A novel model developed for Quantitative Microbial Risk Assessment in the pork food chain

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0.1 Abstract

Food-borne diseases contribute substantially to morbidity and mortality rates worldwide. The deleterious impact of these diseases on human health, concurrent with the associated socioeconomic cost has led to an increased demand for the production of safe food globally. Consequently, agencies such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have resolved to address this issue. In this vein, scientific, risk-based approaches which facilitate estimation of the probability of disease occurrence, the magnitude of the disease and efficacious control measures have been recommended for use internationally.

Many pathogens have been implicated as aetiological agents of food-borne disease. The WHO has identified non-typhoidal *Salmonella*, *Escherichia coli* and thermophilic *Campylobacter* as zoonotic food-borne pathogens of greatest importance. These pathogens can be transmitted to humans through pork consumption. This thesis therefore proposes a suite of novel, mechanistic, semi-stochastic, quantitative, modular process risk models describing the propagation of these three pathogens from the live pig at the abattoir, to pork chops sold at retail. The model is developed for use in risk-based, quantitative microbial exposure assessments in New Zealand and can be employed to explore different intervention strategies targeted at mitigating contamination levels of these pathogens on pork chops.

The models comprise multiple, coupled, differential and difference equations. These equations explicitly describe bacterial growth, inactivation, removal, cross-contamination and food partitioning occurring in continuous and discrete time in abattoirs and at retail. Distributions of pathogen numbers on the surface of carcasses, and prevalence levels are output by the models at different stages of abattoir processing and pork chop production. Both dressed pork carcases exiting abattoirs in New Zealand and pork chops at retail are predicted to contain low surface contamination levels of the pathogens under consideration, while a small percentage is estimated to be highly contaminated.

Median contamination levels on dressed pork exiting the abattoir are predicted to be less than one cfu/cm^2. Generally, there are large reductions in surface bacterial numbers for all three organisms from the time the live pig enters the abattoir, to sale of the pork chop at retail. The introduction of a second singeing procedure immediately post-evisceration in the abattoir is predicted by our models, to be an effective mitigation strat-

egy, with estimated reductions in median pathogen levels of 100%. This control measure is considered to be more effective than coverage of the anal region of the pig during evisceration. This latter mitigation strategy was predicted to result in 10% – 44% reduction of median pathogen contamination levels.

At retail, pork chops are also estimated to contain low numbers of these pathogens. Therefore handling of the raw pork chop soon after purchase from retail outlets may be associated with a low risk of contracting salmonellosis, colibacillosis and campylobacteriosis. This risk can be further reduced by placing pork chops in a blast chiller for 12 hours prior to display. When this mitigation strategy was modelled the outputs indicated a 15% – 61% reduction in the maximum pathogen levels on pork chops, 44 – 100% reduction in the 10^{th} – 90^{th} range and 14% – 50% reduction in pathogen prevalence levels.

Detailed investigation revealed the limitations of a specific modelling approach. We determined that the population-based modelling approach is not an appropriate alternative to the individual-based modelling approach when there is a large disparity in contamination levels between processed carcasses. Therefore the former technique should not be used in the presence of large heterogeneity with respect to the number of bacteria on the food unit of interest, or when bacterial populations input into the model are described with large variances.

This thesis demonstrates the application of a suite of novel risk models in the pork food chain. We propose use in quantitative microbial exposure assessments. The applicability of these models is not only limited to the pork chain or to the above mentioned pathogens, but by modification of parameters, the entire model, or portions thereof can be extrapolated to other animal species undergoing similar abattoir procedures with pathogens of analogous epidemiological patterns. Finally the information provided by the models can be instrumental in assisting risk managers in their decision-making and policy development undertakings and provide guidance to effectively and strategically funnel limited resources.

0.2 Acknowledgements

This PhD was truly a journey, a process - a process of developing a new way of thinking and working; exponentially increasing my knowledge in the field of study; a test of tenacity; determination; personal maturity; tolerance; patience; enduring the seemingly innumerable storms and yet, constantly attempting to maintain my inner peace. My journey through this process has been filled with summits, excitements, thrills, irritations and troughs of utter frustration and agony. But, I came to finally learn not to let the PhD master me, but to master it; not to let it overwhelm me but to take each step at a time, each day at a time until the mountain becomes a plain before me. With this perception, half the battle was already won.

No man is an island and this work could not have been completed without the assistance of many people. I would therefore like to thank the following people for their support and help: All the staff and students in the EpiCentre, IVABS and the Statistics Department that assisted me from 2004 - 2007; the computer software developers in the EpiCentre; the New Zealand Pork Industry Board; Landmeat, Freshpork and Taranaki Abattoirs; The Roger Morris Foundation for providing the needed funds; the New Zealand Commonwealth Scholarship for financially supporting me while I was in New Zealand, the Government of Trinidad and Tobago that allowed me the opportunity to further my education; Graham McBride, a mathematician; my supervisors Nigel French, Mark Stevenson and Roger Morris and special thanks to Jonathan Marshall, another mathematician.

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supported me, for enabling me to complete the task of finishing the thesis. He is truly faithful and helped me ‘keep it together when everything around me seemed to indicate that things were falling apart’. It was His contribution that was the single most significant contributor to my success. Now I can happily say ‘Return to rest O my soul, For the Lord has dealt bountifully with you.’. For ‘all things work together for good to those who love Him and are called according to His purpose’.

0.3 Nomenclature

CAC	Codex Alimentarius Commission
cfu	colony forming units
cm	centimetre
FAO	Food and Agriculture Organization
g	gram
kg	kilogram
ml	millilitre
mm	millimetre
MPRM	Modular Process Risk Model
NZ	New Zealand
NZFSA	New Zealand Food Safety Authority
OIE	Organisation International des Epizooties or World Organisation for Animal Health
QMRA	Quantitative Microbial Risk Assessment
RA	Risk Assessment
sd	Standard deviation
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

0.4 Glossary

In this thesis the following terms are defined as stated below:

- **Contamination**:
  The presence of bacteria on the surface of the pig carcass, which may or may not be associated with faecal material; or the presence of unwanted material on the surface of an object.

- **Cross-contamination**:
  The movement of bacteria from one object to another.

- **Deterministic Model**:
  A model that predicts point estimate outputs and does not incorporate the element of chance or contain randomly varying components.

- **Inactivation**:
  The action of rendering an organism non-pathogenic.

- **Infection**:
  Bacterial colonisation of the gastro-intestinal tract and associated tissues.

- **Model**:
  A simplified representation of a realistic phenomenon.

- **Stochastic Model**:
  A model in which the element of chance is explicitly described so that each realization of the model can output different results for the same initial values.

0.5 Symbols and Units

Symbol	Description	Units
α	rate of pathogen movement from water to carcass in scalding	minute^{-1}
β	rate of pathogen movement from carcass to water in scalding	minute^{-1}
δ	rate of pathogen movement from dehairing machine to carcass	minute^{-1}
ϵ	pathogen inactivation rate on carcass in singeing	minute^{-1}
θ	rate of pathogen movement between carcasses in storage/chilling	hour^{-1}
κ	pathogen inactivation/growth rate on pork chop	day^{-1}
λ	transmission parameter	minute^{-1}
μ	rate of pathogen movement from carcass to the dehairing machine	minute^{-1}
τ_1	pathogen inactivation rate on carcasses in scalding	minute^{-1}
τ_2	pathogen inactivation rate in water in scalding	minute^{-1}
a	probability that each cfu of bacteria moves from the carcass exterior to the knife (pork chop models)	cfu^{-1}
$a_{f,s}$	probability that each cfu of bacteria moves from the faeces to the knife (abattoir models)	cfu^{-1}
area	relative proportion of pork chop surface area with respect to the half carcass	%
$a_{x,s}$	probability that each cfu of bacteria moves from the carcass exterior to the knife (abattoir models)	cfu^{-1}
b	probability that each cfu of bacteria moves from the knife to the carcass exterior (pork chop models)	cfu^{-1}
$b_{e,S}$	probability that each cfu of bacteria moves from the knife to the carcass exterior (pork chop models)	cfu^{-1}
c	probability of inactivation from the knife per cfu of bacteria (pork chop models)	cfu^{-1}

Symbol	Description	Units
$c_{e,S}$	probability of inactivation from the knife per cfu of bacteria (abattoir models)	cfu^{-1}
$c_{x,S}$	probability of inactivation and removal from carcass exterior (abattoir models)	cfu^{-1}
d	probability of bacterial inactivation and removal from carcass exterior (pork chop models)	cfu^{-1}
g_f	concentration of bacteria in faeces	cfu/g
h	smoothing parameter	
k	kernel function	
r	pathogen inactivation rate on carcass in storage/chilling	hour^{-1}
$r_{p,c}$	pathogen inactivation rate on pork chop in storage/chilling	hour^{-1}
t	time	minute^{-1}
t_D	decimal reduction time	minute^{-1}
time	time	days^{-1}
x	pork chop contamination level after cross-contamination	cfu
y	pathogen numbers on skin of the pork chop after partitioning when z$>$30,000	cfu
y_1	pathogen numbers on skin of the pork chop after partitioning when z$<$30,000	cfu
z	bacteria numbers on half carcass	cfu
A	probability that bacteria are present on area of carcass in contact with knife for evisceration cut	cfu^{-1}
$A_{f,d}$	faecal quantity output from pig in dehairing	g
B	pathogen numbers in faeces released from carcass in dehairing	cfu

Symbol	Description	Units
C	probability of transfer of bacteria from carcass surface to the knife	cfu^{-1}
C_1	pathogen numbers on halved carcass in storage	cfu
C_2	pathogen numbers on another halved carcass in storage	cfu
E	probability of bacteria on the region to be trimmed coming into contact with knife	cfu^{-1}
F	probability of transfer of bacteria to knife	cfu^{-1}
G	probability that bacteria are present on area of carcass in contact with knife during halving	cfu^{-1}
H	probability of transfer of bacteria to knife	cfu^{-1}
Inf	probability of inactivation of bacteria on carcass skin	cfu^{-1}
I	number of infected animals	pigs
M	pathogen numbers on dehairing machine	cfu
$N_{e,S}$	bacterial load in environment	cfu
$N_{f,d}$	pathogen concentration in the faecal material in dehairing	cfu/g
$N_{f,l}$	pathogen concentration in infected faeces in lairage	cfu/g
$N_{f,S}$	pathogen numbers in leaking faeces from a carcass	cfu
$N_{p,l}$	total number of bacteria in a pen in the lairage	cfu
N_{saw}	pathogen number on saw after cutting pork chop	cfu
$N_{x,c}$	pathogen number on pork chop after partitioning	cfu
$N_{x,pc}$	pathogen number on skin surface of the pork chop after cross-contamination process	cfu
$N_{p,i}$	pathogen number on skin surface of the pork chop in intervention strategy	cfu
$N_{x,S}$	bacterial load on carcass surface	cfu

Symbol	Description	Units
$N_{x,storage}$	pathogen number on halved carcass from abattoir	cfu
p_{cut}	probability of pathogen present on skin surface of the pork chop	
P_d	pathogen numbers on carcass in dehairing	cfu
P_k	pathogen numbers on carcass in scalding	cfu
P_s	pathogen numbers on carcass in singeing	cfu
$Prob$	probability of an animal being infected in lairage	
R	probability of removal of bacteria on the carcass skin	cfu^{-1}
S	number of susceptible animals	pigs
S_0	pathogen numbers on carcass before singeing	cfu
S_t	pathogen numbers on carcass after singeing	cfu
T	temperature	°C
$T_{f,l}$	total infected faecal material in lairage	g
W	pathogen numbers in water in scalding	cfu
$w_{f,s}$	mass of leaking faeces	g
X	cross-contamination	
Y	inactivation	

Contents

List of Figures

List of Tables

CHAPTER 1

Introduction

Within recent years, food safety has moved to the forefront as a topic of international concern, demanding the attention of governments, members of the food industry and consumers. As such, deliberate efforts targeted at minimizing the transmission of pathogens propagated along food pathways have been undertaken using risk-based techniques.

This thesis embarked on new research aimed at addressing the issue of food safety with respect to microbial zoonotic pathogens in pork originating from New Zealand. To this end, a suite of semi-stochastic, modular process risk models describing the propagation of *Salmonella*, *Escherichia coli* and thermophilic *Campylobacter* through the pork food chain from the abattoir to the retail outlet, and evaluating potential control strategies, was developed. This thesis is presented as a series of papers in differing stages of preparation. Consequently, there may be some degree of repetition between manuscripts.

The thesis begins with a review of the literature describing the different model types and techniques used in microbial risk modelling, background information on the organisms of concern, and finally an explanation of the stages of the food pathway modelled. Chapters 3 to 6 focus on modelling pathogens dynamics through the abattoir. Chapter 3 describes the abattoir models, while Chapter 4 details the events occurring in the scald tank, a stage in abattoir processing. A comparison of different modelling techniques is described in Chapter 5. Chapter 6 provides an evaluation of intervention strategies to minimize pathogen contamination in the abattoir and details sensitivity analyses performed on the models. Chapter 7 presents models describing pathogen dynamics during further processing of the dressed pig carcass into pork chops. The predicted efficacy of a control measure is also included. The final section contains a general discussion and final conclusion.

The models developed in this thesis incorporate a range of techniques to describe

pathogen and food-handling dynamics in risk-based modelling. The results of this research propose a suite of models which elucidate mechanisms by which pathogens are propagated along the food chain. Not only can indications of contamination and prevalence levels in pork products sold at retail outlets be predicted from these models, but fairly rapid investigations into the efficacy of control strategies targeted against zoonotic, food-borne agents, transmitted via pork in New Zealand can be undertaken.

CHAPTER 2

Literature Review

2.1 Introduction

Food safety is imperative for the development and maintenance of a healthy population. As such, methods for establishing the safety of food continue to be debated at local and international forums. The World Health Organization (WHO) has identified major foodborne illnesses caused by zoonotic microbial pathogens (WHO 2002). These include diseases produced by *Escherichia coli*, non-typhoidal *Salmonella* species (spp.) and thermophilic *Campylobacter* spp. The consumption of pork has been implicated in all of the infections from these major zoonotic pathogens (Adak et al. 2005, Bolton et al. 2003, Korsak et al. 1998).

Within the last two decades the incidence risk of reported cases of campylobacteriosis in New Zealand, the disease produced by thermophilic *Campylobacter* spp., has increased to high levels (Baker et al. 2007). Lower incidence risks of diseases arising from non-typhoidal *Salmonella* spp. and *E. coli* O157 infections have also been recorded (ESR 2006). Consequently, concerted efforts instigated by the pork industry have been directed at increasing the safety of pork products destined for human consumption. A major impetus for this is an attempt to develop an export enterprise of pork products from New Zealand to Singapore. One objective of the pork industry, therefore, is to demonstrate that pork products originating from New Zealand have minimal likelihood of contamination, particularly with zoonotic microbes of major public health significance.

This literature review provides an overview of three main topic areas. The first focuses on risk determination, the second section provides an overview of the specific organisms, non-typhoidal *Salmonella* species (spp.), *Escherichia coli*, and thermophilic *Campylobacter*, while the final section reviews the food pathways that will be modelled in subsequent

chapters in this thesis.

2.2 Microbial risk assessment

Salmonellosis, campylobacteriosis and colibacillosis are zoonotic food-borne diseases produced after susceptible individuals consume sufficient quantities of the aetiological pathogens to result in infections and illnesses. Like all food-borne diseases, the risk of developing clinical symptoms following the consumption of the pernicious pathogen is made up of two components:
(1). the probability of developing an adverse health effect, and
(2). the severity or magnitude of that disease (Anonymous 1999). Consequently, multiple factors impact on the risk of contracting food-borne illnesses of microbial aetiology.

Microbial risk assessments (MRA) provide a scientific and systematic method of examining and evaluating disease risk factors. The output of this assessment is a risk estimate which presents a measure of both the likelihood and consequence of disease attributable to the investigated pathogen, for the population under consideration. Microbial risk assessments encompass a wide range of specialised fields including epidemiology, microbiology, virology, parasitology, mathematics and others, depending on the nature of the risk under investigation. The technique of risk assessment is not novel, with application in fields such as engineering (Holloway 1979) and environmental assessment for decades (Anonymous 1983). Only recently has risk assessment been applied to microbial food safety (Ahl 1996).

Risk assessment (RA) forms part of risk analysis. The latter is currently used in food safety to evaluate microbial hazards. Risk analysis is the term given to a multipart process incorporating hazard identification, risk assessment, risk management and risk communication (Figure 2.1) (Anonymous 2004*a*, Ahl 1996). Risk analyses may be quantitative, semi-quantitative or qualitative. Quantitative risk analyses produce numerical results as an output to describe risks and an indication of uncertainties, while qualitative risk analyses rank risks into separate distinct descriptive categories, such as low, medium and high risks. Semi-quantitative risk assessments predict risk estimates based on a mixture of qualitative and quantitative data. It has been stated that semi-quantitative risk analyses have been associated with inconsistent, anomalous and inappropriate out-

comes (Anonymous 2004*c*). Further, they may insufficiently differentiate between risks. Quantitative risk analyses may be difficult to execute under circumstances of limited data, time, knowledge and expertise. In such instances, qualitative analyses may be undertaken. Table 2.1 presents some of the advantages and disadvantages of the three approaches.

Emblemsvag & Kjolstad (2006) cite that a disadvantage of qualitative risk analyses is that widely different results can be obtained on the same assessment, depending on the approach used. One reason for this is the subjective nature of the method. This author suggests that mathematical analysis should be incorporated to reduce subjectivity and increase accuracy of the risk estimates. Cox et al. (2005) conclude that qualitative risk rating systems function well when risks can be clustered into groups, where for instance, components in one group have a higher risk than components in another group. When such clustering fails to exist, the qualitative system performs poorly. These authors further suggest that a simple quantitative assessment is preferred to a qualitative RA as the qualitative risk ranking approach insufficiently distinguishes between quantitatively small and large risks.

Table 2.1: Overview of different analytical approaches for conducting risk analyses.

Model Type	Advantages	Disadvantages
Qualitative risk analysis	Least complex to perform. Gives a general understanding of risk Applicable in the absence of limited data Good initial screening activity	Can introduce subjectivity into analysis
Semi-quantitative risk analysis	Intermediate in complexity Least commonly used	Has been associated with inconsistent inappropriate outcomes and poor differentiation of risk
Quantitative risk analysis	Minimises subjectivity in analysis Can give good indication of risk	Quality of analysis dependent on data accuracy Most complex to perform

2.3 Steps involved in risk analysis

The Organisation International des Epizooties (OIE), also known as the World Organisation for Animal Health, which is concerned with the animal health field, imports concerning animals and animal products defines risk analysis as the four steps of: hazard identification, risk assessment, risk management and risk communication. This system is

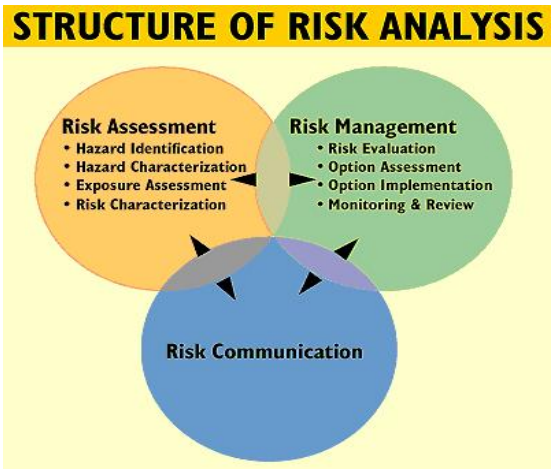


Figure 2.1: Codex Alimentarius Commission schematic for Risk Analysis.Diagram illustrates the interaction between each of the three main compartments comprising Risk Analysis. Figure taken from: Risk Management and Food Safety: Report of a Joint FAO/WHO Consultation, Rome, Italy, 27 to 31 January 1997

based on the Covello and Merkhofer system (Anonymous 2004*a*).

Alternatively, hazard identification may form part of risk assessment, resulting in only three main steps in the risk analysis process — risk assessment, risk management and risk communication (Figure 2.1). This is used by the National Academy of Sciences, National Research Council (NAS-NRC) and has been adopted by the Codex Alimenatarius Commission (CAC), the branch of the Food and Agriculture Organization of the United Nations responsible for development of international food standards. Its use is specifically for microbial food safety risk assessment and consequently will be elaborated on in this thesis.

The first and critical step in conducting a risk analysis, be it by the OIE or CAC classification is to identify a hazard(s). Without identification of a hazard, no risk analysis can be conducted. In MRA, hazard identification is predominantly a qualitative process (Anonymous 1999) which involves identification of a potentially pernicious biological agent. Generally, hazards can refer to physical, chemical and biological agents capable of producing ill-health among individuals (Rocourt et al. 2001, Lammerding 1997). For the purpose of this thesis, only microbial hazards are of interest. Hazards may be identified

from relevant data sources, scientific literature, government agencies and expert opinion.

After identification of the microbial hazard, a risk profile is sometimes conducted. Risk profiling is the process whereby contextual and background information on the food/hazard combination is collated in order to provide decision-makers (e.g. risk managers) with sufficient scientific data to determine whether resources should be allocated to a more detailed scientific study in the form of a risk assessment. The information gathered in the risk profiling may also result in immediate risk management action or the implementation of a programme(s) for further data collection. Information for the ranking of food safety issues is achieved by risk ranking. The scope of a risk profile is dependent on the food safety issue and specific requirements set by risk managers (Anonymous 2002*b*). A profile may include information on the hazard such as: a description of the human—pathogen relationship; details on routes of human exposure and adverse effects on human health, along with other information (Anonymous 1999). On the basis of a risk profile, risk managers decide whether or not a full risk assessment is to be conducted. Pointon et al. (2006) present a comprehensive risk profile of the red meat industry in Australia. In New Zealand, risk profiles have been prepared for *Bacillus* spp. in rice, *Listeria monocytogenes* in ice cream and *Campylobacter* in poultry, among others (NZFSA 2006).

Assuming that the risk manager(s) has agreed to proceed with further scientific investigations, a risk assessment is conducted. The scope is usually determined by the risk managers (Rocourt et al. 2001). Risk assessments can be either quantitative or qualitative. Qualitative RAs are conducted when there is a lack of understanding of the biology of hazard/exposure or insufficient data. Risks are ranked and separated into distinct ordinal categories. A short communication by (Coburn et al. 2005) presented the results of a qualitative risk assessment to determine the risk to human health from handling/consuming wild game in the United Kingdom (UK). The results were categorised into one of four categories of increasing risk: negligible, very low, low and non-negligible. Unfortunately, these do not differentiate between medium and high risk. Despite this, a qualitative assessment was conducted according to the principles of the Codex Alimentarius Commission (Anonymous 2002*b*). Published and unpublished data, as well as expert opinion were used to complete the RA.

Palmer et al. (2005) presented five qualitative RAs of emerging animal diseases — porcine circovirus, porcine hepatitis E, bovine norovirus, borna diseases virus and *Clostrid-*

ium difficile, to determine their zoonotic potential in the UK. The result of the RA for porcine circovirus could have been biased as results were taken from studies with very small sample sizes (50 persons in the United States of America) and therefore lacked statistical power. The shortcoming was however noted by the author. This article systematically explained and demonstrated the steps involved in conducting a qualitative RA. Results were biologically plausible and internally valid. Nevertheless, the external validity of the RA to other populations may be questionable, as different strains of viruses and bacteria existing outside of the UK can possess different characteristics which may influence their zoonotic potential.

Quantitative RAs provide numerical results as an output for describing risks and an indication of uncertainties (Hathaway 1999). They are therefore considered less subjective than qualitative RAs. However, their use is limited to availability of the necessary data, information and resources (Lammerding & Fazil 2000). Before commencing a risk assessment, according to the CAC structure, an unambiguous statement of purpose outlining the goals, scope, limitations and form of output of the risk assessment should be provided.

Microbial Risk Assessments, which follow the CAC classification, estimate the consequences from exposure to an infectious pathogen(s) (Haas et al. 1999) in a four step process elaborated below (Anonymous 1999, Ahl 1996):

1. Hazard identification: As previously discussed, this is predominantly a qualitative process (Anonymous, 1999) which requires identification of the potentially pernicious biological agent. In MRA, a hazard is a biological agent, with the potential to produce an adverse effect in an individual (Lammerding & Fazil 2000). For example, the identified hazards in this thesis are non-typhoidal *Salmonella* species, thermophilic *Campylobacter* spp. and *Escherichia coli*, specifically verocytotoxic *Escherichia coli* O157.

2. Exposure assessment: A qualitative and/or quantitative evaluation determines the likelihood that an individual/population is exposed to or ingests the identified hazard. Clough et al. (2006) conducted a qualitative exposure assessment of *E. coli* O157 in pasteurised milk at the point of retail in the UK, describing possible likelihood of exposure to the population in the UK. If a quantitative exposure assessment is conducted simulation modelling and data collection may predominate this step (Rocourt et al. 2001). All assumptions, sources of data and uncertainties must be clearly stated and identified

(Anonymous 1999). In summary, this step evaluates the degree of exposure/intake likely to occur by an individual in the population under consideration (Cassin et al. 1998). Exposure assessment modelling techniques are fast developing to account for pathogen dynamics in food at different temperatures and under different conditions. A recent article by Bahk & Todd (2007) discusses this and also proposes a model to incorporate estimations of the frequency of food consumption in the exposure models, if the data are available in the country. These authors use a stochastic model to estimate a parametric distribution describing the frequency of consumption of the food that is under investigation in the exposure assessment. This would be an asset to exposure models. However, if their model instead output both parametric and non-parametric distributions, more precise representations of the eating patterns of the population could be predicted. Whereas if this model proposed by the authors should be beneficial, the data needed to inform their model may be unavailable in many countries, making model use limited.

3. Hazard characterisation: This may be a qualitative and/or quantitative evaluation of the nature of adverse health effects associated with the hazard (Rocourt et al. 2001). This step estimates the magnitude of illness for an individual or population, based on exposure to the pathogen(s). Dose-response relationships can be used to determine these outcomes (Downes & Ito 2001). This should take into account microbe virulence, infectivity, interaction with the host, the pathogenesis of disease as well as host factors such as age, genetic factors, physiological status and the food matrix. Alternatively, a ranking system may be used to characterise severity and or duration of disease based on expert opinion (Anonymous 1999). McLauchlin et al. (2004) reviewed the hazard characterisation of *Listeria monocytogenes*. The paper discussed the adverse effects of this organism in humans, transmission, mechanisms of the disease and also presented incidence data from England and Wales stratified by age and risk groups. The authors stated that at least five different mathematical models have been employed to represent the dose-response relationship. However, it was concluded that despite several attempts to elucidate the dose-response relationship, it is still poorly understood.

4. Risk characterisation: This is the qualitative and/or quantitative determination or estimation of the probability of occurrence and severity of the investigated adverse effect in a given population, including uncertainties, using the above listed steps (Rocourt et al. 2001). The information from the above steps is synthesised to produce a risk es-

timate. For example, Uyttendaele et al. (2006) conducted a quantitative risk assessment of *Campylobacter* in poultry in Belgium. These authors determined a risk estimate or mean probability of illness of 784×10^{-4}/portion of 100g of chicken consumed. Risk estimates are highly influenced by the quantity and accuracy of information input into previous steps (Hathaway 1999). The degree of confidence in the risk estimate is dependent on the assumptions made, as well as the variability and uncertainty identified (Anonymous 1999).

Quantitative microbial risk assessments can identify gaps in available data and can be useful in identifying stages that are most influential in determining the risk of food-borne illness, as well as evaluating the efficacy of intervention strategies (Cassin et al. 1998). Risk assessments must be well documented and able to be updated in light of new data (Ahl 1996).

Eleven basic principles of MRA have been described by the Codex Alimentarius Commission are as follows:

1. MRA should be soundly based upon science.

2.There should be a functional separation between risk assessment and risk management.

3. MRA should be conducted according to a structured approach that includes hazard identification, hazard characterisation, exposure assessment, and risk characterization.

4. A MRA should clearly state the purpose of the exercise, including the form of risk estimate that will be the output.

5. The conduct of a MRA should be transparent (that is all the information sources should be given, and processes stated).

6. Any constraints that impact on the risk assessment such as cost, resources or time, should be identified and their possible consequences described.

7. The risk estimate should contain a description of uncertainty and where the uncertainty arose during the risk assessment process.

8. Data should be such that uncertainty in the risk estimate can be determined; as far as possible, data and data collection systems should be of sufficient quality and precision that uncertainty in the risk estimate is minimised.

9. A MRA should explicitly consider the dynamics of microbiological growth, survival, and death in foods and the complexity of the interaction (including sequelae) be-

tween human and agent following consumption as well as the potential for further spread.

10. Wherever possible, risk estimates should be reassessed over time by comparison with independent human illness data.

11. A MRA may need re-evaluation, as new relevant information becomes available (Anonymous 1999).

Peer review and feedback of the risk analysis is encouraged and although not listed as a step, is an important part of the process.

Risk management identifies, selects and implements measures that can be applied to reduce the level of risk (Anonymous 2004*a*). It therefore synthesises the information provided by the risk assessment to influence policy decisions and implement appropriate control and regulatory options (Anonymous 1999). Ideally, this process should be a pragmatic decision-making process that attempts to minimize risk. Risk communication is the exchange of information among the risk assessors, risk mangers, consumers and all other interested or concerned parties (Hathaway 1999).

In general, risk analysis in the food safety context facilitates a pro-active approach, enables addressing the entire farm to fork continuum instead of only end product testing, is systematic, structured and science-based. It is an iterative, on-going process in which modifications are undertaken as new data become available and when further evaluations are needed. The process should be transparent, well documented and peer-reviewed. Regular and frequent communication between risk assessors, risk managers, and stakeholders should form an integral part of risk analysis (FAO/WHO 2005).

2.4 Quantitative microbial risk assessment

This section provides an overview of basic principles, model types, simulations, sampling techniques, parameter estimation and sensitivity analyses used in quantitative microbial risk assessments (QMRA).

2.4.1 Model types

An overview of mathematical models more commonly used in exposure assessments of quantitative microbial risk assessments is provided in this section. There is a range of modelling types available (May 1974) and Table 2.2 provides a summary of those dis-

cussed in this thesis. It should be noted that a single model may be a combination of model types. Therefore the categories discussed here are not mutually exclusive.

Mathematical models

Mathematical modelling in biology uses mathematics to provide a simplified representation of an existing system. Accordingly, models facilitate relatively quick and cost efficient calculation, evaluation and predictions of a system's behaviour and allow the dynamics of biological functions to be quantified. However, the extent to which models reproduce the behaviour of the original system is dependent on and limited to the quality of the data available, the accuracy and detail of the model structure and the current knowledge. Mathematical models allow comprehension of the system under investigation (Brown & Rothery 1993, Neelamkavil 1987). Uyttendaele et al. (2006) describe a mathematical model for the propagation of *Campylobacter* in poultry-based meat preparations from retail to consumption and the probability of infection and illness. The modelling process enabled the authors to clearly identify the food pathway under investigation (from retail to consumption), pathogen dynamics and pathogen—host dynamics that required modelling and the order in which modelling was to occur. Parameter values and factors influencing the model were also identified. This process of developing and executing a risk mathematical model therefore requires in—depth knowledge of the investigated system which can be acquired during model building. A criticism of this paper is that a sensitivity analysis was not conducted. This would have facilitated identification of factors/parameters which are most influential in determining the model output, leading to further comprehension of the modelled system.

Models comprise input parameters or constants. Some models incorporate values (variables) that are calculated and therefore change during the model execution. Simple mathematic models are represented by equations calculating the magnitude of change of one variable in terms of others. Models can be categorised as dynamic, which describe processes unfolding in time and non-dynamic, which do not incorporate time. In the former category of dynamic models, modelled processes can occur in continuous-time or discrete-time. Continuous time models, as the name suggests, considers time to be a continuous variable. It therefore describes processes which can be measured at any moment, employing differential equations to express instantaneous rates of change. Discrete-time

processes on the other hand, are used to model events occurring at equally spaced intervals and are described using difference equations (Adler 2005, Bossel 1994, Brown & Rothery 1993, Hurd & Kaneene 1993). In this thesis both continuous and discrete time processes are modelled.

Deterministic and stochastic

Mathematical models can be classified as deterministic or stochastic. Deterministic models produce point estimate output values and fail to incorporate any element of chance or have no randomly varying component. Therefore, deterministic models predict the same output for a given set of initial conditions. Variable states are described by fixed point values. Stochastic models, on the other hand, incorporate chance and include randomly varying components. As such, every calculation or determination of the model output can produce a different outcome for the same starting values (Matthews & Woolhouse 2005, Brown & Rothery 1993). Stochastic is derived from the Greek word, *stokhastikos*, meaning "capable of guessing". Stochastic models enable integration of random variation, an integral component of many biological systems, into models. Also variable states are described by probability distributions. Deterministic models usually focus on "worst-case" scenarios. However, these extreme values fail to consider the range of probabilities for the occurrence of an investigated event. But, if average values are considered, the extremes are disregarded, and in certain diseases the extremes may represent the high risk portion of the population (Downes & Ito 2001, Brown & Rothery 1993). Deterministic models are therefore limited in their ability to report a range of values over which the event occurs. Nevertheless, they are valid when the population under consideration is large (Ball & Neal 2002, French et al. 1999).

Stochastic models are applicable when smaller populations are used. In these models the influence of chance is explicitly modelled producing different results for each computation of the model. Model outputs can be reported as distributions (Matthews & Woolhouse 2005, Bossel 1994). Armstrong & Haas (2007) utilised a stochastic model to conduct a quantitative risk assessment model for Legionnaires' disease, more specifically, for human exposures at selected spas in The Netherlands. The model structure was well described, assumptions were clearly stated and the exposure assessment risk model output predicted distributions of bacteria per unit area (cfu/m^3), with the arithmetic means,

5^{th} and 95^{th} percentiles reported. However, the model results are to be interpreted with caution, as one of the limitations of the model was the unavailability of relevant data.

Stochastic models take into account the entire distribution of possible values, incorporating both uncertainty and variability. Uncertainty is a lack of knowledge about the modelled system, be it from errors with respect to the parameters, or simplification of realistic processes, which can be reduced by further measurements or data collection (Lindqvist et al. 2002). Model uncertainty may occur as a result of over simplification of modelled processes and the use of inappropriate or unknown variables, while parameter uncertainty can be a consequence of measurement, sampling and/or systematic errors. Variability represents the true heterogeneity of population which is irreducible by further experiments (Vose 2000). Both variability and uncertainty can be described using probability distributions. The separation of variability and uncertainty model parameters is critical in producing an accurate risk estimate (Nauta 2000). This will be elaborated on later in this chapter. A summary of the advantages and disadvantages of these modelling techniques is presented in Table 2.2.

Mechanistic models

Mechanistic models, also known as white box models or explanatory models are derived from a theoretical basis or prior knowledge of the modelled phenomena and equations that can describe the processes involved (Estrada-Flores et al. 2006). These models require an understanding of the processes governing the behaviour of the modelled system. They therefore are constructed to depict or closely resemble realistic scenarios. Mechanistic models can be mathematically complex and usually comprise a series of equations (usually differential equations) which need to be solved either analytically or numerically. Input data for the model are independently measured (Estrada-Flores et al. 2006). Generally, model parameters, of which there are usually many, are obtained either directly from calculated or inferred measurements, or indirectly by estimating data. These models may be deterministic or stochastic, and in the case of the latter, variability and uncertainty can be described (Bossel 1994, Young & Lees 1993).

There are several advantages of mechanistic models. Firstly, they can be used when studying complex phenomena over time. They facilitate the mathematical incorporation of biological and management practices, allow up-dating, and can be used to evaluate

the effect of intervention strategies (Snary et al. 2002, de Jong 1995). These models however, may result in the oversimplification of scenarios that they are meant to depict. Also, model development is time-consuming and limited to the knowledge of the model builder (Young & Lees 1993). Mechanistic models provide mathematical descriptions of biological processes, thereby enabling the prediction of fairly accurate risk estimates. Further, parameter values used in these models have physiological meaning and can be measured by experimentation (Crump 1994). A mechanistic model was used by Nauta, van der Fels-Klerx & Havelaar (2005) to perform a quantitative exposure risk assessment of *Campylobacter* in poultry in The Netherlands. The model mimicked the dynamics and propagation of *Campylobacter* on the surface of chickens throughout the different abattoir stages. This model was used not only to produce an estimate of the probability of infection from consuming chicken, but also to evaluate intervention strategies targeted at reducing the pathogen load in poultry, so as reduce the incidence risk of food-borne disease (Nauta, Jacobs-Reitsma, Evers, Pelt & Havelaar 2005). The model equations were complex, and a large number of parameters were required to inform the model. For some parameters, no data was available, so assumptions were made and expert opinion solicited for parameter estimation. Further, the model equations failed to take into consideration the time for chicken processing events to occur. Despite these shortcomings, and with some modifications, the model presented is a good template on which other exposure assessment models can be based.

Black box models

Black box models are designed without any knowledge of the dynamics of the modelled system, and are not necessarily based on theoretical principles. These models estimate functional forms between inputs and outputs. Black box models are usually employed when mechanisms describing systems are unknown, poorly understood or highly complex, such as in neural networks[1] (Geeraerd et al. 1998). These models are generally stochastic, contain fewer parameters than white box models and are estimated directly from empirical data using statistical methods (Young, 1993). Geeraerd et al. (1998) used black box models to describe bacterial growth in chilled food products, accounting for

[1]Artificial neural network are non-linear statistical models consisting of a composite of functions describing the relationship between inputs and outputs.

temperature, pH and salt percentage. This knowledge can be used in quantitative risk assessments of foods for which chilling is required. Empirical models have been described as black box models (Anonymous 2002*a*). They are developed from 'real world' data and are used to predict an output, not explain the underlying dynamics of a system. These models generally consist of a mathematical function that sufficiently describes a biologically plausible relationship between model inputs and outputs. Data provide the basis for parameter estimation in empirical models. These models are easier to develop and implement than white box models and have been used in determining dose-response models for assessing the range of response behaviours of humans exposed to pathogens. Some empirical model types include logistic, Weibull and exponential (Chenlo et al. 2006, Eits et al. 2005, Fernandez et al. 2002). Strachan et al. (2005) used empirical models to combine data from outbreaks of *E. coli* O157 in the USA, UK and Japan with experimental dose-response data, to determine the most appropriate dose-response model. The beta-Poisson model was found to produce the best fit, describing the relationship between dependent and independent variables. This information can be used in the conduction of quantitative microbial risk assessments for *E. coli* O157.

Compartmental models

Compartmental models sub-divide the system to be modelled into discrete sections, states or compartments, defining different population qualities (Matthews & Woolhouse 2005). These models describe the transfer of an entity between compartments usually with the use of ordinary differential equations (Young 1993). They are frequently employed in modelling the transmission of infectious diseases and can be either stochastic or deterministic (Massad et al. 2005, French et al. 1999, Matis & Wehrly 1979). For example, Morgan et al. (2006) assessed foot-and-mouth disease transmission in Saiga antelope in Kazakhstan using a compartmental model. The disease status of the population investigated was divided into the categories of susceptible, latent infected, infected and recovered. The model was therefore referred to as a SLIR model. Compartmentalisation of the model resulted in simplification of the modelled processes. Transmission of the virus through the population was modelled to predict the occurrence of an epidemic of a persistently infected antelope population. The model predicted that once the total number of susceptible animals was less than 1.5 million, and the transmission parameter or force

of infection was set at a medium value, epidemics were predicted to only occur in autumn. If, on the other hand the susceptible population and force of infection were both high, with the former exceeding 1.5 million, cyclic epidemics could occur at any time. Finally if population size is small, no epidemics were expected to occur unless the force of infection is very high. With respect to QMRA, compartmental models can describe the transmission of the microbe(s) under investigation at the farm level and sometimes at the lairage stage of the slaughter house.

Table 2.2: Overview of model types, applications, advantages and disadvantages used in microbial quantitative assessments.

