresponding points predicted from the regression equation $\mu = 1.4943\ln(T) - 5.0885$ to estimate the specific growth rate constant for campylobacter in response to the temperature of the chicken product.

Similarly, the D-value, is also related to temperature. Data presented by Doyle & Roman (1981) and Gill & Harris (1982) suggests that an exponentially declining function can be used to describe the effect of temperature. Hence, an exponential function was fitted to the experimental data using least squares to quantify the relationship between the D-value and temperature of the product. The data are given in Table 7.4 and the predicted exponential function and the data points are plotted in Figure 7.6. The regression equation is $D = 14926e^{-0.169T}$ and the R^2 value is 0.92.

Table 7.4: Experimental data showing the D-value in minutes as a function of temperature

Temperature	D-value (mins)	Reference
48	7.7	Doyle & Roman (1981)
50	3.3	Doyle & Roman (1981)
53	1.85	Doyle & Roman (1981)
55	0.9	Doyle & Roman (1981)
60	0.345	Gill & Harris (1982)
65	0.22	Gill & Harris (1982)
70	0.185	Gill & Harris (1982)

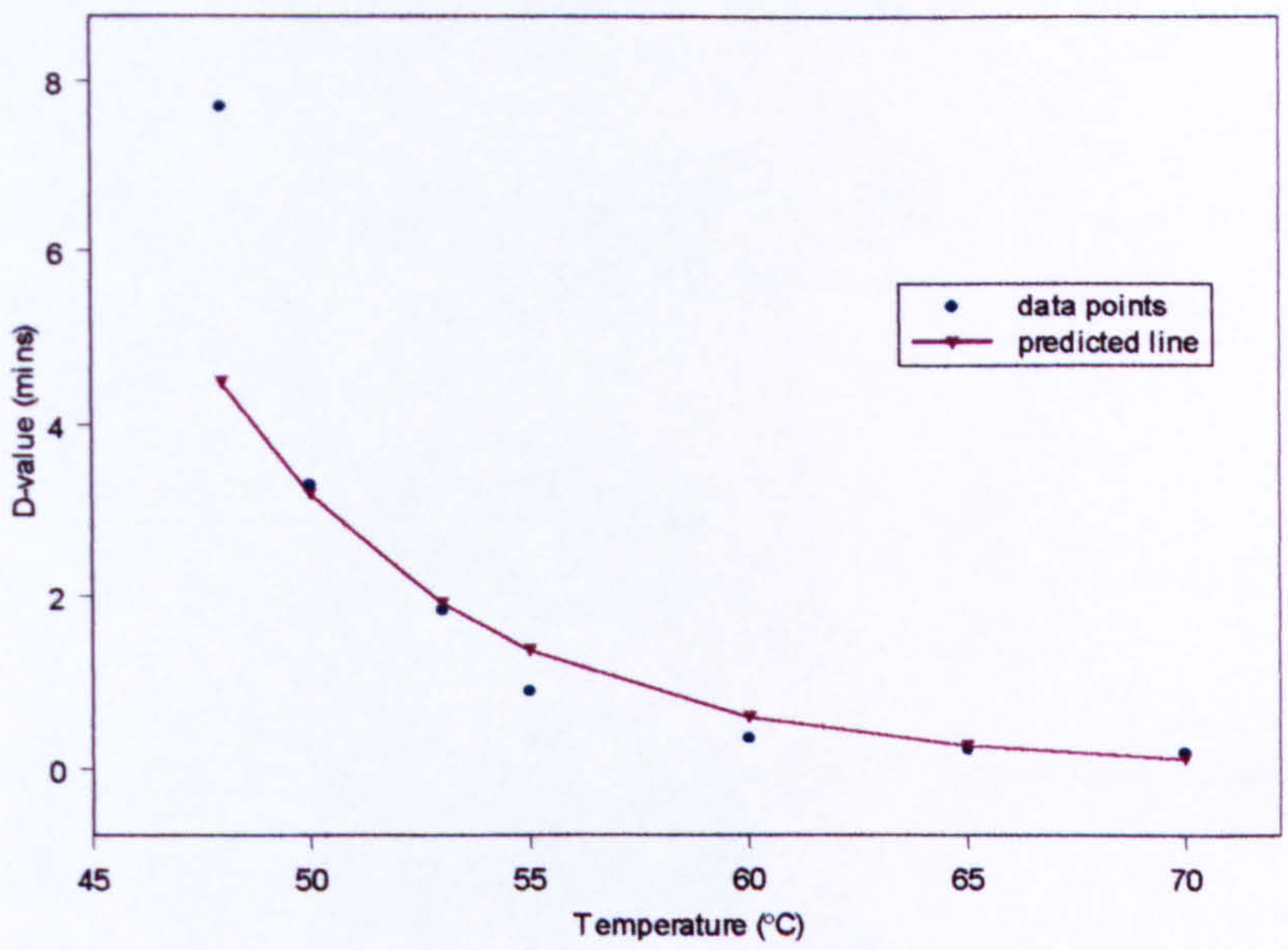


Figure 7.6: Graph showing the experimental data in comparison with the corresponding points predicted from the regression equation $D = 14926e^{-0.169T}$ to estimate the D-value for campylobacter in response to the temperature of the chicken product.

To summarise the model describing the cooking process, Figure 7.7 is a schematic representation of the model to estimate the number of organisms contaminating the product post cooking. The model uses time steps of 5 minutes.

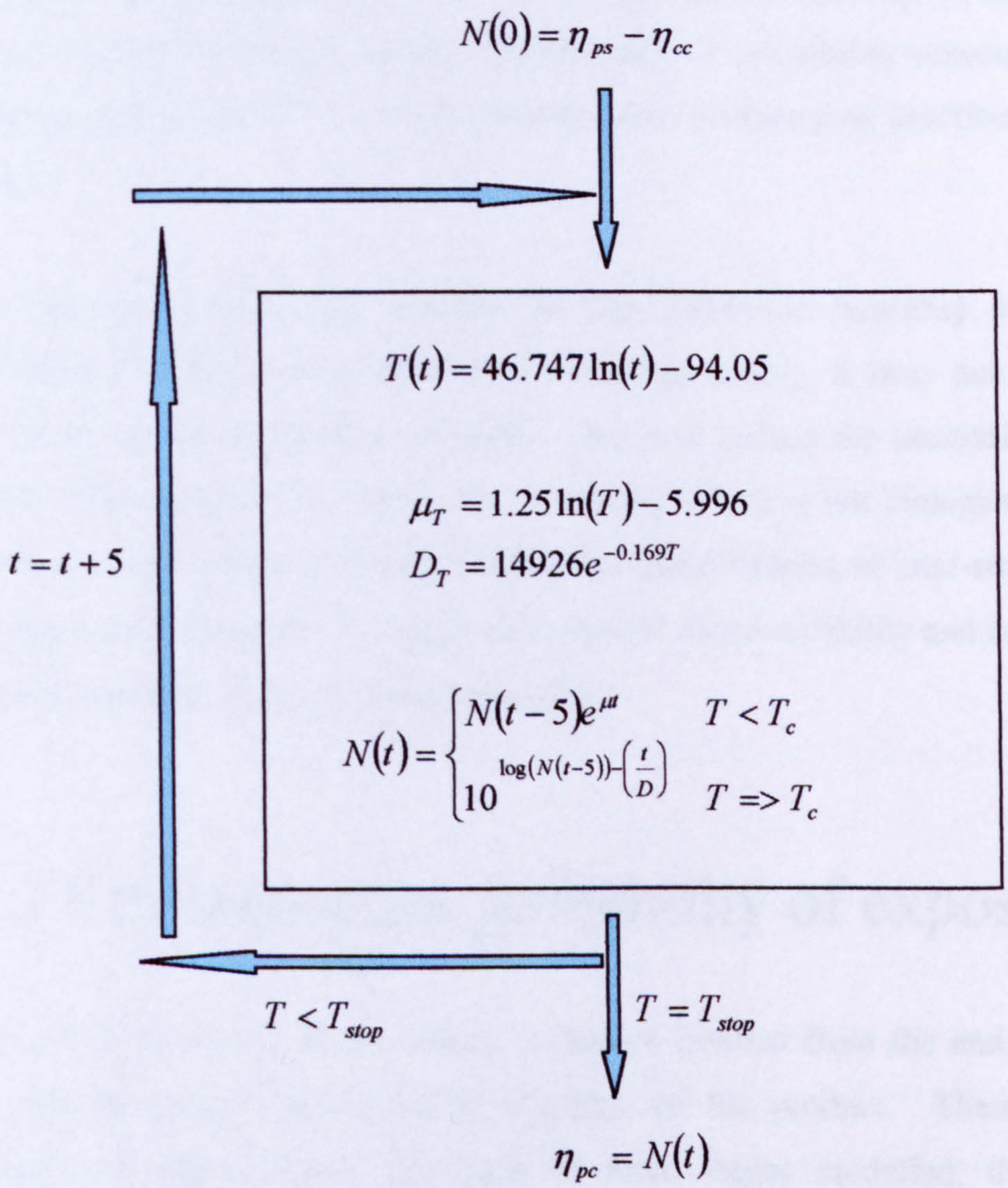


Figure 7.7: schematic representation of the model estimating the number of campylobacter surviving cooking. For details of the mathematical terms see section Estimating η_{pc}.

Incorporating uncertainty in the model framework

Throughout this chapter the method of least squares has been used to fit models to data sets. However, each of these data sets consists of small sample sizes. Further, as discussed previously, there is uncertainty about the manner in which different strains of campylobacter respond to temperature. This results in uncertainty associated with the regression line. To incorporate this uncertainty, each regression model is first linearised, and the distributions of uncertainty associated with the residuals generated by use of a non-parametric bootstrap as described in detail in Chapter 2.

The uncertainty generated through the non-parametric bootstrap is assumed to correspond to inter-strain variability. Although strictly, it does not represent the variability as the acquisition of further data will reduce the uncertainty and, as a result of this assumption, reduce the variability which is not biologically plausible. However, in the absence of data to allow the quantification of inter-strain variability this approach recognises the issues with current data availability and is a first step to reconciling the problem of strain variability.

7.3 Estimating the probability of exposure

The model described above follows a random product from the end of processing through the stages leading up to ingestion of the product. These are storage, preparation and cooking. At each of these stages modelled, the number of contaminating organisms on the product is calculated. This calculation mimics the changes in numbers as a result of the particular step.

At preparation and cooking, a serving of a chicken meal containing the product can be defined as contaminated if it carries at least one organism. By means of a

conditional statement, the model can state whether a selected serving is contaminated or not. The conditional statement is

$$\psi_i = \begin{cases} 1 \text{ if } \eta_{exp} \rangle 1 \\ 0 \text{ if } \eta_{exp} \langle 1 \end{cases}$$

where ψ_i is whether or not the serving is contaminated at the point of consumption. Within a given simulation distributions are sampled n times, on each time the result is either a contaminated or uncontaminated product. Multiple samplings of the distributions represent the storage, preparation and cooking of multiple products. Therefore running the model allowing for n samplings of each distribution the probability that a serving of a chicken meal is contaminated can be calculated by use of equation (7.8).

$$P_{exp} = \frac{\sum_{i=1}^{i=n} \psi_i}{n} \qquad (7.8)$$

where P_{exp} is the probability that serving of a chicken meal results in the ingestion of at least one organism, based upon n samplings within a simulation of the model.

7.4 Model simulation

The model contains several parameters, each of which is described by an appropriate variability distribution or regression model. The variability distributions and regression models have associated uncertainty with respect to the true form of the distribution or regression line. As such the model can be run with different combinations of variability distributions and regression lines with each combination representing one possible realisation of the preparation of a chicken meal. When the model is simulated a number of times the result is multiple distributions describing the variability in the number of campylobacters ingested as a result of 1 serving of a

chicken meal along with multiple estimates of the probability of ingesting at least one campylobacter as a result of a single serving. From this, the level of uncertainty with respect to the outputs of the model η_{exp}, the number ingested, and P_{exp}, the probability of ingesting one or more campylobacters can be quantified.

The model is run for 50 simulations. Upon each simulation a variability distribution or regression line is selected from the associated uncertainty space for each second-order random variable and kept constant for any given simulation. Within each simulation the selected distributions are sampled 15 000 times and η_p calculated as shown in equation (7.1). At the end of each simulation P_{exp} is then given as shown in equation (7.8). Multiple simulations result in the uncertainty distribution for P_{exp}. The number of samples taken within a simulation was chosen according to when the running mean of P_{exp} no longer deviates ±1% from the 'true' mean, defined as the mean of P_{exp} at 20,000 iterations. The deviation of the running mean from the 'true' mean is illustrated in Figure 7.8. It can be seen that 15,000 samples are adequate to fulfil this criteria. The number of simulations was chosen to ensure adequate selection of the variability from the uncertainty interval for each of the second-order random variables.

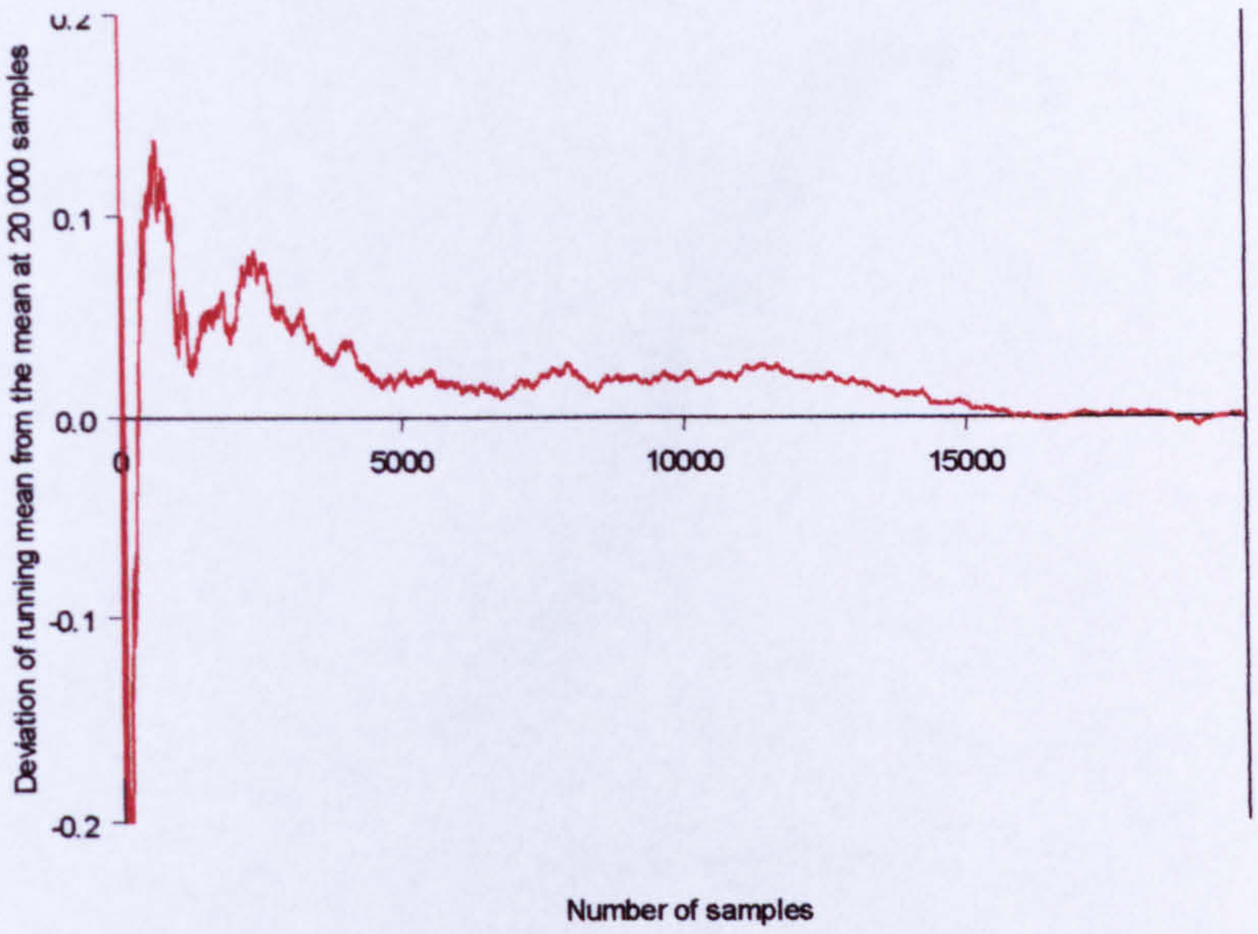


Figure 7.8: Graph to show the deviation of the mean number organisms ingested with a single serving of a chicken meal, P_{exp}, from the 'true' mean of P_{exp} defined as the mean at 20,000 distribution samples (iterations).

7.5 Results

The distribution describing the number of organisms which will be ingested given an individual is exposed to campylobacter from a serving of chicken, based on 50 simulations of 15,000 samples is shown in Figure 7.9. For clarity only 25 simulations are illustrated in Figure 7.9.

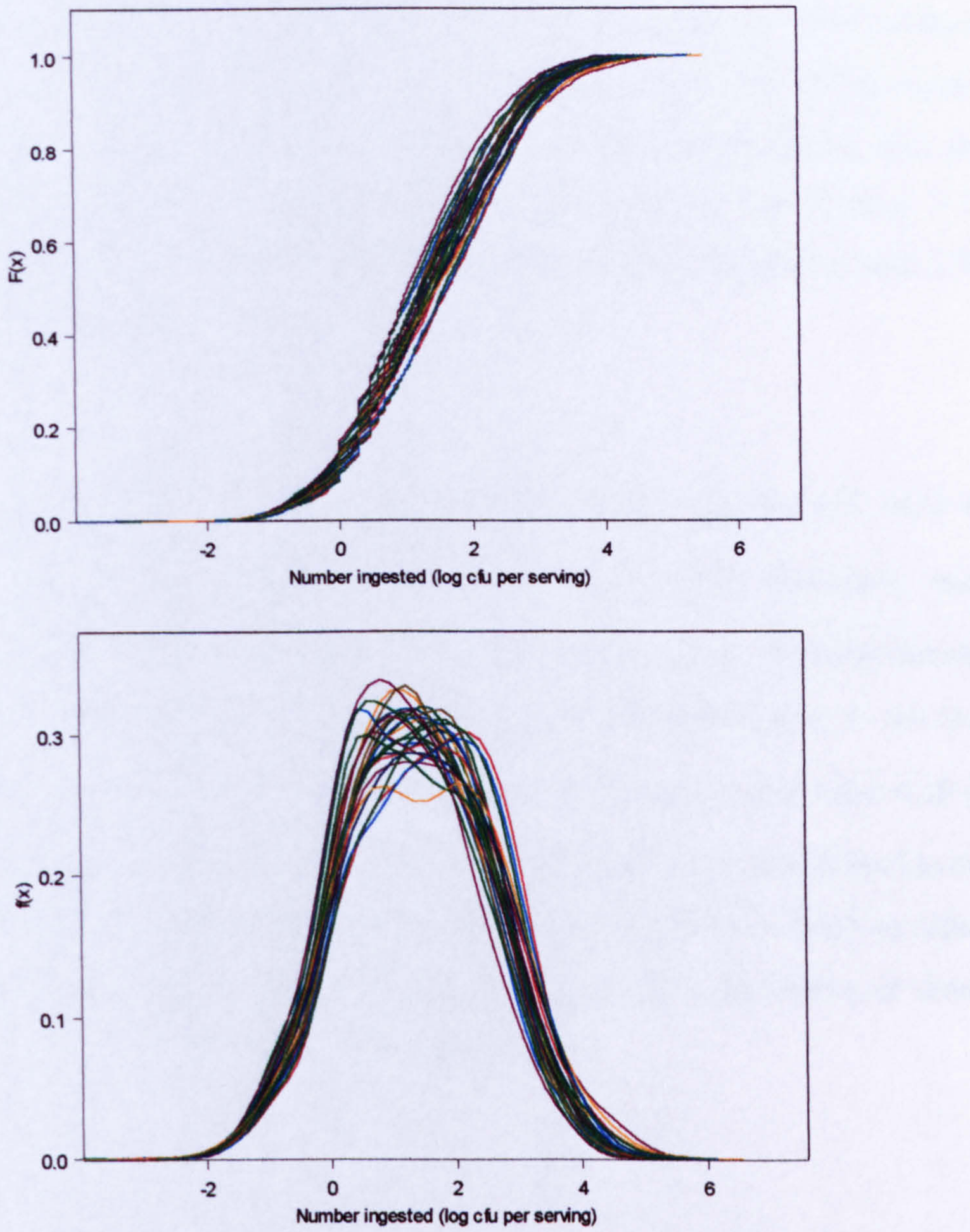


Figure 7.9: Second-order cumulative and density plots describing the number of campylobacter ingested given exposure occurs from a single serving of chicken, estimated using the model, and illustrating the uncertainty that arises due to the variability in the combined process.

This is a second-order distribution and as such, each line describes a single realisation of the variability on the number of organisms ingested. The multiple lines and their locality, generated as a result of multiple simulations of the model, reflect the level of certainty associated with this variability. Considering this, the mean number of campylobacter ingested given exposure ranges from 2.30 to 3.10 log cfu per serving. Further, the 95th percentile of this distribution ranges from 2.94 to 3.60 log cfu per serving.

Based on the distributions for η_{exp}, the probability that a serving will lead to ingestion of at least one campylobacter, that is P_{exp}, is also uncertain, each simulation resulting in a possible value for this probability. The resulting density plot for P_{exp} is shown in Figure 7.10. This figure shows the uncertainty in the true value of P_{exp}. This probability has a mean value of 0.21, and ranges from 0.18 to 0.26. That is on average, 21 servings out of 100 servings of chicken will lead to the ingestion of at least one campylobacter. This exposure could be a result of either indirect exposure that occurs due to poor hygiene practices in the home or direct exposure due to failure to adequately cook the product.

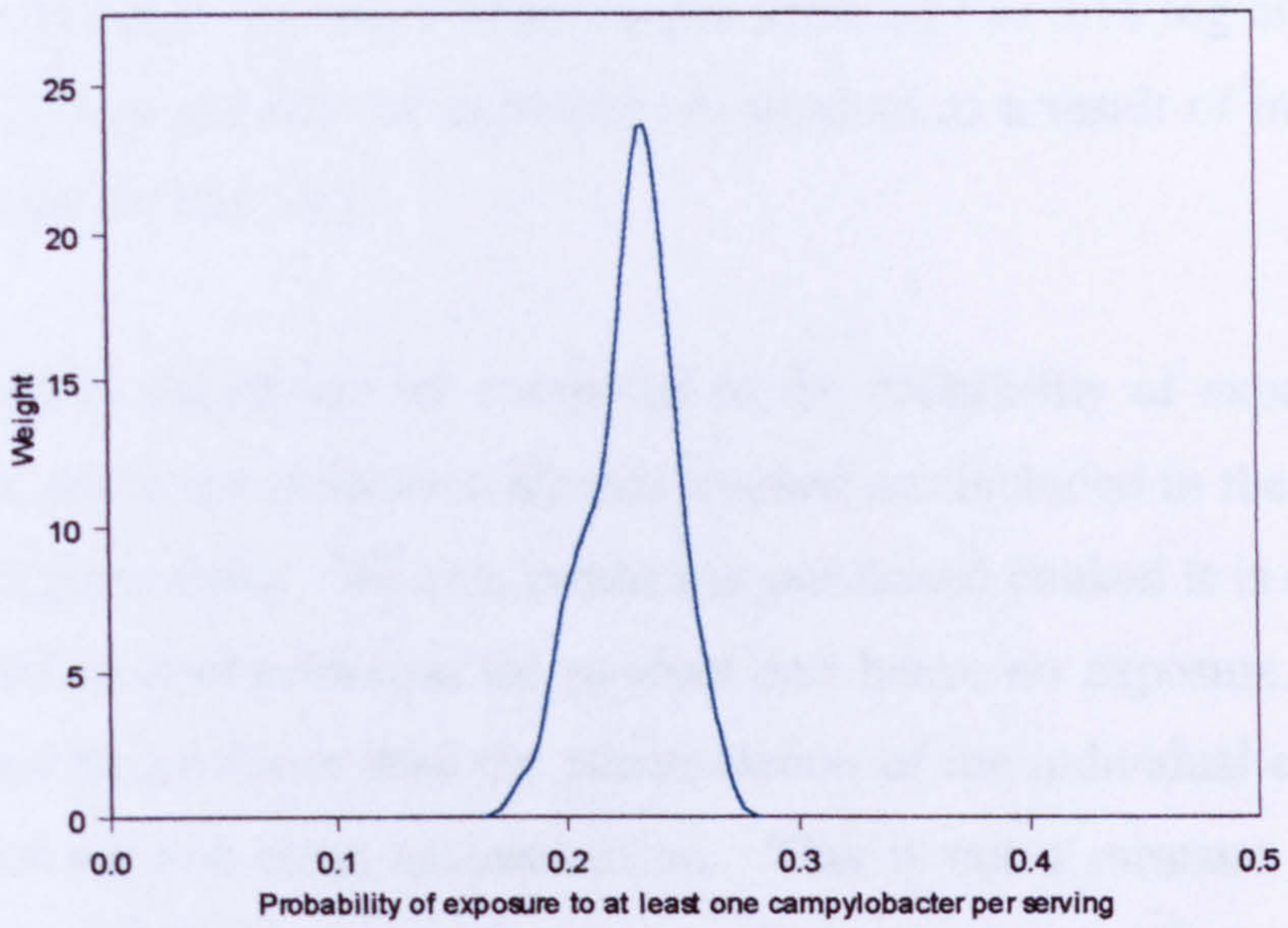


Figure 7.10: Uncertainty distribution describing the probability that an individual will ingest at least one campylobacter from a single serving of chicken.

7.6 Sensitivity of model results to route of exposure

The model described in this chapter incorporates two possible routes of exposure, these are indirect exposure as a result of poor hygiene practices during the preparation of the meal, and direct exposure resulting from failure to adequately cook the product. To investigate the relative importance of each of these routes of exposure on the predicted levels of exposure, the distribution for the number of organisms which are available for ingestion, from a random product as a result each of the two routes are generated. The distribution for the number of campylobacter per product which could potentially be ingested as a result of poor hygiene is shown in Figure 7.11. The mean value ranges from 2.81 to 3.68. Further, the probability of exposure per product due to poor hygiene ranges from 0.23 to 0.32. For comparison, the number of campylobacter per product which could potentially be ingested as a result of failure to adequately cook the product is shown in Figure

7.12. In this case the mean value ranges from 2.07 to 3.18 log cfu per product. In addition, the probability of exposure per product as a result of inadequate cooking ranges from 0.02 to 0.03.

These results should not be compared to the probability of exposure per serving. Products which are purchased already cooked are included in the model results for exposure per serving. When a product is purchased cooked it is assumed there are no organisms contaminating the product and hence no exposure. As such overall model results are lower than the accumulation of the individual exposure pathways from cooking and cross contamination. This is not a measure of the number of organisms ingested per serving from each source, rather it is a measure of the likelihood that organisms contaminating a product will lead to exposure via the two routes.

The comparison of the routes of exposure, that is poor hygiene and inadequate cooking, clearly demonstrate that poor hygiene presents the greatest potential for ingestion of campylobacter to occur with inadequate cooking of a product less likely to lead to exposure. Interestingly, model results suggest that when exposure by either route occurs the mean number of organisms that could potentially be ingested per product are similar in magnitude. However, examination of Figures 7.11 and 7.12 shows, despite similar mean uncertainty intervals, that higher numbers of organisms may be ingested as a result of poor hygiene, compared with inadequate cooking.

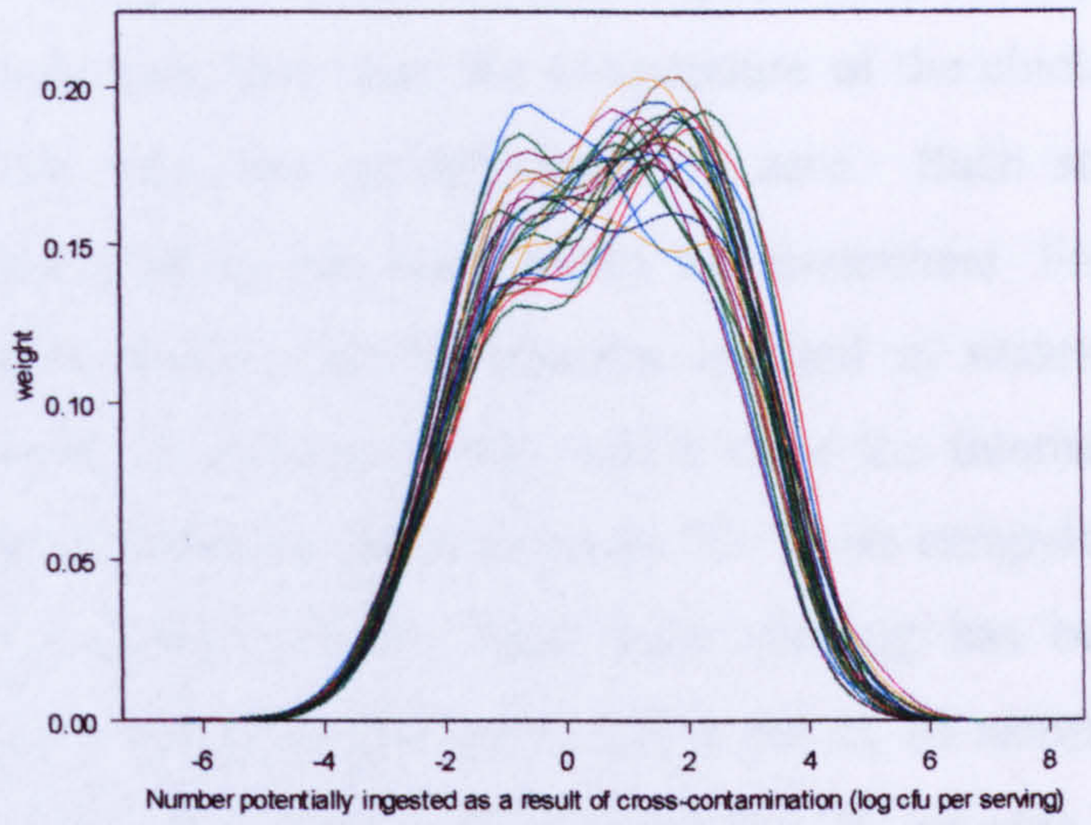


Figure 7.11: Second-order distribution describing the number of organisms per product which may potentially be ingested as a result of poor hygiene practices during preparation of the product.

