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12

RISK ASSESSMENT OF *CAMPYLOBACTER* SPP. IN BROILER CHICKENS

TECHNICAL REPORT

WORLD HEALTH ORGANIZATION
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FOREWORD

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

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

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

Microbiological risk assessment can be considered as a tool that can be used in the management of the risks posed by foodborne pathogens and in the elaboration of standards for food in international trade. However, undertaking a microbiological risk assessment (MRA), particularly quantitative MRA, is recognized as a resource-intensive task requiring a multidisciplinary approach. Nevertheless, foodborne illness is one of the most widespread public health problems, creating social and economic burdens as well as human suffering; it is a concern that all countries need to address. As risk assessment can also be used to justify the introduction of more stringent standards for imported foods, a knowledge of MRA is important for trade purposes, and there is a need to provide countries with the tools for understanding and, if possible, undertaking MRA. This need, combined with that of the Codex Alimentarius for risk-based scientific advice, led FAO and WHO to undertake a programme of activities on MRA at the international level.

The Nutrition and Consumer Protection Division (FAO) and the Department of Food Safety and Zoonoses (WHO) are the lead units responsible for this initiative. The two groups have worked together to develop the area of MRA at the international level for application at both the national and international level. This work has been greatly facilitated by the

contribution of people from around the world with expertise in microbiology, mathematical modelling, epidemiology and food technology, to name but a few.

This Microbiological Risk Assessment series provides a range of data and information to those who need to understand or undertake MRA. It comprises risk assessments of particular pathogen-commodity combinations, interpretative summaries of the risk assessments, guidelines for undertaking and using risk assessment, and reports addressing other pertinent aspects of MRA.

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

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PREFACE

This risk assessment has been elaborated over a number of years. A number of national risk assessments that were available or being finalized when this work was initiated in 2001 were used as a basis from which to elaborate this risk assessment. In the course of its development, the risk assessment has been reviewed by two expert consultations—in 2001 and 2002—and been presented in various forums, including the Codex Committee on Food Hygiene and some international scientific conferences and meetings. Finally, it was subjected to peer review in 2006. Comments and feedback received at each of these steps have been taken into account in the finalization of the risk assessment.

In parallel to the elaboration of this risk assessment, research into *Campylobacter* spp. in broiler chickens and related issues has been ongoing, and risk assessment work at a national level has continued in some countries. Taking this into consideration, the recent literature has been reviewed and incorporated into this work as appropriate to ensure that the risk assessment is current at the time of publication in terms of recent developments in scientific knowledge and data.

ABBREVIATIONS

%	percentage
°C	degree Celsius
AIDS	Acquired Immune Deficiency Syndrome
CARMA	Campylobacter Risk Management and Assessment
CCFH	Codex Committee on Food Hygiene
CDC	Centres for Disease Control and Prevention (USA)
CFIA	Canadian Food Inspection Agency
cfu	colony forming unit
EEC	European Economic Community
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration (USA)
GB	Guillain-Barré Syndrome
HIV	Human Immunodeficiency Virus
MRA	Microbiological Risk Assessment
MRM	Mechanically Recovered Meat
NSAID	Non-Steroid Anti-Inflammatory Drug
PFGE	Pulsed Field Gel Electrophoresis
QMRA	Quantitative Microbiological Risk Assessment
SOD	Superoxide Dismutase
WFP	Within Flock Prevalence
WHO	World Health Organization

EXECUTIVE SUMMARY

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) assembled an expert drafting group to develop a risk assessment for *Campylobacter* spp. in broiler chickens.

The culmination of this work is a risk assessment model and technical document "A Risk Assessment of *Campylobacter* spp. in Broiler Chickens." Comparisons of risk for a variety of scenarios and mitigation measures were conducted. The review and analysis of current scientific information and the description of the risk model can be used by FAO and WHO member countries to conduct country-specific risk assessments or to identify and collect the information and research necessary to conduct a risk assessment for *Campylobacter* spp. in chicken products.

The products considered in this report include fresh intact broilers or whole chickens, and chicken parts produced in commercial processing plants. Frozen chicken was also considered. The exposure scenario includes consumer home preparation, but does not address retail or catering preparation.

The current risk assessment is not intended to describe any one geographical location or system, but rather it was developed and designed to provide relative risk comparisons, and uses data from various geographical areas. It should be understood that the assessment does not provide estimates for the absolute risk attributable to any one system. In order to fully characterize a specific system, the features of that system need to be captured (every system or country is likely to be different to varying degrees), with data specific to that system applied carefully.

Hazard identification

The hazard identification portion of the risk assessment document provides an in-depth review of available scientific data and information that identifies and characterizes the sources of *Campylobacter* infection in the human population arising from the consumption of chicken.

Campylobacter is a leading cause of zoonotic enteric infections in most developed and developing countries (WHO, 2001). The reported incidence of *Campylobacter* infections has markedly increased in many developed countries within the last 20-year period. Under-reporting of *Campylobacter* infections is an issue in many countries and incidence rates only reflect the number of laboratory-confirmed cases. The true rate of infection is considered to be higher than the number of reported cases (from 7.6 to 100 times higher) (Skirrow, 1991; Kapperud, 1994; Wheeler et al., 1999; Mead et al., 1999; Samuel et al., 2004). Cases are usually caused by *Campylobacter jejuni* and to a lesser extent by *Campylobacter coli* (Nielsen, Engberg and Madsen, 1997; Wooldridge and Ketley, 1997; Anon., 1999; Nadeau, Messier and Quessy, 2001; Anon., 2006b). Most *Campylobacter* infections are classified as sporadic cases, or as part of small, family-related outbreaks. Identified outbreaks are relatively uncommon.

The burden of human *Campylobacter* infection is not known for the majority of developing countries, as national surveillance may be limited in these countries. However, it is likely that the rate of campylobacteriosis is highest among children below 5 years of age (Coker et al., 2002), causing substantial morbidity and a lesser rate of mortality.

Bacteria belonging to the genus *Campylobacter* are non-spore forming, oxidase-positive, Gram-negative rods. In general, *Campylobacter* spp. grow at 37°C, but not below 32°C. *C. jejuni* and *C. coli* are distinguished from most other *Campylobacter* spp. by their high optimum growth temperature (42°C). Therefore, one can reasonably assume that *Campylobacter* spp. do not multiply

during slaughter, post-processing, transport and refrigerated storage of chicken products. Though *Campylobacter* spp. may persist for prolonged periods in chilled and frozen products, a reduction in the concentration and viability has been recorded after several weeks of storage at 4°C (Oosterom et al., 1983b; Yogasundram and Shane, 1986) and in frozen poultry after several months (Hänninen, 1981; Oosterom et al., 1983b; Yogasundram and Shane, 1986; Solow, Cloak and Fratamico, 2003; Thorkelsson et al., 2003; Bhaduri and Cottrell, 2004; Georgsson et al., 2006) *C. jejuni* and *C. coli* are rather sensitive to heat (D-values are 0.21-2.25 minutes at 55-60°C) (ICMSF, 1996)

C. jejuni is predominantly associated with poultry (Tauxe, 1992; Anon., 1998a, 1999, 2001a, 2006b; Nadeau, Messier and Quessy, 2001), but has also been isolated from cattle, sheep, goats, dogs and cats (Nielsen, Engberg and Madsen, 1997; Anon., 1999, 2006b). *C. coli* is predominantly found in pigs (Rosef et al., 1983; Nielsen, Engberg and Madsen, 1997; Boes et al., 2005; Jensen et al., 2006), but has also been isolated from poultry, cattle and sheep (Anon., 1999, 2006b).

Risk Assessment Model Overview

The risk assessment, in accordance with CODEX guidelines for conducting microbiological risk assessment, consists of the accumulation of an exposure assessment, a hazard characterization and a risk characterization. A schematic overview of the framework for the *Campylobacter* risk assessment is shown in Figure 1.

The risk-assessment model is a mathematical description (including Monte Carlo simulation) of a number of phenomena that, through a complex web of interactions, contribute to the level of risk associated with consuming broiler chicken products contaminated with *Campylobacter* spp. This assessment is a result of merging models developed prior to this assessment in Canada, Denmark and the United Kingdom. These models were developed for different reasons and their individual structures and emphases varied accordingly.

The model was implemented in Microsoft Excel™, together with the add-in @Risk™ to provide Monte Carlo simulation capability.

Exposure assessment

The exposure assessment considers the occurrence and number of *Campylobacter* that may be present in chicken products throughout the process and up to the point of consumption. As illustrated in Figure 1, the model is based upon a modular structure. The stages from rearing of broilers to the consumption of chicken products are grouped into four main modules. The modules are: 1. *Farm and Transport*; 2. *Processing*; 3. *Storage*; and 4. *Preparation*. The exposure assessment initially evaluates the frequency and levels of *Campylobacter* on the farm, estimating the probability that a random flock is *Campylobacter*-positive, the within-flock prevalence and the levels of colonization and contamination of the broilers (internally and externally). Subsequently, the stages of transport, processing, storage and preparation by the consumer are explored, and combined to predict the overall impact that these stages will have upon the contaminating *Campylobacter* load on a random chicken carcass or product, and to determine the final exposure level.

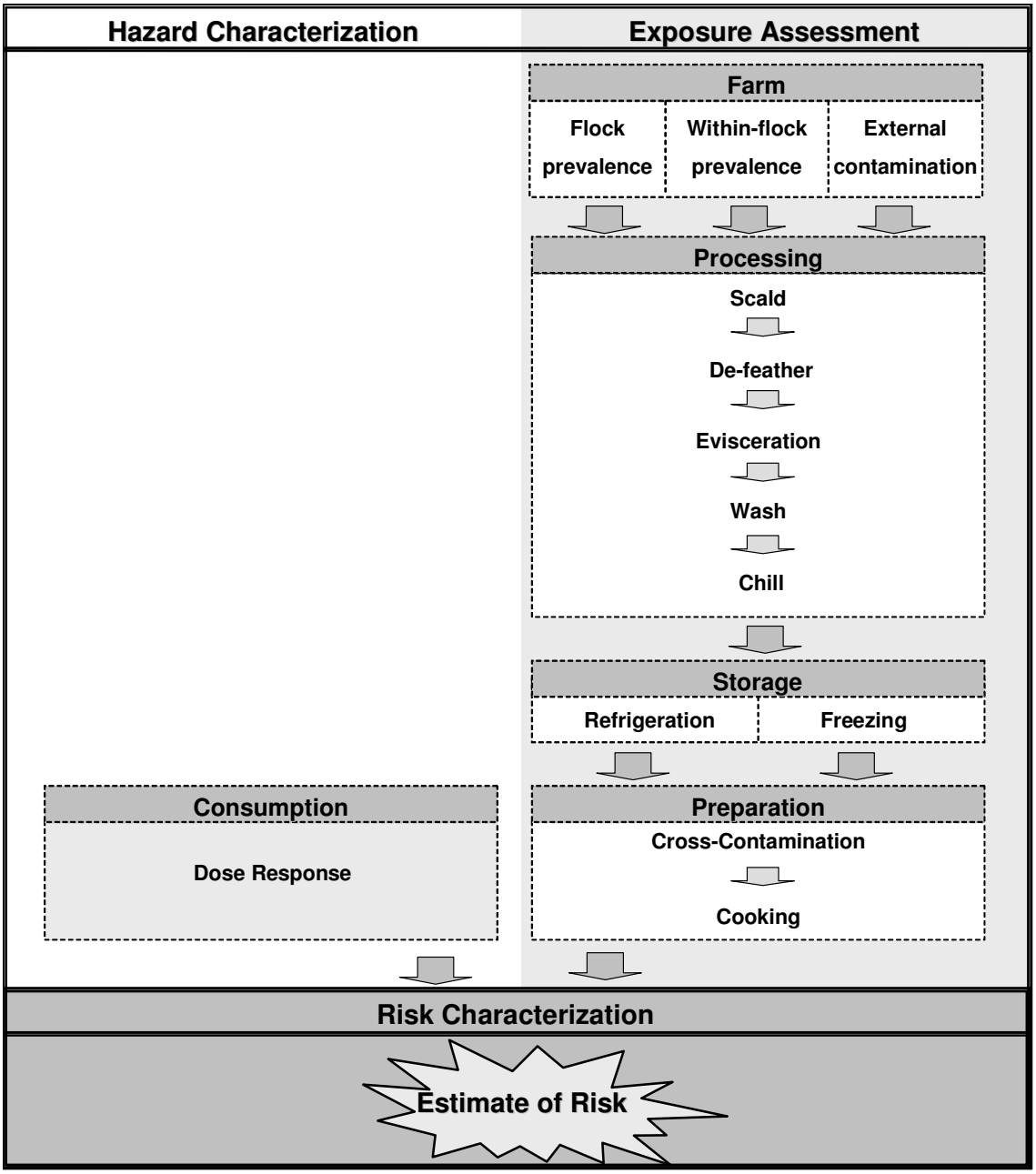

Figure 1. Schematic representation of the risk assessment model for *Campylobacter* in broilers.

Hazard characterization

Hazard characterization describes the adverse health effects of a substance, organism or other entity. This component of the risk assessment usually includes a dose-response relationship. This is represented as a probability that a random member of the population will become infected or ill after exposure to a specific number of *Campylobacter* organisms. The types of data that can be used to establish dose-response relationships include animal and human feeding studies, and epidemiological data, such as data from outbreak investigations.

There is insufficient information in the epidemiological literature to allow a dose-response relationship to be derived using epidemiological data. There is one human feeding trial study reported (Black et al., 1988) using just over one hundred healthy young adult volunteers (in the United States of America). In this risk assessment, the data for *C. jejuni* A3249 and 81-176 were pooled and fitted to the Beta-Poisson dose-response model. For this model, the response was infection. In order to estimate the probability of illness, the conditional probability of illness following infection is required. The human feeding trial data available did not indicate a clear dose-response relationship for the conditional probability of illness following infection. As a result, a conditional probability that is independent of the dose causing infection is applied in this risk assessment. Specifically, once infected, it is assumed that the probability of subsequent illness is not dependent on the original dose ingested.

Risk characterization

The risk characterization step of risk assessment integrates the information collected during the hazard identification, exposure assessment and hazard characterization steps to generate estimates of the probability of adverse events that can be predicted to follow the preparation and consumption of chicken. This step links the probability and magnitude of exposure to *Campylobacter* to the probability of illnesses that might occur.

As stated earlier, the current risk assessment is not intended to describe any one geographical location or system, but rather it was developed and designed to provide relative risk comparisons. It should be understood that the assessment does not provide estimates for the absolute risk attributable to any one system. In order to fully characterize a specific system, the features of that system need to be captured (every system or country is likely to be different to a varying degree), with data specific to that system applied carefully.

For the purposes of this risk assessment, the focus of risk characterization is on scenario analysis. The scenario analysis relies on relative comparisons. Several scenarios were constructed that were intended to reflect and inform the formation of potential risk management strategies. Some of the findings from the scenario analysis are summarized below.

- Reduction in retail prevalence of positive chicken products has a roughly proportional effect in risk reduction.
- Reduction in the contamination level of positive chicken products has a somewhat more complex relationship with the estimate of risk. For highly contaminated products, moderate reductions in the contamination level have relatively mild effects. As the contamination level is further reduced, further reductions have increasing relative impact, and eventually yield significant relative risk reductions.

- Between-flock prevalence is roughly proportional to the risk of illness. The presence of cross-contamination between flocks complicates this slightly due to risk that negative flocks become contaminated by positive flocks during transport and in the slaughter plant.
- Reduction in within-flock prevalence clearly reduces the overall estimate of risk, but with a less-than-proportional rate due to the presence of cross-contamination in the slaughter process, which increases the within-flock prevalence for carcasses during processing.
- A number of scenarios were compared wherein the contamination levels in the processing environment were reduced. The analysis indicates the greatest benefit from reduced total loading of the intestinal tract of birds (thereby reducing the total load on the system). In addition, the benefits of reductions in levels of contamination that take place early in the processing stages can be undermined by cross-contaminating processes later in the processing environment.
- Freezing of poultry will inactivate *Campylobacter* slowly over time. This has been suggested and implemented as a risk mitigation measure, particularly for positive flocks in some countries. The scenario includes the potential that freezing may present countervailing risks through reduced effectiveness of cooking. An example is provided where the net effect of freezing is to increase the risk when both the reduction in numbers and the reduction in the effect of cooking are considered. This is an example of the complex relationships that can be identified and evaluated using such models and which can facilitate more informed risk management decision-making.

These findings represent a sample of the analyses that member countries might conduct by adapting this model of the system. Readers are encouraged to familiarize themselves with the model in order to explore alternate assumptions, the impact of new evidence, and proposed risk management options from production to consumption.

At present, the model can be used in two distinct modes. The first mode is the generation of insight into the complex mechanics of the propagation of the risk of campylobacteriosis through the production, processing and consumer handling subsystems. The development of this insight might be considered a pre-requisite to risk management strategy development. Risk assessors and risk managers could employ the model as a test-bed for ideas on how the system might be managed and how the system reacts to various changes, intentional or otherwise. The use of the model to generate insight into the drivers of risk is limited only by the imagination and resources of the user in adapting or applying, or both, the model to uncover new relationships.

The second mode of use would be to characterize very specific risk mitigation options. The infrastructure to do this is available in the current model. Strategies designed to affect any number of model elements can be evaluated quantitatively by comparing the baseline scenario with the risk estimates that are generated by including the changed model elements caused by the risk management options. Examples of elements that might be modified by risk management strategies include: between-flock prevalence; within-flock prevalence; surface contamination during transport; decontamination through scalding; inactivation effectiveness in chilling water; home storage; or cooking behaviours.

1. INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) assembled an expert drafting group to develop a risk assessment framework for *Campylobacter* spp. in broiler chickens. Initially, the risk management questions posed by the Codex Committee on Food Hygiene (CCFH) were the same as those that were addressed in the risk assessment for *Salmonella* in broiler chickens. The questions requested estimates for:

- the risk of exposure and illness as a result of exposure to *Campylobacter* spp. from broiler chicken products; and
- the change in exposure and illness likely to occur following the implementation of different interventions in primary production, processing and food handling.

The initial framework and the document "Draft Hazard Identification, Hazard Characterization and Exposure Assessment of *Campylobacter* spp. in broiler chickens" were presented to the CCFH for comment in October 2001, in Bangkok, Thailand. In March 2002, work began on completion of the risk characterization portion of the project. The culmination of this work is a risk assessment model and technical document "A Risk Assessment of *Campylobacter* spp. in Broiler Chickens." The model and document include the risk characterization component to complete the risk assessment. Comparisons of risk for a variety of scenarios and mitigation measures were also conducted. The review and analysis of current scientific information and the description of the risk model can be used by FAO and WHO member countries to conduct country-specific risk assessments or to identify and collect the information and research needed to conduct a risk assessment for *Campylobacter* spp. in chicken products.

1.1 Scope of the assessment

In this analysis, the extent of human exposure to *Campylobacter* from consumption of chicken is estimated through the consideration of a variety of pathways. These pathways include practices on the farm, during processing and through consumer handling that may contribute (positively or negatively) to the level of contamination of a chicken product with *Campylobacter*. Furthermore, the risk assessment model is used to assess the effects of mitigation measures at various stages of production and handling. This is performed through examining their impact on the prevalence and levels of *Campylobacter* associated with broiler chickens (or carcasses) through the production to consumption continuum. The changes in prevalence or levels result in changes to the level of risk of illness in the consuming population.

In many countries, *Campylobacter jejuni* is isolated as the agent responsible for more than 90% of cases of campylobacteriosis with *Campylobacter coli* being the second most often isolated species. Except where indicated, when this report is discussing *Campylobacter*, especially human clinical isolates, the reader may assume *Campylobacter jejuni*.

The products considered in this report include fresh whole chickens, and chicken parts (i.e. cut-up, but not comminuted). Frozen chicken is also considered. No evidence was found of different prevalences or concentrations of *Campylobacter* on intact broiler chickens versus broiler parts.

The current risk assessment is not intended to describe any one geographical location or system, but rather it was developed and designed to provide relative risk comparisons, and uses data from various geographical areas. It should be understood that the assessment does not provide estimates for the absolute risk attributable to any one system. In order to fully characterize a specific system,

the features of that system need to be captured (every system or country is likely to differ to a varying degree), with data specific to that system applied carefully.

1.2 Approach: Hazard Identification

The hazard identification provides an in-depth review of available scientific data and information that identifies and qualitatively characterizes evidence regarding the sources of *Campylobacter* infection arising from the consumption of chicken in the human population.

1.3 Approach: Exposure Assessment

The approach for the exposure assessment portion of the model was to develop a model that details the prevalence and concentration of *Campylobacter* throughout the production line from the farm through to consumption, based on the models already prepared in Canada (Fazil et al., 2000), the United Kingdom (Hartnett, 2001; Hartnett et al., 2001), and Denmark (Christensen et al., 2001; Rosenquist et al., 2003). The exposure assessment addresses:

- *Farm and Production Practices* This component considers the pathways on the farm during rearing and transportation by which chickens may become contaminated with *Campylobacter*, and the subsequent spread of the organism through the flock. The impacts of different intervention strategies that may reduce the prevalence of positive flocks or the amount of organism are considered.
- *Slaughterhouse Processing* The processing component considers the main processes involved and their various impacts on both the prevalence and concentration of the organism associated with carcasses. Of special interest are 5 major practices—scalding, de-feathering, evisceration, washing, chilling—that provide opportunities for cross-contamination with *Campylobacter* to broiler carcasses in close proximity. The effectiveness of different intervention strategies that might reduce the prevalence and concentration on slaughtered carcasses are also examined.
- *Post-Processing and Consumer Handling Practices* This can be divided into two sub components, one addressing the impact of storage conditions post-processing and the other considering the impact of cooking and cross-contamination, and their contributions to human exposure to *Campylobacter*.

1.4 Approach: Hazard Characterization

Hazard characterization describes the dose-response relationship and important pathogen and host characteristics that may be associated with infection and illness. Combined with the estimated dose, the result is an estimate of the probability with which individuals may become ill following ingestion of *Campylobacter* at doses estimated in the exposure assessment. In line with current FAO/WHO (2003) guidelines on hazard characterization, this section describes:

- Characteristics of the pathogen, host, and food matrix. These include the influence of infectivity, virulence and pathogenicity on the ability of the organism to elicit infection and illness.
- Host characteristics that could influence acquisition of an infection, such as susceptibility, gender, environmental factors, genetics, underlying disease and concurrent medication. The role of the food matrix is also discussed.

- Public health outcomes, including the likelihood of infection, gastrointestinal illness, septicaemia, and also non-gastrointestinal sequellae such as Guillain-Barré syndrome and reactive arthritis.
- Susceptible populations. Although susceptible populations are an important factor in determining if an illness will progresses to more severe consequences, data are sparse with respect to specific adjustments to the general dose-response relationship. However, the importance of subpopulations is discussed.
- Dose-response model: A detailed description of the dose-response model and related data is provided.

1.5 Approach: Risk Characterization

In the risk characterization, the hazard characterization and exposure assessment are combined to derive estimates for the risks of *Campylobacter* illness from broiler chickens. The overall approach of risk characterization for this document is based on relative risk estimation. A series of scenarios are described that compare different situations and their relative impact on risk compared to a baseline scenario.

2. HAZARD IDENTIFICATION

The hazard identification is an in-depth review of available scientific data and information that identifies and characterizes evidence concerning the sources of *Campylobacter* infection arising from the consumption of chicken in the human population in developed and developing countries. Factors considered include human incidence, characteristics of the organism, sources of infection, risk factors and characteristics of the infection.

2.1 Human incidence of Campylobacter infection in developed countries

In the 1970s, with the development of suitable selective media, it was established that thermotolerant *Campylobacter* was a common cause of bacterial gastroenteritis in humans (Skirrow, 1977). *Campylobacter* is now the leading cause of zoonotic enteric infections in most developed and developing countries (WHO, 2000). The trend for registered human cases per 100 000 inhabitants caused by thermotolerant *Campylobacter* for a number of countries is shown in Figure 2.1. The cases are usually caused by *Campylobacter jejuni,* and to a lesser extent by *Campylobacter coli* (Nielsen, Engberg and Madsen, 1997; Wooldridge and Ketley, 1997; Anon., 1999, 2006b; Nadeau, Messier and Quessy, 2001). In some countries, e.g. United States of America, *C. coli* also contributes to a large part of the human cases (Gillespie et al., 2002). Most human *Campylobacter* infections are classified as sporadic single cases or as part of small, family-related outbreaks. Identified outbreaks are relatively uncommon.

The reported incidence of *Campylobacter* infections has markedly increased in many developed countries since the 1980s and 1990s (Figure 2.1). In some countries, there has been a steady increase during the last decades. In several countries, such as Australia, Denmark, Finland, Ireland, New Zealand, Norway, Sweden, the Netherlands and United Kingdom, the (reported) incidence rate has exceeded that of *Salmonella* (Anon., 2006a, b, c). In a number of countries, the incidence rate has declined in recent years, probably due to interventions in the poultry production chain, e.g. in Denmark, Norway, the Netherlands and United States of America. In Iceland, a significant decrease in the number of human cases between 1999 and 2000 was also attributed to the fact that several interventions and mitigation strategies were introduced in the broiler production industry during this period.

The reported incidence rates of *Campylobacter* infections vary widely among countries (Figure 2.1). In 2004, rates per 100 000 inhabitants ranged from 12.8 cases in United States of America to 299.1 in New Zealand. Some of the variation may in part be explained by differences in surveillance systems, diagnostic methods and means of reporting, so caution should be used when drawing inferences from the data.

It seems likely that there is under-reporting of campylobacteriosis cases, as the incidence rates only reflect the laboratory confirmed cases. Laboratory-confirmed cases are the subset of all cases where the patients have sought treatment through a physician or hospital, and where *Campylobacter* has been detected in a stool sample from the patient. As this represents only a fraction of the true number of infections, the true rate of infection is considered to be much higher (from 7.6 up to 100 times as high) (Skirrow, 1991; Kapperud, 1994; Wheeler et al., 1999; Mead et al., 1999; Samuel et al., 2004).

In 2004, campylobacteriosis was notifiable in 18 of 26 countries in the European Union. Only in France, Germany, Luxembourg, Malta, Poland, Portugal, the Netherlands and United Kingdom is

campylobacteriosis not notifiable in humans (Anon., 2006b). Campylobacteriosis is also notifiable in other countries, including Australia, Canada, New Zealand and Norway.

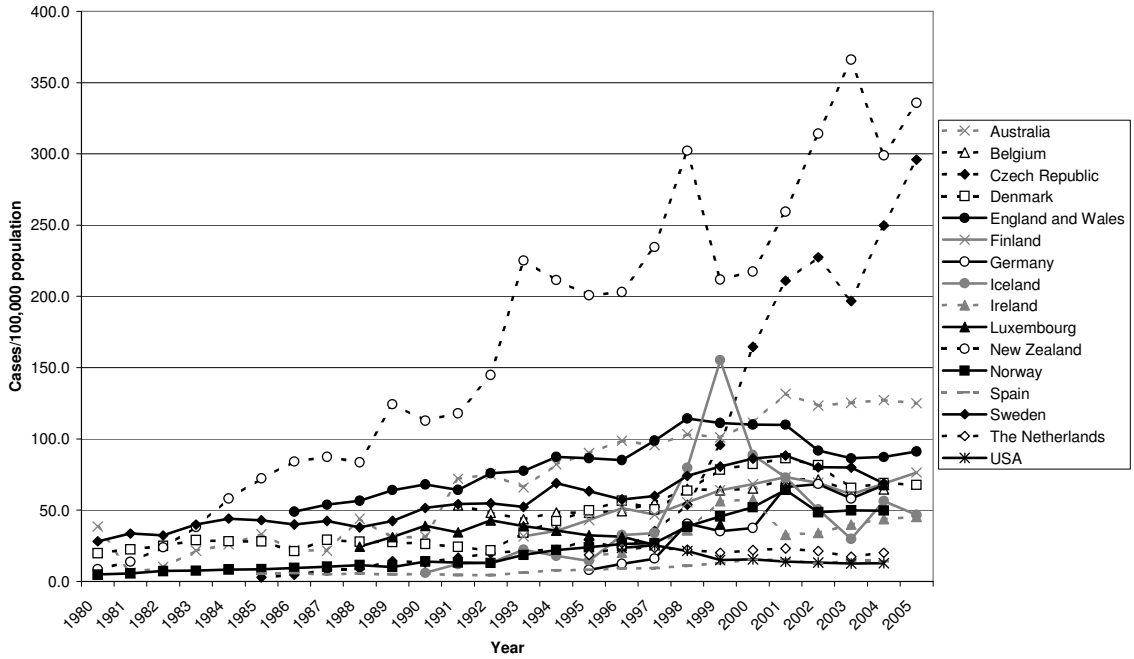

Figure 2.1 The number of registered human cases per 100 000 population caused by *Campylobacter jejuni/coli*. The data presented are those data available to the drafting group in the summer of 2006.

SOURCES: CDC-FoodNet; Anonymous, 1999, 2001a, b, c, d, e, f, g, 2002, 2003, 2004a, 2005a, b, 2006a; Friedman et al., 2000a; Benes, 2001; Kruse, 2001; Georgsson, 2001, pers. comm.

2.2 Human incidence of Campylobacter infection in developing countries

The burden of human *Campylobacter* infections is not known for many developing countries, as national surveillance does not exist. Estimates of incidence in developing countries are from laboratory-based surveillance studies, giving estimates for the general population from 5 to 20% (Coker et al., 2002). This is quite similar to the general incidence in developed countries. However, available data suggest a significantly higher incidence rate for campylobacteriosis in children. Case-control community-based studies have provided estimates of 40 000 to 60 000 per 100 000 population for children below 5 years of age, making campylobacteriosis a paediatric disease in developing countries (Coker et al., 2002). Campylobacteriosis contributes, for example, significantly to malnutrition, as campylobacteriosis is particularly acute during the weaning period (WHO, 2001).

While asymptomatic carriers are considered to be infrequent in developed countries, recovery of *Campylobacter* from children under the age of 5 without diarrhoea is common in developing countries. In a study from Algeria, no significant difference was found in the isolation rates for symptomatic and asymptomatic children (Megraud et al., 1990). In a cohort of 179 children in Mexico under 5 years of age, there was an average infection rate of 66% over a year.

Approximately 22% of these cases were associated with diarrhoea (Calva et al., 1988) suggesting that the incidence of *Campylobacter*-associated diarrhoea in children is greater than 20 000 cases per 100 000.

In a case-control study in 2002, in India, 13.5% of people with diarrhoea tested positive for *Campylobacter*, while only 0.6% of asymptomatic controls were positive. *Campylobacter* infection was significantly higher in children under the age of 5 years (Jain et al., 2005). In Bangladesh, *C. jejuni* was isolated from 25.5% of 102 children with diarrhoea and from 8.6% of children without symptoms. The isolation rate was higher in children under the age of 1 year (32.8%) compared with children above the age of 1 year (15.9%) (Haq and Rahman, 1991). In Cameroon, *Campylobacter* was isolated in 21 of 272 children aged 1 to 4 years (7.8%), while 3.2% of controls were positive for the organism. The researchers found that approximately 28% of the infected children were exposed to live chickens, while 23.8% may have been exposed through stream water (Koulla-Shiro, Loe and Ekoe, 1995). In Harare, Zimbabwe, children under the age of 5 years with diarrhoea were infected with *Campylobacter* 9.3% of the time (Simango and Nyahanana, 1997). In Thailand, *Campylobacter* spp. were isolated from approximately 28% of children under the age of 12 years with diarrhoea, the mean age being 17.9 months. *C. jejuni* and *C. coli* accounted for 80% and 20% of the cases, respectively (Bodhidatta et al., 2002). However, in Singapore, isolation rates of *Campylobacter* were low (1.2%) (Lim and Tay, 1992).

Although *C. jejuni* is responsible for the majority of *Campylobacter* infections in both developing and developed countries, species such as *C. coli*, C. *lari*, *C. upsaliensis*, C. *hyointestinalis* and others may also be responsible for a larger percentage of infections, albeit milder, in developing countries (Georges-Courbot et al*.,* 1986). *C. coli* was isolated in 41% of samples from children in China, Hong Kong Special Administrative Region (Hong Kong SAR) (Ho and Wong, 1985) and in 39% of the samples from children with *Campylobacter* infections in Central African Republic (Georges-Courbot et al., 1986). *Campylobacter* infections from non-*C. jejuni* species in Thailand were found to be more prevalent, milder and usually not associated with bloody diarrhoea (Taylor et al*.,* 1993).

In developing countries, children are constantly exposed to *Campylobacter* and, as a consequence, they develop serum antibodies very early in life. The level of antibodies in children from developing countries tends to be higher compared with the antibody level in children from developed countries. Furthermore, the level of antibodies tends to increase with age. The immunity seen among adults in developing countries is absent in adults in developed countries (Coker et al., 2002).

In summary, in developing countries *Campylobacter* is primarily the cause of campylobacteriosis in children below 5 years of age, and especially children under the age of one. Furthermore, *C. jejuni* accounts for the majority of the incidences.

2.3 Seasonal variation

In developed countries, *Campylobacter* infections are largely sporadic and observed during the warmer months of the summer and autumn, and suggest a seasonal pattern. Seasonal variation has been described in several areas, e.g. Europe, United States of America, and to some degree in New Zealand (Nylen et al., 2002). In developing countries, information is limited on seasonality of campylobacteriosis. However, a seasonal variation does not seem to be observed, perhaps because the temperature variations in some developing countries are not as extreme as in many of the developed countries (Coker et al., 2002). In any case, seasonality of illness in developing countries cannot be discounted at present; the lack of public health infrastructure and epidemiological surveillance data do not permit the detection of any seasonal variation at the current time.

2.4 Characteristics of the organism

Bacteria belonging to the genus *Campylobacter* are non-sporeforming, oxidase-positive, Gram-negative rods. Cells are pleomorphic. Log-phase cells have a characteristic slender, curved or spiral shape and have flagella, usually single, at one or both poles (monotrichate or amphitricate) and are highly motile, spinning around their long axes and frequently reversing direction. As cultures age, spiral or curved forms may be replaced by coccoid forms (Barrow and Feltham, 1993).

In general, *Campylobacter* species do not grow in conventional aerobic or anaerobic culture systems. *Campylobacter* are oxygen-sensitive micro-aerophilic bacteria, with optimal growth in an atmosphere containing 5-10% oxygen and 1-10% carbon dioxide. They do not ferment or oxidize sugars and are sensitive to hydrogen peroxide and superoxide anions produced in media. To neutralize these toxic products of oxygen and to increase the aerotolerance of the organisms, SOD (superoxide dismutase), catalase (Hoffmann et al., 1979), lysed blood, FBP (0.025% each of ferrous sulphate, sodium metabisulphite and sodium pyruvate) (ICMSF, 1996), or CFP (0.4% charcoal, 0.025% ferrous sulphate and 0.025% sodium pyruvate) (Bolton and Coates, 1983) can be added to enrichment broths and selective agars to increase aerotolerance. Oxygen sensitivity is, however, dependent on the growth media, and results obtained in one substrate do not necessarily apply to others (Hodge and Krieg, 1994).

C. jejuni and *C. coli* are distinguished from most other *Campylobacter* species by their high optimum growth temperature (42°C). *C. jejuni* has two subspecies: subsp. *jejuni* – the more common cause of enterocolitis in man; and subsp. *doylei* – a more fastidious and slower growing organism that does not grow at 43°C. *C. upsaliensis* also appears to be enteropathogenic for man and is related to the thermotolerant *Campylobacter,* even though not all strains grow at 42°C. *C. upsaliensis* is seldom detected by conventional methods used for *C. jejuni* and *C. coli*. Primary isolation of this organism usually requires the use of selective filtration, non-selective media and incubation at 37°C. Additionally, *C. upsaliensis* requires H_2 or formate for micro-aerophilic growth (Holt et al*.,* 1994). *C. lari* is thermotolerant like *C. jejuni* and *C. coli,* but is considered to be of low virulence and is only occasionally encountered in man (Barrow and Feltham, 1993; Anon., 2006b).