Model Type	Applications	Advantages	Disadvantages/Limitations	Examples from literature
Deterministic	Large populations	Applicable for use on large populations	Not appropriate for small populations	(Hurd et al. 2004) (French et al. 1999)
Stochastic	Small populations	Incorporates element of chance in models. Output incorporates range of distributions of values	Not applicable for use in large populations	(French et al. 1999) (Nauta 2001) (Armstrong & Haas 2007, Barker et al. 2002)
Mechanistic	Used when studying complex phenomena over time	Allows updating; identifies intervention strategies; incorporates biological and management practices; based on fundamental principles	Mathematically complex	(Crump 1994) (Estrada-Flores et al. 2006)
Black box	Applicable for dose-response models; used to predict system behaviour	Simple in structure, stochastic, less parameters than mechanistic models; understanding underlying mechanisms is not essential	Does not explain system dynamics	(Geeraerd et al. 1998) (McLauchlin et al. 2004) (Buchanan et al. 2000)
Compartmental	Describes the transfer of entities between discrete sections or compartments in the model	Allows simplification of processes	Analytical solutions can require tedious calculations	(Matthews & Woolhouse 2005) (Massad et al. 2005) (Morgan et al. 2006)

2.5 Simulation modelling

Simulation is a technique that calculates a model output multiple times with different input parameters. The aim of this technique is to get a complete range of all possible scenarios. Simulation modelling is usually executed using computer programs designed to facilitate multiple simulations of a mathematical model for particular starting values. The course of development of the model is therefore mimicked on the computer and model results collated for a predetermined number of times, specified by the number of simulations. A single simulation consists of one to any number of iterations. An iteration is one recalculation of the model within a simulation. The result of a single simulation incorporates all the outputs of every iteration within that simulation. Deterministic models require one simulation because the output for a given set of initial input values is always the same. However multiple simulations are preferred in stochastic models, so as to derive a series of potential distributions reflecting the random variation in the inputs. Simulations facilitate investigation of a system in a relatively short space of time. Processes occurring over extended periods of time, such as months or years, can be simulated within minutes, hours or days. New concepts and systems can be tested prior to implementation using this technique. Their results can assist policy makers and provide insight into the dynamics of the investigated systems (Chung 2004). However, there are limitations to this tool. Inadequate and inaccurate data can produce inaccurate results in any type of modelling, and simulation modelling is no exception. Secondly, model development can be time consuming and acquiring accurate data to inform the model may be costly (Chung 2004).

Monte Carlo simulation

Monte Carlo simulation is the random sampling of each input probability distribution in the model for each iteration of a simulation model. All probability distributions of variables are sampled per iteration, so as to reproduce their shape. The model outcome therefore accounts for the range of possible model inputs and weights each possible selection by the probability of its occurrence. At present, this simulation technique is widely used in QMRA modelling and has several strengths. It enables correlation and inter-dependence to be modelled, allows for quick and easy up-dating of the model and is readily accessible in proprietary software packages. However, the precision of Monte Carlo simulation is

directly proportional to the number of iterations performed in each simulation; so many iterations are required for precise results to be outputted. This can be time-consuming. Another disadvantage is that this technique performs poorly when modelling rare events (Vose 2000). Other methods of calculating outcome distributions for QMRA exist, such as method of moments and exact algebraic solutions. These are however, not as commonly used as Monte Carlo simulations. Monte Carlo simulations have been used in risk assessments for *Bacillus cereus*, *Staphylococcus aureus*, human salmonellosis from turkey consumption and *Salmonella* Typhimurium DT104 from pork sausages, among others (Bemrah et al. 2003, Nauta et al. 2003, Alban et al. 2002, Lindqvist et al. 2002).

2.5.1 Simulation sampling

There are several different methods of sampling when using Monte Carlo simulations, but only two of the more commonly used methods will be discussed.

Monte Carlo sampling

Monte Carlo sampling is a random sampling method used to select samples from input probability distributions in simulations. The random sampling method, ironically, is both this method's strength and weakness, as it sometimes permits over and under sampling of various sections of the probability distributions. This concern is addressed in Latin Hypercube sampling (LHS) (Vose 2000).

Latin hypercube sampling

Latin Hypercube sampling sub-divides the probability distribution into many unequally sized sections, equivalent to the number of iterations within the simulation. Sections in the tail regions of the distribution are wider than those at the distribution peaks. Each section is sampled only once, thereby enabling the shape of the samples selected to represent that of the input distribution (Vose 2000). A summary of the sampling types discussed is described in Table 2.3.

Table 2.3: Overview of sampling Model types used in microbial quantitative risk assessments.

Model Type	Advantages	Disadvantages
Monte Carlo sampling	Random sampling; Good for large number of iterations.	At small iteration numbers, over and under sampling of sections within distributions may occur
Latin Hypercube sampling	Good for small numbers of iterations; Samples selected from the distribution are representative of the input distribution	Less useful with large iteration numbers

2.6 Sensitivity analyses

Generally, models are built using current prevailing knowledge. They are not always meant to reproduce every realistic scenario, but increase the understanding of the processes under evaluation and the factors influencing them. Sensitivity analyses of models are mathematical techniques designed to identify the parameters most influential to the model output and therefore the investigated processes (Anonymous 2004*b*). If a model is highly sensitive to a parameter in which there is some degree of concern about its accuracy, this indicates that more information is needed on the parameter to increase the model's robustness and practical value. Generally, knowledge of key inputs describing variability in the model can help identify efficient control measures, while knowledge of key inputs describing uncertainty highlights areas that require further data collection. Sensitivity analyses can therefore be of great assistance to risk managers, responsible for the development of targeted control strategies (Frey & Patil 2002). Furthermore, these analyses can play important roles in model verification (Fraedrich & Goldberg 2000, Saltelli & Scott 2000). Model verification ensures that the model is being executed as planned. Model validation, on the other hand, is the process whereby the model results are compared to independent observations obtained from the system being modelled. Complete validation may not always be possible in risk assessment studies due to data insufficiency (Frey & Patil 2002). This section provides a brief overview of three methods for conducting sensitivity analyses in food-safety risk assessment models.

Methods for conducting sensitivity analyses can be classified as mathematical or statistical. Mathematical analyses assess the model output with a range of variations of input values. These methods fail to address the variance in the output as a result of the variance in the input values. One example of this method is the nominal range sensitivity analysis.

Statistical methods incorporate simulations. Inputs are assigned probability distributions and the effect of the input variance on the output variance can be determined (Andersson et al. 2000, Neter et al. 1996). Statistical methods used for conducting sensitivity analyses include regression analysis and correlation analysis among others. Graphical illustrations, such as scatter-plots and tornado plots provide a visual indication of the effect of the input parameter on the output (Geldermann & Rentz 2001, Stiber et al. 1999). Frey & Patil (2002) discuss various statistical methods for conducting sensitivity analyses, however, with respect to quantitative microbial risk assessments, only correlation and scatterplots are widely used (Perez-Rodriguez et al. 2007).

The nominal range sensitivity analysis is a simple mathematical method, applicable to deterministic models, and therefore not for probabilistic analyses. Very few examples have been found in the literature where the technique has been employed in risk assessment. Dakins et al. (1994) used this technique when conducting an exposure assessment of flounder in a contaminated harbour. The effect of the predicted model output was assessed by varying the entire range of model inputs, one at a time. That is, one input was varied, while all others inputs kept fixed at their nominal values. The output of this sensitivity analysis was presented as the percentage change (both positive and negative) from the nominal solution. This technique is most appropriate in linear models (Frey & Patil 2002). Nonetheless, it is limited to deterministic linear models as it is unable to evaluate interactions between inputs (Cullen & Frey 1999).

Regression analysis as the name suggests, uses regression models to describe the relationship between variables and the predicted model output. If the regression coefficient of input values significantly differs from zero, then the model is sensitive to changes in the input value. The more significantly the coefficient of the input varies from zero, the greater the influence of the corresponding input value on the output value (Frey & Patil 2002). This method produces accurate results when the underlying assumptions of the regression model are met. For example, least squares regression assumes a straight line relationship between input and output variables, and also, that residuals are normally distributed. Regression analysis works well when there is independence of inputs (Neter et al. 1996). Mokhtari et al. (2006) used both correlation and regression techniques to conduct sensitivity analyses and compared these results with sensitivity analyses using classification and regression trees (CART) methodology. CART has previously been used

in the medical field for decision making analyses. Sensitivity analyses using the three techniques were conducted on a microbial food safety risk model of *E. coli* O157 in beef at a theoretical slaughter house. The results of the sensitivity analyses were different using each of the techniques, with CART showing results most similar to the regression analysis technique. The CART technique is available is some statistical software packages.

Correlation analysis is another type of statistical method employed in sensitivity analyses. Both partial rank correlation coefficients and Spearman's rank correlation used by Blower & Dowlatabadi (1994) and Armstrong & Haas (2007) respectively, evaluate the contribution of model inputs with respect to variation in selected model outputs (Brikes & Dodge 1993). The results of these analyses have no units and range from -1 to 1. Weak predictive inputs produce values close to zero (Cassin et al. 1998) and correspondingly, results closer to -1 or 1 represent inputs with more influence on the output (Zwietering & van Gerwen 2000). Negative values represent inverse correlation (Vose 2000). The correlations measured are between the variability of the inputs and outputs. The rank order correlation technique is fast, easy to calculate and has been cited as the preferred method to multivariate stepwise regression by the Organisation International des Epizooties (Anonymous 2004*b*, Vose 2000). Armstrong & Haas (2007) used a rank order correlation coefficient to conduct a sensitivity analysis of the quantitative microbial risk assessment of Legionnaire's disease at selected spa pools. Of the four spa pools assessed, inhalation rate of the pathogen was determined to have the greatest effect on the probability of having an outbreak of the disease.

2.7 Second order modelling

Second order modelling is the separation of variability and uncertainty, which allows a more accurate risk estimate to be produced (Nauta 2000, Vose 2000, Frey & Burmaster 1999). As previously stated, uncertainty refers to a lack of knowledge of data, that can be reduced by acquiring more pertinent data, whereas variability represents the true heterogeneity of the population that is irreducible and unaffected by more data. The incorporation of both uncertainty and variability in a single risk assessment distribution can result in a loss of information regarding the individual contributions of each of these two components. Furthermore, failure to separate the two can lead to difficulty in interpretation

of the output, or worse, meaningless outputs. If, for example, a variability distribution is erroneously used as an uncertainty distribution, the model output may be described as a distribution, when instead, it ought to be a point estimate. Vose (2000) identified two methods to separate uncertainty and variability. One calculates variability and simulates uncertainty, while the other simulates variability, selecting a random sample from the uncertainty distributions for each simulation. Nauta et al. (2003) separated the two by implementing Monte Carlo simulations first sampling from uncertainty distributions, and then sampling from variability distributions. This technique separately propagates uncertainty and variability in risk assessments by nesting multiple realisations of model parameters and iterations of input variables. The output is a collection of distribution functions describing the uncertainty and variability in the results (Wu & Tsang 2004). Second order Monte Carlo simulations (MCS) are also known as two-phase or two-dimensional MCS.

2.8 Parameter estimation

For parameter estimation, information pertaining to the parameter is sourced through the use of published or unpublished data, the conduction of studies targeted at obtaining relevant data or through the use of expert opinion. Meta-analyses can also be conducted to provide a summary measure of effect from published literature. Care should be taken when obtaining data from the literature to ensure that the information is relevant and that it was collected in an appropriate manner from an appropriate study design. Obtaining data from the literature and other available sources allows the acquisition of information that would otherwise be very costly and time-consuming to collect. Unpublished data sources may include government and or company registries. In the absence of both of the above, studies can be conducted to collect the needed data. In the case of microbial studies, data collection can be expensive, thereby limiting the quantity of information obtained. Yet another approach is through the use of expert opinion. This is considered a subjective approach that can potentially introduce bias in the model (Anonymous 2004*b*). However, there are structured protocols for obtaining information from expert opinion. Two documented procedures are the OIE recommended modified Delphi approach (Anonymous 2004*b*) and the European Communities Procedures guide for structured expert judgement (Van der Fels-Klerx et al. 2005, Cooke & Goossens 2000).

2.9 Modelling techniques

The following outlines some modelling techniques for QMRA. These include Bayesian belief networks, probabilistic scenario analysis (PSA), predictive microbiology, process risk models and modular process risk analysis (MPRM).

2.9.1 Bayesian belief network approach

One technique that does not incorporate the use of Monte Carlo simulations but is used to produce a risk estimate for QMRA is the Bayesian belief network (BBN) approach. Comparative analyses of this method and Monte Carlo simulation for one study, produced similar results (Nauta et al. 2003). The basics of Bayesian theory and BBN are briefly described.

Bayesian Networks, also known as Bayesian belief networks (BBN), belief networks, Bayesian nets or graphical probability models are models designed to determine values/distributions for uncertain variables. This technique is based on the Bayes's theorem described by Thomas Bayes in 1763 (Moore & McCabe 2003).

Bayesian theory differs from the "frequentist" theory. "Frequentists" calculate the probability of an event A occurring (denoted as $P(A)$) as the frequency of that event in relation to the total number of possible outcomes. However, if no information is present on the frequency of occurrence of A or the total number of outcomes, according to the frequentist approach, $P(A)$ cannot be calculated. Nonetheless, the Bayesian approach allows calculation of $P(A)$ under these conditions by using an individual's prior knowledge (K) of the probability of *A* (in the absence of data) which is denoted as $P(A|K)$, the probability of *A*, given *K*. This is a conditional probability, or an expression of a "belief measure". In Bayes' theorem, uncertainty is expressed as conditional probabilities and is mathematically written as:

$$P(A|B) = \frac{P(B|A)P(A)}{P(B)} = \frac{L(A|B)P(A)}{P(B)} \tag{2.1}$$

where $L(A|B)$ is the likelihood of *A* given *B*, for a fixed value of *B*. The probability $P(B)$ is known as the normalising constant, while $P(A|B)/P(B)$ is referred to as the standardised likelihood. The probability $P(A)$ is the prior probability and consequently the conditional probability $P(A|B)$ is the posterior probability.

The previous equation is therefore synonymous to:

$$\text{Posterior} = \text{Prior} \times \text{Standardised Likelihood.}$$

The posterior $P(A|K)$ in the previous example therefore can be calculated using the above equation.

Bayesian Belief Networks (BBN) are complex applications of Bayesian statistics (Jensen 1996, Spiegelhalter et al. 1993) which can be applied to risk-based food safety modelling (Nauta et al. 2003, Barker et al. 2002). A BBN consists of a probabilistic, directed, acyclic graph demonstrating relations between uncertain quantities. Each uncertain variable relevant to the problem or pathway under investigation is graphically depicted as nodes and lines indicating relationships (causal or influential) between nodes are arcs. Arcs represent and show the direction of a causal or influential relationship. Each node is described by a discrete probability distribution. The size of the variance of the distribution is dependent on the perceived certainty of the distribution. As such, more certain variables are described using narrower distributions than less certain variables.

Nodes without parents (prior nodes in the network) are assigned initial values or prior probabilities, while for nodes with parents, a table of conditional probabilities is generated that describes the relationship between input and output variables. Generally, probabilities are expressed as discrete distributions (Parsons et al. 2005). The network prior distributions comprise the joint probability distributions of all the nodes derived from the prior distributions of nodes without parents. The prior state of the model can be combined with evidence or information from experience or tests at the level of the node. Any data about nodes are included to produce posterior distributions. The model is executed quickly using appropriate software packages that combine probability calculus with Bayes' theorem to estimate posterior distributions (Barker et al. 2002). Barker (2005) described an exposure assessment of consumers to *Clostridium botulinum* neurotoxin in minimally processed potato product using a BBN. The outcome was a probability distribution representing beliefs of spore concentration in the potato product which was biologically plausible and in keeping with experimental observations. Model assumptions and limitations were stated. Derivation of distributions was through expert opinion as well as data. Sensitivity analyses were conducted. However, no mention of model validation

was made as well as the shortcomings of the BBN technique. Despite this, the paper was informative in explaining the underlying principles of BBN. Malakar et al. (2004) also used BBN to conduct an exposure assessment of *Bacillus cereus* spores in cooked chilled vegetable product. Expert opinion comprised a major part of the information used to determine prior probability distributions, but some data were also used. The output of this paper was comparable with that produced using Monte Carlo simulations (Nauta et al. 2003), thereby indicating that this technique can be used as an alternative to Monte Carlo simulations. Bayesian Belief Networks allow revising probabilities when the need arises. Another advantage of this method is that the visual representation shows the model developer's perception of relationships between different variables (Barker et al. 2002).

2.9.2 Probabilistic scenario analysis (PSA)

Probability scenario analysis (PSA) was initially used as a research tool on work involving atomic bombs (Hammersley & Handscomb 1964). Currently, in fields such as plant and animal health, it has been used to estimate the probability of occurrence of a hazard (Ahl 1996). For this analysis a hazard is first identified. Then the systematic development of a model pathway describing scenarios that may terminate in occurrence of the hazard is created. Each step of the pathway is called a node. The likelihood of the occurrence of each node is determined qualitatively or quantitatively. Data from expert opinion and epidemiological studies may be used to determine these probabilities (Ahl 1996). One strength of this technique is that it facilitates up-dating in the event of new information from further studies (Ahl 1996). Analyses using PSA methodology were employed to assess disease transmission by bovine embryo transfer (Sutmoller 1996), as well as the medical practitioner's role in preventative medicine (Doran et al. 2004). Peeler et al. (2006) used this technique to conduct a qualitative risk assessment on the spread of the parasite *Gyrodactylus salaris* with the movement of Atlantic salmon. Scenario analysis allowed the steps in the model to be clearly identified and visualised. The author presented a description of the hazard, the pathways necessary for introduction of the parasite into non-infected areas and conducted an exposure assessment. One strong point of this paper is that it clearly tabulates the risk factors affecting the introduction and establishment of the parasite at every stage in the scenario analysis which can be targeted in the design of effective control programmes.

2.9.3 Process risk model

Process risk models (PRM) integrate scenario analysis and predictive microbiology (Cassin et al. 1998). Predictive microbiology is used to describe the dynamics of a pathogen through the different stages of the food-pathway, while scenario analysis describes the pathways resulting in food-borne illness. Predictive microbiology has been defined as "*a detailed knowledge of microbial responses to environmental conditions enabling objective evaluation of the effect of processing, distribution and storage operations on microbiological food safety and quality of foods*" (McMeekin et al. 2002). Briefly, this technique uses mathematical models to predict the bacterial dynamics of growth and death/inactivation in foods, taking into account the environmental conditions of the microbe, such as temperature, pH, and water activity, among others. Empirical, black box and mechanistic models can be used in this method (McMeekin et al. 2002). The PRM technique was developed to determine risks and evaluate possible intervening systems to mitigate these risks. Sensitivity analyses are conducted to identify the model parameters which contribute significantly to the risk of disease. It is these factors which are targeted in control strategies. To this end, hypothetical control strategies can be simulated to assess their efficiency. This technique however, fails to incorporate uncertainty and variability (Mokhtari et al. 2006, Delignette-Muller et al. 2006). Its limitation is resolved in the subsequent technique outlined, modular process risk assessment.

2.9.4 Modular process risk model

Modular process risk modelling (MPRM) is derived from process risk modelling and was first documented by Nauta (2001). It incorporates the advantages of process risk modelling, in that it is a structured approach that facilitates identification of different process stages on risk, as well as evaluation of control strategies. However, unlike PRM, it incorporates variability and uncertainty in the model. Furthermore, it can identify gaps in knowledge (Nauta 2002). These can be filled by designing experiments or gathering expert opinion or data from the literature. The aim of this modelling technique is to describe transmission of the hazard along the food pathway (Nauta 2001).

The MPRM technique sub-divides the food pathway of concern into different sections called modules in which occurring events called "processes" are explicitly modelled. Six

processes exist which describe the bacterial and food-handling dynamics of growth, inactivation, partitioning, removal, cross-contamination and mixing. The first two processes refer to bacterial dynamics, the latter, food handling. If the process occurring is too complex or unable to be described under the given processes or the parameters are unknown, a black box model may be used (Nauta 2001). For each module, the input-output relationship is of most concern, with the output consisting of probability distributions of the number of bacteria per unit of food product and the fraction of contaminated units, or the prevalence of bacteria in the food under evaluation. Since the outputs are both distributions of microbial number and prevalence, they incorporate uncertainty and variability, ideal for risk estimate determination. The risk model is stochastic and can be analysed using Monte Carlo simulations. MPRM uses mechanistic models as often as possible. Data to inform the model are collected after the model structure is developed. Gaps in the literature can be identified and overcome through the use of predictive microbiology mathematical models, targeted investigations (inclusive of observations, surveillance and experimental) and expert opinion.

Modular process risk models enable quantitative microbial risk assessments to be conducted even in the event of data scarcity (Nauta 2001). Nauta et al. (2003) used MPRM to conduct an exposure assessment of *B. cercus* in refrigerated processed foods of extended durability, in which the bacterial processes of growth and partitioning were explicitly modelled. The results from this paper were comparable with results from another paper in which the same model was assessed using Bayesian belief networks. Two strains of the bacteria were modelled — the mesophilic and psychotropic strains. The model outputs were distributions of estimated bacteria in vegetable puree prior to cooking. Assumptions were stated and although the model was not validated, it did provide an indication of the bacterial processes and conditions contributing to pathogen exposure. Existing data gaps were identified during model development. One weakness of this technique is that the primary production stage is not addressed (Lindqvist et al. 2002). Another drawback of this method is the assumption that a processing step can be assigned to a module. In some instances the dynamics of part of the modelled process may be too complex to be simplified into any of the six processes. In these situations processes are to be described using black box models (Lindqvist et al. 2002).

In New Zealand, a QMRA was conducted on *Salmonella* (NZFSA 2003) in sheep

meat and a risk profile was undertaken with respect to *Campylobacter* in poultry (Lake et al. 2003). Other risk profiles currently under development include *Campylobacter* in offal, *Campylobacter* in red meat and *Salmonella* and *Campylobacter* in the poultry food chain. At the start of this PhD program, there was no record of any QMRA under development for *Salmonella*, *Campylobacter* or *E. coli* in pork. However, a risk profile for *Campylobacter* and *E. coli* O157 in pork subsequently commenced and is currently under preparation (ESR 2005*b*).

2.10 Applications of risk analysis

In recent years, food safety has become an increasingly important issue worldwide. The reasons for this can be attributed to improved consumer awareness, coupled with the emergence and re-emergence of food-borne pathogens such as *E. coli* in Europe, USA, and Canada causing the deaths in young children and the elderly (Gerner-Smidt et al. 2005, Strachan et al. 2005, Ali 2004, Duffy & Garvey 2000), prions associated with the development of Creutzfeldt-Jakob disease in the UK, a disease for which there is no known cure (Trevitt & Singh 2003, Bradbury 2005) and dioxins in Belgium and Greece and other parts of Europe causing chronic disease, liver damage, neuralgias and has been associated with cancer formation (Fierens et al. 2003, Hayward & Bolger 2005, Papadopoulos et al. 2004).

International trade in food has resulted in the dissemination of pathogens, leading to an increased risk of contracting food-borne disease. As a result there are implications on the food industry and trade can negatively impacted. As such, the CAC, charged with the responsibility of protecting consumers and facilitating trade by promoting international standards for health protection and fair practices in food trade (Garrett et al. 1998) suggested that risk-based assessments be adopted to address this situation. Consequently, risk analysis has been and continues to be developed and has been implemented as the preferred method by which food safety is evaluated.

Risk analysis evolved within the CAC within the last twenty years but was enforced at the Uruguay Round Trade Agreement on the Application of Sanitary and Phytosanitary Measures (SPS) in 1995 (Rocourt et al. 2001). The agreement prohibits World Trade Organisation (WTO) member countries from enforcing unfair and unjustifiable measures

which restrict international trade. Therefore all Sanitary and Phytosanitary measures (measures to protect animal and plant life and health) implemented in member countries have to be scientifically justified (Hathaway 1999). Presently, risk analysis is considered an integral part of the decision-making process of CAC. It was also agreed that guidelines and standards established by the CAC be considered as benchmarks against which international measures are to be evaluated. The step towards harmonisation attempts to eliminate trade barriers between countries. One standard established by the CAC is the "Principles and Guidelines for the Conduct of Microbiological Risk Assessment" which was previously mentioned (Anonymous 1999).

Food safety management is highly influenced by the WTO SPS Agreement. Many governments including those in France, Denmark, The Netherlands, Belgium and USA, use risk-based analyses to inform strategies on programmes designed to establish food safety objectives and mitigate the incidence of food-borne diseases such as *E. coli* O157 and *Campylobacter* (Uyttendaele et al. 2006, Gorris 2005, Rosenquist et al. 2003, Hoornstra & Notermans 2001).

2.11 Risk profiles

This thesis focuses on three organisms, *Salmonella* (with special emphasis on *S.* Typhimurium), thermophilic *Campylobacter* and *E. coli*, particularly VTEC O157, all of which are the aetiological agents of three major zoonotic microbial food-borne diseases. The risk profiles of these organisms, with specific relation to pigs/pork products are presented in this section. Risk profiles as previously described present contextual and background information on the food/hazard combination. This information is collated in order to provide decision-makers (e.g. risk managers) and can result in any of three possible outcomes:
(1) conduction of a risk assessment (2) immediate risk management action or (3) further data collection.

2.11.1 Salmonella

Incidence

New Zealand reported approximately 39.3 cases of salmonellosis per 100,000 head of population during 2005 (ESR 2006). *Salmonella* enterica subspecies enterica serovar Typhimurium is of particular importance as this serotype was identified in more than half of the total number of *Salmonella* serovars isolated in 2005 (ESR, 2005). For this literature review, a risk profile of S. Typhimurium is conducted.

Pathogen of concern: *S.* Typhimurium

Salmonella Typhimurium is one of more than 2,500 serotypes (Smith-Palmer et al. 2003) in the genus *Salmonella* which forms part of the family *Enterobacteriaceae*. This genus contains important food-borne pathogens (Yan Steve et al. 2003). *Salmonella* Typhimurium is one of the most common non-typhoidal *Salmonella* serotypes responsible for food poisoning (Ruiz et al. 2003, Jeoffreys et al. 2001). It is known to persist and cause disease in a wide range of hosts including humans, companion animals, livestock, domestic fowl, rodents and birds (Rabsch et al. 2002).

During the last few decades, *S.* Typhimurium infections have been recognised as a major zoonotic food-borne hazard in most developed countries, with approximately 15% of human salmonellosis cases attributed to pork and pork product consumption (Beloeil et al. 2004, Biendo et al. 2003, Rabsch et al. 2001, Ang-Kucuker et al. 2000, Baggesen et al. 2000, Mhand et al. 1999, Anderson et al. 1977, Felix 1956). The routine isolation of *S.* Typhimurium from pigs worldwide has presented many opportunities for pork products to be contaminated with this organism (Bolton et al. 2003, Giovannacci et al. 2001, Fedorka-Cray et al. 1995).

Pathogen attributes

Salmonella Typhimurium are gram negative, flagellated, non-spore forming, facultative anaerobic bacilli, capable of reducing nitrates to nitrites, fermenting glucose and are oxidase negative (Yan Steve et al. 2003). These organisms possess two major antigens: H (or flagella) antigens and O (or somatic; i.e. cell-wall) antigens which occur on the surface of the outer membrane and are determined by specific sugar sequences on the cell surface.

The bacteria proliferate optimally at 37°C, however growth is seen at 54°C in food. These bacteria can catabolise *D*-glucose and other carbohydrates with the production of

acid and gas. They are oxidase and catalase negative, produce hydrogen sulphide, decarboxylase lysine and fail to hydrolyse urea. These traits form the basis of biochemical tests used in *Salmonella* identification. *Salmonella* isolates would therefore produce acid and hydrogen sulphide gas from glucose in triple sugar iron agar (TSI), produce an alkaline reaction from the decarboxylation of lysine, but fail to hydrolyse urea (D'Aoust 1997).

Long term survival of *Salmonella* in the environment for six years or more increases the likelihood of its passage to host species (Funk & Gebreyes 2004, Winfield & Groisman 2003). *Salmonella* Typhimurium are the most ubiquitous of all *Salmonella* spp. (Fedorka-Cray et al. 1994) proliferating in a pH range from 4.5 to 9.5, with an optimal pH ranging from 6.5 to 7.7. It survives well for prolonged periods of time in freezers and at ambient temperatures. Water activity of less than or equal to 0.93 does not support *Salmonella* growth. *Salmonella* are inhibited by 3 to 4% salt (NaCl), however salt tolerance increases within a range of 10 – 30°C (D'Aoust 1997).

Salmonella Typhimurium — a public health problem

Salmonellosis is induced by the ingestion of non-typhoidal *Salmonella* spp. such as *S.* Typhimurium, and presents in humans as a febrile gastroenteritis accompanied by abdominal cramps, 6 to 72 hours post-ingestion of sufficient quantities of the organism to cause an infection. High probabilities of infection resulting from exposure to 10^0 to 10^4 viable cells have been reported (D'Aoust 1997). In high risk groups, extra-gastrointestinal pathology may occur from haematogenous and or lymphatic dissemination, producing bacteraemias (seen in approximately 5% of individuals) and subsequent focal infections such as meningitis, septic arthritis, osteomyelitis, cholangitis, pneumonia, endovascular infection, deep bone or visceral abscesses. Death may also result from this infection (Hohmann 2001). *Salmonella*-induced chronic conditions include reactive arthritis, Reiter's syndrome, ankylosing spondylitis and chronic rheumatoid diseases. Arthropathies are thought to be linked to Class I HLA-B27 histocompatibility complex (D'Aoust 1997).

Susceptible populations

High risk groups include: the immuno-compromised; persons suffering from malignant conditions; individuals infected with the human immunodeficiency virus (HIV); diabetics; those undergoing corticosteroid therapy and immunotherapy; the very young

and very old; infants with gastric hypoacidity; persons taking H-2 blocker medication or afflicted with altered bowel flora as a result of surgery/antibiotic therapy and rheumatoid disorders. Reticulo-endothelial blockages and anatomical disruptions (such as kidney stones, urinary tract abnormalities, gallstones, atherosclerotic endovascular lesions and prosthetic devices) provide foci for persistent *S*. Typhimurium infection and individuals with these conditions have increased susceptibility to *S*. Typhimurium infection (Hohmann 2001).

Sources of infection for humans

Humans can be infected via pets (particularly reptiles and birds) and after the consumption of contaminated food such as eggs, cheese, pork, chocolate, fruits, juice, fresh vegetables, and water (Barber et al. 2002, Hohmann 2001, van Duijkeren & Houwers 2000). The organism can be isolated on dairy, beef, pig, poultry and fish/seafood farms, as well as slaughtering plants (Fedorka-Cray et al. 1994).

Salmonella Typhimurium in pigs

Sources of infection and farm risk factors

Swine are considered important carriers of the organism. *Salmonella* Typhimurium can be found in the pharynx, tongue, tonsils, ileum, caecum, mandibular, ileocaecal and superficial inguinal lymph nodes, liver, stomach contents and faeces (Hurd et al. 2001, Swanenburg et al. 2001, Fedorka-Cray et al. 1994). Pigs become infected through: the gastro-intestinal or respiratory route from contaminated feed and water; other pigs shedding the organism; cats; the inhalation of aerosols or dust; pests such as infected rodents, birds, wildlife species, beetles, flies and by contact with infected farm workers serving as vectors transporting the agent on their boots and tools or from the environment (Funk & Gebreyes 2004, Barber et al. 2002, Dahl et al. 1996). Vertical transmission has not been confirmed (Funk & Gebreyes 2004, Kranker et al. 2003).

Factors that increase the prevalence of *Salmonella* spp. in pig farms include feed type, stress, stocking densities and type of flooring. Doses of 5 x 10^6 colony forming units (cfu) of *S*. Typhimurium in feed lead to high probabilities of establishment of subclinical infections (*Merck Manual* 2005, Wingstrand et al. 1996). Wet feeding is associated with higher prevalence of *Salmonella* spp. compared with dry feeding. High stocking densities

increase the level of stress of pigs, thereby increasing shedding in carrier pigs, resulting in increased *Salmonella* spp. prevalence. Fenestrated flooring reduces the contact between faecal material and pigs, leading to reduced *Salmonella* spp. prevalence. High ambient temperatures and humidity favour increased numbers and prevalence with the farm (Barber et al. 2002).

Disease in pigs

Two types of disease are seen in swine. Both clinical and asymptomatic infections have been recognised with the latter being more important in the establishment of carrier states and intermittent shedding (Wingstrand et al., 1996; Fedorka-Cray et al., 1994). Clinical disease occurs post-weaning and manifests itself as an enterocolitis or septicaemia with sequelae of pneumonia, enterocolitis, meningo-encephalitis and hepatitis (Fedorka-Cray et al. 1994, Schwartz 1991).

Asymptomatic infections in swine progress to become carriers of *S.* Typhimurium, with intermittent shedding of the pathogen, thereby facilitating spread of the infection and providing a source of contamination in pork (Fedorka-Cray et al. 1994, Barber et al. 2002, Schwartz 1991). The frequency of shedding and the number of bacteria shed in the faeces reduces with age but can persist in low numbers for several weeks or months post clinical infection (Barber et al. 2002, Hume et al. 2001, Dahl et al. 1996, Fedorka-Cray et al. 1994, Wood et al. 1989). Wood (1989) demonstrated that the organism could be shed up to at least 28 weeks post-exposure. Since most pigs in New Zealand are slaughtered at approximately 22 weeks of age, if carrier pigs are present shedding is expected. *Salmonella* Typhimurium persists in several different anatomical sites of the chronic carrier pig including the caecum, colon, ileum, tonsil, mandibular and ileocolic lymph nodes and faeces (Wood & Rose 1992). Infection through the respiratory route can lead to the pathogen localising in the thymus, lung, liver, spleen and blood within 2 hours post infection (Loynachan et al. 2004). Once infected with *S.* Typhimurium, many pigs remain infected, with survivors shedding the pathogen intermittently in faeces (*Merck Manual* 2005).

Up to 30% of pigs may shed *Salmonella* spp. on the farm (Stärk et al. 2002). This percentage increases during transport and in lairage as there is increased stress of the pigs caused by crowding, and transport, leading to increased exposure of naive pigs (Kranker et al. 2003, Barber et al. 2002).

2.11.2 Campylobacter

Incidence

New Zealand has reported an alarmingly high incidence risk of *Campylobacter* infections of 395.3 cases per 100,000 population in 2005 (ESR 2006), with more cases reported in the summer than autumn and winter (Baker et al. 2007).

Pathogen of concern: thermophilic *Campylobacter*

Campylobacter jejuni and *Campylobacter coli* subsequently referred to as *C. jejuni*, and *C. coli* respectively, have been recognised as aetiological agents of gastroenteritis in humans since the 1970s (Murray et al. 2003). *Campylobacter* is reportedly the most common cause of human bacterial enteritis in developed countries (Guevremont et al. 2004) with *C. jejuni* reported as the most common enteric pathogen isolated from diarrhoeic patients in developed countries (Friedman et al. 2000). However, recent research suggests that *C. coli*, which was initially considered to be of minor human health importance, may in fact have a greater impact on the human health burden than previously believed (Tam et al. 2003).

These two thermophilic *Campylobacters* are primarily food-borne, however sporadic cases are known to occur (Guevremont et al. 2004, Pearce et al. 2003). There is a strong association between poultry consumption and campylobacteriosis (Arsenault et al. 2007). However, findings linking this disease to pork consumption are controversial (Adak et al. 2005, Guevremont et al. 2004, Nesbakken et al. 2003, Pearce et al. 2003) due to a reported large reduction in the numbers of *Campylobacter* after chilling at abattoirs (Chang et al. 2003*a*, Nesbakken et al. 2003, Duffy et al. 2001). Based on the reduction of bacterial numbers, some researchers believe that pork at retail does not convey a human risk (Yeh et al. 2005, Nesbakken et al. 2003, Kramer et al. 2000) while other researchers suggest that pork may still carry a risk to the consumers (Payot et al. 2004, Pearce et al. 2003).

Pathogen attributes

Campylobacter are part of the family Campylobacteraceae, with 20 species in the genus *Campylobacter* (Nachamkin 1997). These gram negative, motile, non-spore forming rods, are S-shaped, curved or can appear as spiral rods (Nachamkin 1997). They may

also form coccoid in older cultures or in cultures exposed to the air for extended/prolonged periods of time (Murray et al. 2003). *Campylobacter jejuni* and *Campylobacter coli* are thermophilic, microaerophilic bacteria proliferating at higher temperatures (42°C ideally), but usually at temperatures greater than 30°C (Payot et al. 2004, Young et al. 2000). They are also oxidase positive, fail to ferment glucose and are sensitive to drying, high oxygen environments, freezing, chilling, salinity and low pH (Altekruse et al. 1999, Nachamkin 1997).

Campylobacter — a public health problem

Campylobacteriosis ranges from asymptomatic to severe lower gastrointestinal illness. Symptoms may persist for over 1 week and include pyrexia, abdominal cramps and diarrhoea or dysentery. Infections are usually self-limiting but relapses occur in 5 – 10% of patients. Extra-intestinal infections include: toxic mega-colon, bacteraemias, hepatitis, cholecystitis, pancreatitis, nephritis, prostatitis, bursitis, endocarditis, erythema nodosum, neonatal sepsis, haemolytic uraemic syndrome, urinary tract infections, meningitis, abortion, septic arthritis and abscesses. Risk groups include the immuno-compromised. Death from campylobacteriosis is rare (Murray et al. 2003, Gillespie et al. 2002).

Sequelae include Guillain-Barre syndrome (GBS), reactive arthritis and Reiter's syndrome. Both syndromes are auto-immune disorders. It has been estimated that approximately 20% of affected individuals suffering from GBS suffer long-term disabilities, while an estimated 5% die (Downes & Ito 2001, Altekruse et al. 1999). Fewer than 10^3 cfu of *Campylobacter* organisms are thought to be capable of causing disease (Nachamkin 1997).

Source of infection for humans

People are exposed to these organisms mainly from food consumption and contact with infected pets (Aquino et al. 2002). *Campylobacter jejuni* and *C. coli* are zoonotic, with reservoirs of infection that include rabbits, rodents, wild birds, sheep, horses, cows, flies, pigs, poultry, shellfish and domestic pets (Adak et al. 2005, Nachamkin 1997). *Campylobacter coli* is predominantly found in pigs, while *Campylobacter jejuni* is more widespread (Borch et al. 1996). Excreta from infected animals may contaminate soil, water (Guan & Holley 2003) or vegetables and can serve as a source of infection (Nachamkin

1997). *Campylobacter* spp. have been isolated at low concentrations in rivers, poultry, (both cooked and raw), red meat and offal, raw milk, mushrooms, garlic butter, salads and shellfish in New Zealand (Lake et al. 2003).

Campylobacter in pigs

Pigs can be healthy carriers of *Campylobacter* spp. (Oosterom et al. 1983) which have been isolated from pig faeces in frequencies ranging from 0 – 100 % (Kwiatek & Zasadny 2006, Yeh et al. 2005, Nesbakken et al. 2003, Young et al. 1999, Harvey et al. 1999, Meng & Doyle 1998). *C. coli* is the predominant serotype frequently isolated from the tonsils, stomach, caecum, intestinal contents, faeces and carcass surface of pigs in the abattoir (Pezzotti et al. 2003, Guevremont et al. 2004, Pearce et al. 2003, Borch et al. 1996). In fact, this organism isolated in a study by Weijtens et al. (1997) indicated that piglets are infected at an early age by *Campylobacter* spp., with the number of organisms in the intestine/faeces decreasing as the pig ages. This finding was corroborated by Young et al. (2000). If *Campylobacter* is present in pig faeces, it is usually found in greater numbers than other zoonotic pathogens (Yeh et al. 2005, Oosterom et al. 1985).

2.11.3 Escherichia coli O157:H7

Incidence

In 2004, New Zealand reported 15 cases of *E. coli* O157:H7 in humans (ESR 2006), corresponding to 0.38 cases per 100,000 head of population. The USA reported 1.06 cases per 100,000 in 2005 (FoodNet 2006). However, this incidence is expected to increase as a result of a multi-state outbreak in September 2006 (CDC 2006). In Australia, the incidence risk reported in 2004 for the five different states ranged from 0.05 to 2.04 (Combs 2005).

There are six classes of diarrheagenic *E. coli*, one of which is EHEC, the group in which *E. coli* O157:H7is categorised (Buchanan & Doyle 1997).

Pathogen of concern: *E. coli* O157:H7

Enterohemorrhagic *E. coli* (EHEC) has emerged as a recent zoonotic food-borne organism of considerable importance (Cassin et al. 1998). *E. coli* O157:H7 are gram negative, rod-shaped, facultative anaerobic organisms usually comprising part of the lower

gastrointestinal normal microbial flora of animals and man (Cassin et al. 1998, Chapman 1995). Some strains are invasive and produce a heat-labile toxin that causes irreversible cytotoxic damage to Vero cells and as such have been classified as Verocytotoxin-producing *E. coli* (VTEC). This toxin is similar to a Shiga toxin, produced by Shigella dysenteriae type 1. VTEC *E. coli* O157:H7 was first recognised as pathogenic to humans in 1982 when it caused two major outbreaks of hemorrhagic colitis in the USA (Riley et al. 1983). Since that time it has been reported to be responsible for hundreds of cases of disease in world (Dontorou et al. 2003).

Pathogen attributes

Many *E. coli* spp. are harmless, while others are pathogenic (Robins-Browne 2001). These bacteria possess three major antigens, O (or somatic) antigens, H (or flagella) antigens and K (or capsular) antigens (Robins-Browne 2001). Verocytotoxin-producing *E. coli* O157 fails to ferment sorbitol, decarboxylate lysine and ornithine; but ferments raffinose, dulcitol, rhamnose and produces β-glucuronidase. It does not grow at 4°C (Chapman 1995) nor outside the pH range 4 – 7.5 (Buchanan & Doyle 1997). The organism is resistant to desiccation at refrigeration temperatures, but sensitive to high salt concentrations (Abdul-Raouf et al. 1993, Glass et al. 1992).