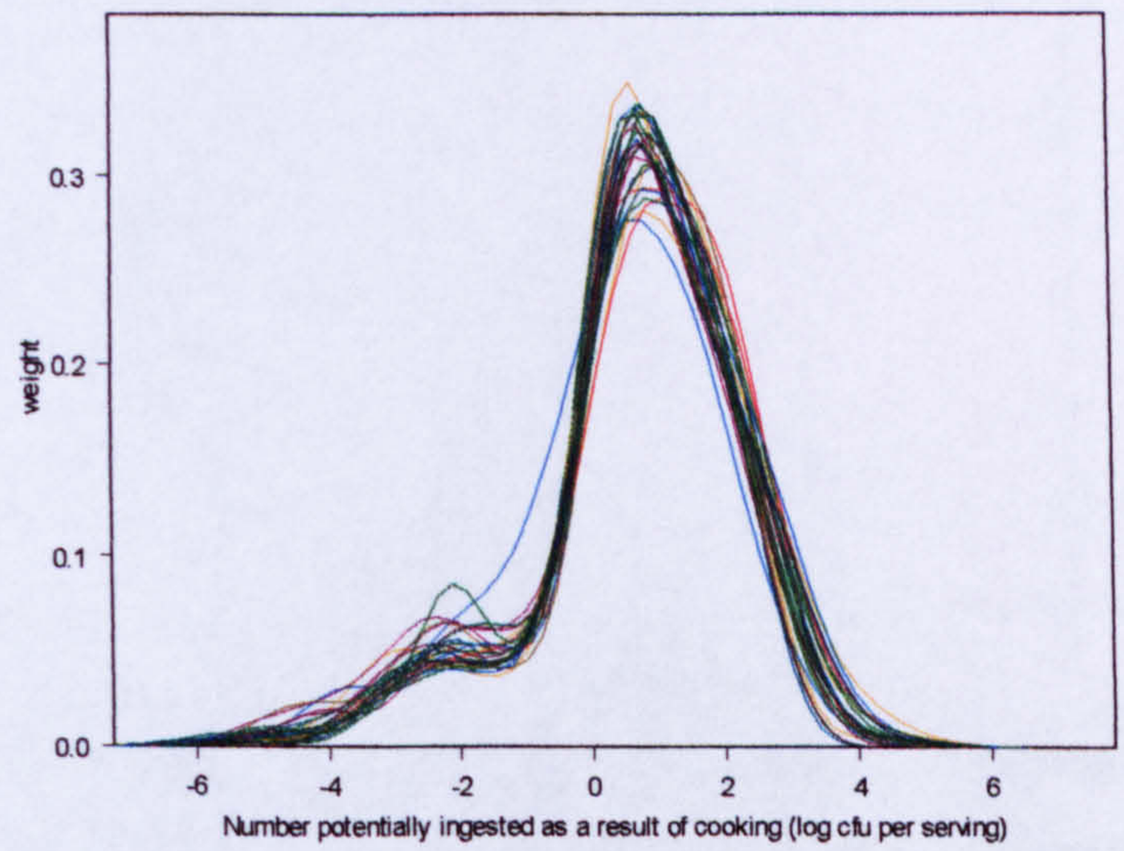


Figure 7.2: Second-order distribution describing the number of organisms per product which may potentially be ingested as a result of the inadequate cooking of the product.

The cooking of the chicken has a significant impact upon the probability of exposure. The predicted response of a campylobacter population of 10^6 cfu to cooking, as predicted by the model is shown graphically in Figure 7.13. From Figure 7.13, it can be seen that once the temperature of the chicken meat exceeds 50°C the population size very quickly drops to zero. Such sensitivity to high temperatures during cooking has been noted by researchers. For example, oven roasting of a whole chicken for 25 minutes resulted in undetectable levels of campylobacter (Doyle & Roman, 1981), while once the internal temperature of inoculated ground beef reached approximately 70 °C no campylobacters could be recovered (Stern & Kotula, 1982). Even poor cooking has been demonstrated sufficient to eradicate contamination with only 4 out of 14 naturally contaminated half carcasses cooked in a conventional oven for 20 minutes at 190°F having detectable levels of campylobacter (Gill and Harris, 1984). These results thus substantiate the findings relating to exposure from cooking (Figure 7.12).

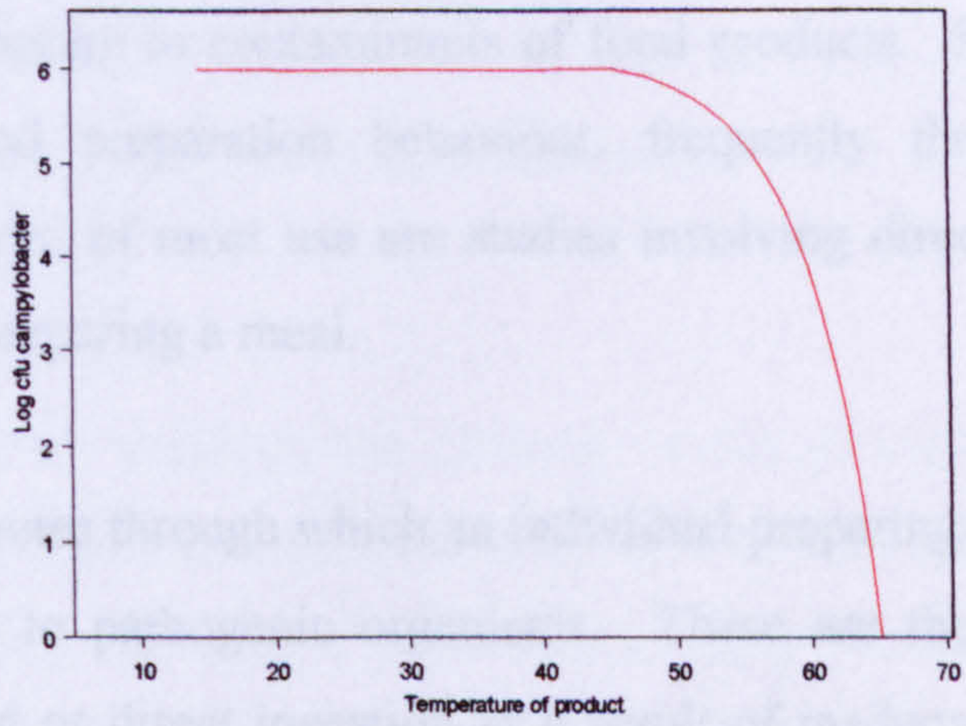


Figure 7.13: Illustration of the decline in a campylobacter population (measured in log cfu per product) of initial size 6 log cfu on a product subject to cooking until a temperature of 74°C is achieved.

MISSING

PAGES

NOT

AVAILABLE

conditions are sparse. There is not enough data to be able to fully parameterise such a predictive model, and hence this is an area of data deficiency. However, measurements of the size of the bacterial population in response to temperature will also include the response which may have occurred due to the changes in physical characteristics of the product during heating. Therefore, although not explicitly modelled, the response of the bacterial population to environmental changes other than temperature that may also occur during heating are described in the model results. It should be noted that unfortunately data with such measurements taken during the heating of chicken meat are currently not available in the published literature. As such an assumption is made that the inoculated medium, for example broth, is equivalent to chicken meat. In the absence of more data specifically for chicken, the impact of this assumption is unknown.

7.8 Conclusions

The manner in which individuals prepare food is crucial to predicting the likelihood and magnitude of exposure to contaminants of food products. Several researchers are investigating food preparation behaviour, frequently through the use of questionnaires, however, of most use are studies involving direct observation of a group of individuals preparing a meal.

There are two main routes through which an individual preparing a meal in the home can become exposed to pathogenic organisms. These are the direct or indirect contamination of food or direct ingestion as a result of inadequate cooking. Both pathways are recognised as causal agents of food related illness. In this chapter a model was described which investigates the levels of exposure that results from the accumulation of these pathways.

Initially the storage of a product is considered. This storage includes both storage at retail and storage in the home prior to preparation. In this way the retail section of

the farm to fork supply chain is incorporated in to the model. Both refrigerated and frozen storage are considered and the result is an estimation of the number of campylobacter contaminating a product prior to preparation

To investigate the effect of preparation three main processes were considered, these are the failure to wash hands after contact with the raw chicken product, failure to wash vegetables, and failure to clean the preparation surface. These events are ordered in time such that a failure to wash the hands after contact allows the transfer of organisms to the vegetables. The outcome of this model is the number of organisms which contaminate the product and the number which are removed from the product is a result of cross-contamination. The proportion of cross-contaminated organisms that will be ingested is unknown. There are numerous pathways via which such organisms can become ingested and the associated likelihood of such pathways will be variable between meals and individuals. Therefore this proportion is assumed to be uniformly distributed. It is not the aim of the model to specify the route via which the organisms are ingested after they have been cross-contaminated. The model assumes that some proportion will be ingested and it does not matter how. More detail information on consumer practices in the home could enable this current framework to be modified to explicitly consider the routes by which such organisms become ingested.

Once the meal has been prepared the cooking of the product is the next stage considered. Here, an empirical model was developed relating the temperature of the product during cooking to time. There are methods available which involve modelling the rate of heat transfer in a product to predict the changes in temperature. However, in the current situation, given data availability, use of such models/methods was not necessary.

Key to both the storage and cooking sections of the model is the response of campylobacter to environmental changes. The data available show that such characteristics are highly variable between campylobacter strains and medium. As

such all data used in the model was from a single strain. No data were available for the response of this strain in chicken meat and hence this is an area of data deficiency. Further, information on a wide range of strains across the temperature ranges the organisms are likely to be subjected to in the home are currently not available. This is a fundamental requirement to enable full assessment of the effects of storage and cooking upon levels of contamination, and hence should be a focus of future research.

The two pathways that lead to the ingestion of campylobacter have been compared, the results clearly show that cross-contamination result in the greatest opportunity for the ingestion of organisms with the probability of cross contamination one or more organism ranging from 0.23 to 0.32 and the probability of at least one campylobacter surviving cooking ranging from 0.02 to 0.03. This does not mean, however, that undercooking poses an insignificant risk. From the model results, on average 2 to 3 individuals out of 100 will undercook their food to an extent that at least one campylobacter will remain to contaminate the product.

In summary, the model described here simulates the storage, preparation and cooking of a random chicken product. On average, the probability that a serving of chicken will lead to the ingestion of at least one campylobacter ranges from 0.18 to 0.26 Further, given a serving is contaminated, the number of organisms ingested is described by a second order distribution. This distribution has a mean value ranging from 2.30 to 3.10, a 5^{th} percentile ranging from –0.70 to –0.36, and a 95^{th} percentile ranging from 2.94 to 3.60 log cfu per serving. Cross-contamination poses the greatest opportunity for the ingestion of campylobacter from a serving of chicken. As such, a public awareness campaign explaining appropriate handling of food items could reduce the risk of exposure to campylobacter from a chicken meal.

Chapter 8

The health consequences of exposure to campylobacter

8.1 Introduction

The ingestion of pathogenic organisms via food is a major cause of illness throughout the world. When an individual is exposed to, and ingests such organisms there are three possible outcomes: organisms may pass through the gut with no consequence, there may be infection, or illness may occur. Here, infection is defined as the establishment or colonisation of pathogens in the exposed individual, but signs of illness are not necessarily present. The probability of infection is a function of several factors. These include the magnitude of the dose ingested, the infectivity and pathogenicity of the organism, the food vehicle via which the individual is exposed, and the vulnerability of the host to infection. Each of these will have an impact which is specific for different species. However, this may also vary from individual exposures to the same species. These factors make fully understanding the mechanisms by which food borne pathogens cause illness a formidable task.

When contaminated food is the vehicle of exposure, the ingested pathogens enter the body by way of the digestive tract. This is therefore the initial site of action by such microbial agents. To protect against infection there are defences at this stage; however, an overwhelming dose or a weakened host resistance may lead to infection. These defences are well documented in numerous medical textbooks and have been summarised by Mims *et al.*, (1993).

Pathogenic micro-organisms represent the result of dynamic evolutionary adaptation of survival strategies. The ability to evade partly or totally one or more of the normal defences of the host is essential to propagate within the individual. Mechanisms used by pathogenic micro-organisms to overcome host protective barriers and hence be successful, include those related to adherence for entry into the host, secretion of toxins, and avoidance of host immune systems. For each individual pathogen the invasion and infection mechanisms will differ and in many cases are not yet fully understood.

Once an individual has been exposed and becomes infected, this may lead to illness. The severity of such an illness depends on several factors, including agent type, amount consumed, individual health status, individual susceptibility and protection by food during passage through the digestive system. Incubation periods for foodborne illnesses range from less than one hour to several weeks and the resulting symptoms are wide and varied ranging from nausea to profuse, watery diarrhoea and chronic pain. Such illness is commonly self-limiting, not requiring treatment except in severe cases. The duration of symptoms can range from a few hours to several months

A number of sectors of the population are at increased risk from food associated illness. In general, this increased risk is associated with an immune impairment of some kind such as infection with HIV or treatment with immune suppressive drugs. Further the young and very old are considered to be at increased risk as the immune systems in such individuals may not be fully developed, or immune responses reduced due to age.

Illness may not be the only consequence of infection. Many enteric pathogens cause chronic sequelae, that is some illness which does not necessarily emerge directly after exposure, but may be several years later. In some cases these conditions may occur in the absence of an acute illness at the time of exposure. As a result the cause of such sequelae may not be linked to a food-borne source in epidemiological studies making the contribution of food borne pathogens to such illnesses difficult to quantify.

To predict and assess the impact of exposure to defined levels of pathogens probability models are currently being widely used. These models are specified by a specific dose-response curve for each pathogen thus assuming that risk depends upon the number micro-organisms ingested.

8.2 Dose Response Modelling

A thorough discussion of the use of mathematical models to describe infection and illness has been presented by WHO/FAO hazard characterisation workshop report WHO/FAO (2000). In summary, a dose-response model is a mathematical function that takes as an argument a measure of dose. This can be any non-negative number and the resulting function yields the probability of the particular adverse effect bounded by zero and one. Because of the variability in both host susceptibility and infectivity of the micro-organisms, models used for dose-response relationships must be flexible. In many cases the modelling of infectious agents may require multiple models. This is often the situation for cancer and non-cancer dose-response models used in chemical risk assessment (Byrd *et al.*, 1998). However, a single model is considered appropriate to represent microbial dose response relations in the human host (Haas, 1993).

There are an infinite number of possible functions that can be used to describe the relationship between dose and effect. The commonest employed functions are monotonic. In particular any cumulative distribution function with domain over $\langle 0,\infty \rangle$ can be a candidate. Conversely any dose response function that is monotonic and bounded by zero and one with domain over $\langle 0,\infty \rangle$ is a cumulative distribution function. This is convenient, since for many distribution functions the mathematical properties have been well studied.

8.2.1 Data for Dose Response modelling

To understand the mechanisms by which pathogens cause illness within the human host, and the consequential relationship between dose, infection and illness experimental feeding trials may be used. Experimental feeding trials are often used to establish the infective dose that may result in infection in an outbreak, yet they differ fundamentally from an outbreak. The test population commonly consists of

healthy young men. In contrast an outbreak affects whom ever is unfortunate enough to have eaten a contaminated meal and most certainly includes highly susceptible members of the population. As a consequence, volunteer feeding studies may report mild or no illnesses for a given number of infectious agents consumed whereas, in actual outbreaks, lower levels of micro-organisms may cause illness.

Data from food-borne illness outbreaks and human feeding studies indicate that the doses of infectious agents required to cause illness differ greatly among types, genera, species and strains of infective micro-organisms (CAST, 1994). Further, these studies demonstrate great inter-individual variability in the way which the body reacts to a given dose. This is illustrated by several members of the test population showing a variety of reactions to a dose of the same magnitude (Coleman & Marks, 1998).

There are many obstacles when attempting to accurately interpret data from outbreaks and feeding trials. In reports from feeding trials there may be no clear distinction between asymptomatic infection and illness as an endpoint, a difference having a major impact on the estimated infective dose. In epidemiological data from outbreaks only illness may be reported, and only in rare cases is a measure of dose known. For example in Japan it is a requirement that a sample of all meals prepared in restaurants is kept frozen for 2 weeks. Hence if an outbreak should occur there is a sample of the food to which sufferers were exposed. This has several problems. If temperature abuse of the food occurs, for example, numbers of pathogens determined at any time will not necessarily reflect number of pathogens actually consumed. Hot holding of foods may cause death of organisms and freezing may result in substantial declines in bacterial populations. Competition from spoilage organisms may also interfere in laboratory assays to determine the occurrence of the pathogens in a suspect food. Thus, after the fact estimates of how many bacteria an affected person has ingested are speculative. However, despite such problems this type of data is beginning to provide insight into the epidemiology of food-borne outbreaks.

Given the current level of knowledge available from outbreaks, dose-response models are generally parameterised using feeding trial data. Generally such data is reported giving the dose and the number of people in the study either infected, or ill or both. Hence a rough relationship can be formulated between dose and infection/illness. There may not be data available for a given organism, however, it may be possible to use surrogate data if it is ascertained that the organisms are comparable in terms of dose and resulting consequences.

8.3 Estimating the probability of infection

Several models are available and have been used to describe microbial dose response data. Such models used to describe dose-response relations can be segregated into three over all categories, those which are based upon mechanistic assumptions, referred to as mechanistic models, those models which are essentially an empirical fit to a given data set, referred to as empirical models, and those which have elements that are both empirical and mechanistic and hence are referred to as semi-mechanistic. However, underlying the frameworks of empirical, mechanistic and semi-mechanistic dose-response models is the assumed mode of infection by the particular organisms. There are two opposing theories available; these are the single-hit theory and the threshold theory.

For an individual to become infected it is necessary for the organisms to posses the capability of establishing themselves within the host. Such an event can only occur after the ingestion of at least one organism. Any such organism that is able to establish within the host is capable of growth, multiplying to produce several clones, which then infect the individual. To prevent this from occurring, the host usually has several defence mechanisms, such as those described previously. These are aimed at killing, removing or inactivating the organisms before they grow to a level sufficient to cause an adverse reaction. Therefore in an immune competent host the probability that any one ingested organism is capable of colonising and hence

infecting the host is very small. The infection of an individual by one ingested organism is referred to as a single hit.

An alternative to the single hit theory is offered by the threshold theory. Under the threshold theory it is assumed that for infection to occur there is a predetermined minimum inoculum size which must be ingested. Exposure below this size will not result in an adverse reaction, and hence carries a zero risk.

8.3.1 Classes of dose-response model

Whether the single-hit theory or threshold theories apply to the pathogen in question, this is then applied within the appropriate model framework. As previously stated the three classes of model are empirical, mechanistic and semi-mechanistic. Each of these will now be described.

Empirical models

Empirical models can be defined as models which are chosen based upon observation and experimental evidence such as laboratory experiments such models are not necessarily supported by any biological action. The model suitability is based upon the goodness of fit to the data. These models are commonly used for the analysis of chemical toxicity.

In brief, these models are based upon the theory that a susceptible population has a pre-determined tolerance level, a dose above this level will always result in an adverse consequence. Therefore, the curve is derived from the data, and hence the dose-response model is assumed to describe the distribution of tolerance for the population (Buchanan *et al.*, 2000). A tolerance distribution is essentially a density function with respect to the average dose, d, that is $f(d)$ and can be defined by the integral

$$P_{\inf}(d) = \int_0^d f(y)dy$$

Here, $P_{inf}(d)$ is the probability of infection (Haas *et al.*, 1999) for a defined dose, d.

When such models were first becoming available in the scientific literature the theory of minimum infectious dose emerged (Buchanan *et al.*, 2000), that is the threshold theory. This led to dose-response relations being described by a single number – the threshold level, above which adverse consequences will occur and below which there is no risk.

There are several threshold models available. In principal any density function which gives support over the data set can be considered as a tolerance distribution. However, there are three models which are most commonly applied in toxicological issues. These models are the Log-logistic, Log-probit, and Weibull models. The log-logistic uses the log-logistic distribution as the tolerance distribution where as the Log-probit model makes use of the lognormal distribution and the Weibull model assumes a Weibull distribution for tolerance. Each of these models are similar at high doses but predict different levels of risk when doses are less than 100 organisms (Haas *et al.*, 1993).

More recently a number of non-threshold models have been used to describe the entire dose-response curve. By use of curve fitting algorithms fitting one of the empirical models to the available data can be a simple task. However, as the models are empirical they cannot be used to infer any underlying biological mechanisms.

In the application of dose-response models empirical models are not commonly utilised. This is attributed to the lack of biological plausibility associated with these models and lack of data to provide a basis upon which to select a model. A preferred choice may be a model which is mechanistic in nature.

Mechanistic models

Mechanistic dose-response models describe the biological processes that occur, resulting in infection. Currently there are no dose-response models in the literature which are solely mechanistic in nature. However in an attempt to more realistically model the infection process, Buchanan *et al.*, (2000) present a possible framework for a mechanistic dose-response model. This is a compartmental model describing the main events that must occur for an individual to become ill from the consumption of pathogenic organisms. The compartments consider the successful passage through the gastric acid barrier, the ability to attach to the gut of the individual and hence result in infection, and finally the resulting infection leading to either illness or death.

This model is a simple example and several more compartments may be required to fully describe the infection process. Further, variation in species makes this a mechanistic model which is host-pathogen specific and therefore it is likely that for each pathogen the model formulation is unique. Although this framework provides advantages in that a more realistic description of the process is offered, developing such models is likely to be a complex process.

There is a third class of models which fall into neither the empirical or mechanistic group of models. These are therefore considered semi-mechanistic models.

Semi-Mechanistic models

The derivation of a semi-mechanistic dose response model is based upon a set of biologically based assumption about the stages which, following the ingestion of organisms, leads to infection. However these assumptions, and hence the chosen model, lead to a dose-response relationship which is known to fit the data. As such these are not truly mechanistic models as they are biased by the form of the data, yet they are not empirical in the sense that the model does incorporate some interpretation of the biological processes occurring during infection.

For an individual to become infected by pathogenic organisms in food, two stages must occur (Coleman & Marks, 1998). First the individual must ingest one or more of the pathogenic organisms which must subsequently evade the individuals immune defences and reach a site where infection can be initiated.

Consider exposure of a population to a food product containing a population of some pathogenic micro-organism. If the mean dose ingested by the population is denoted d, and an individual ingests exactly j organisms the probability that k organisms will survive and reach a site to initiate infection is given by

$$P(k)=\sum_{j=1}^{\infty}P_1(j|d)P_2(k|j)$$

Here P_1 describes the probability of ingesting j organisms and P_2 describes the probability that of the j organisms ingested, k will initiate infection. Further they incorporate the inter-individual variation in number of organisms ingested (P_1) and the factors of host/organisms interaction that must occur for k organisms to survive to initiate infection. For a given individual there will be a minimum number of organisms that must survive for infection to be initiated, this is $k_{\min}$. The probability of infection, $P_{\inf}$, can now be defined in terms of the mean dose (d), the actual dose (j), the number of organisms surviving to initiate infection (k) and the minimum that this number can be to enable infection ($k_{\min}$). More specifically, the overall semi-mechanistic model is given by equation (8.1).

$$P_{\inf}=\sum_{k=k_{\min}}^{\infty}\sum_{j=k}^{\infty}P_1(j|d)P_2(k|j) \qquad (8.1)$$

Currently under debate is the numerical value that the factor $k_{\min}$ should take for a given micro-organism. This is determined by the nature of the infection process in the host. Under the single hit theory, bacteria are assumed act independently to

initiate infection, therefore the survival of only one organism may be sufficient to fulfil the criteria for initiation of infection, as such k_{min}=1. According to the threshold theory, the micro-organisms which are ingested could be acting co-operatively whereby there is a minimum population size of micro-organisms required to initiate infection, and therefore k_{min}>1. Models assuming the threshold theory to be appropriate are becoming less common as epidemiological evidence supporting the single hit theory is mounting (Buchanan *et al.*, 2000).

It can be seen that semi-mechanistic models present a formulation defined according to the assumed mode of infection by the organisms ingested. The choice of semi-mechanistic model is dependent upon whether the single hit theory or the threshold theory is considered appropriate.

8.4 Estimating the probability of illness

Dose response models correlating the ingested dose and probability of illness to date have not been well studied. Of the work available in the literature it would appear that for some pathogens there is no clear relationship between dose and the probability of illness (Dupont *et al*, 1995; Rose *et al*, 1991). In contrast other organisms show a clear pattern with the probability and severity of illness increasing with dose (Haas *et al.*, 1999). Little work has been done on developing this relationship into a dose-response model.

To be able to develop the current level of research on the relationship between dose and illness more data is required for pathogenic species incorporating not only a wide range of doses but also a variety of disease indicators.

8.5 A dose-response model for campylobacter

Several dose-response models for campylobacter appear in the literature (Medema *et al.*,1996). Each of these models utilises the same data set and assumes the same model form. In this thesis the same model is also adopted to describe the dose-response relationship for campylobacter. The data used and model derivation shall now be described.

8.5.1 Human feeding trials with campylobacter

In 1988 Black and colleagues (1988) under took a human volunteer feeding trial using campylobacter species. The study consisted of 68 healthy young adults from Baltimore, USA. The volunteers were admitted to an isolation ward of the local hospital and challenged with doses of *C. jejuni* suspended in milk. Post challenge the volunteers were interviewed daily for 12 days by a physician and all stools collected. Illness was defined as the presence of diarrhoea or fever.

Two strains were used as innnocula, strains A3249 and 81-176. The results of the challenge tests are shown in Table 8.1. From the study involving strain A3249 studies demonstrated that ingestion of even low doses of *C. jejuni* resulted in diarrhoea. This is illustrated in Figure 8.1. Interestingly, all volunteers became infected at high doses, yet the highest number of illnesses was not recorded at the highest dose with the highest number of illnesses recorded at a dose of 9×10^4 cfu. Therefore although rates of infection increase with dose, development of illness does not show a clear dose relationship.

Table 8.1: Results of human volunteer feeding trials with C. jejuni (after Black *et al.*, 1988)

Dose (cfu)	Number of volunteers	% infected	% ill
	C. jejuni A3249		
8×10^2	10	50	10
8×10^3	10	60	10
9×10^4	13	85	46
8×10^5	11	73	9
1×10^6	19	79	11
1×10^8	5	100	0
	C. jejuni 81-176		
1×10^6	7	100	43
2×10^8	10	100	60
2×10^9	22	100	41

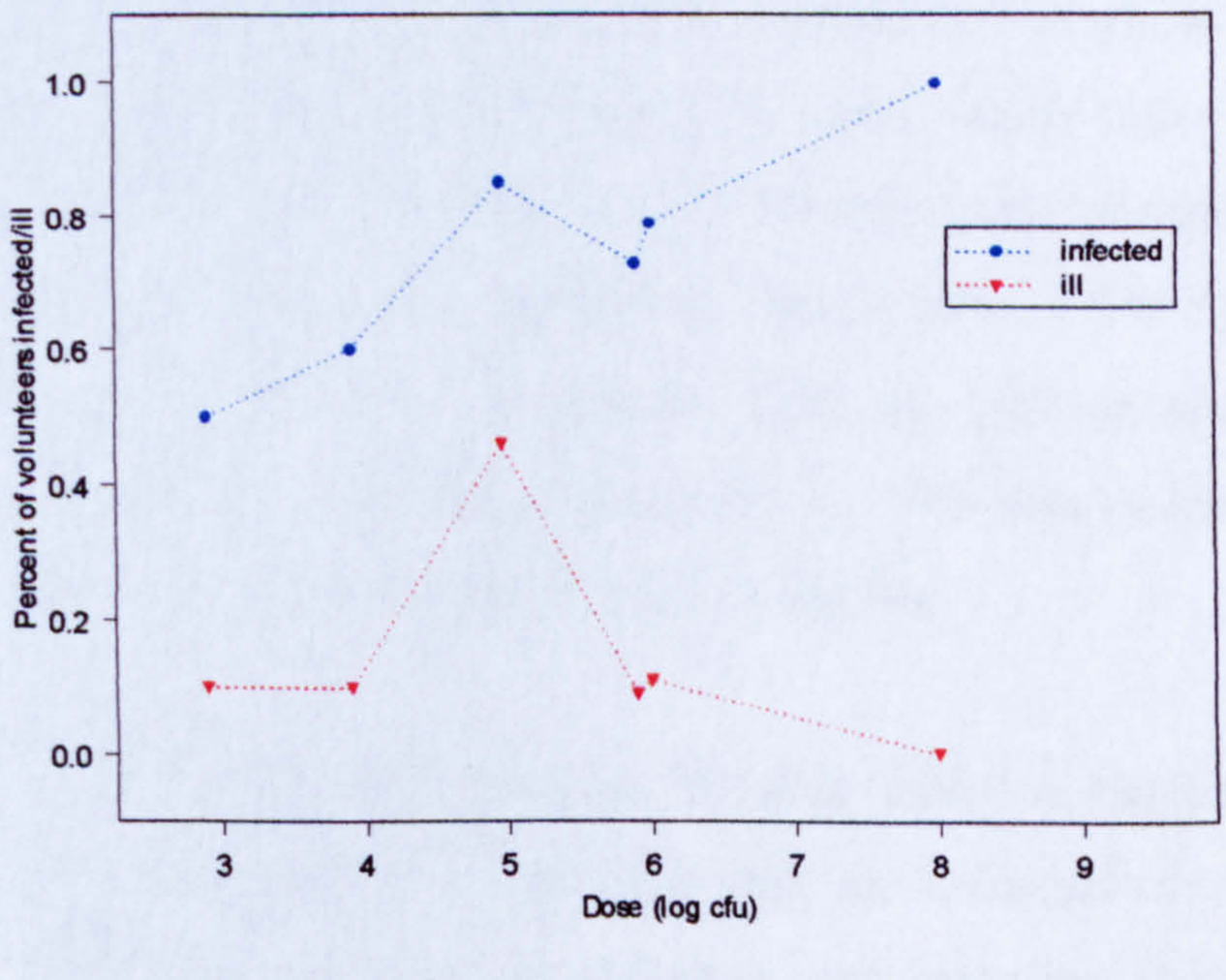


Figure 8.1: Graph showing the results of the human feeding trial with *C. jejuni* A3249

8.5.2 Estimating the probability of infection with campylobacter

When an individual is infected with campylobacter it is currently unknown whether there is a required minimum dose to initiate infection or if a single organism is sufficient. The data available by Black *et al.*, (1988) does not include low doses and therefore does not provide any insight into the validity of the single hit or threshold theory. Dose response models based upon this data appear frequently in the literature (Medema *et al.*, 1996; Teunis *et al.*, 1997). A common feature each of these is the assumption that the single hit theory applies. Further, each of these uses the Beta Poisson model to describe the probability of infection given ingestion of organisms at a defined level based upon the available data.