Due to the different growth requirements of different *Campylobacter* species, detection and isolation of *Campylobacter* from foods and food-animal matrices is dependent on the types of media used and isolation and laboratory method used.

2.4.1 Growth and survival

In general, *Campylobacter* spp. grow at 37°C, but not below 30°C (Table 2.1). It is therefore reasonable to assume that *Campylobacter* spp. do not multiply during processing, post-processing, refrigerated transport and in refrigerated storage. However, the organisms can survive these steps, especially when the temperature is low. On chilled, raw chicken and pork skin, *C. jejuni* and *C. coli* have been found to survive for several weeks (Solow, Cloak and Fratamico, 2003).

The reduction obtained by chilled storage is reported to be between 0.6 and 1.0 $\log_{10}$ cfu/g (Oosterom et al*.,* 1983b; Yogasundram and Shane, 1986). The reduction obtained by freezing at -18°C for 1 to 4 weeks is between 0.1 and 2.87 $\log_{10}$ cfu/g. Further storage at freezing temperatures decreases the *Campylobacter* counts further, but the largest decrease occurs during the initial freezing period (Hänninen, 1981; Oosterom et al., 1983b; Yogasundram and Shane, 1986; Solow, Cloak and Fratamico, 2003; Thorkelsson et al., 2003; Bhaduri and Cottrell, 2004; Georgsson et al*.,* 2006). A recent Norwegian study (Sandberg et al*.,* 2005) revealed a continuous reduction in $\log_{10}$ cfu/g up to 120 days. After 10 days there was a reduction of approximately 1.0 $\log_{10}$ cfu/g (90%), and after 21 days approximately 2.0 $\log_{10}$ cfu/g (99%). Due to the documented reducing effect of

freezing, this technique has been implemented as intervention in broiler processing in Norway (Hofshagen and Kruse, 2003), Iceland (Reiersen et al., 2003) and Denmark.

Table 2.1 Growth characteristics of thermophilic *Campylobacter* species (ICMSF, 1996).

	Minimum	Optimum	Maximum
Temperature (°C)	30	42-43	45
pH	4.9	6.5-7.5	~ 9
NaCl (%)	—	0.5	1.5
Water activity (a_w)	>0.987	0.997	—
Atmosphere	—	5% O_2 + 10% CO_2	—

In water, and other environments with suboptimal growth conditions, *Campylobacter* may convert into a 'viable but non-culturable state'. The importance of this state in the transmission of *Campylobacter* to animals and man is an area of debate. Question remain as to whether the viable but non-culturable organisms are still virulent or if they can revert into a culturable, virulent state after passage through a host. In some studies, viable but non-culturable campylobacters have been shown to regain culturability after passage, for example through chickens (Stern et al., 1994), mice (Jones, Sutcliffe and Curry, 1991; Baffone et al., 2006), rats (Saha, Saha and Sanyal, 1991), and embryonated eggs (Cappelier et al., 1999). In other studies it has not been possible to demonstrate that viable but non-culturable forms can regain culturability in simulated gastric, ileal and colon environments (Beumer, de Vries and Rombouts, 1992), one-day-old chickens (Medema et al., 1992; Fearnley et al., 1996), broth (Boucher et al., 1994), chicken guts (Korsak and Popowski, 1997), and in caeca over two weeks, when given, by gavage, to day-of-hatch chickens and broiler chickens (Ziprin et al., 2003; Ziprin and Harvey, 2004). The possible influence of viable but non-culturable forms of *Campylobacter* on human health is not addressed in the present risk assessment, as their role and relative importance with respect to the contamination of poultry is still unknown. Given the relatively high prevalence and abundant numbers of culturable organisms in poultry production environments, it seems unlikely that viable but non-culturable forms of *Campylobacter* play an important role within the pathways considered in this risk assessment. If this risk assessment were extended to include more detailed consideration of environmental reservoirs of *Campylobacter*, then this exclusion might need to be revised.

2.4.2 Death or inactivation

Campylobacter are particularly sensitive to drying and reduced pH. For example, *Campylobacter* growth minimum is at pH 4.9. Growth optimum is pH 6.5 to 7.5. In addition, *Campylobacter* is sensitive to salt concentrations above 1.5% (ICMSF, 1996). *C. jejuni* and *C. coli* are sensitive to heat and do not survive cooking or pasteurization temperatures with D-values of 0.21–2.25 minutes at 55–60°C (ICMSF, 1996).

2.5 Reservoirs

The principal reservoir of pathogenic *Campylobacter* spp. is the alimentary tract of wild and domesticated mammals and birds. Several countries have monitoring programmes to determine the prevalence of *Campylobacter* in food producing animals and birds. The results of these programmes have earlier been reported to WHO and published by the Community Reference Laboratory on the Epidemiology of Zoonoses (BgVV, Berlin) and are currently being reported to and published by the European Food Safety Authority (EFSA). From these reports, it is evident that *Campylobacter* is

commonly found in broilers, broiler breeder flocks, cattle, pigs, sheep, wild animals, birds and dogs (Anon. 2001a; 2006b). Other investigations have shown that healthy puppies and kittens (Hald and Madsen, 1997), rodents (Cabrita et al., 1992; Berndtson, 1996), beetles (Jacobs-Reitsma et al., 1995), and flies (Rosef and Kapperud, 1983; Berndtson, 1996; Hald et al., 2004; Nichols, 2005) may also carry *Campylobacter*.

C. jejuni is predominantly associated with poultry (Tauxe, 1992; Anon., 1998a; 1999; 2001a; 2006b; Nadeau, Messier and Quessy, 2001), but has also been isolated from cattle, sheep, goats, dogs and cats (Nielsen, Engberg and Madsen, 1997; Anon., 1999; 2006b). *C. coli* is predominantly found in pigs (Rosef et al., 1983; Nielsen, Engberg and Madsen, 1997; Boes et al., 2005; Jensen et al., 2006), but has also been isolated from poultry, cattle, and sheep (Anon., 1999; 2006b).

A seasonality of broiler flock colonization has been observed in some countries, leading to a peak in flock prevalence during the warm summer months (Kapperud et al., 1993; Jacobs-Reitsma, Bolder and Mulder, 1994; Newell et al., 1999; Christensen et al., 2001). However, studies conducted in the United Kingdom, United States of America and Canada (Quebec) have not observed such seasonal influence on the prevalence (Humphrey, Henley and Lanning, 1993; Gregory et al., 1997; Nadeau, Messier and Quessy, 2001). The influence of season may be associated with the increased ventilation of houses and the increased amount of insects during the warm summer and autumn months. If large volumes of air are introduced, it is conceivable that flies with *Campylobacter* from the outside are introduced into the flock.

Water is an important part of the ecology of *Campylobacter*. *Campylobacter* has been isolated from surface water, rivers and lakes at prevalences up to about 50% (Bolton et al., 1987; Carter et al., 1987; Brennhovd, Kapperud and Langeland, 1992; Arvanitidou et al., 1995; Eyles et al., 2003; Kapperud et al., 2003). *Campylobacter* is introduced into the water by sewage and faeces from wild animals and birds. Along the coast of Tel Aviv, *C. jejuni* was isolated at levels ranging from 2 to 13 cfu per 100 ml seawater, and 13 to 20 cfu per g sand (Ghinsberg et al., 1994). In the United Kingdom, *Campylobacter* spp. were isolated in 46 out of 92 samples of sand (50%) from beaches not meeting the EEC Bathing Water Directive standard, and in 36 of 90 samples of sand (40%) from beaches meeting the EEC standard. Further, *C. jejuni* and *C. coli* were isolated more frequently in sand from beaches that did not meet the EEC standard (Bolton et al., 1999).

The isolation frequency of *Campylobacter* from water is highest in cold winter months (Carter et al., 1987; Brennhovd, Kapperud and Langeland, 1992). This may be explained by higher survival persistence at low temperatures around 4°C compared with higher temperatures below growth temperature. A study has shown that *C. jejuni* remains recoverable for up to four months when suspended in aged, filter-sterilized stream water held at 4°C. At 25°C and 37°C the bacteria become non-culturable within 28 and 10 days, respectively (Rollins and Colwell, 1986). Variations in exposure to daylight may also contribute to the high isolation frequency in winter and low isolation frequency in summer. In seawater, *Campylobacter* has been found to survive for 24 h in darkness and 30 to 60 minutes in daylight (Jones, Betaieb and Telford, 1990).

2.6 Contamination during processing

As a common inhabitant of the gastrointestinal tract of warm-blooded animals, *Campylobacter* spp. can be expected to contaminate the meat during slaughter and evisceration as a result of faecal contamination. The more faecal material that is spread, the more *Campylobacter* there will be on the meat. The slaughter of pigs and cattle is generally more hygienic than processing of broilers. Therefore, beef and pork are generally less contaminated with *Campylobacter* than chicken meat (Rosenquist and Boysen, unpublished). The lower contamination rates are also due to differences in processes. For cattle and pigs, the concentration of *Campylobacter* declines during slaughter,

probably due to dehydration of the meat surface during cooling (Oosterom et al., 1983a). An investigation of 600 pig carcasses has, for example, showed that the chilling procedure reduced the prevalence of *Campylobacter* contaminated carcasses from 43–85% to 11–18% (Sørensen and Christensen, 1996). De-hiding of cattle also lowers the contamination of the meat.

During processing of broiler chickens, the level of *Campylobacter* contamination present on the broiler carcasses will fluctuate (Figure 2.2). The process operations that have been found to cause the greatest changes in the contamination are: scalding, de-feathering, evisceration, washing and chilling (Oosterom et al., 1983b; Wempe et al., 1983; Izat et al., 1988; Cason et al., 1997; Berrang and Dickens, 2000; Stern and Robach, 2003; Rosenquist et al., 2006). The relative changes during the processes are almost the same in the various studies, despite the use of different methods for sampling and quantification, and indicate that the concentrations of *Campylobacter* in commercial broiler slaughter plants may be relatively uniform.

2.7 Contamination of retail products

Reports from EU as well as other countries reveal that fresh poultry meat is the food vehicle most frequently contaminated with *Campylobacter* (Anon., 1998a; 1999; 2001a; 2006b; Taremi et al., 2006). In some member states in EU in 2004, the prevalences were as high as 83.0%. In Iran, a prevalence of 63% has been reported (Taremi et al., 2006). In Japan, 45.8% of retail poultry was contaminated with *Campylobacter* (Ono and Yamamoto, 1999).

The species predominantly isolated in chicken meat is *C. jejuni,* as in live poultry. However, high proportions of *C. coli* are observed in some countries, e.g. the Czech Republic (Anon., 2006b) and the United States of America. A high prevalence of *Campylobacter* (77%) has also been found in lamb liver in a study in Northern Ireland (Anon, 2001a). At low frequencies, *Campylobacter* has been found in beef, pork, other meat products, raw milk and milk products, and in fish and fish products (Anon., 2006b; Taremi et al., 2006). Other food products from which *C. jejuni* has been isolated include mushrooms (Doyle and Schoeni, 1986); fresh vegetables, such as spinach, lettuce, radish, green unions, parsley and potatoes (Park and Sanders, 1992); and modified-atmosphere packaged foods, such as unsmoked bacon and salad vegetables (Phillips, 1998).

Recently, a few countries have also reported numbers of thermotolerant *Campylobacter* in food. Counts in fresh, chilled chickens have been reported to range from the detection limit up to 7 log cfu/g, depending on type of product (Dufrenne et al., 2001; Jørgensen et al., 2002; Anon., 2004b; Scherer et al., 2006).

It is generally believed that the contamination of meat with *Campylobacter* predominately is a surface contamination. However, recent studies have shown that *Campylobacter* may also be present in internal tissues of chicken legs, though at very low concentrations. A study in Germany showed that 66% of chicken legs were positive on the skin at a median of 2.4 log cfu/g skin. In the muscles, the prevalence was 27%, the concentration being under the detection limit (<0.3 MPN *Campylobacter* per g) for most samples (Scherer et al., 2006).

Seasonality has been found to influence the *Campylobacter* prevalence in retail chicken meat, as also observed for the flock prevalence (Willis and Murray, 1997; Rosenquist and Nielsen, 1999). Particularly in northern countries, the likelihood of buying a *Campylobacter* contaminated chicken is highest during summer and early autumn.

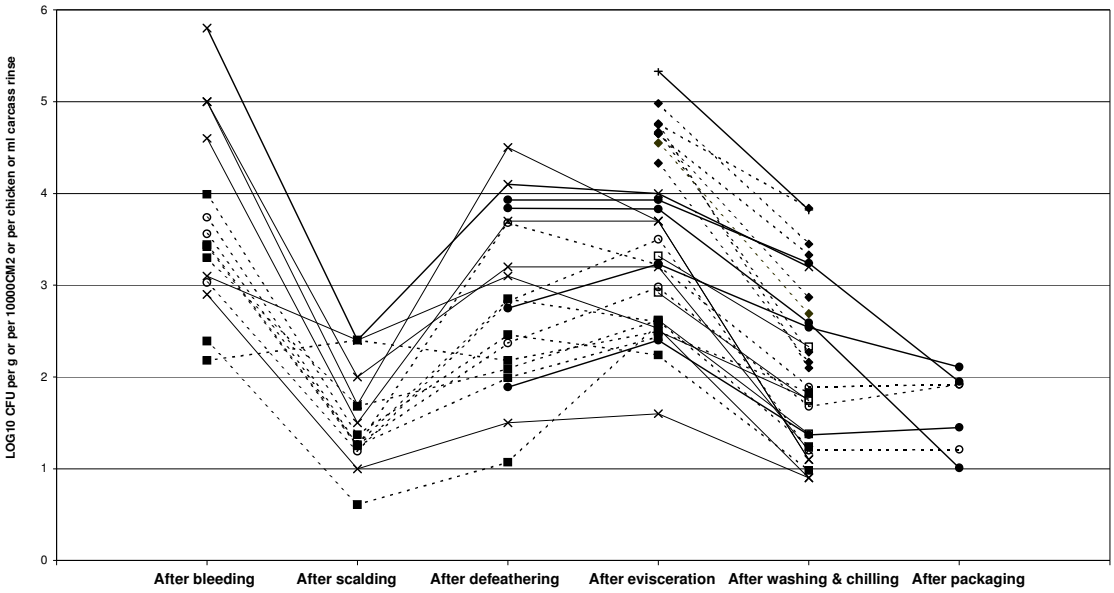

Figure 2.2 Tendencies regarding the influence of selected processing operations on the *Campylobacter* contamination of broiler carcasses.
KEY: ■, Oosterom et al., 1983b; □, Wempe et al., 1983; ○, Izat et al., 1988; +, Cason et al., 1997; ×, Berrang and Dickens, 2000; ♦, Stern and Robach, 2003; ●, Rosenquist et al., 2006.

2.8 Risk factors in developed countries

Campylobacter may be transmitted from the principal reservoirs to humans by direct contact with contaminated animals or animal carcasses, or indirectly through the ingestion of contaminated food or water. For current purposes, the primary risk in developed countries is considered to be the food-related risk factors.

2.8.1 Food-related risk factors

The possible risk factors related to sporadic cases of human campylobacteriosis have been investigated in several case-control studies conducted worldwide. The results are summarized in Table 2.2. Most studies have identified handling of raw poultry and the consumption of poultry products as important risk factors, accounting for a variable percentage of cases. Also, cross-contamination of *Campylobacter* from raw chicken to prepared food has been identified as a risk factor. Harris, Weiss and Nolan (1986) observed an association between infection and not washing the kitchen cutting board with soap. Other food-related risk factors that have repeatedly been identified include consumption of other meat types, undercooked or barbecued meat, raw seafood, drinking untreated surface water, or unpasteurized milk or dairy products. Eating meat cooked outside the home (at restaurants) has also been identified as a risk factor in the United States of America and New Zealand. Other food items that have been related to sporadic cases of human campylobacteriosis are contaminated shellfish (Griffin et al., 1983; Harris, Weiss and Nolan, 1986).

Table 2.2 Overview of case-control studies.

Country	Risk factors	Reference
Australia	Puppies (OR 16.58) Pet chickens (OR 11.80) Consumption of mayonnaise (OR 4.13)	Tenkate and Stafford, 2001
Denmark	Consumption of undercooked poultry (OR 4.5, OR 8.2) Consumption of red meat at a barbecue (OR 2.3, OR 4.1) Consumption of grapes (OR 1.6, OR 2.8) Drinking unpasteurized milk (OR 2.3, OR 11.8)	Neimann et al., 2003
Denmark	Consumption of fresh, unfrozen chicken (5.8)	Wingstrand et al., 2006
Germany	Consumption of poultry (OR -)	Newell, 1982
Netherlands	Eating chicken (OR -) Eating chicken at barbecue (OR -) Pork (OR -)	Oosterom et al., 1984
New Zealand	Eating poultry at a friend's house (OR 3.18) Eating poultry at a barbecue (OR 3.00) Eating undercooked chicken (OR 4.94) Drinking water from a non-urban supply (OR 2.7) Consumption of chicken bought fresh (OR 1.8)	Ikram et al., 1994
New Zealand	Eating raw or undercooked chicken (OR 4.52) Chicken eaten at restaurants (OR 3.85) Overseas travel (OR 4.43) Untreated water (OR 2.2) Consumption of raw dairy product (OR 3.10 or 12.00) Contact with puppies (OR 2.67) Contact with cattle/calves (OR 2.29/2.27)	Eberhart-Phillips et al., 1997
Norway	Barbecuing (OR 7.6) Daily contact with dog (OR 4.3) Eating poultry bought raw (OR 3.2)	Kapperud et al., 1992
Norway	Drinking undisinfected water (OR 1.9, OR 2.5) Barbecuing (OR 3.2, OR 4.1) Eating poultry bought raw (OR 1.3, OR 1.4) Occupational exposure to animals (OR 10.3, OR 19.3) Eating undercooked pork (OR 9.0, OR 37.0)	Kapperud et al., 2003
Sweden	Drinking unpasteurized milk (OR 3.56) Consumption of chicken (OR 2.29) Consumption of pork with bones (OR 2.02) Barbecuing (OR 1.98) Daily contact with chickens (OR 11.83) Living or working on a farm (OR 3.06)	Studahl and Andersson, 2000
Switzerland	Travel abroad (OR 21.2) Foreign citizenship (OR 6.7) Eating chicken liver (OR 5.7)	Schorr et al., 1994
United Kingdom	Drinking milk from bottles with tops pecked by birds (OR 15.24)	Lighton, Kaczmarski and Jones, 1991
United Kingdom	Consumption or handling of milk from bottles attacked by birds (OR 15.5-42.1)	Southern, Smith and Palmer, 1990
United Kingdom	Occupational contact with raw meat (OR 9.37) Pet with diarrhoea in household (OR 2.39) Drinking untreated water (OR 4.16)	Adak et al., 1995
United Kingdom	Foreign travel (OR 3.4) Eating chicken (OR 1.4) Drinking milk from bottles damaged by birds (OR 3.3) Diabetes mellitus (OR 4.1) Consumption of medication omeprazole (OR 3.5, CI 1.1–12) and H_2 and H_2 antagonists (OR 3.7, CI 1.3–15) Contact with puppies (OR 2.67)	Neal and Slack, 1997
USA	Consumption of chicken (OR -) Handling raw chicken (OR -)	Hopkins and Scott, 1983

Country	Risk factors	Reference
USA	Drinking untreated water (OR 10.7) Drinking raw milk (OR 6.9) Eating undercooked chicken (OR 2.8) Cat in household (OR 3.2)	Hopkins, Olmsted and Istre, 1984
USA	Consumption of raw milk (OR 4.6) Consumption of mushrooms (OR 1.5)	Harris, Weiss and Nolan, 1986
USA	Consumption of raw milk (OR -)	Schmid et al., 1987
USA	Eating chicken prepared commercially (OR 1.8) Consumption antibiotics (OR 3.3)	Effler et al., 2001
USA	Contact with poultry (OR 6.9)	Potter, Kaneene and Hall, 2003
USA	Travel abroad Consumption of chicken prepared at restaurant (and other meats) (PAF 24.0)	Friedman et al., 2004

NOTES: OR = odds ratio. PAF = Population attributable fraction.

Some of the risk factors that have been associated with *outbreaks* of campylobacteriosis are the consumption of unpasteurized milk, foods especially chicken, untreated surface water and contaminated public and private water supplies (Finch and Blake, 1985; Pebody, Ryan and Wall, 1997; Engberg et al., 1998; Pearson et al., 2000; Neimann, 2001; CDC, 2002; Allerberger et al., 2003; Kuusi et al., 2004; Schuster et al., 2005; Mazick et al., 2006; Anon., 2006b). Contaminated cucumbers have also caused an outbreak (Kirk et al., 1997).

Outbreaks and sporadic cases may have different epidemiological characteristics. For example, the sporadic cases seem to peak in summer, whereas the outbreaks (based on 57 outbreaks in the USA) seem to culminate in May and October (Tauxe, 1992).

2.8.2 Other risk factors

Other risk factors that have been identified are acquiring an infection during travelling, contact with pets and farm animals, and recreational activities in nature.

Exposure during travel abroad seems to be a common risk factor in the northern European countries and the USA. In Denmark and the United Kingdom, travelling abroad has been estimated to account for 10 to 25% of the reported cases (Cowden, 1992; Neal and Slack, 1995; Mølbak et al., 1999). In Sweden and Norway, the estimated percentage is 40 to 60% (Kapperud and Aasen, 1992; Berndtson, 1996; Anon., 2006b). Campylobacteriosis has primarily been associated with travel to the Mediterranean countries and Asia (Kapperud, 1994; Mølbak et al., 1999; Neimann, 2001; Wingstrand et al., 2006); however, this must be considered in proportion to the relative frequency of travelling to these destinations.

Several investigations have indicated contact with pets, particularly young pets like kittens and puppies, as a behaviour increasing the risk of acquiring infection by *Campylobacter* spp. (Blaser et al., 1978; Hopkins, Olmsted and Istre, 1984; Deming et al., 1987; Brieseman, 1990; Kapperud, 1994; Adak et al., 1995; Neimann, 2001; Tenkate and Stafford, 2001). Hald and Madsen (1997) found that 29% of healthy puppies examined carried *Campylobacter* spp. with a species distribution of 76% *C. jejuni*, 5% *C. coli* and 19% *C. upsaliensis*. Only 5% of 42 healthy kittens examined excreted *C. upsaliensis*.

The information about the risk associated with professional handling of production animals at farm level is contradictory. Saeed, Harris and DiGiacomo (1993) found no increased risk for *Campylobacter* enteritis associated with contact with various animals. However, exposure to

diarrhoegenic animals was associated with a four-fold increase in the risk of human campylobacteriosis. In addition, Brieseman (1990), Skirrow (1987) and Kist and Rossner (1985) described a higher incidence of campylobacteriosis in the rural population than in the population living in urban areas. In a case-control study, Studahl and Andersson (2000) identified living or working on a farm as a potential risk for campylobacteriosis, and Kapperud et al. (2003) found that occupational exposure to animals did increase the risk for human infection. In contrast, Adak et al. (1995) demonstrated that occupational contact with livestock or their faeces was associated with a decrease in the risk of becoming infected by *Campylobacter* spp. Another investigation has revealed a higher incidence among the urban population than in the population living in rural areas (Kapperud and Aasen, 1992).

As a consequence of the presence of *Campylobacter* spp. in the environment, and in particular in untreated water, recreational activities taking place in nature, such as camping, trekking and bathing, could pose a risk of acquiring an infection by *Campylobacter* spp. In a case-control study carried out by Adak et al. (1995), it was found that ingestion of untreated water while participating in recreational activities was associated with an increased risk of acquiring campylobacteriosis. This was also suggested in earlier studies by Hopkins, Olmsted and Istre (1984) and Skirrow (1987). Schönberg-Norio et al. (2004) identified swimming in natural water sources as a risk factor for sporadic *Campylobacter* infection. In contrast to this, another case-control study in Norway found that swimming in the sea, lakes and swimming pools was associated with a reduced risk for *Campylobacter* infection (Kapperud et al., 2003).

2.8.3 Person-to-person transmission

In developed countries, person-to-person transmission is considered to be infrequent (Altekruse et al., 1999; Ethelberg et al., 2004). Some experts explain this by the relatively low percentage of asymptomatic carriers (Engberg et al., 2000; de Wit et al., 2001), but it could also be postulated to be due to a loss of human virulence on passage through the human host, as patients suffering from campylobacteriosis shed large numbers of *Campylobacter,* probably without infecting close relatives. A few examples of person-to-person transmission have, though, been observed. For example, in an outbreak in Kansas (USA), among people attending a school luncheon, the only source of transmission of *Campylobacter* seemed to be the food handler, who happened to have a *Campylobacter* infection (Olsen et al., 2001). In developing countries, human carriage may play a larger role in the transmission of infection, as asymptomatic carriage of *Campylobacter* is more frequent (Blaser, Taylor and Feldman, 1983; Coker et al., 2002).

2.8.4 Relative importance of the risk factors

So far it has not been possible to quantify the number of *Campylobacter* cases related to each of the different risk factors described. However, the relative importance of the potential sources of *C. jejuni* for human cases of campylobacteriosis has been investigated in several studies by applying different subtyping methods to isolates of *C. jejuni* obtained from patients and the possible sources described (see below). In addition, the attributable fraction of risk factors has been investigated in several case-control studies. Friedman et al. (2004) found that, for people who had not travelled, the largest population-attributable fraction (24%) was related to chicken prepared at a restaurant. A Danish case control study indicated that 5 to 8% were attributed to undercooked poultry, and 15 to 20% to barbequing poultry meat, beef and pork (Neiman et al., 2003). From these studies, it seems evident that chickens contribute to a large part of the human cases, as there is overlap between subtypes and as handling and eating chicken are common risk factors. However, the exact attribution to campylobacteriosis from chicken meat is not known.

The importance of poultry as a risk factor for human cases has been demonstrated in countries where interventions have been implemented in the broiler production chain or where poultry has been withdrawn from the market, and where a decline in human cases has followed. For example, in Belgium, due to the dioxin crisis in 1999, where all poultry meat and eggs were withdrawn from the market, the estimated reduction of campylobacteriosis cases following this event was 40% within the crisis period (Vellinga and Van Lock, 2002). Another example is in Iceland, where introduction of fresh poultry meat on the market in the 1990s was followed by a dramatic increase in the incidence of human campylobacteriosis. By introducing strict control measures during 2000, including monitoring of all flocks and freezing of contaminated carcasses, the annual human incidence was reduced by 70%, which documents that poultry was a major determinant for human campylobacteriosis in Iceland in the late 1990s (Stern et al*.,* 2003)

Subtyping investigations have shown the following results. Hudson et al. (1999) reported similarities in the distribution of serotypes of *C. jejuni* isolated from humans, water and chickens. Fricker and Park (1989) demonstrated similarities in the serotypes between isolates of *C. jejuni* originating from humans, offal, beef, sewage and poultry. Bänffer (1985) found a positive correlation in the frequencies of bio- and serotypes of *C. jejuni* isolated from humans and chickens, whereas isolates from humans and pigs showed no correlation. Frost et al. (1999) and Kramer et al. (2000) showed that the distribution of *C. jejuni* serotypes isolated from chicken and lamb was similar to that seen in concurrent human infections. Wareing et al. (1999) have described a strain of *C. jejuni* (Penner serotype HS4, 'complex': Preston phage-group 55) that has frequently been associated with human gastroenteritis in the United Kingdom. This strain seems to have a global distribution and has been shown to be the causative agent in several milk-borne outbreaks of human campylobacteriosis. Using a PFGE subtyping method, Hänninen et al. (2000) demonstrated indistinguishable genotypes of *C. jejuni* isolated from cases of human infections and retail chicken meat in Finland. In Denmark, similarities between *C. jejuni* serotypes have been demonstrated among isolates from humans, broilers and poultry products, and—to a lesser extent—cattle, with serotype O:2 being the most dominant type (Anon., 1998b; Nielsen, Engberg and Madsen, 1997; Nielsen and Nielsen, 1999; Nielsen et al., 2006). For *C. coli,* similarities between serotypes isolated from humans, broilers, pigs and retail poultry products have been described. In a Canadian study, macro-restriction profiling has revealed that approximately 20% of human *Campylobacter* isolates were genetically related to genotypes found in poultry (Nadeau, Messier and Quessy, 2001).

Several studies have identified multiple strains of *C. jejuni* or *C. coli* on contaminated meat (Kramer et al., 2000; Zorman et al., 2006). Furthermore, co-infection with more than one *C. jejuni* strain has been observed in samples from people with diarrhoea (Richardson et al., 2001). Therefore, an exact tracing and account of the source attribution is not feasible when using molecular typing alone.

The frequency with which *C. coli* is isolated from humans and from retail poultry products is, in several countries, low compared with *C. jejuni* (Anon., 2006b). However, in some countries, a large proportion of *C. coli* is also found in poultry products, including USA, Czech Republic, Slovenia and Bosnia Herzegovina. Nevertheless, most human infections are still identified as being caused by *C. jejuni* (Zhao et al., 2001; Gillespie et al., 2002; Anon., 2006b; Zorman et al., 2006).

2.9 Risk factors in developing countries

In both developed and developing countries, *Campylobacter* spp. are a leading cause of gastrointestinal and diarrhoeal disease, but the sources and incidence of illness differ to some degree, sometimes quite dramatically. In developed countries, fresh chicken, poultry and other foods of animal origin are thought to be the most likely sources of infection. In developing

countries, several environmental sources pose risks of *Campylobacter* infections. Waterborne transmission, direct contact with animals, especially chickens, and animals in cooking areas are thought to be the major routes of human infection (Georges-Courbot et al., 1990; Quick et al., 1999; WHO, 2001; Coker et al., 2002). Poultry may reside in the household or in close proximity to the household, and serve as a source of the organism. For instance, chickens living in or near the household may commonly lead to exposure of household members (Koulla-Shiro, Loe and Ekoe, 1995; Harvey et al., 2003). A Peruvian study found that corralling of chickens had a negative impact on the incidence of campylobacteriosis, with twice as many cases of *Campylobacter* infections and seven times the rate when more than 20 chickens were kept in one corral (Oberhelman et al., 2006). Food-production and -preparation workers may be at additional risk of infection. In one study from Brazil, *Campylobacter* could be recovered from 38% of carcasses and 13% of worker's stool specimens (Dias et al., 1990). Ingestion of *Campylobacter*-contaminated foods, including chicken, also pose a threat of infection.

3. EXPOSURE ASSESSMENT

Exposure assessment considers the occurrence and number of *Campylobacter* that may be present in chicken and on the carcass and resulting products that contribute to the dose ingested by the consumer. The information cited in this section is based mainly upon studies carried out in temperate climates under conditions of intensive production that utilize environmentally controlled housing, where many thousands of birds may be kept together.

3.1 Campylobacter on the broiler farm

3.1.1 Introduction

Broiler production is highly specialized and follows a defined structure (ACMSF, 1996). When the birds are newly hatched they are moved to a broiler growing farm, where they remain until they reach slaughter weight at ages between 30 and 60 days. At this point, de-population occurs. Birds are removed from the house and transported to the processing plant.

In a typical grow-out house, the birds are reared on litter, with feed and water liberally available. While on the growing farm, the birds are exposed to a variety of potential sources of *Campylobacter*. These may include insects, rodents and wild birds. Unless rigorous control of hygiene is enforced, farm staff may also introduce campylobacters from external sources. Such external sources of contamination can also appear when the flock is partially de-populated (thinning) prior to reaching the final slaughter weight, or if the flock is de-populated in multiple catch lots.

Sources of *Campylobacter* infection of poultry flocks are still debatable. Vertical transmission via contaminated eggs has been reported, but strong supporting evidence is lacking. Isolation from eggs has been demonstrated as a rare event. In particular, Shanker, Lee and Sorrell (1986) obtained two positive eggs from a sample of 187 eggs from a *Campylobacter*-positive breeder flock. The occurrence of only two positive samples is attributed to faecal contamination of the eggshell. *Campylobacter* have poor survival rates in egg albumen (Jones et al*.,* 1991). *Campylobacter* colonization is rarely evident before flocks are two weeks of age, so vertical transmission is not likely to be a major route of flock infection, unless the bacteria are slow to revive, grow and spread among the birds, after being in the harsh environment of the egg (Annan-Prah and Janc, 1988; Van De Giessen et al*.,* 1992).

Transmission from flock to flock, or 'carry-over', 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). Further sources, such as feed (Humphrey, Henley and Lanning, 1993; Mead and Hinton, 1989) and litter (Pokamunski et al., 1986; Clark and Bueschkens, 1988) are unlikely because campylobacters are fragile organisms that are sensitive to desiccation. As such, they are unlikely to survive well in feed or litter. Most evidence from serotyping and case-control studies (Evans, 1992 and Jacobs-Reitsma et al., 1995) suggest that the external environment is the primary source of contamination. As *Campylobacter* spp. are ubiquitous, this hypothesis has intuitive appeal. Once the flock has been exposed to *Campylobacter*, the water and feed systems can play an important role in its dissemination throughout the flock. When *Campylobacter* is first detected in the birds, the feed soon becomes culture positive. Furthermore, water as a vehicle for transmission of the organism through a flock has been demonstrated experimentally. Interestingly,

chlorination of the water supply has been shown to slow the within-flock transmission of the organism (Pearson et al., 1993).

Farm workers also play a role in the transmission of *Campylobacter*. Case-control studies have indicated that farm staff are a risk factor (Lindblom, Sjorgen and Kailser, 1986; Evans, 1992) and external contamination of a flock by catchers has been demonstrated.

The prevalence of *Campylobacter* colonization 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 may be predictors of the age at which flocks became colonized, but a follow up study reported that intervention methods were only successful in delaying the onset of colonization (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 appear to be colonized. This is consistently seen in commercial flocks (Lindblom, Sjorgen and Kailser, 1986; Mead and Hinton, 1989) but absent in laboratory experiments (Shanker, Lee and Sorrell, 1990).

Seasonality of the colonization of broiler chickens (higher colonization rates during warmer periods) has been reported in certain countries, e.g. in Denmark, Norway, United Kingdom and the Netherlands (Kapperud et al., 1993; Jacobs-Reitsma, Bolder and Mulder, 1994; Newell et al., 1999; Christensen et al., 2001), but in other countries such as USA and Canada (Quebec), no evidence of seasonal variation has been found (Gregory et al., 1997; Nadeau, Messier and Quessy, 2001).

PFGE-typing has revealed a high degree of genetic diversity among poultry isolates. Usually a flock is colonized by a unique genotype, but flocks raised at different grow-out periods often have different genotypes (Nadeau, Messier and Quessy, 2001).

To consider the frequency and level of exposure of humans to contaminated chicken products, and methods of control, the exposure assessment requires estimation of the probability that a random broiler chicken destined for human consumption will be *Campylobacter*-positive at the point of slaughter. To illustrate the on-farm component of the exposure assessment, the model presented here estimates the probability of a random bird from a flock being *Campylobacter*-positive at the time of slaughter, together with an estimation of the uncertainty in this probability.

3.1.2 Risk assessment model description: Farm component

The aim of the rearing module is to estimate the probability that a random bird from the national 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 (3.1)

$$P_{pb} = P_{fp} * P_{wfp} \tag{3.1}$$

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 colonized with *Campylobacter*. Estimation of P_{fp} and P_{wfp} was undertaken as follows.

3.1.2.1 Estimating flock prevalence, P_{fp}

The frequent colonization of poultry flocks with *Campylobacter* is well documented worldwide (Byrd et al., 1998; Gregory et al., 1997). However, the national flock prevalence is likely to be specific for any given nation, and should ideally be based upon available data on the *Campylobacter* prevalence in that country. To illustrate how such data can be used within this risk assessment, sample data obtained from four sources—two fully integrated poultry companies, an

epidemiological study (Evans, 1996) and a published source (Humphrey, Henley and Lanning, 1993) —representing the *Campylobacter* status of broiler flocks in Great Britain were used to obtain an estimate of P_{fp}, as described in Appendix A.

3.1.2.2 Estimating within-flock prevalence, P_{wfp}

Within-flock prevalence (WFP) is a measure based on the number of birds expected to be colonized 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, Lee and Sorrell, 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, Sugawa and Ishii, 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 specialized and follows a defined structure (ACMSF, 1996). Briefly, when the birds are newly hatched they are taken to a broiler-growing farm, where they remain until they reach slaughter weight at ages between 30 and 60 days. At this point, de-population occurs, whereby birds are removed from the house and transported to the slaughter facility for processing.