E. coli O157 — a public health problem

The disease caused by this organism ranges from asymptomatic to severe, with the latter resulting in deaths in the elderly and very young (Cassin et al. 1998). Infections can produce haemorrhagic colitis, presenting as diarrhoea, pyrexia, vomiting, which may or may not be accompanied with blood, acute abdominal cramps lasting 3 to 7 days, and sometimes kidney dysfunction, seizures and coma. (Robins-Browne 2001, Cassin et al. 1998, Chapman 1995). Approximately 10% of all cases develop haemolytic uraemic syndrome (HUS), primarily seen in children less than 10 years of age. Other complications include thrombotic thrombocytopenia purpura, a condition that is more common in older patients (Dontorou et al. 2003), nervous system disorders (seizures and strokes), colonic perforation, pancreatitis and hypertension (Cassin et al. 1998, Buchanan & Doyle 1997, Tarr 1995, Bell et al. 1994). A high probability of infection is associated with exposure to approximately $10^{3.3}$ cfu or less (Buchanan & Doyle 1997).

Source of infection for humans

Ruminants, particularly cattle are considered the primary reservoir of the pathogen (Caprioli et al. 2005, Borzyck et al. 1987). Sheep are also regarded as important reservoirs (Chapman et al. 1997). Transmission to humans can occur through the consumption of contaminated undercooked food such as beef, raw milk and to a lesser extent cheese, sausages made with pork intestines, contaminated apple juice, salads, bean sprouts, melons, lettuce, and yoghurt (Dontorou et al. 2003, FAO/WHO 1996, Chapman et al. 1993, Riley et al. 1983). Other animal sources include pigs, poultry, cats and dogs (Bouvet et al. 2001, Beutin et al. 1995, Beutin et al. 1993). Differences exist regarding the risk that pork products pose to the consuming public in terms of contracting the disease. In European countries, pork products are considered to be a low risk food (Eriksson et al. 2003, Bouvet et al. 2001). A family outbreak of *E. coli* O157 in Italy was found to be caused by the consumption of dry fermented pork salami (Conedera et al. 2007). This was the first case of the disease reported in Europe that was attributable to pork consumption. In Chile and Japan, pork products are considered high risk foods. One possible explanation for this is that in both countries (Chile and Japan), the carriage rate of the organism in pigs is higher than that reported in European countries (Eriksson et al. 2003, Nakazawa & Akiba 1999, Rios et al. 1999, Acheson & Keusch 1996).

Transmission between persons and contact with infected livestock have also been described. These routes are however less important than food transmission (Heuvelink et al. 1999, Chapman 1995).

Verocytogenic *E. coli* 0157 in pigs

Non-verocytogenic *Escherichia* coli 0157 has been isolated from both healthy pigs and piglets suffering from diarrhoea (Chapman 1995, Gannon et al. 1988, Linggood & Thompson 1987). Oedema disease in pigs is caused by VTEC serotypes (Robins-Browne 2001). The organism has been found to be present in pork samples but its source is unknown and it is possible that this can be a result of cross-contamination along the food pathway (Chapman 1995). Verotoxigenic *E. coli* O157 is rarely isolated from pigs at slaughter in Europe (Eriksson et al. 2003).

2.11.4 Summary

The prominence of these three organisms (*S*. Typhimurium, thermophilic *Campylobacter*, and VTEC O157) warrants conduction of exposure assessments and evaluation of efficacious mitigation strategies. The results of these can inform risk managers of procedures that can potentially minimise the risk of contracting diseases associated with these pathogens, as a result of pork consumption. Although reducing the prevalence of these organisms in pigs is ideal, a more immediate reduction may be seen when control strategies are implemented at the processing and further processing stages of the food pathway. These areas are the focus of this thesis. One of the most commonly consumed pork products in New Zealand was selected for analysis, as it is reasonable to assume that mitigation procedures implemented for this product may reduce the number of food-borne illnesses associated with pork consumption.

2.12 Overview of Production processes

2.12.1 Abattoir

Modelling of the entire farm to fork pathway was thought to be complex, intensive and unrealistic to complete given the available resources for this study. As a compromise, a section of the food pathway was identified (the abattoir and further processing until retail), with the express purpose of: (i) determining the possible extent of pork product contamination, (ii) comprehending the processes most influential/critical to determining the risk to consumers, and (iii) evaluating intervention strategies. The main stages involved in pig abattoir processing are outlined below.

Killing and lairage

Prior to slaughter, animals are housed temporarily in pens in the abattoir, in an area called the lairage. This area serves two main purposes: (1) to allow pigs to recover from transport stress and (2) to serve as a reservoir of pigs for the slaughter facility.

Meat quality is affected by the length of time pigs are kept in the lairage. Short periods of time of less than 1 hour are associated with increased prevalence of Pale Soft Exudative (PSE) meat while extended lairage times (in excess of 2 hours) are associated

with increased blemishes and lacerations on pigs due to fighting, reduced carcass yield (even though food is offered), as well as dark firm dry (DFD) meat (Warriss 2003). At temperatures greater than 10°C, showering serves to clean pigs, reduce aggression and fighting, and results in improved meat quality (Knowles et al. 1998). Holding times of no more than 30 minutes are optimal under high ambient temperatures (35°C) as it reduces the incidence of PSE meat (Warriss 2003). Generally with respect to pig quality, a lairage time of 1 – 3 hours is regarded as optimal in the UK (Warriss 2003, Boes et al. 2001).

Pigs are electrically stunned in the head, rendering them unconscious, during which time the animal is shackled and hung up by its hind legs. A knife is then inserted into the mid-line of the neck within thirty seconds of stunning and the anterior vena cava is severed. Death results from exsanguination (Borch et al. 1996).

Scalding

After bleeding is complete, the carcass is immersed in a tank of water between 58 and 65°C for 6 – 10 minutes (Borch et al. 1996), in a process known as scalding. This immersion loosens the hair and bristle in the outer layer of the skin, facilitating dehairing which occurs in a subsequent processing stage. Several pigs are scalded together in the same batch of water, thus resulting in the accumulation of dirt, faeces, ingesta and bacteria in the tank (Bolton et al. 2003). There was no addition of water to the scald tank at any of the plants visited during operations, so the effect of water replacement was not included in the model.

Dehairing

Dehairing is accomplished when one or more carcasses are placed in a rotating drum with in-built metal paddles that scrape the hair and outer layer of the skin off the carcass. Warm water is sprayed on the carcasses and equipment to remove hair and debris. During this process faecal matter may be spread onto the carcass surface and machine. In this case, the machinery becomes contaminated with bacteria and may serve as a source of contamination for other carcasses in the processing chain.

Singeing

The carcass is singed using a blow torch, where the flames remove any remaining hair and tighten the skin. This increases the external temperature of the skin to up to 100°C and has been reported to be very effective in reducing bacterial counts on the surface of the carcass (Pearce et al. 2004, Berends et al. 1997, Borch et al. 1996, Sorqvist & Danielsson-Tham 1990). The time taken to complete this process varies between carcasses and is also dependent on the operator's judgement; but is usually between 10 – 15 seconds. In some abattoirs 'polishing' occurs, where rotating rubber brushes are used to remove the burnt skin from the carcass.

Evisceration, trimming and halving

Evisceration involves removal of the entrails. This procedure is thought to be the main cause of carcass contamination (Bonardi, Brindani, Pizzin, Lucidi, D'Incau, Liebana & Morabito 2003, Bonardi, Pizzin, Lucidi, Brindani, Paterlini & Tagliabue 2003, Hald et al. 2003, Berends et al. 1997) as it is estimated that 55 – 90% of carcass contamination occurs from this procedure (Berends et al. 1997). The critical operations are circumcising of the rectum, which can be done manually or mechanically, followed by cutting the carcass from the anus to the breastbone to allow manual removal of the intestinal tract and pluck set (trachea, lungs, gullet, heart and liver). Removal of the intestines carries the risk of puncturing of the intestine resulting in faecal contamination of the carcass and spread of zoonotic pathogens from the intestine to the carcass. Trimming is the manual removal of visibly contaminated regions on the carcass (Borch et al. 1996). Halving is the process of cutting the carcass into two halves with the aid of an automated saw.

Chilling/Storage

The final processing stage is the chilling of the carcass. Carcasses are chilled to a temperature of approximately 4 – 7°C using blast and/or conventional chillers. This is a critical step for both meat quality and bacterial contamination. Carcasses chilled too rapidly can experience contraction of the muscles, reducing the quality and tenderness of any subsequent meat produced. Chilling can also minimise some bacterial proliferation, however, pathogen growth is very variable and influenced by the type of organism, temperature and

pH (Borch et al. 1996).

At present in New Zealand there are 11 abattoirs that slaughter pigs. Six of these are located in the North Island and five in the South Island. In this thesis three abattoirs were visited, two of which were in the North Island. One abattoir in the North Island slaughters pigs on two days in the week, except for the month of December in which increased market demand led to slaughtering of pigs on 3 days in the week. It is estimated that a total of 15,000 pigs are slaughter per week. The number of pigs slaughtered daily in abattoirs in New Zealand can vary from 150 to over 1,000 pigs. The procedures described above are undertaken in all visited plants. There is a procedure called polishing, that results in the removal of any black rind from singeing. This procedure was not modelled as the methods used in New Zealand to execute this procedure are varied, ranging from the manual use of nylon hand brushes to large automated equipment with rubber flails and this procedure is sometimes absent. There appeared to be no standardisation with which the procedure is executed in abattoirs visited throughout New Zealand.

2.12.2 Further processing processes

The production of pork chops is evaluated in this thesis as it is one of the most commonly purchased and consumed pork products in New Zealand. Pork chops can be produced in some abattoirs and/or retail outlets by cutting the longissimus dorsi muscles into desired sizes.

2.13 Conclusion

This review has outlined different techniques available for conducting exposure assessments in quantitative microbial risk assessments. Important zoonotic pathogens relevant to New Zealand, that will be addressed later in this thesis were discussed so as to provide background knowledge to the reader. Additionally, pork processing in abattoirs and pork chop production were described, since these stages of the farm to fork pathway can be important in targeting control measures to reduce pathogen levels in purchased pork chops. In New Zealand, despite the development of a number of quantitative microbial risk assessments, there is a distinct paucity or absence of QMRAs conducted on locally produced pork products. Given the increased food safety initiatives established world-

wide by the CAC, it is imperative that risk-based analysis be implemented for pathogens transmitted to humans through pork consumption. To this end, this thesis addresses the challenge of developing an exposure assessment model to be used in QMRAs enabling determination of the risk of exposure to *Salmonella* spp., thermophilic *Campylobacter* and *E. coli* from pork chops locally produced and sold at retail outlets in New Zealand.

CHAPTER 3

Modelling pathogen dynamics in the abattoir

3.1 Abstract

The consumption of pork products contaminated with food-borne microbial pathogens such as *Salmonella*, *Campylobacter* and *Escherichia coli* may result in illness in susceptible individuals. Understanding the mechanism(s) by which these pathogens are propagated along the food chain can be critical in formulating and refining efficient control strategies. The purpose of this study is to propose a model simulating the propagation of these three pathogens in New Zealand abattoirs.

To this end, a suite of quantitative, semi-stochastic, modular process risk models (MPRM) sufficiently generic to describe the propagation of *Salmonella*, *Escherichia coli* and *Campylobacter* through the various stages of pork processing in pig abattoir is developed. Dynamics of pathogen inactivation, removal, partitioning and cross-contamination are described and explicitly modelled using a combination of difference and differential equations. Second order modelling is performed to quantify parameter variability and uncertainty. Parameters are estimated from published data and targeted investigations in abattoirs in New Zealand.

Using Monte Carlo simulations, our model predicted that both dehairing and evisceration contributed the most to increased carcass contamination levels. Scalding was demonstrated to be highly effective in reducing pathogen numbers, particularly with respect to *Campylobacter*. The latter abattoir procedures of evisceration and storage were shown to contribute more to increased pathogen variance than the earlier procedure of scalding. Distributions of all three pathogens of interest on carcasses (from the time of killing to storage of the completed dressed product) were estimated to be highly right-skewed with the 10^{th} to 90^{th} percentile predicted to be 1 – 2495, 1 – 245 and 1 – 85cfu/half carcass

for *Salmonella*, *Campylobacter* and *Escherichia coli*, respectively. A small percentage of carcasses were highly contaminated, with most carcasses possessing low levels of surface contamination. The estimated median contamination levels on the dressed carcasses exiting the abattoir were less than one cfu/cm^2 for the three pathogens.

The models predicted that at least one cfu was present on most carcasses, therefore resulting in mean prevalence values on the final product of 98% and 94% for *E. coli* and *Campylobacter* respectively. If the prevalence level of *Salmonella* in New Zealand was similar to levels currently reported in Europe, the model predicted prevalence levels on dressed carcasses leaving the abattoir for this pathogen of 100%. However *Salmonella* in pigs in New Zealand abattoirs is rare. Second order modelling quantified parameter uncertainty and variability for all pathogens. Also, model development permitted identification of many data gaps.

We conclude that although the pathogens of interest were predicted to be present on nearly all carcasses, only a small number of these dressed carcasses would be expected to possess large pathogen numbers on exiting the abattoir. Further, we propose that the models developed are sufficiently rigorous yet flexible to be extrapolated to other species with similar abattoir processing. Additionally, since the model outputs distributions of pathogen numbers and prevalence, it can be used in quantitative microbial exposure assessments and for investigating the efficacy of intervention strategies to reduce pathogen load and the occurrence of food-borne diseases.

3.2 Introduction

Quantitative microbial exposure assessments provide scientific and systematic methods of evaluating the likelihood that an individual/population is exposed to a specific microbe(s). One methodology recently developed for conducting microbial quantitative exposure and risk assessments is the modular process risk model (MPRM) which allows evaluation of food related health risks by describing the propagation of microbial hazards through food pathways (Nauta 2001). These models output distributions describing both the pathogen prevalence and contamination level on foods of interest. Also, numeric estimates of model uncertainty and variability can be obtained. Modular process risk modelling requires compartmentalisation of the food pathway into processing steps known as

"modules". Within each module, any of the six "processes" of growth, inactivation, mixing, partitioning, cross-contamination and removal can be explicitly described. Additionally, MPRM lends itself to evaluation of intervention strategies and has been successfully used for executing quantitative microbial exposure assessments of *Bacillus cereus* in refrigerated processed foods of extended durability; *Escherichia coli* O157 in steak tartare and *Campylobacter* in poultry (Nauta et al. 2001, Nauta 2001, Nauta, van der Fels-Klerx & Havelaar 2005). *Salmonella*, *Campylobacter*, and *E. coli* (particularly EHEC 0157) have been identified as major pathogens, transmissible to humans from the consumption of pork (Bolton et al. 2002, Li et al. 2004, Alban & Stärk 2005). Diseases produced by these organisms can manifest as gastroenteritis with sequelae of Reiter's, Guillain-Barre, and hemolytic uraemic syndromes following salmonellosis (Barth & Segal 1999), campylobacteriosis (McDonald & Gruslin 2001), and colibacillosis (Liu et al. 2005) respectively.

In New Zealand (NZ), the incidence risk of food-borne diseases resulting from these pathogens varies. High incidence risks of 432/100,000 head of population (ESR 2006) have been recorded for campylobacteriosis, with lower values of 38.8/100,000 head of population (ESR 2006) for salmonellosis and 1.8/100,000 head of population for Shiga toxin-producing *E. coli* (ESR 2005*a*, ESR 2006). The impact of these diseases on human health, concurrent with the associated economic cost of food-borne illness to the country, has given rise to increased public concern and the need to mitigate their occurrence. Whereas the human impact of these diseases has been noted in New Zealand, no exposure assessment for these pathogens has been conducted with respect to pork products. Therefore in this paper we present a novel suite of models for use in exposure assessments of non-typhoidal *Salmonella*, thermophilic *Campylobacter* and *E. coli* on pork carcasses in New Zealand abattoirs using the MPRM. These models are referred to as the abattoir models. The risk simulation abattoir models comprise systems of differential and difference equations describing the transmission of each pathogen through the abattoir. By changing relevant input parameters, the model simulates the propagation of the pathogen of choice within the slaughter house. In this way, the relative importance of bacterial dynamics influencing the change in pathogen prevalence and levels of carcass contamination can be quantified.

3.3 Model Description

Definition of scope (pathogen and food type)

In this paper non-typhoidal *Salmonella* refers mainly to *S.* Typhimurium, a serotype responsible for many human salmonellosis cases internationally (Ruiz et al. 2003, Jeoffreys et al. 2001). We define *Campylobacter* as the thermophilic *Campylobacter* spp. of *C. coli*, *C. jejuni* and to a lesser extent *C. lari*. Initially we considered investigating EHEC *E. coli* O157, however, after very low levels of detection were observed in our data collection studies, we modified our research to incorporate all *E. coli*, both pathogenic and non-pathogenic strains. The presence of this pathogen is considered to be an indicator of faecal contamination. For the purpose of this manuscript, the food under investigation is the halved pork carcass as it exits the abattoir.

Model description

Abattoir procedures are referred to as "stages" while "processes" describe any two or more of bacterial growth, inactivation, mixing, partitioning, cross-contamination and removal. The Modular Process Risk Model is used to develop the suite of semi-stochastic, mechanistic models to simulate pathogen numbers and prevalence on each carcass as it progresses through the abattoir. A total of six modules (with their pertinent pathogen processes) are formulated, corresponding to different abattoir stages, from the time the live animal enters the abattoir, to storage of the dressed product (Figure 3.1):

(1) Lairage and killing: The lairage is a holding bay for pigs and is constructed as a series of pens. Pigs are moved from the lairage to the area in which they are killed. Prior to killing, pigs are showered or washed with a power hose.

(2) Scalding: involves placing pigs in a large tank of warm water for six to eight minutes. This procedure facilitates hair removal.

(3) Dehairing: removal of surface hair. Pigs are placed in a machine resembling a cylindrical rotating drum with scrapers that mechanically remove hair from carcasses.

(4) Singeing: removal of remnant fine and coarse hairs present after the dehairing stage using hand-held blow torches.

(5) Evisceration, trimming, and halving: a combination of visceral extraction, removal of visible contamination on the carcass and halving the carcass. For the purpose of this

model, we assume a knife is used for halving.

(6) Storage or chilling: usually in chillers with an average temperature of 3 – 4°C.

The final output from the models predict the prevalence and concentration of bacteria on modelled carcases. As shown in Figure 3.1, the outputs of one module are used as inputs for the following module. In this paper, contamination refers to the presence of bacteria on the carcass surface, while infection refers to bacterial colonisation of the gastro-intestinal tract and associated tissues. Cross-contamination is the movement of bacteria from one object to another; while inactivation is considered to render the organism non-pathogenic. The latter two processes were recognised as the principal bacterial processes in our model. Cross-contamination primarily impacts pathogen prevalence, while inactivation reduces pathogen numbers. Partitioning occurs during the halving abattoir procedure, when there is sub-division of the carcass. The abattoir procedure of polishing in which any black rind resulting from singeing is removed was not incorporated into our model. The methods used in New Zealand to execute this procedure are varied, ranging from the manual use of nylon hand brushes to large automated equipment with rubber flails and this procedure is sometimes absent. There was no standardisation of this polishing procedure in abattoirs throughout New Zealand.

The following assumptions are made in this model:

(1) For all microbes of concern, pathogen populations are uniform with respect to resistance to thermal inactivation

(2) There is no change in the resistance of pathogens to thermal inactivation through prolonged or repeated exposure to elevated temperatures

(3) The temperature is constant within each process and changes in a step-wise fashion between processes

(4) Unless otherwise specified, there is no growth of the pathogens of concern on carcasses.

(5) We assume there is no addition of fresh water to the dehairing machine or scald tank during operation.

and,

(6) Carry-over of water from the scald tank model to the subsequent process is zero.

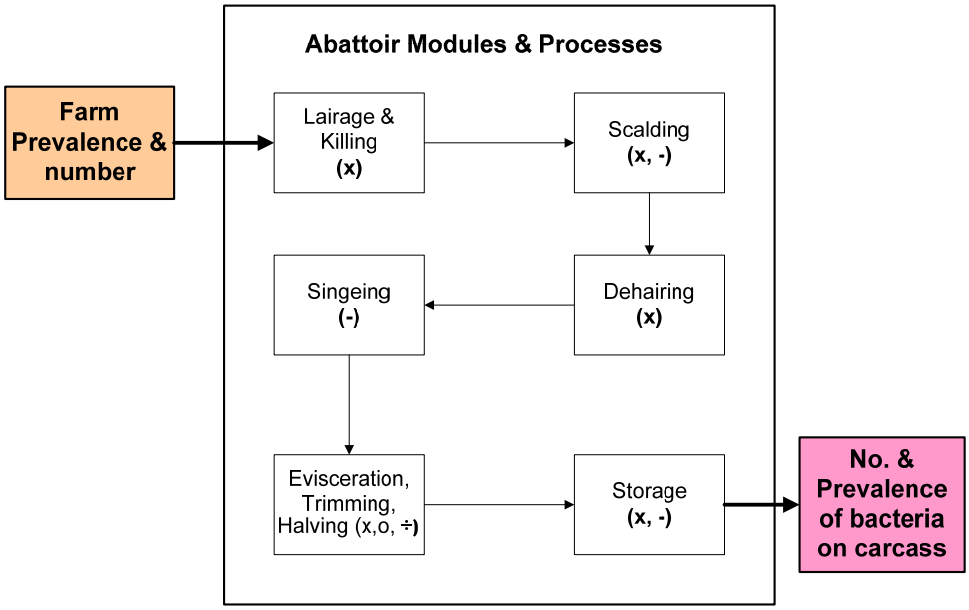


Figure 3.1: Modules and processes in a theoretical slaughter house. The processes are represented as follows: cross-contamination (x), inactivation (-), partitioning (÷) and removal (o). The number of bacteria present at the start of processing is dependent on the prevalence of the pathogen on the farm of origin. Model outputs indicate the prevalence and number of pathogens on carcasses.

Model equations

Both difference and differential equations are used to model the change in bacterial numbers on carcasses throughout the abattoir. This change is modelled in either discrete or continuous time. An abattoir procedural stage is considered to occur in continuous time if its corresponding processes can be observed at any moment. On the other hand, discrete time stages are only observable at distinct time intervals. In this study, evisceration, trimming and halving are identified as discrete stages and therefore modelled using difference equations. All other modules occur in continuous time and are therefore modelled using differential equations.

Difference equations

Difference equations model time as a discrete variable. They are concerned with the input-output relationships of pathogens on carcasses, for processes and events occurring in discrete time. The difference equations used for the evisceration, trimming and halving module are adapted from Nauta, van der Fels-Klerx & Havelaar (2005). For this module, we develop a model consisting of two compartments: (i) the environment which we limit to the knife and (ii) the exterior surface of the pig carcass. There are also three variables and six parameters. Figure 3.2 presents a schematic diagram on which the difference equations are based. On completion of a single stage of processing *S*, each carcass (*i*) carries a bacterial load on its surface (*x*), measured in colony forming units (cfu), and is denoted as $N_{x,S}(i)$. The quantity of bacteria in the environment or the knife (*e*), after passage of a single carcass (*i*) at this stage is represented by $N_{e,S}(i)$. For this model we assume that all knives are dipped in hot water at 70°C before use on a different carcass.

Bacteria numbers in the leaking faeces from a single carcass *(i)* during a processing stage $N_{f,S}(i)$, is the product of the probability of faecal spillage, (described by a Bernoulli distribution), the mass of leaking faeces in grams $w_{f,S}(i)$ and the concentration of bacteria in colony forming units(cfu) per gram of faeces at the processing stage $g_f(i)$. Both $N_{x,S}(i)$ and $N_{e,S}(i)$ are variables determined by bacterial dynamics occurring between the two model compartments and are calculated as described in Equations 3.1 and 3.2. An explanation of the parameters shown in Figure 3.2 and Equation 3.1 is provided in Table 3.1.

With respect to the trimming and halving stages, the environment is a knife and the

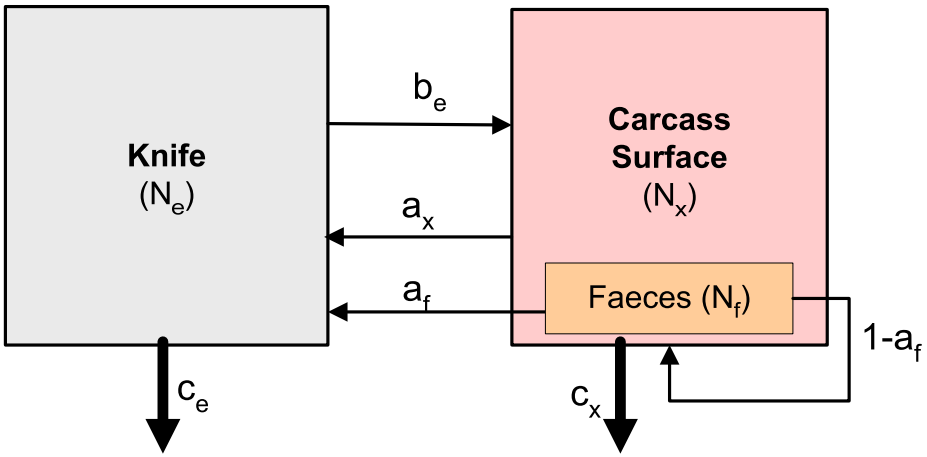


Figure 3.2: Diagram adapted from Nauta, van der Fels-Klerx & Havelaar (2005) showing parameters, variables and compartments modelled using difference equations. Thin arrows indicate the direction of movement of bacteria between compartments (that is, cross-contamination and auto-contamination). Thick arrows represent bacterial inactivation and removal. Table 3.1 provides an explanation of the parameters shown.

Table 3.1: Overview of model parameters for difference equations in the abattoir models."S" subscript indicates that parameter values differ with each processing stage, but during the process, it is assumed to be constant.

Parameter	Description
$a_{x,S}$	Probability that each cfu of bacteria moves from the carcass exterior to the knife.
$a_{f,S}$	Probability that each cfu of bacteria moves from the faeces to the knife.
1-$a_{f,S}$	Probability that each cfu of bacteria moves from the faeces to the carcass exterior.
$c_{x,S}$	Probability of inactivation and removal from the carcass exterior per cfu of bacteria.
$c_{e,S}$	Probability of inactivation from the knife per cfu of bacteria.
$b_{e,S}$	Probability that each cfu of bacteria moves from the knife to the carcass exterior

variable $N_{f,S}(i)$ is omitted from the equations as viscera have been removed from all carcasses. It is assumed that the abattoir is free of all zoonotic pathogens at the commencement of processing on each day.

Cross-contamination is represented by $a_{x,S}$, $a_{f,S}$ and $b_{e,S}$. The first two parameters represent the probability of bacteria moving from the carcass skin, and faeces to the knife, while $b_{e,S}$, depicts the probability of bacteria moving from the knife to the carcass exterior. Inactivation and removal from the knife and exterior of the carcass are parameterised by $c_{e,S}$ and $c_{x,S}$ respectively. Auto-contamination of carcasses by its own faecal material is described by 1-$a_{f,S}$. The variables $N_{f,S}(i)$, $N_{x,S}(i)$ and $N_{e,S}(i)$ and can differ in value with each carcass. On completion of each processing stage, the model equations for a single carcass are as follows:

$$N_{x,S}(i) = (1 - a_{x,S})(1 - c_{x,S})N_{x,S-1}(i) + b_{e,S}N_{e,S}(i-1) + (1 - a_{f,S})N_{f,S}(i) \quad (3.1)$$

$$N_{e,S}(i) = a_{x,S}N_{x,S-1}(i) + (1 - b_{e,S})(1 - c_{e,S})N_{e,S}(i-1) + a_{f,S}N_{f,S}(i) \quad (3.2)$$

Equation 3.1 calculates the number of pathogens on the exterior of each carcass on completion of stage *S*, as the sum of the number of bacteria remaining on the carcass from the previous stage (*S-1*) and that contaminating the carcass from the knife and faeces at stage *S*. Equation 3.2 determines the number of bacteria on the knife after the passage of each carcass by summing the number of bacteria moving onto the knife from the carcass on the previous stage and that remaining on the knife at stage *S*. These equations are implemented once for each of the abattoir procedures of evisceration, trimming and halving.

Differential equations

Differential equations describe the rate of change of pathogen numbers within compartments over time. They are therefore more appropriate for modelling continuously occurring events rather than discrete time events. We use these equations for all modules except evisceration, trimming and halving. For modules described using coupled differential equations, analytical solutions are obtained. However, in the scalding module, where the analytical solution is intractable, the differential equation is solved using nu-

merical solutions derived using the 4th order Runge-Kutta method for solving differential equations (Shi et al. 2004).

Model Structure

As previously stated, the risk model is designed so that the outputs from one module become inputs for subsequent modules. In this way, the contamination level on each individual carcass as it travels through the abattoir is captured. The following sections describe the model structure and differential equations for continuous time processes. Diagrammatic representations of modelled processes considered to occur in continuous time are presented in Figure 3.3. Model worksheets are shown section C of the appendix. We assume that the source of contamination for all pathogens of concern originates from faecal material in the rectum.

Lairage and Killing

The propagation of *Salmonella* infection in the lairage is modelled using a simple Susceptible-Infectious (S-I) model. This module has two outputs. The first is an estimate of the likelihood that each pig becomes infected in the lairage and is described as follows:

$$Prob = Bernoulli(1, 1 - e^{-\lambda pt}) \tag{3.3}$$

where *Prob* is the probability of an animal being infected in lairage; *t* is the time spent in the lairage; λ is the pathogen transmission parameter in the lairage and *p* represents the number of pigs shedding. For this equation we make the assumption that there is a constant rate of transmission which is determined by the number of initially infected animals (*t*=0) in the lairage.

The second output provides an estimate of the total number of bacteria in a pen in the lairage ($N_{p,l}$). This is calculated as a product of the total infected faecal material ($T_{f,l}$) by the concentration of *Salmonella* in infected faeces measured in cfu/g ($N_{f,l}$) as given below:

$$N_{p,l} = T_{f,l} N_{f,l} \tag{3.4}$$

Both outputs are sampled from probability distributions described in Table 3.2. For simplicity, the model assumes pigs from the same farm are housed together in one pen

during lairage. Propagation of *Salmonella* in the lairage was modelled (Fedorka-Cray et al. 1995, Hurd et al. 2001, Loynachan et al. 2004) but since no published literature supports propagation of *Campylobacter* and *E. coli* during lairage, this factor was omitted when modelling these latter organisms. In latter tables and figures this section is referred to only as "Killing".

Scalding

The scalding model is further discussed in Chapter 4 where the change in concentration of *Salmonella* on carcass contamination is explored in detail. At this abattoir stage, pigs are placed in a large tank of warm water for six to eight minutes. Because several pigs are usually in the tank at the same time, pathogen inactivation occurs concurrent with cross contamination. Pathogen dynamics during scalding are described using the following system of differential equations:

$$\begin{aligned} \frac{dP_k}{dt} &= -(\tau_1 + \beta)P_k + (\alpha/n)W \\ \frac{dW}{dt} &= \beta \sum_{k=1}^{n} P_k - (\tau_2 + \alpha)W \end{aligned} \tag{3.5}$$

for *n* pigs (from P_1 to P_n), where P_k and *W* are the number of viable bacteria on the k^{th} pig carcass and in the water respectively. The ratio α/n represents the rate of movement of bacteria from the water to the pig for each carcass, β represents the rate of movement of bacteria from the carcass to the water and τ_1 and τ_2 are two rates of bacterial mortality/inactivation on the carcass and in the water respectively. The initial value of W, (when $t = 0$) is zero. It is assumed that one carcass is added to the scald tank approximately every minute. When modelling this procedure, we assumed that the temperature profile within scalding is constant; the carriage of water from the scald tank is zero and there is water flow into the scald tank — as observed in the visited abattoirs.

Dehairing

For dehairing, we model cross-contamination occurring between the dehairing machine and pig carcasses. The final contamination level on carcasses and on the machine after passage of each carcass is calculated using the analytical solution to the system of differential equations described in Equation 3.6 below:

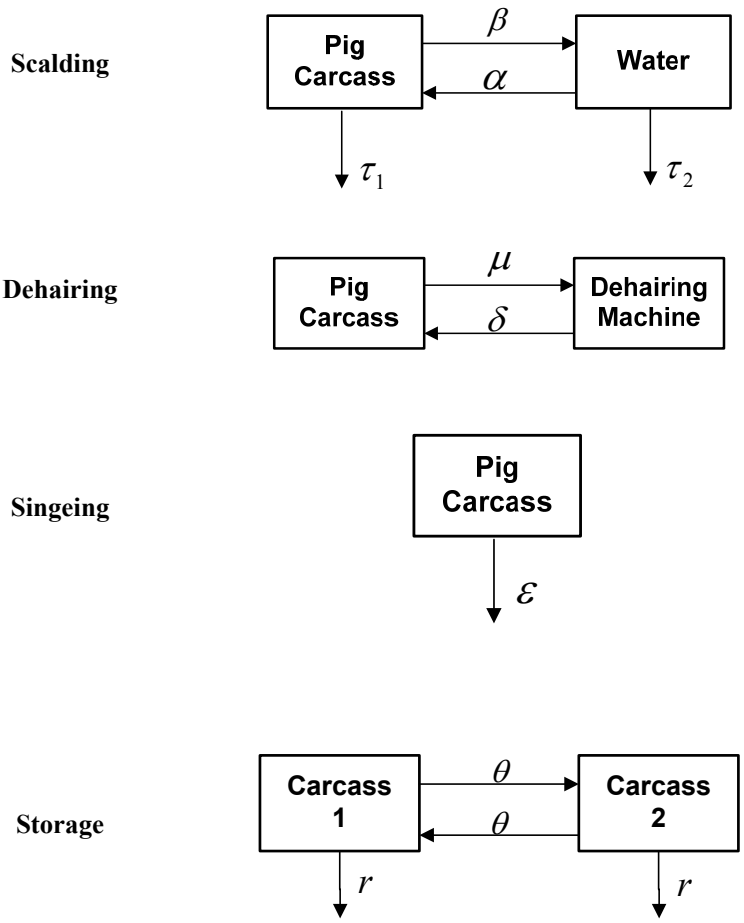


Figure 3.3: Composite diagrams of the pig abattoir modules described by differential equations. Compartments with their respective parameters representing the *per capita* rates and the direction of movement are shown. For scalding τ_1 and τ_2 depict mortality rates on the carcass and in the water respectively while α and β are the rates of movements between the water and the carcass. In the dehairing module, μ and δ are the rates of movement of bacteria between the carcass and dehairing machine. Both singeing and storage incorporate an inactivation step which occurs at rates described by ϵ and *r*, respectively. Cross-contamination between carcasses occurs at the rate depicted by θ.

$$\begin{aligned} \frac{dP_d}{dt} &= -(\mu P_d \ - \delta M) + B \\ \frac{dM}{dt} &= (\mu P_d - \delta M) + B \end{aligned} \tag{3.6}$$

where μ is the rate of surface contamination from the pig to the dehairing machine which is assumed to be constant throughout the process; δ is the rate of bacterial contamination from the dehairing machine to the pig and P_d and *M* are the bacterial loads on the pig and dehairing machine respectively. The initial value of M, (when $t = 0$) is zero. We also added to these equations an amount of faecal material (B) that was assumed to have exited the rectum of each pig and contaminated the dehairing machine and the pig during dehairing. This was calculated using the following equation:

$$B = ProbA_{f,d}N_{f,d} \tag{3.7}$$

where $N_{f,d}$ refers to the concentration of the pathogen of interest in the faecal material, *Prob* is the probability of an animal being infected in the lairage and $A_{f,d}$ is the amount of faecal material extruded by the pig. Derivation of these parameters are described in the section A of the appendix. Pathogen inactivation was not modelled as it was considered to be a very minor process occurring during this abattoir procedure.

Singeing

When modelling singeing, a mathematical equation taking into account only bacterial inactivation is developed. As such, the rate of change in the concentration of bacteria on each carcass surface during singeing (P_s) is influenced by the inactivation rate parameter (ϵ) and the processing time (*t*), as shown in Equation 3.8.

$$\frac{dP_s}{dt} = -\epsilon P_s \tag{3.8}$$

Evisceration, Trimming and Halving

Evisceration and trimming are modelled as previously described, using difference equations. Generally, carcass trimming occurs when there is visible faecal/intestinal slippage. The process of partitioning occurs during the halving abattoir stage. At this stage, we assume that bacterial contamination is uniformly distributed on all carcasses.

Storage

During storage, carcasses are subject to blast chilling which we assume to be used in all pig abattoirs in New Zealand. Cross-contamination and inactivation are modelled during this stage. Cross-contamination is considered to have occurred as a result of contact between carcasses during storage. We let *r* represent the rate of inactivation, *t* the length of time storage occurs, and θ denote the *per capita* rates of bacterial transfer between two adjacent half carcasses (C_1 and C_2). The equations describing pathogen dynamics in storage are:

$$\begin{aligned} \frac{dC_1}{dt} &= \theta C_2 - (\theta + r)C_1 \\ \frac{dC_2}{dt} &= \theta C_1 - (\theta + r)C_2 \end{aligned} \tag{3.9}$$

Parameters and data

There is very little data from New Zealand available to inform our model parameters. As a result, parameter estimates were obtained from published literature originating from Europe and North America, as well as targeted microbiological and observational studies executed in New Zealand.

Table 3.2 presents parameters with respect to *Salmonella* spp. that are modifiable by the model user. Importantly, some of these parameters are components of distributions which are hard coded into a Visual Basic for Applications Macro. These are described in section B of the Appendix of this chapter. This Appendix also presents parameter values pertaining to *E. coli* and *Campylobacter* which may vary from that relating to *Salmonella* in section A. The General Appendix details the Macro for the suite of models describing pathogen transmission in abattoirs. Since *Salmonella* was not detected in the faeces or on any pig carcasses sampled, all parameters pertaining to *Salmonella* are obtained from the literature. The effect of seasonality, which can influence the prevalence of carcass contamination was not incorporated in our model.

Separation of Uncertainty and Variability

The separation of uncertainty and variability is called ”second order modelling” and this is accomplished by identifying distributions representing parameter variability and parameter uncertainty and utilising a double-looping technique to determine the contribution of each in the model. Parameter uncertainty refers to a lack of knowledge about the parameter, that is reducible by the acquisition of more pertinent data. Parameter variability represents the true heterogeneity of the parameter that is irreducible by additional data. The outer loop of the double-looping technique propagates parameter uncertainty while the inner loop propagates parameter variability. In our model we recognise two types of variability, one at the individual pig level and the other at the batch level. Latin Hypercube sampling of parameters representing uncertainty is conducted once only for each simulation. Therefore, during multiple iterations in a simulation, they are held constant. In contrast, Latin Hypercube sampling of parameters representing variability is executed for each iteration. This enables the full range of parameter values to be explored. A non-parametric determination of the distribution of bacteria post-storage using an adaptive density estimation is then conducted in the software package MATLAB® 7. Further detail on this adaptive density estimation is given in section A in the Appendix. Seven parameter distributions are classified as variable, while the others are considered to describe uncertainty. Distributions representing variability are indicated in Table 3.2. For second order modelling the number of pigs was fixed at 50 for all iterations and simulations.

Model implementation

The risk model is executed in Microsoft Excel with Palisade @Risk 4.5 (Palisade Corporation, 2005) using Latin Hypercube sampling and a Visual Basic for Applications Macro, in which the entire model was hard coded. The General Appendix contains a copy of the Macro used for this suite of models. We ran 1,000 simulations of 10 iterations of the risk model. Therefore a total of 10,000 iterations are executed. For practical reasons the model is spread over 14 spreadsheets and represents a real-life scenario, where bacterial numbers in/on equipment after processing of pigs from one farm affect subsequently processed pigs from other farms. To incorporate this feature, each set of 10 iterations are dependent, so pathogen numbers from the environment (for example, the scald tank water, dehairing machine or knife) after modelling of the last pig of an iteration, become

the initial value of the environment for subsequent iterations within a simulation.

Every iteration represents a batch of pigs submitted to an abattoir from a farm. The number of pigs sent from each farm is sampled from a zero truncated Normal distribution (mean=50, standard deviation=10). A simulation therefore mimics the events occurring in the pig abattoir in 24 hours or one day. Model inputs describe animal prevalence and concentration of the selected organism. The output indicates the number and prevalence of pathogens on dressed pork carcasses as it leaves the abattoir. (The model can be viewed on the accompanying compact disc.)