The Beta Poisson model

The Beta Poisson model is commonly chosen to describe the dose response relation ship for enteric pathogens. The model is semi-mechanistic in nature and as such incorporates assumptions about the infection process which takes place in an individual. As defined earlier (section 8.3.1), the basic semi-mechanistic model considers exposure to a dose of d organisms. This results in the ingestion of j organisms; of which k survive the immune defences and are able to initiate infection. This process is described by equation (8.1). The Beta Poisson model is a development of these principles, and is derived as follows.

Assume that the distribution of organisms between doses is random and hence Poisson distributed and that each organism has an independent and identical probability of surviving the immune defences and initiating infection. This probability is defined as r. As it is assumed that the single hit theory applies, $k_{\min}=1$. Therefore it can be seen that the probability of ingesting j organisms given a mean dose of d is Poisson distributed. Further, the probability that of the j

organisms ingested, k survive to initiate infection given a survival probability of r, follows a Binomial distribution. Considering this, equation (8.1) becomes

$$P_{\text{inf}} = \sum_{k=k_{\min}}^{\infty} \sum_{j=k}^{\infty} \left[\frac{d^j}{j!} e^{-d} \right] \left[\frac{j!}{k!(j-k)!} (1-r)^{j-k} r^k \right]$$

This can be re-written in terms of d and r to yield equation (8.2)

$$P_{\text{inf}} = \sum_{k=k_{\min}}^{\infty} \frac{(dr)^k e^{-dr}}{k!} \sum_{j=k}^{\infty} \frac{[d(1-r)]^{j-k}}{(j-k)!} e^{-d(1-r)} \qquad (8.2)$$

Consider the second summation of equation (8.2), that is $\sum_{j=k}^{\infty} \frac{[d(1-r)]^{j-k}}{(j-k)!} e^{-d(1-r)}$.

This has the form of a Poisson distribution, where $P(n) = \frac{e^{-\mu} \mu^n}{n!}$ and is therefore the summation of a Poisson series, that is $\sum_{n=0}^{\infty} \frac{e^{-\mu} \mu^n}{n!}$. As such, the summation is equal to unity. Hence,

$$P_{\text{inf}} = \sum_{k=k_{\min}}^{\infty} \frac{(dr)^k e^{-dr}}{k!}$$

Given that the summation of a Poisson series is equal to unity this can be re-written as

$$P_{\text{inf}} = 1 - \sum_{k=0}^{k=k_{\min}-1} \frac{(dr)^k e^{-dr}}{k!}$$

As the single-hit theory applies $k_{\min} = 1$, this simplifies to give Equation (8.3). This is referred to as the exponential dose response relationship.

$$P_{\text{inf}} = 1 - e^{-rd} \tag{8.3}$$

This model assumes a constant survival probability, however it is reasonable that given variation in host responses and pathogenicity of organisms that there may be variability in this probability. Therefore, the probability of the organisms surviving immune defences and initiating infection, that is r, is described by a probability distribution. Such a technique was first publicised by Furumoto & Mickey (1967) when considering the dose response of a tobacco mosaic leaf to tobacco mosaic virus. They recognised that there was variance amongst the cells of the leaf in the manner they responded to different samples of a given concentration of virus. To introduce such a distribution, the probability that of the number of organisms ingested (j) a given number will survive (k), $P_2(k|j)$, is described by a mixture distribution of the binomial with respect to the probability of the organisms surviving and initiating infection (r).

A mixture distribution is where a set of Poisson distributions is combined with another probability distribution to yield an alternative, discrete distribution (Haas *et al.*, 1999). The general characteristic of alternative distributions is that they provide for greater variability in the expected count among replicates of the same sample than afforded by a Poisson distribution with constant mean density. Such a distribution denoted $P_m(x;V;\beta)$ can be derived from the Poisson distribution $P_p(x:\mu V)$ where μV is the product of the mean density in a single sample and the volume of that sample. The mixture distribution is given by the following integral

$$P_m(x;V;\beta) = \int_0^\infty P_p(x:\mu:\ )h(\mu:\beta)d\mu$$

where h is the mixing distribution which is the probability density function describing the variability of the sample mean density, (μ), and β is a parameter of that distribution.

In the present situation, assuming that the variation in the system is dose-to-dose variation, and that this variation can be described by a Poisson distribution then the mixture distribution for the probability of infection, applied to equation (8.3) is given by

$$P_{\text{inf}} = \int_0^1 \left(1 - e^{-rd}\right) f(r) dr = 1 - \int_0^1 \left(e^{-rd}\right) f(r) dr$$

To describe the variation surrounding the survival probability from dose to dose or even host to host variability resulting in a variation in the magnitude of r, a commonly used distribution is the Beta distribution. Furomoto and Mickey (1967) first adopted such a technique. When the beta distribution is substituted into the above mixture distribution, this yields

$$P_{\text{inf}} = 1 - \int_0^1 \left[\frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} r^{\alpha-1} (1 - r)^{\beta-1} \right] e^{-rd} \, dr$$

The solution of this integral is presented by Furomoto and Mickey (1967), and is obtained as follows:

$$P_{\text{inf}} = 1 - \int_0^1 e^{-rd} \left[\frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} r^{\alpha-1} (1 - r)^{\beta-1} \right] dr$$

Re-writing yields

$$P_{\text{inf}} = 1 - e^{-r} \int_0^1 e^{r(1-d)} \left[\frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} r^{\alpha-1} (1 - r)^{\beta-1} \right] dr$$

Given that $e^{r(1-d)} = \sum_{j=0}^{\infty} \frac{d^j}{j!} (1 - r)^j$

$$P_{\text{inf}} = 1 - e^{-r} \sum_{j=0}^{\infty} \frac{d^j}{j!} \int_0^1 (1 - r)^j \left[\frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} r^{\alpha-1} (1 - r)^{\beta-1} \right] dr$$

which can be simplified to give

$$P_{\text{inf}} = 1 - e^{r} \sum_{j=0}^{\infty} \frac{-d^{j}}{j!} \int_{0}^{1} \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} r^{\alpha-1}(1-r)^{\beta+j-1} dr$$

This can be written in terms of the Gamma Function, where $\Gamma(x) = \int_{0}^{\infty} t^{x-1} e^{-t} dt$, as shown in equation (8.4). This is the Beta-Poisson model.

$$P_{\text{inf}} = 1 - e^{-r} \sum_{j=0}^{\infty} \frac{\Gamma(\alpha+\beta)}{\Gamma(\alpha)\Gamma(\beta)} \cdot \frac{\Gamma(\beta)\Gamma(\beta+j)}{\Gamma(\alpha+\beta+j)} \cdot \frac{d^{j}}{j!} \qquad (8.4)$$

For simplification, equation (8.4) can be expressed as a hypergeometric function. The hypergeometric function is defined as

$$F(a,b;c;x) = 1 + \frac{ab}{c}x + \sum_{j=1}^{\infty} \frac{a(a+1)b(b+1)}{c(c+1)} \cdot \frac{x^{j}}{j!}$$

Therefore the corresponding hypergeometric function for the Beta Poisson equation is given by $F(\beta;\alpha+\beta;d)$. Therefore the solution to the Beta Poisson model is given by equation (8.5).

$$P_{\text{inf}}(d) = 1 - e^{-r} F(\beta;\alpha+\beta;d) \qquad (8.5)$$

To simplify the application of the Beta Poisson model, an approximation to equation (8.5) is available (Furomoto and Mickey, 1967). The approximation is given by equation (8.6).

$$P_{\text{inf}} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha} \qquad (8.6)$$

This approximation only holds under certain conditions. More specifically, when $\beta \gg 1$, and $\alpha \ll \beta$.

Teunis and Havelaar (2000) present the application of approximation to the Beta-Poisson model (equation (8.6)) to describe the relationship between dose and probability of infection in the campylobacter jejuni feeding trial reported by Black *et al.*, (1988), outlined in section 8.5.1. In this work it is shown that the approximation provides a good fit to the data. However, this work highlighted that when trying to quantify the uncertainty associated with the predictions made by the approximation to the Beta Poisson model the results are highly dependent upon the extent to which the data, and hence parameter estimates, obey the conditions $\beta \gg 1$, and $\alpha \ll \beta$.

As previously discussed the data available from Black *et al.*, (1988) doses not include low doses and hence extrapolation is required from the model to predict the probability of infection from low doses. When the 95% confidence limit is estimated for doses less than the minimum dose present in the data set, the level of uncertainty is large. Further, consider the limiting situation, that is the probability that ingestion of 1 organism will lead to infection, $r = 1$. Hence, in this situation the probability of infection is equal to unity for any dose equal to, or greater than 1 organism. When this is compared to the 95% confidence limit of the Beta-Poisson approximation the upper bound of risk predicted from the Beta-Poisson approximation exceeds that predicted by the limiting case, that is $r = 1$. This is shown in Figure 8.2. This suggests that a fraction of an organism may cause infection. It is possible that if exposed to a fraction of an organism which contains the pathogenic mechanisms this could lead to infection, however, until more is known about the mechanisms by which campylobacters cause disease the biological plausibility of this is difficult to assess.

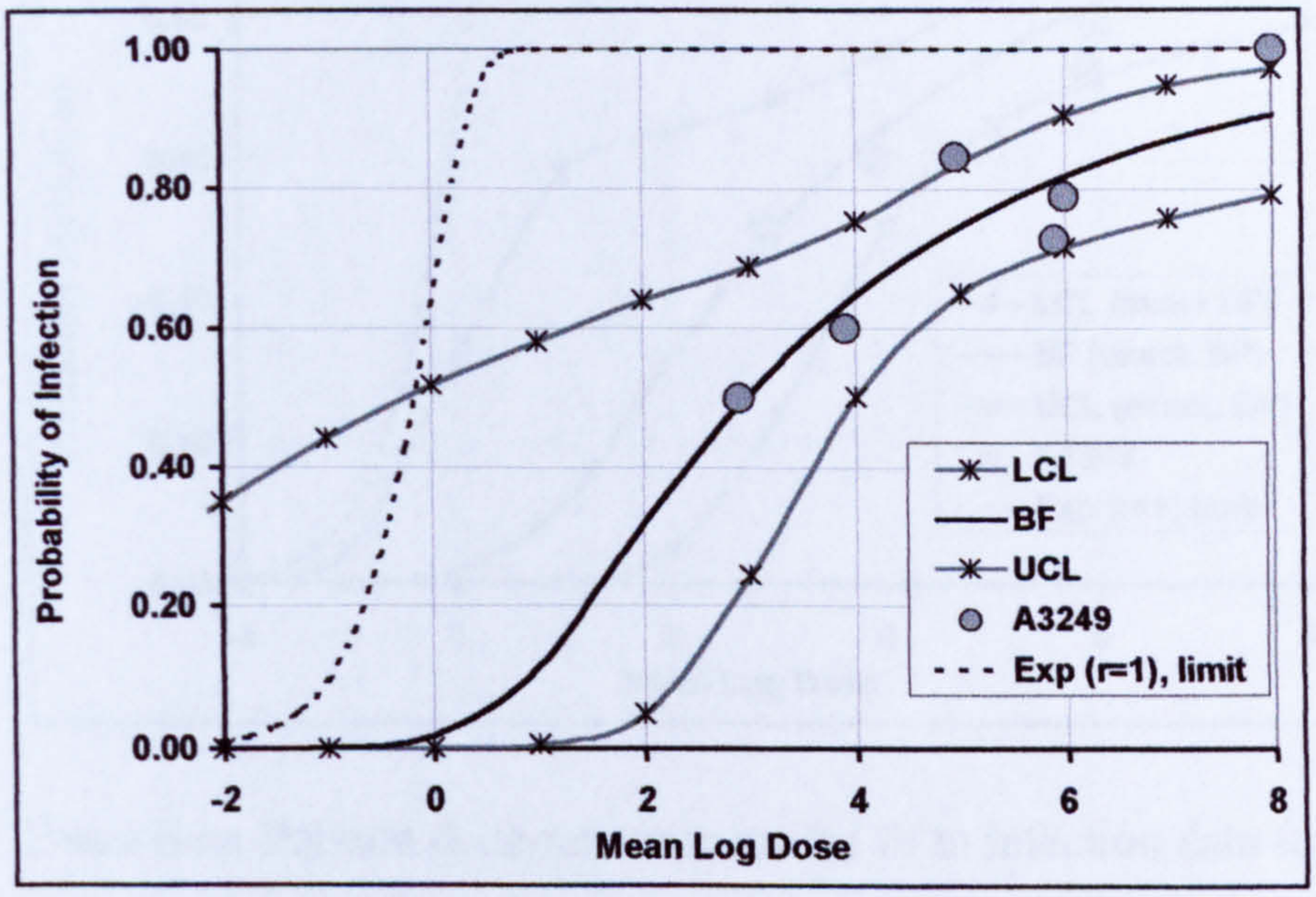


Figure 8.2: Approximate Beta-Poisson dose-response model fit to infection data for *C. jejuni* A3249, after Teunis & Havelaar (2000).

To further investigate the low dose extrapolation as predicted by the Beta-Poisson model the data from Black *et al.*, (1988) were used to parameterise the solution to the Beta-Poisson equation, that is equation (8.5). Interestingly this shows that, although the maximum likelihood parameter values are near the range where the approximate model is valid, and hence applicable, at low doses the difference in confidence intervals is dramatic. In contrast to the approximate model, the upper confidence bound on the Beta-Poisson model solution stays below the limiting case where $r = 1$. This is shown in Figure 8.3.

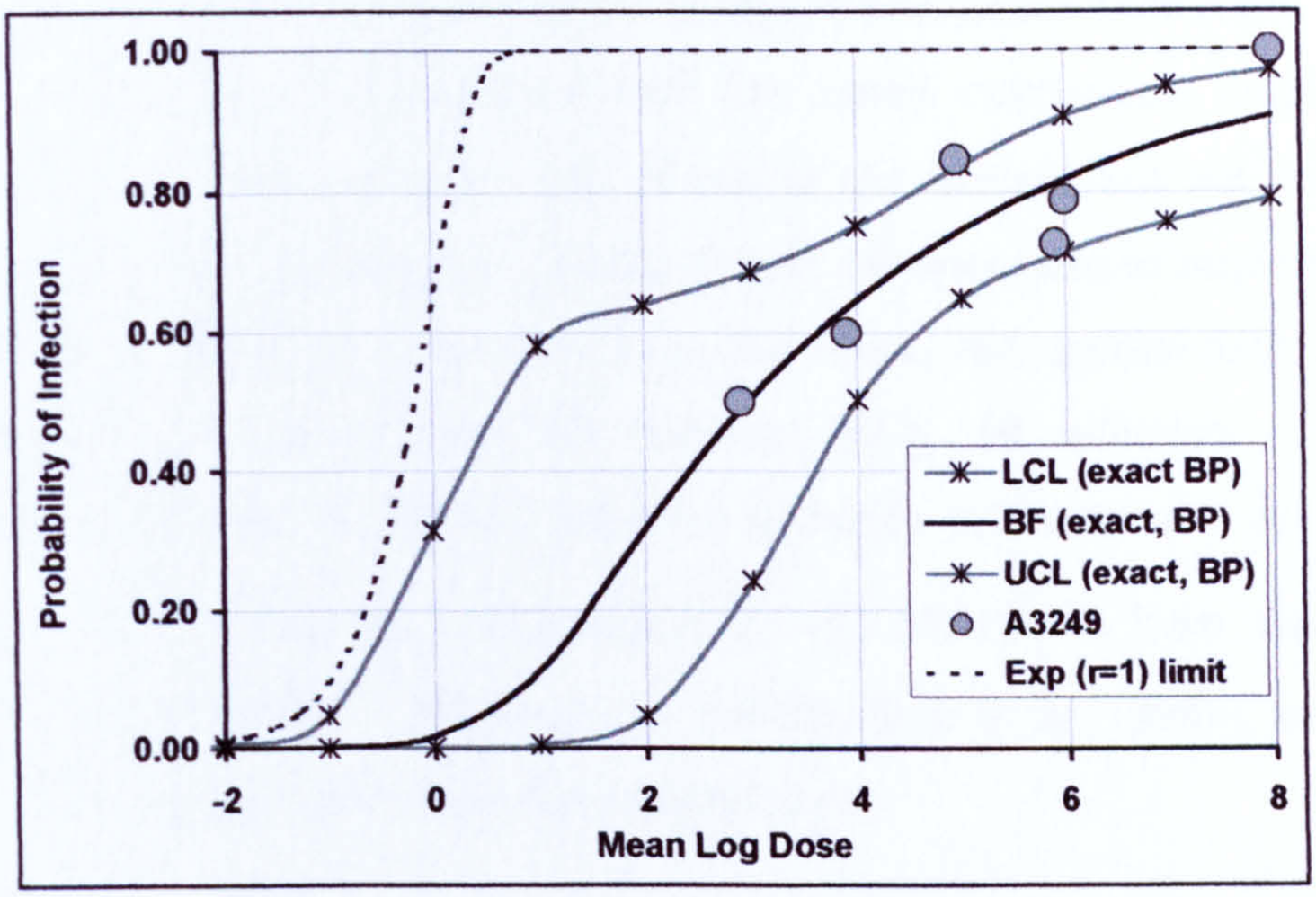


Figure 8.3: Exact Beta-Poisson dose-response model fit to infection data for *C. jejuni* A3249, after Teunis & Havelaar (2000).

The work of Teunis & Havelaar (2000) demonstrates the importance of validating the applicability of the criteria for use of the approximate model. However, given the Beta-Poisson model consists of a hypergeometric function it is more mathematical complex than the associated approximation. This makes the approximate model attractive, as it is a simple function, easier to fit to the data and more efficient to use in terms of computing time. Further, if the mean of the uncertainty in risk is the output of interest it was demonstrated that the two models are comparable.

Due to the discrepancy between the Beta-Poisson model and the approximate model at low doses it is difficult to set a criteria upon which to base a decision with regards to which model is most appropriate to use. There are no data available for the probability of infection at low doses hence neither model can be validated. The biological plausibility of the upper bound of risk of either model can be defended with the argument that only a fraction of the physiology of the bacterial cell may be necessary to induce illness in an individual. This could be refuted. It may be that

the minimum requirement for infection or illness is one, entire bacterium. Neither of these viewpoints can be discarded until the actual mechanisms employed by campylobacters inducing infection and illness in the human host are more fully elucidated. Given this the selection of model is based upon ease of implementation and computer efficiency. As such, within this work, the approximate model is selected to estimate the relationship between dose and infection. There is consistency within the scientific literature between estimates for α and β. Maximum likelihood estimates of $(\hat{\alpha},\beta)$=(0.145,7.589) have been reported by Teunis & Havelaar (2000), Medema *et al.*, (1996), Haas *et al.*, (1999), and others. These parameter estimates are therefore adopted here.

As previously stated, the approximate model determines the average probability of infection given exposure at some pre-determined level. This is based upon a set of data consisting of healthy, young males, exposed to a single strain of campylobacter jejuni. In previous chapters, the variability that exists between strains of campylobacter jejuni has been highlighted. For example in Chapter 7 the rate of decline in population size at a given temperature exhibits inter-strain variability. As such, there is variability present in both the pathogenic potential of the strain ingested by a given individual and also in the response observed by such an individual. To incorporate such variability into the approximate model a formulation was developed by Haas (1983). The formulation is

$$P_{\text{inf}} = 1-(1-\theta)^{D}$$

Here, θ is the probability of infection from ingestion of one organism, $\theta \sim \text{Beta}(\alpha,\beta)$ where the parameters α and β take the same value as applied to the approximate model, and D is the dose ingested. The result of this formulation is a variability distribution in the probability of infection for an individual for a given dose, the mean of which is equal to the response predicted by the approximate model.

8.5.3 Estimating the probability of illness

Currently, there are no models in the scientific literature that relates level of exposure to campylobacter to the probability of illness, illness being defined as the manifestation of symptoms. Commonly diarrhoea and/or fever are used as an indicator of illness in experimental feeding trials. The data presented by Black *et al.*, (1988) can be used to indicate the expected occurrence of illness. However it should be noted that these data do not allow any quantification of the severity of a predicted illness as only two outcomes were reported these being the presence of either diarrheal stools or fever.

Although limited, this data set indicates a distinct difference in the relationship between dose and illness and dose and infection. More specifically, the probability of illness changes with dose in a manner different to that of the probability of infection, it is not monotonically increasing. Rather, the relationship takes the form of a rough bell-shaped curve, as illustrated in Figure 8.1.

To establish the relationship between a given dose and the probability of illness linear interpolation is used to describe the behaviour of the function between data points assuming a linear relationship between any two data points. The interpolating function passes through the data points and is used to predict what the probability of illness would have been if observations had been made at doses between the recorded data.

It should be noted that use of an interpolation function for extrapolating beyond the recorded data set is not recommended. In contrast to regression analyses, the interpolating function may have extreme variations beyond the scope of the data. Thus interpolation is used here to predict function values between, but not beyond the recorded data points. However, given the lack of information given probability of infection at doses below 8×10^{2} an assumption is made that the function intercepts the $(0,0)$ origin.

The probability of illness, P, at a given dose, x, can be estimated from equation (8.7) by use of the empirical data:

$$P - y_i = \frac{y_{i+1} - y_i}{x_{i+1} - x_i}(x - x_i) \qquad x_i < x < x_{i+1} \quad (8.7)$$

Here, x_i is the dose below the actual dose x for which there is a recorded observation and y_i is the associated probability of infection. Similarly, x_{i+1} is the dose above, and y_{i+1} is the associated probability of illness.

8.6 Model simulation

To run the model a dose is generated based on the outputs from the model describing the preparation and cooking of a chicken meal (Chapter 7). From this dose, an estimate of the risk of infection is then calculated by use of equation (8.6). Further an estimate of the probability of illness is also given from equation (8.7). The number of iterations performed is based upon when the probability of illness is stable. That is when the running mean deviates less than 1% from the true mean, defined as the mean at 20,000. Despite this section of the model being first-order the inputs into equation (8.6) are second order and as such multiple simulations are required. The number of simulations is chosen to ensure sufficient sampling from the uncertainty space of the second order inputs.

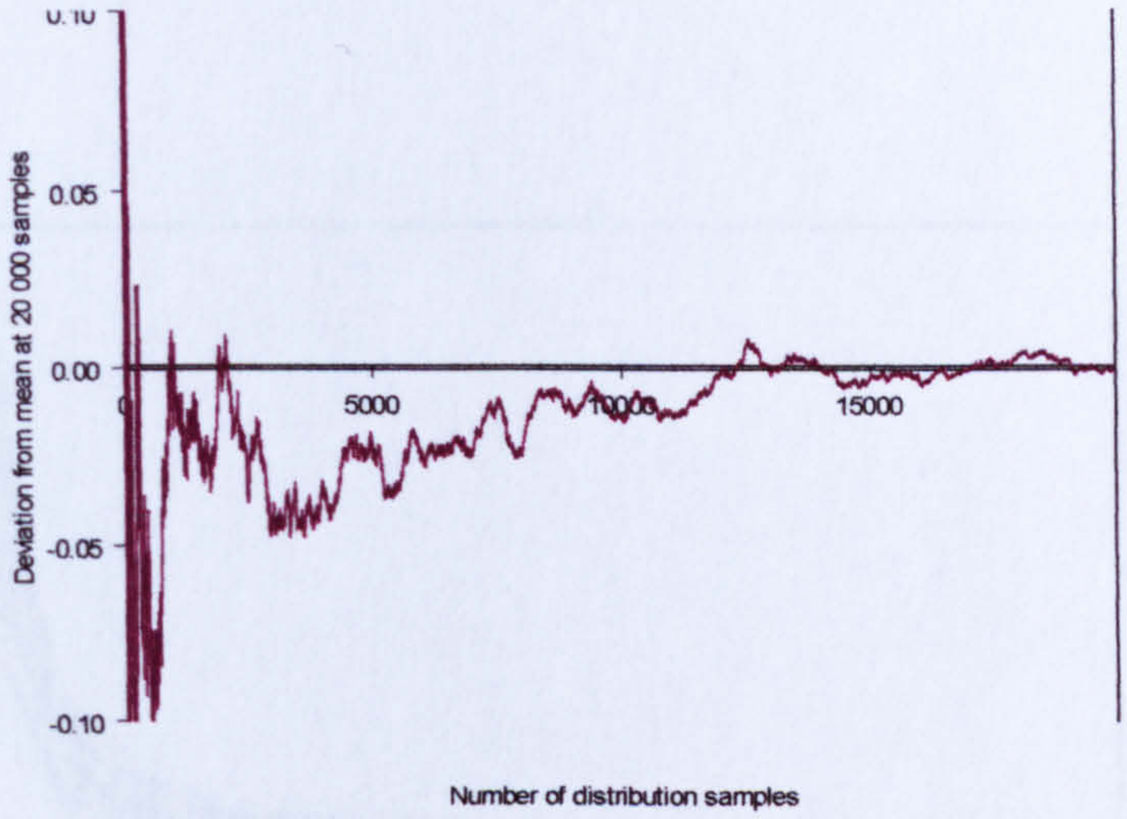


Figure 8.4: Deviation of the running mean of the risk of infection per serving from the mean at 20,000 distribution samples.

8.7 Model results

The density and cumulative distributions for the risk of infection with campylobacter following the consumption of a random chicken meal, P_{inf}, based on 50 simulations made up of 5000 distribution samples are shown in figure 8.4.

In this figure, each individual line represents one possible variability distribution for the risk of infection with campylobacter following the consumption of a random chicken meal, P_{inf}. The multiple curves and their locality, resulting from multiple simulations of the model, indicate the degree of uncertainty surrounding this variability. Considering this, the mean risk of infection has an uncertainty interval ranging from 0.04 to 0.07. That is on average, an individual consuming a chicken meal will have a risk of becoming infected with campylobacter in the range of 0.04 to 0.07. Further, the 95th percentile of this distribution ranges from 0.098 to 0.160. This indicates that 95 times out of 100 the risk per serving may be up to 0.160.