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 and Foenander, 1962; Collias et al., 1966; McBride, Parker and Foenander, 1969; Wood-Gush, Duncan and Savory, 1978; Tribe, 1980; Pamment, Foenander and McBride, 1983) and experimental work suggests a similar social behaviour is displayed by birds in the commercial rearing environment (Preston and Murphy, 1989). The area explored by a given bird diminishes with age (Preston and 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 colonized. The mechanism by which a single bird becomes colonized and the time at which this occurs is generally unknown. Following colonization 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 C*ampylobacter* are excreted in the faeces of positive birds. As broilers are coprophagic, this activity leads to ingestion of the organisms by other birds in the flock, and hence bird-to-bird transmission. In addition to bird-to-bird transmission, excretion of the organism results in the contamination of the feed and water systems. In a relatively short period, a threshold is assumed to be reached where the contamination level of feed and water is sufficient to cause extensive colonization in birds as a result of the ingestion of the contaminated feed and water. This facilitates dissemination of *Campylobacter* throughout the whole flock until all birds are colonized.

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

Within this model it is assumed that the first bird becomes colonized at time $t = t_{ex}$ This time is defined as the age at which the first bird in the flock becomes colonized. 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 colonization of the first bird, *Campylobacter*s can be detected in the feed, water and litter after 3 days (Shanker, Lee and Sorrell, 1990). It is therefore assumed that contamination levels become sufficient to allow widespread dissemination of the organism throughout the flock 4 days following colonization of the first bird. Thus, a model for simple epidemic spread can be used to represent the second stage of the colonization process. Thereafter, transmission continues until either all birds become colonized or de-population occurs at time t_A. The models describing each of the stages are presented by Hartnett et al. (2001) and are detailed in Appendix A.

From these models, the number of colonized birds within a flock, that is $I(t)$, at time t is estimated. Hence, the within-flock prevalence at time t since the time of exposure can be calculated directly as follows, where n is the total number of birds in the flock:

$$P_{wfp}(t) = \frac{I(t)}{n} \tag{3.2}$$

3.1.2.3 Possible model modifications

The assumptions on which the model is based are important in the interpretation of the generated results. Within the current model, it is assumed that a flock initially comprises birds in clusters. Successful colonization occurs from a single bird in one cluster. The organism is then disseminated, initially by direct contact with the colonized bird and then via contaminated feed and water. The validity of the assumption that a single bird becomes colonized will depend on the source of infection. Thus, if campylobacters are introduced into the house as a result of farm staff with, for example, contaminated footwear, 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 colonized 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. *Campylobacter* 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 birds may become colonized and initiate the colonization process throughout the flock. 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, i.e. equation (A1.4), in Appendix A, rather than the chain-binomial model.

It is debatable whether vertical transmission of *Campylobacter* can occur (Cox et al., 1999; Jacobs-Reitsma, 1997). If vertical transmission does occur, it is expected to be a relatively infrequent event at the level of individual birds. However, for large broiler flocks, even a low rate of transmission from parent flocks would result in the regular occurrence of colonized flocks via this pathway. Such an occurrence would result in multiple colonized birds and multiple initial clusters containing colonized birds. This can be modelled by use of multiple chain-binomial models (Ng and Orav, 1990).

In the Netherlands, an alternative farm model has been formulated, which includes not only the dynamics of *Campylobacter* transmission within flocks, but also transmission between flocks, that is between subsequent flocks in the same house or between concurrent flocks in different houses (Fischer et al., 2003). The model uses data from an experimental study on within-flock transmission (Jacobs-Reitsma, 1997) and an observational study of 13–14 cycles on ten broiler farms with 2–7

houses per farm (Jacobs-Reitsma et al., 1999). Due to an associated increase in the probability of contamination, farm size (expressed as the number of houses within a farm) was predicted to be an important risk factor. The probability of introduction of *Campylobacter* into a new flock from a source not associated with other flocks at the same farm or in previous cycles was found to be relatively small.

A novel method to reduce the *Campylobacter* load in poultry is the use of bacteriocins from *Bacillus* and *Paenibacillus,* as a therapeutic treatment for chickens colonized by *Campylobacter* (Svetoch et al*.,* 2003). The intended effect is that through feeding the animals therapeutic feed at the appropriate point in the cycle, levels and frequency of colonization can be reduced, which is presumed to be effective in lowering the human health risk imposed by *Campylobacter*.

An alternative new method to reduce the level of *Campylobacter* on the chicken skin might be the application of host-specific bacteriophages (Goode, Allen and Barrow, 2003; Atterbury et al*.,* 2003b). These bacteriophages can survive on fresh and frozen retail poultry products. As they are found to be naturally present on the chicken skin, their usage as a biocontrol agent would not introduce any entity into food products that is not already present (Atterbury et al*.,* 2003a).

3.1.3 Parameter estimation

The parameters and their estimated distributions are listed in Table 3.1. There is extensive published work on *Campylobacter*, although 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. More specifically, this was implemented using the Discrete function (in the @Risk software) with parameters $(\{E_1,E_2,\ldots,E_n\},\{w_{E1},w_{E2},\ldots,w_{En}\})$, 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. In this analysis, each expert was given equal weighting.

The transmission rate for *Campylobacter*, b, is based upon experimental studies (Shanker, Lee and Sorrell*,* 1990). These studies involved the placing of a colonized bird in a group of un-colonized birds. Samples were then taken daily to measure the change in the number of colonized birds over time. From these studies, two values for the transmission rate were estimated and used to define the extremes of a uniform distribution. Ideally, more information is required, including the most likely value of b within the range of these two values. If this information were available, the use of a triangular distribution or another unimodal 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. The proportionality factor is equal to $1/10n$. Due to the absence of data, experts in the area of the *Campylobacter* colonization of chickens agreed with this factor by inspection of the resulting epidemic curve.

The age at first successful exposure, t_{ex}, is an unknown parameter in the model. Several studies have shown that C*ampylobacter* are rarely isolated from commercial flocks less than three weeks of age. One explanation for this is that the colonization 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 from exposure until the number of birds colonized is large enough to allow detection, 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 de-population.

Finally, distributions for flock size (n), and time of de-population (t_A) are country-specific parameters. To illustrate use of the model, values presented represent the broiler industry of Great Britain. The use of country-specific parameters is recommended.

Table 3.1 Probability distributions and associated parameter values used in the model to estimate the probability distribution for a random bird from a positive flock selected from the United Kingdom national 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	Discrete($\{\alpha,\beta,\gamma\},\{P_\alpha,P_\beta,P_\gamma\}$)
		Where: α~Triangular(12,100,500)*
		β~Triangular(30,50,120)*
		γ~ Triangular(20,45,100)*
Number of times a bird comes into contact with a given bird in one day	R	Discrete($\{\lambda,\sigma\},\{P_\lambda,P_\sigma\}$)
		Where: λ~ Triangular (3,5,6)*
		δ~ Triangular (2,6,8)*
Size of cluster	n_c	Discrete($\{\mu,\omega\},\{P_\mu,P_\omega\}$)
		Where: μ~ Triangular(N/12,N/10,N/8)*
		ω~ Triangular(100,300,1000)*
Industrial Data		
Flock size	N	Triangular(7800,30750,41596)
Age at de-population in days	t_A	Triangular(28,42,64)
Age at first exposure to *Campylobacter* in days	t_{ex}	Uniform(14,t_A)

NOTE: * These parameters are Triangular distributions based on expert estimates

3.2 Contamination level of chickens on the farm and during transport

It is well recognized 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 quantify the level (or concentration) of *Campylobacter* likely to be present in the gut of a colonized bird at the point of entry into the processing facility, where the slaughter process begins. It is also necessary to consider the exterior contamination of birds as a separate pathway of exposure into the processing plant.

When a bird becomes exposed to, and ingests *Campylobacter*, depending on the level of exposure, the organisms may establish, and reproduce within the gut of the bird. This process will continue until equilibrium is reached and the level of colonization will be maintained, leading to a more stable bacterial population.

Once a bird is colonized with *Campylobacter,* it will excrete large numbers of the organism in its faeces. Contact with the faeces of such a bird is one mechanism by which the organisms spread throughout a flock. However, the second consequence of this excretion of organisms is the contamination of the exterior of the birds. This external contamination may occur while the birds are on the farm or during transportation to the slaughter facility.

In this section, a model is described that estimates the number of *Campylobacter* in the faeces of a colonized bird. Further, estimates are made of the level of external contamination that occurs on the farm, and the extent of cross-contamination during transport.

3.2.1 Model development: Extent of colonization and external contamination.

3.2.1.1 Contamination on the farm

Given that a bird is colonized, it seems appropriate to assume that such a bird will also be contaminated on its exterior. However, in a flock that contains colonized birds but has a within-flock prevalence of less than 100%, there is the opportunity for the birds that are not colonized to become contaminated on their exteriors. This can occur as a result of contact with either an externally contaminated bird, or contaminated faeces.

The probability that a non-colonized bird will become contaminated on its exterior can be expected to be related to the within-flock prevalence of the flock. Consider the within-flock transmission dynamics discussed in Section 3.1.2.

In the initial stage, transmission occurs amongst the social cluster with which the first colonized bird interacts. 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. Hence the bird has the opportunity to come into contact with colonized and externally contaminated birds. Once transmission enters the second stage, colonized birds will occur in a random fashion throughout the flock. It then becomes highly likely that a random bird will come into contact with either a contaminated bird or contaminated faeces. It is therefore assumed that the probability that a bird is contaminated during this stage of transmission is equal to 100%.

3.2.1.2 Contamination during transportation to slaughter facility

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 (or crates). The baskets are grouped together in modules, each module containing perhaps three rows of four baskets. The modules are placed in the vehicle in rows, stacked one on top of the other, with the number of modules 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. Multiple vehicles are used for any given flock.

During transportation to the slaughter facility, the stress of the process may result in changes in the consistency of the faeces, to a more liquid nature. This can cause contamination of the exterior of a large proportion of the birds in the transport vehicle, despite the metal sheeting separating the modules. It is likely that in any given section of the vehicle there will be contamination of the birds with faeces resulting from the excrement of 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 that are excreting campylobacters. Therefore, the probability that a bird becomes contaminated during transport is a function of the number of rows that contain colonized birds and the location of these birds within the vehicle in relation to non-colonized birds.

When estimating the level of contamination on the exterior of a bird upon 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 colonized birds, hence no birds are shedding the organism. Each of these situations will now be discussed in turn.

3.2.1.3 Model description: Contamination during transport

The assessment of the distribution of the probability and extent of contamination during transport has been considered by Hartnett (2001). The mathematical model developed by Hartnett (2001) is adopted here to describe the transport components of the chicken supply chain. The model can be summarized as follows.

During rearing, the flock is represented by an a × b lattice structure where a represents a horizontal co-ordinate (expressed as a number of birds) within the house, and b represents a vertical co-ordinate. The total number of birds within the flock is $N = a \times b$

Each flock is assigned a profile, which describes the status of the flock and the birds within the flock. This status is considered over the period of rearing, and hence the colonization status of the birds in the flock changes in relation to the time, t. The components of the profiles are outlined in Table 3.2. Note that once a bird is colonized, $c_x = 1$, and it remains colonized. If the flock is negative and $\theta_f = 0$, then $c_x = 0$ for all $x = (a,b)$ over all t.

Table 3.2 Components of the model describing the contamination during transport.

Component	Description		Determining model variables
θ_f	Flock status	$\theta_f = 0$ indicates a negative flock $\theta_f = 1$ indicates a positive flock	P_{pf}
c_x	Colonization status of bird at location x	$c_x = 0$ indicates a negative bird $c_x = 1$ indicates a positive bird	P_{fp}, P_{wfp}, t
CD_x	Contamination status of bird at location x	$CD_x = 0$ indicates an uncontaminated bird $CD_x = 1$ indicates a contaminated bird	P_{fp}, P_{wfp}, t

At the time of first exposure, t_{ex}, a bird is selected at a random location and designated as the first positive bird, and the cluster around the bird is defined. The birds in this cluster then become positive and change status from $c_x = 0$ to $c_x = 1$, at a rate determined by the model for the first part of within-flock spread. For a given bird, if it has been assigned to the cluster containing the first colonized bird, then it is assumed to be contaminated on the exterior, and $CD_x = 1$. Once four days have passed since first exposure, birds become colonized at random locations throughout the flock, sampling of birds is undertaken, without replacement, as governed by the model for the second stage of within-flock spread. At this time, all birds are assumed to be contaminated on their exteriors. This process continues until $t = t_A$, the time for de-population. The result of the model described above is a coordinate for each bird in the flock and an associated colonization status and contamination status at the point of de-population. Given that a bird is contaminated at de-population, the level of contamination on the exterior is defined as Ω_d.

The next stage of the model is to place the birds into the transport vehicles. It is assumed that for all flocks, a transport crate contains 100 birds. The birds are placed in the order that they are in the house onto the transport vehicles, in groups of 100. One end of the house is allocated as the front of the house, so the further away from the front of the house a bird is, the higher the number vehicle the bird will be transported in. Each vehicle has a maximum capacity of x groups of birds, assuming that all vehicles carry M crates. The number of vehicles required for any flock is therefore N/xM. Once all the birds are placed into the transport vehicles, the probability that a random bird from the flock is contaminated during transport is defined as P_t.. The parameters N, x and M may be country-

, and industry-, specific. For the purpose of illustrating the model, it is assumed that $x = 60$ and $M = 10$.

There are no data available that provide estimates of the probability that a bird will become contaminated during transport in relation to the location of the bird within the vehicle. It is therefore assumed that there are two modes of contamination. The first mode is that which occurs as a result of a bird being physically located below colonized birds. The second mode is contamination from the adjacent sections of the vehicle containing contaminated birds.

Consider a random bird in crate i and row j. The probability that contamination occurs vertically, from the birds in crate i, rows 1 to j is defined as $C_T(d)_{i,j}$. This probability is dependent upon the distance, i.e. number of rows, between the selected bird and the nearest colonized birds above. It is assumed that the probability that a bird becomes contaminated is given by the reciprocal of this distance, more specifically:

$$C_T(d)_{i,j} = \frac{1}{i - \max\{C_{z,j}.z\}} \qquad z = 1,..,i-1 \qquad (3.3)$$

Next, consider cross-contamination from the adjacent birds in row j, crates 1 to M. 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 crates 1 to M is contaminated and the reciprocal of the distance between this module and the selected module. More specifically:

$$C_T(V)_{i,j} = \left[\sum_{z=1}^{j-1} C_T(d)_{i,z}.\frac{1}{j-z+1}\right] + \left[\sum_{z=j+1}^{M} C_T(d)_{i,z}.\frac{1}{z-j+1}\right] \qquad (3.4)$$

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

$$P(CT)_{i,j} = C_T(V)_{i,j} + C_T(d)_{i,j} - C_T(V)_{i,j}.C_T(d)_{i,j} \qquad (3.5)$$

Hence, on arrival at the slaughter facility, each bird has an associated status for the occurrence of contamination during transport, defined as $CT_{i,j}$ where $CT_{i,j} = 1$ 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, dependent upon $P(CT)_{i,j}$. Given a bird that has become contaminated during transport, the level of contamination on the birds exterior is defined as Ω_t.

3.2.1.4 Levels of contamination at slaughter for positive flocks

The level of external contamination that is present upon a bird on arrival at the slaughter facility is significantly different ($p<0.05$) to the level of external contamination that which is present before the flock is transported (Stern et al., 1995), thus supporting the assumption that cross-contamination within the flock occurs during transportation. However, as previously described, the probability that a random bird will become contaminated during transport is a function of the location of the colonized birds in the flock within the transport vehicles, in relation to the location of the selected bird. As such the level of contamination on the exterior of a bird is governed by the probability that the bird became contaminated during transport. When the birds are placed in the transport vehicle each bird has a contamination status at the point of de-population, (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 follows.

$$\eta_{ext} = \begin{cases} 0 & CD = 0; CT = 0 \\ \Omega_d & CD = 1; CT = 0 \\ \Omega_t & CT = 1 \end{cases} \quad (3.6)$$

Here, Ω_d is the level of contamination on the exterior of a bird at de-population, 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.

3.2.1.5 The transportation of negative flocks

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

Given the absence of colonized birds it may be assumed that within such a flock there is no opportunity for a bird to become contaminated on their exteriors. This is not the case. Experimental data suggests that there are at least two occasions when birds in negative flocks may become contaminated. First, it has been hypothesized 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, the crates within which the birds are transported may be contaminated. The baskets may or may not be routinely cleaned once the birds are removed at the slaughter facility and such cleaning may not be adequate to remove all the organisms present.

3.2.1.6 Levels of contamination at slaughter for negative flocks

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 can be seen 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 negative flock becomes contaminated is related to the national flock prevalence, and number of flocks transported that day by $P_{nc} = 1 - (1 - P_{pf})^n$. Here n is the number of flocks likely to be transported in a truck per day and P_{nc} is the probability that a *Campylobacter* negative flock becomes contaminated during transport. The flock prevalence, P_{pf}, is as described in Section 3.1.

The extent to which a bird from a negative flock will become contaminated is related to the level of contamination in the positive flocks. Consider contamination by catcher's hands. Experimental work looking at the cross-contamination of organisms from surfaces to hands and hands to surfaces suggests a transfer rate of 10% (Zhao et al., 1998). For a bird to become contaminated via catcher's hands, two things must occur. First, the catchers hands come into contact with a contaminated bird and hence become contaminated. Second, the contaminated hands 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. The same assumption is made with regard to 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 of a positive flock.

Therefore, 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 condition (3.7) (Hartnett, 2001).

$$\eta_{ext} = \begin{cases} \Omega_s & \theta_f = 1 \\ 0.01\Omega_s & \theta_f = 0 \end{cases} \quad (3.7)$$

3.2.2 Parameter estimation and simulation

The level of colonization within the caeca of several birds within random flocks is reported by Stern et al. (1995). This data set recorded levels of colonization before and after the birds had been transported. These data suggest that there is no significant difference in colonization levels before and after the birds have been transported. There are several other sources in the literature that give an indication of colonization levels in positive birds (Stern, 1988; Aho and Hirn, 1988; Jacobs-Reitsma, Kan and Bolder, 1994; Berndtson, Danielsson-Tham and Engvall, 1996; Atabay and Corry, 1997). However, such reports commonly record only the mean value, or maximum colonization observed. These data do not enable the definition of a variability distribution to describe colonization levels in random birds. Therefore, only the data set from Stern et al. (1995) is utilized to define the colonization level at slaughter, Λ_s. This data set is shown in Table 3.3. It can be seen by comparison with reports of colonization levels in the literature that this data set is consistent with other findings.

Table 3.3 The number of *Campylobacter*s colonizing the caeca of broilers at slaughter.

Farm number	Mean Log cfu/gram caecal 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

SOURCE: Data from Stern et al. 1995.

There is little information in the published literature with regard to the level of contamination on the exterior of birds either before or after transport. An investigation by Stern and colleagues (Stern et al., 1995) recorded measurements of external contamination with *Campylobacter*s both before and after transport. This data set is shown in Table 3.4. 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, and 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 3.4 that, on seven occasions, no *Campylobacter* was 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 transportation.

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 3.4.

The importance of the length of time of transport and holding should be noted. It is intuitive that if birds are subjected to transport times of, for example, 30 minutes, the extent of external contamination occurring during transport and holding may be much lower than if the birds were subjected to longer transport and holding times. The transport time is likely to be highly variable, not only between producers, but also between countries. Further, there is currently no data available

to quantify this effect. However, the model described above can be adjusted to accommodate this data.

Table 3.4 The number of *Campylobacter*s (mean log cfu per carcass) contaminating the exterior of broilers prior and post transport.

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

NOTE: * denotes data from a randomly collected farm not under experimental control. See text for details.
ND = not detected.
SOURCE: Data taken from Stern et al., 1995.

3.3 The slaughter and processing of chicken

In many nations, most poultry meat is produced in modern, large-scale processing plants that operate at line-speeds of up to 6 000 birds per hour. The process is now almost fully mechanized and is carried out as a well-defined sequence of operations that begins with the stunning and slaughter of live birds and ends with transportation of the final product to distribution centres, retail outlets or plants for further processing.

On entering the processing plant, birds carry large numbers of different microorganisms on the skin, among the feathers and in the alimentary tract. During processing, the microbial load of the bird is progressively reduced but, because of the close proximity of neighbouring carcasses on the processing line and the nature of processing operations, opportunities exist for microbial contaminants to spread among the carcasses at virtually every stage, via aerosols, process water, contaminated surfaces and equipment, and the hands of the plant workers. Because *Campylobacter*

occurs at relatively high levels in the gut and on external surfaces of carrier birds, there is potential for cross-contamination of *Campylobacter*-negative flocks that are processed subsequently.

Despite the spread of microorganisms, overall levels of carcass contamination can be controlled to a certain extent by Good Manufacturing Practices and the application of principles embodied in the Hazard Analysis Critical Control Point system (Mead, 2000). There is, however, no current means of completely eliminating foodborne pathogens from contaminated carcasses. As a result, full control of this type of contamination would be highly dependent on the ability to exclude contamination from the live birds on the farm and during transport to slaughter.

For pathogens like *Campylobacter* that are carried asymptomatically in the alimentary tract of the bird, the control of faecal contamination of carcasses is critical, especially during the evisceration stage. Nevertheless, levels of *Campylobacter* on carcass surfaces are likely to be reduced during scalding, washing, mechanical water chilling and freezing of carcasses, with a further decline possible during frozen storage. However, taken together, these processes do not result in total elimination. An important factor in the persistence of the organisms is their tendency to become attached to or entrapped in the skin surface (Notermans and Kampelmacher, 1974; Thomas and McMeekin, 1980). These phenomena appear to offer a degree of protection from environmental stresses encountered during heating, chilling and exposure to chlorinated water. They also limit the removal of microbial contaminants during carcass washing.

Unlike certain other organisms, *Campylobacter* appears unable to multiply in the processing plant since the minimum growth temperature is 30 to 35°C and the optimum is 42°C. Also, growth outside the alimentary tract requires a reduced concentration of atmospheric oxygen and is favoured by 10% carbon dioxide.

This stage of the risk assessment describes the processing of chicken, considering each of the stages and modelling their impact on product contamination. The outcome of the model is an estimate of the probability that a random chicken product will be contaminated with *Campylobacter* and the number of such organisms that may be present.

3.3.1 The stages of chicken processing

There are nine major stages in primary processing, beginning with the stunning and slaughter of the birds and ending with the packaging of oven-ready carcasses or cut-up pieces, prior to final chilling and distribution. The main stages are considered below.

3.3.1.1 Stunning and slaughter

On arrival at the processing plant, birds are removed manually from the transport crates and hung by the feet on shackles of the continuously moving slaughter line. This part of the process must be physically separated from the stages that follow because there is a degree of wing-flapping that disseminates both dust and microorganisms. The birds pass first into the stunning equipment, which commonly comprises an electrically charged water bath in which the bird's head is partly immersed. The stunning process has few microbiological implications, although birds may sometimes inhale contaminated water, which can reach internal tissues (Gregory and Whittington, 1992). This potential pathway is not included in this assessment. After the stunning stage, the neck of each bird is cut and the bird is allowed to bleed out for a few minutes.

3.3.1.2 Scalding

When the birds have been adequately bled, they are passed through a tank of hot water in order to loosen the feathers and facilitate mechanical plucking. Carcasses that will be used for fresh, chilled

products often undergo a 'soft' scald at 50–52°C for up to 3.5 minutes, thus avoiding aesthetic damage to the product. For carcasses used to prepare frozen products, a 'hard' scald may be applied at 58–60°C for up to 2.0 minutes where skin appearance may be less important. Alternatively, lower temperatures of 56–57°C may be used. Scalding practices will vary from country to country or depend on the type of product being produced. Prior to scalding, carcasses are heavily contaminated with microorganisms on the skin and among the feathers. Many of these are washed off and accumulate in the scald water, while further contamination may arise from involuntary defecation. After an initial build-up, however, microbial levels in the water become relatively constant in systems where fresh water is added continuously to replenish that retained in the feathers of carcasses leaving the scald tank.

Although immersion scalding results in a net reduction in carcass contamination, the highly contaminated state of the water offers ample opportunity for microbial transmission between carcasses. Also, some internal contamination may result if any water is inhaled initially (Lillard, 1973). Survival of vegetative bacteria in scald-water is influenced by the water temperature, but *Campylobacter jejuni* is relatively heat-sensitive and a D_{10} -value of only 2–3 minute has been reported at 52°C and pH 6–7 (Bolder, 1998). Nevertheless, those bacterial cells that become attached to the carcass surface or within the skin structure are more protected from the lethal effect and therefore may survive the scalding process (Notermans and Kampelmacher, 1975). Thus, different scalding regimes may be assumed to have little direct effect on surface contamination with *Campylobacter* (Slavik, Kim and Walker, 1995), although poorer survival in the water at higher temperatures may reduce dissemination of the organisms at this stage of the process.

Increasingly, scalding systems are designed to reduce the hygiene problems outlined above. This has been achieved by moving carcasses against the flow of incoming water (counter-flow), so that they meet the cleanest water at the carcass-exit end, and using several successive tanks operated in the same way, to provide a dilution effect (Veerkamp, 1991). With such a system, Berrang and Dickens (2000) obtained almost a thousand-fold reduction in *Campylobacter* contamination of carcasses, though much of the advantage was reported to be lost during subsequent stages of processing.

3.3.1.3 De-feathering

De-feathering is a mechanical process that is carried out immediately after scalding, while the carcasses remain warm. It involves a series of in-line machines containing banks of counter-rotating metal domes or discs bearing multiple rubber 'fingers' that scour the surface of each carcass. These machines incorporate continuous water sprays that help to flush away the feathers as they are removed. Any remaining feathers are removed by hand. The principal microbiological problem with de-feathering is cross-contamination of carcasses associated with the mechanical action of the machines, and the tendency to disperse microbial contaminants in all directions via aerosols. In most cases, the levels and numbers of carcasses contaminated can significantly increase (ICMSF, 1980).

3.3.1.4 Evisceration

The evisceration process involves removal of the feet, head and viscera of the birds, and the harvesting of edible offal. All these operations are carried out at stages that involve either manual labour or use of appropriate machinery. Of major importance is the need to minimize rupture of the exposed intestines and prevent the spread of faecal bacteria such as *Campylobacter,* which occur in relatively high numbers in the intestines of positive birds.

Mechanized evisceration systems can cause damage to the intestines during their exposure and partial removal prior to carcass inspection, and this may result in extensive faecal contamination of carcasses. The problem arises because the machines are occasionally unable to adjust to the natural variation in carcass size that is associated with all poultry flocks, and the contents of damaged viscera then leak onto the underlying carcasses. More modern systems provide immediate removal of viscera onto a separate, but parallel processing line for inspection purposes. There is, however, no evidence that this necessarily leads to lower levels of carcass contamination. Control of contamination depends upon careful setting of evisceration machinery, strategic washing of carcasses at points close to sites of contamination, in order to avoid microbial attachment (Notermans, Terbijhe and van Schothorst, 1980), and, where legislation allows, spraying of contact surfaces with chlorinated water (Bailey et al*.,* 1986). Control is also improved by use of automatic transfer of carcasses from the slaughter line to the evisceration line and, where possible, on to the chilling line. In this way, microbial contamination is reduced by avoidance of product handling.

In some countries, carcasses may be sold uneviscerated or only partially eviscerated (*effilé*), or evisceration may be delayed to allow a period of storage at up to 4°C for meat-flavour development. While *Campylobacter* may survive the storage period, growth is highly unlikely under the conditions that occur. However, only complete evisceration on the day of production is considered within the risk assessment model, since the microbial implications of the other products are not fully understood.

3.3.1.5 Washing

On reaching its final form, the carcass is often cleaned by spray washing prior to chilling. The process is carried out primarily to eliminate any blood spots or other organic debris. High-pressure washers may also remove significant numbers of microbial contaminants from both the inner and outer surfaces of the carcass. The extent to which contamination is reduced at this stage depends upon the frequency and degree of washing at earlier stages. Washing has been found to produce a ten-fold reduction in *Campylobacter* (Cudjoe et al*.,* 1991) but would be expected to have little effect on cells attached to carcass surfaces. There does not appear to be any published evidence that washing carcasses in super-chlorinated water causes additional reduction in microbial contamination.

3.3.1.6 Chilling

Freshly eviscerated carcasses are still relatively warm and must be chilled as soon as possible to inhibit microbial growth. Chilling is carried out in two stages, one before packaging of oven-ready carcasses (discussed in this section), and the other prior to distribution (section 3.3.1.8). Alternatively, packaged carcasses are frozen at the second stage.

In primary chilling, the deep muscle is usually cooled to about 8°C and one of two types of chilling system is used. The first is air chilling, which, in larger processing plants, is a continuous process involving a cold-air blast, with or without a fine water spray initially to assist cooling by evaporation or to avoid excessive weight loss by the carcasses, or both. The process is usually complete in approximately one hour, and generally has no significant effect on levels of *Campylobacter* contamination (Cudjoe et al*.,* 1991).

In some countries, continuous water-immersion chilling is used only for carcasses that will be frozen, while in other countries water-immersion chilling is also used for fresh, chilled products. The chilling system comprises one or more tanks of cold water in which many carcasses are cooled together and are propelled mechanically through the water. During chilling, the water is kept in a state of agitation to assist cooling and there is a net removal of organisms from both inner and outer

carcass surfaces due to the washing effect. Reductions in carcass contamination of about ten-fold can be expected, and have been demonstrated for *Campylobacter* (Laisney, Colin and Jacobs-Reitsma, 1991). Super-chlorination of the water is thought to have little further effect on microbial contamination of carcasses, but is of value in controlling cross-contamination via the water itself (Mead and Thomas, 1973a, b).

3.3.1.7 Grading and packaging

Following the chilling stage, carcasses are graded, weighed and packaged. In preparation for packaging, whole carcasses are trussed by hand and, again, opportunities arise for cross-contamination, although few studies have addressed the problem and information is lacking on any changes in levels of carcass contamination at these post-chilling stages. Methods of packaging and product presentation vary according to requirements. In the United Kingdom, fresh, chilled carcasses are usually packed without insertion of a giblet pack, whereas giblets are often included with frozen carcasses.

3.3.1.8 Secondary chilling and distribution

In the interest of a longer shelf life for fresh, chilled products, the ultimate temperature may be further reduced to 0–2°C. This is achieved in a period of chill storage prior to distribution by refrigerated transport. Where freezing is required, carcasses may be blast frozen at –40°C instead of being chilled further.

3.3.1.9 Portioning and mechanically recovered meat

There is now a considerable demand in both retail and catering sectors of the food industry for cut portions of various kinds rather than whole carcasses. Increasingly, jointing is carried out using mechanical or semi-mechanical methods, which allow faster line-speeds and higher throughputs than entirely manual systems. Typical cuts include the following:

- *Half* – half the carcass obtained by a longitudinal cut in plane through the sternum and the backbone;
- *Quarter* – a half divided by a transversal cut, by which the leg and breast quarters are obtained;
- *Breast* – sternum and the ribs distributed on both sides of it, together with the surrounding musculature;
- *Leg* – femur, tibia, and fibula, together with the surrounding musculature;
- *Thigh* – femur together with the surrounding musculature; and
- *Drumstick* – tibia, and fibula together with the surrounding musculature.

During processing, the meat is potentially exposed to microbial contamination from contact with machinery, tools, work surfaces and the hands of plant workers, so that cross-contamination of portions can occur. The degree of contamination reflects the extent and duration of exposure to the processing environment.

For some purposes in portioning and product manufacture, the bones are removed, but these will retain a small amount of meat that is still of potential value commercially. In order to harvest the material, machines have been developed to separate meat and bone. The meat thus obtained is in a finely divided state and is highly prone to microbial spoilage, so it is either used within 48 hours in the chilled state or stored frozen until required. This mechanically recovered meat (MRM) is widely used in a range of further processed products, including burgers and sausages, and its microbial

content depends upon both the type and quality of the raw material, and on control of the recovery process. There is no information at present on the behaviour of *Campylobacter* during either portioning of carcasses or production of MRM.

3.3.2 Model description for the slaughter and processing of chicken

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. Each of the stages of processing has been described in detail in Section 3.3.1. The model to describe the slaughter and processing of chicken adopts the work of Hartnett (2001). The model considers the stages of processing that may have an impact upon the level of *Campylobacter* contaminating a carcass. As detailed in Section 3.3.1, 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 Great Britain. Based on the outputs from the models describing the rearing and transport stages of broiler production, 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 colonization. Once carcass history has been designated, the position of the flock in the flocks to be processed that day is allocated. The number of flocks processed in any given day may be country specific. For illustrative purposes, 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 processed, up to $\theta_p = 5$, the fifth flock to be processed.

Following characterization of history, 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. While there is potential for microbial contamination during portioning from machine contact, this process is reported to have little impact on the contamination on a carcass (Holder, Corry and Hinton, 1997). It is thus currently assumed that the process of portioning has no effect on the microbial load. 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. Subsequent to these steps, a random bird is selected from the group and followed through the remaining stages of processing: evisceration, washing and chilling.

The model estimates the stochastic effect of each of the processing stages on the contamination levels on the carcasses. 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, as well as the probability that a product is contaminated at the point of sale, are generated.

3.3.2.1 Level of contamination

The model considers what happens during all stages of processing. From the description of chicken processing presented in Section 3.3.1, it can be seen that scalding results in a proportion of organisms being washed off the carcass. De-feathering causes both a proportion to be washed off 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 self-contamination (from the intestinal tract), 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 (3.8) where the contamination level on a selected carcass *i*, defined as η_{pi} is given by:

$$\eta_{p_i} = \tau_i \nu_i \left(\mu_i \eta_{ext\,i} + \varphi_i + \xi_i\right) \tag{3.8}$$

where $\eta_{ext\,i}$ is the number of *Campylobacter* contaminating bird *i* at the point of entry into the processing plant, μ_i is the change in numbers due to scalding, φ_i is the change in numbers due to defeathering, ξ_i is the change in numbers due to evisceration, ν_i is the proportion remaining in the numbers of *Campylobacter* achieved by 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 measure the levels of contamination on groups of carcasses before and after a given process. There are several methods available to make such measurements, such as 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 that the parameters of Equation 3.8 are measures of proportional changes in the number of 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 measure levels of contamination before and after sampling in a consistent manner can be utilized to estimate model parameters. Accordingly, throughout this model, contamination on a carcass in a data set is referred to as mean log cfu per unit because each study has used a different sampling strategy and hence units of measurement may vary.

Data on numbers of *Campylobacter* on carcasses at different poultry processing stages are collected in the United Kingdom (Corry et al*.,* 2003). It was found that carcasses derived from *Campylobacter*-infected broiler chickens will be contaminated with log 5–6 cfu *Campylobacter* per carcass, with no or only slight reduction in numbers during processing.

3.3.2.2 Estimation of changes in number of organisms after scalding, μ_i

The probable proportion of organisms remaining after the scalding process, μ_i is dependent upon whether a carcass undergoes hard or soft scald. The proportion of products that are scalded each way will be country dependent, and the model here should be adjusted accordingly. To illustrate the model, the assumption is made here that this is governed by product type θ_s under the following condition:

$$\mu_i = \begin{cases} SS & \theta_s = \alpha \\ HS & \theta_s = \beta \end{cases} \tag{3.9}$$

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*. The data points and calculated proportions are given in Table 3.5.

Table 3.5 Measured mean log cfu *Campylobacter* on sets of carcasses before and after soft and hard scald, and the assumed proportion remaining as a result of the scalding process.