For second order modelling, 100 simulations consisting of 100 iterations are implemented. Unlike the previously described implementation technique, iterations are independent, so that the model simulates abattoir processing of multiple realisations of a single batch over multiple days with no transference of bacteria values to subsequent iterations. This independence between iterations allows estimation of the relative contribution of parameter variability and uncertainty in the model.

Data Collection

Microbial numbers, proportion of animals shedding specific pathogens and other model parameters were determined by conducting observational and microbial studies in three abattoirs in New Zealand, as the available data sets were very limited. Both quantitative microbial and presence/absence microbial testing were undertaken on faecal samples. For these studies, a total of 134 faecal samples were collected. Carcass contamination levels were determined by swabbing 130 carcasses in two different abattoirs in New Zealand. Pig carcasses were swabbed prior to and after completion of abattoir procedural stages.

Presence/absence testing:

To determine the proportion of animals shedding the pathogens of concern, 140 swabs of faecal samples were taken in the lairage from different sites of the faecal pat. For *Campylobacter*, samples were placed in Bolton's selective enrichment broth at 42°C for 48 hours, followed by isolation on Modified Campylobacter Charcoal Differential Agar (mCCDA) at 42°C after 48 hours. For growth of *Campylobacter*, all samples were incubated under microaerophilic conditions in a commercial cabinet which maintained a gaseous atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen. To identify *Salmonella*, samples were placed in Rappaport-Vassiliadis Broth (RVS) enrichment broth

for 24 hours at 42°C, followed by isolation on xylose-lysine-deoxycholate (XLD) agar after 24 hours at 37°C. In order to detect *E. coli*, samples were incubated in Lactose broth for 24 hours at 37°C, followed by isolation on Eosin Methylene-blue Lactose Sucrose (EMB) agar incubated at 37°C. Confirmation of colonies for *Salmonella* was accomplished using biochemical tests (indole, citrate, urease and Methyl Red Voges-Proskauer tests). *Campylobacter* colonies were confirmed using a combination of biochemical and molecular techniques (gram stain, oxidase and genus specific polymerase chain reaction). The polymerase chain reaction (PCR) amplified the 894 base pair *cadF* gene conserved in *Campylobacter* using the primer sequences of GATCTTTTTGTTTTGTGCTGC and CCTGCTACGGTGAAAGTTTTGC.

During these visits to the abattoir, we recorded the following: (a) the number of farms that submitted pigs, (b) the number of pigs per pen, (c) the number of pigs submitted per farm and (d) the time spent in lairage, to inform relevant parameters.

Quantitative testing:

For the quantitative testing of carcass contamination levels, 225cm^2 of the dorsal or ventral midline regions of pig carcass were swabbed placed in 5ml of buffered peptone water and stored on ice. These were then transported to the laboratory and logarithmically plated out in duplicates of 200μl using a spiral plater onto XLD agar for *Salmonella*, mCCDA agar for *Campylobacter* and (EMB) plates for *E. coli*. Both *Salmonella* and *E. coli* plates were incubated for 24 hours at 37°C. Confirmation of colonies was done as stated for the presence/absence testing. *E. coli* colonies were confirmed using biochemical tests (indole, citrate, urease and Methyl Red Voges-Proskauer tests). All samples were processed the same day.

For faecal samples, 10g of faeces was homogenised in 90ml buffered peptone water for 30 s using a stomacher. Serial dilutions were made and plated onto appropriate media for the desired organism. The number of colonies per plate was counted and the initial pathogen concentration calculated. During these visits to the abattoir, we documented the estimated time carcasses spent in storage as this information was required for parameter estimation.

Other studies conducted

1. In order to determine the amount of leaking faecal material moving onto the knife during evisceration, 30 pieces of gauze were cut approximately 25cm^2 in size, and

weighed. These were then individually wrapped and autoclaved. The knife used during carcass evisceration was wiped with one of the weighed, sterilised pieces of gauze, then repackaged in a ziploc bag, transported on ice to the laboratory and re-weighed. The difference in weight was considered to be an estimate of the amount of leaking faecal material moving onto the knife during evisceration.

2. As part of the abattoir procedure, pigs are weighed immediately post-evisceration. These weights were documented. The length of the carcass and incision made for evisceration were also recorded. We also measured the size of trimmed areas removed as a result of faecal contamination.

3. Any faecal material observed that leaked onto the carcass during evisceration at the abattoir was collected, placed in a pre-weighed plastic bag, transported on ice to the laboratory, where it was re-weighed. The difference in weight was considered to be an estimate of the amount of leaking faecal material moving onto the carcass surface.

Table 3.2: Parameter values used in the model for *Salmonella spp*.

Parameters for *Salmonella*	Distribution	Input values	Comments/References
Lairage			
[2] Number of pigs in lairage	Normal	mean:50 sd:10	Numbers from the NZ pig abattoirs, described in Appendix
Prevalence of infected pigs in lairage	Beta	alpha:44 beta:120	(Stärk et al. 2002)
Proportion of infected pigs shedding	Beta	alpha:60 beta:99	(Stärk et al. 2002)
[2] Time spent in lairage (minutes)	Uniform	min:30 max:1440	estimated in NZ, described in Appendix
[2] Amount of faecal material shed (grams) per pig in lairage/min	Uniform	min:1.38 max:4.86	(IPPC 2006)
[2] Concentration of Salmonella in infected faeces (cfu/g)	Uniform	min:10 max:38,000	(O'Connor et al. 2006)
Transmission parameter in lairage/min		0.001	derived using maximum likelihood estimates described in Appendix
Killing			
Proportion of faecal material on each pig (cfu)	Beta	alpha:2 beta:500	estimate shown in Appendix
Scalding			
Rate of movement of bacteria from water to pig/minute		0.002	(Yang & Johnson 2001, Notermans & Kampelmacher 1974)
Rate of movement of bacteria from pig to water/minute		0.4	(Berends et al. 1997)
Rate of bacteria inactivated from pig surface/minute		0.37	(Murphy, Beard, Martin, Duncan & Marcy 2004, Murray et al. 2003)
Rate of bacteria inactivated in water/minute		1.6	Calculated from (Bolton et al. 2003)
Dehairing			
Amount of faeces extruded/pig (g)	Gamma	alpha:1 beta:1	described in Appendix

Table 3.2: Parameter values used in the model for *Salmonella spp*.

Parameters for *Salmonella*	Distribution	Input values	Comments/References
Rate of bacterial movement from pig to dehairing machine/minute		0.10	(Rivas et al. 2000)
Rate of bacterial movement from dehairing machine to the pig/minute		3.95	(Rivas et al. 2000)
[2] Time (minutes)	Uniform	min:0.13 max:0.25	(Borch et al. 1996)
Singeing			
Inactivation rate/minute		11.8	(Pearce et al. 2004)
[2] Time (minutes)	Uniform	min:0.16 max:0.25	(Borch et al. 1996)
Evisceration			
[1] Probability of bacteria on carcass moving to knife/cfu	Beta	alpha:7 beta:25	Parameter estimation shown in appendix
[1] Probability of bacteria in knife moving on carcass/cfu	Uniform	min:0.0 max:0.1	Parameter estimation shown in appendix
Probability of inactivation of bacteria on carcass/cfu	Uniform	min:0.05 max:0.7	Parameter estimation shown in appendix
Probability of inactivation of bacteria in knife/cfu	Uniform	min:0.9 max:1	Parameter estimation shown in appendix
[1] Probability of bacteria in leaking faeces moving to knife/cfu	Uniform	min:0.0125 max:0.5	Parameter estimation shown in appendix
Amount of faeces (g), leaking from carcass	Uniform	min:0.0125 max:20.5	Parameter estimation shown in appendix
Bacterial concentration in faeces of leaking carcass (cfu/g)	Uniform	min:10 max:38,000	(O'Connor et al. 2006)

Table 3.2: Parameter values used in the model for *Salmonella spp*.

Parameters for *Salmonella*	Distribution	Input values	Comments/References
Trimming			
[1]Probability of bacteria on carcass moving to knife/cfu	Beta	alpha:7 beta:25	Parameter estimation shown in appendix
[1]Probability of bacteria in knife moving on carcass/cfu	Uniform	min:0.0 max:0.1	Parameter estimation shown in appendix
[1]Probability of inactivation of bacteria on carcass/cfu	Uniform	min:0.05 max:0.7	Parameter estimation shown in appendix
Probability of inactivation of bacteria in knife/cfu	Uniform	min:0.9 max:1	Parameter estimation shown in appendix
Bacterial concentration in faeces of leaking carcass (cfu/g)	Uniform	min:0 max:0	
Halving			
[1]Probability of bacteria on carcass moving to knife/cfu	Beta	alpha:7 beta:25	Parameter estimation shown in appendix
[1]Probability of bacteria in knife moving on carcass/cfu	Uniform	min:0.0 max:0.1	Parameter estimation shown in appendix
Probability of inactivation of bacteria on carcass/cfu	Uniform	min:0.05 max:0.7	Parameter estimation shown in appendix
Probability of inactivation of bacteria in knife/cfu	Uniform	min:0.9 max:1	Parameter estimation shown in appendix
Bacterial concentration in faeces of leaking carcass (cfu/g)	Uniform	min:0 max:0	

Table 3.2: Parameter values used in the model for *Salmonella spp*.

Parameters for *Salmonella*	Distribution	Input values	Comments/References
Storage			
Cross-contamination: Rate of movement of bacteria between adjacent half carcasses/minute		0.006	(Yang & Johnson 2001, Notermans & Kampelmacher 1974)
[2] Time (hours)	Uniform	min:12	Estimated from pig abattoirs in NZ
		max:24	
Inactivation: Rate of inactivation/hour		0.035	for carcass numbers less than 1000cfu (Chang et al. 2003*b*)
		0.048	for carcass numbers greater less than 1000cfu (Chang et al. 2003*b*)

All bacterial numbers are counted in colony forming units (cfu). [1] These parameters are components of distributions which are hard coded into the Visual Basic for Applications Macro and detailed in the Appendix.
[2] Parameters representing variability.

3.4 Results

Figure 3.4 shows the predicted median pathogen numbers with the 10^{th} and 90^{th} percentiles for *Salmonella*, *E. coli* and *Campylobacter* at different stages in the abattoir. The models predict high bacterial numbers on carcasses in the lairage and during killing for all three zoonotic pathogens. *Campylobacter* is estimated to be present in the highest concentration at killing, however the concentration of this pathogen plummets dramatically during scalding to values lower than those estimated for both *Salmonella* and *E. coli*. We estimate a reduction in the median value of *Campylobacter* from $\log_{10}$ 6.8 to $\log_{10}$ 1.3 during scalding as compared to reductions from $\log_{10}$ 6 to $\log_{10}$ 3.2 and from $\log_{10}$ 5.9 to $\log_{10}$ 2.7 for *Salmonella* and *E. coli* respectively. *Salmonella* is estimated to be present in the largest numbers immediately after scalding.

During dehairing, when faecal material is dislodged from the pig anus moves onto the machine, and then to the surface of pigs, *Campylobacter* numbers were predicted to increase to levels that exceed those for *E. coli* and *Salmonella*. The numbers of *Campylobacter* remained consistently higher than the other pathogens until storage where it approximated levels similar to those predicted for *Salmonella*. Abattoir stages occurring post-dehairing resulted in a reduction of all pathogens with the exception of evisceration, in which median pathogen levels either reduced, as was the case for *Campylobacter* and *E. coli* or remained relatively unchanged, which was observed for *Salmonella*. From the singeing module to the storage module, *E. coli* was predicted to be present in the lowest numbers. A summary of the predicted outputs for the different abattoir stages is presented in Table 3.3. In general, our models predict scalding to be the most effective abattoir procedure to reduce pathogen contamination levels on pig carcasses. Dehairing, the subsequent procedural step is estimated to result in the greatest increase in pathogen levels. Storage is more effective in reducing *Campylobacter* numbers than *Salmonella* or *E. coli*. Although trimming is important in pathogen reduction, it is only executed on a small percentage of carcases and therefore the effect of this procedure was not graphically displayed.

The median pathogen load for each organism on dressed pig carcasses exiting the abattoir is less than 100 cfu per half carcass. The distributions of pathogens at this stage are predicted to be skewed to the right. Therefore, most carcasses possessed low levels of contamination, with a small percentage possessing high surface bacteria counts. A further

description of these pathogen distributions was executed by fitting parametric distributions to the data, using Palisade @Risk software. *E. coli*, *Salmonella* and *Campylobacter* followed Loglogistic, Lognormal and Inverse Gaussian distributions, respectively. These distributions are presented in Figure 3.5.

Our models not only predict the extent of contamination on carcasses with respect to the pathogens of interest, but the prevalence level as well. The estimated predicted prevalence levels ranged from 98 – 100%, 84 – 100% and 83 – 100%, with means of 100%, 98% and 94% for *Salmonella*, *E. coli* and *Campylobacter*, respectively at the end of storage. Therefore based on the model outputs, most pig carcasses emanating from the abattoir in New Zealand are estimated to be contaminated with low levels of these pathogens. The values for *Salmonella* are based on data from Europe and the USA which have higher prevalence and contamination levels of this organism than New Zealand in pigs and on pig carcasses.

Figure 3.6 shows the predicted changes in contamination levels on individual pigs processed in the abattoir and by extension, changes in pathogen variance. This figure demonstrates the predicted levels of *Salmonella* on individual carcasses at different stages in the slaughter house for three hypothetical farms submitting 50 pigs each to the abattoir, with different initial pathogen prevalence levels.

In general, pigs from Farm 1 were estimated to have the highest level of contamination of *Salmonella* and the least contaminated pigs originated from Farm 3. Contamination levels on individual pigs are shown to vary both between and within farms. Scalding reduced pathogen levels by similar extents for all farms, but had little impact on pathogen variance. Therefore on completion of scalding, pig contamination levels were still highest in Farm 1 and lowest in Farm 3 as shown in Figure 3.6b. During evisceration, where there is occasional spillage of contaminated faecal material onto the surface of carcasses, the models predicted elevated contamination levels on some pig carcasses (recognised as 'peaks' in the graph), thereby increasing pathogen variance. Faecal spillage was predicted to occur least frequently in Farm 3 and at similar rates for Farms 1 and 2. This reflects the real-life scenario, where the extent and frequency of contamination of pig carcasses during evisceration is varied. Bacterial numbers on dressed carcasses are reduced as a result of storage. For the storage module, final pathogen levels on carcasses were a result of the extent of cross-contamination, which can result in both increased and decreased surface

pathogen numbers, as well as inactivation. Farm 3 was predicted to display the greatest amount of pathogen variance as a result of these two processes, however pathogen contamination levels on pigs from this farm, generally remained consistently lower than that for the other farms. Storage and evisceration stages resulted in the alteration of pathogen numbers and increased variance within farms, while scalding primarily impacted contamination levels.

Second order modelling

We present the results of four types of parameter variability/uncertainty. To accomplish this, parameter uncertainty was held constant while parameter variability was assessed and the converse also applies, parameter variability was held constant while parameter uncertainty was assessed. We demonstrate: parameter variability between pig carcasses (pig variability) within a batch of 50 pigs; parameter variability between batches of pigs (batch variability); parameter variability between simulations; and parameter uncertainty.

Pig and batch parameter variability

'Spaghetti-looking' plots for *Salmonella, E. coli* and *Campylobacter* are shown in Figures 3.7a, 3.8a and 3.9a respectively. We show pig and batch variability for 100 iterations in a single simulation. Simulation number 50 was selected, however any simulation number could have been chosen. The distribution of pathogen numbers on the surface of pigs exiting the abattoir from each iteration is represented by a green line in the graphs. The area under the curve for each iteration equals one. Pig variability is shown by the number of bacteria on the dressed pig carcass exiting the abattoir, which corresponds to the values along the x-axis. Batch variability is demonstrated as the vertical difference between green lines or the difference between pathogen distributions from independent iterations. The median, 5^{th} and 95^{th} percentiles of the distributions of pathogens from all iterations are shown. The difference between the two percentiles (5^{th} and 95^{th}) represents 90% of the variability existing between batches within a single simulation of 100 iterations. Batch variability within a single simulation was found to be similar for all investigated pathogens with the parameters describing the propagation of *Salmonella* having the greatest quantity and parameters for the model pertaining to *E. coli* displaying the smallest quantity (Figures 3.7, 3.8, 3.9).

Parameter variability between simulations

To examine parameter variability between simulations, we plotted the distribution of pathogens output from the 10^{th} iteration, for each of the 100 simulations. Every green line in the graph therefore corresponds the 10^{th} iteration of a simulation. Any iteration number could have been selected, but the same iteration number needed to be selected for each simulation. We also plot the median value of these iterations, which represents the median of the distribution of pathogens output from the 10^{th} iteration for all 100 simulations. This process resulted in uncertainty being held constant. Figures 3.7b, 3.8b and 3.9b show the variability between simulations, which is demonstrated as the vertical difference between iterations. From examining the graphs, we show that this variability is the smallest among all parameter variabilities. Models describing *E. coli* and *Campylobacter* were predicted to show less variability between simulations than *Salmonella*.

Parameter uncertainty

Finally, all variability was held constant and parameter uncertainty evaluated. Therefore, we obtained a median distribution from each of the 100 iterations, within a single simulation. The median distribution was obtained in the same manner as described for the batch and pig variability. We then obtained median distributions for all 100 simulations which are plotted as shown in Figures 3.7c, 3.8c and 3.9c. Each green line represents one median distribution from a single simulation. The median of all the plotted median distributions is shown. Uncertainty is measured as the vertical difference or span between iterations. Therefore the amount of parameter uncertainty in the model for each pathogen is seen by comparing the uppermost median distribution for a single simulation and lowermost median distribution for a single simulation. Parameter uncertainty was greatest for the model pertaining to *Salmonella* and the least for the model describing *E. coli*.

Table 3.3: Summary of descriptive statistics of the predicted number of pathogens on carcasses on completion of the abattoir stages of killing, scalding, dehairing, evisceration and storage.

	Salmonella (cfu)	*E*. coli (cfu)	*Campylobacter* (cfu)
Killing			
Mean	925476	1799062	11361306
Median	428458	951272	6678921
10^{th}–90^{th} Decile	104319-2469041	185005-5100701	561093-29805266
Scalding			
Mean	4908	1366	39
Median	1745	493	22
10^{th}–90^{th} Decile	348-14585	105-3873	1-107
Dehairing			
Mean	5375	3178	22478
Median	2062	2158	8941
10^{th}–90^{th} Decile	419-15749	722-6717	323-59914
Singeing			
Mean	497	295	2104
Median	182	183	797
10^{th}–90^{th} Decile	34-1332	60-633	24-5254
Evisceration			
Mean	15916	3413	43017
Median	150	116	497
10^{th}–90^{th} Decile	23-7498	17-838	17-5869
Trimming			
Mean	8637	1527	20079
Median	150	116	453
10^{th}–90^{th} Decile	127-5270	99-722	442-3196
Halving			
Mean	6119	1262	18342
Median	56	42	145
10^{th}–90^{th} Decile	47-2816	37-282	140-1684
Storage			
Mean	2714	95	1874
Median	30	9	38
10^{th}–90^{th} Decile	1-2495	1-85	1-245

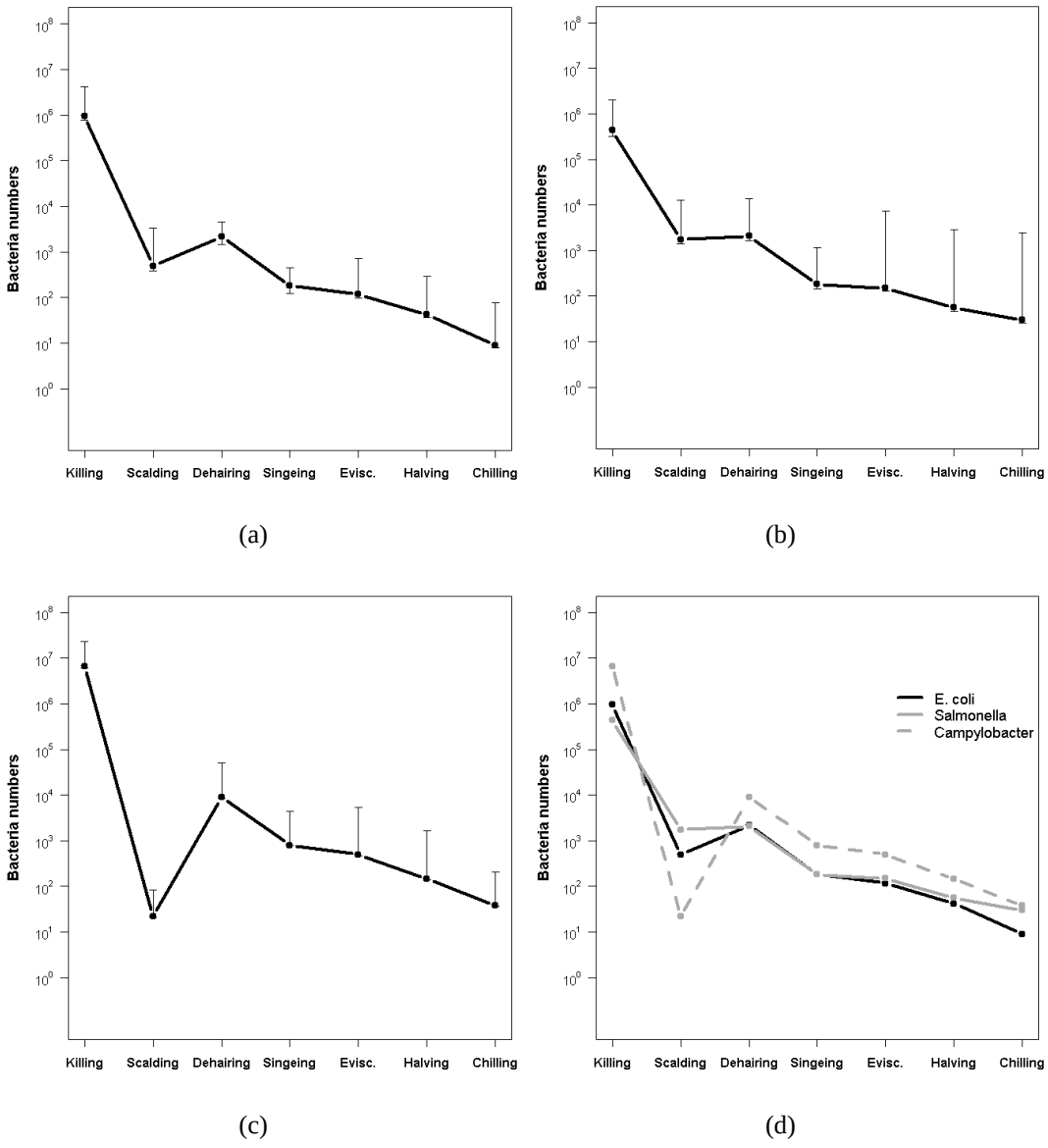
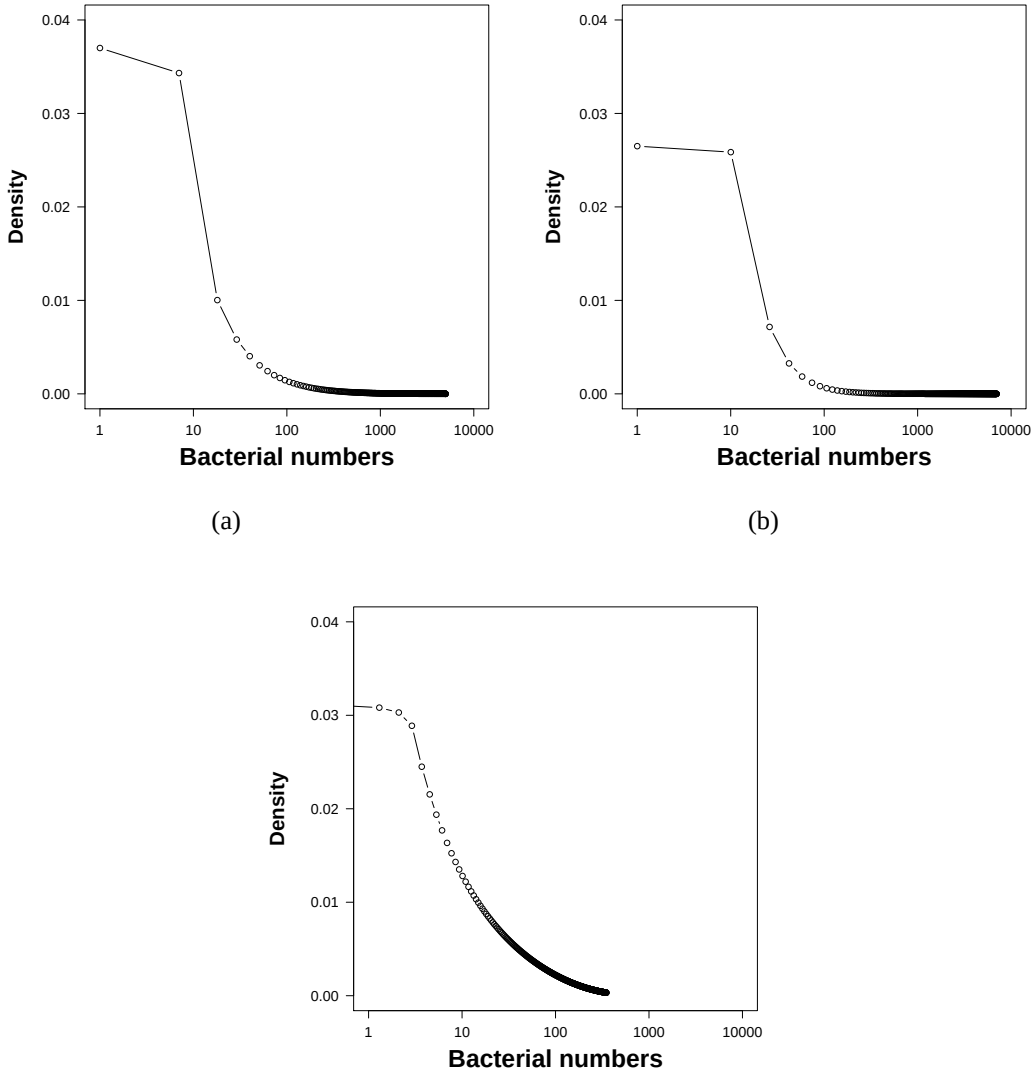


Figure 3.4: Predicted median bacterial numbers on the surface of pig carcasses, with 10^{th} and 90^{th} percentiles displayed at different stages in the abattoir for *E. coli* (a), *Salmonella* (b), *Campylobacter* (c) and a combination of all three pathogens (d).



(a)

(b)

(c)

Figure 3.5: Predicted distributions of the surface contamination levels on carcasses at different stages of the abattoir predicted by the suite of models for *Salmonella* (a), *E. coli* (b) and *Campylobacter* (c) at the end of storage in the abattoir.

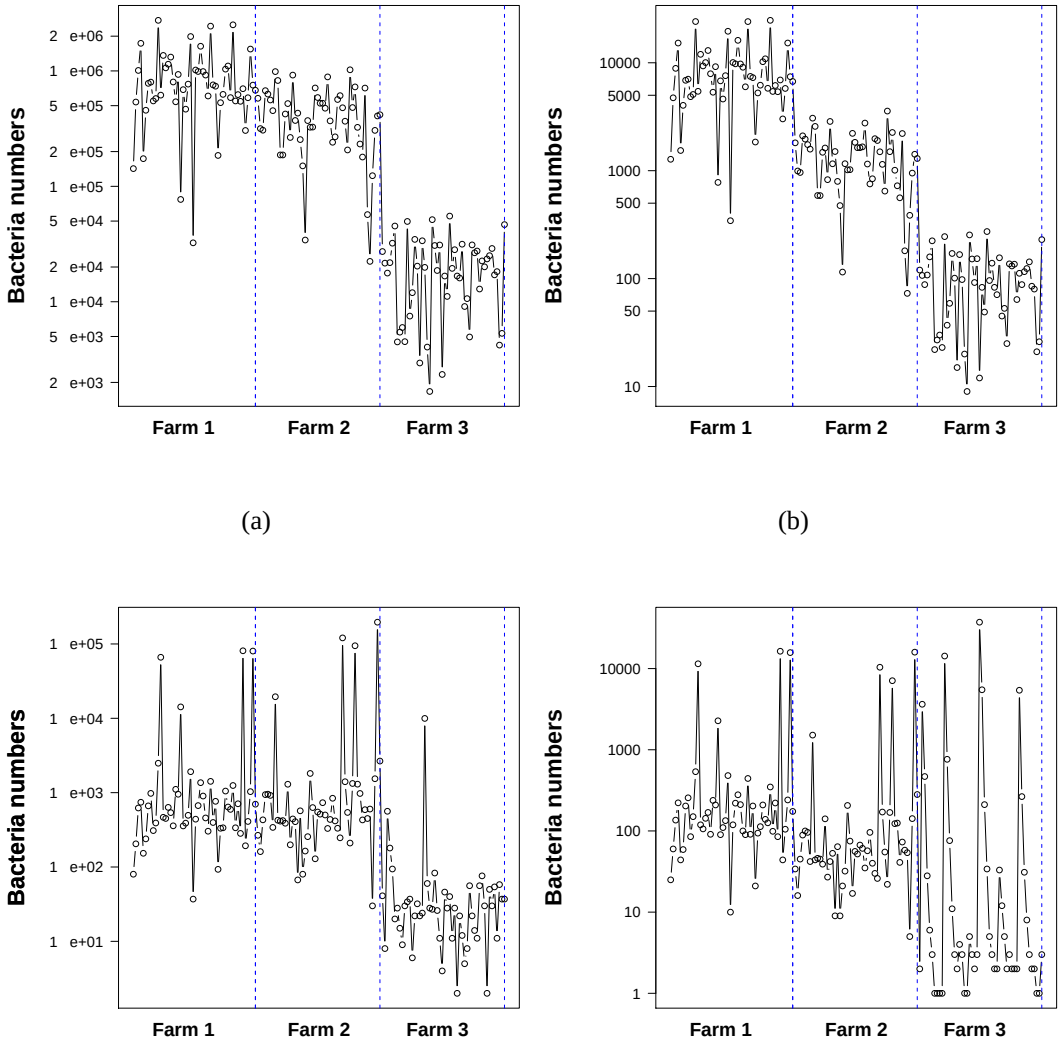
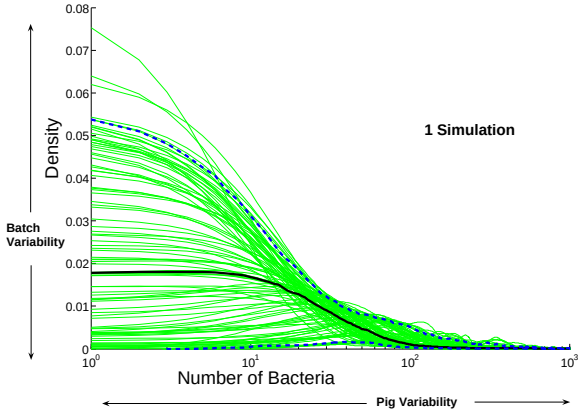
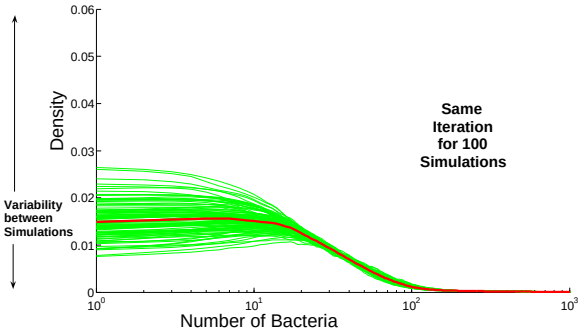


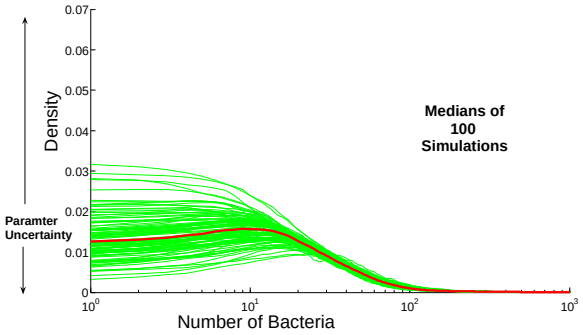
Figure 3.6: Time Series of predicted surface contamination levels of *Salmonella* on pig carcasses from three different farms at the killing (a), scalding (b), evisceration (c) and storage (d) stages of the abattoir. Each point in the graphs corresponds to a predicted pathogen number on a pig carcass. For storage, a value of one was added to all predicted output values.



(a)

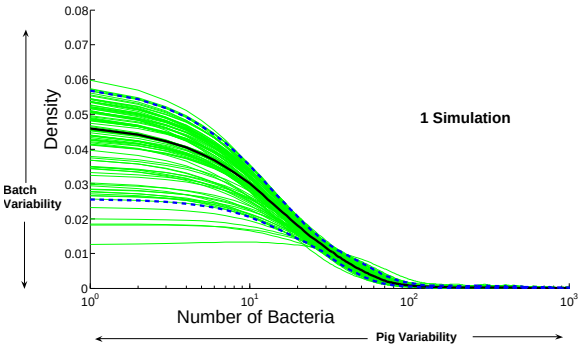


(b)

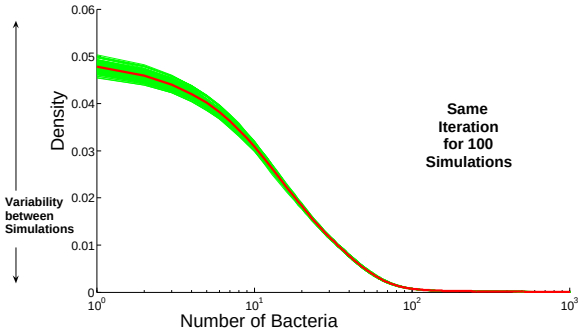


(c)

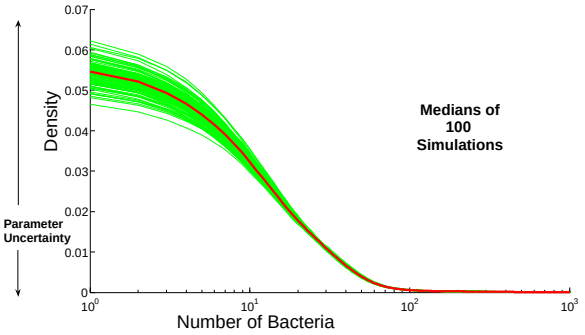
Figure 3.7: Outcome of the second order analysis showing the medians (red, solid line) for: one simulation of 100 iterations (each iteration is coloured green) (a); the 10^{th} iteration (each iteration is coloured green) for each of 100 simulations (b); and 100 simulation medians (each median from one simulation is coloured green) (c), for *Salmonella spp.* Dashed lines in (a) indicate the 5^{th} and 95^{th} percentiles. For graphs (a) and (b), each green line corresponds to a non-parametric distribution of the number of pathogens on carcasses at the end of storage in the abattoir. For (c) each green line corresponds to the non-parametric distribution of the median number of pathogens on carcasses at the end of storage from one simulation in the abattoir.



(a)

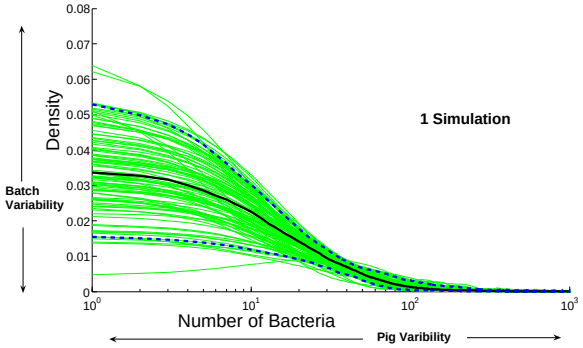


(b)

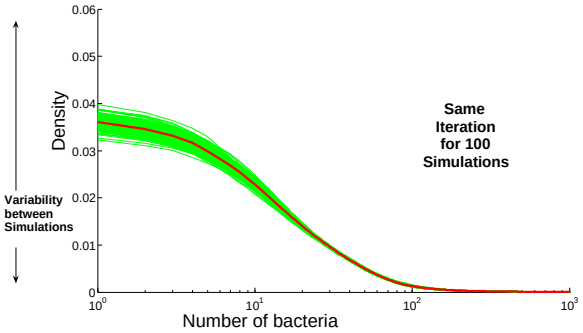


(c)

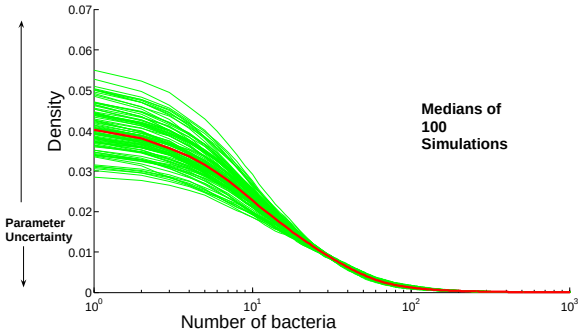
Figure 3.8: Outcome of the second order analysis showing the medians (red, solid line) for: one simulation of 100 iterations (each iteration is coloured green) (a); the 10^{th} iteration (each iteration is coloured green) for each of 100 simulations (b); and 100 simulation medians (each median from one simulation is coloured green) (c), for *E. coli*. Dashed lines in (a) indicate the 5^{th} and 95^{th} percentiles. For graphs (a) and (b), each green line corresponds to a non-parametric distribution of the number of pathogens on carcasses at the end of storage in the abattoir. For (c) each green line corresponds to the non-parametric distribution of the median number of pathogens on carcasses at the end of storage from one simulation in the abattoir.



(a)



(b)



(c)

Figure 3.9: Outcome of the second order analysis showing the medians (red, solid line) for: one simulation of 100 iterations (each iteration is coloured green) (a); the 10^{th} iteration (each iteration is coloured green) for each of 100 simulations (b); and 100 simulation medians (each median from one simulation is coloured green) (c), for *Campylobacter*. Dashed lines in (a) indicate the 5^{th} and 95^{th} percentiles. For graphs (a) and (b), each green line corresponds to a non-parametric distribution of the number of pathogens on carcasses at the end of storage in the abattoir. For (c) each green line corresponds to the non-parametric distribution of the median number of pathogens on carcasses at the end of storage from one simulation in the abattoir.

3.5 Discussion

This article presents a suite of semi-stochastic, mechanistic, quantitative risk models that describe the propagation of *Salmonella*, *E. coli* and *Campylobacter* in pork processing. The models output distributions of surface contamination levels on pig carcasses as well as pathogen prevalence, at the end of storage in the abattoir. It can therefore be used in exposure assessments, which form part of quantitative microbial risk assessments (QMRA). Although our models were developed specifically for use in New Zealand, with modification of specific parameters, its use can be extrapolated to other countries. Additionally, the models are sufficiently rigorous yet flexible to be used as a template for pathogen propagation in other species with similar abattoir processing. Also unlike other traditional mechanistic models, our models allow quantification of variability and uncertainty.

Minimisation of human exposure to *Salmonella*, *E. coli* and *Campylobacter* can be achieved at multiple points along the farm to fork continuum. We investigate one part of the food pathway, the abattoir, with the aim of describing the extent of human exposure to these pathogens, to eventually predict the effectiveness of different interventions strategies for their control. As such, we developed a suite of models describing pathogen dynamics so as to quantify the effect of abattoir processing on pork contamination and elucidate the relative importance of pathogen processes in determining prevalence and levels of contamination on pork. From the distributions of contamination levels output from the models, we see that both dehairing and to a lesser extent evisceration procedures were found to be the two most important abattoir stages, contributing to the increased levels of carcass contamination for all three investigated organisms (Figures 3.4,3.6). This is in agreement with empirical studies which also identify these two abattoir procedures as important in contributing to elevated carcass surface bacterial numbers (Malakauskas et al. 2006, Pearce et al. 2004, Gill & Bryant 1993).

Post-evisceration contamination levels have greater implications for the contamination of dressed pig carcasses with zoonotic pathogens, than post-dehairing contamination levels. After dehairing, pig carcasses are subjected to singeing, an abattoir procedure which has been found to significantly reduce the numbers of viable surface pathogen numbers (Berends et al. 1997, Borch et al. 1996). However, no comparable step resulting in pathogen reduction of a similar extent is conducted post-evisceration. Blast chilling has nevertheless been found to be efficient in reducing *Campylobacter* numbers

(Yeh et al. 2005). As previously stated, this system of chilling is currently used in New Zealand.