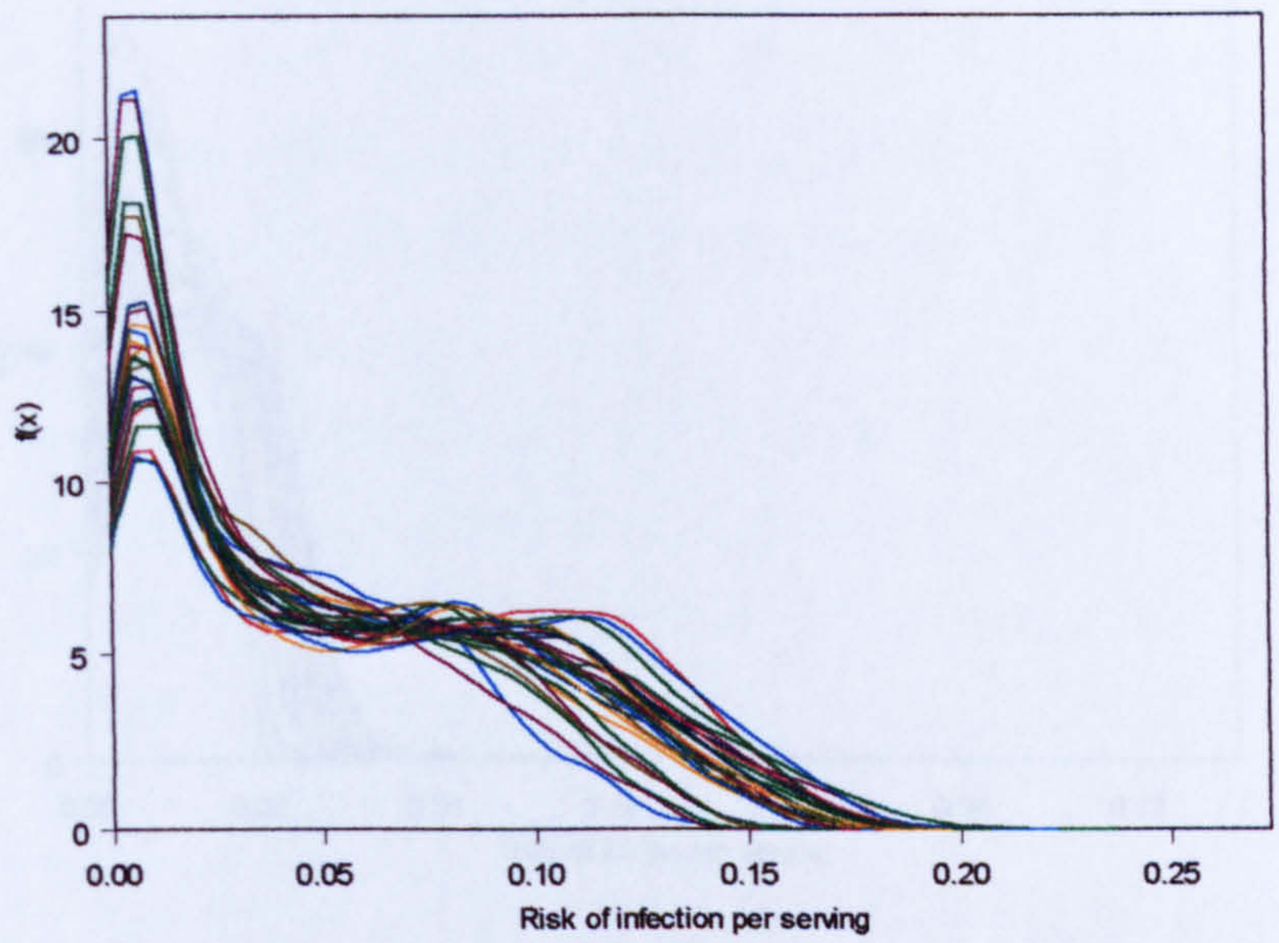


Figure 8.4: Distribution for the risk of infection with campylobacter per chicken meal.

The distribution for the risk of developing illness following the consumption of a random chicken meal is given in Figure 8.5. As for the risk of infection, this is a second order distribution and hence reflects the level of uncertainty in the model results. The mean risk of illness ranges from 0.012 to 0.019. This indicates that on average, an individual consuming a random chicken meal has a probability of developing a campylobacter related illness in the range of 0.012 to 0.019.

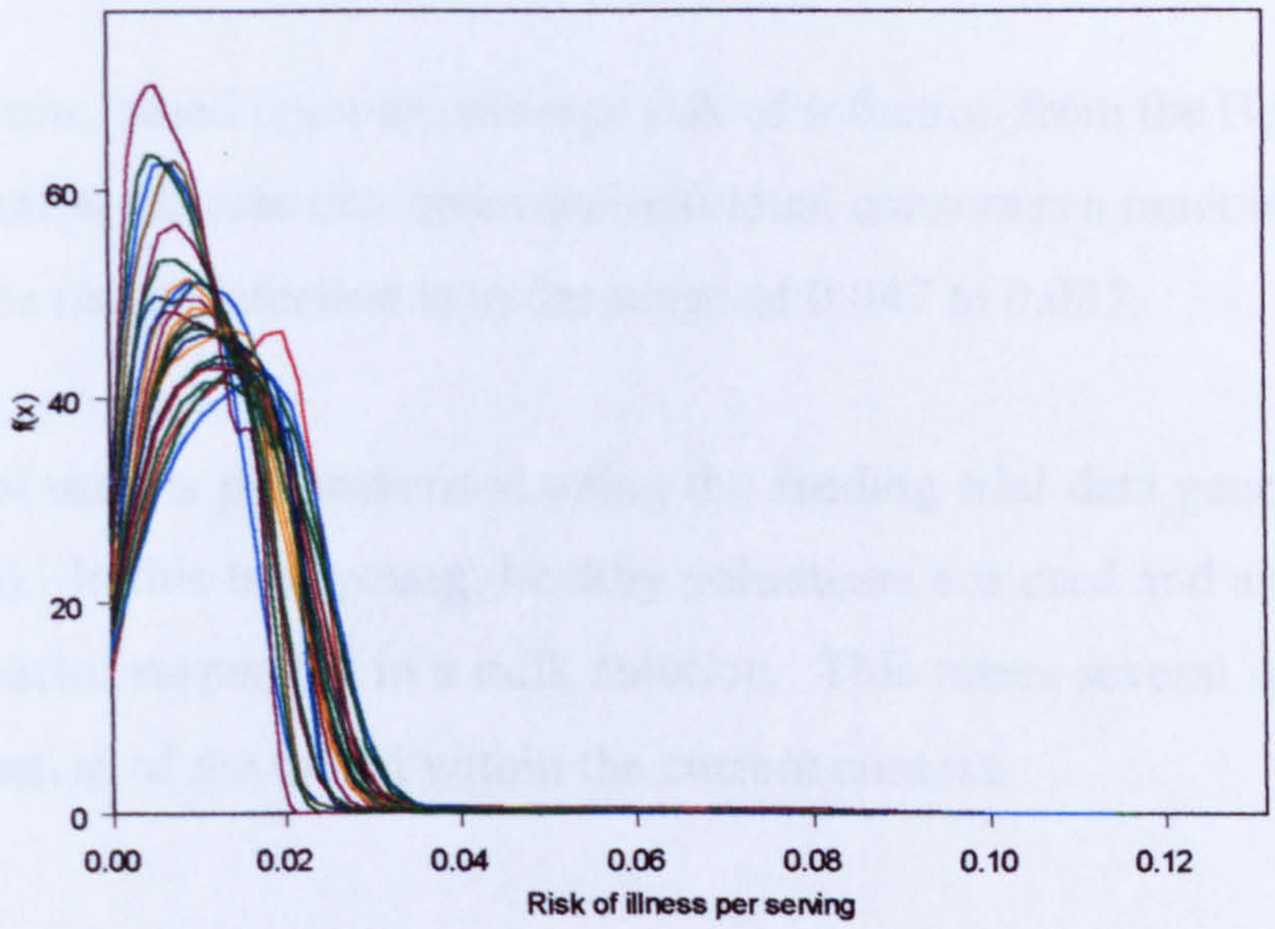


Figure 8.5: Distribution of the probability of developing a campylobacter related illness from consuming a random chicken meal.

8.8 Discussion

It has been assumed that the Beta-Poisson model describes the probability of infection given the ingestion of a dose of campylobacter. This assumption is based upon the goodness of fit of the model to data from a volunteer feeding trial *using C. jejuni* as the challenge organism. Unfortunately these data do not include challenges of doses less than 8×10^2. As such it is necessary to use the model to extrapolate the possible dose-response relationship for doses below this level. This results in a high level of uncertainty with regards to the probability of infection at doses below 8×10^2. The average value for the probability of infection as a result from the ingestion of a single organism is 0.018. That is on average approximately one out of ten exposures to a single organism will result in an infection. Further, the 95% confidence limits at this dose are 0 and 0.6, however, seventy percent of the time this probability will be less than 0.1 (Medema *et al*, 1996). As a result of the high

level of uncertainty present no uncertainty is incorporated into the parameter estimates for the approximate Beta-Poisson model.

Model results, based upon the average risk of infection from the Beta-Poisson model approximation indicate that given an individual consumes a random chicken meal on average the risk of infection is in the range of 0.047 to 0.081.

The model used is parameterised using the feeding trial data generated by Black *et al.*, (1988). In this trial young, healthy volunteers are used and are challenged with campylobacter suspended in a milk solution. This raises several issues with regards the application of the model within the current context.

The probability that a person will become infected given exposure to campylobacters is dependent upon numerous factors including the amount of food ingested, type of food ingested, immune status, gender, age, and possibly other factors such as nationality, and geographical location. The wide variability in person to person characteristics makes the description of infection of an individual selected at random by use of current dose-response models hazardous. Further, the data upon which current campylobacter dose-response models are based consists of young, healthy males, dosed with a known level of campylobacter administered in buffered milk. How representative this method of infection is compared to infection due to chicken consumption must be carefully considered. The predicted results of such models are difficult, if not impossible to validate without the implementation of large scale feeding trials, a method which has strong ethical implications.

No models are currently available in the literature that investigate the relationship between magnitude of dose of campylobacter and the probability of illness. Therefore, interpolation is used to develop a function describing the relationship between dose and the probability of illness. It is assumed that the function between any two adjacent data points is linear. The impact of the assumption of linear interpolation upon true estimates of illness is unknown. In the absence of further data points it is difficult to determine the form of the function between reported data

points cannot be determined. An option would be to assume some parabola links data points rather than a straight line. In this instance the curvature of the parabola may provide more information about the function between points than a straight line. For example, consider the form of the function for probability of illness linking a dose of zero and 1×10^2. Given biological phenomena are often reported as being represented by a smooth curve it may be appropriate to assume that this function is a parabola, monotonically increasing between zero and 1×10^2. This would result in estimates based upon linear interpolation made in this range overestimates of the risk of illness.

A further issue raised when considering the probability of illness is from examining the data (Table 8.1) it can be seen that the probability of illness would appear to have a different form to that of the probability of infection with increasing dose. It is not monotonically increasing. Without conducting more experimental work investigating the relationship between the probability of illness and dose it is not possible to validate the form of the relationship presented in the available data. To investigate the impact of assuming a monotonically increasing risk of illness the probability of illness between a dose of 9×10^4 up to a dose of 1×10^8 was set to linearly increase such that the probability of illness at a dose of 1×10^8 is equal to 1. A comparison of the results for the probability of illness with campylobacter under the two model frameworks, that is assuming the data are correct, and assuming that the risk of illness should increase with dose, has no effect upon the overall distribution of the probability of illness. This can be attributed to all the distribution of exposure. The 95th percentile of the distribution of ingested organisms ranges from 3.34 to 4.04 log cfu per serving. As such, the majority of exposures are below the point where the two model frameworks differ, that is a dose of 9×10^4.

Throughout this chapter, the uncertainty associated with developing dose-response models has been highlighted. Such uncertainties have led to the investigation of alternative methods of relating exposure to infection. A model developed by Vose (Unpublished) to consider the risk of infection with antibiotic campylobacters as a

result of chicken consumption circumvents the use of a dose-response model by assuming that the risk of infection is proportional to the probability of exposure. The proportionality factor is estimated from survey data measuring the prevalence of contaminated products at retail and relating this to the number of illnesses in the community. Such a model has limitations. More specifically, the model framework prevents the investigation of critical control points which may affect the proportionality factor. As such the applicability of a framework of this kind will be specific to the situation in question and aims of the risk assessment.

8.9 Conclusions

When an individual ingests pathogenic organisms, the potential health consequences are three fold:

* Ingestion of organisms has no consequence
* The organisms survive and colonise the individual, this process is referred to as infection, but this colonisation is non-symptomatic
* The individual becomes infected and consequently displays signs of illness

When the organism ingested is from the genus campylobacter, any resulting illness can manifest in a variety of symptoms ranging from nausea to sever pain and chronic diarrhoea. For some individuals with lessened immune response illness may be more severe. Further, infection with campylobacter may result in some sequelea which can develop a number of years following infection. Such conditions include reactive arthritis, and Guillian Barré syndrome.

To predict the impact of exposure to pathogenic organisms dose-response models are used. These models are commonly parameterised using data from human feeding trials. This has a number of difficulties when extrapolating to the general population. Specifically, human feeding trials commonly include only one gender, a small age range and all volunteers are known to be healthy. As such, they do not

represent the general population. However, there is currently no alternative available.

Dose response models commonly fall into two categories, these are empirical and mechanistic. Empirical models are chosen upon observation and experimental evidence and the type of model based upon the goodness of fit to the data and as such no biological implications can be inferred. In contrast, mechanistic models describe the processes occurring which result in infection. However, all the dose response models in common use do not fall into one of these two categories and it is more appropriate to consider the models in terms of three categories, empirical, semi-mechanistic, and mechanistic. Semi-mechanistic models are not solely empirical as some description of the biological process is involved, however, the final model form is biased by experimental evidence.

Several authors have presented dose response models for infection with campylobacter species. Common to each of these is the choice of the Beta-Poisson model. The beta-Poisson model is a semi-mechanistic model. The derivation of this model was presented in this chapter.

In this chapter, using the levels of exposure determined in Chapter 7, the risk of an individual will become infected with campylobacter given the ingestion of a serving of a chicken meal was estimated. This risk has a mean value ranging from 0.04 to 0.07, with a 95^{th} percentile from 0.098 to 0.160.

Dose response models can also be used to estimate the probability of illness occurring following infection. This is an area of research not fully exploited and there are few such models in the scientific literature. It is debatable whether there is a relationship between dose and illness with some organisms showing clear relationships and others showing no relationship at all. In this chapter, from the data of Black *et al.*, (1988) a relationship has been formulated. As a result, the risk of developing a campylobacter related illness following the consumption of a chicken product has a mean value from 0.012 to 0.019, with a 95^{th} percentile of 0.019 to 0.028. However, the data would suggest that the risk of illness takes a different

form to that of infection, that is it is not monotonically increasing with dose. Whether or not this is biologically plausible in currently unknown, and has led some authors to hypothesise that an increased dose results in a heightened immune response and hence a lower probability of illness. To investigate this, the model derived from the data was compared with a model which assumes that the probability of illness increases with dose. It was demonstrated that within the current model framework, both model produce the same result.

In summary, in this chapter a model has been presented which utilises the estimates of exposure developed in chapter 7 to predict the risk of infection, and illness with campylobacter as a result of the consumption of a chicken product produced in the UK. The model predicts that, on average, the risk of infection ranges from 0.04 to 0.07, and the risk of illness ranges from 0.012 to 0.019. Interpretation of these results is a complex task as experimental data is used to parameterise the model. The suitability of this data for extrapolation to the general population is currently unknown, and accumulation of further data to validate current assumptions should be a focus of future research.

Chapter 9

The integrated simulation model estimating the risk of campylobacter infection & model results

9.1 Introduction

In this thesis a model has been described which investigates the risk of human infection with campylobacter. The model considers all stages involved in the production of chicken products, beginning with the placement of the birds on the farm through to the consumption of a chicken meal by a random individual and any subsequent health consequences. The model is developed in a modular fashion such that each stage of the supply chain is described by a distinct model which provides inputs into the next stage. However, each of the models can be used in isolation from the models describing the rest of the supply chain hence providing a versatile product for decision-makers.

In this chapter, the integration of the models presented in Chapters 3 to 8 is discussed. The overall model results are presented and an investigation into the importance of the route of exposure is carried out. The chapter then concludes with a discussion focusing on the validation of these results.

9.2 Model implementation

The model presented in this thesis essentially describes the variability inherent in the rearing, processing, preparation and consumption of chicken products. Numerous data sets have been used to parameterise the model, many of which consist of small sample sizes. As such there is uncertainty associated with several variable parameters. The importance of separating variability and uncertainty in model frameworks has been discussed in Chapter 2. It was noted that failure to distinguish between these characteristics can lead to erroneous results. Therefore, to ensure that the variability and uncertainty remain distinct throughout the model as far as possible the uncertainty is generated using second-order modelling through the generation of non-parametric second-order distributions, as described in Chapter 2.

This results in a model which provides the variability present in the model outputs along with a visualisation of the degree of certainty associated with such results. A measure of the certainty associated with model results is invaluable when policy decisions are made upon the basis of such information.

The second-order nature of the model is such that the model can be run with different combinations of variability distributions with each combination representing one possible realisation of the infection of humans from the consumption of chicken meat. If there was no associated uncertainty there would be only one possible combination. These different realisations are mimicked by simulating the model a number of different times and the result is multiple distributions describing the variability in the number of campylobacters contaminating a product and multiple estimates of the prevalence of contaminated products at the end of processing. This allows the quantification of the level of uncertainty with respect to the outputs of the model P_{inf}, the risk of infection with campylobacter, and P_{ill}, the probability of developing a campylobacter related illness as a result of the consumption of chicken produced within the UK. As such a complete run of the model should include all possible permutations of variability distributions to represent the true extent of the uncertainty. However, due to current computer limitations this is not possible as it requires storing every sample taken on each simulation and hence is a large computer burden. Therefore, the model is run with only one combination for each possible variability distribution, with an assumption made that this adequately reflects the true level of uncertainty present

The distributions for the model parameters and the calculations described in this thesis were formulated in the simulation package @RISK (©Palisade Corp.) and the risk of human infection with campylobacter as a result of the consumption of chicken obtained as described in equation (8.6). The number of samples is chosen according to when the model output no longer deviates from the 'true mean' by ±1%

defined as the mean at 20,000 samples. This occurs at 13,000 samples and is illustrated in Figure 9.1.

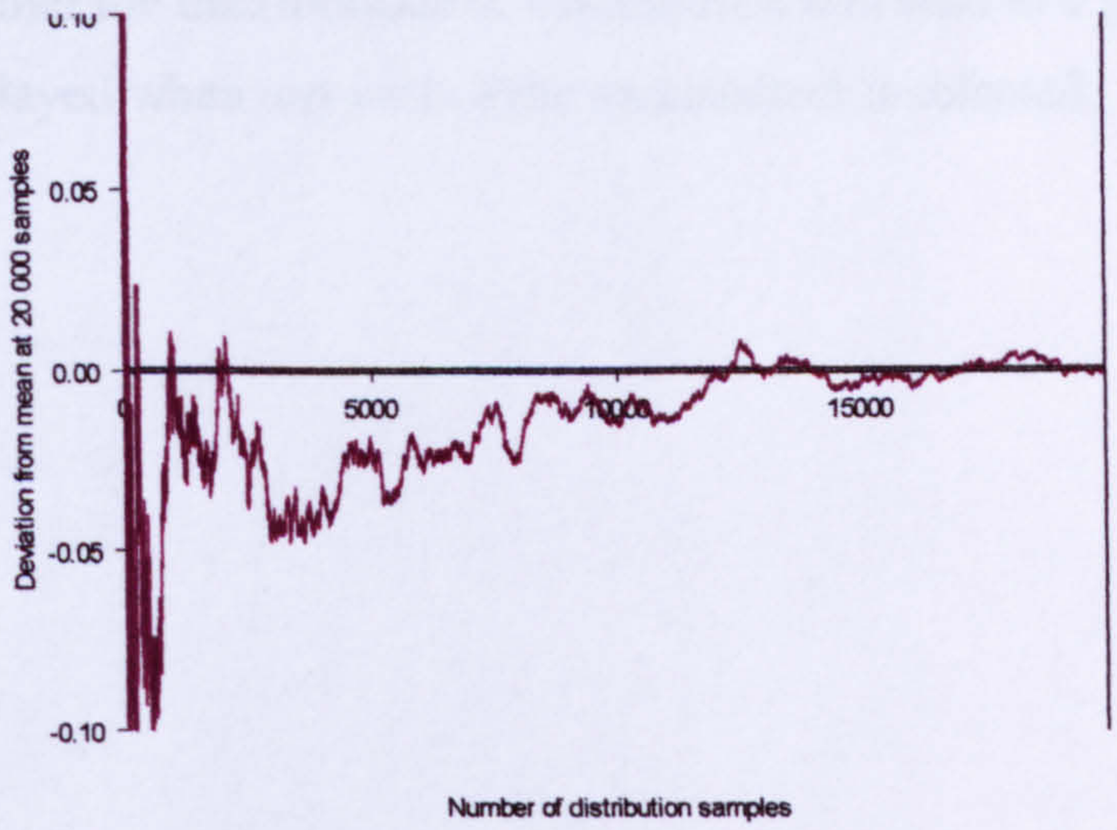


Figure 9.1: Deviation of the running mean of the risk of infection per serving from the mean at 20,000 distribution samples.

Transparency of not only model results, but also model form and parameter values is crucial to any model which is intended to be used in the policy framework. Thus enabling any decision to be made with a full understanding of not necessarily the mathematical details of the model but the processes described but the model which give the final output. Further, the impact of key assumptions on model outputs should also be recognisable.

The model described in this thesis is developed in a spreadsheet environment. As such transparency is evident in the modelling process and model parameters. This enables an individual without detailed mathematical or modelling knowledge to

grasp the concepts and processes the model addresses. For clarity, a separate spreadsheet is used to describe each of the stages of the supply chain. The spreadsheet models describing the farm level model (described in Chapter 3), the processing model (described in Chapter 6) and the preparation and consumption models (described in Chapters 7 and 8) are shown in Figures 9.2 to 9.4 respectively. For each parameter there is a brief description and the value of the parameter can easily be seen. Further the distributions or calculations that lead to a given parameter value are also displayed when any cell of the spreadsheet is selected.

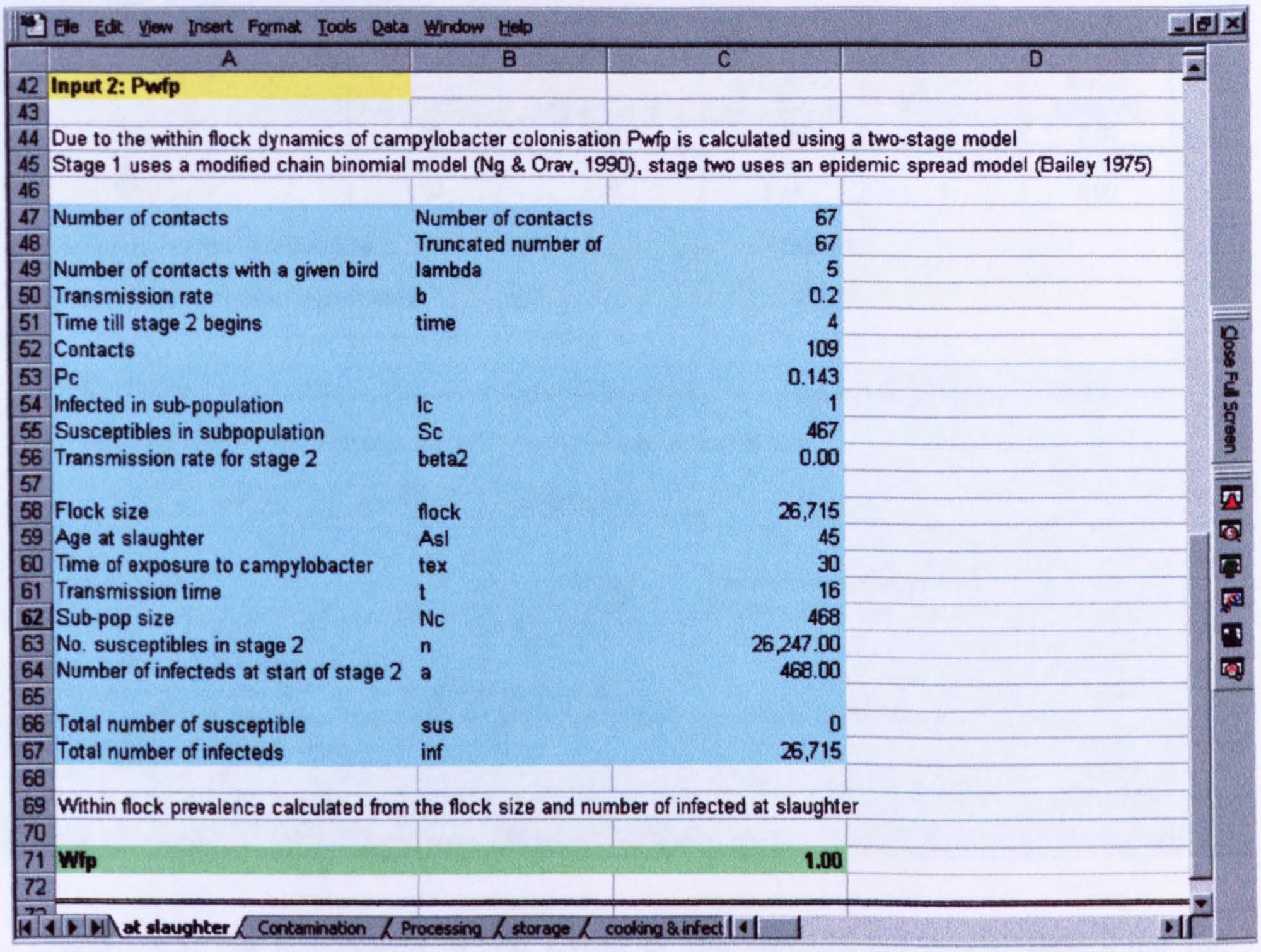


Figure 9.2: An illustration of the layout of the spreadsheet model estimating the within-flock prevalence of a positive flock at slaughter described in detail in Chapter 3.

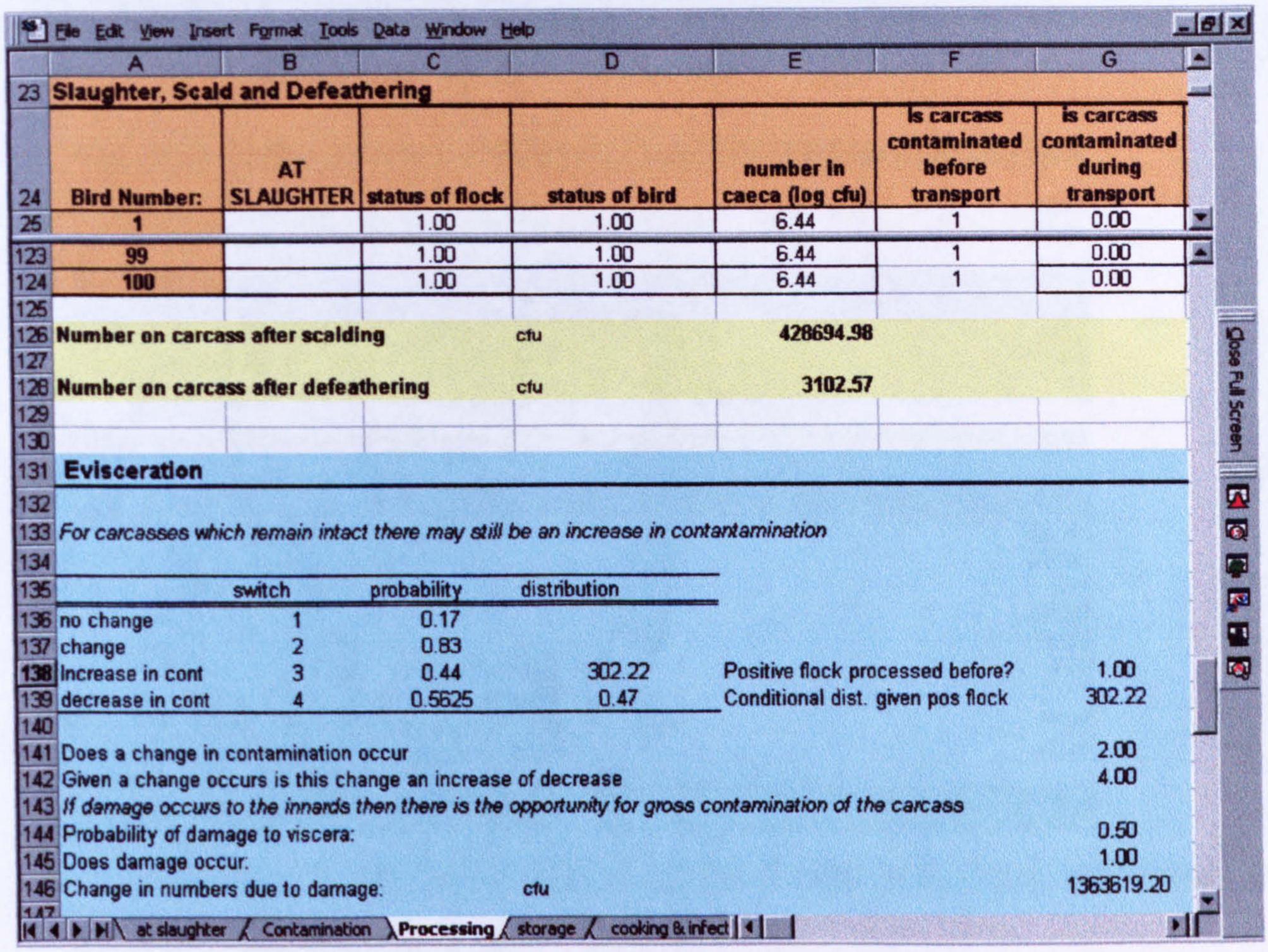


Figure 9.3: An illustration of the layout of the spreadsheet model describing the slaughter and processing of chicken. Specifically, elements of the scald, defeathering and evisceration sections are displayed. Note the allocation of a 'history' to the group of 100 birds at the start of the section and subsequent selection of a random bird from the flock, as described in Chapter 6.