Type of scald	Mean log cfu per unit before scald	Mean log cfu per unit after scald	Calculated proportion of organisms remaining post scald	Reference
Soft	3.99	1.37	0.002	Oosterom et al., 1983b
Soft	3.30	1.68	0.020	Oosterom et al., 1983b
Soft	2.18	2.40	1.66	Oosterom et al., 1983b
Soft	3.74	<1.26	0.003	Izat et al., 1988
Soft	3.56	1.26	0.005	Izat et al., 1988
Soft	3.03	1.19	0.014	Izat et al., 1988
Soft	2.9.0	1.00	0.012	Berrang and Dickens, 2000
Soft	5.00	2.00	0.001	Berrang and Dickens, 2000
Soft	5.00	1.70	0.001	Berrang and Dickens, 2000
Soft	3.10	2.40	0.199	Berrang and Dickens, 2000
Soft	5.80	2.40	0.0003	Berrang and Dickens, 2000
Soft	4.60	1.50	0.001	Berrang and Dickens, 2000
Hard	2.39	0.61	0.016	Oosterom et al., 1983b
Hard	3.42	1.25	0.007	Oosterom et al., 1983b
Hard	3.44	1.26	0.007	Oosterom et al., 1983b

For soft scald, the calculated proportions were used to derive a non-parametric distribution to describe the variability in the proportion remaining.

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 Log-Uniform with parameters (-4,-1). This dictates a minimum proportion remaining of 0.0001 and a maximum of 0.1.

It should be noted that the use of these data are problematic for a number of reasons:

- the "before" and "after" data represent the means of two different sets of carcasses, rather than observed changes in individual carcasses. This adds considerably to the underlying noise in the data; and
- the use of the differences between means on the log scale is not the most appropriate to infer the change in the numbers of organisms remaining. The change in the arithmetic mean is preferred to estimate the proportion remaining.

3.3.2.3 Estimating change in contamination after de-feathering, φ_i

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 (Mead, Hudson and Hinton, 1994). 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.

When carcasses originate from a positive flock, the nearest positive carcass will most likely be the one next to it. This is not the case for carcasses originating from a negative flock. Within such a group, there may be a proportion of carcasses that are contaminated. However, only low-level contamination may occur and the nearest contaminated carcass may be several carcasses away.

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 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. The extent of cross-contamination is related to the distance (i.e. the number of birds) any given carcass is from the carcass being defeathered. 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 3.6.

Table 3.6 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 after first 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

Taking this information, 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, using the data in Table 3.6. The regression equation is $y = 0.0113(i - j)^{-1.9067}$ 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, (R^2 value is 0.91). Here i is the position of the selected carcass and j is the position of the carcass 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 subsequent birds as a result of de-feathering, and the reduction that results from de-feathering due to organisms being lost via the removal of feathers and the 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 3.10.

$$\varphi_i = \left(\sum_{j=1}^{j=i-1} \eta_{c,d(i-j)} \left(0.0113(i-j)^{-1.9067}\right) \right) - \left(\sum_{j=i+1}^{j=100} \eta_{c,d_i} \left(0.0113\, j^{-1.9067}\right) + r_i \right) \quad (3.10)$$

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 $\eta_{c,d}$ is the level of contamination on carcass i at the point of de-feathering, given by $\mu_i\, \eta_{ext}$.

The model described above describes solely the cross-contamination that occurs during the process of de-feathering as a result of the external contamination of the birds. However, given the nature of the process, it is likely that there may be some leakage of caecal contents hence increasing the number of organisms available to contaminate the surface of the birds. At present there are no data sets available that allow the quantification of this effect. It may be assumed that, when compared to the evisceration process, this route of additional contamination is relatively minor.

3.3.2.4 Estimation of changes in numbers of *Campylobacter* due to evisceration, ξ_i

The probable change in numbers due to evisceration, ξ_i, is dependent upon the colonization 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 re-distribution of contaminating organisms within a given flock. During evisceration, the potential for cross-contamination between flocks is considered. 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. The overall effect of evisceration is given by the change in carcass contamination due to the process of evisceration, C_c, and the change resulting from any damage that may occur to the innards of the carcass, C_d. This effect is determined by permutations of the variables Da and κ, binary variables that are in turn dependent upon the probabilities P_{Da}, the probability that damage occurs to the innards, and P_κ, the probability that, disregarding damage, some change in contamination level on the carcass occurs during evisceration, respectively.

From published data (Table 3.7), evisceration has mixed effects on the level of carcass contamination (Oosterom et al., 1983b; Izat et al., 1988; Abu-Ruwaida et al., 1994; Berrang and Dickens, 2000). If an increase in contamination occurs during evisceration, then $C_c = d_\zeta$, the number of organisms added. If a decrease occurs then $C_c = \eta_{ce}.d_\varphi$, where d_φ is the proportion of organisms remaining, and η_{ce} is the number of campylobacters contaminating a carcass at the start of evisceration, which is given by $\eta_{ext\,i}\,\mu_i + \varphi_i$.

Given damage occurs to the innards during evisceration, the increase in contamination is given by C_d. The variable C_d is assumed to be a uniform random variable with a minimum value of zero and a maximum value of Λ_s, that is the number of campylobacters colonizing 1 gram of the caecal contents of a bird.

$$\xi_i = \begin{cases} C_c + C_d & Da = 1\ \kappa = 1 \\ C_c & Da = 0\ \kappa = 1 \\ C_d & Da = 1\ \kappa = 0 \\ 0 & Da = 0\ \kappa = 0 \end{cases} \quad (3.11)$$

Table 3.7 Sample data measuring the levels of *Campylobacter* contamination on a carcass before and after evisceration.

Log cfu per unit before evisceration	Log cfu per unit after evisceration	Change observed	Change in contamination level	Reference
		Increase log cfu per carcass		
1.99	2.44	increase	1.98	Oosterom et al., 1983b
1.07	2.58	increase	0.96	Oosterom et al., 1983b
2.09	2.62	increase	2.08	Oosterom et al., 1983b
2.18	2.5	increase	2.17	Oosterom et al., 1983b
2.37	3.12	increase	2.36	Izat et al., 1988
2.82	3.49	increase	2.82	Izat et al., 1988
2.82	3.49	increase	2.82	Izat et al., 1988
3.2	3.2	none	0	Berrang and Dickens, 2000
3.7	3.7	none	0	Berrang and Dickens, 2000
		Proportion of organisms remaining post evisceration		
4.5	3.7	decrease	0.16	Berrang and Dickens, 2000
3.1	2.53	decrease	0.27	Berrang and Dickens, 2000
4.1	4	decrease	0.79	Berrang and Dickens, 2000
<3	1.6	decrease	0.04	Berrang and Dickens, 2000
5.75	5.7	decrease	0.89	Abu-Ruwaida et al., 1994
3.68	3.49	decrease	0.65	Izat et al., 1988
2.46	2.24	decrease	0.60	Oosterom et al., 1983b
2.85	2.6	decrease	0.56	Oosterom et al., 1983b

3.3.2.5 Estimation of change in number of *Campylobacter* after washing, v_I

Washing reduces the level of contamination on a carcass. This can be seen in the data shown in Table 3.8. This table consists of measurements of the level of carcass contamination (for two distinct groups of carcasses) taken before and after the washing process. Given the data in Table 3.8 the proportion of organisms remaining after a wash of the carcass, defined as $v_{i,}$ was estimated and a non-parametric distribution describing the variability in the remaining proportion defined.

3.3.2.6 Estimation of changes in *Campylobacter* after chilling, τ_I

Air chilling is assumed to have no effect on the organism levels on the carcass (Cudjoe et al*.,* 1991). However, water chilling can decrease the levels of contamination. For modelling purposes, the assumption is made that if a carcass is to be sold as frozen products water chilling is used, and for fresh products air chilling is used. When water chilling is used, chlorine may be used as an additive. This affects the carcass contamination, as can be seen in Table 3.9. This effect is due to chlorine controlling cross-contamination from the water to the carcasses. Here carcass contamination was measured before and after chilling with water. Procedures both with and without chlorine are included in this data set, as currently available in the published literature.

The frequency with which chlorine is used will be specific to a country. This frequency is defined as P_{Cl} and, for illustrative purposes, 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*.

Table 3.8 Sample data showing measures of *Campylobacter* before and after carcass washes.

Mean log cfu before washing	Mean log cfu after washing	Proportion of organisms remaining
1.60	1.00	0.25
3.20	2.10	0.08
3.70	3.30	0.39
2.53	2.00	0.29
4.00	1.60	0.003
3.70	2.70	0.10
2.83	1.71	0.08
2.94	2.39	0.28
3.50	3.04	0.35
5.70	5.10	0.25

SOURCE: Data from Berrang and Dickens, 2000.

Table 3.9 Data measuring the levels of *Campylobacter* contamination on a carcass before and after water chilling with and without chlorine added to the chill water.

Chlorine Presence	Mean log cfu before chilling	Mean log cfu after chilling	Reference
-	1.71	1.43	Izat et al, 1988
-	2.39	1.85	Izat et al, 1988
-	3.04	1.18	Izat et al, 1988
-	2.92	1.74	Wempe et al, 1983
-	2.62	1.38	Wempe et al, 1983
-	3.32	2.33	Wempe et al, 1983
-	2.50	1.76	Wempe et al, 1983
+	2.10	1.20	Berrang and Dickens, 2000
+	3.30	1.10	Berrang and Dickens,2000
+	2.00	0.90	Berrang and Dickens, 2000
+	1.60	3.20	Berrang and Dickens, 2000
+	2.70	1.10	Berrang and Dickens, 2000
+	5.35	3.86	Cason et al. 1997

In the model described here, 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, $\theta_s = \beta$, as 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 by:

$$\tau_i = \begin{cases} 1 & \theta_s = \alpha \\ \tau_{cl} & \theta_s = \beta; \quad Cl = 1 \\ \tau_{ncl} & \theta_s = \beta; \quad Cl = 0 \end{cases} \tag{3.12}$$

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 that has chlorine added to the water. The variables τ_{cl} and are τ_{ncl} estimated, initially by calculating the reduction in contamination in the samples shown in Table 3.9 with and without chlorine addition to the water. These data are then combined to give a non-parametric distribution.

Stern and Robach (2003) reported *Campylobacter* count data for faecal samples and carcass rinses of broiler chicken flocks in Georgia, USA, comparing the situation in 1995 to that in 2001. Levels of *Campylobacter* on freshly processed broiler carcasses showed a significant decrease from an average of $10^{4.1}$ per carcass in 1995, to $10^{3.1}$ in 2001. Results of their study suggest that, in the United States of America, the implementation of antimicrobial interventions (i.e. water chilling with added chlorine) has reduced consumer exposure to *Campylobacter*.

3.3.3 Estimating the prevalence of contaminated products, *Ppp*

The model described above follows initially a group of 100 birds from a randomly selected flock and subsequently a random bird from within this group through the processing plant. At each of the processing stages, 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

$$\psi_i = \begin{cases} 1 \text{ if } \eta_p \rangle 1 \\ 0 \text{ if } \eta_p \langle 1 \end{cases} \tag{3.13}$$

where ψ_i is whether or not the product is contaminated at retail. Within a given simulation, distributions are sampled n times. Each time, the result is either a contaminated or an uncontaminated product. Multiple samplings of the distributions represent the production of multiple products. Therefore, running the model allows for n samplings of each distribution. The probability that a random product is contaminated can be calculated by use of Equation 3.14.

$$P_{pp} = \frac{\sum_{i=1}^{i=n} \psi_i}{n} \tag{3.14}$$

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

3.3.3.1 Alternative processing models

As an example of the adaptation and extension of the current model to a national context, a poultry processing model has been developed for use in risk management in the Netherlands. This models a more mechanistic description of cross-contamination, inactivation and removal for each processing stage (Nauta et al., 2003). It differentiates between *Campylobacter*s from the carcass exterior and

*Campylobacter*s in the faeces that leaks during processing. It uses the same model structure for all processing stages, which enhances the transparency of the model. Except for the stages of de-feathering and evisceration, the processing model presented in this report assumes a linear input-output relation on a log scale of concentrations of *Campylobacter* on the carcass exterior. Nauta et al.'s model provides an alternative approach, which may be particularly relevant where cross-contamination between carcasses is considered important.

The current model, as well as the Netherlands model, confirms the need for adequate quantitative data on chicken and carcass contamination before and during processing. Of particular value is knowledge of the arithmetic mean of counts and the attending variability between individual chicken carcasses. The use of the "mean of log counts" is far from ideal, but is often the only information available. In the case of the Netherlands risk assessment, to address missing data and knowledge on the parameters of the processing model, a formal expert judgement study was performed to obtain information on the parameters of the processing model (Van der Fels-Klerx et al., 2005; Cooke et al., 2004).

3.3.3.2 Storage of the products

Following estimation of the prevalence of contaminated chicken products post-processing, the storage of products is considered. Two models are adopted, one to describe refrigerated storage and one for frozen storage. To describe refrigerated storage, the D-values presented by Koidis and Doyle (1983) are used within the Bigelow model. To describe frozen storage, the experimental studies of Aho and Hirn (1988), involving enumeration on frozen chicken products over a period, are utilized directly within a regression model to determine the relationship between the proportion of organisms contaminating a product that remain and the period for which that product has been frozen. The storage time will be country specific. For illustrative purposes, the time data used here are based upon consumer surveys carried out in the United Kingdom.

3.4 Home preparation and handling of chicken

In the home, during meal preparation, individuals can be exposed to *Campylobacter* from fresh chicken through a large number of pathways. These pathways could include: direct contamination from the chicken to any food commodities not undergoing a subsequent cooking step before ingestion; indirect contamination of surfaces upon which cooked products or ready-to-eat food are placed; contamination directly onto hands and subsequent ingestion; insufficient cooking; and a wide variety of other potential contamination events.

The model assumes that some amount of liquid that was part of the broiler or chicken product brought into the home, and which contains *Campylobacter*, may cross-contaminate and expose the consumer. The transfer of liquid may be back to the already cooked chicken or other foods prepared and consumed during the same meal. Alternatively, it may be ingested directly, for example, from contaminated hands. Transfer can be facilitated by liquid carried on hands, utensils and cutting boards, and these mechanisms may be a significant contributor to exposure and foodborne illness. Unsafe food handling procedures in private kitchens are assumed to be responsible for a large number of cases of foodborne diseases in most countries (Zhao et al., 1998; Worsfold and Griffith, 1997). In the United States of America, it was estimated that 21% of 7219 cases of foodborne diseases were related to private households in the period from 1973 to 1987 (Williamson, Gravani and Lawless, 1992). Furthermore, in England, it was estimated that 35% and 28%, respectively, of 101 outbreaks of foodborne diseases were related to insufficient heat treatment and cross-contamination of foods during preparation of meals in private households (Ryan et al., 1996). In

Sweden, the authorities have estimated that half of the number of foodborne cases were acquired in private households (Anderson, Norberg and de Jong, 1994).

Modelling of consumer handling and preparation in private kitchens has been divided into two parts: (1) Cross-contamination of a meal due to unsafe food handling procedures; and (2) the survival of *Campylobacter* due to undercooking of the chicken.

It should be noted that, depending upon the goals of the modeling exercise, different modelling approaches could be used. For instance, if a mechanistic understanding of the entire process is desired, and there is data or information that can allow the mechanistic model to be developed, then alternative approaches can be used. The models used in the current document were deemed to be most appropriate given the nature of information available at the time this model and document were prepared.

3.4.1 Cross-contamination

There is a large degree of uncertainty and variability associated with the food handling procedures in the kitchen. Estimating the risk of infection via cross-contamination is a difficult exercise and the data describing this area are extremely limited when compared with the diversity of possible behaviours and exposure pathways. The studies that currently exist report primarily on the presence of contamination on various surfaces following preparation activities, but little information is provided on the degree of contamination. These studies thus provide insight on the possibility of contamination, but do not provide a quantitative estimate of the transfer of *Campylobacter* from a contaminated product.

It is assumed that during the preparation of a chicken meal, a single event will occur that will result in the transfer of *Campylobacter* from the chicken to a utensil or cutting board, and also a subsequent transfer from the kitchen surface to a food that is consumed during the meal, such as cooked chicken, salad, vegetables or breads. Although it is acknowledged that during the preparation of a chicken meal an individual may cross-contaminate several surfaces and foods with *Campylobacter*, there are insufficient data to estimate the frequency of multiple cross-contamination events. In any case, the task would be to estimate the number of bacteria ingested across multiple pathways.

Therefore, at present we do not believe that it is possible to separate kitchen processes into different contamination routes and to quantify to what extent each of these routes contribute to the overall risk. The current document uses “the drip-fluid model”, which is based on a model previously described (Fazil et al., 2000), and is the most general of the models that were reviewed.

3.4.1.1 Description of the drip-fluid model

The drip-fluid model is an approach related to the water a chicken gains through processing. The model is based on the fact that when water is applied to chickens during processing, such as in washing and in an immersion chiller, a proportion of the load that is loosely attached is diluted in the volume of water, some of which may be absorbed by the chicken or form a thin layer around the carcass. A certain portion of the fluid subsequently drips off the chicken during processing; however, the concentration of loosely attached organisms in the fluid that remains on the carcass is the same, provided that additional cells do not subsequently go into suspension in the fluid. For example, if the concentration on a chicken is estimated to be 3 log cfu/carcass, and 5% of the load is assumed to be loosely attached, and if it is further assumed that the loosely attached cells are diluted in 200 ml of fluid as a result of immersion chilling, and that after the chill tank, approximately 75 ml of fluid remain on the carcass and the rest drips off at the processing plant, then the concentration in the fluid contained on the chicken can be crudely estimated as follows:

- Concentration on Chicken = 3 log cfu/Chicken = 1000 cfu/Chicken
- Fraction of loosely attached = 0.05
- Number of cells loosely attached = 1000 × 0.05 = 50 cfu/Chicken
- Volume of fluid diluting loosely attached cells = 200 ml
- Number of cells in 200 ml of fluid = 50 cfu
- Concentration in fluid = 50cfu/200 ml = 0.25 cfu/ml

If only 75 ml of the fluid remain on the carcass when it is packaged, the concentration in this volume of fluid would still be 0.25 cfu/ml. There would be approximately 19 cfu in the fluid on the chicken (0.25 cfu/ml × 75 ml) and approximately 31 cfu would have 'dripped' off in the fluid that did not remain on the carcass.

Figure 3.1 summarizes the steps taken to estimate the concentration of *C. jejuni* in drip fluid in the home, based on the concentration on the chicken during processing.

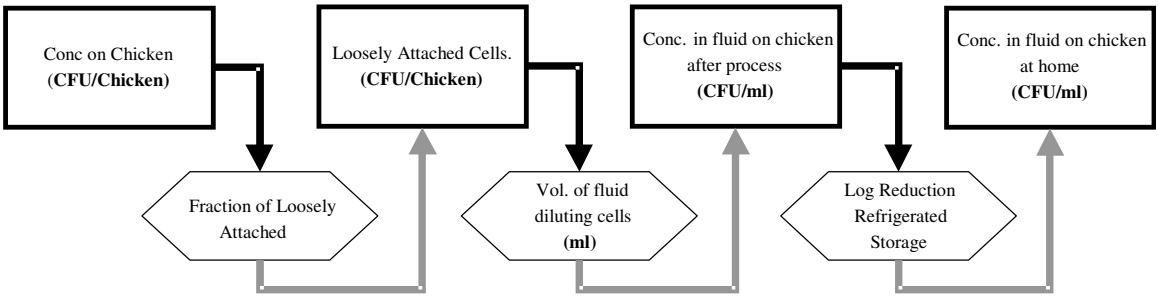

Figure 3.1 Steps in estimating the concentration of *C. jejuni* in fluid on a chicken.

As an example, the Canadian Food Inspection Agency (CFIA) has regulations in place for the permissible weight increase for chicken from the addition of water during processing, permitting up to 8% for chickens under 2.3 kg. In the USA, similar guidelines exist, with chickens of 4.5 lb. (2 kg) or less allowed to absorb no more than 8% of their weight in water during processing (FSIS, 1996). A broiler of 1400 g could thus have approximately 100 ml of water contributing to its weight. The volume of water on a chicken could be quite substantial and the assumptions for the dilution volume are quite feasible. However, research looking at issues such as the amount of fluid on a chicken and the concentration in the fluid in relation to the concentration on the carcass would provide valuable information towards a better estimation of the risk associated with cross-contamination. This current assessment provides an estimate of the potential risk from exposure to drip fluid from chicken. The assumptions used to estimate the concentration in the fluid may be reasonable estimates, although it should be noted that there are no 'hard' data to support the assumptions. As stated earlier, there exists a substantial data gap in this area, which might be significantly reduced through research activities.

The risk estimated from the drip-fluid module is based on the consumer ingesting, through a single or multiple pathways, between 0.5 and 1.5 ml of drip fluid. Exposure in this case assumes ingestion of this volume of fluid without further reduction in the concentration (through cooking, for instance). The route of exposure to this volume of drip fluid is not explicitly specified, but the

volume of fluid used in the estimate is relatively small and could be transferred to the consumer along many pathways during meal preparation (some of which were noted earlier in this section).

The distributions used in the drip-fluid model and their descriptions are summarized in Table 3.10.

Table 3.10 Model parameters in drip-fluid model

Parameter	Description	Unit	Distribution/expression
F_{loose}	Fraction of loosely attached *Campylobacter* cells		Uniform(0.01,0.1)*
V_{Drip}	Volume of drip fluid ingested	ml	Uniform(0.5,1.5)*
V_{Dilute}	Volume of fluid assumed to dilute loosely attached cells	ml	Uniform(150,250)*

NOTE: * = Uniform(min,max)

Quantitative research on cross-contamination due to food handling in the private kitchen has been receiving increasing attention during the course of this risk assessment. Although the variability in consumer behaviour remains a crucial complicating factor for risk assessment, the increased availability of data and models will allow improved analyses for risk assessment in the near future (e.g. Chen et al., 2001; Gorman, Bloomfield and Adley, 2002; Kusumaningrum et al., 2003; Mylius, Nauta and Havelaar, 2003; Moore, Sheldon and Jaykus, 2003; Mattick et al., 2003). See also Redmond and Griffith (2003) for a review of food safety studies dealing with consumer food handling in the home.

3.4.1.2 Cooked chicken

This section addresses the exposure pathway of ingestion of *Campylobacter* that survive a roasting process applied to a whole carcass in a domestic kitchen. The goal of this analysis is to determine the frequency with which carcasses will remain contaminated following cooking and the number of surviving cells. In order to generate a model to fully characterize this exposure, the following information is required:

- the number of cells on the carcass;
- the distribution of those cells throughout the carcass surface and mass;
- the extent to which there are areas of the carcass that provide protection of the cells from the heat of the oven ('protected areas');
- the time-temperature profile on the surface of the chicken and within the mass of the chicken;
- the time-temperature profile within any protected areas during the cooking process;
- the rate of deactivation of cells as a function of time and temperature;
- variability of each of the above phenomena across carcasses, consumers, ovens and strains; and
- variations in the mode of preparation (e.g. baking, broiling, grilling, microwaving or stir-frying) and the resulting impact on the time-temperature profiles listed above.

Several modelling approaches have been pursued, with alternative assumptions and varying levels of complexity. Three approaches were described in the development of this risk assessment, but further work is required to provide support for conclusions on the overall efficacy of domestic cooking processes.

3.4.1.3 Approaches to modelling of cooking – overview

Campylobacter is sensitive to the effects of thermal processing, and cooking is likely to result in substantial log reductions. One of the reasons for this expectation is the fact that cooking regimens for poultry have, in many jurisdictions, been designed for the inactivation of *Salmonella* spp., which are significantly more tolerant of heat. Due to this somewhat 'over-designed' heat treatment with respect to *Campylobacter*, it seems reasonable to forego calculation of the reduction of *Campylobacter* on the surface of the carcass, since the sustained high surface temperatures (>70°C) will generate more than enough log-reductions to eliminate them. Instead, the focus of the modelling described here is to discover the conditions whereby some *Campylobacter* might survive and to attempt to characterize this exposure. The goal is to provide a basis for reasoned attribution of the home exposure risk between the 'undercooked' and 'cross-contamination' pathways. This type of analysis is intended to complement the results of epidemiological approaches, which attempt to attribute risk between undercooking and food handling practices.

Given the list of information requirements listed in the previous section, it is clear that modelling of cooking retains a number of imposed shortcomings. However, modelling assists in the management of this risk by providing a mechanistic explanation of the relatively large log reductions associated with cooking, and by describing alternative explanations of the means by which *Campylobacter* might survive what would otherwise be considered a thorough heat treatment (e.g. in thermally protected areas such as cracks, air pockets under wings, on surfaces that receive less direct heat, or deep within the carcass mass).

Internal temperature approach (summary)

In this approach, a representative point is chosen in the mass of the chicken and it is assumed that this represents an area of the chicken that can be expected to receive the mildest heat treatment. In the approach described below, a point within the mass of the drumstick portion of the chicken is selected. This selection is partially based on the existence of a cooking temperature survey, which measured the internal temperature of chicken drumsticks. The reduction in the number of cells at this point is then calculated by calculating a step-wise time-temperature profile and applying reductions at each time-step. This approach also includes the possibility of an increase in cell numbers in the period where the chicken is being heated through the range of temperatures that might allow growth of *Campylobacter*. The variability in consumer practices is implemented in this approach by varying the stopping point (i.e. the final temperature) in the simulated time-temperature profile. The variation in the stopping point is based on survey data measuring this final temperature.

Protected areas approach (summary)

This approach employs four main assumptions:

- assume that only some fraction of the carcasses will experience the type of undercooking described in this approach;
- assume that the only cells that have any possibility of survival are within an area that is relatively protected (or insulated) from the heat of the oven;
- assume some fraction of the total number of cells to be located in the protected areas; and
- assume a maximum temperature reached within this protected area and the time for which this maximum temperature is applied.

With these assumptions, a reduction is calculated based on the assumed final temperature stage within the protected area.

The heat transfer approach (summary)

This approach attempts to calculate the internal time-temperature profile at a number of different depths into the meat of the carcass. This requires use of transient heat transfer models and parameters of thermal properties of chicken, which are generally available in food engineering texts. This approach requires the following assumptions:

- the proportion of the bacterial load found at the surface and at various depths into the carcass;
- a simplified characterization of the roasting of a carcass with respect to specifying heat transfer assumptions; and
- the oven temperature and the time at which the chicken is removed from the oven.

The reduction in cell numbers can be characterized at each depth into the chicken meat by considering the reduction in each simulated time-step.

Ultimately, for this risk assessment, a combination of the internal temperature and protected areas approaches was applied.

3.4.1.4 Description of the cooking model

In order to estimate the log reductions from cooking, the effects of temperature on the organism were modelled using experimentally determined D and z values. The D-value is the time required at a specific temperature to destroy 90% (1 log decrease) of the population. The z-value is the temperature increase required to reduce the D-value by 90%, or a factor of 10.

Blankenship and Craven (1982) studied the thermal sensitivity of *C. jejuni* in poultry meat. The thermal death times for a five-strain composite and strain H-840 in autoclaved ground chicken were determined (Table 3.11). The z-values for the five-strain composite and strain H-840 were reported as 6.35°C and 5.91°C, respectively. It is evident from these data that large log reductions can be expected during normal cooking (e.g. greater than a 5 log reduction if temperature exceeds 57°C for 5 minutes).

Table 3.11 Thermal death times for strain H-840 and a 5-strain *C. jejuni* composite.

Temperature (°C)	**H-840** z-value = 5.91 C D-value (minutes)	**5 strain composite** z-value = 6.35 C D-value (minutes)
49	20.5	ND
51	8.77	9.27
53	4.85	4.89
55	2.12	2.25
57	0.79	0.98

NOTE: ND = non detected.
SOURCE: Data from Blankenship and Craven, 1982.

To estimate the log reductions at different times and temperatures, a linear regression was performed on the data. The regression used the log transformed D values, using an equation of the form shown in Equation 3.15:

$$Log(D) = (-a \times Temp) + b \qquad (3.15)$$

"*a*" and "*b*" are constants that are estimated through the regression procedure. However, within this equation the term "a" is equivalent to the inverse of the z-value. Therefore the published z-value for the study was used and fixed, while adjusting the "b" coefficient in order to provide a "least squares" fit to the data. In the current analysis, only the data for the 5-strain composite in chicken meat was used in the linear regression, although the data could be pooled and a linear regression performed on this data set as well. The results of both analyses (composite and pooled) are shown in Figures 3.2 and 3.3.

The equation used is:

$$Log(D) = (-0.1575 \times Temp) + 9.004 \tag{3.16}$$

The D-value at that temperature is then simply the log transform of this value:

$$D = 10^{(-0.1575 \times Temp)+9.004} \tag{3.17}$$

Finally, given the D-value, and recalling the definition of the D-value given earlier, the log reduction that would occur at that temperature for a given period of time (t), was estimated using Equation 3.18.

$$LogR = \frac{t}{10^{(-0.1575 \times Temp)+9.004}} \tag{3.18}$$

The sensitivity of *C. jejuni* to temperature effects suggests that organisms exposed to the heat without significant protection are unlikely to survive cooking. Those cells that are present on the surface of chicken are likely to be inactivated with even moderate heat, unless the chicken is relatively grossly undercooked. Thus, it may only be those cells that are in an area of the carcass or product that affords them some level of protection from direct heat that will actually survive. These areas may include visceral cavities, crevices, and areas around joints or in cut and bruised tissues. It was assumed that 10 to 20% of the organisms would be located in such "protected" areas (Table 3.12).

Table 3.12 Distribution for proportion of cells in "protected" areas of chicken carcass

Distribution	Min	Mode	Max	Expected Value
TRIANGULAR	0.10	0.15	0.20	0.15

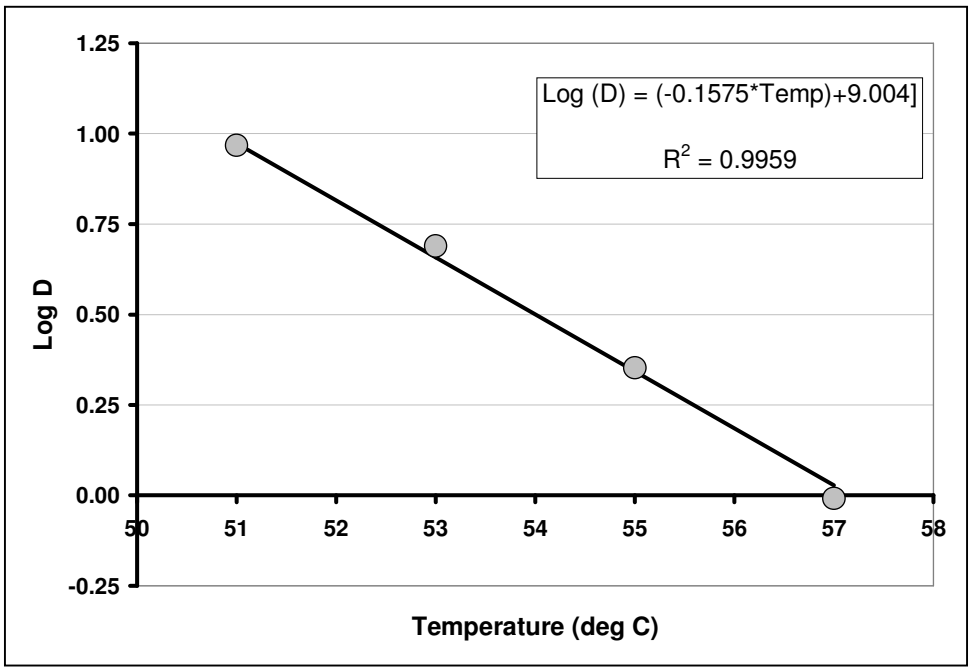

Figure 3.2 Linear regression using the given Z-value and 5-strain composite sample in chicken.
SOURCE: Data from Blankenship and Craven, 1982.

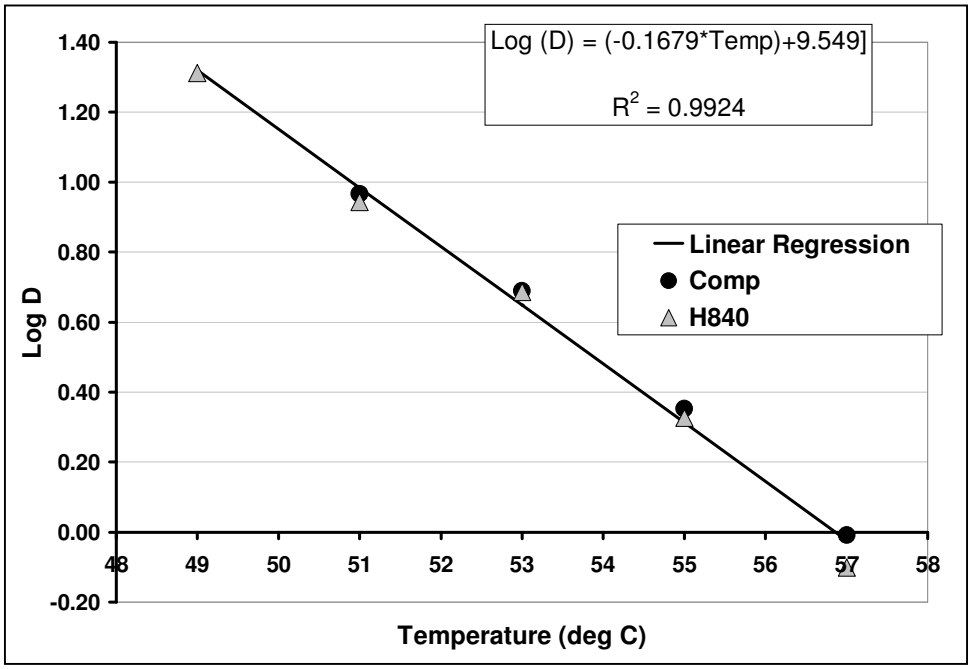

Figure 3.3 Linear regression using 5-strain composite and H-840 in chicken meat.
SOURCE: Data from Blankenship and Craven, 1982.

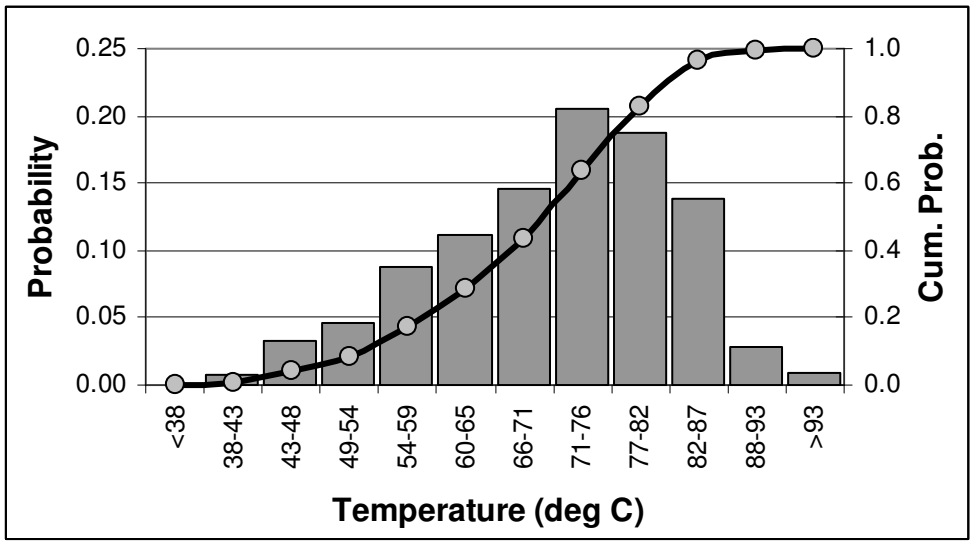

Figure 3.4 Audits International data for poultry final cooking temperature.

In order to estimate the log reductions that could occur in areas of the chicken that are "protected", it was assumed that during cooking these "protected" areas would be exposed to a temperature represented by data reported in the "FDA Home Cooking Temperature Database" compiled by Audits International and summarized in Figure 3.4. The time that these areas experience this temperature was assumed to range from 0.5 to 1.5 minutes (Table 3.13). There are no data indicating what the true time and temperature combinations might be in protected areas within the chicken during moderate cooking scenarios. This parameter may be an important determinant of risk and additional experimentation into this area may be valuable.

Table 3.13 Distribution for time "protected" area is exposed to maximum heat.