Model estimates indicated that the procedure of evisceration had little effect on carcass contamination. This is not consistent with some empirical studies, in which evisceration has been identified as a major source of surface bacterial contamination (Pearce et al. 2004, Lo Fo Wong et al. 2002, Berends et al. 1997). However, it should be noted that our models predicted highly skewed distributions of pathogens on carcasses as a result of evisceration (Figure 3.6). Therefore it is possibly that the effect of this procedure in increasing carcass contamination levels is only evidenced in a relatively small proportion of the carcasses. The right skewed distribution indicated that although most carcasses possessed low surface bacterial levels, a small number possessed relatively high levels. It is this latter group of highly contaminated carcasses that may serve as possible sources of contamination for other carcasses as well as present possible microbial hazards to the consuming public. The level of surface bacterial contamination during evisceration is dependent on the degree of auto-contamination, cross-contamination, pathogen prevalence and concentration. Results from one of our quantitative targeted microbial studies, which ascertained the surface contamination of sampled carcasses in New Zealand abattoirs, indicated that in general, bacterial counts pertaining to the three pathogens of interest were low. (The results of this study are presented in the Appendix.) One possible explanation for this is attributed to the fact, in New Zealand there is a slower abattoir chain speed as a result of the lower numbers of carcasses processed per day in comparison to other developed countries. In Spain, for example, 200 pig carcasses are processed per hour (Rivas et al. 2000), and in the USA, some 800 carcasses per hour (Yu et al. 1999) in comparison to approximately 163 – 1000 each processing day in New Zealand. Therefore, in New Zealand the operator has comparatively more time to spend on each carcass and therefore there is less prone to erroneously cause faecal spillage onto the carcass. Another possible reason may stem from the fact that experienced operators are usually employed and these are less likely to incise the intestine during evisceration (Borch et al. 1996). Both of the above possible explanations result in the low levels of auto and cross-contamination observed in our study.

The median contamination level of *Campylobacter* from evisceration was 0.04 cfu/cm^2, with 0.004 cfu/cm^2 – 0.13 cfu/cm^2 recorded as the 10^{th} – 90^{th} decile range. Yeh et al.

(2005) reported isolating mean low numbers of *Campylobacter*, post-evisceration (0.7cfu/cm^2) in a microbial surveillance study conducted in Taiwan. This reported value was not very different from the median value predicted from our model for this organism. For storage, our model estimated the median level of *Campylobacter* on carcasses to be 0.005 cfu/cm^2 with 10^{th} and 90^{th} deciles of 0.0001 cfu/cm^2 and 0.035 cfu/cm^2 respectfully. Oosterom et al. (1983) reported reductions of *Campylobacter* numbers on the surface of pig carcasses in the abattoir to values below detection after chilling overnight. This reduction was explained to result from the sensitivity of the organism to desiccation. Using the predicted median values from our model, an area the size of 200cm^2 needs to be swabbed to detect a single organism. The possibility of detecting the organism at these concentrations is very low. It is however unexpected that the predicted level of *Campylobacter* is higher than the other pathogens. One reason for this finding may be attributed to the large initial concentration of this organism in pigs faeces, which was at least 2 - 18 times higher than that for *Salmonella* and *E. coli*. This high level of *Campylobacter* in faecal material, exceeding counts for other pathogenic organisms was also reported by Yeh et al. (2005). Teunis et al. (2005) predicted a probability of infection ranging from 0.6 to 0.8 after exposure to 100cfu of the organism, based on a dose-response assessment informed by data from a human feeding study and two milk outbreaks.

Within Europe and North America, *Salmonella* has been routinely isolated from pigs and on pork carcasses (Berends et al. 1996, Snijders & Collins 2004, Vieira-Pinto et al. 2006). Our microbial studies indicated that *Salmonella* is rare on pig carcasses and in pig faeces in the lairage. (The results of these quantitative studies are presented in the Appendix). Therefore pork may not currently be a significant vehicle for transference of this pathogen to the New Zealand population. Nevertheless, given the international importance and the possibility of it emerging as an important pathogen in New Zealand, we described pathogen propagation of this organism in our suite of models. Available published literature was used to determine model parameters and variables. Since our models allows updating, in the event that this zoonotic organism does become prevalent in pigs in New Zealand, country specific parameters can replace those currently in use. Model outputs predicted that pig carcasses will be contaminated with a median value of 0.004 cfu/cm^2 post-storage. Haas et al. (1999) estimated that consumption of 2.36×10^4 cfu can result in a 0.5 probability of illness. This assessment was based on data from nine

different non-typhoidal *Salmonella* strains investigated in human feeding studies.

The predicted median value of *E. coli* on carcasses exiting the slaughter house was 0.001 cfu/cm^2. Our results are consistent with that found by Chang et al. (2003*b*), who identified blast chilling, currently used in New Zealand abattoirs, to be effective in reducing the number of *E. coli* on the pork skin. Chang et al. (2003*b*) demonstrated that pig carcasses contaminated with low numbers of *E. coli* pre-storage would be reduced to undetectable levels after blast chilling for 24 hours. From our estimated median value, an area of 1000cm^2 will have to be swabbed to detect a single *E. coli* cfu. We can therefore assume that the predicted level of contamination is close to or below the detection level. From an empirical study, Tamplin et al. (2001) reported mean values for *E. coli* after 24 hours post-chilling at storage, of 0.1 – 6cfu/cm^2 which is much higher than the median value predicted by our model.

Our model predicted mean prevalence levels of 100%, 98% and 94% for *Salmonella*, *E. coli* and *Campylobacter* respectively. These values appear to be greater than others obtained from microbial studies reported in the literature (Pezzotti et al. 2003, Tamplin et al. 2001). Tamplin et al. (2001) reported prevalence values of 58% for *E. coli* and a mere 7% for *Salmonella* post-chilling. For *Campylobacter*, Pezzotti et al. (2003) reported a prevalence of 10.3% in pork at retail, however the storage time was unknown. The reason(s) for the discrepancy may stem from limitations of microbiological tests to detect very low bacteria numbers. It must be remembered that in these cited studies, the entire carcass or half carcass was not swabbed and therefore there is a chance of non-detection of bacteria. In our studies an area of 225cm^2 was swabbed. This represents only approximately 1.6% of the total surface area of the average pig carcass. There is also the possibility that our model over-estimates the prevalence level. This can be modified by using more precise parameters which can be obtained by conducting further microbial tests.

The model development process facilitated identification of the dominant bacterial processes of cross-contamination and inactivation. One novel feature of this suite of models is the use of a variety of different methods to model cross-contamination and inactivation. Cross-contamination in abattoirs is complex. Of the six MPRM processes, it is the most difficult to model and consequently relatively few studies have addressed this issue (Aziza et al. 2006). Cross-contamination has previously been modelled both deter-

ministically and stochastically using a variety of techniques including simple difference equations (Aziza et al. 2006, Nauta, van der Fels-Klerx & Havelaar 2005); difference equations based on the Reed-Frost model (Ivanek et al. 2004) and using fixed transfer rates (Kusumaningrum et al. 2004) to determine the effect on pathogen prevalence and level of contamination. Our models employ a mixture of techniques including differential equations that incorporate the element of time when modelling continuous-time processes; difference equations when describing discrete-time processes, as well as stochastic transfer rates. We also describe the processes of cross-contamination occurring between two or more surfaces sequentially as well as simultaneously. It is this feature that makes our models both complex and unique, and can therefore be used as a template for future modelling of cross-contamination in food pathways.

Another aspect of our models is the mechanistic nature. Using this approach, the contamination level on each pig is calculated and output as it progresses through the abattoir. Also, the number of bacteria output at the end of one module becomes the input value for the same pig as it enters subsequent modules. In this way the individual carcass contamination level on each pig can be followed throughout the slaughter house. A distribution of surface bacterial levels for the specific pathogens is output by our models. This methodology allows the between carcass heterogeneity of surface contamination to be captured. A similar mechanistic approach was conducted in other studies. One such as example is by Nauta, van der Fels-Klerx & Havelaar (2005) in which a quantitative risk assessment of *Campylobacter* in poultry in the Netherlands was performed.

During model development several data gaps were identified. The most important was the lack of quantitative data on pathogen prevalence and contamination levels on pig carcasses in abattoirs in New Zealand. Also, there is less quantitative information in the published literature in comparison with prevalence values. This dearth of New Zealand specific data affected parameter estimation which proved to be challenging and very time consuming. As far as possible, data from the literature was used and targeted observational and microbial studies were undertaken in order to determine parameters applicable to New Zealand. However, results from our microbial studies demonstrated that the contamination levels of pork, with the organisms of interest in the abattoir were relatively low, thus making bacterial enumeration challenging. (The results are presented in the Appendix.) Additionally, there were difficulties in obtaining some parameters from

the abattoir, particularly with respect to scalding and dehairing. The arrangement of the processing chain in abattoirs makes sampling of carcasses post-killing and pre-scalding difficult. Also, the physical arrangement of the machinery makes access to carcasses between scalding and dehairing virtually impossible.

For simplicity, our definition of the environment did not include the air in the abattoir and consequently our models failed to incorporate carcass contamination with aerosolised bacteria. These bacteria can contribute to carcass contamination levels particularly with respect to *Salmonella*, leading to increased carcass contamination levels (Pearce et al. 2006).

Separation of uncertainty and variability results in the production of more mathematically correct risk estimates (Wu & Tsang 2004, Vose 2000). Second order modelling demonstrated that the relative contribution of parameter variability was greater than parameter uncertainty, in the final model output, that is the pathogen levels on carcasses at the end of storage.

In our model we assumed the rate of addition of pigs to the scald tank was approximately one per minute. If however, this rate were decreased, then it is possible that carcass contamination levels on carcasses exiting the scald tank would be lower than currently predicted. Correspondingly, it is expected that increasing the frequency of scalding so that more than one pig is introduced into the scald tank per minute may result in a higher level of contamination than that currently predicted.

Unfortunately, severely limited resources and time did not allow model validation. The data required for model validation is currently unavailable in New Zealand. For model validation, data from several abattoirs regarding pathogen prevalence and contamination levels on pig carcasses both before and after multiple abattoir procedures would be needed. The model can then be evaluated by comparing model predictions to collected empirical data. In the absence of model validation, model predictions should be interpreted with caution although some model outputs are congruent with other studies. In the event that additional time and resources are allocated to this study, priority should be placed on model validation. The model could have been further optimised by using database software packages instead of the spreadsheet application. Alternatively other programming languages such as Visual Basic, C++ and others which facilitate code compilation may also reduce model computation time. Some degree of model optimisation

was performed within the model code in an attempt to expedite model execution time. The suite of models did not include an abattoir procedural step called polishing. This omission may affect the ability of the models to be generalised to all abattoirs in New Zealand. However, it is not possible to include in the models all the unique aspects of all abattoirs and therefore the most practical approach was taken. Furthermore, it must be remembered that inherently, models are simplified, incomplete mathematical representations of realistic systems.

In conclusion, the semi-stochastic, mechanistic quantitative risk models of pork processing provide insight into the effect of the different abattoir stages on carcass contamination levels, which is not readily obtained from microbiological prevalence data. The abattoir processes of dehairing and evisceration contribute the most to increased carcass surface pathogen load. The models predict bacteria levels on every pig in every module and also outputs distributions of these contamination levels for every module. It is this aspect of our models, in addition to the fact that a distribution of pathogen prevalence is also produced, that enables their applicability in quantitative microbial risk assessment models. Further, our models can be adapted for use in quantitative exposure assessments of other species.

3.6 Acknowledgements

We would like to thank Greg Bolton and Nicolai Moles-Benfall for their assistance in computer programming of the mathematical models.

3.7 Appendix

The appendix contains three sections, the results of microbial studies previously described and details of parameter and variable estimations (A); parameters values used for *Campylobacter* and *E. coli* (B); and finally snapshots of the model worksheets for *E. coli* (C). Model parameters may vary for the different organisms and under conditions where this occurs, we state the method for derivation of parameters for all organisms but predominantly parameters pertaining to *Salmonella* are reported in section A.

A. Model parameter estimation with results of microbial studies

Summary of results for faecal testing

No *Salmonellae* were isolated from any of the tested faecal samples. The zero prevalence level found in our study was also reported for New Zealand by Wong et al. (2007). *E. coli* was cultured from 74% of faecal samples, and *Campylobacter*, 44%. The counts of these two later pathogens ranged from 100 – 13,000cfu/g and 200 – 240,000cfu/g for *E. coli* and *Campylobacter* respectively. The prevalence of *E.coli* was not 100%, possibly as a result of the inability of the media to sufficiently promote growth of the organism.

Results for carcass testing

Quantitative microbial testing of the surface of carcasses in the abattoir revealed that there were low carcass contamination levels. *Salmonella* was not isolated from any of the carcasses in the abattoir. *E. coli* however, was isolated in 2% of the carcasses after dehairing, none after singeing, 1% after evisceration and 1% after carcass halving. Counts of *E. coli* on carcasses ranged from 0.11 – 0.3cfu/cm^2 with three exceptions. Bacteria were isolated from three carcasses at counts equating to 8.6cfu/cm^2, 4.3cfu/cm^2 and 23cfu/cm^2 post-dehairing, post-evisceration and post-halving respectively.

Campylobacter was isolated in 1% of the sampled carcasses after dehairing, 1% after singeing and 0% after both evisceration and halving. Counts of *Campylobacter* on carcasses ranged from 0.11 – 0.3cfu/cm^2.

A table of raw data is presented below.

Table 3.4: Results of quantitative faecal testing for *Campylobacter* and *E. coli*.

Pathogen	Pathogen Counts (cfu)			
Campylobacter	150000	110000	130000	50000
	198000	240000	230000	30000
	1500	33000	200	20000
	15000	80000	3500	59000
	2000	4000	90000	15000
	40000	4000	4000	20000
	54000	25000	1400	13000
	70000	31000		
E. coli	1000	1500	2000	100
	5000	1300	8500	7000
	3700	2000	3650	1450
	3500	6500	1700	2000
	12400	13000	3000	2500

Results for carcass testing

Quantitative microbial testing of the surface of carcasses in the abattoir revealed that there were low carcass contamination levels. *Salmonella* was not isolated from any of the carcasses in the abattoir. *E. coli* however, was isolated in 2% of the carcasses after dehairing, none after singeing, 1% after evisceration and 1% after carcass halving. Counts of *E. coli* on carcasses ranged from 0.11 – 0.3cfu/cm^2 with three exceptions. Bacteria were isolated from three carcasses at counts equating to 8.6cfu/cm^2, 4.3cfu/cm^2 and 23cfu/cm^2 post-dehairing, post-evisceration and post-halving respectively.

Campylobacter was isolated in 1% of the sampled carcasses after dehairing, 1% after singeing and 0% after both evisceration and halving. Counts of *Campylobacter* on carcasses ranged from 0.11 – 0.3cfu/cm^2. A table of raw data is presented below.

Table 3.5: Results showing contamination levels on carcasses sampled in an abattoir in New Zealand

Site	Pathogen	Counts (cfu/cm^2)										Mean
Before Singeing	*Salmonella*	0	0	0	0	0	0	0	0	0	0	0
	E. coli	0.06	0	0	0	0	0	0	8.4	0	0	0.846
	Campylobacter	0	0	0	0	0	0	0	0.28	0	0	0.028
After Singeing	*Salmonella*	0	0	0	0	0	0	0	0	0	0	0
	E. coli	0.06	0.11	0	0	0	0	0	0	0	0	0.017
	Campylobacter	0	0	0	0.06	0.17	0.06	0	0	0	0	0.029
Before Evisceration	*Salmonella*	0	0	0	0	0	0	0	0	0	0	0
	E. coli	0	0	0	0	0	0	0	0	0	0	0
	Campylobacter	0	0	0	0	0	0	0	0	0	0	0
After Evisceration	*Salmonella*	0	0	0	0	0	0	0	0	0	0	0
	E. coli	0.11	4.3	0	0	0	0	0	0	0	0	0.441
	Campylobacter	0	0	0	0	0	0	0	0	0	0	0
Before Halving	*Salmonella*	0	0	0	0	0	0	0	0	0	0	0
	E. coli	0	0	0	0	0	0	0	0	0	24.4	2.44
	Campylobacter	0.06	0	0	0	0	0	0	0	0	0	0.006
After Halving	*Salmonella*	0	0	0	0	0	0	0	0	0	0	0
	E. coli	0.16	0	0	0	0	0	0	0	0	0	0.016
	Campylobacter	0	0.06	0	0	0	0	0	0	0	0	0.006

Seventy other samples from carcasses were collected. However it was presumed that volume of diluent/transport media was too large and therefore no bacteria were detected from any of these samples. This result does indicate that if the pathogens of interest were present on swabbed carcasses, the expected contamination levels were $<$50cfu/cm^2, which was the lower detection limit of the test.

Data for other results from observational studies are tabulated below:

Table 3.6: Results of observed carcass weights in abattoir, quantities of faecal material on knife; quantities of faecal material leaked on carcass and size of trimmed area observed in NZ abattoirs.

	Observed results			
Carcass weights in abattoir (g)	71.8	62.8	71.8	70.0
	72.0	72	69	63.4
	76.6	60.8	61.8	75
	59.8	86.2	77.6	58.4
	70.8	66.8	78.4	72.8
Quantities of faecal material on evisceration knife (g)	0.147	0.096	0.123	0.122
	0.094	0.325	0.118	0.140
	0.139	0.13	0.231	0.236
	0.254	0.115	0.109	0.495
	0.013			
Quantities of faecal material leaked onto carcass (g)	19.8	12.6	19.4	7.1
	14.8	6.6	7.3	13.5
	15.9	10.4		
Size of trimmed area (cm^2)	62	51	43	65
	59	70	63	58

Lairage section

Number of pigs in a pen in the Lairage:

The visited abattoirs received submissions from approximately 10 farms for each scheduled slaughter day. The number of pigs submitted from each farm varied considerably from 6 to 120 pigs. Pen sizes varied within a single lairage and between lairages in different abattoirs. Accordingly, the number of pigs in each pen varied depending on the numbers submitted by the farms and the sizes of the pens in lairage. Our records indicate a range of 1 to 103 pigs per pen. For the purpose of the model, we approximated the mean number of pigs per pen as 50. Since most pens contained 25 to 80 pigs, we described the number of pigs in the lairage as a zero-truncated, Normal distribution of mean = 50 and standard deviation = 10.

From observational studies and discussions with abattoir plant managers, it was determined that the time pigs spend in lairage is very variable ranging from less than one hour to 24 hours. One of the of the reasons for this stems from the fact that there is a variable period from the time pigs arrive and are kept in abattoirs (this can be during the day or night), to commencement of slaughter operations and the time in which pigs are slaugh-

tered. Pigs can arrive at abattoirs at variable times in the day and night. Additionally. pig slaughter in abattoirs can commence from as early as 6.00a.m. to as late as early afternoon (approximately 2 p.m.). There were differences in the operations of some plants. In one instance it was observed that pigs were slaughtered soon after arrival at one abattoir, yet at another abattoir, pigs were slaughtered on the following day of arrival, even though the pigs arrived at the abattoir during operational hours when carcass processing was occurring. Consequently, the time in lairage spent by pigs was modelled using a Uniform distribution with minimum and maximum times of 30minutes to 1 day.

Pathogen prevalence:

In the absence of available data from New Zealand, parameter values were estimated from published literature for all three pathogens. Data from other countries with comparable climatic conditions in Europe (Stärk et al. 2002, Pesti 1979) and the USA Harvey et al. (1999) were used for estimating parameter values for *Salmonella, E. coli* and *Campylobacter* respectively. Using the BetaBuster software program, information from Stärk et al. (2002) Harvey et al. (1999) and Pesti (1979) were used to produce Beta distributions bounded between 0 and 1 with α = 44, β = 120 for *Salmonella*, α = 53, β = 8 for *Campylobacter* and α = 40, β = 1 for *E. coli*, respectively.

Proportion of infected pigs shedding:

Since no *Salmonella* was isolated from faecal samples, we estimated the proportion of infected animals from Stärk et al. (2002). This uncertainty parameter was described by Beta $\sim$(60,99). *Campylobacter* was isolated from 44% of the sampled faecal material and *E. coli* was isolated from 74%. These uncertainty parameters were describing by Beta distributions with α = 18, β = 7 for *E. coli* and α = 45, β =56 for *Campylobacter*. All of these Beta distributions were bounded between 0 and 1.

Time spent in lairage:

The time spent in lairage was recorded as varying from 30 minutes to one day at the visited abattoirs. We represented this parameter as a Uniform distribution, with a minimum value = 30 minutes and a maximum = 1440 minutes, the latter value corresponds to one day. The time spent in the lairage depended on the location of the farm submitted pigs, with respect to the abattoir; the time at which pig slaughter commences, which in some abattoirs was quite variable and the number of pigs needed to be processed in a day. In one abattoir

visited, pigs that arrived during the working hours in one day were processed on the subsequent day. The time each pig spends in lairage is very variable. We make the assumption that there is an equal probability of pigs spending from 30 minutes to 1 day in the lairage.

Amount of faecal material shed per pig in the lairage/minute:
This parameter was calculated from IPPC (2006), using a Uniform distribution with minimum value = 1.38g and maximum value = 4.86g.

Pathogen concentration in infected faeces (cfu/g):
A study conducted by O'Connor et al. (2006) was used to determine the distribution of *Salmonella* in faecal material in the lairage from three different pens in a swine abattoir. Each pen contained a different distribution of bacteria, the distributions followed Normal, left-skewed and right-skewed patterns for each of the three pens in the same lairage. We therefore chose a Uniform distribution to represent this parameter in our model. Data from this study was also used to determine the minimum and maximum levels of the Uniform distribution, which were 10cfu and 38,000cfu respectively. From our quantitative studies, counts of *Campylobacter* ranged from 200 – 240,000cfu/g of faeces and for *E. coli*, we detected counts from 100 to 13,000cfu/g. As for *Salmonella*, Uniform distributions were used to describe the concentrations of the other pathogens in faecal material.

Transmission Parameter for *Salmonella* in the lairage:
This parameter was derived using maximum likelihood estimates. Data from three studies on the transmission of *Salmonella* in pig lairages were used to estimate this parameter (Hurd et al. 2001, Larsen et al. 2003, Larsen et al. 2004). The literature provided information on the number of animals becoming infected and the number of animals remaining susceptible, on completion of their time in the lairage. The likelihood estimate determines the probability that data results are obtained, for a given parameter, that is: Likelihood = P(Data results, for a given parameter). The parameter of interest is the transmission parameter. For ease of calculation, the equation is natural log (ln) transformed to produce a log likelihood estimate. The equation for the log likelihood estimate is:

$$ln[(1-e^{-\lambda t})^{I} nf (e^{-\lambda t})^{S}] \tag{3.10}$$

where *Inf* is the number of infected animals at the end of the time in the lairage, *S* is the number of susceptible animals at the end of the time in the lairage, β is the transmission parameter and *t* is the time spent in the lairage. The probability that a pig does not become infected is $e^{-\lambda t}$, while the probability of a pig becoming being infected is $1 - e^{-\lambda t}$.

Using the data provided by the studies, we calculated the log likelihood estimate for each study using an estimate for the transmission parameter. The sum of all three log likelihood estimates corresponding to each study is obtained. Using Excel solver, the value for λ which corresponds to the maximum value of the total log likelihood was obtained. The value for λ was determined to be 0.001/minute.

Killing section

Proportion of faecal material on each pig:

Firstly, we calculated the average surface area of the pigs using the equation developed by Kelley et al. (1973):

$$SurfaceArea = 734(BodyWeightinkg)^{0.656} \tag{3.11}$$

From our observational studies, the weight of pigs ranged from 58.4kg – 86.2kg. Therefore the surface area of pigs ranged from approximately 10,550cm^2 – 14,000cm^2. Assuming that the body of the pig is in the shape of a box, and that the underside is primarily in contact with the lairage floor and faecal material, we assume that approximately one quarter of the surface area can be contaminated with faecal material. We further assume that the underside of each pig has a maximum faecal carriage capacity of 0.1g/cm^2. Therefore, if a pig remained for 1 day in the lairage, we calculated the range of proportions of faecal material using the maximum quantity of faecal contamination a pig can carry the amount of faecal material shed in the lairage, assuming the pen contained 50 pigs (from the previous parameter) as 0.001 – 0.004. If however, a pig remains for only 30 minutes in the lairage, and assuming a faecal carriage of one tenth of the maximum (at most), we calculated the range of proportions of faecal material (assuming the pen contained 50

pigs) as 0.01 – 0.005. These two calculated proportions capture the range of proportions of faecal material on each pig. We therefore used a Beta distribution with α = 2 and β = 500 to represent the range of above proportions as determined using the BetaBuster software program.

Assuming one quarter of the above calculated surface areas of pigs can be contaminated with faecal material, we calculate that 2637.5–3500cm^2 can potentially carry faecal material in the lairage. Using the assumed maximum faecal carriage value of 0.1g/cm^2, we estimate that 263.75 –350g are present on pigs. The estimated amount of faecal material shed by each pig in the lairage (previously described) in 24 hours is 1987.2–6998.4g. This approximates to a maximum of 4 –18% of faecal excreted by each pig in a day that can be attached to its underside in the lairage.

Scalding section

Parameters used for scalding are described in detail in the following chapter. In summary, the rate of bacterial inactivation from the pig surface (τ_1) and in the water (τ_2) were estimated from decimal reduction time values (*D*-values) using the following equations:

$$\begin{aligned} \tau_1 &= -ln0.1/t_{D1} \\ \tau_2 &= -ln0.1/t_{D2} \end{aligned} \tag{3.12}$$

where t_{D1} and t_{D2} represent the *D*-values of pathogens per minute on the surface of the pig carcasses and in the scald tank water respectively. The *D*-value is the time required to achieve a $\log_{10}$ reduction of the organism at a fixed temperature, and these are extrapolated from the literature. We used a temperature of 60°C. For *Salmonella*, data from Murray et al. (2003) and Murphy, Beard, Martin, Duncan & Marcy (2004) were used to determine the rate of bacterial inactivation on the pig. This is further elaborated in Chapter 4. Data from Nguyen et al. (2006) was used to establish parameter values for the rate of bacteria inactivation from the pig surface with respect to *Campylobacter* and data from Murphy, Beard, Martin, Duncan & Marcy (2004) was used for *E. coli*. Data from Bolton et al. (2003), D'Sa & Harrison (2005) and Blackburn et al. (1997) were used to estimate the parameters for the rate of bacterial inactivation in the water for *Salmonella*, *Campylobacter* and *E. coli* respectively.

Values for α were extrapolated from data presented by Notermans & Kampelmacher (1974) on attachment rates of *E. coli* to chicken skin at temperatures ranging from 0.6°C

– 40°C. We assumed the attachment rate to chicken skin is similar to that onto pork skin. Since there is a 0.5 fold reduction in attachment rate from 33 – 40°C for *E. coli*, assuming the reduction to be relatively constant, we estimated the attachment rate at 60°C for scalding to be approximately 0.001/minute. Fabrizio & Cutter (2004) presents an attachment experiment of *Campylobacter* onto pork skin at room temperature, which was assumed to be 21°C. Since this calculated value is approximately two fold greater than the attachment rate for *E. coli* at this temperature, we assumed that the attachment rate of *Campylobacter* to also be two fold greater than *E. coli* at 60°C. Therefore we estimated the attachment rate of *Campylobacter* at 60°C to be 0.0022/minute.

The attachment rate of *Salmonella* to chicken skin at 21°C approximates 0.012/minute (Yang & Johnson 2001). Given that *E. coli* and *Salmonella* are both gram negative, flagellated, *Enterobacteriaceae* with similar mechanisms of attachment and that there is an approximate estimated six-fold reduction in the attachment rate of *E. coli* from 21°C to 60°C in chicken skin (Notermans & Kampelmacher 1974), a similar situation is thought to apply to *Salmonella*. We therefore estimated α to be 0.002/minute for *Salmonella*.

The values for β are estimated using data from Berends et al. (1997), where scalding results in a reduction of *Enterobacteriaceae* from approximately $\log_{10}4$ to $\log_{10}2$. Assuming that this reduction is applicable only to *Salmonella* and attributable only to β, the maximum estimated value of this parameter would be 0.54 /minute. On the other hand, if we assume a one log reduction of *Salmonella* on the carcass surface during scalding; the value for β is estimated at 0.25 /minute assuming that scalding lasts 6 to 8 minutes. Parameter values for β per minute are chosen from a uniform distribution (minimum = 0.25, maximum = 0.54), with an average of 0.4 per minute, as this value varies depending on multiple factors such as the size, location and consistency of faecal material on the surface of the pig, as well as the bacterial location within the faecal material and location of bacteria attached to the pig skin. The value of 0.4/minute may be over-estimated as the extent and rate of simultaneous inactivation occurring during the scalding procedure cited by Berends et al. (1997) is unknown.

Dehairing section

Amount of faeces emanating from each pig in the dehairing machine: We assumed that at any single period in time, approximately 50% of pigs at the farm have faecal material in their rectum. After transportation and a minimum of 30 minutes in the lairage, which is

stressful to pigs, some pigs defecate and we assume that approximately 30% of pigs have faecal material in their rectum after lairage. During dehairing, faecal material is dislodged from the rectum of pigs and for the purposes of parameter estimation, we assumed that all of the faeces dislodged from pigs goes onto the dehairing machine.

Let us assume that 1000 pigs are slaughtered in a day, therefore by our calculation 300 should contribute to faecal material going onto the dehairing machine. If each of these 300 pigs outputs 10g of faecal material, there would be 3kg of faecal material on the dehairing machine at the end of the day. This amount of faecal material is too large for that seen in reality. We therefore assume that most pigs output low quantities of faecal material, less than 10g. Also, we considered that since approximately 70% of the pigs are estimated to output no faecal matter, we describe the uncertainty distribution of the amount of faeces emanating from each pig in the dehairing machine as a Gamma distribution with α = 1 and β = 1.

Rate of bacterial movement from pig to dehairing machine and rate of bacterial movement from dehairing machine to pig:
Using data from Rivas et al. (2000), which presented the quantitative change of *E. coli* on carcasses and on the machine before and after dehairing, employing the assistance of a mathematician, the previously described Equation 3.6 was solved assuming that t = 1 minute. The time of one minute was used because the pigs mentioned in this paper are Iberian pigs, which have more abundant and tougher hair than the other traditional breeds. For this calculation to determine δ and μ we assumed that every pig output infected faecal material in the dehairing machine and that the pathogen number in the faecal material (B) was a fixed number of 10cfu. The values for δ, the rate of bacterial contamination from the dehairing machine to the pig and μ the rate of bacterial contamination from the pig to the dehairing machine were therefore estimated to be 3.95/minute and 0.1/minute respectively. We assumed this to be the same for all pathogens.

Singeing section

The equation describing pathogen inactivation on carcasses during singeing is:

$$S_t = S_0 e^{-\epsilon t} \tag{3.13}$$

where S_0 and S_t are the number of bacteria on the pig carcass before and after singeing respectively, and *t* is the singeing time. Using data from Pearce et al. (2004) that presented quantitative values for *E. coli* on pig carcasses before and after singeing, we substituted these values into the above equation and estimated the inactivation rate (κ) to be 11.8/minute. We assumed that at the high temperature of singeing, *Salmonella* and *Campylobacter* would have the same inactivation rate.

Evisceration section

Probability of bacteria (cfu) on carcass skin moving to knife:

We let the probability of bacteria (cfu) on carcass skin moving to knife equate *P(A) * P(C)* where *A* is the probability that bacteria are present on the area of the carcass that would be in contact with the knife during the evisceration cut. *C* is the probability of transfer of bacteria from the carcass surface to the knife. For *A*, we assumed that all bacteria were evenly distributed on the carcass surface and therefore considering the length of the incision made during evisceration, which from our observational studies ranged from 129cm –146.1cm with an average of 137cm, and the width of the knife, which was estimated to be 1mm in width, the range of surface areas of skin in contact with the knife during evisceration was calculated. The total surface area of the pig carcass was estimated in a previous parameter determining the proportion of faecal material on each pig. Using the range of surface areas of skin in contact with the knife, and the range of total surface areas of pig carcasses, the probability that bacteria are present on the area of the carcass that would be in contact with the knife during the evisceration incision ranged from 0.001 – 0.002. This is represented by a Uniform distribution with minimum value = 0.001 and maximum value = 0.002.

For *C* a transfer probability described by a Beta distrbution (alpha = 7, beta = 25, bounded between 0 and 1) was used for *Salmonella*. We assume the same probability for *E. coli* since both pathogens are both *Enterobacteriaceae* and have similar means of attachment to surfaces. The transfer probability for *Campylobacter* is described by Beta $\sim$ (10, 26, bounded between 0 and 1) (Kusumaningrum et al. 2003). These probabilities were estimated from data indicating the percentage of transfer of *Salmonella* and *Campylobacter* from sponges to a stainless steel surface as described by Kusumaningrum et al. (2003) and the BetaBuster software program. The percentage transfer value from Kusumaningrum et al. (2003) was calculated by dividing the number of bacteria on the

stainless steel surface by the total number of bacteria in experiment, which is a proportion. Assuming this sample proportion is the same as the population proportion, a probability can be obtained. Although the sponge has a different consistency of surface from a carcass, in the absence of the ideal probability, we can obtain a reasonable estimate of the possible probabilities of transfer of bacteria occurring between a contaminated surface and the stainless steel portion of the knife.

Probability of bacteria (cfu) on knife moving on carcass skin:
This parameter is calculated from the multiplication of the probability of survival of bacteria in water at 70°C with the probability of transfer of bacteria from the knife to the skin. From Spinks et al. (2006), a minimum of a 1 $\log_{10}$ reduction in pathogen numbers is expected. In terms of probability distributions this means that the probability that an organism will survive a 1 $\log_{10}$ lethality reduction can be described using a maximum value of $1/10^1$ and an estimated minimum probability value of 0. The probability of transfer from the knife to the skin is described by Uniform $\sim$ (0.9, 1), which was derived from Kusumaningrum et al. (2003). We used the percentage transfer of bacteria of *Salmonella* and *Campylobacter* from a stainless steel surface to chicken with the application of 500g of pressure.

Probability of inactivation of bacteria (cfu) on carcass skin:
The reduction in pathogen numbers of *Salmonella* and *E. coli* between 55°C – 65°C in ground pork as determined by Murphy, Beard, Martin, Duncan & Marcy (2004) ranged from $0.002\log_{10} - 0.5\log_{10}$ and $0.5\log_{10} - 0.6\log_{10}$ respectively. The reduction in *Campylobacter* numbers at the same temperature in chicken meat was estimated from the literature to range from $0.09\log_{10} - 1.6\log_{10}$ (ICMSF 1996, Grau 1989). Assuming the reduction in ground pork and chicken meat to be the same as that on the carcass skin, we use these estimates of the extent of pathogen reduction to determine the probability of inactivation on the carcass skin. The probability that an organism will survive an x $\log_{10}$ lethality reduction is $1/10^x$. The probability of inactivation is therefore calculated as 1 - $1/10^x$. We therefore estimated the probability of inactivation for the three pathogens to be as follows:

Salmonella	: Uniform (0.05, 0.7)
E. coli	: Uniform (0.67, 0.75)
Campylobacter	: Uniform (0.18, 0.97)

Probability of inactivation of bacteria (cfu) on knife:
This parameter is estimated from the probability of survival of bacteria in water at 70°C. From Spinks et al. (2006), a minimum of a 1 $\log_{10}$ reduction in pathogen numbers is expected. In terms of probability distributions, this means that the probability that an organism will survive a 1 $\log_{10}$ lethality reduction (p) will have a maximum value of $1/10^1$ and an estimated minimum probability value of 0. The probability of inactivation of bacteria (cfu) on knife is therefore calculated as 1 - p, resulting in a range of 0.9–1.

Based on observational studies and personal communication with quality assurance abattoir personnel, we estimated the probability of faecal spillage to be 0.1. This was incorporated into the Visual Basic for Applications Macro by using the following distribution: Binomial $\sim$ (1, 0.1). The parameter incorporated spillage of faecal material on the knife used for evisceration, as well as the carcass. The proportion of carcasses stored in blast chillers that had sections of the skin removed during trimming which were indicative of resulting from faecal spillage was obtained as 0.03.

Probability of bacteria (cfu) in leaking faeces moving to knife:
First we estimated the amount of leaking faecal material on the knife from a quantitative study previously described and conducted at abattoirs in New Zealand. For this study, when faecal leakage was thought to have occurred, the knife used during evisceration was immediately wiped with a weighed piece of gauze and the difference in weights was estimated to be the amount of leaking faecal material on the knife. This procedure will only give a rough estimate of the weight of faecal leakage as the presence of small quantities of blood and fat were also present on the knife. Furthermore it was not possible to remove all the faecal material on the knife by this technique. The amount of leaking faecal material on the knife was estimated to range from 0.0125g to 0.5g. This parameter was described by a Uniform distribution with a minimum value = 0.0125g and a maximum value = 0.5g. From the available published literature we ascertained that each gram of faecal material could contain *Salmonella* per cfu distributed in the following manner:

Uniform (10, 38,000) (O'Connor et al. 2006). We therefore estimated the number of *Salmonella* (cfu) on the knife by a simple calculation: Uniform(0.25, 1) * Uniform(10, 38,000)).

Secondly, we estimated the total amount of leaking faecal material from the results of our observational studies. This is a combination of the faecal material leaked onto the carcass, and that leaked onto the knife. From another quantitative study, also previously described, we used the maximum and minimum weight of faecal material collected from carcasses, these corresponded to quantities of leaking faeces ranging from 6.6 – 19.8g. The range of the total amount of leaking faecal material therefore ranged from 0.0125g – 20.3g. We calculated the number of *Salmonella* in the total amount of faecal as (Uniform (0.0125, 20.3) * Uniform(10, 38,000)). The latter distribution representing the pathogen concentration in one gram of faecal material. The range of values for the probability of bacteria (cfu) in leaking faeces moving onto knife was obtained by dividing the first equation by possible values of the second. Similar methods were used to calculate parameter values for the other two pathogens.

Trimming section

For our model, it was assumed that the process of trimming occurred when there was visible faecal leakage onto the carcass, which was observed to be 6.6 – 19.8g of faecal material. The number of bacteria per gram of faeces is very variable, but we decided to initiate trimming when values of 19,020 cfu of *Salmonella*; 120,100 cfu of *Campylobacter* and 6,550 cfu of *E. coli* were present on the carcass surface. These trimming threshold values corresponded to the mean concentration of bacteria per gramme in infected faeces. These threshold values also correspond to faecal quantities ranging from 6.6 – 19.8g.

Probability of bacteria (cfu) on carcass skin moving to knife:
Let this equate *P(E)*P(F)* where *E* is the probability of bacteria on the region that is to be trimmed coming into contact with knife and *F* is the probability of transfer of bacteria to the knife. For *E*, we assumed the surface area in the region to be trimmed is a percentage of the total surface area described by: Uniform $\sim$ (0.004,0.006). The average size of the area trimmed as a result of faecal contamination which was determined from observational studies to be 59cm^2 (the range of the sizes of the trimmed areas was 43 – 70cm^2), which is approximately 0.004 – 0.006 of the total surface area of the halved carcass. For *F* we

used the transfer probability described by Beta (alpha = 7, beta = 25, bounded between 0 and 1) for *Salmonella* and *E. coli*, and Beta (alpha = 10, beta = 26, bounded between 0 and 1) for *Campylobacter* as previously explained (Kusumaningrum et al. 2003).

Probability of bacteria (cfu) on knife moving on carcass skin:
This is calculated as described in the evisceration section.

Probability of inactivation and removal of bacteria (cfu) on carcass skin:
Let the probability of inactivation of bacteria on the carcass skin be *P(I)*. As previously described in the parameter "Probability of inactivation of bacteria (cfu) on carcass skin", the values for *P(I)* are described as follows:

Salmonella	: Uniform (0.05, 0.7)
E. coli	: Uniform (0.67, 0.75)
Campylobacter	: Uniform (0.18, 0.97)

We let the probability of removal of bacteria on the carcass skin be *P(R)*. From (Yeh et al. 2005), we derive the approximate contamination level on carcasses to be 0.12 – 0.24 cfu/cm^2. The average surface area of the pigs was calculated from an equation developed by Kelley et al. (1973): Surface Area = 734(Body Weight in kg)$^{0.656}$. From our observational studies, the weight of pigs ranged from 58.4kg – 86.2kg. Therefore the surface area of pigs ranged from approximately 10,550 cm^2 – 14,000 cm^2. The contamination level of *Salmonella* on carcasses, in the absence of any visible faecal spillage, immediately after evisceration is expected to therefore range from 1266 – 2800 cfu. Since we estimated the total amount of faecal material spilled on the carcass to range from 6.6 to 19.8g and using our knowledge of the range of pathogen numbers that can be contained in that quantity of faecal material, which is 70 – 760,000, we estimated that 0.4% – 98% of the total surface bacteria are located in the contaminated region. Since this contaminated region is removed during trimming, *P(R)* follows the distribution: Uniform (0.04, 0.98). This percentage range was obtained by dividing the bacterial contamination from spilled faecal matter by the average of the total surface contamination on carcasses. The probability of inactivation and removal of bacteria (cfu) on carcass skin which is bounded between 0 and 1 is calculated as follows:

$$P(I) + P(R) - P(I) * P(R) \tag{3.14}$$

Using the results from our microbial carcass contamination studies, *Campylobacter* numbers ranged from 0 – 0.5 cfu/cm^2, which approximates to 0 – 7,000cfu on a carcass. Since the concentration of *Campylobacter* per gram of faecal material ranges from 200 – 240,000cfu, we estimated *P(R)* for this organism to follow the distribution: Uniform (0.16, 1). From our microbial studies, *E. coli* was isolated in numbers ranging from 0 – 212cfu/cm^2, which results in contamination levels of 0 – 2968000cfu on a carcass. For *E. coli*, *P(R)* is described by a Uniform distribution, with min = 0, max = 0.08.