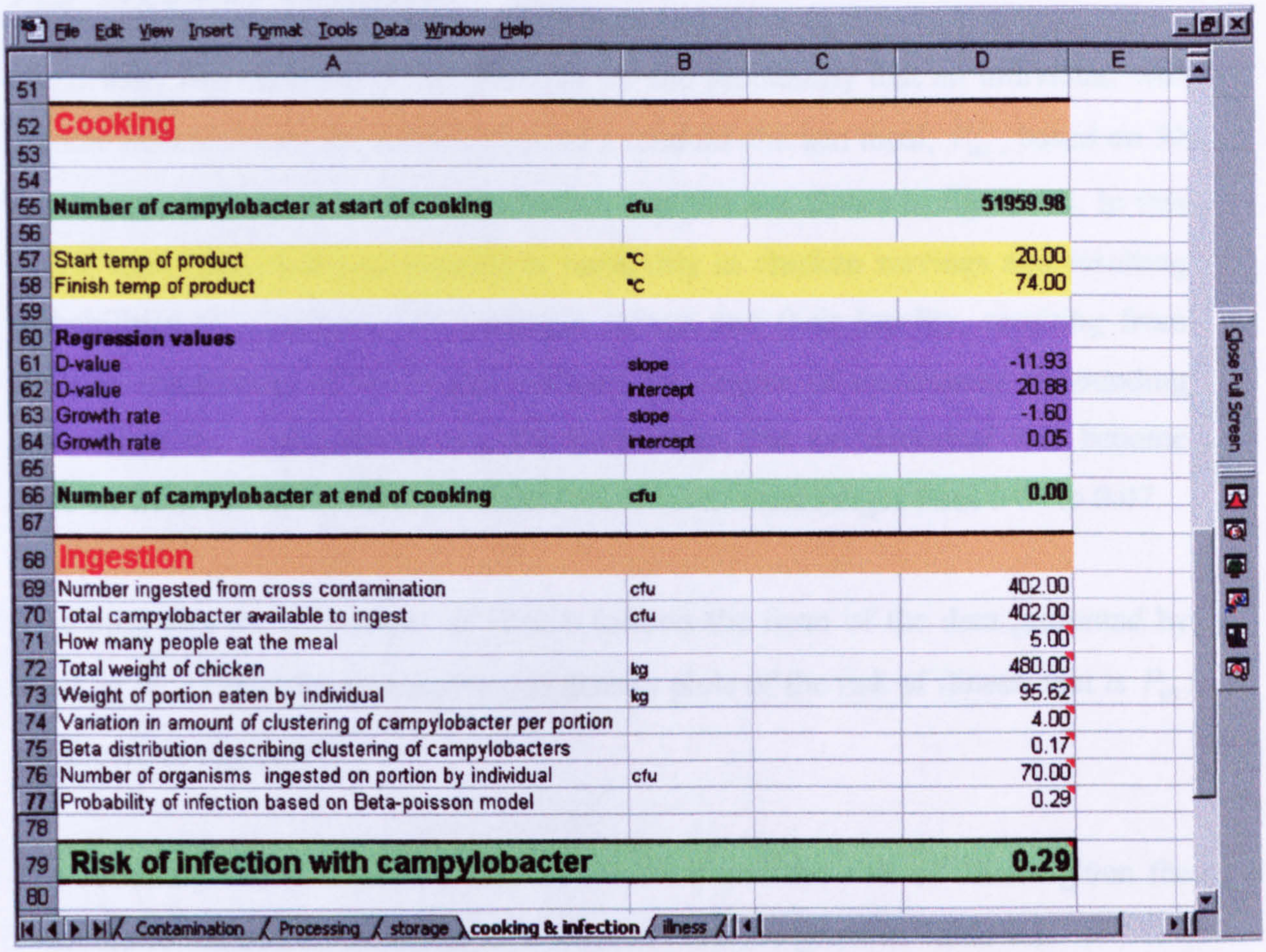


Figure 9.4: An illustration of the layout of the spreadsheet models describing the cooking, consumption and risk of infection resulting from a chicken product. For model details see Chapter 7 and 8.

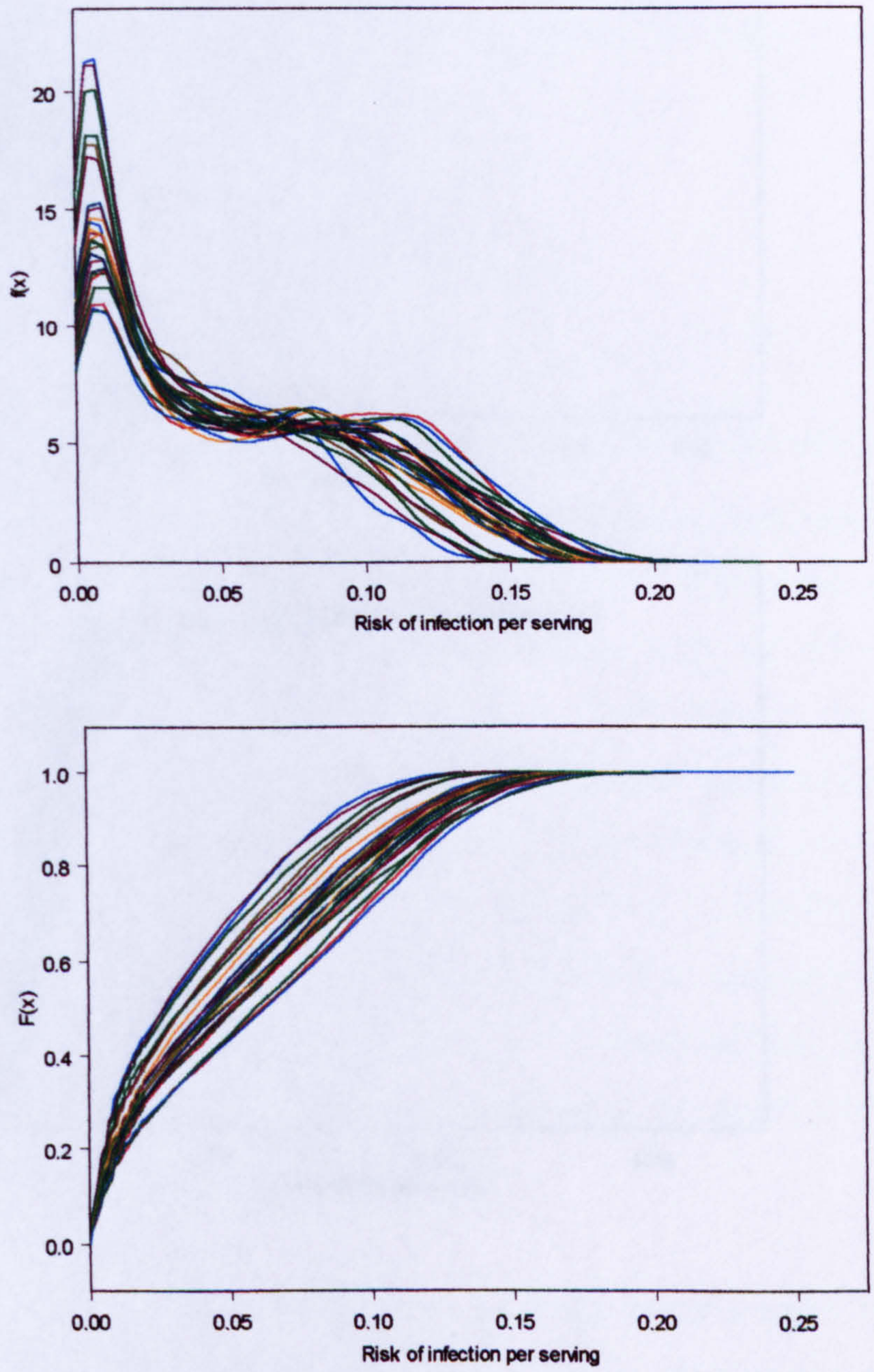


Figure 9.5: The density and cumulative second order distributions for the risk of infection with campylobacter from the consumption of a random chicken meal.

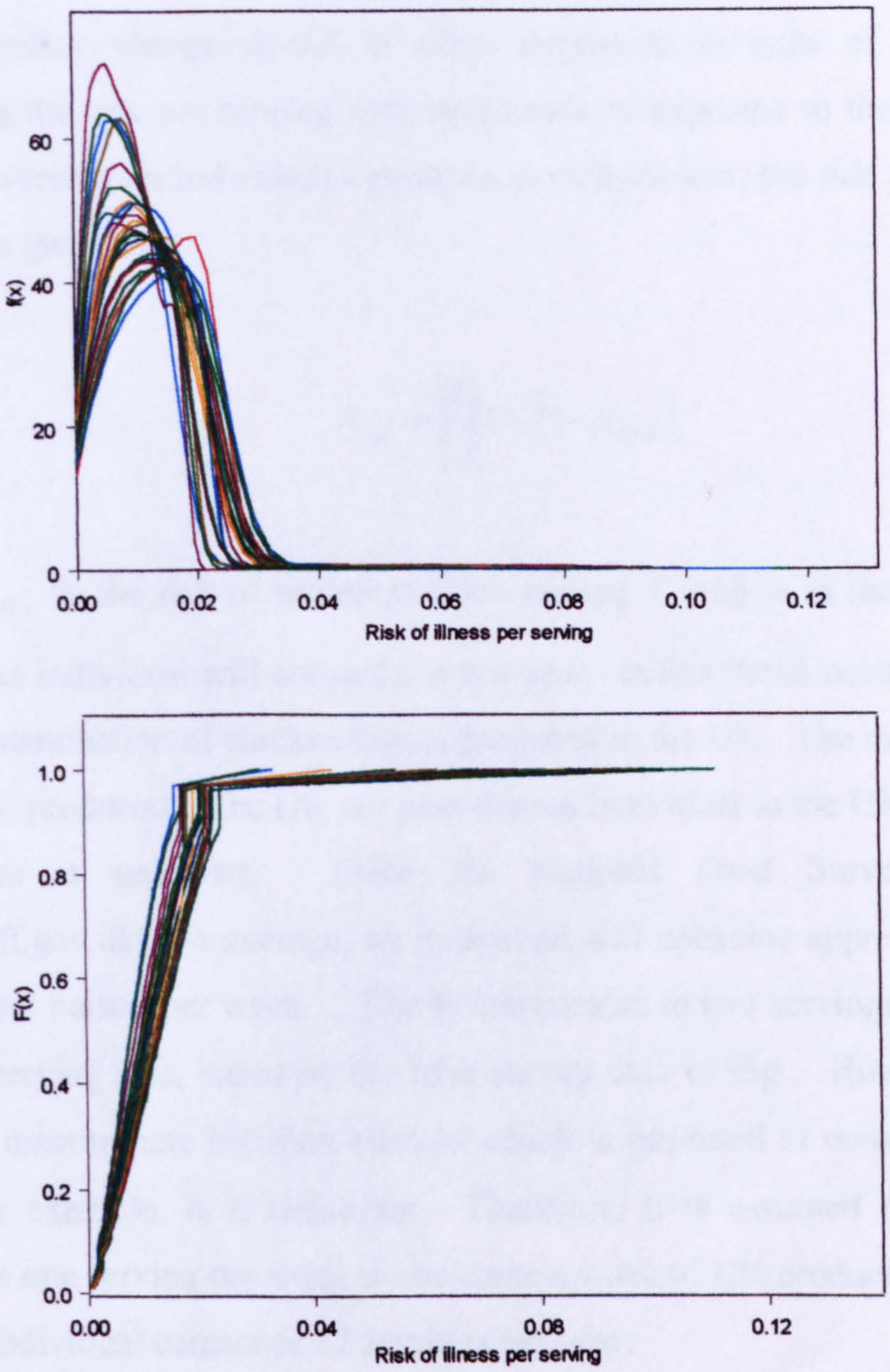


Figure 9.6: The density and cumulative second order distributions for the risk of developing a campylobacter related illness from the consumption of a chicken serving.

From a policy viewpoint risk is often expressed in units of year^{-1} and thus integrating the risk per serving with magnitude of exposure to the risk. Assuming that each serving an individual consumes is independent, the risk per year, defined as R_{py}, is given by

$$R_{py} = \prod_{i=1}^{i=n} 1 - \left[1 - R_{ps,i}\right]$$

where $R_{ps,i}$ is the risk of infection from serving i, and n is the total number of servings an individual will consume in one year. In this thesis consideration is given to the contamination of chicken that is produced in the UK. The number of servings of chicken produced in the UK per year that an individual in the UK will consume in the home is unknown. From the National Food Survey (available at www.maff.gov.uk), on average, an individual will consume approximately 200g of chicken per person per week. This is comparable to two servings per week, as the average serving size, based on the Irish survey data is 95g . However, this survey does not discriminate between chicken which is imported or consumed outside the home for example, in a restaurant. Therefore, it is assumed that an individual consumes one serving per week in the home a week of UK produced chicken, and as such an individual consumes 52 servings per year.

The distribution for the risk of infection per year with campylobacter is shown in Figure 9.7. Each line represents that variability in the risk per year that results from the variability in the processes leading to infection. This distribution has a mean value from 0.85 to 0.97.

Further, the risk of developing a campylobacter related illness has a mean value from 0.36 to 0.67. The density and cumulative graphs for the risk of illness per year are shown in Figure 9.8. The summary statistics for the risk of infection and the risk

of illness given the consumption of a random serving of a chicken meal are given in Table 9.2.

Table 9.2: Summary of model results for the risk of infection and the risk of illness with campylobacter following the consumption of a serving of a random chicken meal.

Model Output	5^{th} percentile	50^{th} percentile	95^{th} percentile
Risk of infection per year	0.79-0.95	0.86-0.97	0.91-0.99
Risk of illness per year	0.32-0.50	0.35-0.56	0.40-0.64

A striking feature of Figures 9.7 and 9.8 is the high degree of uncertainty associated with the variability in risk. This is a result of the amplification of the uncertainty present per serving, over the period of a year. The incorporation of more data to reduce the uncertainty associated with estimates of risk for a single serving would result in a reduction in the uncertainty in estimates made for a year period.

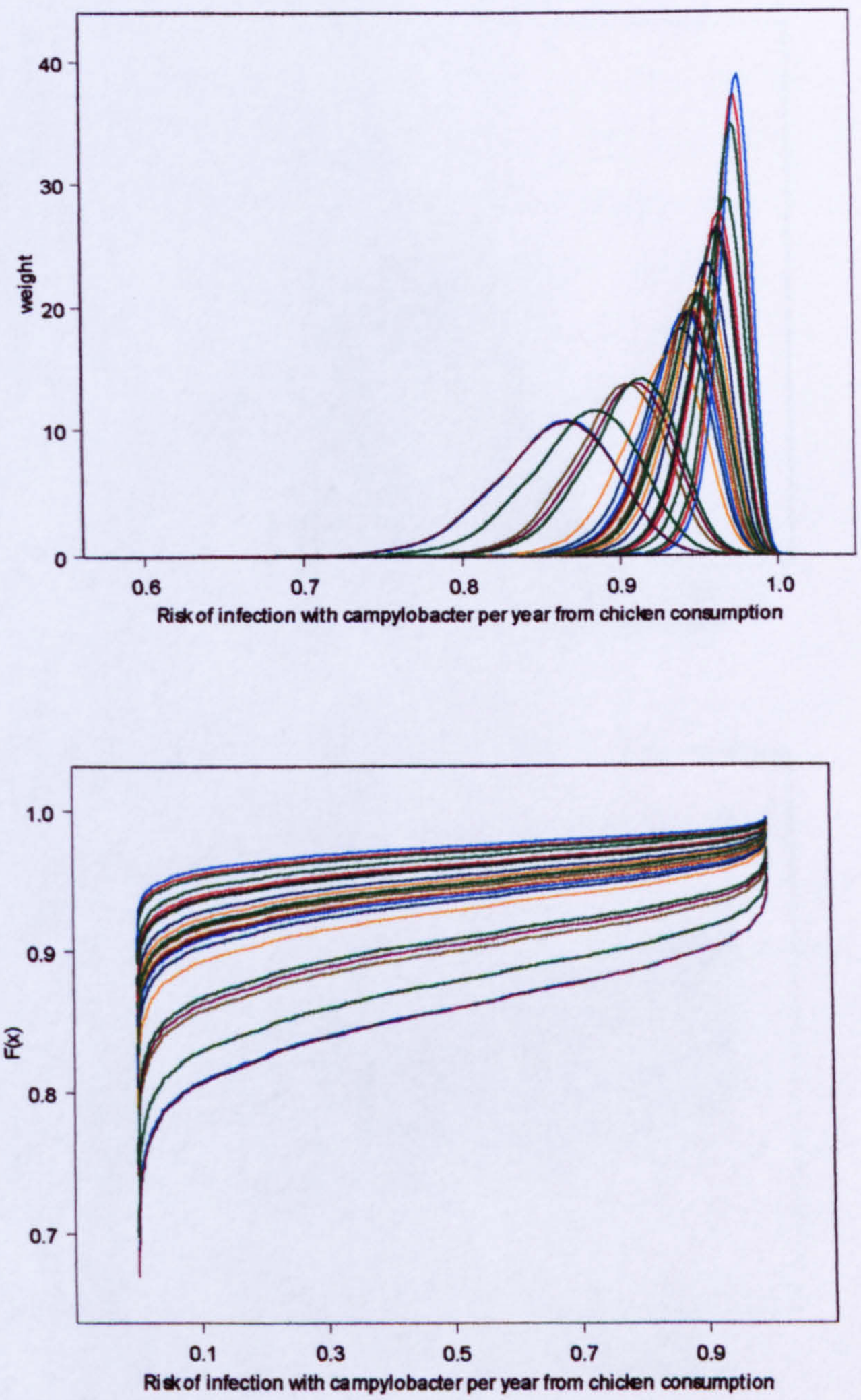
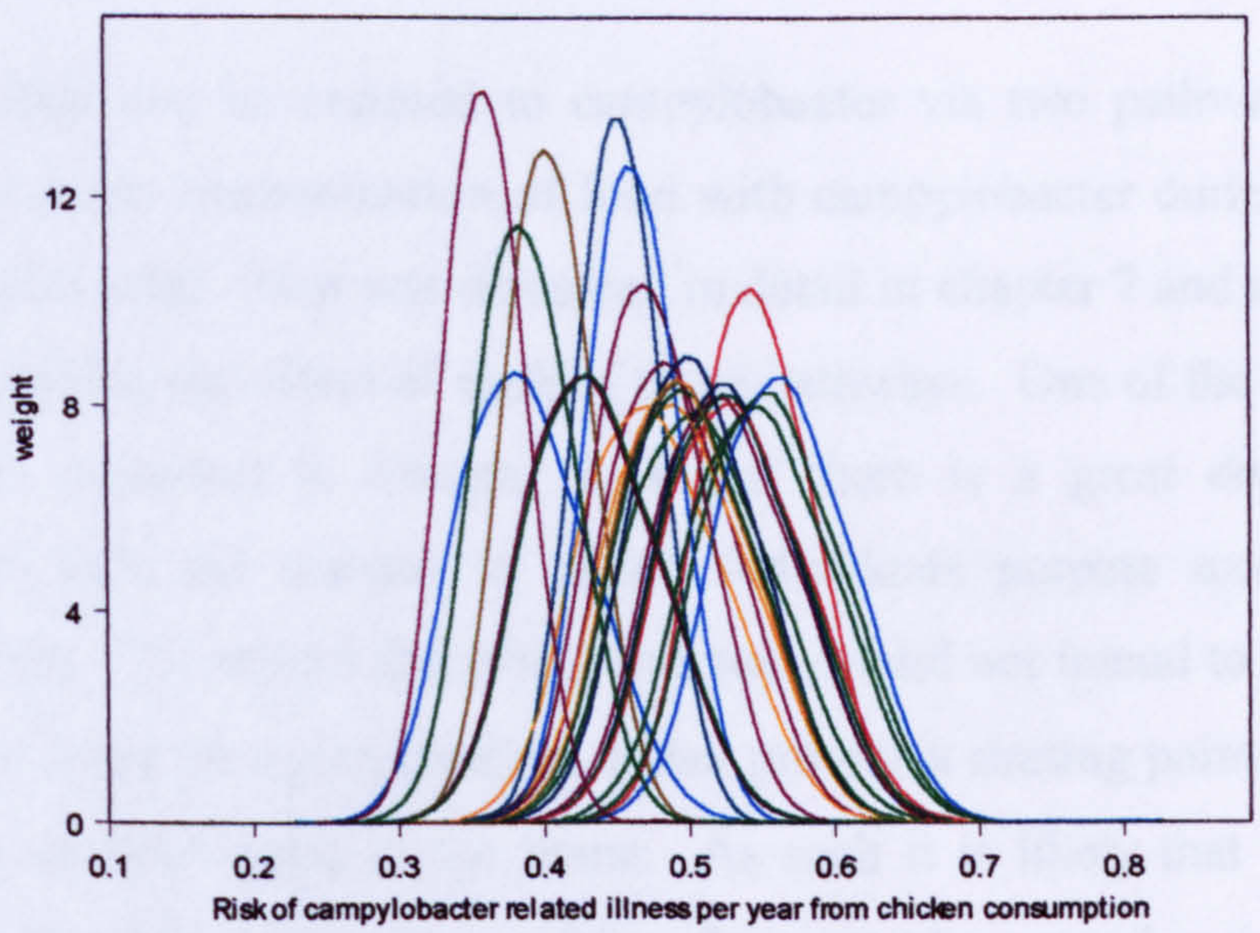


Figure 9.7: The density and cumulative second order distributions for the risk of developing a campylobacter related illness from the consumption of a chicken serving.



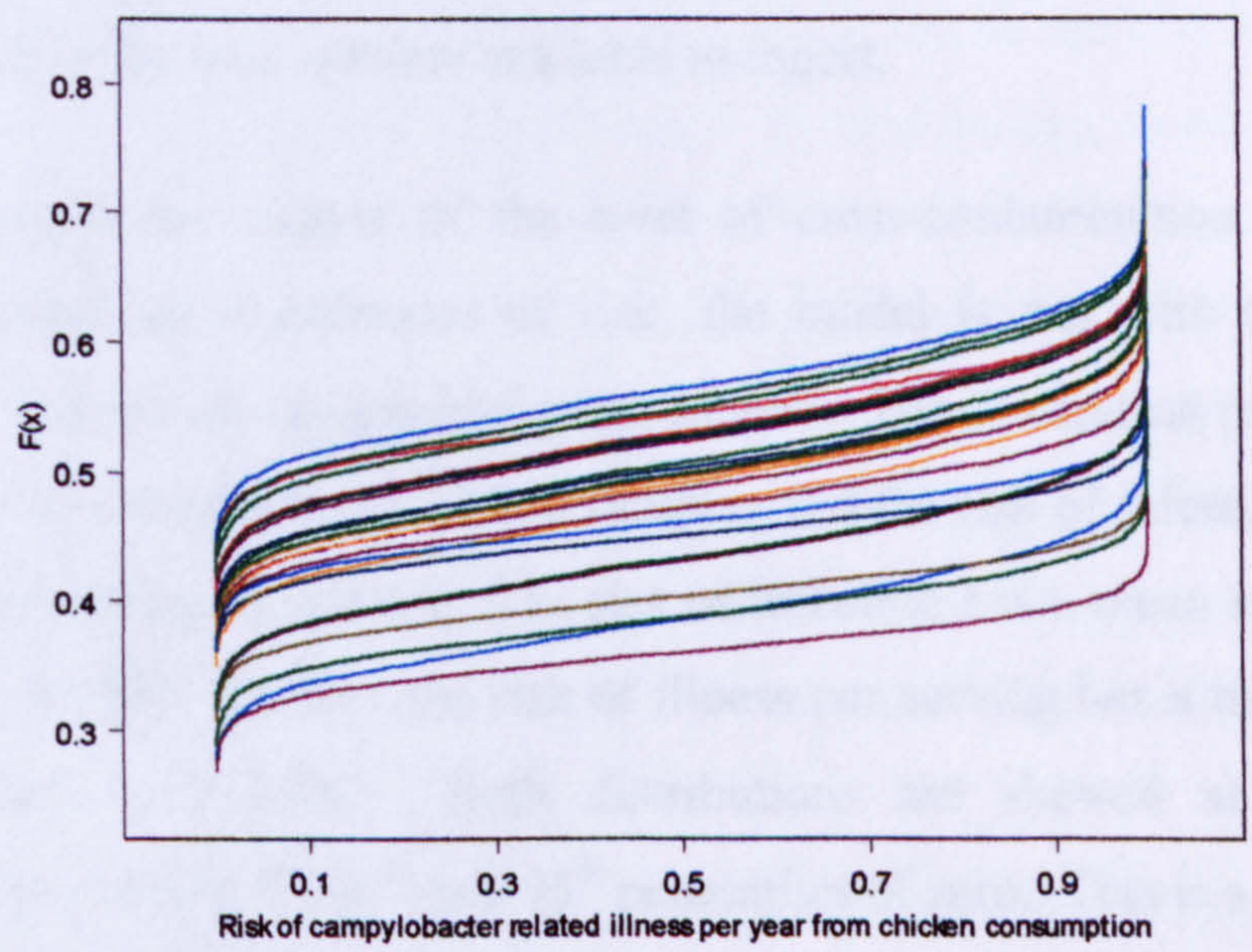


Figure 9.8: Cumulative and density plots for the risk of developing a campylobacter related illness per year for the consumption of chicken.

9.4 Sensitivity of results to exposure pathway

An individual can be exposed to campylobacter via two pathways, these are the indirect or direct contamination of food with campylobacter during the preparation of a chicken meal. This was discussed in detail in chapter 7 and a model presented which quantifies the effect of each of these pathways. One of the conclusions from the model described in Chapter 7 is that there is a great deal of uncertainty associated with the manner in which individuals prepare meals in the home environment. The model describing this section did not intend to fully describe the stages that occur during preparation, rather provide a starting point for modelling the behaviour of individuals in the home. As such it is likely that exposure via this route is over estimated by the model as there may be several actions and processes which occur before cross-contaminated organisms are ingested and this may result in a reduction in the total number available to ingest.

To investigate the impact of the level of cross-contamination estimated by the current model upon estimates of risk, the model is run with the probability of exposure from cross-contamination set to zero. For illustration purposes the model is run for 10 simulations of 13,000 samples and the risk of infection and the risk of illness per serving calculated. The risk of infection has a mean value ranging from 0.0013 to 0.0037. Further the risk of illness per serving has a mean value ranging from 0.0003 to 0.0009. Both distributions are skewed at zero, with both distributions having 5^{th}, 50^{th} and 95^{th} percentiles of zero. This is a dramatic decrease in risk from compared to the model results presented in Table 9.2, where cross-contamination is incorporated in to the model framework.

Using these results, the risk per year is then calculated as described in Section 9.3. The resulting density plots for the risk per year of infection and illness are shown in

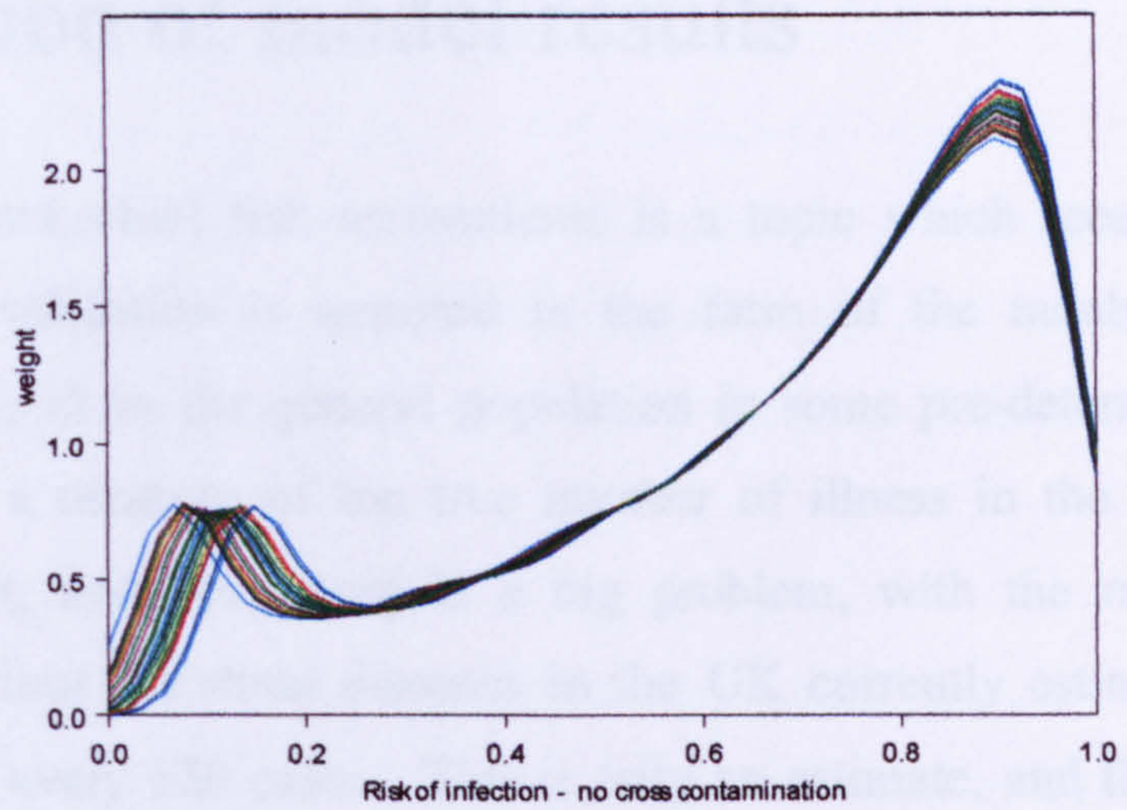
Figure 9. The risk of infection has a mean value ranging from 0.48 to 0.53, and the risk of illness has a mean value ranging from 0.02 to 0.04. The summary statistics for each of these risks are given in Table 9.3.

Table 9.3: Summary of model results for the risk of infection per year with campylobacter in the absence of cross-contamination.