Distribution	Min	Mode	Max	Expected Value
TRIANGULAR	0.50	1.00	1.50	1.00

4. HAZARD CHARACTERIZATION

Hazard characterization describes the adverse health effects of a substance, organism or other entity. This component of the risk assessment usually includes a dose-response relationship, which is represented as the probability that a random person will become infected or ill after exposure to a specific quantity of *Campylobacter* organisms. The types of data that can be used to establish dose-response relationships include animal and human feeding studies and epidemiological data such as data from outbreak investigations where the number of organisms consumed was estimated.

4.1 Pathogen, host and food matrix factors

4.1.1 Infectivity, virulence and pathogenicity of the organism

The dose that may cause infection (infection, for present purposes, being colonization with or without signs of illness) depends upon a number of factors, including the virulence of the strain, the vehicle with which it is ingested and the susceptibility of the individual.

The pathogenesis of *Campylobacter* has been reviewed (Ketley, 1995; 1997; Wooldridge and Ketley, 1997; Smith, 1996). In general, the mechanisms involved in the pathogenesis of *Campylobacter* are rather poorly understood. Motility, chemotaxis and the flagella are known to be important factors in the virulence as they are required for attachment and colonization of the intestinal epithelium (Ketley, 1997). Once colonization has occurred, *Campylobacter* may perturb the normal absorptive capacity of the intestine by damaging epithelial cell function either directly, by cell invasion and/or production of toxin(s), or indirectly, following the initiation of an inflammatory response (Wooldridge and Ketley, 1997). Several virulence determinants have been described to be involved in the induction of diarrhoea: adhesion and invasion molecules; outer membrane proteins; lipopolysaccharides; stress proteins; flagella and motility; M cells; iron acquiring mechanisms; and cytotonic and cytotoxic factors (Smith, 1996). However, their relative role and importance for development of diarrhoea is not quite clear. The ability of *Campylobacter* to invade host cells *in vitro* is well established and cytotoxin production is consistently reported (Ketley, 1997). Early reports of enterotoxin production have not been confirmed and thus the opinion that *Campylobacter* produce an enterotoxin is no longer widely held (Allos and Blaser, 1995; Wooldridge and Ketley, 1997). Not all strains involved in human enteritis produce toxins, and no correlation has been found between serotype and toxin production (Fricker and Park, 1989).

4.1.2 Host characteristics

4.1.2.1 Susceptibility

Populations at risk with respect to infectious diseases often include the elderly, children and individuals suffering from illnesses that compromise their immune systems (e.g. AIDS and cancer patients). For campylobacteriosis, young adults (around 15–25 years old) appear to be more frequently exposed or more susceptible than other age groups (Blaser, Taylor and Feldman, 1983; Engberg and Nielsen, 1998; Kapperud and Aasen, 1992).

4.1.2.2 Age

In developed countries, all age groups may become infected with *Campylobacter*. However, in most countries the reporting rate of campylobacteriosis is higher in young children (0–4 years old) and young adults, as seen in Norway (Kapperud and Aasen, 1992; Kruse, 2001), Denmark (Anon., 2001b), Iceland (Thorkelsson et al., 2001), Finland (Anon., 2001d), New Zealand (Brieseman, 1990; Perks, 2001), England and Wales (PHLS, 2001), and the United States of America (CDC-FoodNet; Friedman et al., 2000). The high incidence rate in children may be a result of higher susceptibility, more frequent exposure to pets, or a higher notification rate compared to adults, reflecting that parents more frequently seek medical care for their children compared with illness among adults. The high incidence rate in young adults has been suggested to be due to higher travel activity in this age group compared to other age groups (Kapperud and Aasen, 1992); higher recreational activity, including participation in water sports (Skirrow, 1987); and an increased exposure to high-risk food items (Engberg and Nielsen, 1998). The higher incidence may also be a result of unsafe food handling practices among persons that may be in the process of learning how to prepare food for themselves.

In developing countries, illness is more common among infants and children and it may be assumed that young adults and adults have some level of acquired immunity following repeated exposure (WHO, 2000).

4.1.2.3 Sex

In general, males seem to have a higher incidence rate of *Campylobacter* infections than females. In the United States of America in 1998 (CDC-FoodNet), 30% of the infected persons were women and 70% were men. It has been reported that young boys have a higher incidence rate than young girls (Skirrow, 1987; Kapperud and Aasen, 1992; Statens Seruminstitut, unpublished results). For adolescents and young adults, the sex differences seem to vary between countries. In Denmark, the incidence rate is higher for young females than young males (Statens Seruminstitut, unpublished results). In United States of America, New Zealand and Norway, young males are more frequently infected (Skirrow, 1987; Brieseman, 1990; Kapperud and Aasen, 1992). The reason for this sex difference has not been explained.

4.1.3 Demographic and socio-economic factors

4.1.3.1 Ethnicity

Differences in infection rates between different ethnicity groups have been observed. In New Zealand, the rate of infection has been calculated for people belonging to different ethnicity groups. Pacific people had a lower rate (50.8) compared with Europeans (245.0) and other ethnicities (216.2). The reason for the difference was not explained (NZ Ministry of Health, 2001).

4.1.3.2 Local environmental factors

The incidence of campylobacteriosis seems to be area dependent, i.e. some areas in, for example, Denmark, Norway, the United Kingdom and New Zealand have a much higher incidence than the rest of the country (Engberg and Nielsen, 1998; Brieseman, 1990; Kapperud, 1994; Jones, Betaieb and Telford, 1990). In the United Kingdom and New Zealand, *Campylobacter* infections have occurred at a higher incidence in rural than urban areas (Skirrow, 1987; Brieseman, 1990). In Norway, the opposite has been observed (Kapperud and Aasen, 1992). The higher incidence in

urban areas was explained by a higher proportion of imported cases (e.g. involving travel) in these areas in comparison with rural areas (Kapperud and Aasen, 1992).

4.1.3.3 Poultry slaughterhouse workers

Several investigations have revealed that new workers at slaughterhouses are part of the population with an increased risk of becoming infected by *Campylobacter* spp. (Jones and Robinson, 1981; Christenson et al., 1983; Mancinelli et al., 1988; Berndtson, Danielsson-Tham and Engvall, 1996). This is presumably due to the heavily contaminated environment at the slaughterhouse. The presence of *C. jejuni* in the air at broiler slaughterhouses has been investigated. Berndtson, Danielsson-Tham and Engvall (1996) demonstrated that 40% to 75% of air samples from the surroundings of a processing line were contaminated with *C. jejuni*, and Oosterom et al. (1983a) found that the number of *C. jejuni* per m^3 air was in the range $\log_{10}$ 1.70 to $\log_{10}$ 4.20. The presence of *C. jejuni* in the air along the processing line could pose a risk to the workers through contact with contaminated aerosols. Further, contamination of the hands of processing line workers by *C. jejuni* at levels up to $\log_{10}$ 4.26 *C. jejuni* per hand has been demonstrated (Oosterom et al., 1983a; Ono and Yamamoto, 1999). This may pose a risk to the health of the exposed person and may enhance the possibilities of cross-contamination of the products.

4.1.3.4 Season

A seasonal variation in the number of human cases has been noticed in several countries, including Denmark, Sweden, Norway, Finland, Iceland, The Netherlands, United Kingdom, the United States of America and New Zealand, with more than doubling of the incidences in late summer (Statens Seruminstitut, unpublished results; Brieseman, 1990; Kapperud and Aasen, 1992; Skirrow, 1991; Newell et al., 1999; Friedman et al., 2000; Anon., 2001d; Kruse, 2001; Thorkelsson et al., 2001; Waagenar, 2001). The significance of seasonality seems to increase with increasing latitude (Kapperud and Aasen, 1992). The late summer peak coincides with seasonal habits of travelling abroad, but domestically acquired infections also increase in number during this period (Kapperud, 1994; Engberg and Nielsen, 1998).

In countries such as Denmark, Norway, Sweden, the United Kingdom and the Netherlands, the seasonality in the human cases is coincident with the seasonality in the number of infected broiler flocks (Danish Veterinary Laboratory, unpublished data; Kapperud et al., 1993; Berndtson, 1996; Newell et al., 1999; Waagenar, 2001). This may indicate that humans acquire *Campylobacter* from eating chickens or that humans and broilers are becoming infected from a common source.

4.1.3.5 Developing and developed countries

The clinical manifestations of infections seem to differ between developed countries and developing countries, both in the age of the affected populations and in the severity of infection. In developed countries, *Campylobacter* enteritis often affects older children and young adults, and the effects can be severe, being characterized by fever, abdominal cramping and bloody diarrhoea that may require treatment with antimicrobials. In contrast, *Campylobacter* infections in developing countries tend to affect children under one year of age with severe symptoms and illness, but the symptoms are often milder in older children.

In developed countries, serious infection often results in the excretion of the organism for 2 weeks or more (Karmali and Fleming, 1979). For developing countries, a similar period is often observed for children under one year of age, but the period appears to be shorter in older children and individuals. In an Egyptian study, a cohort of 397 children below the age of 3 years was studied over a three-year period. Convalescent excretion of *Campylobacter* was on average 14 days after a

diarrhoeal episode (Rao et al., 2001). Researchers in Thailand showed that during recovery from a *Campylobacter* infection, excretion of the organism occurred for approximately 14 days in children under one year of age, but only for an average of 8 days for children between the ages of 1 and 5 years (Taylor et al., 1988). Subsequent studies in Thailand showed that the amount of *C. jejuni* organism excreted also declined with increasing age (Taylor et al., 1993).

4.1.4 Health factors

Different health factors may affect the susceptibility of the host, including immunity, concurrent infections, medication and underlying disease.

4.1.4.1 Acquired immunity

Patients suffering from campylobacteriosis may develop acquired immunity for the causative *Campylobacter* strain (for a period). This was demonstrated by Black et al. (1988), where the volunteers who became ill developed a serum antigen response to the *Campylobacter* strain they had ingested and hence were protected from subsequent illness but not infection with the same strain. Acquired immunity may explain why employees in broiler slaughterhouses who develop campylobacteriosis at the beginning of their employment, are less prone to campylobacteriosis as their employment progresses (Christenson et al., 1983). In addition, the complement fixing antibody against *Campylobacter* was found more often in poultry and meat process workers than the normal population (Jones and Robinson, 1981). This was not observed in a Danish study, where Lings, Lander and Lebech (1994) found no significant differences in the prevalence of serum antibodies against *C. jejuni* between a group of 217 Danish slaughterhouse workers and a group of 113 Danish greenhouse workers.

4.1.4.2 Underlying disease

Underlying disease has been described as a predisposing factor for acquiring enteric infections. In addition, underlying disease seems to enhance the severity of such infections. In a study carried out in Spain, 93% of 58 patients with bacteraemia caused by *Campylobacter* spp. had an underlying disorder, including liver cirrhosis, neoplasia, immunosuppressive therapy and human immunodeficiency virus infection (Pigrau et al., 1997). In a similar study carried out in Denmark, Schonheyder, Sogaard and Frederiksen (1995) described 15 cases of bacteraemia caused by *Campylobacter* spp. Eleven of the 15 patients in this investigation had underlying disorders, including immunological, neoplastic and vascular disease. In a Danish case-control study, Neimann (2001) reported that underlying disease like kidney, vascular and intestinal disorders were dominating among patients with campylobacteriosis. The disease diabetes melitus is also recognized as a factor increasing the risk related to infections by enteric pathogens (Neal and Slack, 1997).

Persons infected with Human Immunodeficiency Virus (HIV) are also at increased risk of acquiring *Campylobacter* infections. A study in Los Angeles (USA), 1983–1987, showed that the reported incidence of laboratory-confirmed campylobacteriosis in persons with AIDS was 519 per 100 000, much higher than the reported rate in the general population (Sorvillo, Lieb and Waterman, 1991).

4.1.4.3 Concurrent medication

Medication with antisecretory drugs like omeprazole and H_2-antagonists has been shown to increase the risk for acquiring campylobacteriosis, presumably due to a rise in the pH of the stomach contents (Neal et al., 1996; Neal and Slack, 1997). Results of case-control studies suggest that the

use of antibiotics and hormones will increase the risk of acquiring infection by *Campylobacter* spp. (Neal et al., 1996; Effler et al., 2001; Neimann, 2001).

4.1.5 Factors related to the matrix and conditions of ingestion

The vehicle with which *Campylobacter* bacteria are ingested may be important for development of illness. In a volunteer feeding experiment, the illness rate was higher in volunteers given the organisms in bicarbonate compared with in milk (Black et al., 1988). This can be explained by the barrier effect of the gastric acid, which is reduced when *Campylobacter* bacteria are ingested with a buffering vehicle.

4.2 Adverse health effects

4.2.1 Acute gastrointestinal manifestations

Enteropathogenic *Campylobacter* can cause an acute enterocolitis, which is not easily distinguished from illness caused by other enteric pathogens. The incubation period may vary from 1 to 11 days, typically 1 to 3 days. The main symptoms are malaise, fever, severe abdominal pain and diarrhoea. Vomiting is not common. The diarrhoea may produce stools that can vary from profuse and watery to bloody and dysenteric. In most cases, the diarrhoea is self-limiting and may persist for up to a week, although mild relapses often occur. In 20% of cases, symptoms may last from one to three weeks (Allos and Blaser, 1995). Excretion of the organism may continue for up to 2 to 3 weeks.

4.2.2 Non-gastrointestinal sequelae

Campylobacter infections may be followed by rare but severe non-gastrointestinal sequellae that may afflict the normal population, namely reactive arthritis, the Guillain-Barré syndrome (GBS) and the Miller Fisher Syndrome. These complications show different pictures of symptoms or disorders.

Reactive arthritis (incomplete Reiters Syndrome) has been estimated to occur in approximately 1% of patients with campylobacteriosis. Reactive arthritis is a sterile post-infectious process, which may affect multiple joints, particularly the knee joint. The symptoms occur seven to ten days after onset of diarrhoea (Peterson, 1994). Pain and incapacitation can last for months or become chronic. Reactive arthritis is often associated with the tissue phenotype HLA-B27 and cannot be separated from the affection of the joints that may follow from a *Yersinia*, *Salmonella* or *Shigella* infection (Peterson, 1994; Allos and Blaser, 1995). The condition is immunological and cannot be treated with antibiotics. The medical treatment may consist of a non-steroid anti-inflammatory drug (NSAID). The pathogenesis of this entity is unknown (Allos and Blaser, 1995).

Evidence suggests an association between *Campylobacter* illness and a rare but serious paralytic condition, GBS, a demyelinating disorder of the peripheral nervous system resulting in weakness, usually symmetrical, of the limbs, weakness of the respiratory muscles and loss of reflexes (areflexia). Early symptoms of GBS include burning sensations and numbness that can progress to flaccid paralysis. GBS has been estimated to occur about once in every 1000 cases of campylobacteriosis, i.e. up to 40% of all GBS cases in the USA occur after *Campylobacter* infections (Mishu and Blaser, 1993; Mishu et al., 1993; Allos, 1997). GBS seems to be more common in males than females (Mishu et al., 1993). Although most GBS patients recover (about 70%), chronic complications and death may occur (Blaser, Allos and Lang, 1997). There is no relation between the severity of the gastrointestinal symptoms and the likelihood of developing GBS after infection with *C. jejuni*; in fact, even asymptomatic *Campylobacter* infections can trigger GBS (Allos and Blaser, 1995). The pathogenesis of GBS is only partly known. GBS is presumably caused by an immunological cross-reaction between *Campylobacter* anti-genes

(lipopolysaccharides) and glycolipids or myelin proteins in the peripheral nervous system. The serotype O:19 seems to be more often involved in this condition than other *Campylobacter* serotypes (Allos and Blaser, 1995; Allos, 1997).

In some cases, campylobacteriosis has also been associated with the Miller Fisher Syndrome, which is considered to be a variant of the Guillain-Barré syndrome. The Miller Fisher syndrome is characterized by opthalmoplegia, ataxia and areflexia (Othsuka et al., 1998).

4.2.3 Mortality

In general, very few deaths are related to *Campylobacter* infections and these deaths usually occur among infants, elderly and immuno-suppressed individuals (Tauxe, 1992; Altekruse et al., 1999). In England and Wales, fewer than 10 deaths in approximately 280 000 cases were reported from 1981 to 1991 (<0.0036%) (Phillips, 1995). In 1999 in the United States of America, 2 of 4025 registered patients died (0.05%) (CDC-FoodNet). The average annual number of deaths related to *Campylobacter* in the United States of America has been estimated to be 124 of 2 453 926 estimated campylobacteriosis cases (0.005%) (Mead et al., 1999). A recent Danish analysis of mortality at 30-days post infection suggests that in Denmark the case-fatality rate may be 4 per 100 000 (0.004%). HIV infection may contribute to this mortality (WHO, 2000). In New Zealand, 2 deaths were reported in 1997, giving a case-fatality rate of 0.02% (NZ Ministry of Health, 2001).

4.2.4 Effect of antimicrobial resistance

Development of antimicrobial resistance may compromise treatment of patients with severe diarrhoea and bacteraemia. In the beginning of the 1990s, fluoroquinolone-resistant *C. jejuni* and *C. coli* emerged in human populations in Europe, as reported in the United Kingdom, Austria, Finland and the Netherlands (Piddock, 1995; Endtz et al., 1991). This resistance has been linked to the approval of enrofloxacin for treatment of diseases of broiler chickens, as investigations have shown that fluoroquinolone-sensitive *C. jejuni* strains were able to convert to resistant forms when fluoroquinolone was added to broiler chicken feed (Jacobs-Reitsma, Kan and Bolder, 1994). In general, most human *Campylobacter* infections are self-limiting and do not need antimicrobial therapy. However, in severe cases, medication may be necessary. In such cases, the drug of choice is usually erythromycin, though fluoroquinolones such as ciprofloxacin and norfloxacin are also used (Blaser, Taylor and Feldman, 1983).

Fluoroquinolone-resistant *Campylobacter* from chicken and other poultry is an emerging public health problem. A study by Smith and colleagues (Smith et al., 1999) found that patients with resistant *C. jejuni* infections had a longer duration of diarrhoea than patients infected with susceptible strains. Although lower frequencies of resistance are reported in many countries, the problem of fluoroquinolone-resistant *Campylobacter* is particularly acute in Taiwan Province of China, Thailand and Spain, where resistance levels are 56.9%, 84% and 88%, respectively (Gallardo et al., 1998; Hoge et al., 1998; Li et al., 1998). Treatment can also be complicated by the emergence of multidrug resistance. In Thailand, Hoge et al. (1998) found 100% resistance to both fluoroquinolones ciprofloxacin and azithromycin. Although resistance to many drugs is mounting in *Campylobacter*, many bacteria classified as resistant to a certain antibiotic in laboratory tests may nevertheless be amenable to antimicrobial therapy. This has been shown by Piddock (1999), who noted that only 1 of 39 patients with ciprofloxacin-resistant *Campylobacter* enteritis did not respond to ciprofloxacin therapy.

4.3 Campylobacter dose-response analysis

The probability of illness is dependent on the occurrence of three conditional probabilities: the probability that the organism is ingested; the probability that the organism is able to survive and infect the host once it is ingested; and the probability of the host becoming ill once infected. The environment, the pathogen and the host are all variables that play an important role in the probability of illness. Environmental influences include the food vehicle and the stability of the gastrointestinal tract ecosystem. Pathogen influences include the dose, virulence, and the colonization potential in the host gastrointestinal tract. Host influences include immune status, age and stomach contents (Coleman and Marks, 1998).

The dose-response analysis translates the number of organisms to which an individual is exposed, into an estimate of the individual's probability of infection. In developing a relationship for the quantitative dose-response analysis, there are two types of data that can be used if they are available: epidemiological, outbreak data; and feeding trials with human subjects. Epidemiological data, if collected well and if information such as attack rate and ingested dose are provided, can be an ideal data set. These data would essentially provide a "real-world" response using subjects that are representative of the population at large. Although there are an abundant number of epidemiological studies, the investigations rarely analyse the foods to enumerate the disease-causing doses. The second type of data, feeding trials using human subjects, can provide useful dose-response analysis data, but the doses applied in these studies are usually high, and the subjects are predominantly healthy individuals. Furthermore, these studies often use one or a limited number of strains, which may not represent all the virulence characteristics.

In the epidemiological literature that has been reviewed, there is insufficient information to allow a dose-response relationship to be derived using this type of data. There is one human feeding trial study that has been reported (Black et al., 1988). This study used healthy young adult volunteers from the Baltimore, USA, community. The challenge dose was administered in milk, and the volunteers fasted for 90 minutes before and after ingesting the organism. This study involved the use of two strains of *C. jejuni* (A3249 and 81-176). Strain A3249 was isolated from a 16-year old boy with a sporadic infection after an outbreak at a camp in Connecticut, USA. Strain 81-176 was isolated from an ill nine-year old girl in an outbreak in Minnesota, USA. The results of the feeding trial study are presented in Table 4.1 and in Figures 4.1 and 4.2.

Two distinct hypotheses have been proposed for the nature of the dose-response relationship for foodborne pathogens. The first is based on a historical notion that there is a threshold number of organisms, or minimum infectious dose, that must be ingested before any infection or adverse effects occur. The second hypothesis is that a single pathogen cell has the ability to initiate an infection or illness (Haas, 1983; Rubin, 1987; Rubin and Moxon, 1984). In the second hypothesis, there is no threshold number, and the probability of causing infection increases as the levels of the biological agent increases.

Several investigators have examined the available data and proposed non-threshold models for a number of pathogens (Haas, 1983; Teunis et al., 1996). The sufficiency of these models to describe the data, and—more importantly—the acceptance of the theory underpinning the models, has resulted in non-threshold dose-response models being the currently accepted models for describing the dose-response relationship. In addition, with low dose linearity, they are recommended by FAO/WHO for use in hazard characterization (FAO/WHO, 2003). The primary non-threshold single-hit models currently used in microbial risk assessment are the exponential and Beta-Poisson dose-response models.

In the exponential model, it is assumed that all of the ingested organisms have the same probability, r, of causing an infection. The dose ingested is assumed to be Poisson distributed with a mean of N organisms per portion (Haas, 1983).

$$P_{inf} = 1 - \exp(-r \times N) \quad (4.1)$$

Where:

P_{inf} is the probability of infection

r is the probability of one cell initiating an infection

N is the dose.

Table 4.1 Human feeding trial data.

Strain	Dose	Log dose	Total No. of trials	Positive stool (infection)	Proportion (infection)	Diarrhoea or fever (illness)	Proportion (illness)
A3249	8.00E+02	2.90	10	5	0.50	1	0.10
A3249	8.00E+03	3.90	10	6	0.60	1	0.10
A3249	9.00E+04	4.95	13	11	0.85	6	0.46
A3249	8.00E+05	5.90	11	8	0.73	1	0.09
A3249	1.00E+06	6.00	19	15	0.79	2	0.11
A3249	1.00E+08	8.00	5	5	1.00	0	0.00
81-176	1.00E+06	6.00	7	7	1.00	3	0.43
81-176	2.00E+08	8.30	10	10	1.00	6	0.60
81-176	2.00E+09	9.30	22	22	1.00	9	0.41

SOURCE: Black et al., 1988.

In the Beta-Poisson model, heterogeneity in the organism-host interaction is introduced and r, the probability of an organism initiating an infection given a successful introduction in the host, is assumed to follow a Beta-distribution (Furomoto and Mickey, 1967; Haas, 1983). In the derivation of this model, a complex function results. However, under the assumption that β is much larger than both α and 1, the following approximation can be used:

$$P_{inf} = 1 - (1 + N/\beta)^{-\alpha} \quad (4.2)$$

Where:

P_{inf} is the probability of infection,

N is the dose ingested, and

α and β are the dose response parameters.

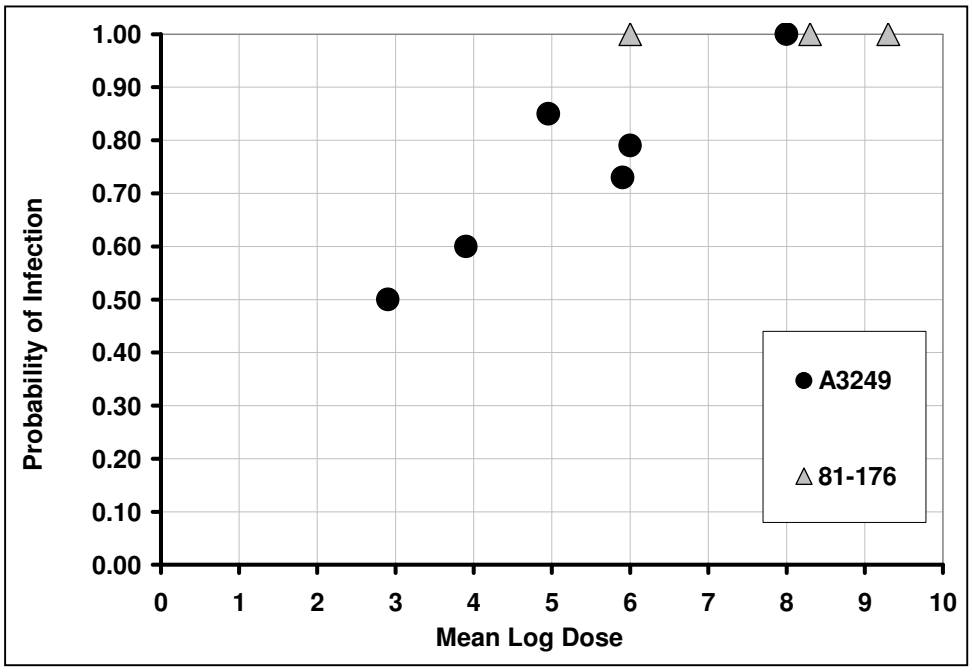

Figure 4.1 Human feeding trial data and probability of infection.

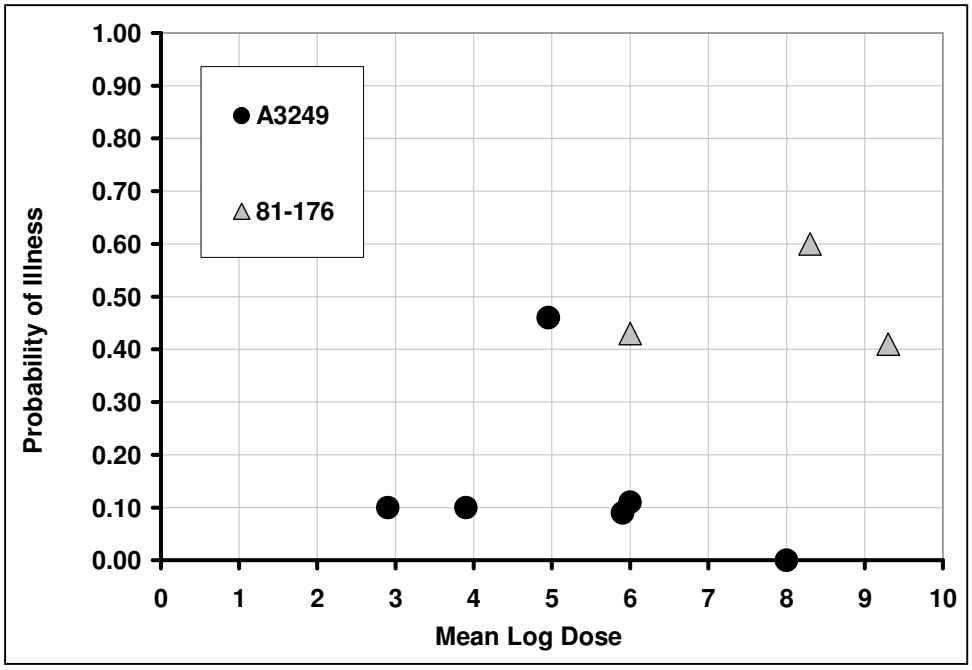

Figure 4.2 Human feeding trial data and probability of illness

Some of the human feeding trial data of Black et al. (1988) has been fitted to dose-response models. The dose-response data for infection for strain A3249 have been fitted to the dose-response models presented using maximum likelihood techniques. The Beta-Poisson model has been reported to provide a statistically significant fit to the data with parameters $\alpha = 0.145$ and $\beta = 7.59$ (Medema et al., 1996; Teunis et al., 1996). The value of these parameters, estimated using the approximate form of the Beta-Poisson model, which can be considered questionable with regard to the conditions for the approximated solution (β much larger than both α and 1), motivated Teunis and Havelaar (2000) to estimate the parameters using the exact solution to the Beta-Poisson. The parameters generated using the exact approach are $\alpha = 0.145$ and $\beta = 8.007$. These parameters and the exact form of the Beta-Poisson equation do not deviate very much from the approximate solution for the best fitting curve. However, the confidence limits for the two approaches have been shown to be very different. The approximate solution with this set of parameters produces a likelihood-based confidence limit that is greater than should be theoretically possible. The approximate Beta-Poisson dose-response model fitted to the A3249 infection data is shown in Figure 4.3, from which the overestimation in the upper confidence limit can be seen. The limiting curve represents the probability of infection with a single hit hypothesis and the probability of one cell initiating an infection set at 1. For comparison, the exact Beta-Poisson dose-response model is presented in Figure 4.4.

In addition to human feeding trial data for A3249, Black et al. (1988) also studied the dose-response relationship for *C. jejuni* 81-176. The response, at the three doses tested for this strain, were 100%. As a result, it is not possible to derive a dose response relationship for 81-176 by itself. However, if our goal is to generate a dose-response relationship for *C. jejuni*, it may be reasonable to combine the data for the two strains and generate a dose-response relationship for this new data set. Since, in the risk assessment, no distinction is made for different strains, it would seem illogical to distinguish between two strains at the dose-response level. Furthermore, a visual comparison of the probability of infection for the two strains (Figure 4.1) does not indicate that one strain is more or less infective than the other.

In the current exercise, the data for *C. jejuni* A3249 and 81-176 were pooled and fitted to the Beta-Poisson dose-response model. The fit to the data was found to be statistically significant at the 95% confidence level (2 log likelihood = 4.67, with 6 degrees of freedom). The parameters of the Beta-Poisson dose-response model were estimated to be $\alpha = 0.21$ and $\beta = 59.95$. The values of the parameters, when the data for both strains are pooled, allow the approximate and simpler form of the Beta-Poisson equation to be used. Furthermore, the upper confidence limit in this case does not exceed the theoretical maximum, as was the case when the approximate solution was used with the data for A3249 alone. The best fitting dose-response curve and the 95% confidence limits generated for the two-strain pooled data are shown in Figure 4.5. The confidence region, on a log scale, for the parameters of the dose-response model are shown in Figure 4.6.

The Beta-Poisson model, in the form expressed in Equation 4.2, estimates the average risk to a population following the ingestion of an average dose. In order to estimate the probability of infection for an individual consuming a meal with a specific dose, the Beta-Poisson model needs to be expressed in a format that will allow it to be simulated in a similar manner to the exposure assessment. Equation 4.3 reflects the same assumptions as the original Beta-Poisson model, but variability for the probability of infection from a particular dose is incorporated within the simulations so that the model estimates the risk of infection for an individual consuming a specific dose.

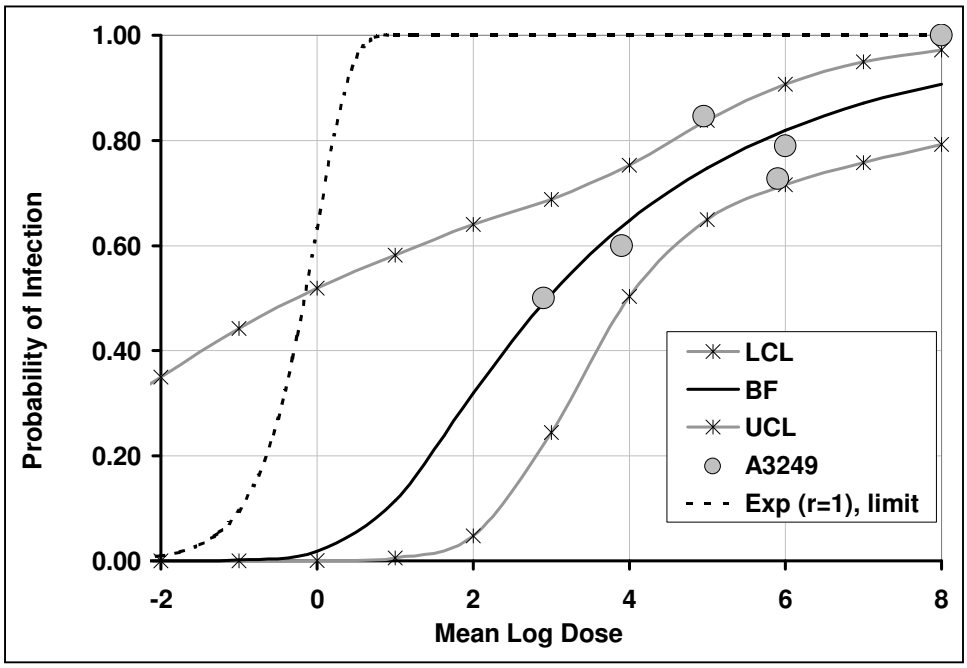

Figure 4.3 Approximate Beta-Poisson dose-response model fitted to infection data for *C. jejuni* A3249.
Source: Black et al., 1988.

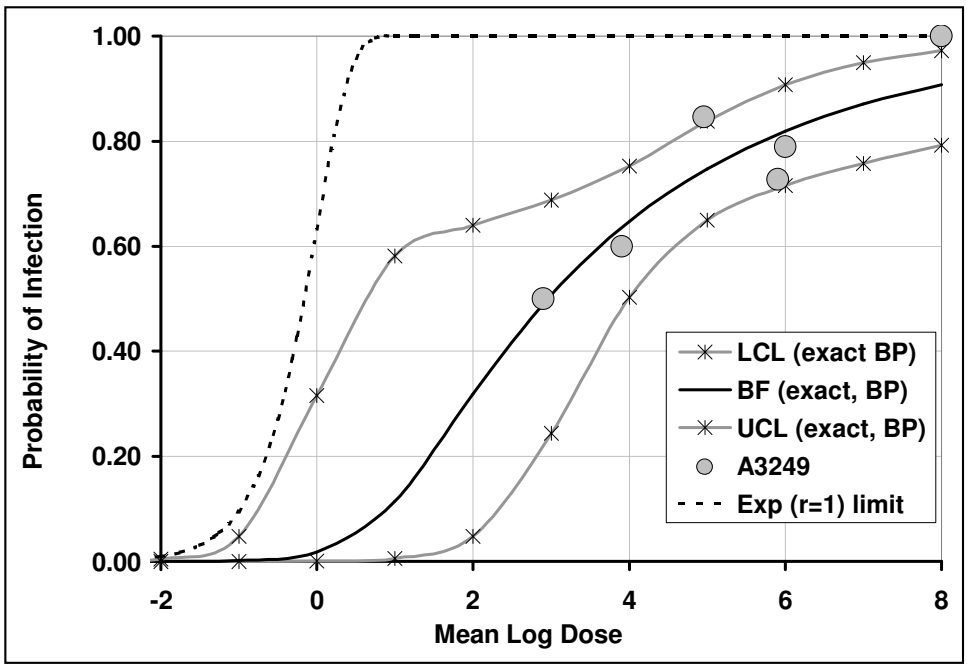

Figure 4.4 Exact Beta-Poisson dose-response model fitted to infection data for *C. jejuni* A3249.
Source: Black et al., 1988.