Probability of inactivation of bacteria (cfu) on the knife is derived as shown in the evisceration section.

Halving section

Probability of bacteria (cfu) on carcass skin moving to knife:

We let this equate *P(G)* * *P(H)* where *G* is the probability that bacteria are present on the area of the carcass that would be in contact with the knife during halving. *H* is the probability of transfer of bacteria to the knife. For *G*, we assumed that all bacteria were evenly distributed on the carcass surface and therefore considering the length of the carcass, (which from our observational studies ranged from 137.7cm – 164.5cm with an average of 152cm), and the width of the knife, which was estimated to be 1mm in width, the range of surface areas of skin in contact with the knife during evisceration was calculated to be 12.9 –14.61cm^2. The total surface area of the pig carcass, was estimated in a previous parameter describing the proportion of faecal material on each pig (10,550 cm^2 – 14,000 cm^2). Using the range of surface areas of skin in contact with the knife, and the range of total surface areas of pig carcasses, the probability that bacteria present in the area of the carcass that would be in contact with the knife during halving is 0.001. The derivation and parameter values for *H*, the probability of transfer of bacteria has been previously described in the Trimming section (probability of bacteria on carcass skin moving to knife).

Parameter estimation for the probability of bacteria (cfu) on knife moving on carcass skin, the probability of inactivation of bacteria (cfu) on knife; and the probability of inactivation of bacteria (cfu) on carcass skin, are calculated as in the evisceration section.

Storage section

The rate of transfer of bacteria between carcasses was determined by estimating the rate of attachment of the organisms to pork surfaces. The rate of attachment of *E. coli* to broiler skin at the storage temperature of 6°C is 0.00147/minute (Notermans & Kampelmacher 1974). We assume that the attachment rate to pork skin is similar. At 21°C the attachment rate of *Salmonella* to broiler skin is four times greater than for *E. coli* (Yang & Johnson 2001, Notermans & Kampelmacher 1974) and assuming the same conditions apply at 6°C, we estimate the attachment rate of *Salmonella* during storage to be 0.00588/minute. From Atterbury et al. (2003), it was determined that the rate of attachment of *Campylobacter* to defeathered broiler skin at 4°C is 0.004/min. We assumed for the purposes of this model that the attachment to pork skin at 6°C would be the same. Additionally we determined the attachment rate for *Campylobacter* by another method. From (Fabrizio and Cutter 2004), we calculated an attachment rate of 0.007/minute to pork skin at room temperature, which is assumed to be 21°C (Fabrizio & Cutter 2004). Since this calculated value is approximately two fold greater than for *E. coli* at this temperature, again we assume that the attachment rate of *Campylobacter* to be consistently two fold greater than *E. coli* at 6°C. We therefore estimated that at storage, the attachment rate for *Campylobacter* is approximately 0.003, similar to the previous estimation. For the purposes of modelling 0.004/min was used.

Inactivation in storage was determined using data from Chang et al. (2003*a*). This article considers inactivation of pig carcasses in blast chillers in abattoirs. Two inactivation rates were determined, one for pathogen numbers less than 1000cfu, while the other is applicable when pathogen numbers exceed 1000cfu.

The data collected from abattoir visits indicated that the duration of pig carcasses in storage varied from approximately 12 to 24 hours. These values were used in the model.

Non-parametric density estimator

A non-parametric density estimator or adaptive kernel smoothing technique was used to describe the pathogen distributions output by the model from second order modelling. The kernel smoothing technique or density estimator allows visualisation of the distribu-

tion of data as a probability density function. This technique uses the following equation:

$$\hat{f}(x) = \frac{1}{n}\sum_{i=1}^{n}\frac{1}{h_i}k\left(\frac{x - x_i}{h_i}\right) \tag{3.15}$$

where x_i refers to each data point, k is the kernel function that defines the shape of the distribution, which for our plots was Gaussian and h_i depicts the smoothing parameters or bandwidths. Smoothing can be increased or decreased by enlarging or reducing the value of h_i. Over-smoothing can result in the masking or obscuring of patterns while under-smoothing can produce spurious patterns. When data are distributed in a highly variable manner, adaptive smoothing is best as it reduces over and under smoothing within the same data set by adjusting h_i. Inherent in this technique is the "edge effect". This phenomenon can result in the production of spurious patterns at data boundaries (Vidal Rodeiro & Lawson 2005, Lawson et al. 1999). This effect was minimised by modification of the kernel function to produce a boundary corrected, non-parametric, adaptive density estimator of the distribution of pathogens on the carcass post-storage. The assistance of Jonathan Marshall, a mathematician was used to produce these distributions.

B. Model worksheets; Parameters used for *Campylobacter* and *E. coli*

Table 3.7: Inputs and parameter values used in the model for *Campylobacter*.

Parameters for *Campylobacter*	Distribution	Input values	Comments/References
Lairage			
[2] Number of pigs in lairage	Normal	mean:50 sd:10	Numbers from the NZ pig abattoirs, described in Appendix
Prevalence of infected pigs in lairage	Beta	alpha:53 beta:8	(Harvey et al. 1999)
Proportion of infected pigs shedding	Beta	alpha:45 beta:56	Numbers from the NZ pig abattoirs, described in Appendix
[2] Time spent in lairage (minutes)	Uniform	min:30 max:1440	estimated in NZ
[2] Amount of faecal material shed (grams) per pig in lairage/min	Uniform	min:1.38 max:4.86	(IPPC 2006)
[2] Concentration of *Campylobacter* in infected faeces (cfu/g)	Uniform	min:200 max:240,000	Numbers from the NZ studies used, results described in Appendix
Killing			
Proportion of faecal material on each pig (cfu)	Beta	alpha:2 beta:500	estimate shown in Appendix
Scalding			
Rate of movement of bacteria from water to pig/minute	Uniform	min:0.0022 max:0.0022	(Fabrizio & Cutter 2004)
Rate of movement of bacteria from pig to water/minute		0.4	(Berends et al. 1997)
Rate of bacterial inactivated from pig surface/minute		2	(Nguyen et al. 2006)
Rate of bacterial inactivated in water/minute		8.08	Calculated from (D'Sa & Harrison 2005)
Dehairing			
Amount of faeces extruded/pig (g)	Gamma	alpha:1 beta:1	described in Appendix

Table 3.7: Inputs and parameter values used in the model for *Campylobacter*.

Parameters for *Campylobacter*	Distribution	Input values	Comments/References
Rate of bacterial movement from pig to dehairing machine		0.10	(Rivas et al. 2000)
Rate of bacterial movement from dehairing machine to the pig		3.95	(Rivas et al. 2000)
[2]Time (minutes)	Uniform	min:0.13(Borch et al. 1996)	
		max:0.25	
Singeing			
Inactivation rate/parameter		11.8	(Pearce et al. 2004)
[2]Time (minutes)	Uniform	min:0.16	(Borch et al. 1996)
		max:0.25	
Evisceration			
[1] Probability of bacteria on carcass moving to knife/cfu	Beta	alpha:10	Parameter estimation shown in appendix
		beta:26	
[1] Probability of bacteria in knife moving on carcass/cfu	Uniform	min:0.0	Parameter estimation shown in appendix
		max:0.1	
Probability of inactivation of bacteria on carcass/cfu	Uniform	min:0.18	Parameter estimation shown in appendix
		max:0.97	
Probability of inactivation of bacteria in knife/cfu	Uniform	min:0.9	Parameter estimation shown in appendix
		max:1	
[1] Probability of bacteria in leaking faeces moving to knife/cfu	Uniform	min:0.0125	Parameter estimation shown in appendix
		max:0.5	
Amount of faeces (g), leaking from carcass	Uniform	min:0.0125	Parameter estimation shown in appendix
		max:20.5	
Bacterial concentration in faeces of leaking carcass(cfu/g)	Uniform	min:200	Numbers from NZ studies used, results described in Appendix
		max:240,000	

Table 3.7: Inputs and parameter values used in the model for *Campylobacter*.

Parameters for *Campylobacter*	Distribution	Input values	Comments/References
Trimming			
[1]Probability of bacteria on carcass moving to knife/cfu	Beta	alpha:10 beta:26	Parameter estimation shown in appendix
[1]Probability of bacteria in knife moving on carcass/cfu	Uniform	min:0.0 max:0.1	Parameter estimation shown in appendix
[1]Probability of inactivation of bacteria on carcass/cfu	Uniform	min:0.18 max:0.97	Parameter estimation shown in appendix
Probability of inactivation of bacteria in knife/cfu	Uniform	min:0.9 max:1	Parameter estimation shown in appendix
Bacterial concentration in faeces of leaking carcass (cfu/g)	Uniform	min:0 max:0	
Halving			
[1]Probability of bacteria on carcass moving to knife/cfu	Beta	alpha:10 beta:26	Parameter estimation shown in appendix
[1]Probability of bacteria in knife moving on carcass/cfu	Uniform	min:0.0 max:0.1	Parameter estimation shown in appendix
Probability of inactivation of bacteria on carcass/cfu	Uniform	min:0.18 max:0.97	Parameter estimation shown in appendix
Probability of inactivation of bacteria in knife/cfu	Uniform	min:0.9 max:1	Parameter estimation shown in appendix
Bacterial concentration in faeces of leaking carcass (cfu/g)	Uniform	min:0 max:0	

Table 3.7: Inputs and parameter values used in the model for *Campylobacter*.

Parameters for *Campylobacter*	Distribution	Input values	Comments/References
Storage			
Cross-contamination: Rate of movement of bacteria between adjacent half carcass/minute		0.004	(Fabrizio & Cutter 2004, Atterbury et al. 2003)
[2]Time (hours)	Uniform	min:12	Estimated from pig abattoirs in NZ
		max:24	
Inactivation: Rate of inactivation/hour		0.08	for carcass numbers less than 1000cfu (Chang et al. 2003*b*)
		0.136	for carcass numbers greater less than 1000cfu (Chang et al. 2003*b*)

All bacterial numbers are counted in colony forming units (cfu). [1] These parameters are components of distributions which are hard coded into the Visual Basic for Applications Macro and detailed in the Appendix.

[2] Parameters representing variability.

Table 3.8: Inputs and parameter values used in the model for *E. coli*.

Parameters for *E. coli*	Distribution	Input values	Comments/References
Lairage			
[2]Number of pigs in lairage	Normal	mean:50 sd:10	Numbers from the NZ pig abattoirs used
Prevalence of infected pigs in lairage	Beta	alpha:40 beta:1	(Pesti 1979)
Proportion of infected pigs shedding	Beta	alpha:18 beta:7	Estimated from study conducted in NZ, detailed in Appendix
[2]Time spent in lairage (minutes)	Uniform	min:30 max:1440	estimated in NZ
[2]Amount of faecal material shed (grams) per pig in lairage/min	Uniform	min:1.38 max:4.86	(IPPC 2006)
[2]Concentration of *E. coli* in infected faeces (cfu/g)	Uniform	min:100 max:13,000	Estimated from study conducted in NZ, detailed in Appendix
Killing			
Proportion of faecal material on each pig (cfu)	Beta	alpha:2 beta:500	estimate shown in Appendix
Scalding			
Rate of movement of bacteria from water to pig/minute		min:0.001	(Notermans & Kampelmacher 1974)
Rate of movement of bacteria from pig to water/minute		0.4	(Berends et al. 1997)
Rate of bacteria inactivated from pig surface/minute		0.7	(Murphy, Beard, Martin, Duncan & Marcy 2004)
Rate of bacteria inactivated in water/minute		1.15	Calculated from (Blackburn et al. 1997)
Dehairing			
Amount of faeces extruded/pig (g)	Gamma	alpha:1 beta:1	described in Appendix

Table 3.8: Inputs and parameter values used in the model for *E. coli*.

Parameters for *E. coli*	Distribution	Input values	Comments/References
Rate of bacterial movement from pig to dehairing machine/minute		0.10	(Rivas et al. 2000)
Rate of bacterial movement from dehairing machine to the pig/minute		3.95	(Rivas et al. 2000)
[2]Time (minutes)	Uniform	min:0.13 max:0.25	(Borch et al. 1996)
Singeing			
Inactivation rate/minute		11.8	(Pearce et al. 2004)
[2]Time (minutes)	Uniform	min:0.16 max:0.25	(Borch et al. 1996)
Evisceration			
[1]Probability of bacteria on carcass moving to knife/cfu	Beta	alpha:7 beta:25	Parameter estimation shown in appendix
[1]Probability of bacteria in knife moving on carcass/cfu	Uniform	min:0.0 max:0.1	Parameter estimation shown in appendix
Probability of inactivation of bacteria on carcass/cfu	Uniform	min:0.67 max:0.75	Parameter estimation shown in appendix
Probability of inactivation of bacteria in knife/cfu	Uniform	min:0.9 max:1	Parameter estimation shown in appendix
*[1]Probability of bacteria in leaking faeces moving to knife/cfu	Uniform	min:0.0125 max:0.5	Parameter estimation shown in appendix
Amount of faeces (g), leaking from carcass	Uniform	min:0.0125 max:20.5	Parameter estimation shown in appendix
Bacterial concentration in faeces of leaking carcass (cfu/g)	Uniform	min:100 max:13,000	Estimated from study conducted in NZ, detailed in Appendix

Table 3.9: Inputs and parameter values used in the model for *E. coli*.

Parameters for *E. coli*	Distribution	Input values	Comments/References
Trimming			
[1]Probability of bacteria on carcass moving to knife/cfu	Beta	alpha:7 beta:25	Parameter estimation shown in appendix
[1]Probability of bacteria in knife moving on carcass/cfu	Uniform	min:0.0 max:0.1	Parameter estimation shown in appendix
[1]Probability of inactivation of bacteria on carcass/cfu	Uniform	min:0.67 max:0.75	Parameter estimation shown in appendix
Probability of inactivation of bacteria in knife/cfu	Uniform	min:0.9 max:1	Parameter estimation shown in appendix
Bacterial concentration in faeces of leaking carcass (cfu/g)	Uniform	min:0 max:0	
Halving			
[1]Probability of bacteria on carcass moving to knife/cfu	Beta	alpha:7 beta:25	Parameter estimation shown in appendix
[1]Probability of bacteria in knife moving on carcass/cfu	Uniform	min:0.0 max:0.1	Parameter estimation shown in appendix
Probability of inactivation of bacteria on carcass/cfu	Uniform	min:0.67 max:0.75	Parameter estimation shown in appendix
Probability of inactivation of bacteria on knife/cfu	Uniform	min:0.9 max:1	Parameter estimation shown in appendix
Bacterial concentration in faeces of leaking carcass (cfu/g)	Uniform	min:0 max:0	

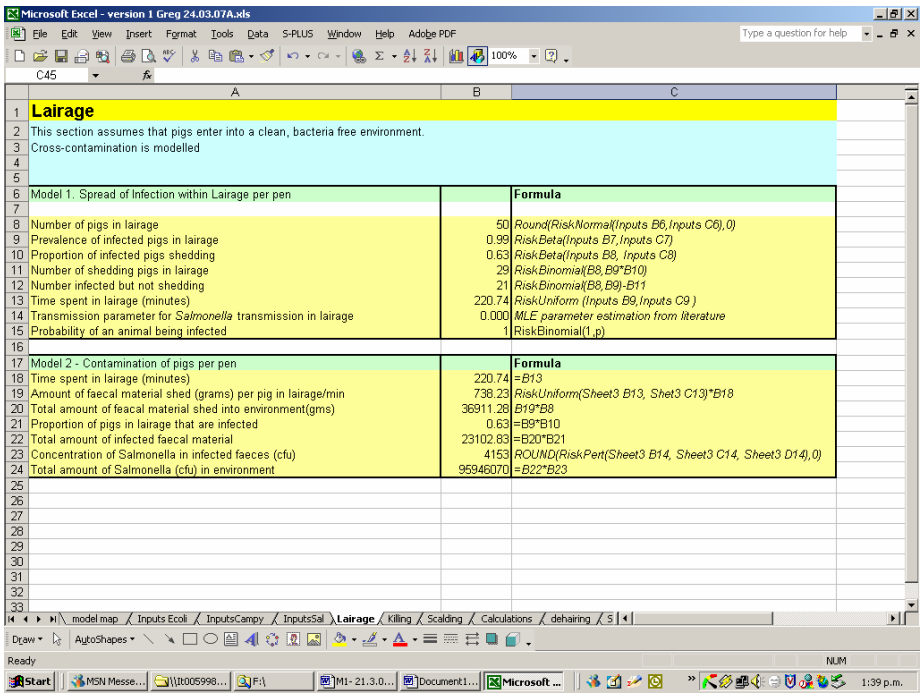
Table 3.9: Inputs and parameter values used in the model for *E. coli*.

Parameters for *E. coli*	Distribution	Input values	Comments/References
Storage			
Cross-contamination: Rate of movement of bacteria between adjacent half carcasses/minute		0.0015	(Notermans & Kampelmacher 1974)
Time (hours)	Uniform	min:12	Estimated from pig abattoirs in NZ
		max:24	
Inactivation: Rate of inactivation/hour	Uniform	0.009	for carcass numbers less than 1000cfu (Chang et al. 2003*b*)
		0.16	for carcass numbers greater less than 1000cfu (Chang et al. 2003*b*)

All bacterial numbers are counted in colony forming units (cfu). [1] These parameters are components of distributions which are hard coded into the Visual Basic for Applications Macro and detailed in the Appendix.

[2] Parameters representing variability.

This section presents the layout of the different modules of the models after one model was run for demonstration purposes.



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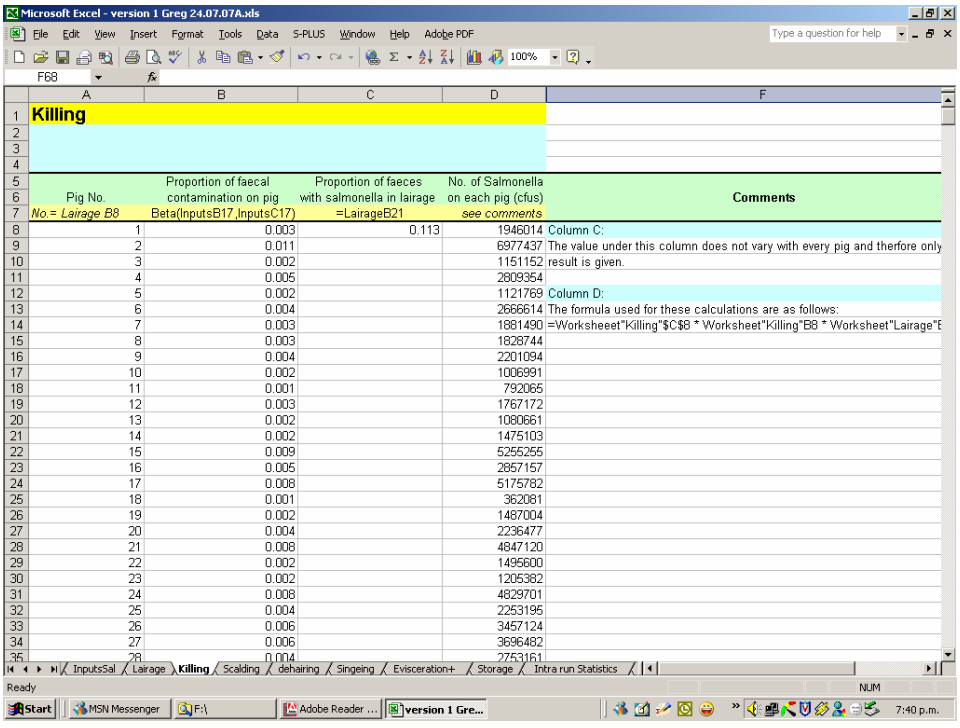
Lairage

This section assumes that pigs enter into a clean, bacteria free environment.
Cross-contamination is modelled

Model 1. Spread of Infection within Lairage per pen		Formula
Number of pigs in lairage	50	*Round(RiskNormal(Inputs B6,Inputs C6),0)*
Prevalence of infected pigs in lairage	0.99	*RiskBeta(Inputs B7,Inputs C7)*
Proportion of infected pigs shedding	0.63	*RiskBeta(Inputs B8, Inputs C8)*
Number of shedding pigs in lairage	29	*RiskBinomial(B8,B9*B10)*
Number infected but not shedding	21	*RiskBinomial(B8,B9)-B11*
Time spent in lairage (minutes)	220.74	*RiskUniform (Inputs B9,Inputs C9)*
Transmission parameter for *Salmonella* transmission in lairage	0.000	*MLE parameter estimation from literature*
Probability of an animal being infected	1	RiskBinomial(1,p)

Model 2 - Contamination of pigs per pen		Formula
Time spent in lairage (minutes)	220.74	*=B13*
Amount of faecal material shed (grams) per pig in lairage/min	738.23	*RiskUniform(Sheet3 B13, Shet3 C13)*B18*
Total amount of feacal material shed into environment(gms)	36911.26	*B19*B8*
Proportion of pigs in lairage that are infected	0.63	*=B9*B10*
Total amount of infected faecal material	23102.83	*=B20*B21*
Concentration of Salmonella in infected faeces (cfu)	4153	*ROUND(RiskPert(Sheet3 B14, Sheet3 C14, Sheet3 D14),0)*
Total amount of Salmonella (cfu) in environment	95946070	*=B22*B23*

(a)



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Killing

Pig No.	Proportion of faecal contamination on pig	Proportion of faeces with salmonella in lairage	No. of Salmonella on each pig (cfus)	Comments
No.= Lairage B8	Beta(InputsB17,InputsC17)	=LairageB21	*see comments*	
1	0.003	0.113	1946014	Column C:
2	0.011		6977437	The value under this column does not vary with every pig and therfore only
3	0.002		1151152	result is given.
4	0.005		2809354	
5	0.002		1121769	Column D:
6	0.004		2666614	The formula used for these calculations are as follows:
7	0.003		1881490	=Worksheeet"Killing"C8 * Worksheet"Killing"B8 * Worksheet"Lairage"E
8	0.003		1828744	
9	0.004		2201094	
10	0.002		1006991	
11	0.001		792065	
12	0.003		1767172	
13	0.002		1080661	
14	0.002		1475103	
15	0.009		5255255	
16	0.005		2857157	
17	0.008		5175782	
18	0.001		362081	
19	0.002		1487004	
20	0.004		2236477	
21	0.008		4847120	
22	0.002		1496600	
23	0.002		1205382	
24	0.008		4829701	
25	0.004		2253195	
26	0.006		3457124	
27	0.006		3696482	
28	0.004		2753161	

(b)

Figure 3.10: Lairage (a) and Killing (b)module of abattoir model.

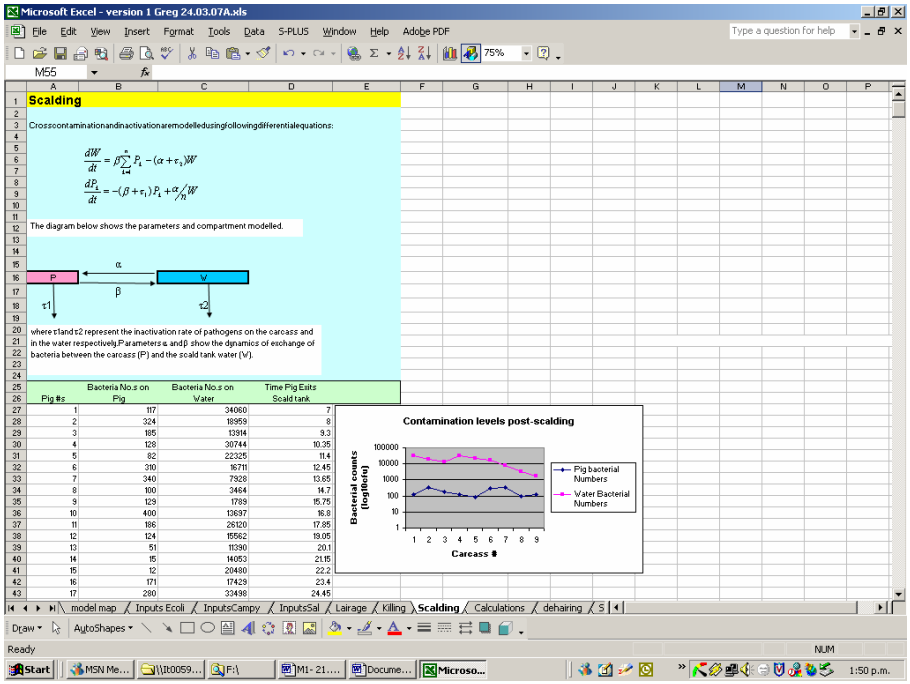


Figure 3.11: Scalding module of abattoir model.

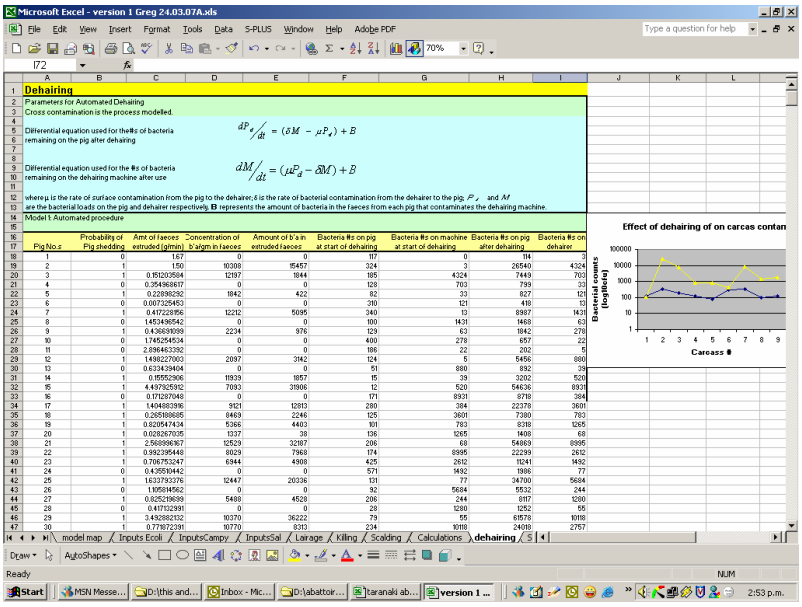
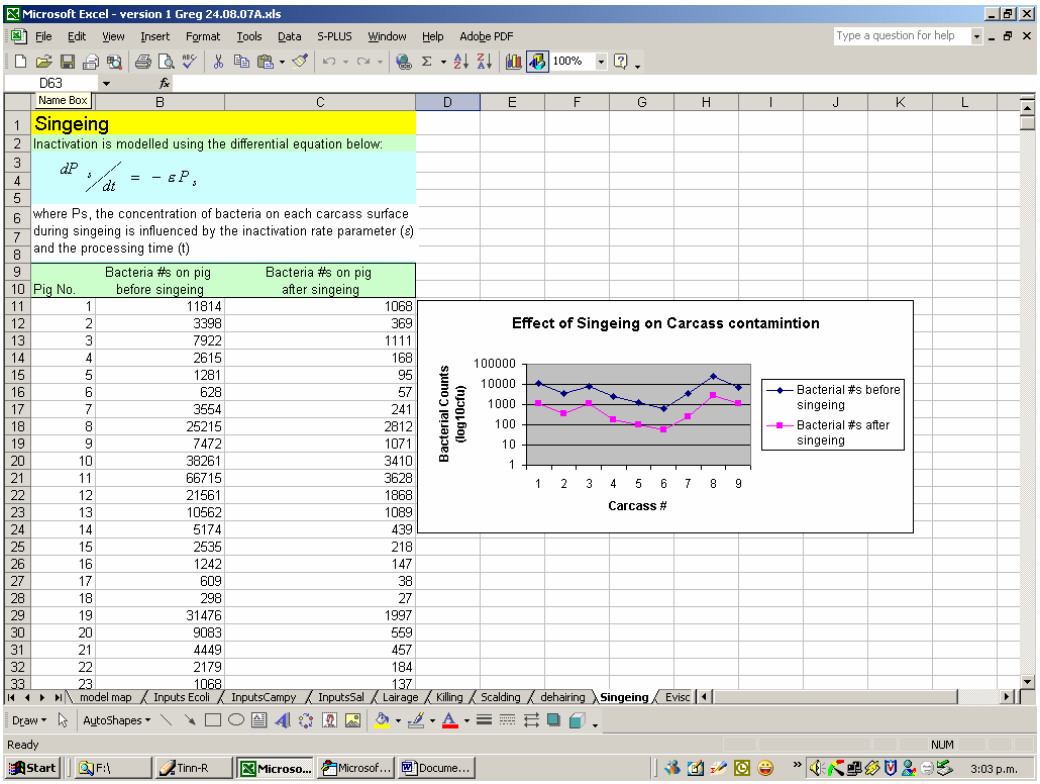
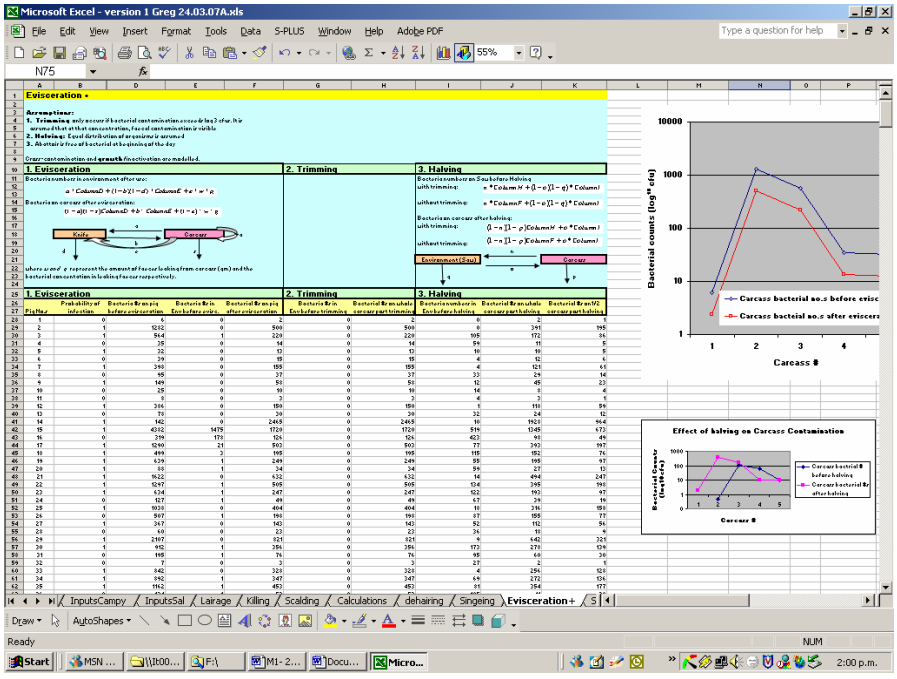


Figure 3.12: Dehairing module of abattoir model.



(a)



(b)

Figure 3.13: Singeing (a) and Evisceration (b) modules of abattoir model.

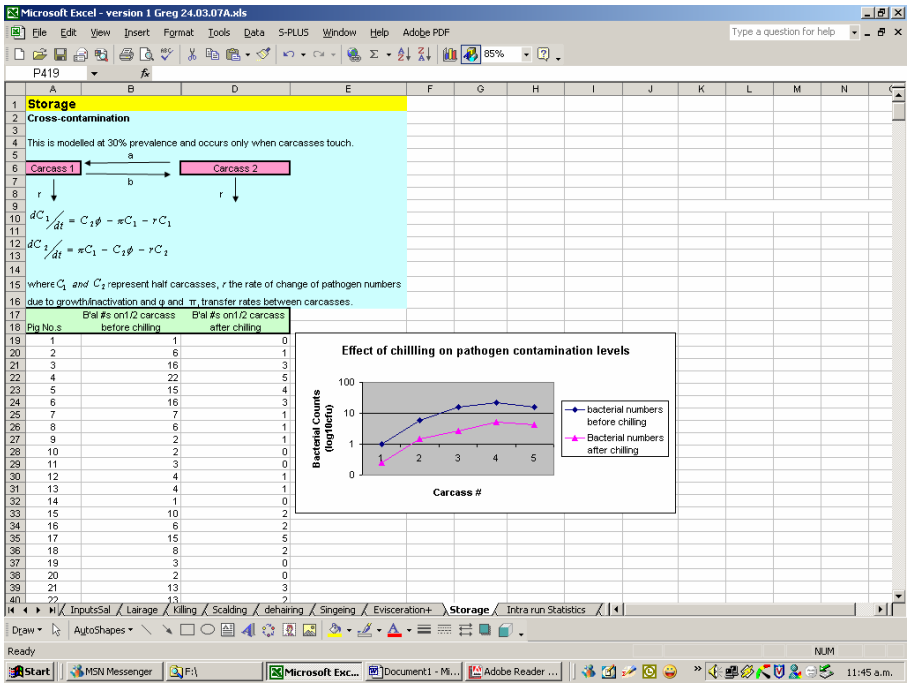


Figure 3.14: Storage module of abattoir model.

CHAPTER 4

Modelling pathogen dynamics during Scalding in the Pig Abattoir

4.1 Abstract

Salmonella contamination is a major problem in the pork industry in many developed countries. We developed a model framework for examining the quantitative effects of scalding on *Salmonella* pathogen dynamics. To this end, deterministic and semi-stochastic models of single and multiple pig scalding were used to describe explicitly the bacterial dynamics of inactivation and cross-contamination occurring between carcasses and the scald tank water. Our models capture both the movement of bacteria from the pig carcass to the water, and thermal inactivation, and identifies them as dominant processes occurring during scalding, resulting in approximately 90% reductions of carcass surface contamination levels. We demonstrate intervention strategies such as increasing scalding temperatures, extending scalding times and reducing initial contamination levels can result in 30 fold, five-fold and nine-fold reduction of *Salmonella* contamination on carcasses post-scalding. Our semi-stochastic model is sufficiently generic to be applicable to a wide range of pig pathogens and can be extrapolated for use in other species, such as chicken, sheep and goat scalding. Additionally, we propose that our semi-stochastic version of the model is applicable for use in exposure assessments, a component of quantitative microbial risk assessments, as the model outputs provide a distribution of bacterial numbers post-scalding.

4.2 Introduction

Non-typhoidal salmonellae are major food-borne pathogens (Loynachan et al. 2004, Hald et al. 2003, Giovannacci et al. 2001, Hohmann 2001, Swanenburg et al. 2001) responsible for cases of gastroenteritis, bacteremia, and death (Hohmann 2001). In the USA, salmonellosis is the second most common aetiological agent of bacterial origin causing food poisoning (Funk & Gebreyes 2004), and accounts for approximately 30% of deaths from food-borne diseases (Hohmann 2001).

Pigs are carriers of a variety of *Salmonella* species (Bolton et al. 2003, Bonardi, Brindani, Pizzin, Lucidi, D'Incau, Liebana & Morabito 2003, van der Wolf & Peperkamp 2001, Fedorka-Cray et al. 1995) and the organism can be routinely isolated from the gastro-intestinal tract, pharynx, tonsils and mesenteric lymph nodes of slaughtered pigs (Giovannacci et al. 2001, Hurd et al. 2001). Pigs acquire salmonellosis from contaminated feed, carrier pigs shedding the organism, pests such as rodents, flies and wild birds, and infected farm workers (Funk & Gebreyes 2004, Giovannacci et al. 2001). Consequently, pork and pork products are major sources of human salmonellosis in several countries (Giovannacci et al. 2001). It is estimated that approximately 10%, 15% and 20% of salmonellosis cases in Denmark, The Netherlands and Germany respectively, originate from pork consumption (Pearce et al. 2004, Hald et al. 2003). The impact of this food-borne pathogen on human health has resulted in a need to implement measures aimed at minimizing food contamination.

Abattoirs present a ready opportunity for controlling the impact of pork-derived salmonellosis on human health by reducing carcass contamination through the modification of slaughter procedures (Lo Fo Wong et al. 2002). Scalding is used in pork abattoir processing to facilitate hair removal. During this procedure, carcasses are sequentially placed in a large tank of warm water, for 6 to 8 minutes (Nauta, van der Fels-Klerx & Havelaar 2005, Bolton et al. 2003, Borch et al. 1996, Sorqvist & Danielsson-Tham 1990) and removed in similar fashion resulting in the processing of multiple pigs simultaneously in the same batch of water (Borch et al. 1996). This procedure causes dirt, faeces, ingesta and bacterial accumulation in the tank over time (Borch et al. 1996) which can serve as a source of bacterial contamination for pig carcasses (Sorqvist & Danielsson-Tham 1990, Simonsen et al. 1987).

Although research has been undertaken on the significance of scalding in pig carcass

contamination (Sorqvist & Danielsson-Tham 1990), no studies have explicitly modelled the changes in carcass surface bacterial counts during scalding to gain insight into or further our understanding of pathogen dynamics. The aim of this study therefore, is to mathematically model the events occurring in the scald tank in order to assess the effect of this process on *Salmonella* numbers on the surface of pig carcasses. In this way, the relative importance of bacterial dynamics influencing the change in pathogen prevalence and levels of carcass contamination could be quantified, allowing targeted model investigations to quickly determine the most efficient risk mitigation strategy to minimize carcass contamination during scalding.

This study presents two models describing cross-contamination of *Salmonella* between pig carcasses and the environment of the scald tank water, as well as inactivation both on carcasses and in the scalding water. The first model is deterministic, simulating bacterial dynamics when only one pig is scalded. Although this situation is oversimplified, it contributes to our understanding of pathogen dynamics between a carcass and the environment of the scald tank water. The second, parallel, semi-stochastic model quantifies pathogen dynamics when 6 to 8 pigs are scalded at the same time. This latter model reflects current commercial abattoir practices. Both models aim to provide a framework for examining the quantitative effects of scalding on pathogen dynamics.

4.3 Model Description

The Simple Model

First we construct a simple deterministic model that incorporates bacterial inactivation, and cross-contamination, the movement of bacteria between the scald tank water and the pig carcass surface, during the scalding of only one pig carcass. Figure 4.1 illustrates the structure of this model, where *P* is the number of viable bacteria on the carcass and *W* is the number of viable bacteria in the water.

Cross-contamination is modelled using two parameters: α and β, representing the *per capita* rates of movement of bacteria from the water to the pig (α) and from the pig to the water (β). Also, the coefficient rates of inactivation on the carcass and in the water are described using τ_1 and τ_2, respectively. Pathogen dynamics occurring during the single-pig scalding are described by the following coupled differential equations:

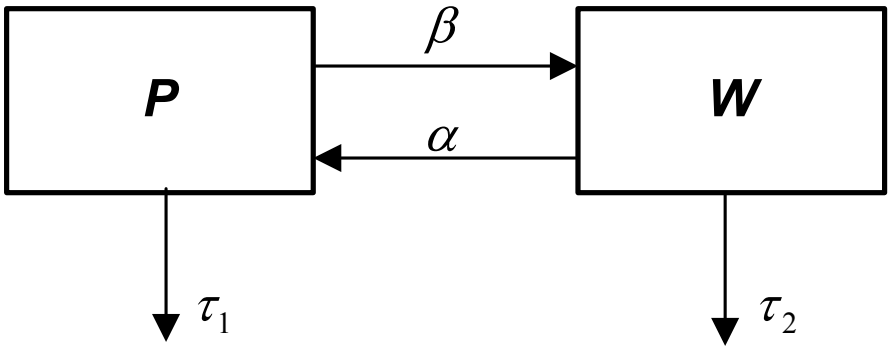


Figure 4.1: Schematic of modelled scalding processes where α represents the rate of movement of *Salmonella* from the scald tank water to the pig and β is the rate of movement from the carcass to the water. Parameters τ_1 and τ_2 which were derived from *D*-values for *Salmonella*, depict the mortality rates of the organisms on the surface of the pig and in the water, respectively.

$$\begin{aligned} \frac{dP}{dt} &= -(\tau_1 + \beta)P + \alpha W \\ \frac{dW}{dt} &= \beta P - (\tau_2 + \alpha)W \end{aligned} \tag{4.1}$$

where *dP/dt* computes the rate of change of bacterial numbers on the pig carcass, while *dW/dt* calculates the rate of change of bacterial numbers in the scald tank water. Assuming that $\tau_1 = \tau_2$, the analytical solution to Equation 4.1 is:

$$\begin{aligned} P(t) &= [(P_0\beta - \alpha W_0)e^{(-(\tau_1+\beta+\alpha)t)} + (P_0 + W_0)\alpha e^{-\tau t})]/(\beta + \alpha) \\ W(t) &= [(W_0\alpha - \beta P_0)e^{(-(\tau_1+\beta+\alpha)t)} + \beta e^{-\tau t}(P_0 + W_0))]/(\beta + \alpha) \end{aligned} \tag{4.2}$$

where P_0 and W_0 depict bacterial numbers on a pig carcass and in the water at the start of scalding and *t* represents time. The analytical solution to Equation 4.1 for $\tau_1 \neq \tau_2$ is

provided in the Appendix and is used to describe the change in carcass and water bacterial levels during scalding. This model is used to investigate the dynamics of *Salmonella* spp. on scalding for 8 minutes, at different temperatures (55°C and 60°C). Firstly, we evaluate pathogen dynamics when a carcass with an initial surface contamination of 1,000 colony forming units (cfu) is placed in a scald tank with 1 cfu of *Salmonella*. Secondly, we predict pathogen dynamics when a non-contaminated carcass is scalded in water with an initial pathogen level of 10,000 cfu.