Output	5^{th} percentile	50^{th} percentile	95^{th} percentile
Risk of infection	0.05 – 0.15	0.73 – 0.76	0.94 – 0.97

The impact that the model quantifying the impact of cross-contamination upon model results can clearly be seen. The current model predicts that on average an individual has a probability of 0.85 to 0.97 of developing a campylobacter infection per year. However, when only cooking is considered as the route of exposure, this is reduced, ranging from 0.65 to 0.68. Further, the uncertainty in the estimate of the risk of illness is reduced as can be seen by inspection of Figure 9.9, compared with Figure 9.7. In addition, the variability is dramatically increased, indicating that for a random individual, in the absence of cross contamination, an individual has a probability of developing an infection with campylobacter ranging from zero to one.

This clearly illustrates the importance of the acquiring good data to allow more thorough quantification of the pathways other than cooking that may lead to exposure during the preparation of a chicken meal.



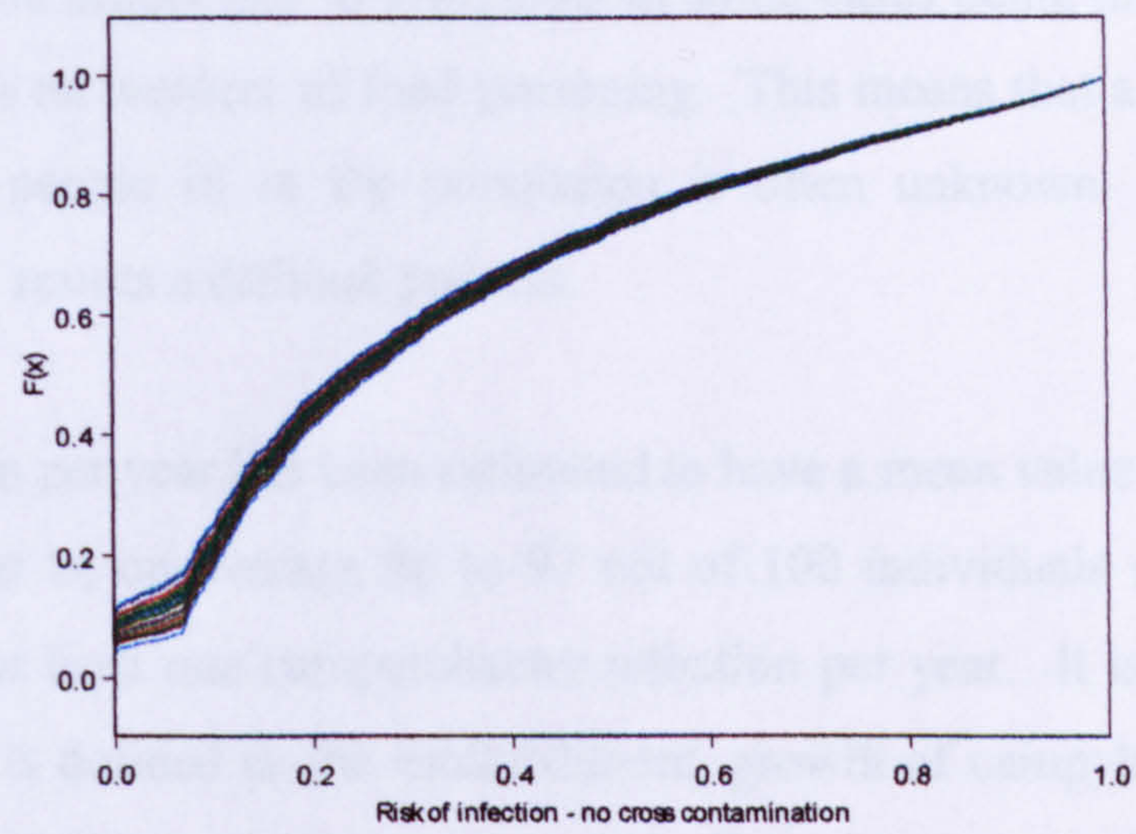


Figure 9.9: Density and cumulative plots for the risk of campylobacter infection per year in the absence of cross contamination in the kitchen.

9.5 Validation of model results

The validation of microbial risk assessments is a topic which receives a lot of attention. Often, validation is required in the form of the number of people predicted to become ill in the general population in some pre-determined unit of time. To do this, a measure of the true number of illness in the population is required. However, under-reporting is a big problem, with the rates of under reporting for infectious intestinal diseases in the UK currently estimated to be 1 illness reported for every 136 cases. This is only an estimate, and the true rate is unknown and may be higher due to symptoms in some cases being mild enough to not be recognised as an incident of food poisoning. This means that a true measure of the number of people ill in the population is often unknown. This makes validation of model results a difficult process.

The risk of infection per year has been estimated to have a mean value ranging from 0.86 to 0.97. That is, on average 86 to 97 out of 100 individuals who consume chicken will have at least one campylobacter infection per year. It is important to note that infection is defined as the establishment, growth of campylobacter in the gut of the individual but symptoms are not necessarily present. As such it is difficult to say what this measure means in terms of cases of human illness. As discussed at length in chapter 8 the connection between infection and illness is not yet evident for campylobacter related illness. As such it is difficult to validate this result.

The rate of campylobacter infection in the human population per year is currently unknown. To date there have not been any surveys which have measured infection rate. The model, results suggest high rates of infection resulting from chicken consumption, however, in the absence of survey data the validity of this result is unknown. The model describing the slaughter and processing of chicken predicted that, the prevalence of contaminated products at the end of processing ranges from

0.73 to 0.90 with a mean value of 0.80. Following storage, preparation and cooking the probability of exposure to campylobacter is estimated to be 0.21. This is exposure only from chicken products. An individual can also be exposed to campylobacter form a number of other sources such as contaminated water, raw milk, pets and wild birds. As such, it is biologically plausible that the population could have very high campylobacter infection rates, without necessarily displaying symptoms.

Once an individual is infected with campylobacter, further exposures will have no effect as the individual is already infected. Currently the model assumes that each exposure has the potential to result in an infection. A more realistic description would include details of the previous exposure to campylobacter and hence result in the generation of a campylobacter status of an individual detailing whether or not they are currently infected.

A further complication for validation is individuals can become immune to infection following exposure to campylobacter. This has been demonstrated both experimentally (Kist, 1982) and in the general population where poultry plant workers commonly get an illness in the first few weeks of employment, but rarely become ill again. However, such individuals are likely to be exposed to campylobacter on a daily basis, suggesting frequent exposure results in immunity to illness. In the current model framework, each exposure has an equal probability of resulting in infection. If individuals become immune following exposure, then the risk of infection on each exposure is a function of the frequency and magnitude of exposures which the individual has already experienced. This is not currently incorporated in the model. To include such factors in the model information detailing the manner which exposures affect the immune status of individuals would be required. Further, the time since infection and hence current immune status of an individual at the point of exposure would also be desirable. However the integration

of such information into the current framework would result in a more realistic representation of the infection process.

Given these difficulties it is likely that the current model results for rates of infection and illness are overestimates as individuals which in reality would be either already infected or immune at the point of exposure to campylobacter are becoming re-infected. As such it is necessary to validate the model at points in the model prior to exposure, for example post processing. Model results presented here estimate that the probability that a product is contaminated was estimated to range 0.73 to 0.9, with a mean value of 0.8. It has been reported from experimental studies that 80% of products at retail are contaminated with campylobacter (Corry & Atabay, 2001). It would be expected that the model would estimate higher levels of contamination than experimental studies as such results are limited by minimum detection levels, below which products may or may not be identified in laboratory investigations. However, results from experimental studies should be used with caution to validate model results. Sample sizes are very small as they require a high level of resources to carry out large scale sampling of retail products. This is a result of the cost and technical expertise required to carry out such investigations. As such, experimental investigations may not fully represent the variability in the contamination levels of products on the market.

The model presented in this thesis makes two general assumptions. First, all campylobacter are pathogenic to humans, and second, all campylobacters have the same survival characteristics. Work by Clow (cited in Newell & Wagenaar, 2000) has demonstrated that there would appear to be two populations of campylobacter which are relevant to this model. These are campylobacters which are found in chickens and campylobacters which cause disease in humans. This work has shown that these population overlap, however there are strains which are exclusive to either poultry or humans. This would indicate either not all campylobacter in chickens are capable of causing disease in humans or are unable to persist through the supply

chain and hence do not get the opportunity to cause disease. The assumption that all campylobacter are able to cause disease will result in an overestimate of the risk as some of the products which are contaminated will not be contaminated with pathogenic campylobacter and therefore carry no risk from campylobacter infection or a related illness. Further, assuming that all campylobacter are equally likely to persist through the supply chain also results in an over estimate of the risk. It may be that there are strains of campylobacter that are highly pathogenic to humans but are unable to survive the stages of processing and therefore are not associated with human disease via this route. Information detailing the pathogenic and survival characteristics of different species and strains of campylobacter would enable the current model parameters to be refined representing only campylobacter contamination of chicken products which poses a risk. As such a more realistic representation of infection and illness via the chicken supply chain would be the result.

As a result of the difficulties described above, the model results presented here should not be taken as absolute estimates of the risk of infection, or illness from the consumption of chicken. Given the current level of knowledge and data availability regarding the chicken supply chain and campylobacter it is not possible to accurately estimate this risk. However, in 2000, when dioxin contaminated supplies of chicken feed in Belgium, the sale of chicken meat and chicken products dropped dramatically (MAFF, 2001). It is yet to be seen what effect this will have on the reported rate of campylobacter related illness in the population. A similar situation has also occurred as a result of the outbreak of Foot and Mouth disease in the UK in 2001. This outbreak has led to increased consumption rates of chicken and chicken products, as well as an increase in the amount of chicken imported (MAFF, 2001). These situations could provide an indirect way to validate model results, specifically the importance of chicken as a source of campylobacter infection in the general population.

This is a common outcome of microbial risk assessments of this type and focus is beginning to turn to the notion of relative risk rather determination of an actual risk. In this way the main aim is not to quantify the absolute risk, rather a measure of risk, that is as good an estimate that is possible given all the information available. This can then be used as a baseline and the model used to investigate strategies which may result in a reduction of this risk. The most effective strategy being the one which has the greatest positive impact upon the base line estimate of risk. The use of the model presented in this thesis to identify risk reduction strategies is presented in the following chapter, Chapter 10.

9.6 Conclusions

In this chapter the models presented in Chapters 3 to 8 have been integrated to give the overall estimate of the risk of infection and illness with campylobacter as a result of consuming chicken produced in the UK. The models are developed in a spreadsheet environment and as a result are transparent regarding both model form and parameter values. This provides a useable tool for decision makers, in the absence of detailed mathematical knowledge.

The model predicts that an individual has a mean risk per serving of infection with campylobacter from 0.036 to 0.067. Further, for a random individual, the risk of developing a campylobacter related illness per serving of chicken ranges from 0.010 to 0.016. From a policy view point it is often helpful to translate this to risk per year. Consequently, for a random individual consuming UK produced chicken, the risk of infection per year ranges has a mean value from 0.856 to 0.973. The risk of developing a campylobacter related illness, per year, translates to a mean value of 0.360 to 0.567.

Individuals can be exposed, and hence develop and infection or illness via two pathways which may occur in combination of exclusively, that is exposure as a

result of cross-contamination or exposure as a result of inadequate cooking. The importance of each of these exposure routes on the estimates if risk has been investigated and results indicate that the cross-contamination route of exposure has a large influence on current estimates of risk. As such, future research should focus upon the provision and collection of data which enables this section of the model to be thoroughly quantified.

Validation of the results presented in this chapter is a complex task. Such validation requires a baseline for comparison, this should ideally be the risk of infection or illness per year in the UK population. The rate of infection in the UK is unknown, further, the true rate of illness is also unknown, with under-reporting the main obstacle in the estimation of the number of illnesses per year. As such, it is not appropriate to use current estimates of rates of illness in the population to validate current model results.

The model makes several assumptions which would suggest that the current results over estimate the risk. These assumptions were made as a result of lack of data and knowledge with regards to aspects of the infection of humans via the chicken supply chain. As such, it is not currently possible to accurately estimate the risk posed to the population in the UK from the consumption of UK produced chicken. However, current estimates can be used as a baseline, and as such, the model presented in this thesis can be used to investigate the relative impact different mitigation strategies may have upon the risk of infection and illness. Such impacts are likely to translate to the real situation.

Chapter 10

Investigation of mitigation strategies to reduce the risk to the human population

10.1 Introduction

In this thesis a risk assessment model quantifying the risk of human infection with campylobacter, consequent upon the consumption of a random chicken meal has been presented. This model considers all stages of the poultry supply chain including rearing of the birds, the slaughter and processing, preparation and cooking of a chicken meal, consumption of the meal and any subsequent illness. The model results are presented in Chapter 9. These results indicate that the presence of campylobacter in the gut of birds on the farm poses a risk to the human population.

There are several areas of data deficiency in the model framework. as a results of these deficiencies, assumptions have been made regarding the pathogenicicty and survival characteristics of any Campylobacter spp. present in the chicken supply chain. The incorporation of these assumptions makes the interpretation of the predicted risk a complex process. Further, validation of the model results is not appropriate at this time. As such, the results presented in Chapter 9 should not be taken as absolute estimates of risk, rather a baseline, which is as close to the true risk as is currently possible to estimate. This can then be used to investigate the efficacy of mitigation strategies on a relative scale, hence identifying which strategies are likley to have a the largest impact upon the risk of infection with campylobacter.

In this Chapter such an investigation is undertaken, with the aim to identify mitigation strategies which will reduce the risk of infection with campylobacter as a result of the consumption of a serving UK produced chicken. Such investigations will thus increase the current level of knowledge with respect to the management of campylobacter infection in humans. As such, recommendations can be made allowing future risk management strategies to be formulated based upon a wider knowledge base.

10.2 Investigation into potential mitigation strategies

To incorporate the heterogeneity naturally present in this system and uncertainty due to data deficiencies the model takes the form of a stochastic simulation model. The flexibility of this type of model enables it to be altered to investigate any particular scenario which may occur during the stages leading up to the ingestion of a chicken meal. In particular, there may be processes which can be modified such that a reduction in current levels of risk occur, and hence are of interest to decision and policy makers.

The predominant strategy to reduce the risk posed to the human population from campylobacter in chicken is to rid the national flock of the organisms. Research into occurrence of the colonisation of chicken flocks with campylobacter is extensive. Investigations in to the mechanisms by which flocks are exposed to, and subsequently colonised with campylobacter are on going. The hypothesis driving such research is the potential for the eradication of the organism in the live birds to remove the risk to humans, from the consumption of chicken. However, this has yet to be demonstrated.

To investigate the extent to which the national flock prevalence, P_{pf}, and the within-flock prevalence of a positive flock, P_{wfp}, influence the risk posed to the human population the model was adapted such that the value of P_{pf} and P_{wfp} varied from zero to one. All other parameters remained as described by their associated distributions. The way in which the mean probability of infection varies with P_{pf} and P_{wfp} is shown in Figure 10.1

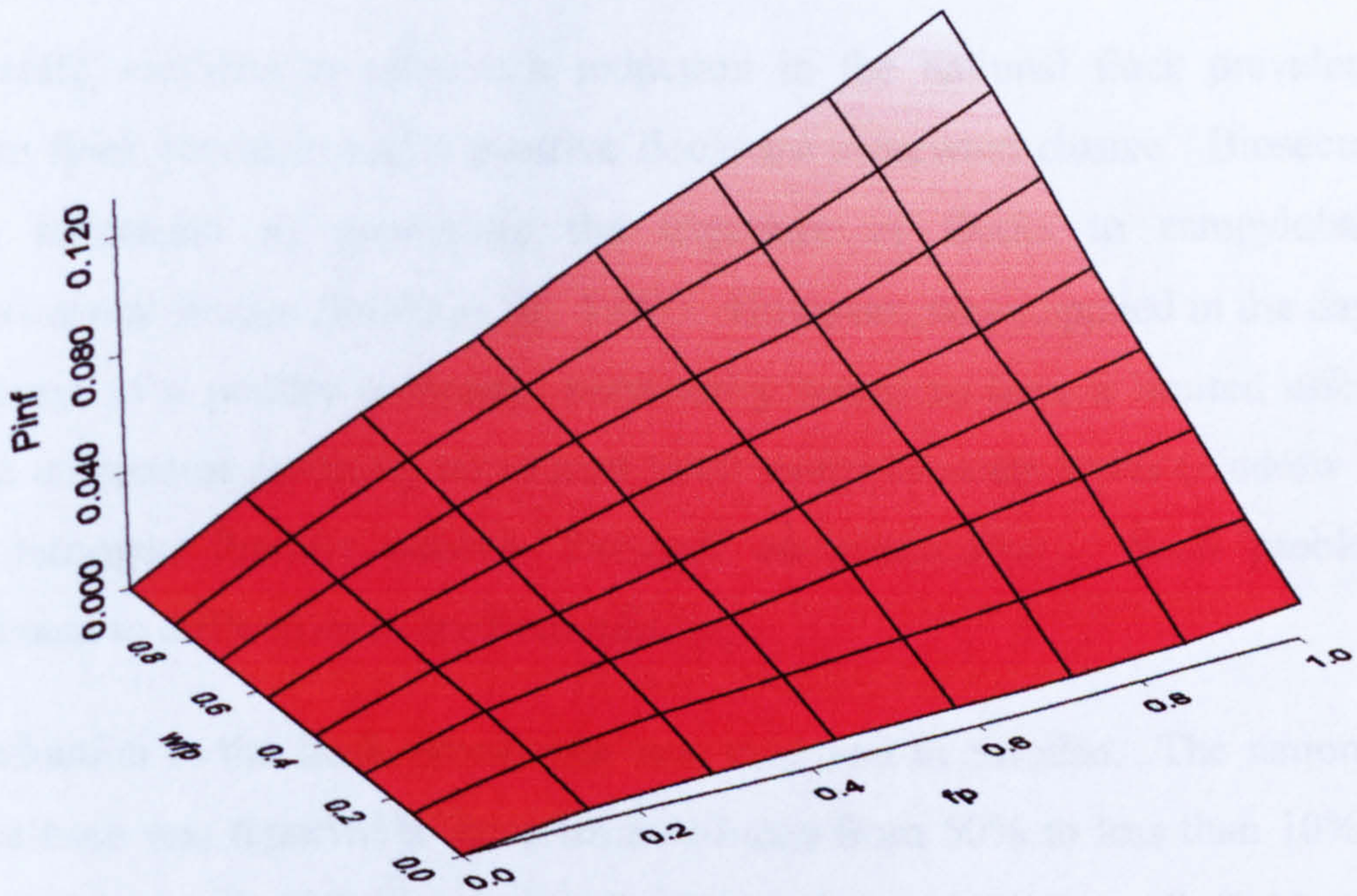


Figure 10.1: Graph showing the relationship between the national flock prevalence (FP), the within flock prevalence (WFP) and the mean of the distribution for the risk of infection with campylobacter ($P\text{inf}$).

From Figure 10.1, it can be seen that both the flock prevalence and within flock prevalence have a positive impact upon the risk of infection. More specifically, a reduction in the flock prevalence results in a reduction in the risk. Further, for a given flock prevalence, a reduction in the within-flock prevalence of the positive flocks results in a further reduction of the risk. These results indicate that a dual strategy at the farm level will have the greatest influence upon the risk posed to humans. Such a strategy should incorporate both measures to reduce the flock prevalence, such as biosecurity, and measures which reduce the degree to which a positive flock is colonised at slaughter. This may include approaches such as vaccination and competitive exclusion.

Currently, methods to achieve a reduction in the national flock prevalence and within flock prevalence of a positive flock are somewhat elusive. Biosecurity has been successful in preventing the exposure of flocks to campylobacter in experimental studies (Evans *et al.*, 1996). However, when applied in the day to day workings of a poultry company, biosecurity seems to have a limited effect, with some companies reporting no impact upon campylobacter levels (Andrew Gibson, QA manager, Premier poultry, Personal communication). Such problems are attributed to difficulties in staff compliance.

A reduction in the flock prevalence was achieved in Sweden. The national flock prevalence was reported to have been reduced from 50% to less than 10%, yet no impact upon rates of human illness has been observed (E. Engvall, Epidemiologist, Personal communication). The potential reduction in risk, achieved as a result of a reduction in the national flock prevalence and the within flock prevalence of a positive flock illustrated in Figure 10.1, assumes that the probability that a bird form a negative flock becomes contaminated, $P(Cnf)$, is given by $P(Cnf) = P_{pf}\text{Uniform}(0,1)$, for all values of P_{pf}. This assumption may not be true; however, there are currently no data available to allow the relationship between $P(Cnf)$ and P_{pf} to be determined. It is important to note that as the national flock prevalence is reduced, the contamination of negative flocks becomes a more important factor in the estimation of risk to the population and hence the validity of this assumption also increases in importance. As discussed in Chapter 3, there are currently no published data which quantify either the probability of the occurrence of the contamination of a negative bird or a negative flock, along with the extent of such contamination. As such, these data gaps complicate the estimation of the risk as the flock prevalence and within flock prevalence are reduced, and hence estimates presented here may not realistically reflect the impact on risk that a reduction in flock prevalence will have.

A further complication is as the flock prevalence increases the rate of exposure and infection in the human population will increase. It has been demonstrated that immunity results from infection (Newell & Wagenaar, 2000), hence it could be

expected that the surface shown in Figure 10.1 would flatten out, and possibly decrease, as the national flock prevalence reaches a maximum. It is possible that such a situation accounts for the lower illness rates reported in the developing world, compared to the developed world.

Thus far, it has been demonstrated that a reduction in the levels of flock prevalence and within flock prevalence can have a dramatic impact upon the risk to human infection. Research is currently ongoing to identify an efficient manner by which this reduction can be achieved. It may be that techniques to reduce either the flock prevalence or levels of flock colonisation may not be available for several years. Given the magnitude of risk that chicken consumption poses to the human population other strategies, which can be implemented currently and are more readily available would be desirable until the time which eradication of campylobacter at the source can be achieved. Therefore, the model is used to quantitatively assess the likely impact of five key mitigation strategies, taking the impact the strategy has upon the distribution of the risk of infection as the measure of comparison.

The supply chain consists of several, integrated stages. These are rearing and transport, slaughter and processing, preparation and consumption in the home and any resulting infection. Each of these stages contributes to the overall risk to the human population. As such there are numerous potential mitigation strategies available. However, it is important that any mitigation strategy investigated feasible in practical terms ensuring that all information acquired is of value to the poultry producer, consumer and policy maker. The strategies investigated are as follows:

- Reduction in probability that birds are contaminated on their exteriors at slaughter
- Reduction in the level of contamination on the exterior of birds at slaughter
- Prevention of carry-over of contamination in the house from flock to flock, identified specifically during evisceration

* Freeze all chicken prior to consumption
* Reduction in the occurrence of cross contamination in the home

Each of these will now be described.

Reduction in probability that birds are contaminated on their exteriors at slaughter

The contamination of the exterior of birds at slaughter is described in Chapter 4. The contamination of birds during transport results in an increase in the level of contamination on the exterior of the birds. This results in a magnification of the reservoir of organisms available to enter the food chain. Here, the impact upon the risk of infection resulting from a reduction in the probability that a bird becomes contaminated during transport is investigated. To measure the impact a mitigation strategy aimed at reducing the external contamination at slaughter has, the probability that a bird becomes contaminated during transport is reduced by half.

Reduction in the level of contamination on the exterior of birds at slaughter

The transportation of birds from the farm to the slaughter facility is a key stage in predicting the level of contamination on the exterior of a bird at slaughter. This is due to the stress of the process resulting in the excrement of the birds becoming more liquid in nature (Mulder, 1995). The consequence is the dispersal of excrement throughout the transport vehicle and hence the potential to contaminate the exteriors of the birds with any campylobacters that may be excreted. To investigate the impact upon risk as a result of some action which reduces the contamination level on birds at slaughter, the levels of contamination predicted by the model are reduced by half.

Prevention of carry-over of contamination between flocks on a daily basis

When a colonised or contaminated flock is processed the result is the contamination of the processing equipment. This is well recognised for several organisms present in poultry and as a result the whole processing plant is cleaned at the end of each day. It has been identified that despite this cleaning, some contamination may

persist. This therefore provides a reservoir from which birds processed on the following day can become contaminated. This factor is incorporated in the section of the model describing the evisceration process as described in Chapter 6. Specifically, if a given flock was the first flock of the day to be processed, or no positive flocks have been processed so far a reservoir of contamination is available as a direct result of the persistence of organisms from the previous day. To investigate the impact this has upon the risk of infection, the number of campylobacter which survive cleaning and hence can contaminate flocks processed on the next day is set to zero.

Within the model describing evisceration a further source of contamination is also available. This reservoir is a result of the processing the birds that day which contaminate the processing environment. It is likely that a reduction in the level of cross contamination between flocks processed on any given day would have a positive impact upon the risk to humans. This could be achieved by cleaning the equipment between the processing of individual flocks. However, given the high intensity of processing and market demands on the quantity of chicken meat produced it is unlikely that processors are able to incorporate further cleaning stages during a given processing day. As a result, this potential mitigation is not considered here.

Freezing all chicken prior to consumption

Currently, approximately 30% of all chicken is sold frozen (BPMF, 1998). However, in Chapter 7 it was shown that campylobacters are sensitive to freezing, with no campylobacter contamination detectable on artificially inoculated chicken products after four weeks of frozen storage. As such, it is possible that the freezing of all chicken prior to consumption could have a positive impact upon the risk of infection. To investigate the impact of freezing chicken prior to sale, the probability that a chicken carcass is destined for frozen sale is set to 1.

Reduce the probability of cross contamination in the home

Poor hygiene practices during the preparation of a chicken meal can result in the cross contamination of organisms and hence lead to the indirect ingestion of campylobacters. It was demonstrated in Chapter 7 that this route of exposure carries the greatest opportunity for ingestion of campylobacter compared with exposure resulting from inadequate cooking. The manner in which people behave during the preparation of a meal is poorly understood and the frequency of the occurrence of poor hygiene practices in the home is poorly quantified. However, several studies have identified that poor knowledge of and poor compliance with hygiene practices in the kitchen is not an uncommon event.

The occurrence of cross contamination in the home could be reduced through education schemes. Such schemes could include advertisements and labelling of foods providing information on how to handle foods appropriately. To investigate the potential impact a reduction of the probability of exposure may have upon the risk of infection the probability of occurrence of each of the cross contamination events considered in the model were set half their current values. The stages considered are failure to wash hands after handling the poultry, failure to wash vegetables, and failure to clean he preparation surface after contact with the chicken product as described in Chapter 7.

Running the model to investigate the mitigation strategies

To run the model all of the variability distributions which have associated uncertainty were replaced with the mean variability distribution from the uncertainty space. The result is a model which produces the average response of the simulation model described in Chapter 3 through to 8. For each mitigation the appropriate distribution is changed according to the description above and the model run until the mean of the distribution for the risk of infection reaches convergence.

The cumulative distributions for the risk of infection under the current model assumptions and each of the 5 mitigation strategies is shown in Figure 10.2. The effect of the mitigations upon the mean probability of infection and the probability

of exposure. These results are for products bought fresh or frozen and do not include products which are bought cooked, and hence are assumed to pose no risk. It can be seen clearly that freezing the chicken prior to consumption has the most dramatic effect upon the risk of infection. Freezing the chicken prior to sale reduces the probability of infection from 0.079 to 0.013. This is a reflection of the reaction in the probability of exposure from 0.32 to 0.11 and a reduction in the level of exposure due to inactivation of the organisms during freezing. The freezing of the chicken could be carried out by the producer and sufficient labelling with information on thawing practices makes this a potential option. However, currently it may be unacceptable to the consumer who preferentially purchases fresh chickens but through communication of the benefits afforded by freezing the chicken this could be overcome.