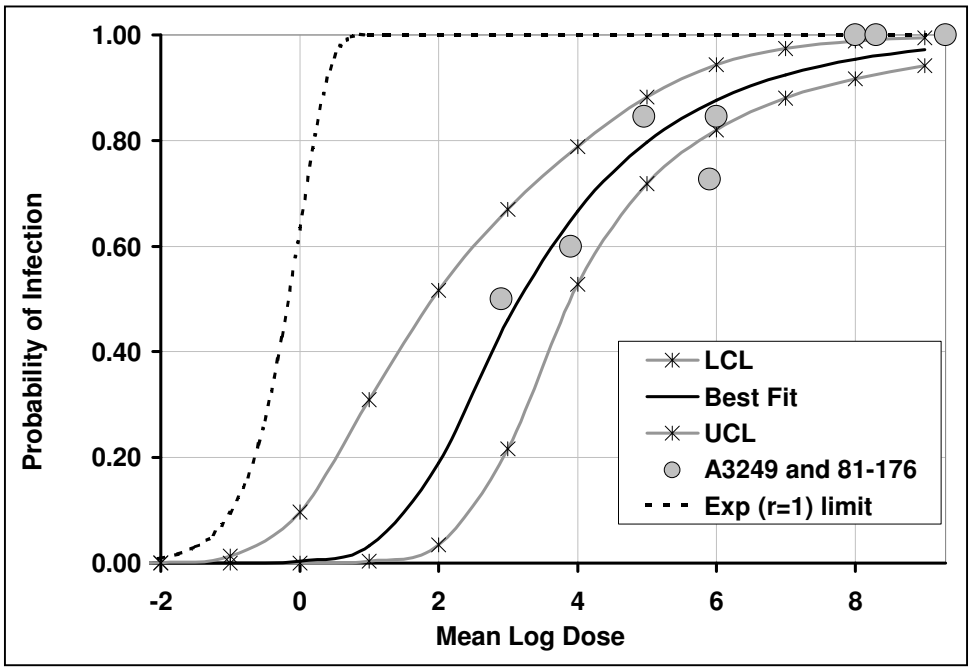

Figure 4.5 Beta-Poisson model fitted to human feeding trial data for *C. jejuni*, strains A3249 and 81-176.

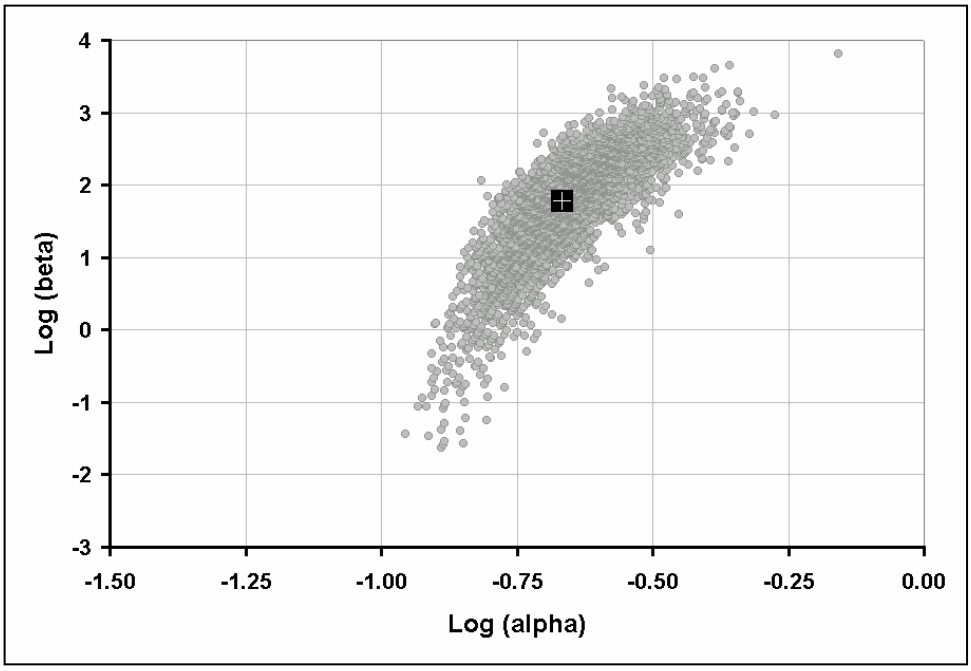

Figure 4.6 Confidence region for parameters (alpha and beta) of the Beta-Poisson dose-response model fitted to feeding trial data for *C. jejuni* A3249 and 81-176.

The simulated Beta-Poisson model samples the beta distribution, using the parameters generated (e.g. alpha = 0.21, and beta = 59.95), to estimate the probability of infection from one organism. The dose ingested is estimated using a Poisson sample, which assumes the organisms on the chicken with some mean concentration are randomly distributed. Finally, the probability of infection from the dose ingested is estimated assuming a binomial process with the number of trials equal to the dose ingested and the probability of 'success' at each trial equal to the value returned from the beta distribution.

$$P_{INF} = \mathbf{1} - \left[\mathbf{1} - P_{INF}(\mathbf{1})\right]^{D} \tag{4.3}$$

Where:

P_{INF} is the probability of infection from the dose

$P_{INF}(1)$ is the probability of infection from one organism (Beta Distribution)

D is the number of organisms estimated to be in the meal (Dose).

The simulated Beta-Poisson model can be interpreted as estimating, during a simulation, the probability of infection for different individuals at every iteration. The host-pathogen relationship, or the probability of infection for an individual from one cell, is assumed to vary according to a beta distribution. Some host-pathogen combinations may have a high probability of infection and some may have a low probability of infection. The range and frequency with which the various probabilities of infection upon exposure to one cell occur during a simulation is dictated by the parameters of the beta distribution.

The probability of illness upon exposure to a dose of a pathogen is conditional upon the probability of infection. Stated another way, in order for an individual to become sick, the individual has to first become infected. The dose-response relationships described so far have estimated the probability of infection upon exposure to a dose. In order to estimate the probability of illness, the conditional probability of illness following infection is required.

The human feeding trial data does not indicate a clear dose-response relationship for the conditional probability of illness following infection. For strain A3249, the data in the human feeding trials actually shows a decreasing trend for the conditional probability of illness with increasing dose. This observation has motivated some researchers (Teunis, Nagelkerke and Haas, 1999) to postulate that perhaps upon exposure to a larger dose of some pathogens, the elicited host defences are stronger, therefore reducing the probability of illness upon exposure to a very large dose compared with a moderate dose. The other alternatives that exist for the relationship of the conditional probability of illness following infection are that the probability increases with increasing dose or the probability is independent of dose. The three alternatives, using hypothetical illustrative data, are shown in Figure 4.7.

In the case of the feeding trial data for *C. jejuni* A3249, the probability of illness decreases with increasing dose, and, as such, a decreasing hazard function has been estimated (Teunis, Nagelkerke and Haas, 1999). However, when the data for both strains are pooled, the conditional probability of illness following infection does not exhibit a dose relationship, but rather is randomly distributed (Figure 4.8).

It may be appropriate in this case to use a dose-independent ratio to estimate the conditional probability of illness. The conditional probability can be estimated from the feeding trial data. For strain A3249, out of 50 people that were infected at various doses, 11 became sick (22%), while for strain 81-176, out of 39 people that were infected at different doses, 18 became sick (46%). Overall, pooling all the data, a total of 29 people became sick out of 89 individuals that were infected (33%).

In order to account for the uncertainty in the conditional probability, it is possible to use a beta distribution, with parameters that are based on the observations (alpha = 30 {number ill +1} and beta = 61 {number infected – number ill +1}). This distribution is shown in Figure 4.9.

In addition to the feeding trial data, Havelaar, de Wit and van Koningsveld (2000) report on an outbreak investigation conducted by Bremell, Bjelle and Svedhem (1991) in which 35 individuals became sick out of a total of 66 that were infected (53%). This estimate would place the conditional probability at the tail of the distribution shown in Figure 4.9.

In conclusion, several issues remain uncertain with respect to application of the dose-response model:

- The acceptability of pooling data from the two strains in order to estimate the probability of infection.
- Use of the approximate and exact Beta-Poisson equation if only data from *C. jejuni* A3249 is used. This should not be an issue for the pooled data set, since the approximate solution is sufficient in that case.
- The appropriate conditional probability for infection following illness, including assumptions on the dose relationship and the value and range of the probability.

4.4 Prevalence-based models of hazard characterization

An alternative approach to hazard characterization (for instance, for management purposes in a country that lacks enumeration data), relies on a simple translation of the prevalence of contaminated chicken to human health consequences, essentially providing a one-parameter hazard characterization model. In this approach, several pieces of information are required for a specific country. Data is needed on the number of *Campylobacter* illnesses that can be attributed to chicken in that country in a specified time interval (e.g. per year). An estimate of the prevalence of contaminated chickens at a point in the farm-to-fork chain is also required, for the matching period. The further down-stream the prevalence estimation (i.e. closer to the consumer), the more utility in the approach for assessing risk mitigation strategies. This approach is the method of hazard characterization (prevalence-response, as opposed to dose-response) employed in the FDA risk assessment addressing fluoroquinolone-resistance for *Campylobacter* in broiler meat (FDA, 2001).

This approach should be used with some caution, as the impact of the attribution of campylobacteriosis to chicken essentially determines the overall risk estimate. The approach essentially embeds a strong assumption of causality. Also, it should only be used to study risk mitigations where the effect is known to affect prevalence, and *will not affect the concentration of organisms* in the product. For most interventions, this condition would not be met. Possible exceptions include risk mitigations based on final product sampling (e.g. where positive final product is diverted to ready-to-eat production) and those that affect between-flock prevalence (e.g. where the proportion of positive flocks is reduced, and the contamination levels of positive flocks are unchanged). Whether the assumption (no change in concentration) is appropriate for mitigations that would reduce the prevalence of fluoroquinolone resistance is a matter of continuing debate in the antimicrobial resistance risk assessment arena.

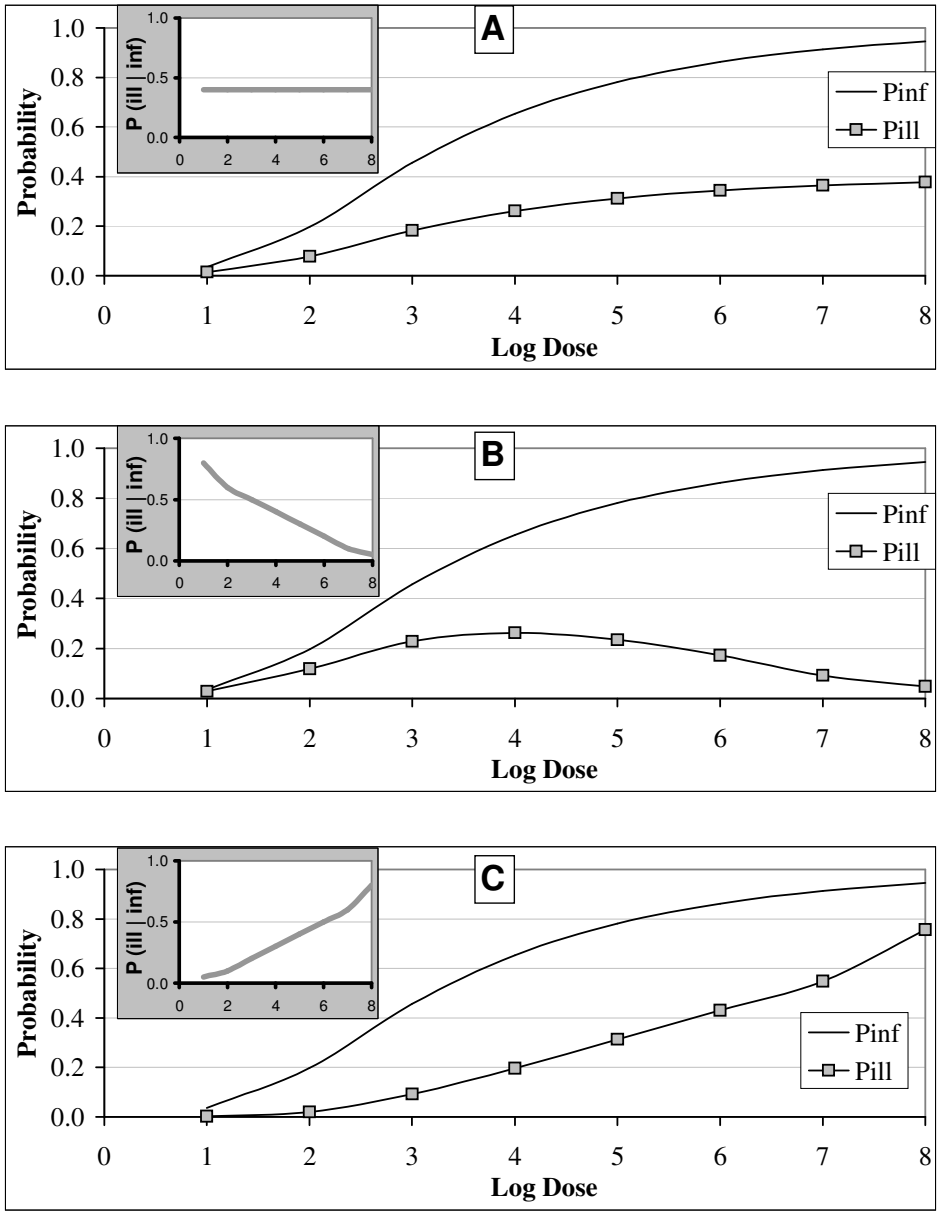

Figure 4.7 Hypothetical probability of illness curves, influenced by three alternative conditional probabilities. Conditional probability assumption shown in inset curves. (A) Probability independent of dose; (B) Probability decreasing with dose; (C) Probability increasing with dose.

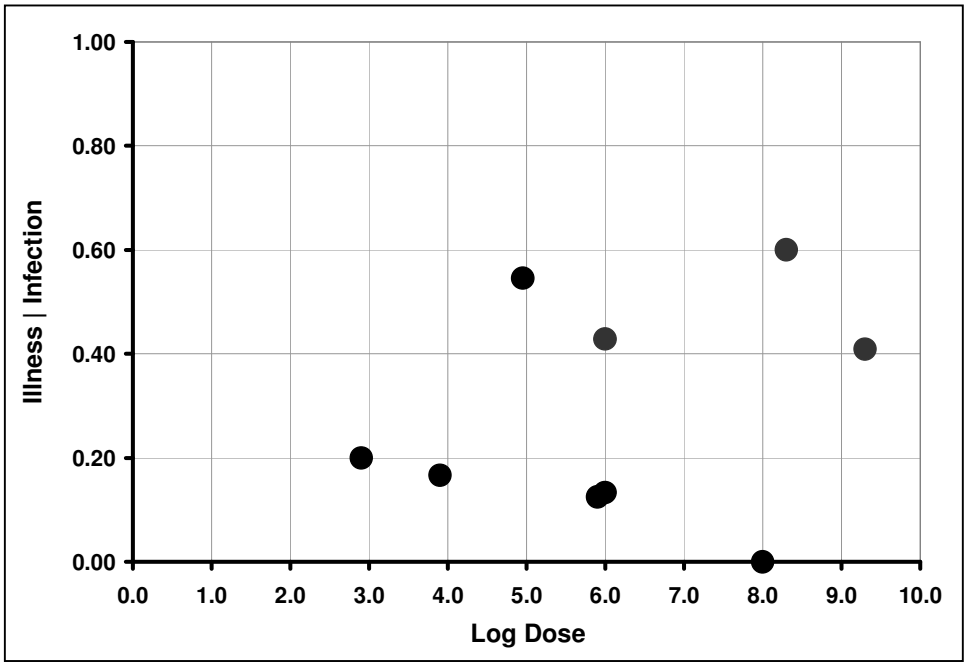

Figure 4.8 Conditional probability of illness following infection for *C. jejuni* A3249 and 81-176.

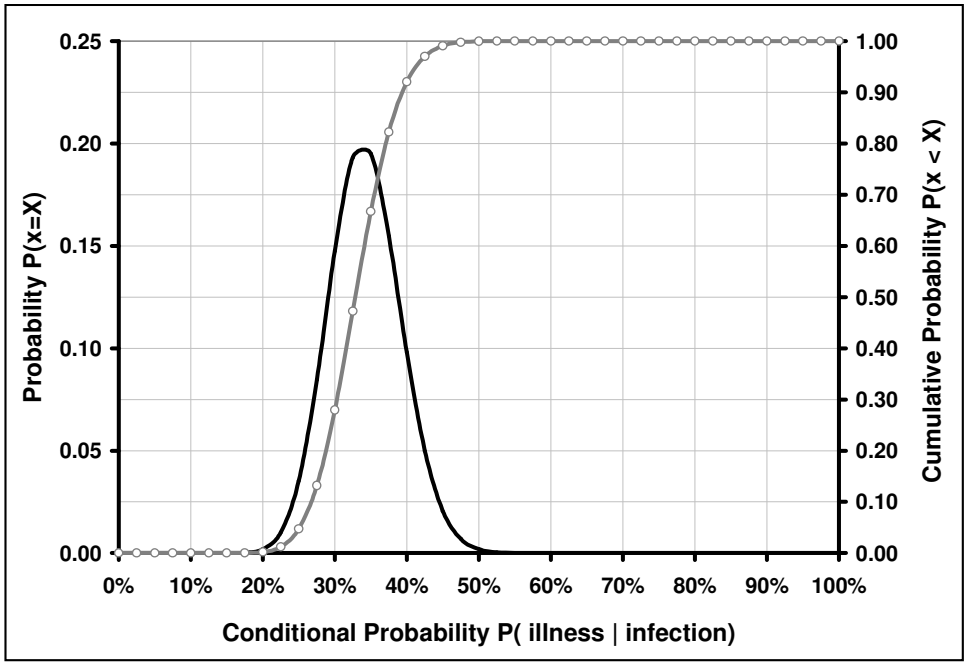

Figure 4.9 Distribution for conditional probability of illness following infection.

5. RISK CHARACTERIZATION

The risk characterization integrates the information collected during the hazard identification, hazard characterization and exposure assessment steps to arrive at estimates of adverse events that might occur due to the consumption of chicken. The resulting risk is expressed as individual risk or the risk per serving of chicken.

The model presented here considers all stages of the chicken production chain, beginning with birds on the farm through to the consequences from the consumption of a chicken meal by a random individual. The model is developed in a modular fashion, with each stage of the supply chain described by a distinct model, which provides inputs into the next stage. However, each of these models can be used separately so that the product is versatile and potentially adaptable to applications that are country specific (with appropriate modification to describe the country-specific characteristics)

5.1 Baseline model

The current risk assessment is not intended to describe any one geographical location or system, but rather it was developed and designed to provide relative risk comparisons. Specifically, the assessment does not provide estimates for the absolute risk attributable to any one system, and every attempt has been made to avoid conveying that message. In order to fully characterize a specific system, the features of that system need to be captured (every system or country is likely to be different to varying degrees), and data specific to that system applied, or if using alternative data, then these data needs to be carefully vetted.

This risk characterization does not focus on a specific system or region. As a result, most of the focus in this section is on the scenario analysis. The scenario analysis relies on relative comparisons. Even though the scenario analysis forms the focus of this risk characterization, some basic background information and insight can be gained by looking at a "baseline" model, which represents a point of reference. The baseline model in this section is defined as a system with an overall flock prevalence of 80%, chickens are water chilled without free chlorine, and sold fresh (refrigerated but not frozen). The model, using this baseline prevalence level, was run for 10 000 iterations.

The results for the estimated change in levels of contamination on the surface of the chicken, as the chickens progress from the farm to the chilling stage (water chill without chlorine) at the end of process, are shown in Figures 5.1 and 5.2. The model simulates chickens coming from positive and negative flocks separately. In Figure 5.1 the change in level of contamination is for chickens coming from positive flocks, while in Figure 5.2 the results are for chickens from negative flocks.

In Figures 5.1 and 5.2, a contamination level of –6 log cfu indicates no contamination or 0 cfu (it is not possible to take a log of 0, and so a value of –6 was used as an indication of this value and to allow the data to be plotted). In general, for positive flocks, apart from the initial increase in contamination during the transportation of chickens, the mean level of contamination decreases through the process. Of note, the "min", "max" and other percentile (10^{th} and 50^{th} percentiles shown) levels for contamination are closest together during de-feathering, indicating a reduced level of variation in the contamination level. Although the mean level drops at this stage, the de-feathering process acts such that contamination is spread from one bird to another in a mixing process so that chickens with very little contamination get contaminated from material drawn off

the heavily contaminated carcasses. Keep in mind, however, that the y-axis of the figure is plotted on the log scale, which makes the shrinking of the range of contamination enhanced visually.

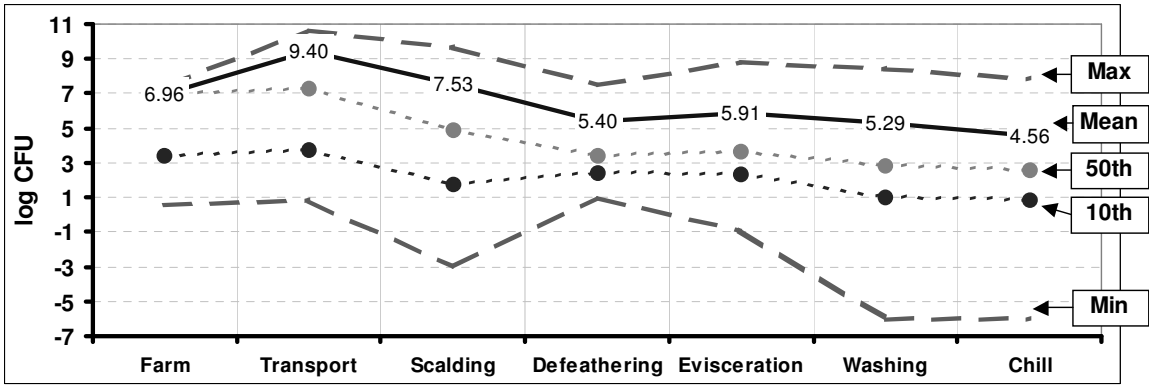

Figure 5.1 Change in contamination levels on chickens from positive flocks. Baseline model with 80% of flocks positive.

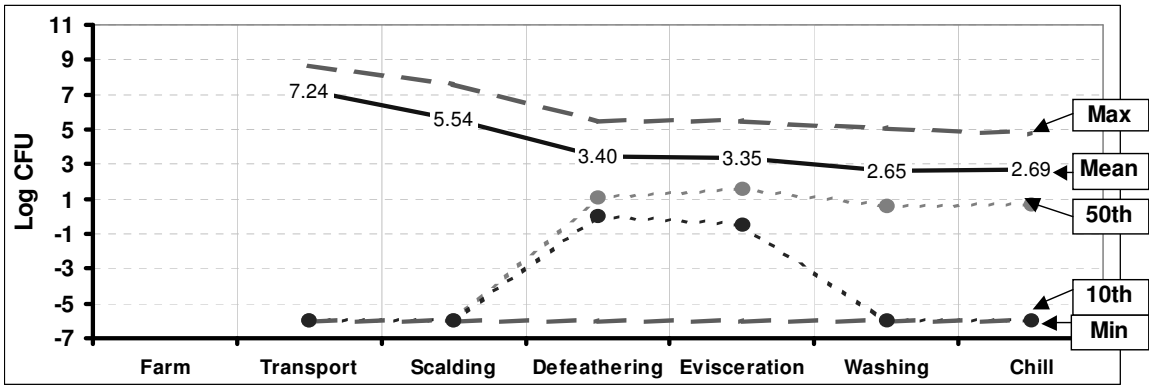

Figure 5.2 Change in contamination levels on chickens from negative flocks. Baseline model with 80% of flocks positive.

The current model simulates a system in which flocks arrive at slaughter in a random fashion, so that a positive flock might arrive first followed by a negative flock and then followed by another positive flock. In the model, the changes in contamination on birds from positive flocks, and on birds from negative flocks as they progress through the process, are simulated separately. The separation of positive and negative flocks in the simulation can help to give insight into the differences that exist when previously positive birds enter the system and when previously negative birds enter the system.

In Figure 5.2, there are no markers shown at the farm level because the flock is negative and as such the contamination level is 0. During transport, however, contamination does occur. The frequency with which contamination occurs at this stage is clearly a function of several factors: the overall prevalence of positive flocks (dictating the probability that a negative flock will be transported in trucks previously used to transport positive flocks); refinement in the logistics employed by the industry (e.g. transporting and processing negative flocks first); and the management strategies employed in the industry (e.g. cleaning and disinfection of trucks between

loads). These are explored in a little more detail later. Even though the mean level of contamination spikes at the point of transport , the implication is not that all the birds are contaminated. In fact, from the percentiles shown on the figure, it can be seen that the 50th percentile value does not spike at transportation, indicating that more than 50% of the birds from negative flocks do not get contaminated at that stage. The high mean value is primarily the result of a few birds getting in contact with very high levels of contamination.

The impact of different between-flock prevalence (prevalence of contaminated flocks) values on the potential for contamination of negative flocks is explored in Figure 5.3. This figure shows three different hypothetical systems: one in which 80% of the flocks are positive; one in which 20% of the flocks are positive; and one where 5% of the flocks are positive.

The mean level of contamination on flocks that are negative at the farm level spikes from no contamination to a value greater than 0 during transport in all three cases shown. This is because, as long as there are positive flocks in the system, there exists some probability that a negative flock will be transported after a positive flock in a truck in which cross-contamination could occur. Obviously, the probability of this happening becomes less as the prevalence of positive flocks in the system decreases.

Although not shown in Figure 5.3, when the transportation of flocks is purely random, and when the overall flock prevalence is 80%, it was calculated that there is slightly less than a 0.5 probability that the birds from negative flocks will become contaminated during transportation. When the overall flock prevalence is 20%, the probability decreases to just under 0.2, and when the overall flock prevalence is further reduced to 5%, the probability is approximately 0.05. The implementation of management strategies, such as those listed earlier (cleaning, processing negative flocks first, etc.), will have similar effects in terms of reducing the probability of negative birds becoming positive due to cross-contamination. In the example shown above, targeting the prevalence reduced the probability, although any of the management strategies would effectively be doing the same thing in different ways and with perhaps different degrees of effectiveness. Employing a combination of strategies will have a synergistic effect, so that a reduction in prevalence, coupled with truck disinfection and the processing of negative flocks first, would have a much greater effect than any one strategy alone. The cost-benefit, efficiency and feasibility of an approach or appropriate mixture of approaches will need to be factored into the overall decision.

5.2 Scenario analysis

The construction of a simulation model of a system (in this case a chicken production and processing system) allows us to gain insights into the system that may otherwise not be possible. We can gain these insights by simulating the model for various scenarios (scenario analysis) that reflect the realities we want to explore. The scenario analysis allows potential mitigation strategies and the expected outcomes, or complications, to be investigated.

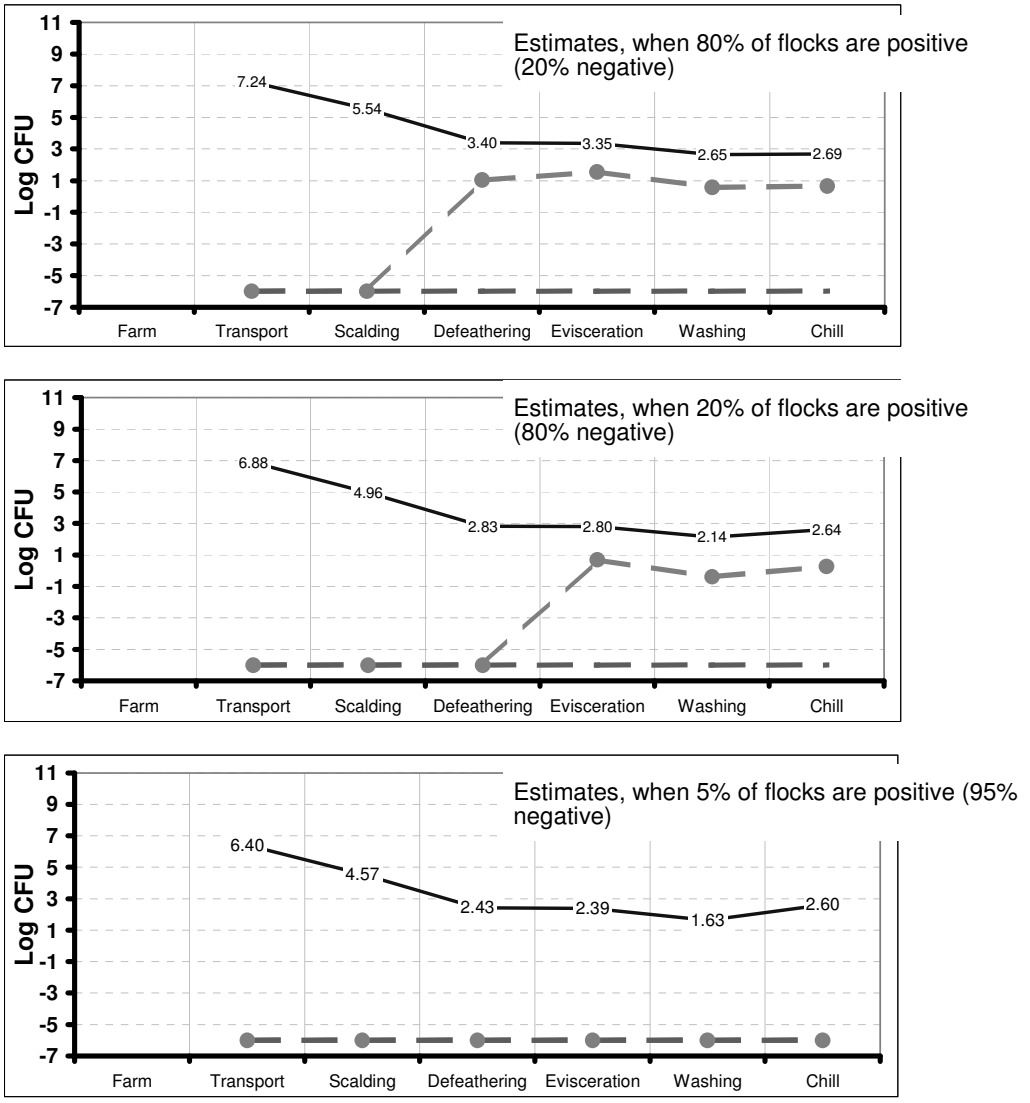

Figure 5.3 Change in contamination level for birds from negative flocks processed in conjunction with positive flocks (individual birds from positive and negative flocks not mixed, but arrival of positive and negative flocks at process on a random basis).

KEY: Top solid line with values represents mean level, middle curve represents 50th percentile, bottom curve represents minimum value from simulations

In the current assessment, several scenarios were constructed that were intended to reflect and inform the formation of potential management strategies. The scenarios could be classified as "general" and "specific". The "general" scenarios provide insight into potential approaches that might be used to reduce the risk, without defining and testing out a specific strategy. The "specific" scenarios are ones that can be interpreted as reflecting the testing out of a candidate strategy to see

how it is expected to perform or to determine if there are any complications, caveats, or issues that need to be considered when implementing the strategy.

It is important to recognize, for any of the scenarios presented, that the results should be used on a relative basis as opposed to an absolute basis. Specifically, the model does not represent any one geographical location and is intended to provide a generic evaluation of the situation. Using absolute risk estimates would be nonsensical, although when we work with relative risk estimates the generic components are typically factored out because they are kept constant from scenario to scenario. Nevertheless, there are also situations where the relative risk comparison may not be as strong as indicated, and so it is essential that if fine management distinctions are to be drawn the specifics of an individual country making the decisions needs to be factored in.

5.3 Scenario 1: Change in prevalence of chickens going to retail

The first set of scenarios investigated looked at the effect of altering the prevalence of contaminated chickens at the retail level. These scenarios can be classified as general scenarios, because they do not specify how the prevalence at the retail level is reduced, but rather they estimate the effect that could be expected should a strategy be implemented that reduces the prevalence. In this scenario, the prevalence level effects are assumed to be measured at the end of processing, or as the products are going to retail. As a result, the results presented in this scenario capture mitigation strategies anywhere along the food production chain prior to retail that are expected to reduce the prevalence.

In order to evaluate the prevalence scenarios, the model was simulated using several different post-process prevalence levels and the risk estimated. Prevalence levels from 0% to 100% were tested, in 5% intervals, resulting in 21 scenarios. The model was simulated for 5000 iterations at each prevalence level, and the expected risk from the consumption of refrigerated and frozen chicken was estimated.

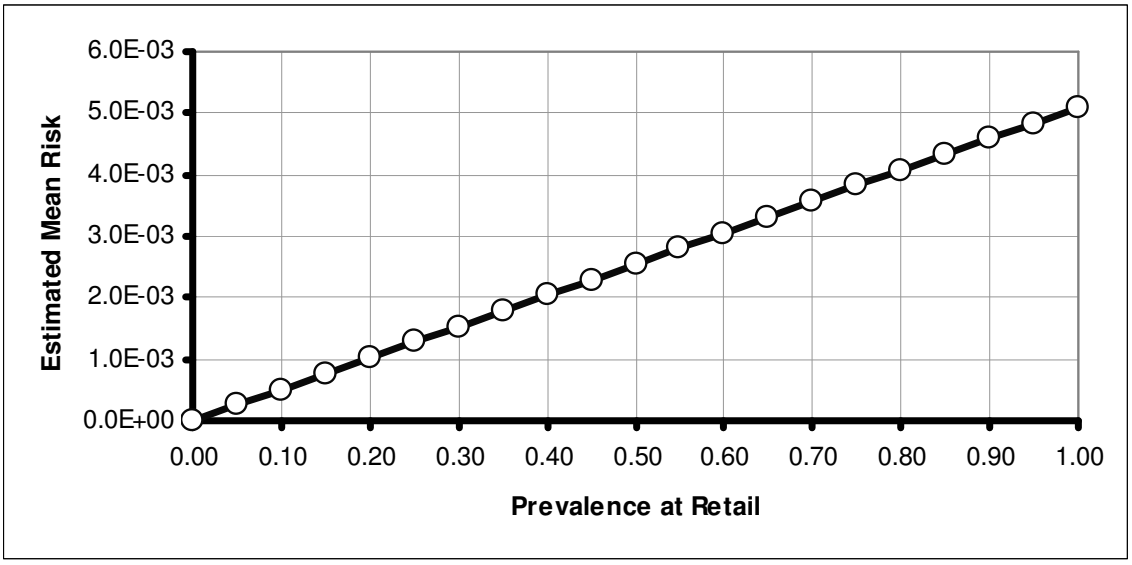

Figure 5.4 Prevalence at retail versus estimated mean risk from consumption of refrigerated chicken.

Figure 5.4 shows the estimated mean risk (y-axis) corresponding to a prevalence level (x-axis). The results are based on an assumed contamination level of approximately 4.0 log cfu/chicken, although the relationship applies regardless of the level of contamination.

From a risk-management perspective, the corresponding reduction in risk as a result of a reduction in prevalence is often the basis upon which a reasonable decision can be made. So, for instance, it is often desirable to know how much the relative risk reduction will be, given a prevalence reduction. This information can be readily calculated and is shown in Figure 5.5, in which the y-axis shows the % change in the estimated mean risk and the x-axis shows the % change in the retail level prevalence. The linear relationship for this chart indicates that the percent reduction in retail level prevalence (x-axis) is estimated to correspond with a comparable % reduction in the mean risk.

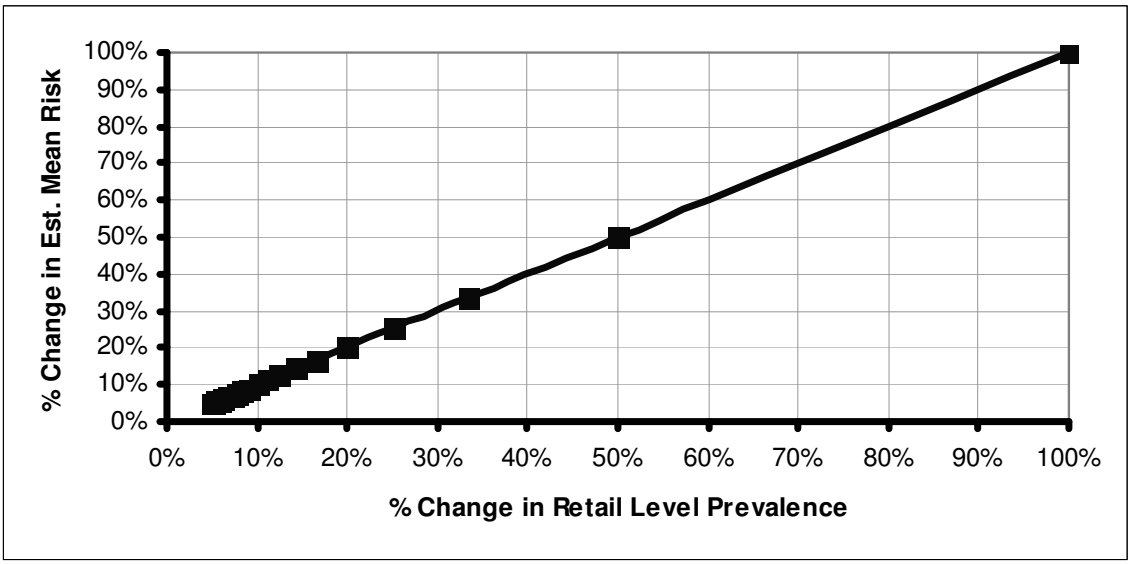

Figure 5.5 Percent reduction in prevalence versus percent reduction in estimated mean risk.