From Equation 4.1, we derive threshold values with respect to the pig and water for the following stated conditions, when $\tau_1 \neq \tau_2$: (i) when a dynamic equilibrium exists during cross-contamination, that is, between bacterial flux on and off the pig (i.e., $\beta P = \alpha W$), in which case the first threshold applicable to the pig and water is as follows:

$$P = \alpha W/\beta \tag{4.3}$$

Movement of pathogens from the water onto the pig dominates when $P < \alpha W/\beta$ and conversely, pathogen movement from the pig to the water is dominant when $P > \alpha W/\beta$; (ii) The second threshold pertaining to the pig carcass applies when the rate of loss of pathogens from inactivation on the pig ($\tau_1 P$) equals the net cross-contamination or bacterial flux from the carcass into the water ($\beta P - \alpha W$). Accordingly, the second pig threshold is described by 2 equations:

$$P = \alpha W/(\beta + \tau_1) \tag{4.4}$$

$$P = \alpha W/(\beta - \tau_1) \tag{4.5}$$

These will be called thresholds 2a and 2b respectively. The choice of equation usage is determined by the dominant pathogen process occurring with regards to threshold (i) (the net pathogen flux on and off the pig). So in the case where there is a net pathogen movement from the water onto the pig, $P > \alpha W/(\beta + \tau_1)$ indicates that cross-contamination on the pig dominates inactivation and $P < \alpha W/(\beta + \tau_1)$ corresponds to the process of inactivation dominating on the pig (Table 4.1). However, when there is a net bacterial flux from the pig to the water, inactivation on the pig is dominant under conditions where $P > \alpha W/(\beta - \tau_1)$, while cross-contamination on the pig predominates when $P < \alpha W/(\beta - \tau_1)$.

For thresholds pertaining to the water, the first threshold is the same as that for the pig. The other water threshold occurs when the rate of loss of pathogens from inactivation in the water ($\tau_2 W$) equals the net flux into the water ($\beta P - \alpha W$), and is described by:

$$P = (\alpha - \tau_2)W/\beta \tag{4.6}$$

$$P = (\alpha + \tau_2)W/\beta \tag{4.7}$$

which we will call threshold 3a and 3b respectively. Equation use for this water threshold is determined by the dominant process of bacterial flux on and off the pig carcass (Threshold 1). Consequently, when there is net pathogen movement from the water to the pig, inactivation in the water predominates and is described by $P > (\alpha - \tau_2)W/\beta$. On the other hand, cross-contamination in the water dominates inactivation when $P < (\alpha + \tau_2)W/\beta$. In the case where pathogen movement from the pig to the water is dominant, inactivation is the primary process occurring in the water when $P > (\alpha + \tau_2)W/\beta$, while cross-contamination is dominant in the water when $P < (\alpha + \tau_2)W/\beta$. Table 4.1 provides details of the thresholds used in our model.

The Parallel Semi-Stochastic Model

The dynamic, parallel, semi-stochastic model quantifies bacterial levels during the simultaneous scalding of multiple carcasses (with a maximum of eight pigs at any one time). For this model we assume that carcasses enter and exit the scald tank at a rate of one/minute. Modification of the this rate would influence pathogen levels in the water and on the pig, however, this was not investigated in the model.

Using the same model structure as presented in Figure 4.1, the model equations for the parallel semi-stochastic model are as follows:

$$\begin{aligned} \frac{dP}{dt} &= -(\tau_1 + \beta)P_k + (\alpha/n)W \\ \frac{dW}{dt} &= \beta \sum_{k=1}^{n} P - (\tau_2 + \alpha)W \end{aligned} \tag{4.8}$$

for *n* pigs (from P_1 to P_n), where P_k and *W* are the number of viable bacteria on the k^{th} pig carcass and in the water, respectively. The term α/n represents the rate of

Table 4.1: Threshold values and corresponding dominant pathogen processes in scalding.

Pig Dynamics:	
Threshold 1: $P = \alpha W/\beta$	
When $P < \alpha W/\beta$	Net flux from water to pig
When $P > \alpha W/\beta$	Net flux from pig to water
Threshold 2: (a)$P = \alpha W/(\beta + \tau_1)$ and (b) $P = \alpha W/(\beta - \tau_1)$	
For (a) $P < \alpha W/(\beta + \tau_1)$	Cross-contamination dominates on pig
$P > \alpha W/(\beta + \tau_1)$	Inactivation dominates on pig
For (b)$P < \alpha W/(\beta - \tau_1)$	Inactivation dominates on pig
$P > \alpha W/(\beta - \tau_1)$	Cross-contamination dominates on pig
Water Dynamics:	
Threshold 1: $P = \alpha W/\beta$	
When $P < \alpha W/\beta$	Net flux from water to pig
When $P > \alpha W/\beta$	Net flux from pig to water
Threshold 3: (a)$P = (\alpha - \tau_2)W/\beta$ and(b) $P = (\alpha + \tau_2)W/\beta$	
For (a)$P < (\alpha - \tau_2)W/\beta$	Cross-contamination dominates in the water
$P > (\alpha - \tau_2)W/\beta$	Inactivation dominates in the water
For (b)$P < (\alpha + \tau_2)W/\beta$	Inactivation dominates in the water
$P > (\alpha + \tau_2)W/\beta$	Cross-contamination dominates in the water

movement of bacteria from the water to the pig for each carcass and as in the previous model, β, τ_1 and τ_2 represent the rate of movement of bacteria from the carcass to the water and bacterial death rates on the carcass and in the water, respectively.

For this model, we demonstrate the effect of cross-contamination and inactivation during scalding for eight minutes, on two populations of pigs (A and B) with differing initial surface contamination levels: (1) Population A: Normal $\sim$ (mean = 1000, standard deviation (s.d.) = 300) cfu and (2) Population B: Lognormal $\sim$ (mean = 1000, s.d. of the naturally log-transformed data = 1000) cfu (Hurd et al. 2001, Fedorka-Cray et al. 1994, Wood & Rose 1992). Since it is common procedure to clean and sanitize abattoirs at the end of each day's operations, in this paper we assume that the scald tank and water are pathogen free at the commencement of each day's activities. Since there was no addition of water to the scald tank at any of the plants visited during operations, the effect of water replacement was not included in the model. Personal communication with senior employees of abattoirs in New Zealand revealed that additives such as sodium hydroxide are sometimes added to the scald tank water to enhance or whiten the colour of the skin of pigs. However the effect of the addition of these additives, which would change the pH of the scald tank and may result in increased pathogen inactivation was not included in the model.

Two sensitivity analyses are conducted to indicate the parameters and initial values most influential in determination of the predicted model output (Anonymous 2004*b*). For both analyses 10,000 model iterations employing Latin hypercube sampling of distributions for parameters and variables are used to perform a rank order correlation between output values and their associated inputs. The first sensitivity analysis explores the importance of the parameters and time on the model output. The values of the initial contamination level on the pig carcass and water are kept constant at 1000 cfu and 1 cfu, respectively. Uniform distributions are used for all parameters and time. Assigned values for the inactivation parameters (τ_1 and τ_2) range from that corresponding to 55°C (minimum) to 62°C (maximum) (Table 4.2) and time values range from 6 to 12 minutes. Parameter values ranging from 0.0001 to 0.003 per minute and 0.25 to 0.54 per minute are used for α and β, respectively. The second sensitivity analysis explores the effect of the initial contamination level on the pig carcass and water, on the model output. For this analysis, parameters values and time are held constant assuming a temperature of 60°C.

We assume values of 0.002 per minute for α and 0.4 per minute for β. Initial pathogen levels in the water and on carcasses are assumed to be Uniform $\sim$ (minimum = 0, maximum = 2000) and Normal $\sim$ (mean = 1000, s.d. = 300), respectively.

Initial values and parameters identified by the sensitivity analyses are then manipulated to investigate the efficacy of different control measures on minimizing carcass contamination during scalding.

Parameter Estimates

Both models contain two compartments: the pig carcass surface and water and two variables: (i) the number of viable Salmonellae on the pig carcass (*P*) and (ii) the number of viable organisms in the scald tank water (*W*). Four parameters (α, β, τ_1, τ_2) describing the rates of movement of *Salmonella* between compartments (α, β) and thermal death rates within compartments (τ_1, τ_2) are also included (Figure 4.1). Parameter values for τ_1, and τ_2 are estimated from decimal reduction time values (*D*-values) using the following equations:

$$\begin{aligned} \tau_1 &= -ln0.1/t_{D1} \\ \tau_2 &= -ln0.1/t_{D2} \end{aligned} \tag{4.9}$$

where t_{D1} and t_{D2} represent the *D*-values of *Salmonella* per minute on the surface of the pig carcasses and in the scald tank water respectively. The *D*-value is the time required to achieve a $\log_{10}$ reduction of the organism at a fixed temperature and these are extrapolated from the literature for τ_1 and τ_2. For each inactivation parameter, *D*-values of *Salmonella* in the appropriate media over a range of temperatures are sourced from the literature and used to plot exponential trendlines that best described the data. The equations producing these trendlines, which are then used to determine *D*-values at 55°C, 60°C and 62°C are as follows:

$$\begin{aligned} t_{D1} &= 4e^{-0.4013T}10^{10} \\ t_{D2} &= 2e^{-0.403T}10^{11} \end{aligned} \tag{4.10}$$

where T is the temperature between 50°C and 62°C. Estimated parameter values of τ_1 are derived from *D*-values of *Salmonella* on poultry skin and ground pork (Murphy, Beard, Martin, Duncan & Marcy 2004, Murphy, Martin, Duncan, Beard & Marcy 2004, Bonardi, Brindani, Pizzin, Lucidi, D'Incau, Liebana & Morabito 2003). Thermal death rates for τ_2 are estimated from studies of *D*-values of *Salmonella* in the scald tank (Bonardi, Brindani, Pizzin, Lucidi, D'Incau, Liebana & Morabito 2003).

Values for α and β are unknown as there is insufficient data in the literature. However we estimated these parameters using the following assumptions. Firstly, we assumed homogenous mixing of bacteria in the scald tank and that the rate of attachment of *E. coli* to chicken skin is assumed to be similar to pork skin. The attachment rate of *Salmonella* to chicken skin at 21°C approximates 0.012/minute (Yang & Johnson 2001). Given that *E. coli* and *Salmonella* are both gram negative, flagellated, *Enterobacteriaceae* with similar mechanisms of attachment and that there is an approximate estimated six-fold reduction in the attachment rate of *E. coli* from 21°C to 60°C in chicken skin (Notermans & Kampelmacher 1974), a similar situation was assumed to apply to *Salmonella*. We therefore estimated a minimum value of α per minute to be 0.001, with a maximum value of 0.003 per minute, and an average of 0.002 per minute over the temperature range 55°C – 62°C. Our estimated value of α does not include pathogens in the water which are on the pig carcass as it is removed from the scald tank. Parameter values for β per minute are chosen from a uniform distribution (minimum = 0.25, maximum = 0.54), with an average of 0.4 per minute, as this value varies depending on multiple factors such as the size, location and consistency of faecal material on the surface of the pig, as well as the bacterial location within the faecal material and location of bacteria attached to the pig skin (Table 4.2). The values for β are estimated using data from Berends et al. (1997), where scalding results in a reduction of *Enterobacteriaceae* from approximately $\log_{10}4$ to $\log_{10}2$. Assuming that this reduction is applicable only to *Salmonella* and attributable only to β, the maximum estimated value of this parameter would be 0.54 /minute. On the other hand, if we assume a one log reduction of *Salmonella* on the carcass surface during scalding; the value for β is estimated at 0.25 /minute assuming that scalding lasts 6 to 8 minutes.

Model Implementation

Models are executed in Microsoft Excel with Palisade @Risk 4.5 (Palisade Corporation, 2005) and a Visual Basic for Applications Macro. For the semi-stochastic model, one simulation consists of 10 iterations of a number of pigs sampled from a zero truncated Normal distribution (mean=50, standard deviation=10), using Latin Hypercube sampling. In this way, a single iteration mimics the events occurring in the scald tank for a batch of pigs from one farm. A simulation approximates pathogen dynamics for pigs scalded in a day from a hypothetical abattoir in New Zealand. Sensitivity analyses are executed using Palisade @Risk 4.5 (Palisade Corporation, 2005).

4.4 Results

Deterministic model

The deterministic model, which assumes that pigs are contaminated with 1000 cfu predicts a large reduction of viable pathogens on the carcass surface and variable pathogen levels in the water during scalding at 55°C and 60°C (Figure 4.2). Scalding at 60°C for 8 minutes results in minimal post-scalding carcass contamination with only 2 cfu of viable pathogens remaining on the carcass compared to 29 cfu when the scald tank was set to 55°C. Bacterial numbers in the water peaked at 447 cfu and 126 cfu and diminished to 251 cfu and 1 cfu for scalding at 55°C and at 60°C, respectively. Our deterministic model also predicts that clean carcasses scalded in water containing 10,000 cfu remain relatively clean, as bacteria numbers on the pig increase to 1 cfu during scalding at 55°C but remain at 0 cfu when scalding is conducted at 60°C.

We evaluate the dominant processes occurring during scalding under different conditions. Cross-contamination, and more specifically the movement of bacteria off the carcass into the water is predicted by our model to be the dominant pathogen dynamic occurring at both 55°C and 60°C, when the carcass contamination level is high and that in the water is low (Figure 4.3). In the water, cross-contamination predominates first and is subsequently replaced by inactivation, which occurs for a longer period of time at 60°C. The net bacterial flux is from the pig carcass to the water therefore pig thresholds 2b and 3b are employed.

When the bacterial load in the water is high and the carcass contamination low, conditions satisfy use of both thresholds 2a and 2b. Our model predicts cross-contamination as the main pathogen process on the carcass at both temperatures, while inactivation predominates in the water (Figure 4.4). At 55°C, there is a brief period of inactivation as the pathogen flux onto and off the carcass nearly equate.

The sensitivity analysis performed on all parameters highlights τ_1 (death rate on the pig) and time as the most influential factors in determining carcass contamination levels post-scalding. The most significant determinants of pathogen levels in the water are the death rates of *Salmonella* (both on the carcass and in the scald tank) and time (Figure 4.5). The second sensitivity analysis indicated that the initial contamination levels of carcasses are more important than the initial contamination levels in the water, in determining the final bacterial levels both on the carcass and in the water at 60°C.

The the deterministic model uses an analytical solution and the semi-stochastic model uses the Runge-Kutta approximation method for solving differential equations.

Intervention Strategies

Based on the results of the sensitivity analysis, inactivation rates, scalding times and initial values of pig carcasses are varied to assess their effect on reducing carcass *Salmonella* levels post-scalding (Table 4.3). Scalding times of 8, 10 and 12 minutes are evaluated, in addition to varying initial pig carcass contamination levels which are sampled from 3 different distributions: right skewed (Lognormal); left skewed (triangle) and not skewed (normal). Inactivation rates are varied by changing the temperature at which the process occurs. Consequently, we examined the post-scalding contamination levels when scalding is conducted at 55°C, 60°C and 62°C. Our model predicts that lower initial values of carcass contamination, coupled with extension of scalding times and increased pathogen death rates (τ_1 and τ_2), would result in reduced carcass contamination levels post-scalding, with the latter being most effective in minimizing the bacterial load on the carcass. These interventions can result in *Salmonella* carcass contamination diminishing nine-fold, five-fold and 30 fold respectively. The effect of extending scalding time is more evident at the lowest scalding temperature of 55°C. Further, lower initial values of pig contamination, as seen in the right skewed distribution contribute to lower post-scalding contamination levels.

The Parallel Semi-Stochastic Model

This model predicts considerable inactivation of surface carcass contamination during scalding irrespective of the initial pathogen population distributions (Figures 4.6 to 4.8). Generally, scalding at 60°C produces carcasses that are less contaminated than those scalded at 55°C (Figures 4.7 and 4.8). An approximate ten fold increase in carcass pathogen levels was predicted when the scalding temperature was decreased from 60°C to 55°C as well as an estimated five-fold increase in pathogen levels in the water. Pathogen numbers in the water during the scalding of carcasses with an initial contamination originating from a distribution of large variance (Population B: Lognormal $\sim$ (1000,1000) cfu), produces a wider range of and records higher contamination levels than carcasses in which initial surface contamination levels are sampled from smaller variance distributions of Normal $\sim$ (1000,300) cfu. Despite the intermittent higher pathogen levels predicted in the water during scalding of population B, carcass contamination levels post-scalding from this group are similar to post-scalding contamination levels from population A. Scalding at 55°C and 60°C failed to alter the distribution of pathogen contamination on carcasses, while it did reduce the pathogen numbers by 93% and 99%.

Figure 4.7 shows the pathogen load in the water when 250 pigs are scalded for approximately 4.5 hours at 55°C, with new pigs introduced approximately every minute. The model predicts that the bacteria numbers in the water generally fluctuates within a range of 350–725cfu.

Table 4.2: Parameter and initial values used in scalding models.

Paramater/Initial value	Deterministic model	Semi-stochastic model
α	0.002/min	0.002/min
β	0.4/min	0.4/min
τ_1	0.05/min[a]	0.05/min[a]
	0.37/min[b]	0.37/min[b]
	0.82/min[c]	0.82/min[c]
τ_2	0.22/min[a]	0.22/min[a]
	1.6/min[b]	1.6/min[b]
	3.7/min[c]	3.7/min[c]
P_0	1000 cfu	A: Normal $\sim$ ($\log_{10}3$,$\log_{10}2.27$) cfu[d]
		B: Lognormal $\sim$ ($\log_{10}3$,$\log_{10}3$) cfu[d]
W_0	1 cfu;10000 cfu	0

[a] Values for scalding at 55°C
[b] Values for scalding at 60°C
[c] Values for scalding at 62°C
[d] These refer to two populations of pigs:Population A and Population B.

Table 4.3: Effect of adjusting parameter and variables on carcass contamination levels post-scalding for 400 pigs.

Temperature (τ) (τ)	Scalding time (t)	Final no. on pig (DM)	Final no. on pig (ND)	Final no. on pig (LD)	Final no. on pig (TD)
55(τ_1:0.05,τ_2:0.22)	8	29	30(6 – 57)	6(0 – 694)	54(3 – 86)
	10	12	13((2 – 23)	5(0 – 355)	23(3 – 38)
	12	5	6(2 – 9)	3(0 – 106)	11(2 – 17)
60(τ_1:0.37,τ_2:1.60)	8	1	2(0 – 4)	4(0 – 7)	4(0 – 6)
	10	0	0(1 – 1)	0(0 – 2)	1(0 – 1)
	12	0	0(0 – 2)	0(0 – 2)	0(0 – 0)
62(τ_1:0.60,τ_2:3.70	8	0	0(0 – 1)	0(0 – 4)	1(0 – 1)
	10	0	0(0 – 0)	0(0 – 1)	0(0 – 0)
	12	0	0(0 – 0)	0(0 – 0)	0(0 – 0)

DM: Deterministic model, initial carcass contamination level of 1000cfu
Parallel model, median values with corresponding range (in brackets) reported. Initial contamination level on all pigs sampled from Normal $\sim$ (1000, 300)cfu (ND); Lognormal $\sim$ (500, 100) cfu (LD) and Triangular $\sim$ (0, 2000, 3000)cfu (TD).

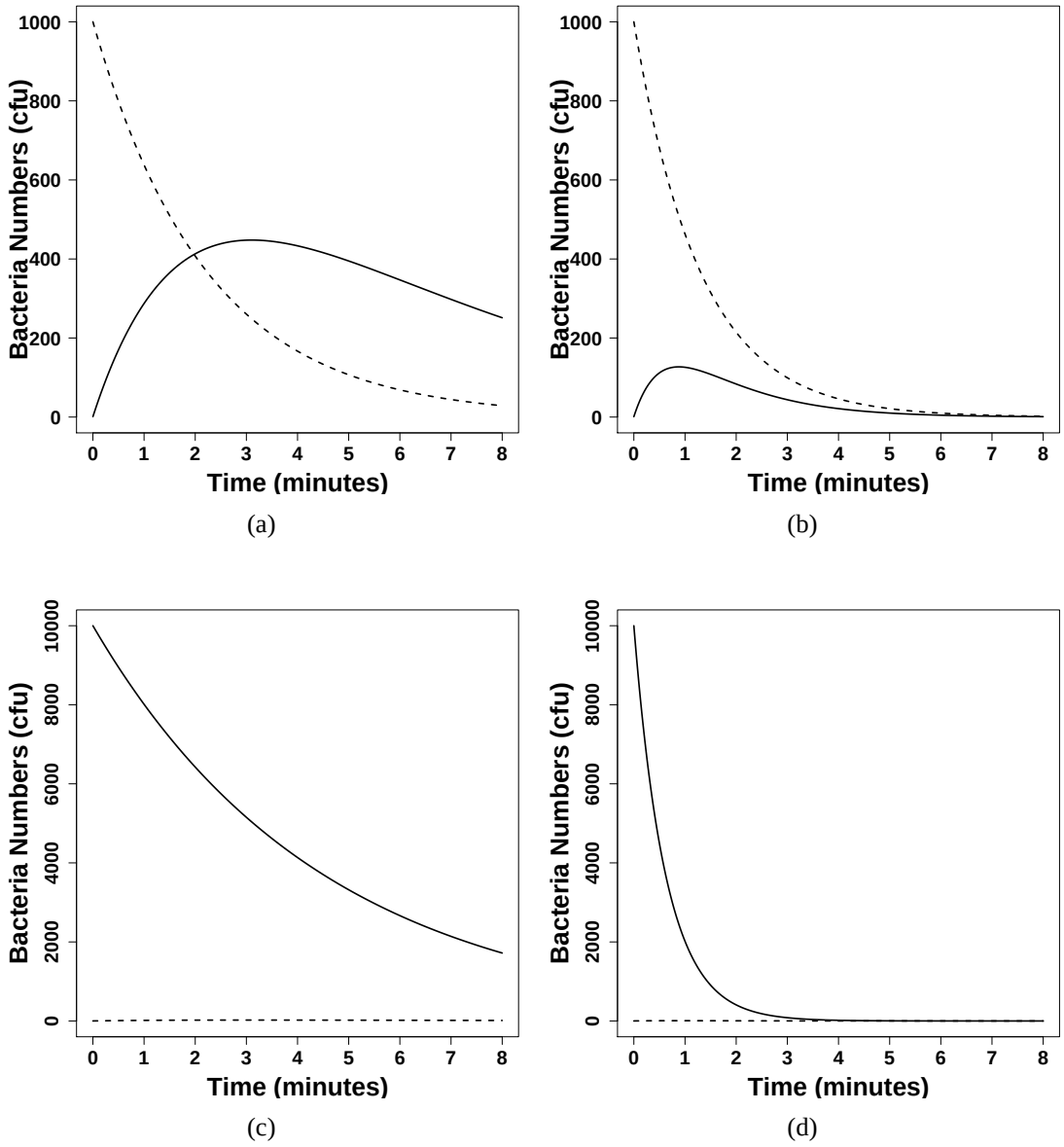


Figure 4.2: Rate of change of pathogens on the carcass (dashed line) and in the water (solid line) during scalding at 55°C (a and c) and 60°C (b and d). Plots a and b demonstrate scalding of a carcass with an initial surface contamination level of 1,000 cfu. Plots c and d show scalding of a non-contaminated carcass in water containing 10,000 cfu of *Salmonella*.

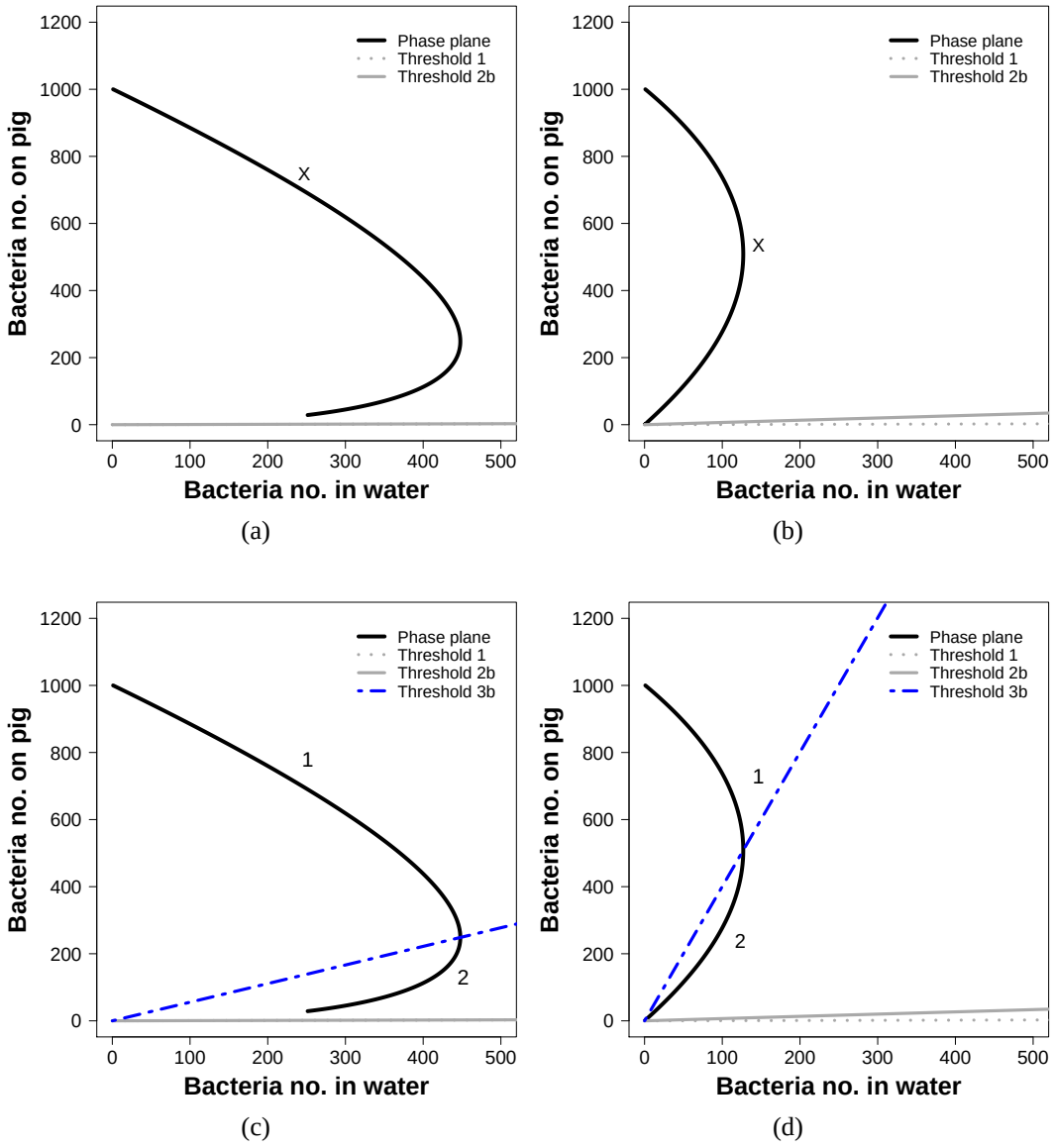


Figure 4.3: Phase plane plots showing pathogen dynamics at 55°C (a and c) and 60°C (b and d) for a carcass contaminated with 1000 cfu scalded in water initially containing a pathogen load of 1 cfu. Pig and water threshold levels delineate dominant regions of bacterial cross-contamination (X) on the pig and cross-contamination (1) and inactivation (2) in the water.

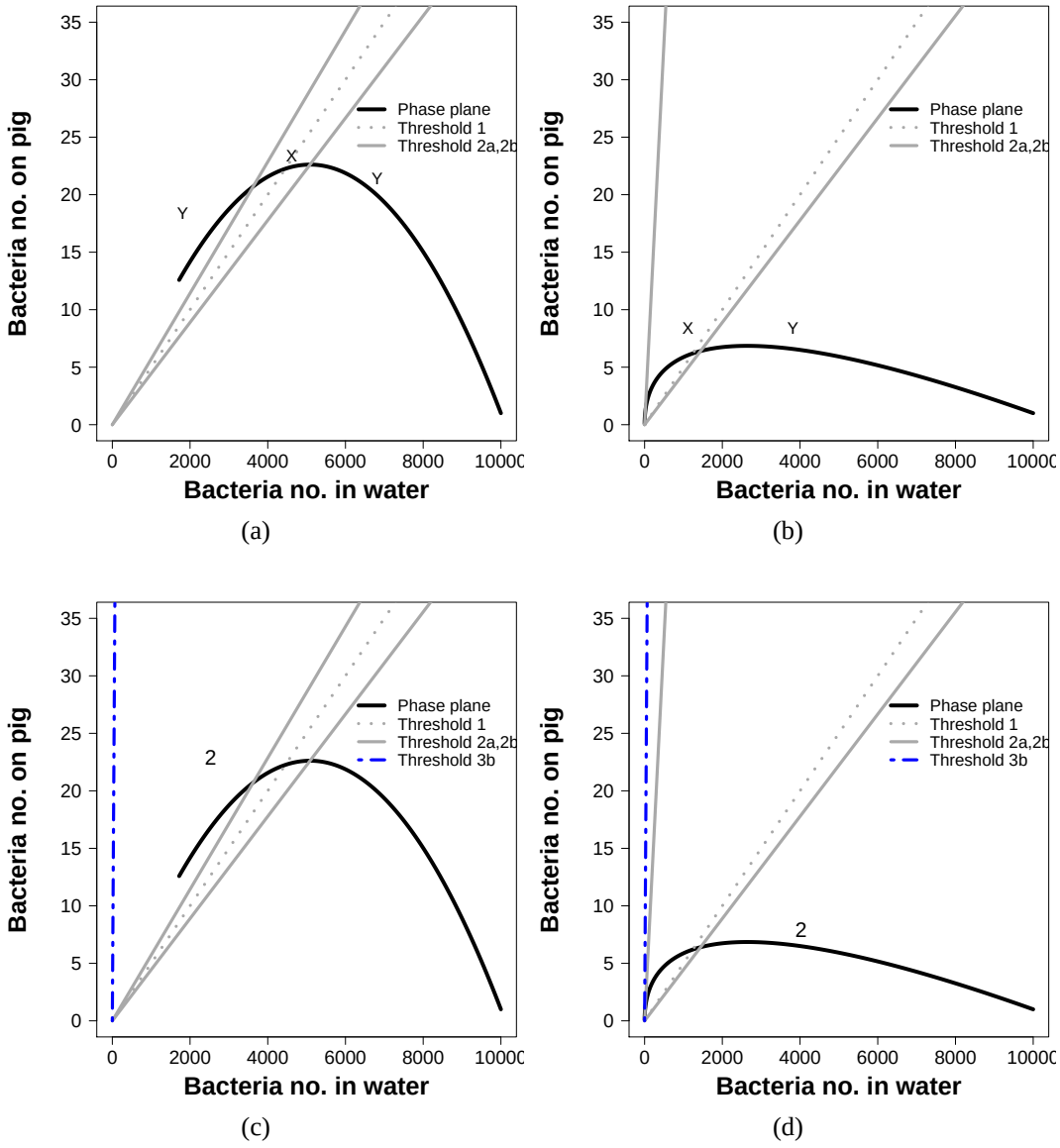


Figure 4.4: Phase plane plots showing pathogen dynamics at 55°C (a and c) and 60°C (b and d) for a non-contaminated carcass scalded in water initially containing 10,000 cfu of *Salmonella*. Pig and water threshold levels delineate dominant regions of bacterial cross-contamination (X) and inactivation (Y) on the pig and inactivation (2) in the water.

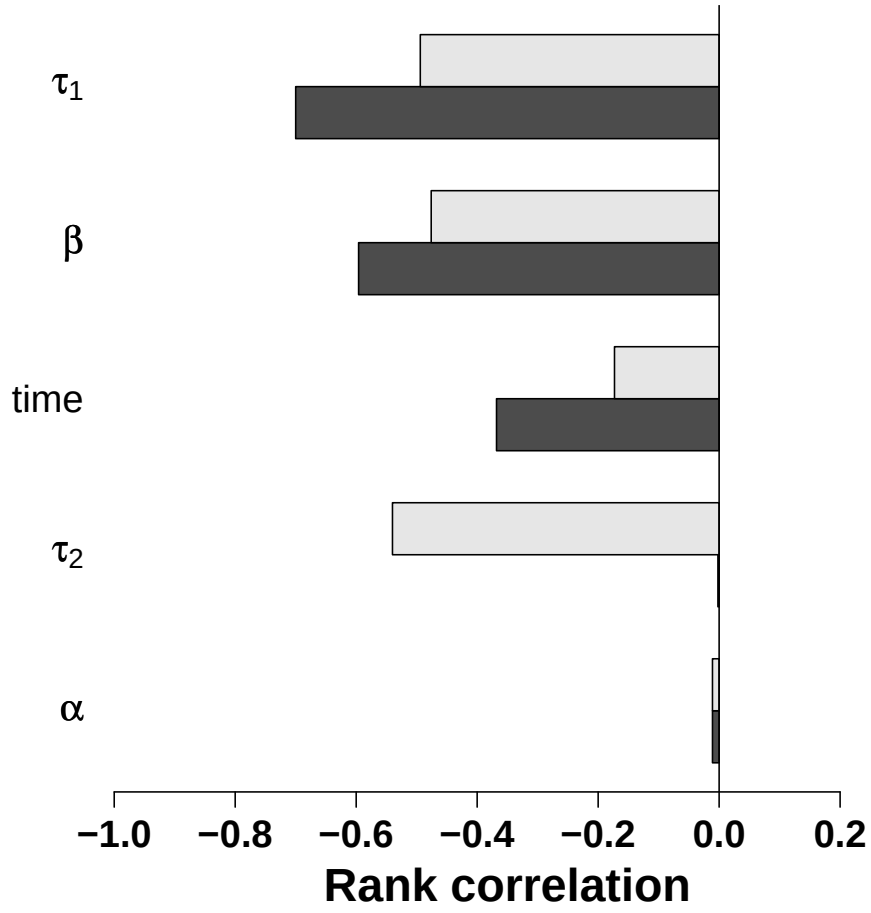
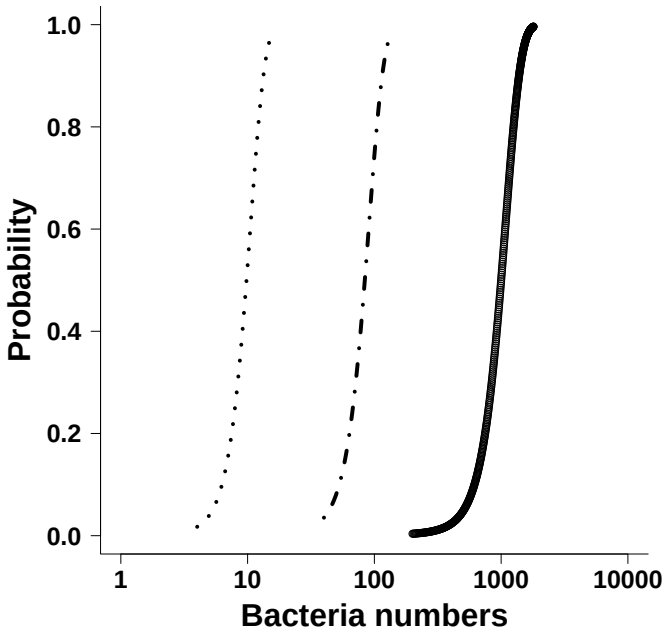
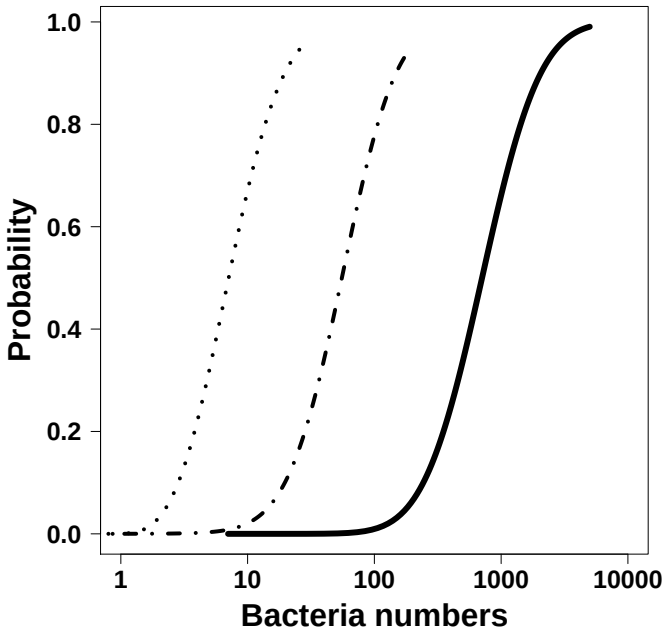


Figure 4.5: Tornado plot of sensitivity analyses results for the parallel, semi-stochastic scalding model. The relative importance of predictive factors on final bacterial contamination levels on the pig (black) and in the water (grey) at temperatures ranging from 55°C to 62°C inclusive is presented.



(a)



(b)

Figure 4.6: Cumulative distributions of *Salmonella* on carcasses with (a) surface bacterial levels sampled from Normal $\sim$ (1000, 300) and (b) surface bacterial levels sampled from Lognormal $\sim$ (1000, 1000). Dotted, dashed and solid lines represent post-scalding at 62°C, 55°C and pre-scalding respectively.