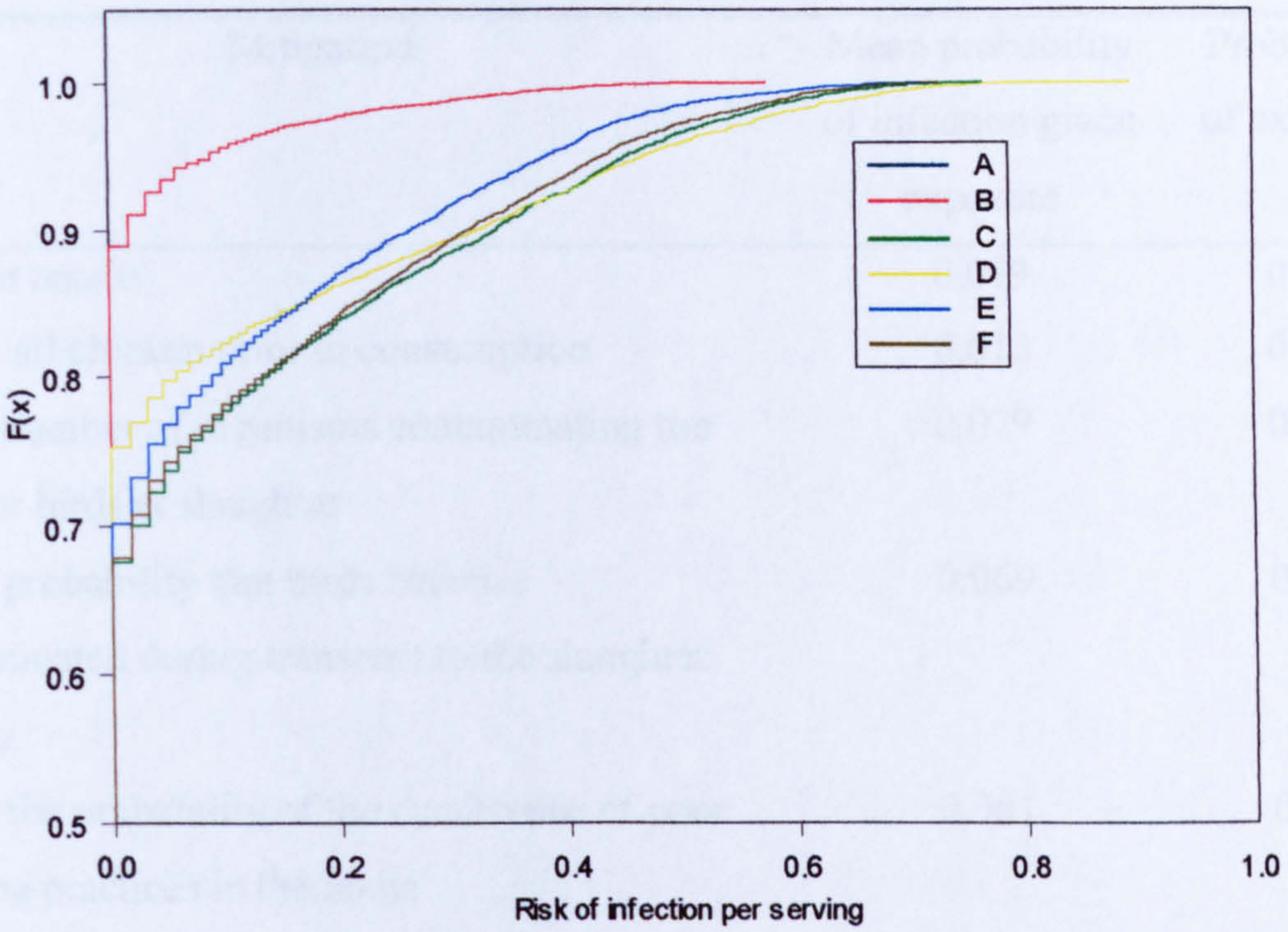


Figure 10.2: Cumulative distributions for the risk of infection per serving of chicken for the current model results (A) and the mitigation strategies: freeze all chicken prior to consumption (B), halve number of organisms contaminating the exterior birds at slaughter (C), halve probability that birds become contaminated during transport to the slaughter facility (D), halve the probability of the occurrence of poor hygiene practices in the home (E), and remove carry over of contamination in the processing plant (F).

Table 10.1: The mean probability of infection given exposure, and the probability of exposure, that is ingestion of at least one organism per serving. Results shown here do not include products which are bought cooked and hence are assumed to pose no risk.

Mitigation	Mean probability of infection given exposure	Probability of exposure
Current results	0.079	0.32
Freeze all chicken prior to consumption	0.013	0.11
Halve number of organisms contaminating the exterior birds at slaughter	0.079	0.32
Halve probability that birds become contaminated during transport to the slaughter facility	0.069	0.25
Halve the probability of the occurrence of poor hygiene practices in the home	0.061	0.29
Remove carry over of contamination in the processing plant	0.075	0.32

The next most effective mitigation strategy is a reduction of the probability of the occurrence of cross contamination events in the home during the preparation of a chicken meal. This results in a reduction of the probability of infection per serving from 0.079 to 0.061. The probability of exposure is reduced from 0.32 to 0.29. If the probability of exposure is used as the measure of effectiveness, this would not be the second most effective measure. Rather, halving the probability of contamination of the exterior of birds during transportation to the slaughter facility. For this mitigation the probability of exposure is 0.25. However the probability of infection is 0.69. This is because halving the probability of the contamination of birds during

the transport to the slaughter facility reduces the number of birds which are contaminated during processing and hence results in a reduction of the number of products at retail which are contaminated. However the levels of contamination and hence levels ingested remain the same. In contrast, a reduction in the occurrence of each of the hygiene practices will reduce the number of organisms ingested as well as the probability of exposure. However this reduction in exposure rate is less than that afforded by reducing the number of contaminated birds entering the processing plant.

A reduction in the probability of the occurrence of the hygiene practices considered in the model could be achieved though education of the public about how raw chicken should be handled and prepared. Further, labelling of foods with instructions on hygiene during preparation could also have an impact.

A reduction in the probability of the occurrence eof cross contamination during transport would require some adjustment to current transport systems preventing the vertical and horizontal spread of faeces throughout the transport vehicle. This may be a difficult goal as the transport of birds to the slaughter facility is governed by bird welfare and any strategy that may threaten bird welfare is not feasible.

Despite the impact a reduction of the occurrence of contaminated birds at slaughter has upon the mean probability of infection, a reduction in the levels of organisms contaminating these birds does not change either the probability of infection or the probability of exposure. This is due to the mixing and re-distribution of the organisms during the processing of the birds.

Interestingly, removing the carry over of contamination has no impact upon the mean risk of infection and the probability of exposure.

Given the above findings it may be that the most effective risk reduction strategy could be achieved by combining the most efficacious strategies. However, when freezing all the chicken is combined with a reduction in the probability of the

occurrence of cross contamination events in the kitchen the mean risk of infection given exposure is 0.011 and the probability of exposure is 0.12. From Table 10.1 the risk of infection given exposure when all chicken is frozen is 0.013 and the probability of exposure is 0.11. Thus, in this circumstance, the accumulation of mitigation strategies would not have a significantly increased impact upon the risk of infection, as predicted by the model. As such, the cost of the implication of multiple strategies should be compared to the magnitude in the reduction in risk such further strategies achieves prior to implementation resulting in the most efficient risk management process.

The freezing of chicken products prior to consumption has been carried out in Iceland. This action was implemented in 2001 as a result of an acute increase in the rates of illness which was seen to follow an increase in the levels of consumption of fresh chicken (D. Newell, Epidemiologist, Personal Communication). The effect of this strategy upon illness rates will be seen in the following years. As Iceland does not import any chicken products, this will provide a direct method of validating the use of the model to predict mitigation strategies.

10.2 Conclusions

In this chapter, the estimates of risk generated from the risk assessment model, presented in Chapter 9, have been used to investigate the efficacy of potential mitigation strategies. The impact of the strategy was measured against the current estimate of the average risk.

A reduction in the flock prevalence can dramatically reduce the risk of infection. However, as such reductions occur, the importance of birds which are not colonised but become contaminated by other means increases. There are several areas of data deficiency associated with the external contamination of non-colonised birds. More specifically, these deficiencies include the probability of such contamination occurring and the extent to which it occurs. Thus complicating the estimation of the impact of a reduction in the flock prevalence/within flock prevalence. In addition to

these difficulties, as discussed in Chapter 3, at present it is not clear how such reductions can practically be achieved, and maintained. As such, 5 alternative strategies were considered. These were

* Reduction in probability that birds are contaminated on their exteriors at slaughter
* Reduction in the level of contamination on the exterior of birds at slaughter
* Prevention of carry-over of contamination in the house from flock to flock, identified specifically during evisceration
* Freeze all chicken prior to consumption
* Reduction in the occurrence of cross contamination in the home

Based upon a comparison of the generated average risk to current estimates, the most effective strategy by far was freezing all chicken prior to consumption. This is due to the inactivation of the organisms that results from the freezing process as described in Chapter 7.

Then next most effective strategy is to halve the probability of the occurrence of poor hygiene practices in the home. This could be achieved by an education strategy communicating the importance of good hygiene.

A reduction in the occurrence of cross contamination during transport also impacts the risk of infection, however, animal welfare is paramount when transport systems are designed, hence achieving a reduction in the cross-contamination during transport may not be a feasible option.

Finally, Removal of carry over of contamination and a reduction in the level of contamination on birds when entering the plant do not impact the risk.

Chapter 11

Discussion

11.1 Introduction

Campylobacters were first identified as human pathogens in 1970s and since this time the breadth of scientific research focusing upon sources of human infection continues to expand. Despite such research, the number of human cases reported each year continues to increase and methods of controlling the current public health threat are elusive. One obstacle in the prevention of human illness is the major source of infection is currently unknown. Epidemiological studies have implicated chicken meat as responsible for a significant contribution of illness. However, the true extent to which chicken is responsible for the current public health threat is unknown. Throughout this thesis the infection of humans with campylobacter, consequent on the consumption of chicken meat produced in the UK, has been considered from mathematical perspective through the development of a quantitative risk assessment model.

The model is stochastic in nature, and represents uncertainty and variability through the use of Monte-Carlo simulation. The variability and uncertainty are distinct within the model framework. In Chapter 2 modelling techniques available to distinguish between variability and uncertainty were discussed and several approaches for generating both variability and uncertainty were presented. Such techniques involve the choice of either a parametric or a non-parametric approach. The model described in this thesis is essentially a non-parametric model. This decision was a result of the lack of data available for numerous parameters, and hence the implementation of parametric assumptions to describe these variables was not appropriate. Further, the lack of data availability led to uncertainty regarding the true form of the variability, hence non-parametric, second-order distributions were used. Through the use of two-stage Monte-Carlo, the variability and uncertainty remained distinct. The result is an estimate of the risk of infection to a random individual, with a description of the variability in this risk between individuals and individual servings along

with a quantitative measure of the level of certainty associated with this estimate of risk.

11.2 Key findings

The model presented here considers all stages of the chicken supply chain in a modular fashion. The modules considered are Rearing and Transport, Slaughter and Processing, Preparation and Consumption, and finally Health consequences. At each stage of the supply chain the model estimates the probability that a bird/carcass/product is colonised/contaminated with campylobacters, and the associated microbial levels. Distinct models describe each of these modules, and the key findings of each module can be summarised as follows.

Initially, the rearing and transport module is described in Chapters 3 and 4. In Chapter 3, the rearing of the birds on the farm was considered. Specifically, the probability that a bird, selected at random from the UK national flock, would be colonised with campylobacter at slaughter was estimated. This estimation involved the accumulation of farm level prevalence data and the development of a dynamic model describing the spread of campylobacter in a flock of chickens following the colonisation of a single bird. This then led to estimates of the within-flock prevalence of a chicken flock at slaughter. The combination of the flock prevalence and within-flock prevalence resulted in an estimate of the probability a bird will be colonised at slaughter. This probability has a mean value of 0.53, with a 5^{th} and 95^{th} percentile of 0.51 and 0.55 respectively (Hartnett *et al.*, 2001).

The manner in which campylobacter colonises a flock could be dependent upon the source of the organism. For example, it is likely that if a flock is colonised as a result of vertical transmission the transmission dynamics will differ compared to a flock which is colonised as a result of exposure to contaminated

feed and water. To allow for such differences, possible model modifications were presented. The frequency with which different sources and hence different modes of flock colonisation occur is currently unknown. The accumulation of such information could enable different modes of transmission to be weighted within the current framework, and hence result in a more realistic description of the colonisation process of a random flock, providing a more realistic estimate of the probability that a bird is colonised at slaughter (Hartnett *et al.*, 2001b).

The levels of colonisation and external contamination associated with a random bird at the point of slaughter are estimated in Chapter 4. Colonisation levels were generated from published data and the model predicts that a colonised bird will carry, on average, in the range of 3.7 to 5.5 cfu per gram of ceacal contents. The 95th percentile for this estimate ranges from 7.2 to 8.6 cfu per gram of ceacal contents. No such data are available to estimate the contamination level of a random bird. The contamination status of a bird at slaughter will be a function of both the contamination status of the bird at depopulation and whether or not any further contamination occurs during transport to the slaughter facility. Both of these factors are related to the location of a given bird in relation to colonised birds. This is because colonised birds will be excreting large numbers of campylobacter in their faeces. To incorporate this, a simulation model was developed which considers the location of the colonised birds in a flock at depopulation, and the location of these birds in the vehicle during transport. To achieve this, the model assumes that the colonisation process of a flock and subsequent cross-contamination during transport can be considered spatially.

Contamination of the exterior of birds is not unique to positive flocks. Inadequate crate cleaning and the process of catching can lead to the contamination of birds from negative flocks. There are no data available to estimate the level of contamination of birds from flocks which are campylobacter negative. Hence, this is an area of data deficiency. As such, assumptions were made regarding both the probability that contamination will occur, and the extent of such contamination present at slaughter. The impact of

these assumptions on the overall estimate is unknown. However, should information become available on the contamination of negative flocks, these model assumptions can be refined.

The model predicts the probability a bird will be contaminated at slaughter has a mean value of 0.83. The distribution describing the uncertainty associated with this probability ranges from 0.8 to 0.85. Given a bird is contaminated, on average the level of contamination will be in the range from 6.08 to 7.23 log cfu per bird, and the 95th percentile ranges from 8.33 to 10.62 log cfu per bird.

The slaughter and processing of chicken was approached in Chapters 5 and 6. It is well recognised that poultry processing has numerous microbiological implications. However, the impact of processing upon the level of contamination of a processed product is specific to the organism under consideration. To identify the stages of processing which are most likely to influence the campylobacter contamination of a product at the end of processing a qualitative assessment was carried out in Chapter 5. This assessment identified scald, de-feathering, evisceration, wash and chill as stages which will contribute when determining the campylobacter contamination status of a product post-processing.

The effect of scald, de-feathering, evisceration, wash and chill upon the contamination status of a product post processing was quantified via the development of a simulation model describing each of these stages, presented in Chapter 6. Each stage of processing was described separately. After each of these stages, the number of campylobacter contaminating a carcass was re-evaluated, resulting in an estimate of the level of campylobacter contaminating a carcass post processing. This estimate was then used to generate the probability that a random carcass will be contaminated at the end of processing (Hartnett *et al.*, 2001b). This model assumed that all *campylobacter* spp. behave in the same manner during processing. Should more information become available, the effects of processing on different strains present on a carcass can be modelled

and therefore more realistic estimates of the likelihood and magnitude of contaminated products post processing would be produced. The number of campylobacter on a contaminated product was estimated, on average, to be in the range of 5.43 to 6.28 log cfu per product, with a 95th percentile ranging from 5.96 to 6.65. Consequently, the probability that a product will be contaminated post processing was estimated to have a mean value of 0.8, with an uncertainty interval ranging from 0.73 to 0.9.

Following processing, the storage, preparation and cooking of a serving of a chicken meal were investigated in Chapter 7. This chapter described models for cross contamination during preparation of a meal. Development of this model recognised that this is an area of data deficiency, with very few studies focussing on the behaviour of an individual during the preparation of a chicken meal. As such there is a high degree of model uncertainty associated with this section of the risk assessment. From Chapter 7, the probability that an individual will ingest at least one campylobacter as a result of a single serving of chicken ranges from 0.18 to 0.26, with a mean value of 0.21. Further, the level of exposure is, on average in the range of 2.4 to 3.3 log cfu per serving, with a 95th percentile from 3.02 to 3.71 log cfu. Comparisons of the risk of exposure posed from inadequate hygiene during preparation and inadequate cooking indicate that the greatest opportunity for the ingestion of organisms is presented as a result of poor hygiene during preparation. As such, it is crucial that future research focuses on providing data to enable a thorough description of the behaviour of an individual during preparation of a chicken meal. Such information will allow a more realistic description of the processes leading to exposure and hence allow more realistic estimates of risk to be formulated.

Estimates of exposure were used in Chapter 8 to predict the risk of infection following the consumption of a chicken meal. Exposure estimates were also used to estimate the risk of developing a campylobacter-related illness. Dose-response modelling has been used by several authors to describe the risk of infection following exposure at a pre-determined level. In each case the Beta-

Poisson model is used to relate the risk of infection to dose ingested. This model is selected based upon the goodness of fit to human feeding trial data (Black *et al.*, 1988). This data involves a small sample size and healthy, young males. As such this data is not a representative sample of the population. Unfortunately, no alternative data are currently available; hence this is an area of data deficiency.

The estimation of the probability of illness in relation to dose is not well researched. Currently there are no models which estimate the risk of illness following consumption of a pre-defined number of campylobacter. As such, estimation as carried out through the use of an empirical model based upon the data of Black *et al.* (1988). As with infection, the data for rates of illness is not representative of the population. The model presented in Chapter 8 does not consider infection and illness in susceptible populations. The rate of infection and illness in such populations is an area of data deficiency. However, since illness in susceptible population is commonly associated with increased morbidity this is an area that future research should not ignore. The risk of infection with campylobacter associated with the consumption of a single serving of chicken has a mean value ranging from 0.04 to 0.07 with a 95^{th} percentile ranging from 0.098 to 0.16. Further, the risk of developing a campylobacter related illness is, on average, in the range from 0.012 to 0.019, with a 95^{th} percentile from 0.019 to 0.028.

11.3 Validation of the risk of infection

Validation of these results is a complex task and, as discussed in depth in Chapter 9, is currently not appropriate. As a result of the current knowledge base, there are several obstacles to validating these results. Of most importance is the importance of the true rates of infection and illness in the population. These are currently unknown as illness statistics are plagued with issues of under-reporting. Further, very little is known about the strains of campylobacter

which result in human illness. As a result, underlying the model development process were two key assumptions, these were:

* All strains present in chickens have the potential to cause disease in humans.

* All strains display the same survival and pathogenic characteristics.

The consequence of these assumptions on model results is currently unknown. however, work has demonstrated that not all strains associated with chicken flocks have been observed in a human infection. This would indicate that the risk will be over-estimated as flocks which are colonised with non-pathogenic campylobacters will be included in current parameter estimates. Further, as discussed in Chapter 7, different strains of campylobacter display a wide range of survival characteristics. As such, some strains may be better at surviving processing and cooking than the strains for which the data used to parameterise the model were obtained. Similarly some strains will no persist as well as this data indicates.

As a result of these difficulties, the results presented in this thesis should not be taken as absolute estimates of the risk of infection with campylobacter in GB. Rather, current estimates can be used as a benchmark to which the impact of mitigation strategies can be compared. However, several current situations may provide opportunity to validate the model results once all the epidemiological data have been collected, reported and analysed. These are the decrease of the consumption of chicken in 2000 as a result of the contamination of chicken feed with Dioxin in Belgium, and the increase in consumption rates in UK following an outbreak of foot and mouth disease in 2001.

11.4 Mitigation strategies

An investigation into the efficacy of possible mitigation strategies was undertaken in Chapter 10. Results clearly show that a reduction in the national flock prevalence, combined with a reduction in the within flock prevalence of positive can have a dramatic impact upon the risk of infection. There is currently no proven method available to achieve, and sustain such reductions. However, the level of research into this area continues to increase with the hope that strategies will be forthcoming. Given the magnitude of the public health threat posed by campylobacter, it would be desirable to reduce the risk of infection trough the implementation of alternative strategies until such time that the flock prevalence and within flock prevalence can be reduced. Therefore, with the aim to identify such strategies, predictions of the risk of infection following the implementation of 5 mitigations were compared with current estimates of risk. The mitigations considered were

* Reduction in probability that birds are contaminated on their exteriors at slaughter
* Reduction in the level of contamination on the exterior of birds at slaughter
* Prevention of carry-over of contamination in the house from flock to flock, identified specifically during evisceration
* Freeze all chicken prior to consumption
* Reduction in the occurrence of cross contamination in the home

Results show that the most effective strategy considered is the freezing of chicken prior to consumption. The freezing of all chicken prior to consumption resulted in a decrease in the average risk given exposure from 0.079 to 0.013. This may be a difficult measure to implement as there will be members of the population who prefer to purchase and consume chicken fresh. However, following an appropriate risk communication strategy, the potential importance of freezing chicken could increase in appreciation. Methods to effectively

reduce current levels of campylobacter related illness are currently unknown. However, this is a huge public health threat with thousands of individuals suffering some campylobacter related illness each year. The model results presented here suggest that freezing chicken prior to consumption could reduce the current risk of infection, and subsequently the rate of illness.

The freezing of all chicken products has been implemented as a risk reduction strategy in Iceland. In the next two years the impact of such a strategy will be evident, and hence will provide the opportunity to validate the model as a risk reduction tool.

11.4 Future work

The mathematical models presented in this thesis will provide invaluable for numerous areas of future research. There are several issues that can be investigated by use of the current model. These are

* Estimate the risk from specific strains of campylobacter
* Estimate the risk from chicken consumed outside the home
* Estimate the risk in other countries
* Estimate the risk from poultry other than chicken

As a result of the underlying model assumption that all campylobacter present in chickens are pathogenic to humans. This assumption was a result of insufficient knowledge regarding which strains are important in human infection. However, should such information become available the model can be refined and hence used to estimate the risk for any given strain. Of current interest is the risk of infection with antibiotic resistant strains of campylobacter, following the use of antibiotics in chickens during rearing. This problem has been approached from a

risk assessment perspective by the US Food and Drug Administration (Vose *et al*, Unpublished), but has yet to be considered for the UK situation.

The estimates of risk presented here are associated with the consumption of chicken meat produced in GB. Several of the variables used in the model are country specific such as rearing practices and consumer behaviour. Given information allowing the parameterisation of such variables the model can be applied to estimate the risk in nations other than GB, which have similar chicken production systems. Such modifications are currently being undertaken as part of the WHO/FAO initiative (Hartnett *et al.*, 2001b) with the aim to produce a risk assessment model which can be used by all member countries.

Epidemiological investigations have implicated the consumption of chicken as a risk factor for campylobacter related illness. However, it is recognised that other poultry are also colonised with campylobacters and as such may also play a role in human infection. The model framework presented here focuses on the chicken supply chain. With modification of the processing section the use of the model is not isolated to chicken meat and may easily be adapted to consider other poultry such as turkeys, geese or ducks.

There are several human pathogens which have been associated with the consumption of chicken, meat for example *Salmonella* spp. The model presented here provides a framework by which pathogens present in chicken meat may be investigated allowing the estimate of risk of a wide range of public health risks.

The current model solely considers chicken which is prepared in the home. However, the model can easily be adapted to allow estimation of the risk associated with chicken prepared outside the home setting such as catering outlets. The situation posed by catering outlets is different to that in the home. If an individual preparing a meal has a poor level of hygiene and cross-contaminates organisms, there is a risk that numerous individuals will be

exposed as a result of the preparation of a single serving of chicken. To enable such estimation, modification to the section of the model which considers the preparation of a chicken meal is necessary, to allow for one individual preparing several meals. Further, such an estimate, combined with estimates for the preparation of a meal in the home would provide a more realistic description of the infection process for a random individual.

11.5 Conclusions

For several years campylobacter has been recognised as a cause of suffering in the population. The number of cases of illness continues to increase throughout the world, and methods of control are elusive. This is attributed to the source of the organism being unknown, but studies indicate that the ingestion of chicken meat may be an important contributor. The work in this thesis investigates the contribution of the consumption of chicken to the rate of campylobacter infection, and illness in the population of GB. As a result of several areas of data deficiency it is not currently possible to estimate the absolute risk posed to the population from the consumption of chicken. However, the model presented in this thesis provides insight in to the way in which the colonisation of chickens on the farm can contribute to human illness. This work is a valuable tool which can be adapted to research several areas of not only campylobacter infection from chicken in GB but also other poultry, other organisms, and other countries.

The model presented in this thesis, and resulting estimates of risk can be used to investigate ways in which the risk can be reduced. Such investigations clearly show that a reduction in the national flock prevalence will reduce the risk to humans. However, the manner by which this can be achieved is currently unknown but the model results indicate this is a key area of research. An alternative method to reduce the risk is to freeze all the chicken prior to consumption. Such a strategy may receive opposition as it impinges upon the consumer's right to choose. However, if implemented, this may have a dramatic

impact on the risk of infection. Given the magnitude of the public health risk posed by campylobacter, such drastic measures may be necessary to protect the population's health.

References

Adak, G.K., Cowden, J.M., Nicholas, S. & Evans, H.S. (1995). The Public Health Laboratory Service national case control study of primary indigenous cases of campylobacter infection . *Epidemiology and Infection* **115**, 15-22.

Advisory Committee on the Microbiological Safety of Food (ACMSF) (1993). Interim report on campylobacter, HMSO, London.

Advisory committee on the microbiological safety of food (ACMSF) (1996). Report on poultry meat, HMSO, London.

Aho, M. & Hirn, J. (1988). Prevalence of campylobacteria in the Finnish broiler chicken chain from producer to the consumer. *Acta Vet. Scand.* **29**, 451-462.

Altekruse, S.F., Hunt, J.M., Tollefson, L.K. & Madden, J.M. (1994). Food and animal sources of human *Campylobacter jejuni* infection. *Journal of the American veterinary medical association* **204**, 57-61.

Altekruse, S.F., Swerdlow, D.L. & Wells, S.J. (1998). Factors in the emergence of food borne disaeses. *Veterinary Clinics of North America: Food Animal Practice* **14**, 1-15.

Annan-Prah, A. & Janc, M. (1988). The mode of spread of *Campylobacter jejuni/coli* to broiler flocks. *Journal Veterinary Medicine* **35**, 11-18.

Annan-Prah, A. & Janc, M. (1998). Chicken-to-human infection with *Campylobacter jejuni* and *Campylobacter coli*: Biotype and serotype correlation. *Journal of Food Protection* **51**, 562-564.

Ano, M., Kurki, M., Rautelin, M. and Kosunen, T.U. (1989). Waterborne outbreak of Campylobacter enteritis after outdoors infantry drill in Utti, Finland. *Epidemiology and Infection* **103**, 133-141.

Atabay, H.I. & Corry, J.E.L. (1997). The prevalence of Campylobacters and Arcobacters in broiler chickens. *Journal of Applied Microbiology* **83**, 619-626.

Audits (2000). Consumer cooking temperatures, retrieved 1st June 2000 http://www.audits.com.html

Bailey, N.T.J. (1975). The mathematical theory of infectious diseases and it's applications, Charles Griffin & Company Ltd.

Baird-Parker, A.C. (1994). Foods and Microbiological risks. *Microbiology* **140**, 687-695.

Bandres, J.C., Mathewson, J.J. & Dupont, H.L. (1988). Heat susceptibility of bacterial enteropathogens implications for the prevention of traveller's diarrhoea. *Archives of Internal Medicine* **148**, 2261-2263.

Berndston, E., Emanuelson, U., Engvall, A. & Danielsson-Tham, M.-L. (1996). A 1-year study of Campylobacters in 18 Swedish chicken farms. *Preventive Veterinary Medicine* **26**, 167-185.

Berrang, M.E. & dickens, J.A. (2000). Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. *Journal of Applied Poultry Research* **9**, 43-47.

Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P. & Blaser, M.J. (1988). Experimental *Campylobacter jejuni* infection in humans. *The Journal of Infectious Diseases* **157**, 472-479.

Blankenship, L.C. and Craven, S.E. (1982). *Campylobacter jejuni* survival in chicken meat as a function of temperature. *Applied and Environmental Microbiology* **44**, 88-92.

Blaser, M.J., Feldmen, R.A. and Wells, J.G. (1982). Epidemiology of endemic and epidemic Campylobacter infection in the united states. In: Campylobacter: Epidemiology, Pathogenesis, and Biochemistry, pp. 3-4. Lancaster: MTP Press.

Bogen, K.T & Spear, R.C. (1987). Integrating Uncertainty and Interindividual Variability in Environmental Risk Assessment. *Risk Analysis* **7** (4), 427-436.

Brattin, W.J., Barry, T.M., Chiu, N. (1996). Monte Carlo modeling with uncertain probability density functions. *Human and Ecololgical Risk Assessment*, **2** (4), 820-840

British Poultry Meat Federation (BPMF) (1998). British retail chicken market review.

Broczyk, A., Thompson, S., Smith, D. & Lior, H. (1987). Water-borne outbreak of *Campylobacter laridis*-associated gasteronenteritis. *The Lancet* **January**, 164-165.

Brouwer, R., Mertens, M.J.A., Siem, T.H. & Katchaki, J. (1979). An explosive outbreak of Campylobacter enteritis in soldiers. *Antonie van Leewenhoek* **45**, 517-519.

Bruce, D., Zochowski, W. & Ferguson, L.R. (1977). Campylobacter enteritis. *British Medical Journal* **2**, 1219-1220.

Bryan, F. & McKinley, W. (1974). Prevention of foodborne illness by time-temperature control of thawing, cooking, chilling, and preheating turkeys in school lunch kitchens. *Journal of Milk Food Technology*, **37**, 420-429

Bryan, F. (1971). Use of time-temperature evaluations in detecting the responsible vehicle and contributing factors of foodborne disease outbreaks. *Journal of Food Protection*, **61**, 1446-1453

Buchanan, R.L. & Whiting, R.C. (1996). Risk assessment and predictive microbiology. *Journal of Food Protection* **Supplement**, 31-36.

Buchanan, R.L. (1995). The role of microbiological criteria and risk assessment and HACCP. *Food Microbiology* **12**, 421-424.

Buchanan, R.L., Whiting, R.C. & Damert, W.C. (1997). When is simple good enough: a comparison of the Gompertz, Baranyi and three-phase linear models for fitting bacterial growth curves. *Food Microbiology* **14**, 313-326.

Byrd, D.M., Allen, D.O. & Beamer, R.L.(1998). The dose-response model for dioxin. *Risk Analysis* **18** , 1

Byrd, J.A., Corrier, D.E., Hume, M.E., Bailey, R.H., Stanker, L.H. & Hargis, B.M. (1998). Incidence of campylobacter in crops of preharvest market-age broiler chickens. *Poultry Science* **77**, 1303-1305.

Cason, J.A., Bailey, J.S., Stern, N.J., Whittlemore, A.D. & Cox, N.A. (1997). Relationship between aerobic bacteria, Salmonella and Campylobacter on broiler carcasses. *Poultry Science* **76**, 1037-1041.

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 (1994). Dose response assessment, In: Foodborne Pathogens Risks and Consequences.