So, from a risk-management perspective, the statement could be made that if the prevalence, or frequency with which chickens are contaminated at the retail stage, is reduced, the corresponding risk reduction will be proportional. Stated in another way: a 50% reduction in prevalence is estimated to result in a 50% reduction in expected risk.

5.4 Scenario 2: Change in level of contamination

A second risk management scenario that was investigated looked at the effect of changes in the contamination level on chickens. Whereas the previous scenario was interested in altering the frequency with which products are contaminated, the current scenario was interested in looking at altering the level of contamination when the product is in fact contaminated.

In order to evaluate the effect that a change in the level of contamination would have on the risk estimates, the model was re-simulated using various contamination levels. The contamination levels in these scenarios are defined to be those that occur at the retail level. As a result, these scenarios could reflect the results of any risk-management strategy from farm to the retail level able to achieve this degree of reduction in contamination levels. The scenarios do not specify what the specific strategy might be or where exactly prior to retail it might be implemented. Those

determinations are left to the discretion of the risk manager, who could perhaps commission further studies to determine the technical basis for achieving those reductions should they be of interest.

Several contamination levels were selected, and the model re-run at each level. The risk associated with each contamination level was then collected and the percent reduction in risk for a corresponding reduction in contamination level was calculated. Figure 5.6 summarizes the results of the simulations. The horizontal line shows the percent reduction in concentration between successive simulations. Since it is a horizontal line, the interpretation is that the concentration was reduced by a constant percentage for every simulation, approximately 44%, starting at a level of 10 000 000 cfu (7.0 log10 cfu). The increasing line shows the percent reduction in risk as a result of the corresponding reduction in the contamination level.

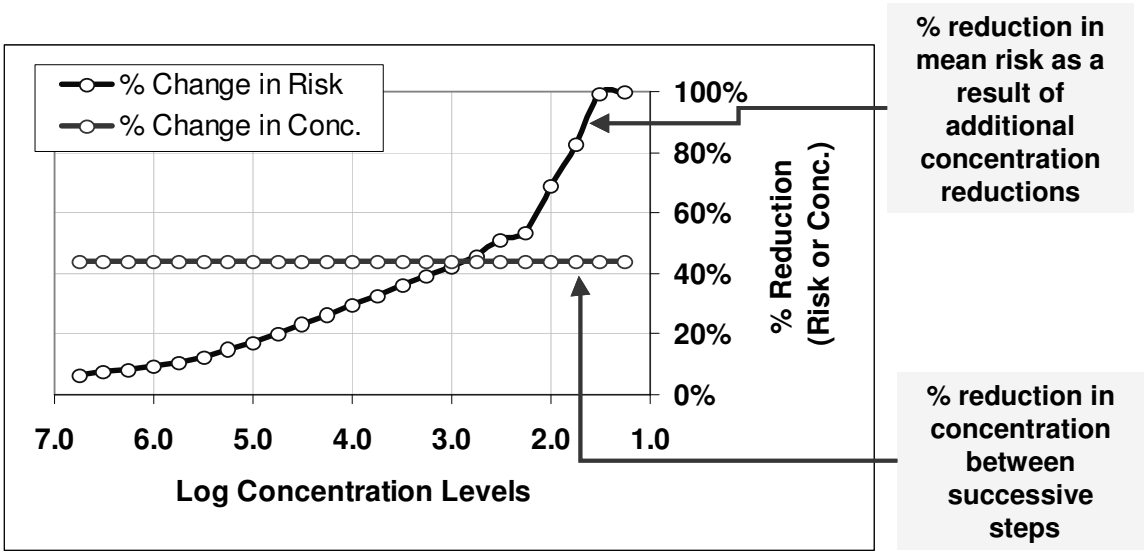

Figure 5.6 Percent reduction in risk corresponding to percent reduction in concentration for various concentration levels.

Of significance in this figure are the regions above and below the horizontal line representing the percent change in concentration. When the percent reduction in mean risk line is below the percent reduction in concentration line, the implication is that a contamination level decrease in this area does not translate to an equivalent risk reduction level. However, in the region where the percent reduction in mean risk line is above the percent reduction in concentration line, reductions in concentration translate to a greater than equivalent percent risk reduction.

Figure 5.7 helps explain the results for this scenario by presenting the information in a slightly different form. In the figure, there are four panels labelled A to D. Each of the four panels has two bars that reflect the initial and final concentration before and after an intervention strategy, and two points that show the corresponding percent reduction in concentration and the resulting reduction in risk. Panel A indicates that when the level of contamination is approximately 6 log cfu, and is reduced by 44%, the risk is reduced by approximately 11%. When the level of contamination on the chickens is lower to start with, the subsequent additional reduction in risk begins to increase. This can be seen in panels B, C and D. In panel B, with a starting contamination level of log 3 cfu, the risk reduction for a 44% concentration reduction is 46%. The risk reduction becomes even more dramatic the lower the initial contamination level. The risk is reduced by 69% for a 44% concentration reduction when the contamination level is just greater than 3 log cfu (panel C) and 82% when the initial contamination level is approximately 2 log cfu (panel D).

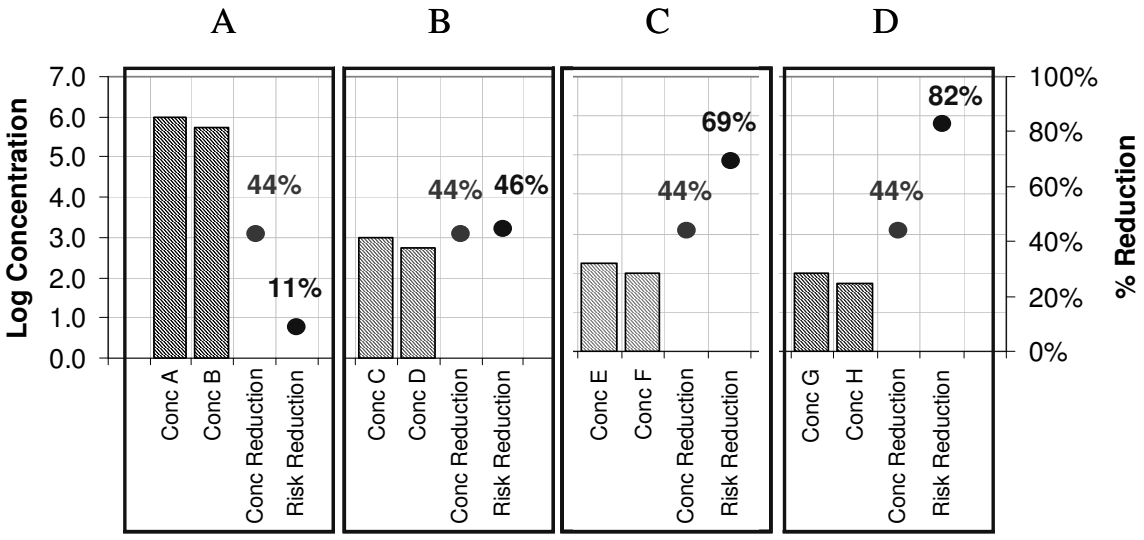

Figure 5.7 Four representative illustrations of the effect on the reduction in risk of reducing the contamination level.

Scenarios 1 and 2 can be summarized as follows: Any management strategy that alters the prevalence of contaminated chicken at retail would be estimated to have a correspondingly proportional impact on the mean risk. A management strategy that alters the level of contamination has a non-linear relationship with different returns on investment, depending upon the starting level of contamination. If the level of contamination is high, small additional reductions will have only a small effect. If the mean level of contamination is lower, additional contamination level reductions will have a greater than proportional effect on risk. From a management perspective, the indication is clear that an assessment of where in the spectrum the system is sitting needs to be done prior to selecting an appropriate strategy. If the level of contamination is reasonably high, reducing the frequency with which chickens are contaminated will have a greater return on investment. It would be more beneficial in this case to try to reduce the actual number of contaminated chickens entering the system or going to market. However, if the level of contamination is reasonably low, reducing the frequency of contamination is likely to be less effective than reducing the level of contamination further. Of course, as in any risk-management decision, the feasibility and technology available to tackle the frequency of contamination or the level of contamination need to be a central consideration in any deliberation.

5.5 Scenario 3: Changing between-flock and within-flock prevalence

The third scenario investigated the effect of altering the between-flock and within-flock prevalences. The between-flock prevalence refers to the prevalence of contaminated flocks at the farm. Altering the between-flock prevalence could be achieved, for instance, by the use of colonization-resistant breeds, if this were a technically feasible option. It should be noted that the purpose of this assessment has not been to specify what technologies or techniques exist or are feasible, but rather to work in general terms to illustrate the estimated risk reductions that could be expected should methods exist or be developed that could be implemented. In a full, well-initiated, risk assessment, the risk manager at the onset could articulate the options, and the specifics could then be incorporated into the assessment. The within-flock prevalence refers to the prevalence of contaminated birds within a contaminated flock. The within-flock prevalence is usually assumed to eventually converge on 100% since contamination will eventually spread through the contaminated flock. However, the within-flock prevalence could be less than 100% when birds head off to slaughter, if for example, biosecurity measures are implemented that delay the introduction of contamination for as long as possible prior to farm exit, such that insufficient contact occurs, preventing all birds becoming positive.

To gain insight into the effect of within-flock and between-flock prevalence on the health risk outcome, three scenarios were investigated and compared with a baseline level. Apart from the changes in between-flock and within-flock prevalence, the other parameters of the model were maintained between simulations. The three scenarios simulated were:

Baseline:	80% between-flock	100% within-flock
Strategy 1:	40% between-flock	100% within-flock
Strategy 2:	80% between-flock	50% within-flock
Strategy 3:	40% between-flock	50% within-flock

The model was simulated for 10 000 iterations at each scenario and the results shown in Figure 5.8.

The results in Figure 5.8 are not extremely surprising, indicating effects that we might expect to see, and perhaps lends to the credibility that we might translate to the model itself. There are some interesting observations that can be made, based upon the results produced, and the mechanisms underlying the process. First, the effect of reducing between-flock prevalence at the farm (Strategy 1) appears to translate to a slightly greater than one-to-one (50% between-flock reduction estimated to produce greater than 50% risk reduction) relationship in risk reduction. Specifically, when the between-flock prevalence is reduced, not only is the probability of a bird from a contaminated flock reaching the consumer reduced, but the probability of a contaminated flock being processed prior to the current one is also reduced, thereby reducing the probability of cross-contamination from the previous flock carrying through to the next batch, during either transport or processing.

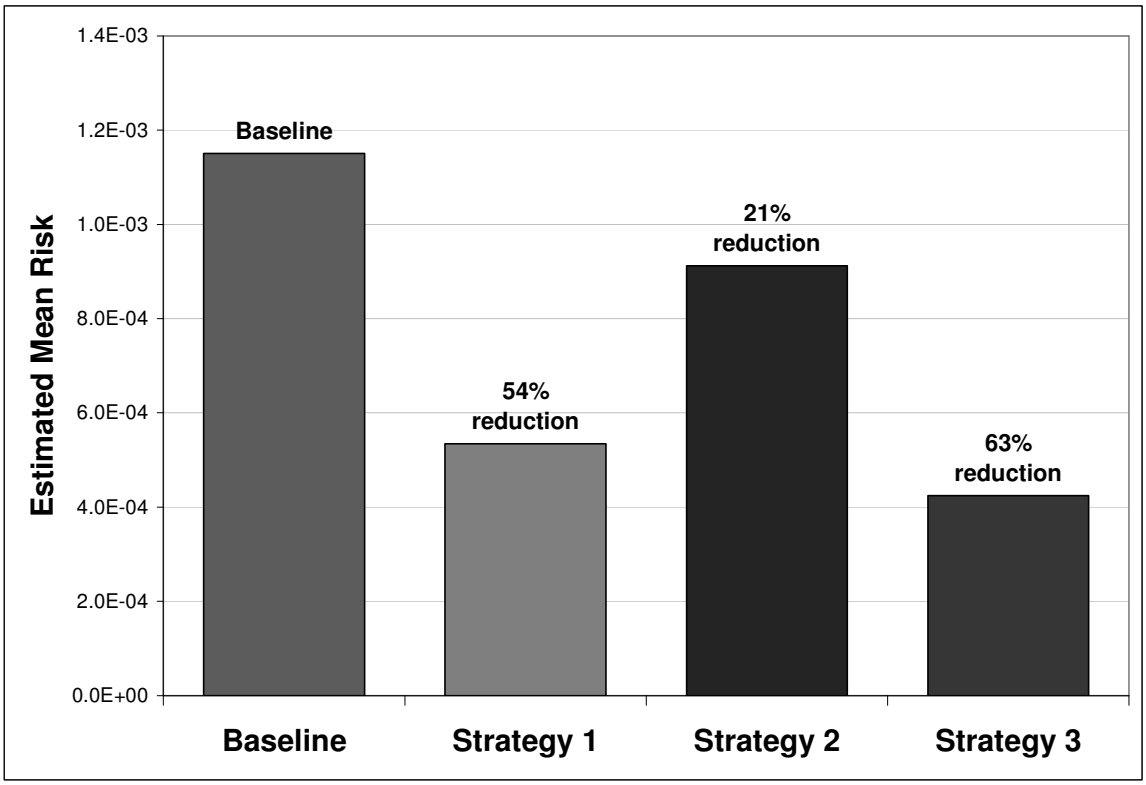

Figure 5.8 Scenario results: testing 3 different strategies changing between-flock and within-flock prevalence. in comparison with a baseline value.

The second strategy focused on the reduction of within-flock prevalence while maintaining the overall between-flock prevalence. In this case, the assumption is that the overall flock prevalence remains unchanged, but that those flocks that would have been positive are managed in such a way that the number of birds within the positive flock are kept to some value less than 100%. In the current scenario, this is set to a value of 50% (half the birds in a positive flock are actually positive, while half are not). The end result of this strategy translates to approximately a 20% reduction in risk. The reduced effectiveness of this strategy is primarily due to the fact that the birds from these flocks are in fact being processed in an environment in which they are surrounded by positive birds and thus subject to high cross-contamination probabilities. A substantial reduction in the within-flock prevalence would be required (reducing cross-contamination probabilities) in order to produce equally substantial risk reductions. Nevertheless, as has been stated previously, the performance of any one strategy needs to be weighed in conjunction with other parameters such as cost, efficiency, time or feasibility. When these are factored in, it might be the case that within-flock prevalence reductions could be achieved very easily, cheaply or quickly, and as such the resulting 20% risk reduction could be a very good investment and in fact turn out to be the preferred option. The final strategy simply illustrates the synergic nature of the individual previous scenarios. A reduction in both within-flock and between-flock prevalence translates to a greater net effect than either of the other two individually.

5.6 Scenario 4: Changing internal and surface contamination levels before and through processing

The fourth scenario investigated comprised several strategies to reflect changes in the level of contamination before and throughout processing. The four alternative strategies investigated were:

Strategy 1: 90% reduction in surface contamination level after transport.

Strategy 2: 90% reduction in levels contaminating carcasses at evisceration.

Strategy 3: 90% reduction in surface contamination post-evisceration.

Strategy 4: 90% reduction in initial internal contamination levels (overall reduction in contamination entering the system).

The first strategy is to bring about a reduction in the level of surface contamination after transport. In this strategy, there is no reduction in the internal contamination levels, but rather the strategy is such that after transport a process is implemented that reduces the amount of contamination on the surface by 90%. With the second strategy, the level that contaminates carcasses at evisceration is reduced by 90%. This strategy assumes that the evisceration process is modified, either through new technology, or through a change in process, such that the amount of contamination from internal damage or from cross-contamination is reduced by 90%. Strategy 3 can be interpreted to mean that after evisceration, there is a step or process introduced that reduces the level of contamination by 90%. Similar to strategy 1, but while strategy 1 was implemented after transport, strategy 3 is applied further along in the process after evisceration. Finally, strategy 4 is assumed to be a strategy that reduces the overall contamination level entering the system. In essence, the scenario can be interpreted to be one in which a strategy is employed that translates to birds that are colonized internally at the farm level, at a low level such that subsequent cross-contamination and surface contamination are at an equivalently lowered level.

The results of the scenario, shown in Figure 5.9, with a baseline mean risk level for comparison, show that the impact of altering the contamination level can be quite varied.

In the first strategy, the impact of reducing the contamination level after transport is not as dramatic as other strategies. The 35% reduction in risk is significant, but it is less significant than others because when simply reducing the contamination level on the surface at this early point in the process, it is possible for contamination through cross-contamination and as a result of potential viscera damage later in the process to occur, and can quite easily counteract the reductions, thus diminishing its effectiveness.

The second strategy produced the least risk reduction of the four strategies tested. However, the strategy was designed to explore the impact of reducing the level of contamination occurring during the evisceration stage. In that context, it could be a reasonably easy step to apply, both technologically and in cost terms, and the relative return could be quite attractive. So, a strategy implemented that reduced the amount of contamination spread to the chicken currently being processed, or subsequent chickens, by 90%, would reduce the overall mean risk by 25%.

The effect of reducing surface contamination after evisceration, as depicted in strategy 3, is estimated to have quite a significant effect. A 90% reduction in the surface contamination level after evisceration translates to a 63% reduction in the mean risk overall. Unlike the first strategy, in which the surface contamination after transport was reduced by 90%, in this case the reduction occurs at evisceration, with fewer subsequent contamination or re-contamination stages in existence after that step, and thus fewer opportunities to negate the strategy.

Finally, strategy 4 reflects a strategy that is a result of an overall reduction in contamination, both internally and as a result of being transferred onto the surface. This strategy could be interpreted as one that targets the internal contamination level of chickens at the farm level and thus the overall contamination level entering the system. The key in this strategy, which is important for the management of *Campylobacter* in general, is that in order to have an impact on the risk by controlling contamination levels, it is essential that either the overall contamination entering the system is reduced, or a step is introduced late in the process to eliminate contamination. An individual surface contamination reduction step early in the process tends to be negated by subsequent steps if in fact there are chickens carrying heavy loads entering the process.

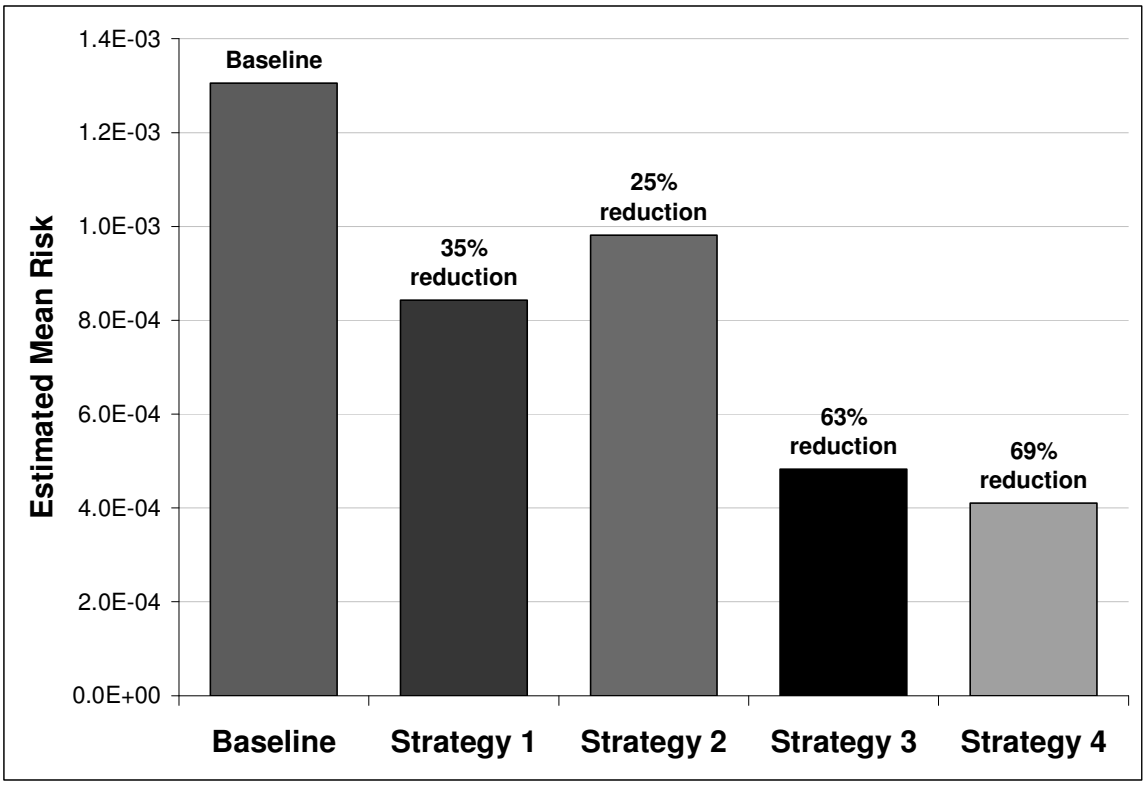

Figure 5.9 Scenario results, testing four strategies that alter the level of contamination before and during processing, compared with a baseline value.

5.7 Scenario 5: Risk-mitigation impacts from freezing of fresh chicken

The fifth scenario investigates the effects of freezing as a risk mitigation strategy. In this scenario, comparisons were made between fresh product stored refrigerated for up to 9 days, and product held frozen for up to 6 weeks prior to consumption. The risk associated with each of these strategies was estimated for a range of contamination levels so that the effects of each strategy could be measured as a function of the concentration. The results of this scenario are shown in Figure 5.10.

Freezing has been found to have a killing-off effect on the level of *Campylobacter* contamination. As a result, and as expected, it is estimated that the chickens that are frozen have a lower risk than those that are sold and stored refrigerated. From Figure 5.10, it can be seen that an

equivalent level of risk can be maintained for more heavily contaminated product that is frozen. For instance the mean risk for refrigerated chicken at a mean contamination level of 4.5 log cfu, is approximately the same as for frozen chicken at a mean contamination level of approximately 5.25 log cfu.

In general the results are expected; however, the magnitude is quantifiable (in an approximate manner) in the current approach. Using the risk modelling approach not only can it be inferred that the risk would be less for frozen versus fresh chicken, but it can also be estimated how much less this risk would be. The second part of the current scenario was to explore in more detail the potential complications that could arise as a result of a freezing strategy.

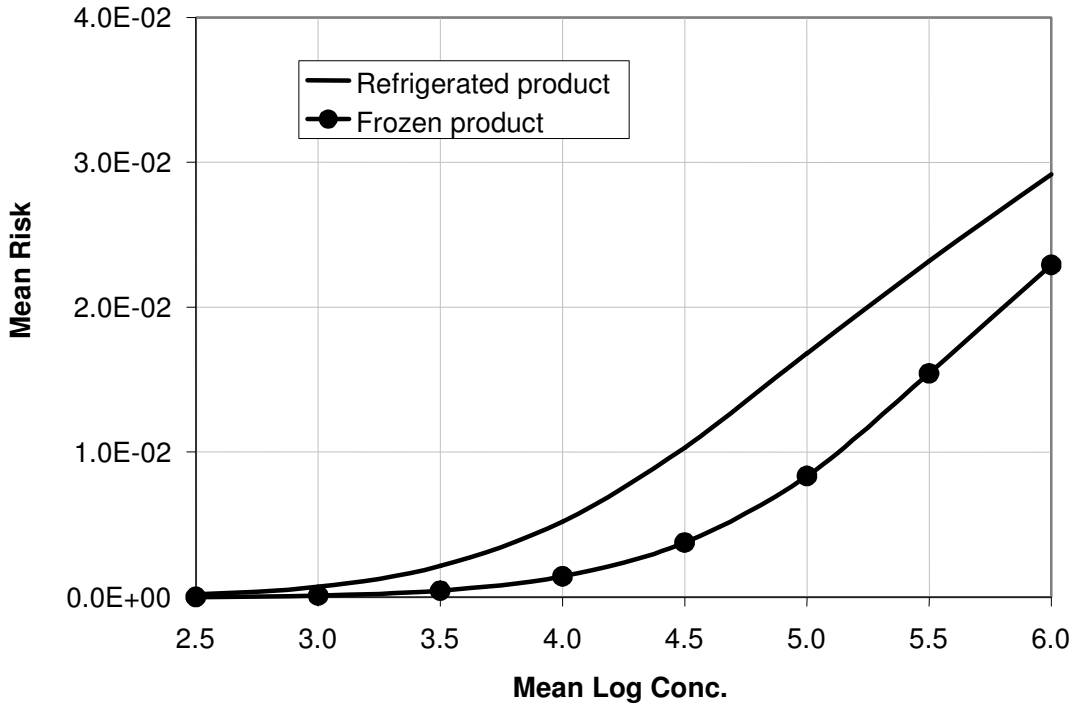

Figure 5.10 Risk estimates for frozen and refrigerated chicken as a function of contamination level.

The consumers' preparation practices could have a dramatic effect on the effectiveness of the risk-reduction strategy employed, especially if the strategy employed has a direct impact on the performance of a practice that the consumer will undertake. When a product is frozen, it is possible that the cooking effectiveness could be reduced due to insufficient thawing, resulting in a lower temperature being attained in parts of the chicken. Two cooking reduction effectiveness scenarios as a result of freezing were explored:

- Effect 1: Final cooking temperature is 2°C cooler in cold spots for frozen chicken.
- Effect 2: Final cooking temperature is 5°C cooler in cold spots for frozen chicken.

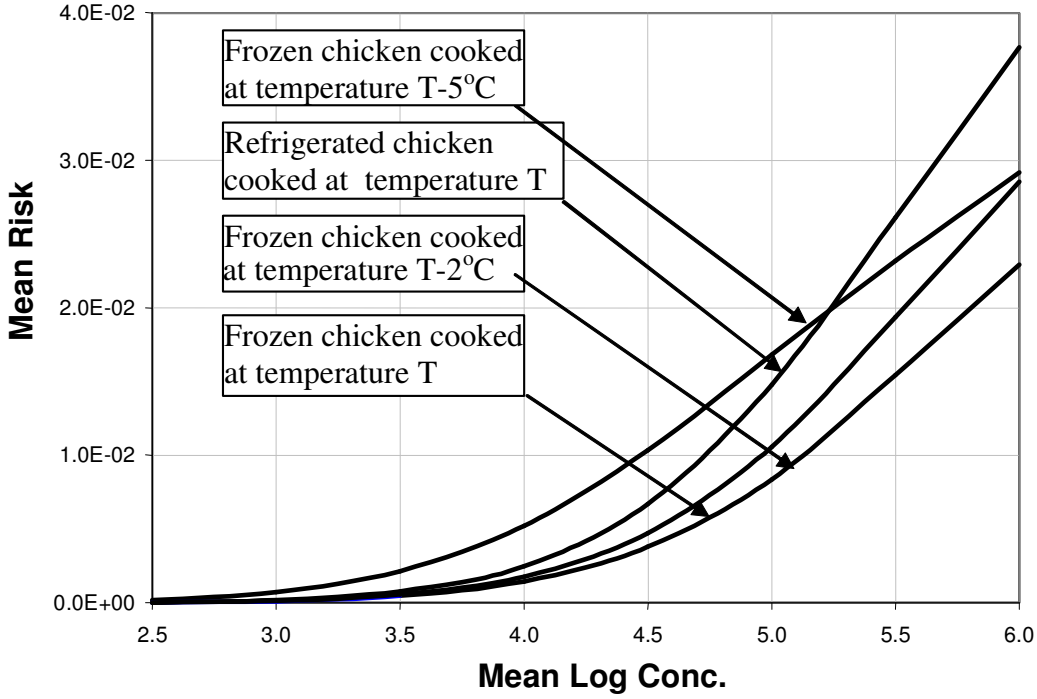

Figure 5.11 Potential complications in risk reduction as a function of contamination level as a result of pursuing a freezing strategy

Figure 5.11 shows the risk estimates for the refrigerated and frozen strategies together with modifications which assume the effect of freezing lowers the cooking effectiveness by reducing the temperature in the coolest spots of the chicken by 2°C and 5°C. If freezing negatively impacts the achievement of optimal temperatures during cooking the difference in risk reduction begins to decrease. As the level of contamination increases, the balance between freezing reductions and impact on cooking effectiveness begins to diminish. At a mean contamination level of approximately 6 log cfu, the risk from fresh refrigerated chicken is estimated to be the same as that of frozen chicken subjected to lower cooking effectiveness. The value of a freezing strategy, without considering the consumers' practices, is more evident when the impact of freezing is assumed to reduce the effectiveness of cooking even further. When the effect of freezing reduces the temperature in the cooler spots of the chicken by 5°C, the mean risk is estimated to start out lower. As the contamination level increases, the risk also increases until eventually it is estimated that the risk from frozen chicken could in fact turn out to be higher than the risk from refrigerated

chicken. When the level of contamination on chicken is greater than approximately 5.25 log cfu, the risk from frozen chicken exceeds that of fresh, refrigerated chicken.

The important consideration from these scenarios and the additional effects is that a strategy that is implemented without consideration for the subsequent potential side effects could produce unexpected results, and make a situation worse. Clearly, it is virtually impossible to predict every potential complication. Risk modelling and the risk assessment approach shown in these scenarios only serves to help bring to the surface and shed light on relationships and potential complications that might result that might otherwise be difficult to see and perhaps easily missed. There will always be repercussions that no one can predict, and, ultimately, the task is to use the tools and information we have as effectively as possible, so that the predictable complications are considered.

5.8 Examples of risk management options employed by member countries

5.8.1 The campylobacteriosis epidemic in Iceland

From 1998 to 2000, Iceland experienced an epidemic of human campylobacteriosis, which was traced to the consumption of fresh chicken. Due to the geographical isolation of the island, this offered a unique opportunity to characterize contributing factors leading to poultry colonization and transmission to humans (Stern et al., 2003).

The increase in incidence of human campylobacteriosis of domestic origin, from about 10 cases per 100 000 in 1990–1995 to 116 per 100 000 in 1999, coincided with an increase of the consumption of fresh poultry meat, which had not generally been sold at retail stores in Iceland prior to 1996 (Reiersen et al., 2003). Therefore, in 2000, carcasses from flocks that tested positive on the farms were subsequently frozen prior to distribution. In that year, the incidence of domestic cases dropped again to 33 cases per 100 000.

These data suggest a strong impact of freezing poultry meat as a risk mitigation strategy (for recent data see Georgsson et al., 2006). However, analysis showed that the peak in human cases of campylobacteriosis could not solely be attributed to eating fresh or frozen poultry meat: additional risk mitigations were taken simultaneously and other unrelated factors (such as the weather) could also have influenced the observations. Stern et al. (2003) conclude there is no immediate basis for assigning credit to any specific intervention

5.8.2 Developments in Denmark and Norway

Denmark, a company has introduced *Campylobacter*-free chicken meat, from broilers that test negative prior to and immediately after slaughter, and then frozen (H. Wegener, unpublished). This meat has not been a successful product so far, as it did not encounter much enthusiasm from the side of the consumers. With the common diagnostic test, freezing is required to tide over the time until the test results are available. Recently, the development of a rapid diagnostic test has allowed the sale of fresh *Campylobacter*-free chicken in Denmark.

In Norway, an action plan against *Campylobacter* spp. in broilers was implemented in May 2001. It consists of a surveillance programme that included all Norwegian broiler flocks slaughtered before 50 days of age, a survey of broiler meat products, and a follow-up advisory service on farms with flocks tested positive for *Campylobacter*. Logistic slaughtering has been performed and the carcasses from *positive* flocks are frozen before being marketed (Hofshagen and Kruse, 2003). However, based on, among others, the findings of Johannessen et al. (2007), logistic slaughter is no longer practised.

6. RISK ASSESSMENT AND DEVELOPING COUNTRIES

Campylobacter is a leading cause of diarrhoeal diseases in developing countries. Although it is a significant public health problem, the disease burden of *Campylobacter* in developing countries is currently not known.

In 2000, a WHO expert meeting noted that surveillance systems in most developing countries, and even in many developed countries, are inadequate to determine the burden of human campylobacteriosis (WHO, 2001). Implementation and development of a quantitative risk assessment, which requires national surveillance data and modelling experience, is a complicated process requiring focused efforts and resources. The ability of developing countries to muster the scientific and technical expertise and other resources needed to tackle the problem is often limited. However, some developing nations have begun implementing food safety control programmes where no national food policies previously existed. In this section, we offer key recommendations that might be used by developing countries to bolster their current risk assessment activities, and that could be used by others as a guidance in developing and implementing risk assessment and risk management steps that might aid in the evaluation and reduction of risks associated with *Campylobacter*.

In developing countries, the public health impact of *Campylobacter* is most dramatic in the very young. Among the pathogens causing paediatric diarrhoeal diseases, *Campylobacter* is a leading cause, being especially acute during weaning. Children under 1 year of age are highly susceptible to *Campylobacter* infections and may suffer the affects of malnutrition. Overall, children under the age of 4 are at high risk and may suffer several bouts of *Campylobacter* illness. However, the occurrence is significantly lower in older children and adults, but the incidence is still many times higher than that observed for developed nations.

The question is whether it is possible for developing countries to apply the concepts and use the components from the present model and risk assessment to conduct a quantitative risk assessment.

The present model is relatively complex, being based on work from previous risk assessments produced in three developed countries (Canada, Denmark and United Kingdom). The common thread among these countries is their relatively similar production methods and consumption patterns, and, although still limited, data are available describing levels and prevalence of *Campylobacter* through most of the production system. In addition, both export and home market chickens pass through similar farming and slaughter processes.

Many of the exposure elements—the consumption patterns, slaughter processes and farming practices—may be quite different from those in developing countries, and thus have limited applicability. Furthermore, there are few or no data on exposure routes, risk factors and human illness associated with *Campylobacter* in developing countries. Thus, the possibility of performing a detailed national quantitative microbial risk assessment (QMRA) may require a capacity that does not currently exist in many developing countries.

There are steps that developing countries can take to aid future risk assessment efforts and to reduce some of the risks associated with *Campylobacter*. First, it is recommended that a process for the collection of data be initiated that is structured and performed in a manner that ultimately will provide information that can be used in a risk assessment. The guidelines provided by the Kiel documents (WHO, 2000; FAO/WHO, 2002) on preliminary risk management activities may be a great help in structuring such a process. Data collection is very time consuming and resource intensive. The process needs to be structured so that the data do not provide misleading conclusions

in an early phase of the process. Based on the experiences from the present work, it is suggested that the following prioritized list of issues be addressed.

6.1 Priority areas

6.1.1 Epidemiology of Campylobacter sources

1. Evaluate possible *Campylobacter* sources, including water, milk, cattle, pigs, pets, poultry, vegetables, processing and food handlers, and other environmental sources. Prevalence data (yes/no) will be sufficient at this stage. It is preferable that sampling be performed at the retail level or on raw food products prior to preparation either by the consumer or in retail settings.
2. If chicken is a significant source of *Campylobacter* survey the retail market to determine the types and quantities of different chicken products consumed by the population. Note that food products that do not undergo any heat treatment before ingestion, such as ready-to-eat products, may be a more important source than expected, despite low prevalence at retail.

6.1.2 Potential pathways of human exposure during preparation, processing and production of chicken

Identify and prioritize possible exposure route of various types of processing and production methods.

1. Types of preparation and food habits (prepared at home, prepared at restaurants, etc.).

2. Types of slaughter processes (home slaughter, retail and local slaughter, industrial slaughter, etc.).

   A. Identify critical points in processing for sampling. With respect to poultry and chicken this could be:

      (i) prior to slaughter;

      (ii) after slaughter or post-processing; or

      (iii) at retail.