Chang, H., Carpenter, J., & Toledo, R. (1998). Temperature histories at critical points and recommended cooking town for whole turkeys baked in a conventional oven. *Journal of Food Science*, **62**, 262-266.

Chen, H.Q., Marks, B.P., Murphy, R.Y. (1999). Modeling coupled heat and mass transfer for convection cooking of chicken patties. *Journal of Food Engineering*, **42** (3), 139-146.

Christensen, B., Sommer, H., Nielsen, N., and Rosenquist, H. (Unpublished) Risk Assessment of *Campylobacter jejuni* in chicken products. The Danish Veterinary and Food Administration.

Christopher, F.M., Smith, G.C. & Vanderzant, C. (1982). Effect of temperature and pH on the survival of *Campylobacter fetus*. *Journal of Food Protection* **45**, 253-259.

Clark, A.G. & Bueschkens, D.H. (1988). Horizontal spread of human and poultry-derived strains of *Campylobacter jejuni* among broiler chicks held in incubators and shipping boxes. *Journal of Food Protection* **51**, 438-441.

Cohen, J. T; Lampson, M. A, & Bowers, T. S. (1996). The Use of Two-Stage Monte Carlo Simulation Techniques to Characterize Variability and Uncertainty in Risk Analysis . *Human and Ecological Risk Assessment*. **2** (4), 939-971.

Coleman, M. and Marks, H. (1998). Topics in dose-response moelling. *Journal of Food Protection* **61**, 1550-1559.

Collias, N.E., Collias, E.C., Hunsaker, D. & Minning, L. (1966). Locality fixation, mobility and social organisation within an unconfined population of Red Jungle fowl. *Animal Behaviour* **14**, 550-559.

Comer, P.J. & Spouge, J.R. (1998). Risk assessment of BSE infectivity in the environment from rendering of over thirty month scheme cattle. *Journal of Risk Research* **1**, 281-293.

Communicable Disease Surveillance Center (CDSC). (1999). Communicable disease report, 9(2).

Correy, J.E.L. & Atabay, H.I. (2001). Poultry as a source of campylobacter and related organisms. *Journal of Applied Microbiology* 90(S6), 96S-145S

Covello, V.T. & Merkhofer, M.W. (1993). Risk assessment methods: approaches for assessing health and environmental risks, Plenum press. New York.

Cox, N.A., Stern, N.J., Hiett, K.L. & Berrang, M.E. (1999). Transmission of *Campylobacter jejuni* from breeders to commercial broiler chickens. Poster presentation at 10th International Workshop on Campylobacter, Helicobacter and Related Organisms, Baltimore.

Darling, W.M., Peel, R.N., Skirrow, M.B. & Mulira, A.E.J.L. (1979). Campylobacter cholecystitis. *The Lancet* **June**, 1089

Davies, J.S. & Penfold, J.B. (1979). Campylobacter urinary tract infection. *The lancet* **June**,1091-1092.

Davison, A. C. & Hinkley, D. V. (1997). Bootstrap Methods and Their Application (Cambridge Series in Statistical and Probabilistic Mathematics , No 1) . Cambrige Univ Press.

DeBoer, E. & Hahne, M. (1990). Cross-contamination with *Campylobacter jejuni* and *Salmonella spp.* from raw chicken products during food preparation. *Journal of Food Protection* **53**, 1067-1068.

Deming, M.S., Tauxe, R.V., Black, P.A., Dixon, S.E., Fowler, B.S., Jones, T.S., Lockamy, E.A., Patton, C.M. & Sikes, R.O. (1987). Campylobacter enteritis at a university: transmission from eating chicken and from cats. *American Journal of Epidemiology* **126**, 526-534.

Doyle, M.P. & Roman, D.J. (1981). Growth and survival of *Campylobacter fetus* subsp. *jejuni* as a function of temperature and pH. *Journal of Food Protection* **44**, 596-601.

Doyle, M.P. & Roman, D.J. (1982). Sensitivity of *Campylobacter jejuni* to drying. *Journal of Food Protection* **45**, 507-510.

Doyle, M.P. (1985). Campylobacter in foods. In: Campylobacter infection in man and animals, CRC press.

Doyle, M.P. (1984). Association of *Campylobacter jejuni* with laying hens and eggs. *Applied and Environmental Microbiology* **47**, 533-536.

Dupont, H.L., Chappel, C.L., Sterling, C.R., Okhuysen, P.L., Rose, J.B. & Jakubowski, W. (1995). The infectivity of *Cryptosporidium parvum* in healthy volunteers. *New England Journal of Medicine* **332**, 855-859.

Ebright, J.R. & Ryan, L.M. (1984). Acute erosive reactive arthritis associated with *C. jejuni*-induced colitis. *American Journal of Medicine* **76**, 321-323.

Efron, B., Tibshirani, & Efron, B. (1994). An Introduction to the Bootstrap (Monographs on Statistics and Applied Probability, No 57). CRC Press.

Engvall, A., Bergqvist, A., Sandstedt, K. & Danielsson-Tham, M.-L. (1986). Colonisation of broilers with Campylobacter in conventional broiler-chicken flocks. *Acta Vet Scandinavia* **27**, 540-547.

European Union (1997). Concerted Action CT94-1456: Microbial control in the meat industry. Volume 2: Management in poultry processing before and after harvest.

Evans, S. (1992). Introduction and spread of thermophilic campylobacters in broiler flocks. *The Veterinary Record* **131**, 574-576.

Evans, S. (1996). A longitudinal study of thermophilic campylobacter infection of poultry broiler flocks in GB. *Report for Animal Health Veterinary Group, MAFF, Tolworth*

Fazil, A., Lowman, R., Stern, N., & Lammerding, A. (Unpublished). A quantitative risk assessment model for *C. jejuni* in fresh poultry.

Frey, H.C., & Rhodes, D.S. (1996). Characterising, simulating, and analysing variability and uncertainty: an illustration of methods using an air toxics emissions example. *Human Health and Ecological Risk Assessment: an International Journal*, 2(4):762-797

Frey, H.C., & Rhodes, D.S. (1998). Characterisation and simulation of uncertain frequency distributions: effects of distribution choice, variability, uncertainty, and parameter dependence. *Human and Ecological Risk Assessment*, 4(2):423-468

Fricker, C.R. & Park, R.W.A. (1989). A two-year study of the distribution of thermophilic Campylobacters in human, environmental and food samples from the Reading area with particular reference to toxin production and heat-stable serotype. *Journal of Applied Bacteriology* **66**, 477-490.

Fukuda K., Sugawa, K. & Ishii, K. (1984). Simulation of infectious disease by Reed-Frost model with proportion of immune and innapparent infection. *Computational Biology in Medicine* **14**, 209-215.

Genigeorgis, C., Hassuneh, M. & Collins, P. (1986). *Campylobacter jejuni* infection on poultry farms and it's effect on poultry meat contamination during slaughtering. *Journal of Food Protection* **49**, 895-903.

Gill, C.O. & Harris, L.M. (1982). Survival and growth of *Campylobacter fetus* subsp *jejuni* on meat and in cooked foods. *Applied and Environmental Microbiology* **50**, 259-263.

Gill, C.O. (1999). HACCP by guesswork or by the numbers? *Food quality* **6**, 28

Gregory, E., Barnhart, H., Dreesen, D.W., Stern, N.J. & Corn, J.L. (1997). Epidemiological study of *Campylobacter* spp. in broilers: source, time of colonisation, and prevalence. *Avian Diseases* **41**, 890-898.

Griffith, C., Worsfold, D., Mitchell, R. (1998). Food preparation, risk communication and the consumer. *Food Control*, **9** (4), 225-232

Griffiths, P.H. & Park, R.W.A. (1990). Campylobacters associated with human diarrhoeal disease. *Journal of Applied Bacteriology* **69**, 281-301.

Griffiths, P.L. & Park, R.W.A. (1990). A review: Campylobacters associated with human diarrhoeal disease. *Journal of Applied Microbiology* **69**, 281-301.

Haas, C.N. (1983). Estimation of risk due to low doses of micro-organisms: a comparison of alternative methodologies. *American Journal of Epidemiology* **118**, 573-582.

Haas, C.N., Rose, J.B., Gerba, C. & Regli, S. (1993). Risk assessment of virus in drinking water. *Risk Analysis* **13**, 545-552.

Haas, C.N., Rose, J.B., Gerba, C.P. (1999). Quantitative microbial risk assessment. John Wiley and Sons.

Hanninen, M.L. (1981). Survival of *Campylobacter jejuni/coli* in ground frozen beef liver and in frozen broiler carcasses. *Acta Vet Scandinavia* **22**, 566-577.

Harris, N.V., Thompson, D., Martin, D.C. and Nolan, C.M. (1986). A survey of campylobacter and other bacterial contaminants of pre-market chicken on retail poultry and meats, King County, Washington. *American Journal of Public Health* **76**, 401-406.

Harris, N.V., Weiss, N.S. and Nolan, C.M. (1986). The role of poultry and meats in the etiology of *Campylobacter jejuni/coli* enteritis. *American Journal of Public health* **76**, 407-411.

Hartnett E., Kelly, L., Wooldridge, M., In Press. A quantitative risk assessment for campylobacter in broilers: work in progress. *Biodeterioration and degradation.*

Hartnett, E., Kelly, L., Newell, D., Wooldridge, M., & Gettinby, G. (2001). The occurrence of campylobacter in broilers at the point of slaughter, a quantitative risk assessment. *Epidemiology and Infection*, **127**, 195-206

Hartnett, E., Paoli, G., Fazil, A., Lammerding, A., Anderson, S., Rosenquist, H., Christiensen, B.B., Nauta, M. (2001b). Hazard identification, hazard characterisation and exposure assessment for *Campylobacter* spp. in broilers. Report prepared for Joint WHO/FAO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods. Geneva, Switzerland July 2001. Available at http://www.who.int

Hathaway, S.C. (1997). development of food safety risk assessment guidelines for foods of animal origin in international trade. *Journal of Food Protection* **60**, 1432-1438.

Hay D.J., & Ganguli L.A. (1980). Campylobacter-enteritis presenting as an acute abdomen, *Postgraduate Medical Journal* **56** (6553), 205-206

Helton, J.C. & Shiver, A.W. (1996). A Monte Carlo procedure for the construction of complementary cumulative distribution functions for comparison with the EPA release limits for radioactive waste disposal. *Risk Analysis* **16** (1), 43-55.

Hinton, M. H., Allen, V. M., Tinker, D. B., Gibson, C., & Wathes, C. M. (1996). The dispersal of bacteria during the defeathering of poultry. Factors affecting the microbial quality of meat, concerted action CT94-1456 volume 2: Slaughter and dressing. 113-123.

Hoffman, F. O & Hammonds, J. S. (1994). Propagation of uncertainty in risk assessments: The need to distinguish between uncertainty due to lack of knowledge ad uncertainty due to variability. *Risk Analysis* **14** (5), 707-712.

Holman, J. P. (1996). Heat Transfer. 8th Edition. McGraw-Hill Higher Education.

Hood, A.M., Pearson, A.D. & Shahamat, M. (1988). The extent of surface contamination of retailed chickens with *Campylobacter jejuni* serogroups. *Epidemiology and Infection* **100**, 17-25.

Hopkins, R.S. & Scott, A.S. (1983). Handling raw chicken as a source for sporadic *Campylobacter jejuni* infections. *Journal of Infectious Diseases* **148**, 770

Hudson, P.J., Vogt, R.L., Brondum, J. & Patton, C.M. (1984). Isolation of *Campylobacter jejuni* from raw milk during an outbreak of Campylobacteriosis. *Journal of Infectious Diseases* **150**, 789

Humphrey, T.J., Henley, A. & Lanning, D.G. (1993). The colonisation of broiler flocks with *Campylobacter jejuni*: some epidemiological investigations. *Epidemiology and Infection* **110**, 601-607.

Hutchinson, D. N., Bolton, F. J., Hinchliffe, P. M. *et al.* (1985). Evidence of udder excretion of *Campylobacter jejuni* as the cause of milk-borne campylobacter outbreak. *Journal of Hygiene, Cambridge* 94 (2), 205-215

International Commission on Microbial Specifications of Foods (ICMSF) (1996). Microorganisms in foods, Volume 5. Characteristics of microbial pathogens, Blackie Academic & Professional, London, pp. 45-65.

Izat, A.L., Gardner, F.A., Denton, J.H. & Golan, F.A. (1988). Incidence and level of *Campylobacter jejuni* in broiler processing. *Poultry Science* **67**, 1568-1572.

Jacobs-Reitsma, W.F., Bolder, N.M. & Mulder, R.W.A.W. (1994). Cecal carriage of campylobacter and salmmonella in Dutch broiler flocks at slaughter: a 1-year study. *Poultry Science* **73**, 1260-1266.

Jacobs-Reitsma, W.F., Van De Giessen, A.W., Bolder, N.M. & Mulder, R.W.A.W. (1995). Epidemiology of *Campylobacter* spp. at two Dutch broiler farms. *Epidemiology and Infection* **114**, 413-421.

Jacquez, J.A. (1987). A note on chain-binomial models of epidemic spread: what is wrong with the Reed-Frost formulation? *Mathematical Biosciences* **87**, 73-82.

Jay, L.S., Comar, D. & Govenlock, L. (1999). A video study of Australian food-handling practices. *Journal of Food Protection* **62**, 1285-1296.

Jhonsen, K., Ostensen, M., Christine, A., Melby, S. & Melby, K. (1983). HLA-B27 negative arthritis related to *Campylobacter jejuni* enteritis in three children and two adults. *Acta Medica Scandinavica* **214**, 165-168.

Jones, D.M., Sutcliffe, E.M. & Curry, A. (1991). Recovery of viable but non-culturable *Campylobacter jejuni*. *Journal of General Microbiology* **137**, 2477-2482.

Kaldor, J. & Speed, B.R. (1984). Guillain-Barre syndrome and *Campylobacter jejuni*: a serological study. *British Medical Journal* **288**, 1867-1868.

Kapperud, G., Skjerve, E., Vik, L., Hauge, K., Lysaker, A., Aalmen, I., Ostroff, S.M. & Potter, M. (1993). Epidemiological investigation of risk factors for campylobacter colonization in Norwegian broiler flocks. *Epidemiology and Infection* **111**, 245-255.

Kelly, L., Anderson, W., Snarey, E. (2000). Exposure assessment for *Salmonella* spp. in broilers. Report prepared for Joint WHO/FAO Expert Consultation on Risk Assessment of Microbiological Hazards in Foods. Rome, Italy 17-21 July 2000. Available at http://www.who.int

Kist, M. (1982). Campylobacter enteritis: epidemiological and clinical data from recent isolations in the region of Freiburg, West Germany. In: Newell, D.G., Campylobacter: epidemiology, pathogenesis, and biochemistry, pp. 138-143. Lancaster: MTP Press.

Koidis, P. & Doyle, M.P. (1983). Survival of *Campylobacter jejuni* in fresh and heated red meat. *Journal of Food Protection* **46**, 771-774.

Kwiatek, K., Wojton, B. & Stern, N.J. (1990). Prevalence and distribution of *Campylobacter* spp. on poutry and selected red meat carcasses in Poland. *Journal of Food Protection* **53**, 127-130.

Lee, P. M. (1997). Bayesian Statistics: An Introduction. Edward Arnold.

Lillard, H.S. (1973). Contamination of blood system and edible parts of poultry with *clostridium perfringens* during water scalding. *Journal of Food Science* **38**, 151-154.

Lindblom, G.B., Sjorgen, E. & Kailser, B. (1986). Natural campylobacter colonization in chickens raised under different environmental conditions. *Journal of Hygiene, Cambridge* **96**, 385-391.

Line, J.E., Bailey. J. S., Cox, N.A. & Stern, N.J. (1997). Yeast treatment to reduce Salmonella and Campylobacter populations associated with broiler chickens subjected to transport stress. *Poultry Science* **76**, 1227-1231.

Lyon, C., Lyon, B., Klose, A., & Hudspeth, J. (1975). Effects of temperature-time combinations on doneness and yields of water-cooked broiler thighs. *Journal of Food Science*, **40**, 129.

Macintosh, D. L., Suter, G. W., Hoffman, F. O. (1994). Uses of probabilistic exposure models in ecological risk assessment of contaminated sites. *Risk Analysis* **14** (4), 405-419

Mackintosh, C.A. & Hoffman, P.N. (1984). An extended model for transfer of micro-organisms via the hands: differences between organisms and the effect of alcohol disinfection. *Journal of Hygiene* **92**, 345-355.

McBride, G. & Foenander, F.(1962). Territorial behaviour in flocks of domestic fowls. *Nature* **192**, 102

McBride, G., Parker, I.P. & Foenander, F. (1969). The social organisation and behaviour of the feral domestic fowl. Part 1. Field studies on North West Island. *Animal Behaviour Monograph* **2**, 127-181.

McMeekin, T.A., Olley, J.N., Ross, T. & Ratkowsky, D.A. (1993). Predictive microbiology: theory and application, Research Studies press Ltd., England

Mead, G.C. & Hinton, M.H. (1989). Behaviour of campylobacters in production, processing and storage of poultry. Hohenheimer Gefugel Symposiun

Medema, G.J., Teunis, P.F.M., Havelaar, A.H. & Haas, C. (1996). Assessment of the dose-response relationship of *Campylobacter jejuni*. *International Journal of Food Microbiology* **30** , 101-111.

Ministry of Agriculture Fisheries and Food (MAFF) (1991). Consumer handling of chilled foods. MAFF.

Ministry of Agriculture Fisheries and Food (MAFF) (1999). Ministry of Agriculture Fisheries and Food (MAFF) statistics: Poultry and Poultry meat statistics notice.

Ministry of Agriculture Fisheries and Food (MAFF) (2001). Ministry of Agriculture Fisheries and Food (MAFF) statistics: Poultry and Poultry meat statistics notice.

Morgan, M. G. & Henrion, M. (1992). Uncertainty: A Guide to Dealing With Uncertainty in Quantitative Risk and Policy Analysis. Cambridge University Press.

Mulder, R.W.A.W. (1995). Impact of transport and related stresses on the incidence and extent of human pathogens in pigmeat and poultry. *Journal of Food Safety* **15**, 239-246.

Mulder, W.A.W., Dorresteijn, L.W.J. & Van Der Broek, J. (1978). Cross-contamination during the scalding and plucking of broilers. *British Poultry Science* **19**, 61-70.

Nauta, M.J. & Heuvelink, A.E. (1998). Exposure assessment of Shiga toxin producing *E. coli* O157 via beef and beef products in The Netherlands. Poster presentation at Society for Risk Analysis Annual Meeting, Arizona.

Nauta, M.J. (2001). Exposure assessment of *E.coli* in steak tatare in the Netherlands. Report prepared for RIVM.

Nauta, M.J. (2000). Separation of uncertainty and variability in quantitative risk assessment models. *International Journal of Food Microbiology* **57**, 9-18.

Newell, D., Hartnett, E., Madsen, M., Engberg, J., Hald, T., Wedderkopp, A., & Engvall, A. (1999). The comparison of seasonality in *Campylobacter* infections in humans and chickens from three European countries. 10th International Workshop on *Campylobacter*, *Helicobacter* and related organisms, Baltimore, Maryland, pp.41

Newell, D.G. & Wagenaar, J.A. (2000). Poultry infections and their control at the farm level. In: Campylobacter, 2nd Edition, pp 497-509, American society of microbiology, Washington D.C.

Ng, J. & Orav, E.J. (1990). A generalized chain binomial model with application to HIV infection. *Mathematical Biosciences* **101**, 99-119.

Ngadi, M. O., Watts, K. C., Correia, L. R. (1997). Finite element method modelling of moisture transfer in chicken drum during deep-fat frying. *Journal of Food Engineering*, **32** (1), 11-20.

Notermans, S. & Jouve, J.L. (1995). Quantitative risk analysis and HACCP: some remarks. *Food Microbiology* **12**, 425-429.

Notermans, S., Gallhoff, G., Zwietering, M.H. & Mead, G.C. (1995). The HACCP concept: specification of criteria using quantitative risk assessment. *Food Microbiology* **12**, 81-90.

Oosterom, J., De Wilde, G.J.A., De Boer, E., Blaauw, L.H. & Karman, H. (1983). Survival of *Campylobacter jejuni* during poultry processing and pig slaughtering. *Journal of Food Protection* **46**, 702-706.

Oosterom, J., Notermans, S., Karman, H. & Engels, G.B. (1983). Origin and prevalence of *Campylobacter jejuni* in poultry processing . *Journal of Food Protection* **46**, 339-344.

Pamment, P., Foenander, F. & McBride, G. (1983). The social and spatial organisation of male behaviour in mated domestic fowl. *Applied Animal Ethology* **9**, 341-349.

Pearson, A.D., Greenwood, M., Healing, T.D., Rollins, D., Shahamat, M., Donaldson, J. & Colwell, R.R. (1993). Colonization of broiler chickens by waterborne *Campylobacter jejuni*. *Applied and Environmental Microbiology* **59**, 987-996.

Pearson, A.D., Greenwood, M.H., Sockett, P.N., Donaldson, P., Hooper, W.L., *et al.* (1987). Sporadic human Campylobacter: evidence for transmission from fresh chickens. In Kaijser, B. and Falsen, E., Campylobacter IV, pg 307-310.

Pitts, D.R., & Sissom, L.E. (1998). Schaum's Outline of Theory and Problems of Heat Transfer (Schaum's Outlines Series). McGraw-Hill Professional Publishing.

Pokamunski, S., Kass, N., Borochovich, E., Marantz, B. & Rogol, M. (1986). Incidence of *Campylobacter* spp. in broiler flocks monitored from hatching to slaughter. *Avian Pathology* **15**, 83-92.

Preston, A.P. & Murphy, L.B. (1989). Movement of broiler chickens reared in commercial conditions. *British Poultry Science* **30**, 519-532.

Randell, A.W. (1997). Codex Alimentarius: food quality and safety standards for international trade. *Revue Scientifique et Technique Office Internationale des Epizooites.* **16**, 313-321.

Roels, T.H., Wickus, B., Bostrom, H.H., Kazmierczak, J.J., Nicholson, M.A., Kurzynski, T.A. & Davis, J.P. (1998). A food-borne outbreak of *Campylobacter jejuni* (O:33) infection associated with tuna salad: a rare strain in an unusual vehicle. *Epidemiology and Infection* **121**, 281-287.

Rollins, D.M., & Colwell, R.R. (1986). Viable but non-culturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. *Applied and Environmental Microbiology*. **52** (3): 531-538

Rose, J.B., Haas, C.N. & Regli, S. (1991). Risk assessment and control of waterborne giardiasis. *American Journal of Public Health* **81**, 709-712.

Rosef, O., Gondrosen, B. & Kapperud, G. (1984). *Campylobacter jejuni* and *campylobacter coli* as surface contaminants of fresh and frozen poultry carcasses. *International Journal of Food Microbiology* **1**, 205-215.

Ross, T., & McMeekin, T.A. (1994). Predictive microbiology. *International Journal of Food Microbiology* **23** (3-4), 241-264

Saleha, A.A., Mead, G.C. & Ibrahim, A.L. (1998). *Campylobacter jejuni* in poultry production and processing in relation to public health. *World's Poultry Science Journal* **54**, 49-58.

Schervish, M. J. (1995). Theory of Statistics (Springer Series in Statistics). 2nd Edition. Springer Verlag.

Shanker, S., Lee, A. & Sorrell, T.C. (1982). *Campylobacter jejuni*: incidence in processed broilers and biotype distribution in human broiler isolates. *Applied and Environmental Microbiology* **43**, 1219-1220.

Shanker, S., Lee, A. & Sorrell, T.C. (1990). Horizontal transmission of *Campylobacter jejuni* amongst broiler chicks: experimental studies. *Epidemiology and Infection* **104**, 101-110.

Shanker, S., Lee. A., & Sorrell, T. C. (1986). *Campylobacter jejuni* in broilers – the role of vertical transmission. *Journal of Hygiene, Cambridge* **96** (2), 153-159

Shreeve, J.E., Toszeghy, M., Pattison, M. & Newell, D.G. (2000). Sequential spread of campylobacter infection in a multipen broiler house. *Avian Diseases* **44**, 983-988.

Shreeve, J.E., Toszeghy, T., Pattison, M. & Newell, D.G. (1999). Sequential spread of campylobacter infection through a multi-pen broiler house. Poster presentation at 10th International Workshop on Campylobacter, Helicobacter and Related Organisms, Baltimore.

Simmons, N.A. & Gibbs, F.J. (1979). *Campylobacter* spp. in oven ready poultry. *Journal of Infection* **1**, 159-162.

Skirrow, M.B. (1982). Campylobacter enteritis - the first five years. *Journal of Hygiene* **89**, 175-184.

Skovgaard, N. (2000). Address by the representative of the "International Union of Microbiological Societies", IUMS, and the "International Committee on Food Microbiology", ICFMH, to the "World Conference on Science" (WCS), Budapest, Hungary, 26 June-1 July 1999, *International Journal of Food Microbiology* **58** (1-2): 135-135

Sorqvist, S. (1989). Heat resistance of *Campylobacter* and *Yersinia* strains by three methods. *Journal of Applied Bacteriology* **67**, 543-549.

Stern, N.J. & Kotula, A.W. (1982). Survival of *Campylobacter jejuni* inoculated into ground beef. *Applied and Environmental Microbiology* **44**, 1150-1153.

Stern, N.J. (1988). Colonization of pouiltry by *Campylobacter jejuni* and the potential for intervention. *Journal of Food Protection* **51**, 831

Stern, N.J., Clavero, M.R.S., Bailey, J.S., Cox, N.A. & Robach, M.C. (1995). *Campylobacter* spp. in broilers on the farm and after transport. *Poultry Science* **74**, 937-941.

Stern, N.J., Meinersmann, R.J., Cox, N.A., Bailey, J.S. & Blankenship, L.C. (1990). Influence of host lineage on cecal colonisation by *Campylobacter jejuni* in chickens. *Avian diseases* **34**, 602-606.

Stern, N.J., Myszewski, M.A., Barnhart, H.M., *et al.* (1997). Flagellin A gene restriction fragment length polymorphism patterns of *Campylobacter* spp. isolates from broiler production sources. *Avian Diseases* **41** (4): 899-905

Stuart, J., Sufi, F., McNulty, C. & Park, P. (1997). Outbreak of campylobacter enteritis in a residential school associated with bird pecked bottle tops. *Communicable Disease Report* **7**, R38-R40

Teunis, P.F.M. & Havelaar, A.H. (2000). The Beta Poisson dose-response model is not a single-hit model. *Risk Analysis* **20** (4), 513-520

Tompkins, D.S., Hudson, M.J., Smith, H.R., Eglin, R.P., Wheeler, J.G., Brett, M.M., Owen, R.J., Brazier, J.S., Cumberland, P., King, V. & Cook, P.E. (1999). A study of infectious intestinal disease in England: microbiological findings in cases and controls. *Communicable Disease and Public Health* **2**, 108

Tribe, A. (1980). Environmental design and site attachment in flocks of broiler chickens. *M.Sc. Thesis, University of Queensland*

Van De Giessen, A., Mazurier, S.-I., Jacobs-Reitsma, W., Jansen, W., Berkers, P., Ritmeester, W. & Wernars, K. (1992). Study on the epidemiology and control of *Campylobacter jejuni* in poultry broiler flocks. *Applied and Environmental Microbiology* **58**, 1913-1917.

Vogt, R.L., Sours, H.E., Barrett, T., Feldman, R.A., Dickinson, R.J. & Witherall, L.E.A. (1982). Campylobacter enteritis associated with contaminated water. *Annals of Internal Medicine* **96**, 292-296.

Vose, D. (2000). Risk Analysis: A Quantitative Guide. 2nd edition. John wiley & sons.

Waterman, S.C. (1982). The heat sensitivity of *Campylobacter jejuni* in milk. *Journal of Hygiene, Cambridge* **88**, 529-533.

Wempe, J.M., Genigeorgis, C.A., Farver, T.B. & Yusufu, H.I. (1983). Prevalence of *Campylobacter jejuni* in two California chicken processing plants. *Applied and Environmental Microbiology* **45**, 355-359.

Whiting, R.C. & Buchanan, R.L. (1997). Development of a quantitative risk assessment model for *Salmonella enteritidis* in pasteurized liquid eggs. *International Journal of Food Microbiology* **36**, 111-125.

Whiting, R.C., & Cygnarowicz-provost, M. (1992). A quantitative model for bacterial growth and decline. *Food Microbiology* **9** (4), 269-277

World Health Organisation (WHO), 2001. Information gathered at http:www.who.int

Wood-Gush, D.G.M., Duncan, I.J.H. & Savory, C.J. (1978). Observations on the social behaviour of domestic fowl in the wild. *Biology of Behaviour* **3**, 193-205.

Wooldridge, M. (1996). Risk analysis, risk assessment, animal health and the decision making process. *State Veterinary Journal* **6**, 4-6.

Worsfold, D. & Griffith, C. (1997). Assessment of the standard of consumer food safety behaviour. *Journal of Food Protection* **60**, 399-406.

Wright, E.P. (1979). Meningism associated with *Campylobacter jejuni* enteritis. *The Lancet* **May**, 1092

Zhao, P., Zhao, T., Doyle, M.P., Rubino, J.R. & Meng, J. (1998). Development of a model for evaluation of microbial cross-contamination in the kitchen. *Journal of Food Protection* **61**, 960-963.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M. & VanT Reit, K. (1990). Modelling of the bacterial growth curve. *Applied and Environmental Microbiology* **56**, 1857-1881.