   B. Acquire data on prevalence and levels (numbers) on birds or products at the critical points.

3. Types of farms (small private home farms, larger-scale farms):

      (i) flock prevalence; and

      (ii) prevalence among birds on-farm.

6.1.3 Data collection and use

During and after the collection of prevalence data and enumeration of *Campylobacter* on chicken products, the information can be used to:

1. Evaluate the importance of the identified exposure routes. Which routes are associated with the greatest levels of the organism?
2. Detail the processes of potential major exposure routes and acquire the relevant data.
3. Perform quantitative risk assessment on major exposure routes.

6.2 Mitigations that could be implemented to potentially reduce Campylobacter *in chicken*

Certain processes can be used to reduce the levels of *Campylobacter* and perhaps the prevalence of the organism on chicken products. These mitigations can potentially reduce *Campylobacter* in chicken products both for the domestic market and for products destined for export.

1. Freezing of chicken products during post-processing. Freezing of poultry products have been shown to reduce the levels of *Campylobacter* by one to three orders of magnitude (1 to 3 $\log_{10}$) (Aho and Hirn, 1988).
2. Control of processing and production steps.

6.3 Additional considerations

Along with this risk assessment process, it is also important to consider a training programme to enhance the understanding and use of QMRA models in risk assessment, and it is recommended that collaboration be established among different core groups of public health professionals (biologists, microbiologists, food safety technologists, food processing and production technologists, veterinarians, modellers, statisticians and other experts) in order to generate data and perform the risk assessment in the most efficient manner.

Finally, it should be stressed that there may be several exceptions where the present risk assessment, or modules from the risk assessment, may be useful. For example, several developing countries produce chickens for export. These chickens are in many countries slaughtered at highly modern slaughterhouses, with implemented hygiene standards similar to those seen in the developed countries. In such cases, the present model may be highly relevant and the slaughterhouse module may be implemented directly in a risk assessment. In such scenarios, the food habits and retail conditions in the exporting country become unimportant, thus leaving the the prevalence of farms as the only factor of importance in terms of national data.

7. GAPS IN THE DATA

An important role of risk assessment is that it can identify areas where data are lacking or very limited. Lack of data or the availability of very limited data contribute to the uncertainty of exposure and risk estimates. Thus, collection of additional data and new research directed at critical parameters of a risk assessment can vastly reduce uncertainty. Combined with sensitivity analysis, which identifies the importance of specific parameters on the final risk estimate, research priorities can more efficiently and effectively target areas that improve the accuracy of the risk estimate or relative-risk-reduction estimate. We identify a number of data gaps where additional data may be very useful in a risk assessment for *Campylobacter* in broilers.

7.1 Hazard identification

- National surveillance data on the number of *Campylobacter* infections per 100 000 inhabitants in developing countries.
- Survey data on the load of *Campylobacter* in chicken products in developing countries.
- The risk factors for campylobacteriosis in developed and developing countries.

7.2 Exposure assessment

7.2.1 On-Farm

- Evidence regarding the routes of *Campylobacter* infection of broilers.
- Survey data on the prevalence of flocks, both within- flock and between-flock.
- Data on the probability and level of contamination of a bird during transport.
- Studies on the dynamics of within-flock transmission.

7.2.2 Processing

- Prevalence and enumeration data for poultry before and after various processing steps, such as scalding, de-feathering, evisceration, washing and chilling
- Prevalence and enumeration data comparing various methods of chilling: air chilling, water chilling, water chilling with chlorine, etc.
- Data describing the actual cross-contamination between positive and negative flocks and within positive flocks during the different slaughter processes.
- Prevalence and enumeration data comparing different scalding temperatures and different packaging methods[1].
- Data on the relationship between the concentration on neck skin samples and the concentration on the whole chicken. in order to calculate a conversion factor.

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1. The survival of *Campylobacter* may be increased or favoured by packaging in a modified gas atmosphere (Blankenship and Craven, 1982). This may be important as more and more chicken parts are packed in this way. Data

- Data on the microbial implications of carcass de-boning.

7.2.3 Post-processing and consumer handling

- Survey data and direct observational data on consumer practices in preparation and handling of chicken that especially detail the number of times transfer of *Campylobacter* could occur during handling and preparation.
- Research data detailing amounts of *Campylobacter* that are transferred to and from surfaces during preparation of chicken and the meal.
- Survey data and direct observational data on preparation and handling practices of chicken in restaurants and other food service establishments and retail outlets.
- Additional data on cooking of chicken that addresses areas of the chicken where *Campylobacter* may be protected from heat.

7.3 Hazard characterization

- Data on strain variability regarding virulence and pathogenicity.
- Studies on the mechanisms of infectivity, virulence and pathogenicity.
- While it would be helpful to have additional human volunteer studies, the ethical issues associated with such research mean that this cannot be done. However, non-human primate studies might be an option, including:
    - studies with other strains of *Campylobacter jejuni,* at dosages ranging from 10^1 to 10^9 organisms; and
    - studies that focus on lower doses of the organism, from 1 to 10^3 organisms.
- Epidemiological data available from outbreak studies that have enumerated the number of *Campylobacter* in suspected food items and include information on attack rates, illnesses, etc.
- Additional epidemiological data on susceptible subpopulations, including immunocompromised, children under the age of 5 years and the elderly.
- Additional epidemiological data on susceptibility of children under 1 year of age.
- Data describing the impact of immunity.
- Studies on the true number of human infections, and other sequellae caused by *Campylobacter*, including Guillain-Barré syndrome (GBS).
- Studies on the true number of human infections caused by *Campylobacter* from different sources, including chicken products.

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need to be generated that elucidate the effect of modified-atmosphere packaging on the prevalence and concentration in such products.

8. CONCLUSIONS

This risk assessment has been prepared in response to a request for advice from the Codex Committee on Food Hygiene (CCFH). The risk assessment has adopted the standard terminology of the Codex Alimentarius Commission guidelines for microbiological risk assessment. It is structured in the stages of hazard identification, exposure assessment, hazard characterization and risk characterization. An overall schematic of the risk assessment model is provided in Figure 1, in the Executive Summary.

The risk assessment model is a mathematical description (including Monte Carlo simulation) of a number of phenomena that, through a complex web of interactions, contribute to the level of risk associated with consuming broiler chicken products contaminated with *Campylobacter*. This assessment is a result of merging models developed in Canada, Denmark and the United Kingdom prior to this assessment. These risk assessments were carried out for different reasons, and their individual structures and emphases varied accordingly.

8.1 Model use and adaptation

The actual simulation model, developed using the Microsoft Excel spreadsheet program, is not designed for direct use by member countries. However, the description of the various modules can be used to construct similar models in various software packages of the user's choice. In addition, the authors do not advocate the exact structure of the model as the preferred model for any given decision. The questions posed by CCFH were sufficiently vague that the process was best served by the development of a model that contained the major elements from the three 'parent' risk assessments, but did not focus on detailed measurement of the risk reduction impact of any specific risk-management strategy.

In some cases, the model elements are quite complex and in others they are relatively simple. These model elements could be customized or replaced by other elements as required for a particular decision context. As an example, the model contains a module that provides detailed computation of the spread of *Campylobacter* colonization within a commercial broiler flock. This module, and other complex elements in the model, can be customized to a particular situation or, with due consideration, replaced by a much simplified model element that serves the same functional role in the simulation (e.g. employing a single fixed number, such as 80%, as an estimate of the prevalence of *Campylobacter* within positive flocks). In a similar fashion, the very simple current module for exposure via cross-contamination in the home could be replaced by an alternative simple module, or a highly complex sub-model that characterizes cross-contamination behaviours, pathways and frequencies in considerable detail, if the user had access to the data and expertise required to establish such a cross-contamination model.

The risk assessment model does not describe any particular closed system (i.e. the production and consumption of poultry in a specific country). Rather, it provides a series of generic production, processing and handling elements that could be adapted or customized to describe a variety of different situations. The model is best viewed as providing a model infrastructure that can be applied by FAO and WHO member nations, including CCFH risk-management working groups, to support a wide-variety of risk-management decisions. This is evident from the breadth and generality of the scenarios that were analysed as part of the risk characterization.

At present, the model can be used in two distinct modes. The first mode is the generation of insight into the complex mechanics of the propagation of the risk of campylobacteriosis through the

production, processing and consumer handling subsystems. The development of this insight might be considered a prerequisite to the development of risk-management strategy. Risk assessors and risk managers could employ the model as a test bed for ideas on how the system might be managed, and on how the system reacts to various changes, intentional or otherwise. The use of the model to generate insight into the drivers of risk is limited only by the imagination and resources of the user in adapting or applying, or both, the model to uncover new relationships.

The second mode of use would be to characterize very specific risk-mitigation options. The infrastructure to do this is available in the current model. Strategies designed to affect any number of model elements can be evaluated quantitatively by comparing the baseline scenario with the risk estimates that are generated by including the changed model elements caused by the risk-management options. Examples of elements that might be modified by risk-management strategies include between-flock prevalence; within-flock prevalence; surface contamination during transport; decontamination through scalding; inactivation effectiveness in chilling water; home storage; or cooking behaviour.

As an example, if a risk manager were to impose a requirement for the freezing of any product that comes from a flock that has tested positive, the impact of this policy could be measured in comparison with a baseline where freezing of broilers is not related to the status of the flock. An addition model element could be added to take into account the sensitivity of the flock testing, or other relevant parameters. Accordingly, the logic that imposes the freezing requirement on test-positive lots would need to be added to the risk assessment. As a result of such adaptations, the model would gradually accumulate a richer description of implemented risk-management options.

8.2 Key findings

The risk assessment provides a tool for discovery and measurement of complex relationships determining the risk of illness upon preparation and consumption of chicken products contaminated by *Campylobacter jejuni*. The five scenarios described above and in more detail in the main report constitute a sample of a large array of potential scenarios that can be studied and evaluated. These scenarios led to the following findings:

- Reduction in retail prevalence of test-positive chicken products has a roughly proportional effect in risk reduction.
- Reduction in the contamination level of test-positive chicken products has a somewhat more complex relationship with the estimate of risk. For highly contaminated products, moderate reductions in the contamination level have relatively mild effects. As the contamination level is further reduced, further reductions have increasing relative impact, and eventually yield significant relative-risk reductions.
- Between-flock prevalence is roughly proportional to the risk of illness. The presence of cross-contamination between flocks complicates this slightly due to risk from test-negative flocks that become contaminated by test-positive flocks during transport and in the slaughter plant.
- Reduction in within-flock prevalence clearly reduces the overall estimate of risk, but with a less-than-proportional rate due to the presence of cross-contamination in the slaughter process, which increases the within-flock prevalence for carcasses during processing.
- A number of scenarios were compared wherein the contamination levels in the processing environment were reduced. The analysis indicates the greatest benefit from reduced total loading of the intestinal tract of birds (thereby reducing the total load on the system). In addition, the

benefits of reductions in levels of contamination that take place early in the processing stages can be undermined by cross-contaminating processes later in the processing environment.

- Freezing of poultry will inactivate *Campylobacter* slowly over time. This has been suggested and implemented as a risk mitigation measure, particularly for test-positive flocks in some countries. The scenario includes the potential that freezing may present countervailing risks through reduced effectiveness of cooking. An example is provided where the net effect of freezing is to increase risk when both the reduction in numbers and the reduction in the effect of cooking are considered. This is an example of complex relationships that can emerge from such models, and which can facilitate more informed risk-management decision-making.

These findings represent a sample of the analyses that member nations might conduct by adapting this computational model of the system. Readers are encouraged to familiarize themselves with the model in order to explore alternative assumptions, the impact of new evidence, and proposed risk-management options from farm to fork.

9. RECENT DEVELOPMENTS IN RISK ASSESSMENT OF *CAMPYLOBACTER* IN BROILERS

Risk assessments of *Campylobacter* in poultry meat, which have been conducted in several countries of the world, were used in writing the current report (Fazil et al., 1999; Hartnett et al., 2002; Rosenquist et al., 2003). During the completion of this report, after the expert consultation held in Bangkok, Thailand, in 2002, additional work on risk assessment of *Campylobacter* in broilers became available. This has led to the development of new risk models and the acquisition of new data that could not be integrated in the models described in this report. Also, in some countries, risk managers have enforced interventions in the poultry production chain to tackle the *Campylobacter* problem.

The overview of recent developments given below serves as an additional resource to the reader. It should be viewed as a brief summary, which does not pretend to be complete. Nevertheless, it provides references to recent research and illustrates the ongoing development of quantitative microbiological risk assessment as a dynamic field of research.

9.1 QMRA: models and data

9.1.1 New risk assessments

In the Netherlands, a risk assessment of *Campylobacter* in poultry meat was performed as part of the *Campylobacter* Risk Management and Assessment (CARMA) project, which started in 2001 and was finalized in 2005 (Havelaar et al., 2007). The project includes risk assessment, economic analysis and research on stakeholder perceptions of the problem and possible interventions.

The broiler chicken model developed in CARMA builds on the models presented in this FAO/WHO report. An alternative farm model is formulated, which includes not only the dynamics of *Campylobacter* transmission within flocks, but also transmission between flocks, that is between subsequent flocks in the same house or between concurrent flocks in different houses (Katsma et al., 2007). A poultry processing model has been developed, that explicitly includes the basic mechanistics of cross-contamination, inactivation and removal for each processing stage (Nauta, van der Fels-Klerx and Havelaar, 2005; Nauta, Jacobs-Reitsma and Havelaar, 2007). It differentiates between *Campylobacter*s from the carcass exterior and *Campylobacter*s in the faeces that leaks to the processing environment during processing, and therefore allows an evaluation of the effect of interventions aiming to reduce faecal contamination of carcasses during processing. Also, it incorporates the non-linear effects of cross-contamination that cannot be derived from data on changes in concentrations of *Campylobacter* through the different processing steps alone.

The CARMA poultry processing model confirms the need for adequate quantitative data on chicken and carcass contamination before and during processing. Additionally, it is shown that, for risk assessment, not only knowledge on the mean of log counts, but also on the arithmetic mean of counts and the attending variability between individual chickens carcasses, are essential to predict changes in levels of contamination of carcasses.

Within the CARMA project, a set of risk management interventions are compared for efficiency in terms of human health risk reduction and in terms of economic efficiency. It is found that interventions during processing, like chemical decontamination by adding lactate to the washing water after de-feathering, are most promising, compared with interventions at the farm stage, or educational information campaigns aiming at improving hygiene practices in the domestic kitchen.

Another promising strategy is to aim at reducing the level of contamination from faeces leaking from the carcasses during processing. The production of fresh broiler meat that is guaranteed *Campylobacter*-free is not a realistic option in the Netherlands (or elsewhere), but, disregarding the effects on trade, a considerable relative risk reduction may be achieved without extreme economic costs.

In Germany, a risk assessment of campylobacteriosis due to the consumption of chicken prepared in the home focuses on the consumer phase (Brynestad et al.,2008). It applies new data on *Campylobacter* concentrations on chicken products at retail, and experimental data on cross-contamination in the domestic environment (Luber et al., 2006). Like other risk assessments, the model shows that reducing *Campylobacter* load on the chicken may result in a greater reduction in the incidence of human illness than reducing prevalence of contaminated products.

A French risk assessment report (Mégraud and Bultel, 2003) describes models developed elsewhere and concludes that the available (quantitative) data sets are insufficient, which, for their purposes, does not allow the completion of a quantitative risk assessment.

In New Zealand, a quantitative risk model covering the whole food chain was finalized in 2006 (Lake et al., 2006). Next to exposure via cross-contamination to a secondary food during food preparation and undercooking, it also explicitly explores cross-contamination of the exterior of packages and hands. As in other risk assessments, cross-contamination to a secondary food is the dominant source of infection. Also, the finding that cross-contamination between flocks during processing has a negligible effect is confirmed (see also Rosenquist et al., 2003; Havelaar et al., 2007; Johannessen et al., 2007).

Finally a new resource entitled the "Campylobacter Risk Assessment Framework (CRAF)"has been made available on the internet which provides information on the modelling structure of quantitative microbiological risk assessments for Campylobacter in broiler meat, developed in the UK (VLA), Denmark (Food-DTU), the Netherlands (RIVM and CVI), Germany (BfR) and New Zealand (NZFSA). This product which is the output of MedVetNet, an EU-funded Network of Excellence provides a general overview of the models, allows the comparison of modules of the different models as well as enabling the user to explore the possibilities of combining the different risk assessments (RIVM, 2009)

9.1.2 New chicken processing data

Stern and Robach (2003) reported *Campylobacter* count data of faecal samples and carcass rinses of broiler chicken flocks in Georgia, USA, comparing the situation in 1995 to that in 2001. Levels of *Campylobacter* on freshly processed broiler carcasses showed a significant decrease, from an average $10^{4.1}$ per carcass in 1995 to $10^{3.1}$ in 2001. Results of their study suggest that, in the USA, the implementation of antimicrobial interventions (i.e. water chilling with added chlorine) has reduced consumer exposure to *Campylobacter*. New *Campylobacter* enumeration data have also more recently been collected (Stern and Pretanik, 2006).

Additional data on numbers of *Campylobacter* on carcasses at different poultry processing stages have been collected in the United Kingdom (Corry et al., 2003). It was found that carcasses derived from *Campylobacter*-infected broiler chickens will be contaminated with log 5-6 cfu *Campylobacter* per carcass, with no or only slight reduction in numbers during processing. Allen et al. (2007) provide novel data on *Campylobacter* concentrations at consecutive stages of broiler chicken processing. They find higher concentrations in flocks with a high animal prevalence.

In Denmark. the effect of slaughter operations on broiler carcass contamination with *Campylobacter* has been quantified (Rosenquist et al., 2006). Their findings indicate that reducing the concentration of *Campylobacter* in the intestines of living birds may be a promising intervention.

9.1.3 New data on consumer food handling

Quantitative research on cross-contamination due to food handling in the domestic kitchen is gaining increasing attention. Although the variability in consumer behaviour remains a crucial complicating factor for risk assessment, the increased availability of data and models will allow improved analyses for risk assessment in the near future (e.g. Chen et al., 2001; Gorman, Bloomfield and Adley, 2002; Kusumaningrum et al., 2003; Moore, Sheldon and Jaykus, 2003; Mattick et al., 2003; Luber et al., 2006; Mylius, Nauta and Havelaar, 2007). See also Redmond and Griffith (2003) for a review of food safety studies dealing with consumer food handling in the home.

9.1.4 Dose-response relationship

Recently the *Campylobacter* dose-response relationship has been updated, based on two independent outbreaks of campylobacteriosis among school children after drinking raw milk at a farm (Evans et al., 1996; Van den Brandhof, Wagenaar and Van den Kerkhof, 2003; Teunis et al., 2005). According to the analysis of these outbreaks, the infectivity and pathogenicity of *Campylobacter* would be more severe than assumed so far. It stresses the need for further research on hazard characterization of *Campylobacter*.

9.2 Risk mitigation

In addition to the examples presented in section 5.8 some novel approaches to risk mitigation are now being tested.

A novel method to reduce the *Campylobacter* load in poultry is the use of bacteriocins from *Bacillus* and *Paenibacillus,* as a therapeutic treatment for chickens colonized by *Campylobacter* (Svetoch et al., 2003). By feeding the animals therapeutic feed at the appropriate moment in the cycle, levels and frequency of colonization can be reduced, which may be effective in lowering the human health risk imposed by *Campylobacter*.

An alternative new method to reduce the level of *Campylobacter* on the chicken skin might be the application of host-specific bacteriophages (Goode, Allen and Barrow, 2003; Atterbury et al., 2003b). These bacteriophages can survive on fresh and frozen retail poultry products. As they are found to be naturally present on the chicken skin, their usage as a biocontrol agent would not introduce any entity into food products that is not already present (Atterbury et al., 2003a). Similarly, experiments suggest that treating live birds with specific bacteriophages shortly prior to slaughter may be an effective control measure (Havelaar et al., 2007).

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Appendix A

A1.1 *Campylobacter* on the farm

A1.1.1 Estimating P_{fp}

Sample data obtained from two fully-integrated poultry companies, an epidemiological study (Evans, 1996) and a published source (Humphrey, Henley and Lanning, 1993) were used to obtain an estimate of P_{fp}. More specifically, individual estimates of flock prevalence were derived for each of the four sources 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 Great Britain 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, Henley and Lanning, 1993). In each case, r denotes the number of positive flocks and s the number of flocks sampled. The beta distribution is used to characterize 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 (Evans, 1996).

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 $w1$, $w2$, $w3$ and $w4$ are the associated weights. The values for $w1$, $w2$, $w3$ and $w4$ are based on the companies' market shares using denominator data derived from MAFF statistics (MAFF, 1999).

A1.1.2 Estimating P_{wfp}

Stage 1: Chain Binomial

In the 1920s, Reed and Frost developed chain-binomial models of epidemic spread (Jacquez, 1987). Although this work was not published, the theory was popularized by Bailey (1975) and these models have frequently appeared in the literature, for example to study HIV epidemics (Ng and 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 colonized birds within the cluster containing the first positive bird.

In the situation presented here, the basic chain-binomial model describes the colonization of a random susceptible bird that becomes colonized after a fixed constant time. The colonized 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 (A1.2)

$$I_c(t+1) = I_c(t) + NI_c(t+1) \qquad \text{(A1.2)}$$

where $I_c(t)$ is the number of colonized birds in the cluster at t, and $NI_c(t+1)$ is the number of newly colonized birds in the period $(t, t+1]$ where $(t, t+1]$ is defined as one day. The number of newly colonized birds at each time point will follow a binomial distribution that 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:

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

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

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

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

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 colonized is dependent upon the transmission rate, the social need to make contact with other birds, and the probability of contact with a colonized 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 and 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 and Orav, 1990):

- 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;
- a bird, which becomes colonized at time t, cannot transmit the organism to another bird until time $t+1$, this allows for a fixed latent period of one day;
- birds within the cluster act independently; and

- each non-colonized bird has the same probability of being colonized at time *t*.

Let *b* equal the probability of transmission given a single contact of a susceptible bird with a colonized bird; *A* equals the number of birds a given bird comes into contact with in one day, that is (*t, t*+1]; and *R* equals 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 that 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 colonized in the period (*t, t+1*], *p*(*t*), is derived as follows:

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

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

P(no transmission occurs □R contacts with a random bird in cluster)

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

Therefore, the probability that a susceptible bird becomes colonized in the period (*t, t+1*], *p*(*t*), is given by equation (A1.3):

$$p(t) = 1 - \Phi_A\left[1 - \left\{ \left[\frac{I_c(t)}{n_c(t)} \right] [1 - \Phi_R(1-b)] \right\} \right] \qquad \text{(A1.3)}$$

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 (A1.3), the probability that a non-colonized bird becomes colonized 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 colonized birds is then given by:

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

Stage 2: Epidemic spread

As previously discussed during the process of *Campylobacter* colonization 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 colonized, and colonization 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 colonized birds at any time point is dependent upon the initial number of colonized birds, that is, the number of birds colonized within the cluster at the time when stage 2 begins ($I_c(t_4)$) and the transmission rate. Under this assumption, the colonization 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 colonized birds in the cluster modelled in stage 1. The colonization process begins with $I_c(t_4)$ colonized 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 colonized birds is proportional to both the numbers of colonized and susceptible birds. Therefore the process can be described by the differential equation (A1.4)

$$\frac{dS_B}{dt'} = -b_B S_B(t')\left[n - S_B(t')\right] \qquad \text{(A1.4)}$$

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 stage 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, colonized birds contaminate feed and water, which then leads to exposure and subsequent colonization 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 (A1.4) for the number of susceptibles gives equation (A1.5):

$$S_B(t') = \frac{S_B(t_4)n}{S_B(t_4) + I_c(t_4)\exp^{[nb_B t']}} \qquad \text{(A1.5)}$$

After completion of the first and second stages, the total number of colonized 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 (A1.6):

$$P_{wfp}(t) = \frac{I(t)}{n} \qquad \text{(A1.6)}$$

A1.2 Consideration of *Campylobacter* sources within a risk assessment framework

For contamination of broiler flocks, a number of sources and contamination pathways have been investigated. Numerous epidemiological investigations have sought to identify sources and risk factors within various countries, including the United Kingdom (Evans and Sayers, 2000; Humphrey, Henley and Lanning, 1993), the Netherlands (Jacobs-Reitsma et al., 1995; Van de Giessen et al., 1998), Norway (Kapperud et al., 1993), Sweden (Berndtson, Danielsson-Tham and Engvall, 1996; Engvall et al., 1986) and in France (Refrégier-Petton et al., 2001). Some investigations have attempted to identify the source of repeated contamination of particular flocks (Pearson et al., 1996). As discussed above, the studies collectively cover a range of risk factors, including season, farm personnel, on-farm hygiene, surface water, other on-farm animals and the poultry house surroundings. Most of the above studies have included a subset of these risk factors, while the French study includes components of each risk factor.

Other experimental investigations have focused on specific sources and reservoirs for broiler flocks. Shanker, Lee and Sorrell (1986) and Cox et al. (1999) have investigated vertical (egg-borne) transmission from breeder flocks to broiler flocks, with opposing conclusions on the plausibility of this pathway of transmission. Feed and water as a source of contamination has been demonstrated (Pearson et al., 1993). Other investigators have performed descriptive bacteriological studies of insect vectors (Rosef and Kapperud, 1983); mice (Van de Giessen, 1996); the ground surrounding the broiler house (Studer, Lüthy and Hübner, 1999) and cross-contamination during transport (Stern et al., 1995). A major study of multiple broiler operations in the USA (Stern et al., 2001) isolated *Campylobacter* in a wide variety of reservoirs within the broiler house environment. An interesting finding was the inability to detect *Campylobacter* in these reservoirs until after detection in the faeces of the birds. Another study in the USA (Nesbit et al., 2001), using molecular methods, found that isolates from within the broiler population were genetically distinct from environmental isolates, with no clear source for the broiler contamination.

In summary, the matter of the dominant source, pathway and risk factors for *Campylobacter* contamination of flocks has yet to be resolved. The accumulated literature underscores the complexity of the exposure situation for broiler populations. Even if it were to become known (or even strongly demonstrated) in some particular situation, it is not clear that any generalization of such findings should be assumed.

A1.2.1 Module for between-flock prevalence

A common practice in farm-to-fork risk assessments has been to model the between-flock prevalence as an observed or assumed entity, as opposed to being predicted from some set of risk factors or other model inputs. For modelling purposes, characterization of between-flock prevalence has been limited to expressing the uncertainty (and occasionally the seasonal variability) in the estimate of between-flock prevalence. To date, it has been uncommon to include predictive modelling for the between-flock prevalence. As a result, risk characterization and subsequent decision support has been limited in the capacity to describe the importance of risk factors, or to suggest mitigations, that affect the between-group prevalence.

The absence of predictive modelling for between-group prevalence is partially explained by:

- The lack of dominant case-control studies, which clearly distinguish key risk factors, as discussed above.
- Expectations that the risk factors would vary according to regional, climatic and farm management scenarios.
- Resulting concerns about extrapolating the measured strength of risk factors outside of the studied population.
- The assumption that the relationship between between-flock prevalence and risk is essentially linear, with resulting clarity in the implications of risk mitigations that reduce prevalence.
- Difficulty in describing the uncertainty, for example, in a logistic regression equation without access to the full data set and statistical results.
- Limitations in the knowledge and experience of modellers in including epidemiological findings into a risk assessment model.

A module to allow for mitigations that would reduce the between-flock prevalence of *Campylobacter* has been developed. This is a separate module, without direct linkage to the calculations in the rest of the risk-assessment model.

The module is intended as an example framework for:

- compiling evidence on the impact of risk factors;
- computing parameters for use in regression;
- dis-aggregating the population of flocks into groups sharing the same risk factors;
- predicting flock prevalence based on the risk factors in each group;
- predicting production-weighted flock prevalence to better account for the share of the population risk associated with larger flocks; and
- describing population-level risk mitigations by shifting the proportion of flocks toward patterns of risk factors that are associated with a reduced risk of flock contamination.

A1.2.2 Risk factor characterization

To use the between-flock prevalence model, evidence regarding the impact of various on-farm and management-risk factors must be accumulated. For present purposes, the evidence is assumed to be in the form of case-control studies. Ideally, a single large study with complete statistical analysis would be employed. Based on an epidemiological study, the prevalence for some control group could be calculated. Given the epidemiological study tables, calculations for the odds-ratios (OR) associated with each risk factor level (or, preferably, using the odds-ratios computed by the study authors) could be performed. For risk factors with multiple levels (low, medium, high), these can be broken down into discrete combinations of binary risk factors (low = 0,0, medium = 1,0, high= 0,1). For present purposes, the logarithms of the calculated odds-ratios become the coefficients in a predictive equation, with each risk factor having an effect on the final odds-ratio of between-flock prevalence.

The output of the predictive equation is an odds-ratio (relative to the control group) for the group of flocks sharing a particular set of risk factors. This allows computation of a flock prevalence based on predicted changes from the control-group prevalence. The set of risk factors that match the control group would yield an odds-ratio of 1, which would reproduce the prevalence in the control group.

A1.2.3 Population-prevalence estimates

The goal is to predict the overall between-flock prevalence for the population of birds in, for example, a country. The set of all flocks must be segregated into distinct groups identified by a shared combination of risk factors. Each group is identified by the pattern of risk factors that best describe the group, by the proportion of all flocks that are in this group and the average size of the flocks in the group.

For each risk factor group, the pattern of risk factors predicts its between-flock prevalence, which is an OR-based multiple of the control group prevalence. The overall unadjusted flock prevalence is a weighted sum of these groups' prevalence estimates, with the weights corresponding to the proportion of all flocks that are in each risk factor group. The adjusted between-flock prevalence is weighted according to both the proportion of flocks in each group, as well as the average flock size, thus corresponding to the proportion of birds produced in conditions described in each risk factor group.

A1.2.4 Mitigations

Mitigations aimed at reducing between-flock prevalence can have an impact in two ways:

- by reducing the proportion of flocks exposed to certain risk factors; and
- by reducing the magnitude of the risk posed by exposure to certain hazards (this would be equivalent to reducing the magnitude of the coefficient associated with each risk factor).

In the current module, the potential for mitigation is limited to the shifts in the proportion of flocks exposed in each risk factor group. For example, if 10% of flocks are in a high-risk group, mitigations could be simulated whereby half of these flocks are moved to a lower-risk group.

To explore the second type of mitigation, additional risk-factor levels could be included, based on estimates of the impact of changes to risk-factor intensity, expressed as a reduced odds-ratio for the added risk-factor levels. To estimate the impact of the mitigation on population level between-

flock prevalence, an estimate of the proportion of flocks that would experience this added risk-factor level would need to be included.

A1.2.5 Limitations of the approach

The current module does not explicitly allow for any uncertainty or variability associated with these risk factors. Since the key risk factors and the intensity of their effects are not readily identifiable from the literature, expressions of uncertainty and variability regarding these effects are not available. Full integration of this type of evidence into the risk assessment would suggest that the uncertainty and variability of the impact of these risk factors (and the proportion of flocks exposed) would need to be characterized.

Uncertainty analysis would have to included, not only regarding the confidence intervals, which may be available from the regression model of the case-control study, but also the uncertainty associated with extrapolation of these results from the study environment to the situation to be modelled. The extrapolation of the case-control findings from the study environment to another environment would probably constitute the dominant source of uncertainty in this type of prediction.

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Appendix B

FAO/WHO have received information from several countries in relation to the *Campylobacter* risk assessment work. Some of this information is listed here.

B1.1 Farm Data

Country	Num Pos	Num Tested	Prevalence	Sample Type	Prev Type	Species
Denmark			95	Cloacal Swab	Flock	Duck
Denmark			60	Cloacal Swab	Flock	Chicken
Denmark			45	Cloacal Swab	Flock	Broiler
Finland	33	1 132	2.9	Faecal	Flock	Chicken
Ireland	82	100	82		Bird	Broiler
Netherlands			29.8		Flock	Broiler
Netherlands	405	936	43.27		Flock	Chicken
New Zealand	7	33	21.2		Flock	Chicken

B1.2 Processing Data

Country	Number Positive	Number Tested	Prevalence	Sample Type	Prev Type	Species	Processing Step
Brazil	15	30	50		Bird	Chicken	End product
Brazil	17	30	36.6		Bird	Chicken	After slaughter
Brazil		34	73.5		Bird	Chicken	
Brazil	14	40	35		Bird	Porcine	
Brazil	19	40	47.5		Bird	Broiler	Final rinsing
Brazil		45	62.2	Surface Swab	Bird	Broiler	Chilling
Brazil		45	51.1	Intestinal Swab	Bird	Broiler	
Cuba	15	30	50	Intestinal Swab	Bird	Broiler	
Cuba	24	30	80	Carcass	Bird	Broiler	Post-evisceration
Cuba	20	30	66.7	Carcass	Bird	Broiler	Chilling
Cyprus	11	27	41		Bird	Broiler	End of processing line
Cyprus	17	57	30		Bird	Broiler	End of processing line
Cyprus	80	118	68		Bird	Broiler	End of processing line
Cyprus	22	51	43		Bird	Broiler	End of processing line
Cyprus	128	288	44		Bird	Broiler	End of processing line
Cyprus	23	41	56		Bird	Quail	End of processing line
Cyprus	52	64	81		Bird	Quail	End of processing line
Cyprus	85	93	91		Bird	Quail	End of processing line
Cyprus	32	42	76		Bird	Broiler	End of processing line
Finland	606 000	19 7 $\times 10^6$	3	Faecal	Bird	Broiler	
Ireland	384	400	87.3	Neck	Bird	Broiler	
Ireland					Bird	Broiler	Post-wash - Pre-chill
Ireland					Bird	Broiler	Post-evisceration
Ireland					Bird	Broiler	After de-feathering
Ireland					Bird	Broiler	Post stun and bleed
New Zealand	15	15	100	Misc. Giblets	Bird	Chicken	
New Zealand	15	15	100	Carcass	Bird	Chicken	

B1.3 Retail Data

Country	Number Positive	Number Tested	Prevalence	Sample Type	Prev Type	Species	Sample Treatment
Brazil	4	27	14.8	Heart	Bird	Chicken	
Brazil		200	13.5	Parts	Bird	Chicken	Raw, refrigerated
Brazil	1	5	20	Carcass	Bird	Chicken	
Brazil	15	82	18.3	Parts	Bird	Chicken	
Brazil	0	32	0	Liver	Bird	Chicken	
Brazil	1	4	25	Feet	Bird	Chicken	
Brazil	40	64	62.5		Bird	Chicken	Fresh
Brazil	30	64	46.9		Bird	Chicken	
Brazil		62	42.1	Carcass	Bird	Chicken	
Brazil	34	50	68	Carcass	Bird	Chicken	
Brazil	6	50	12	Gizzard	Bird	Chicken	
Denmark			34		Bird	Broiler	
Finland	16	174	9.2		Bird	Chicken	
Finland	7	145	15.6		Bird	Chicken	
Netherlands			34	Carcass	Bird	Broiler	Fresh
Netherlands	1	35	2.9		Bird	Guinea-fowl	
Netherlands	1	27	3.7		Bird	Pheasant	
Netherlands	3	52	5.8		Bird	Duck	
Netherlands	1	145	0.7		Bird	Turkey	
Netherlands	1381	4574	30.2		Bird	Chicken	
Netherlands			71	Carcass	Bird	Broiler	Frozen
Netherlands			38	Carcass	Bird	Broiler	Fresh
Netherlands			9	Carcass	Bird	Broiler	Frozen
New Zealand	20	50	40	Wings	Bird	Chicken	
Norway	1	255	0.4		Bird	Broiler	
Norway			10.2		Bird	Poultry	Fresh
Norway			2.3		Bird	Poultry	Frozen
Norway			4.5		Bird	Poultry	Fresh
Norway	9	101	8.9	Parts	Bird	Poultry	Fresh
Norway	0	4	0		Bird	Poultry	
Norway	17	133	12.8	Parts	Bird	Poultry	Fresh
United Kingdom	18	51	35.3	Parts	Bird	Chicken	
Switzerland		302	33		Bird		
Switzerland	22	144	16	Liver	Bird	Poultry	Frozen
Switzerland	43	139	31	Liver	Bird	Poultry	Fresh
Switzerland		302	33		Bird		

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