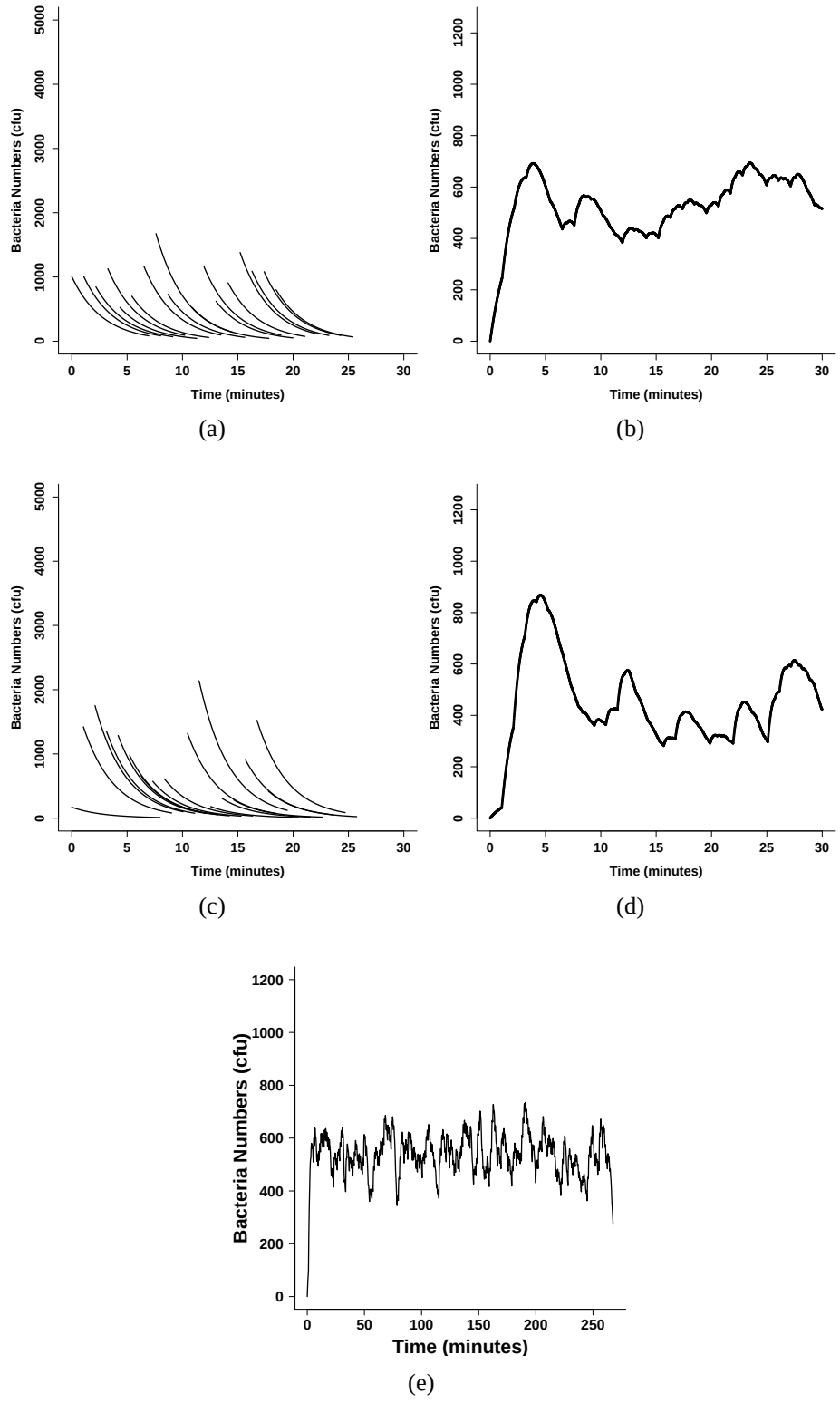
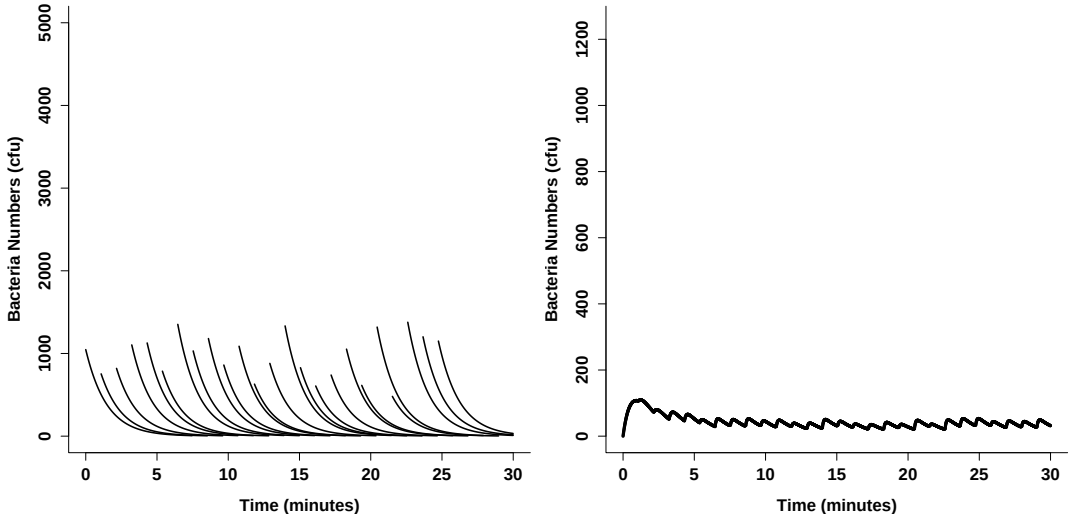
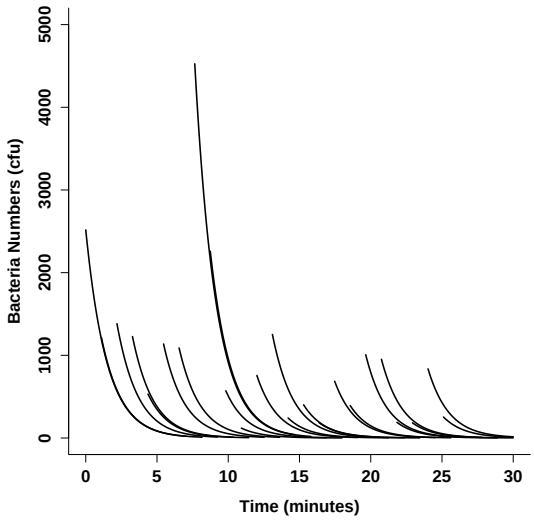


Figure 4.7: Time Series of predicted contamination levels on pig carcasses (a and c) with their corresponding changes in the scald tank water (b and d) at 55°C. Initial bacterial contamination levels on pigs were sampled from Normal $\sim$ (1000, 300), (a and b) and Lognormal $\sim$ (1000, 1000) (c and d). Pathogen levels in the water during the scalding of 250 pigs for 4.46 hours at 55°C with an initial bacterial contamination levels on pigs sampled from Normal $\sim$ (1000, 300) is shown (e).

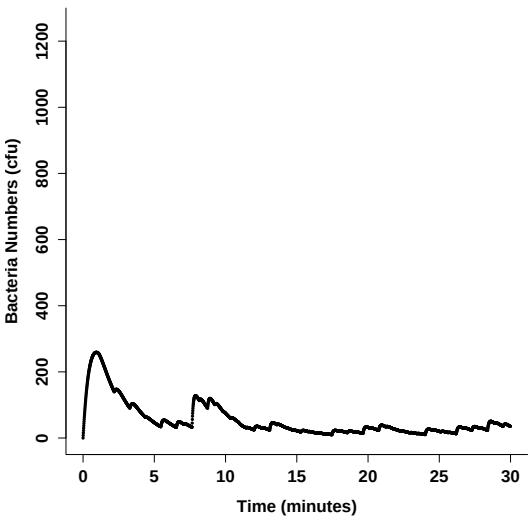


(a)

(b)



(c)



(d)

Figure 4.8: Time Series of predicted contamination levels on pig carcasses (a and c) with their corresponding changes in the scald tank water (b and d) at 60°C. Initial bacterial contamination levels on pigs were sampled from Normal $\sim$ (1000, 300), (a and b) and Lognormal $\sim$ (1000, 1000) (c and d).

4.5 Discussion

This study is the first to describe and evaluate the scalding of pig carcasses in abattoirs by modelling inactivation and cross-contamination processes. Although our models are parameterized for *Salmonella* spp., they are sufficiently generic to be used for specific *Salmonella* strains and other enteric, food-borne, zoonotic, microbes harboured by pigs as well as enteric, zoonotic organisms pertinent to chickens, sheep and goats that undergo scalding during abattoir processing. Input distributions and parameter values will require modification to represent the pathogen and strain under consideration and these can vary depending on farm, transport and abattoir management practices, as well as country prevalence.

Pig scalding has been implicated as an unhygienic abattoir process (Berends et al. 1997), facilitating contamination of the carcass surface, pluck and vasculature (Hald et al. 2003, Jones et al. 1984, Jones et al. 1979). However, both our deterministic and semi-stochastic models indicate that endpoint carcass contamination levels appear to be inversely proportional to temperature and predict that scalding at both 55°C and 62°C considerably reduces carcass surface contamination levels. This ability of scalding to reduce bacterial loads on the pig carcass was also reported by Gill & Bryant (1992) and Sorqvist & Danielsson-Tham (1990). Our models predict that increasing scalding from 55°C to 60°C results in lower bacterial numbers in the scald tank water. This has implications on contamination levels of carcasses exiting the abattoir. Hald et al. (2003) demonstrated that the presence of *Salmonella* in both the scald tank water and pluck led to an increased probability of detection of *Salmonella* on pig carcasses after abattoir processing. Accordingly, scalding at higher temperatures has definite beneficial effects on pig carcass contamination levels immediately after scalding and for carcasses leaving the abattoir. Also, our single pig model predicts a $\log_{10}3$ reduction in the pathogen level on the pig carcass. A similar decrease in carcass pathogen load was also observed a microbial study on pig scalding by Rivas et al. (2000). Our semi-stochastic model however demonstrated a $\log_{10}2$ reduction in the pathogen level on the pig carcass, this is probably because our model does not consider increased pathogen death in the water as a result of additives usually placed in the scald tank water. Another possible explanation is that the model over-estimates carcass contamination arising from the continuous release of pathogens from the frequent introduction of pigs to the scald tank.

The pathogen contamination levels in the scald tank water when the scalding procedure occurs at 55°C does not increase steadily over time. For situations in which carcasses with high carcass contamination levels enter the scald tank containing low pathogen numbers, our model predicts cross-contamination to be the dominant process on the pig and in the water for a large portion of the scalding process. However the reverse is predicted for carcasses with low contamination levels, entering a highly contaminated scald tank — inactivation predominates on both the carcass and in the water. Since pig carcasses with variable contamination levels are introduced into the scald tank water with variable pathogen loads, the dynamics of inactivation and cross-contamination are complex, with a mixture of both inactivation and cross-contamination occurring at different times. The pathogen levels in the scald tank eventually fluctuate within a range, showing neither a steady increase nor decrease in pathogen levels.

One novel feature of this study is the insight it provides into pathogen dynamics in relation to the pig carcass and water during scalding with the use of threshold values. Our model predicts that for highly contaminated carcasses entering a relatively clean scald tank, the increased bacterial death at higher scalding temperatures of 60°C is primarily a result of increased pathogen death in the water, rather than on the pig. We further deduce that cross-contamination, not inactivation on the carcass is the dominant pathogen process. On the other hand, when the initial bacterial load in the water is high and the carcass is free of contamination pre-scalding, carcasses may exit scalding contaminated at 55°C as a consequence of cross-contamination of pathogens from the water. At the higher temperature of 60°C, increased bacterial inactivation in the water reduces the number of viable bacteria available for movement onto the pig and this probably accounts for the lowered carcass post-scalding contamination level. These calculated thresholds which highlight the dominant processes are highly dependent on α, β and τ_1 parameter values. For inactivation to be a dominant process, the following equation must apply: $\beta > \tau_1$. Unfortunately determination of accurate values for α and β may be time-consuming, but once estimated, pathogen dynamics can be predicted. In cases where flux into the water is dominant, additives can be placed in the water to increase destruction of detached pathogens. This may not be necessary under conditions where carcass bacteria inactivation is dominant. Elucidating pathogen dynamics can therefore result in cost-effective measures to reduce carcass contamination levels post-scalding.

Extension of scalding times from 8 to 12 minutes can result in a definite improvement in carcass quality with respect to the microbial load. This influence of time is less noticeable at higher temperatures and may be explained by the increasing dominant effect of the inactivation rate which increases with rising temperature. Consequently, extending the scalding time may be un-necessary if the death rate values on the pig and in the water are increased. Furthermore, much caution needs to be taken as scalding for prolonged periods of time can result in cooking of the outer surface of the carcass. The carcass then becomes friable and renders other abattoir processes difficult to perform. Nevertheless, it may be possible for abattoirs to scald dirty pigs for longer periods of time if possible, as a control strategy for *Salmonella* species.

Increasing pathogen death rates (τ_1 and τ_2) by elevating scalding temperature was found to be the most effective strategy in minimizing post-scalding contamination levels. Temperature has a considerable effect on the inactivation of *Salmonella* both on the carcass and in the water. Bolton et al. (2003) demonstrated that the *D*-value of *Salmonella* strains isolated from pigs at 60°C is 1.4 minutes and at 65°C is 0.18 minutes. Other factors found to influence *Salmonella* survival in the scald tank water include scalding pH (which can be altered through the addition of chemical agents to the water (Humphrey 1981)), carcass immersion intervals and the number of organisms entering the tank (Bolton et al. 2003, Borch et al. 1996). This last factor corresponded to our initial carcass contamination level variable. All factors impacting on *Salmonella* survival and consequently *D*-values, will lead to changes in τ_1 and/or τ_2, and therefore impact model outputs.

Other options available to management to minimize carcass surface contamination levels post-scalding include methods of reducing the initial carcass contamination levels pre-scalding. These can include the washing of pigs prior to processing by showering or use of a hose. We observed this practice being performed in slaughter houses in New Zealand and as our model predictions and sensitivity analysis indicate, may have more of an impact on scalding at lower temperatures. Logistic slaughter has been considered as an option to minimize carcass contamination during abattoir processing (Swanenburg et al. 2001). In this method, clean or less contaminated pigs are processed prior to dirty or more contaminated pigs. This procedure aims to prevent dirty pigs from contaminating subsequent less dirty carcasses. However, the usefulness of this technique may be ques-

tionable when scalding temperatures are greater than or equal to 55°C, according to our model predictions. When we compared the final carcass contamination levels of the two populations of pigs (A and B) post-scalding using our model, there was little difference. This may suggest that when scalding occurs at 55°C and higher, a highly contaminated pig does not result in elevated contamination levels of subsequent pigs. Logistic slaughter however, may have a greater impact on bacterial levels in water and consequently, the quality of pork carcass exiting the abattoir.

Data needed to inform the model included distributions of surface contamination levels on pigs. In New Zealand this data is not readily available and targeted quantitative investigative microbial studies in abattoirs are required for model validation. Pilot studies indicated that these contamination levels are relatively low. However, determination of numerical values for low contamination levels is challenging. Several methods are available for quantitative analyses, but these tests invariably exhibit reduced accuracy and are less sensitive in detecting bacteria at low concentrations. Determination of the detection limit of tests can therefore be important.

There is some uncertainty surrounding model parameters and consequently model predictions. If the values of α and β were remarkably different from that proposed, the outputs of the model pathogen dynamics may differ. Further work is needed to validate the models, therefore despite our model results being consistent with much of the current literature, model outputs should be received with caution. Nevertheless, our models provide a good indication of pathogen dynamics during scalding and contamination levels post-scalding.

Our model did not incorporate the addition of bacteria sometimes extruded from the anus of the pig in faeces, nor the fact that these organisms possess increased thermal resistance as a result of the buffering effect of the organic material, such as dirt, blood and faeces (Sorqvist & Danielsson-Tham 1990, Grosklaus & Lessing 1964). Organic material may create a microenvironment for the bacteria and alter the scald water pH, both of which impact pathogen survival (Sorqvist & Danielsson-Tham 1990, Humphrey 1981). This resistance to inactivation displayed by these microbes may lead to under-estimation of cross-contamination levels and under-estimation of inactivation rates by our model. Our study nevertheless did address and incorporate the increased thermal resistance of *Salmonellae* on the carcass surface (Humphrey et al. 1984).

It can be concluded that the parallel model presented here can be used to model scalding of pig carcasses. Large reductions in carcass surface contamination result from pathogen inactivation and cross-contamination when this process occurs between temperatures of 55 – 62°C. However, the extent of post-carcass contamination levels is inversely proportional to scalding temperature as a result of an over-riding flux of bacteria from the pig carcass to the water and bacterial inactivation. These models provide insight into pathogen dynamics occurring during scalding under different conditions. Since the parallel, semi-stochastic model outputs the distribution of the numbers of bacteria on the carcass post-scalding, it can be used in exposure assessment models of quantitative risk microbial assessments. Furthermore our models facilitate assessment of intervention strategies targeted at minimizing contamination during scalding. As such, the parallel semi-stochastic model has great potential to be used as a tool in the assessment of food safety management practices in pig abattoir processing.

4.6 Appendix

Analytical Solution

The analytical solution to Equation 4.1 when $\tau_1 \neq \tau_2$ is as follows:

$$P(t) = \frac{0.5}{\sqrt{\Phi}}[P_0((\sqrt{\Phi} - B)\Delta(t) + (\sqrt{\Phi} + B)\Psi(t) - 2\alpha W_0(\Psi(t) - \Delta(t))] \quad (4.11)$$

$$W(t) = \frac{0.5}{\sqrt{\Phi}}[2\beta P_0(\Delta(t) - \Psi(t)) + W_0((\sqrt{\Phi} + B)\Delta(t) + (\sqrt{\Phi} - B)\Psi(t))] \quad (4.12)$$

where $\Phi = (\tau_1 + \beta)^2 + (\tau_2 + \alpha)^2 - 2(\tau_1 \tau_2 + \tau_1 \alpha + \beta \tau_2 - \alpha \beta)$

$$\Delta(t) = e^{-[(A-\sqrt{\Phi})/2]t}$$

$$\Psi(t) = e^{-[(A+\sqrt{\Phi})/2]t}$$

$$A = \tau_1 + \beta + \tau_2 + \alpha$$

$$B = \tau_1 + \beta - \tau_2 - \alpha$$

CHAPTER 5

An example of assessing model uncertainty

5.1 Abstract

Risk modelling can be conducted using a variety of approaches including the individual-based and population-approximation methods. In this paper a comparison of these two approaches was undertaken to determine the conditions under which the population-approximation approach is an appropriate alternative to the individual-based method. For this purpose, the two modelling approaches were used in a stochastic quantitative risk model describing the pathogen dynamics of *Salmonella* in a pig abattoir during the trimming procedure.

Different input distributions of Lognormal and zero-inflated Poisson distributions were used for the comparative analysis. These distributions described theoretical pathogen contamination levels on the surface of pig carcasses prior to trimming. We show that the population-based approach is an appropriate alternative to the individual-based technique when zero-inflated Poisson input distributions are used, as both approaches predicted similar results. However, when pre-trimming contamination levels were described by Lognormal distributions with large variances, the predicted outputs from the population-based approach and individual-based methodology varied considerably. Therefore under these conditions the population-based approach it is not an appropriate alternative to the individual-based technique.

We therefore conclude that the population-based method, although useful in reducing computation time, failed to adequately capture the complex dynamics of cross-contamination when there was a high degree of between carcass heterogeneity with respect to carcass contamination levels.

5.2 Introduction

There are several techniques available for modelling exposure assessments in quantitative microbial risk assessments. These include process risk models, modular process risk models (MPRM), and Bayesian belief networks. Process risk models (PRM) synthesize both scenario analysis and predictive microbiology to describe the dynamics of pathogens through the different stages of the food pathway that result in food-borne disease (Cassin et al. 1998). The modular process risk model technique requires compartmentalisation of the food pathway of concern into a series of different units called modules. Within each module any one or more of six "processes" of bacterial growth and inactivation, as well as partitioning, removal, cross-contamination and mixing can be identified and explicitly modelled. This technique facilitates evaluation of the effect of pathogen dynamics on contamination levels on the food product of interest. The outputs from this technique consist of probability distributions describing bacterial numbers on the food of concern as well as the prevalence of bacteria in the food (Parsons et al. 2005, Nauta et al. 2003, Barker et al. 2002, van Gerwen et al. 2000, Cassin et al. 1998). Bayesian belief networks are probabilistic, directed, acyclic graphs demonstrating relations between uncertain variables. These variables are graphically depicted as nodes, with lines indicating the relationship between nodes. Nodes are assigned probabilities describing the relationship between input and output variables (Parsons et al. 2005). Bayesian belief models are executed using appropriate software packages which output probability distributions representing beliefs concerning the investigated pathogen.

Modelling the propagation of microbes throughout the food-pathway using MPRM can be executed using the population-approximation (Nauta, van der Fels-Klerx & Havelaar 2005) and individual based methods. The latter method is tedious and requires modelling the microbe(s) under investigation for each food unit of interest in the pathway. Examples of the food unit of interest can be carcasses in the abattoir or selected portions of meat in further processing. On the other hand, the population-based approach describes the relationship between the predicted model outputs with respect to bacteria numbers and prevalence, as a function of the inputs, at a population level. This is referred to as the input-output relationship at the population level. The population-based approach is computationally less demanding than the individual-based approach. Nauta, van der Fels-Klerx & Havelaar (2005) used the population-based approach in an exposure assessment

model predicting *Campylobacter* numbers in chickens in The Netherlands. To accomplish this, pathogen dynamics on the chicken, in the environment and between the chicken and the environment were described. During model development, it was mathematically determined that an equilibrium soon existed, resulting in equal numbers of pathogens entering and exiting the environment. The existence of this equilibrium therefore is one assumption associated with the population-based approximation modelling technique.

The appropriate use of the individual and population methodologies can help reduce model uncertainty. Uncertainty with respect to mathematical models is defined as the lack of knowledge about the modelled system, which is reducible by conducting further research or engaging in data gathering procedures (Vose 2000). Model uncertainty arises from the lack of knowledge about, or inappropriate model structure, such as misspecification or oversimplification, or inappropriate use of distributions or equations, such as those representing the individual and population-based methodologies, in the design of the mathematical model (Haas et al. 1999).

Whereas both techniques have been used in modelling, little research has been undertaken to investigate model uncertainty pertaining to these two methods, that is, to compare the two approaches and determine the conditions under which the population-approximation approach is an appropriate alternative to the individual-based method. To address this, a model describing the propagation of *Salmonella* species through the pig abattoir is used to compare the outputs given by the individual and population-based approaches under varying conditions. This evaluation would enable more realistic risk estimates to be obtained through the judicious application of the population-approximation based approach. For simplicity, we restricted our comparison of the two techniques to the single abattoir procedure, trimming. The paper begins with an explanation of the modelled section of the pig slaughter house, followed by a description of the modelling techniques evaluated. Model results are then presented.

5.3 Model Description

Pork abattoir processing consists of several procedures in which pigs are stunned, killed, hung on a production line and subjected to a series of procedures or stages. We focus on the trimming stage. During this stage, occasional intestinal spillage resulting from

evisceration is removed by manually cutting away contaminated regions. Knives, which are used to remove the undesirable regions of the carcass during trimming are frequently dipped in warm water to increase bacterial death and minimise transference to other surfaces. Although the trimming process is designed to reduce carcass contamination levels, it can also result in contamination of equipment, which in turn can lead to contamination of other subsequently processed carcasses (Alban & Stärk 2005). Consequently, the bacterial processes of cross-contamination and inactivation are both modelled during trimming.

In this paper two modelling approaches are investigated. Both approaches use difference equations to describe pathogen dynamics, as the trimming process is considered to occur in discrete time. The trimming model consists of two compartments: the environment and the carcass surface (Figure 5.1). In this paper the knife used for trimming is considered the environment. Cross-contamination and inactivation are modelled at the individual level using Model 1 which is described by two equations (Equation 5.2). The first equation describes bacterial numbers on each carcass. The second equation predicts pathogen contamination levels on the knife. On completion of the trimming stage (S), each carcass (i) is contaminated with a quantity of bacteria, $N_{x,S}(i)$, measured in colony forming units on the pig surface. The bacterial concentration (cfu) in the environment at this processing stage, for each carcass is represented by $N_{e,S}(i)$. The contamination level present on each carcass prior to trimming is $N_{x,S-1}(i)$ and the number of pathogens left in the environment from the previous carcass is denoted as $N_{e,S}(i-1)$. We assume that the environment in the abattoir is free of all bacteria at the beginning of each day's activities.

The population approximation approach is modelled using Equation 5.3 and describes cross-contamination at the population level. Carcass bacterial numbers are calculated based on the assumption that the number of bacteria entering and leaving the environment from all carcasses is approximately the same, as the environment exists in a state of stochastic equilibrium. Calculation of the expected number of pathogens in the environment $E(N_{e,S})$ is derived from Nauta, van der Fels-Klerx & Havelaar (2005) and is as follows:

$$E(N_{e,S}) = \frac{a_{x,S} N_{x,S-1}}{b_{e,S} + c_{e,S} - b_{e,S} c_{e,S}} \tag{5.1}$$

where parameter $b_{e,S}$ indicates the probability of movement of bacteria from the environment to the carcass and $a_{x,S}$ depicts the probability of movement of bacteria from the

carcass to the environment. Environmental and carcass surface inactivation parameters are depicted by $c_{e,S}$ and $c_{x,S}$ respectively. As in Model 1 (Equation 5.2), $N_{x,S-1}(i)$ refers to bacteria numbers on each carcass entering the trimming stage. In Model 2 (Equation 5.3), contamination on individual carcasses is not modelled; instead probability distributions describing contamination levels are used.

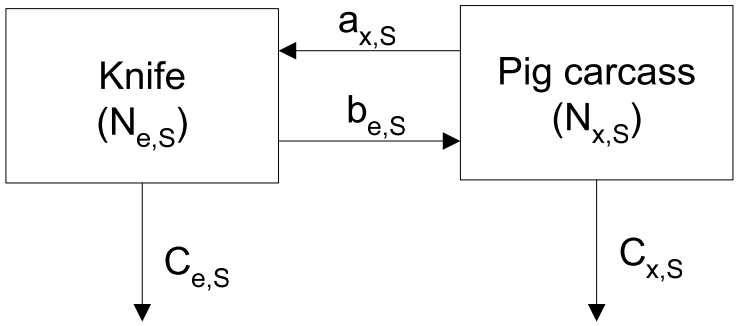


Figure 5.1: Schematic of trimming process. Diagram adapted from Nauta, van der Fels-Klerx & Havelaar (2005) showing parameters, variables and compartments modelled using difference equations. Parameters $b_{e,S}$ and $a_{x,S}$ indicate the probability of bacterial movement between compartments in the direction of the arrows, while $c_{e,S}$ and $c_{x,S}$ represent the probability of inactivation from the environment and carcass surface respectively.

Table 5.1: Overview of description of model parameters for individual and population-based model approaches.

Parameter	Description	Value
$a_{x,S}$	Probability that bacteria per cfu in contact with the knife move from the carcass exterior to the knife.	0.001
$b_{e,S}$	Probability that bacteria per cfu move from the knife to the carcass exterior.	0.048
$c_{x,S}$	Probability of inactivation and removal per cfu of bacteria from the carcass exterior.	0.56
$c_{e,S}$	Probability of inactivation per cfu of bacteria on the knife.	0.94

Model 1:

$$\begin{aligned} N_{x,S}(i) &= (1-a_{x,S})(1-c_{x,S})N_{x,S-1}(i) + b_{e,S}N_{e,S}(i-1) \\ N_{e,S}(i) &= a_{x,S}N_{x,S-1}(i) + (1-b_{e,S})(1-c_{e,S})N_{e,S}(i-1) \end{aligned} \tag{5.2}$$

Model 2:

$$N_{x,S}(i) = (1-ax,S)(1-c_{x,S})N_{x,S-1} + b_{e,S}E(N_{e,S}) \tag{5.3}$$

Parameter values for both models are provided in Table 5.1. These are estimated from published literature (Spinks et al. 2006, Murphy, Beard, Martin, Duncan & Marcy 2004, Kusumaningrum et al. 2003, Grau 1989) and observational studies conducted at abattoirs. Parameter determination is detailed in the Appendix.

Model implementation

The periphery of the regions of visible faecal contamination are trimmed and for comparative purposes, we assume the size and location of this to be the same for all pigs. The individual and population based approaches are executed in the trimming module using two different types of distributions describing initial bacterial contamination levels on the surface of pigs. First we choose the Lognormal distribution to describe the initial carcass contamination level. This distribution is right skewed, which is similar to the distribution predicted by our model in Chapter 3. The second distribution is referred to as the Zero-inflated Poisson (ZIP). This distribution was chosen as it most closely describes the distribution of zoonotic pathogens on carcasses sampled in the New Zealand abattoirs. No *Salmonella* were isolated in our studies, however, the distributions of both *Campylobacter* and *E. coli* isolated from carcasses in New Zealand can be best described by ZIP distributions. We therefore for the purpose of this paper also assumed that *Salmonella*, if present would also be similarly distributed. Furthermore, the ZIP distributions have been associated with bacterial counts (Robinson et al. 2005). A synopsis of the results obtained from carcass contamination studies conducted in New Zealand abattoirs is described in the Appendix.

Five different Lognormal distributions are described, representing five different populations of pigs, with differing degrees of heterogeneity of the initial contamination level ($N_{x,S-1}$). For comparative and demonstrative purposes we assumed the surface contamination level is sampled from Lognormal distributions with means of 1000 cfu, and variances of: 10, 100, 1000, 10000 and 100000 cfu. This wide range of variances was chosen so as to represent low, medium and high levels of between carcass heterogeneity with respect to contamination levels. For each distribution, the output for 10,000 pigs is simulated.

Zero-inflated Poisson distributions contain many zero values, with the non-zero data following a Poisson distribution. This type of data is not uncommon in microbial data

sets. We therefore describe this data dispersion using a combination of two distributions. The first is a Bernoulli distribution that describes the proportion of carcasses with no contamination; the higher the probability of the distribution, the less the number of zero values present in the data set. The other distribution is the Poisson distribution. Both distributions are multiplied, to produce a single distribution that we shall refer to as the as Zero-inflated Poisson distribution (Robinson et al. 2005, Dohoo et al. 2003). Again for comparative and demonstrative purposes, a total of six ZIP distributions describing initial carcass surface bacterial loads for both the individual and population-based approaches are as follows:

a. Bernoulli distribution with a high probability of contamination (p = 0.8) and Poisson distribution with a small lambda (λ=100) [hblp];

b. Bernoulli distribution with a high probability of contamination (p = 0.8) and Poisson distribution with a large lambda (λ=1000) [hbhp];

c. Bernoulli distribution with a low probability of contamination (p = 0.2) and Poisson distribution with a large lambda (λ=1000) [lbhp];

d. Bernoulli distribution with a low probability of contamination (p = 0.2) and Poisson distribution with a small lambda (λ=100) [lblp];

e. Bernoulli distribution with p = 0.5 and Poisson distribution with a large lambda (λ=1000) [mbhp] and

f. Bernoulli distribution with = 0.5 and Poisson with a small lambda (λ=100) [mblp].

Models are executed using Microsoft Excel with @Risk 4.5.5 Add-in (Palisade Corporation, 2005). For the population-based approach, one simulation consists of 10,000 iterations using Latin Hypercube sampling, to ensure convergence. Each iteration corresponds to the modelling of a single pig carcass. For the individual-based approach, we also model the contamination level for each of 10,000 carcasses, where the mean value of $N_{x,S-1}$ is used for the calculating $E(N_{e,S})$.

Stochastic Equilibrium Determination

In addition to a comparative study regarding the use of the two modelling techniques, we also determine when the environmental equilibrium is reached during the pig carcass trimming process for the different distributions. This is calculated by executing the individual-based model (Model 1) for 10,000 pigs contaminated with pathogens described

using the Lognormal and ZIP distributions. We then examine the predicted pathogen levels in the environment, specifically the number of carcasses that are trimmed before the pathogens numbers in the environment are show small fluctuations about a constant value. The mean value of distributions were used for these calculations.

5.4 Results

Stochastic Equilibrium

Equilibrium in the environment was determined to be achieved after the passage of just one pig carcass irrespective of the initial variance describing the carcass contamination levels of the input Lognormal and ZIP distributions.

Comparison of individual and population based modelling approaches with Lognormal input distributions

The predicted outputs for the comparison of the individual and population-based approaches using input Lognormal distributions are presented in Figures 5.2 and 5.3. Both modelling techniques output similar results when the input Lognormal distributions are described by variances of 10, 100 and 1000 cfu. However, as the distribution variance increases to 10,000 and larger values, the population-based approach predicts results that vary considerably from the individual-based approach, with the former approach estimating a greater dispersion of output values than the individual-based method.

Comparison of individual and population based modelling approaches with ZIP input distributions

There was little difference seen in the predicted model outputs from the individual and population-based approaches when the initial contamination levels followed ZIP distributions (Figures 5.4 and 5.5). For the lbhp, mbhp, lblp and mblp ZIP distributions, the population-based approach underestimated predicted values, when compared to the individual-based method when the model output values were low (Figures 5.4b,c and 5.5b,c).

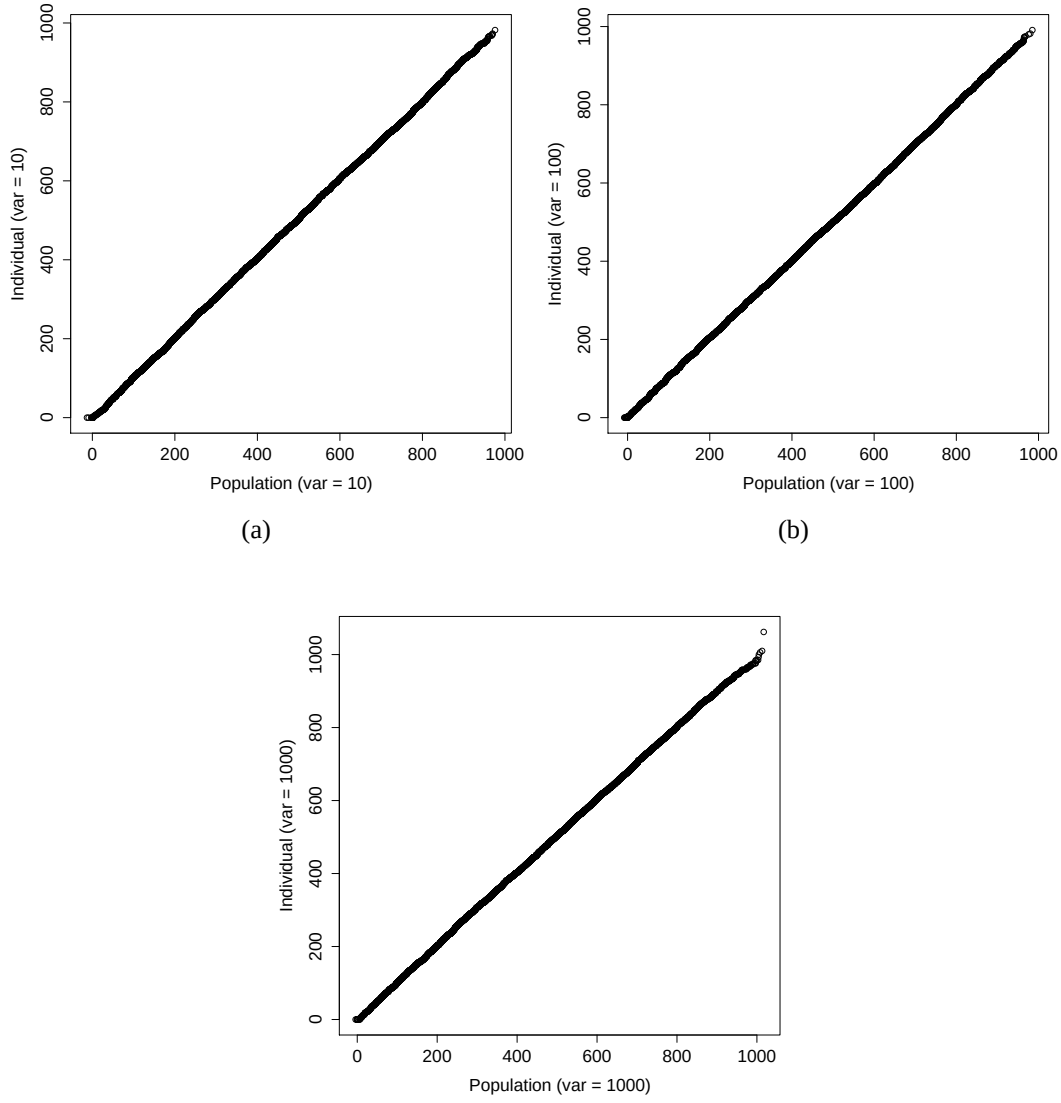
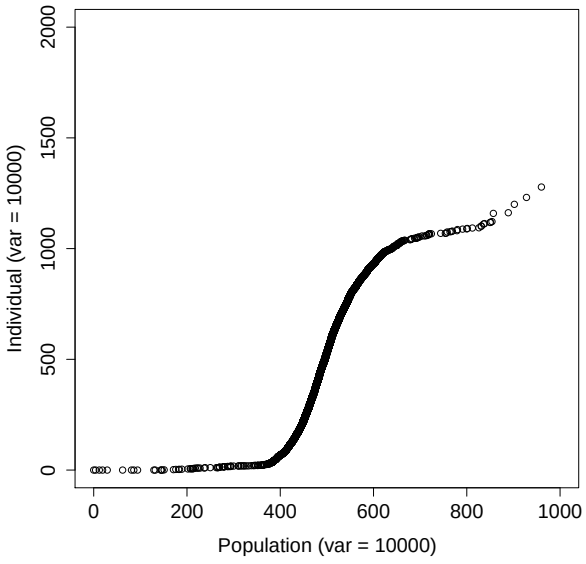
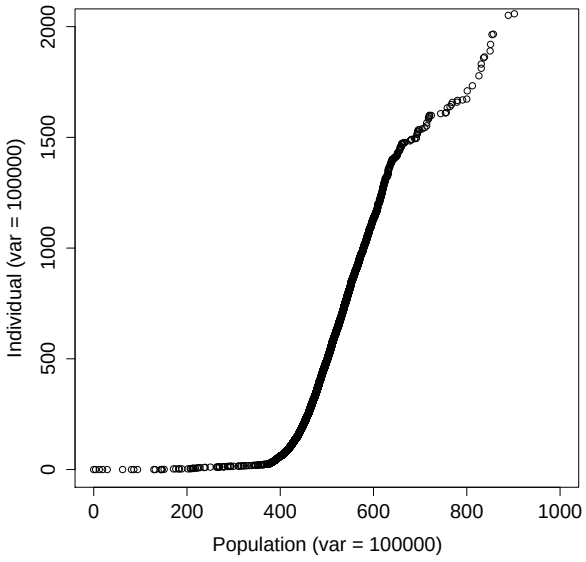


Figure 5.2: Quantile-quantile plots of the predicted distributions of carcass contamination levels after trimming from the individual and population based approaches using initial input Lognormal distributions at variance 10 (a), variance 100 (b) and variance 1,000 (c).



(a)



(b)

Figure 5.3: Quantile-quantile plots of the predicted distributions of carcass contamination levels after trimming from the individual and population based approaches using initial input Lognormal distributions at variance 10,0000 (a) and variance 100,000 (b)

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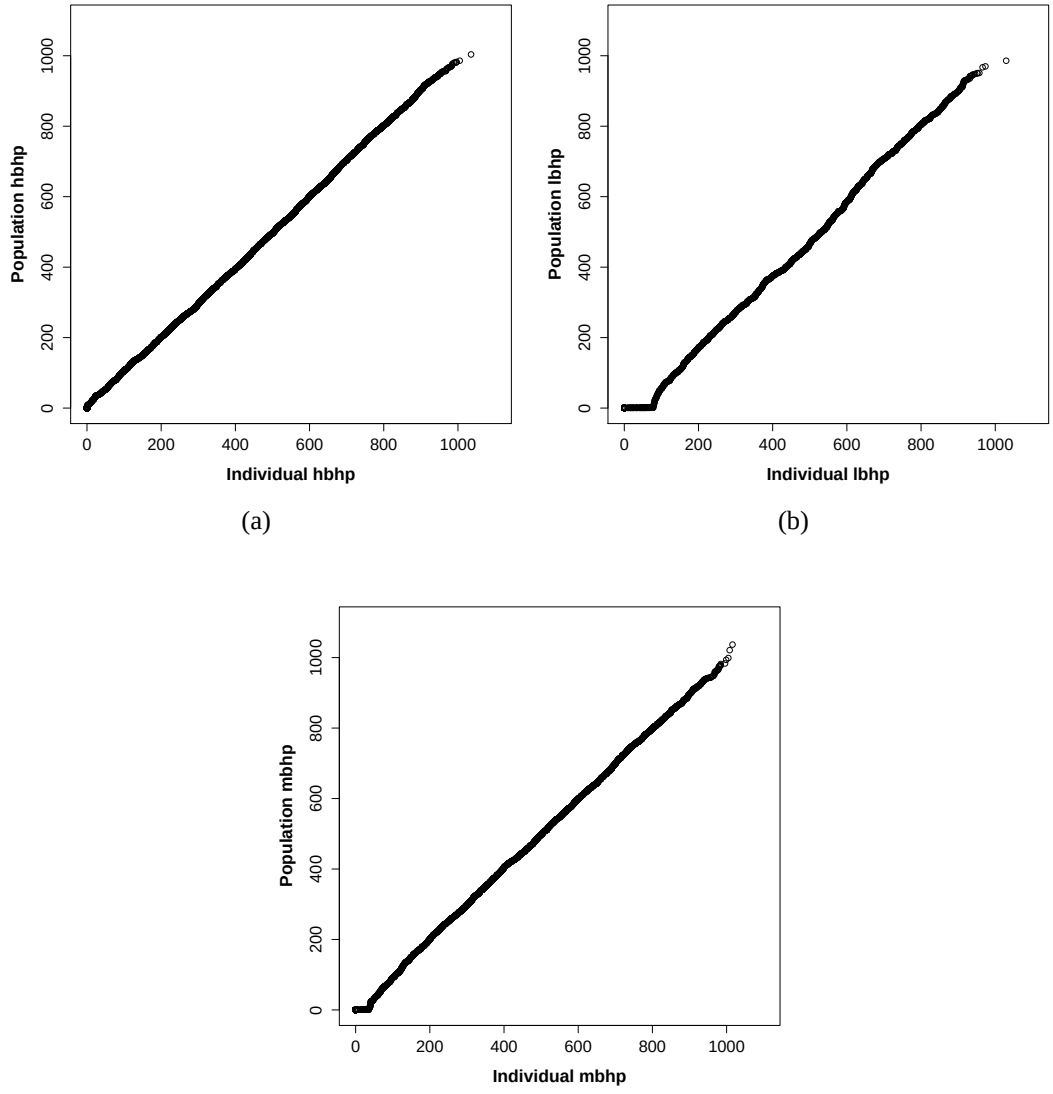
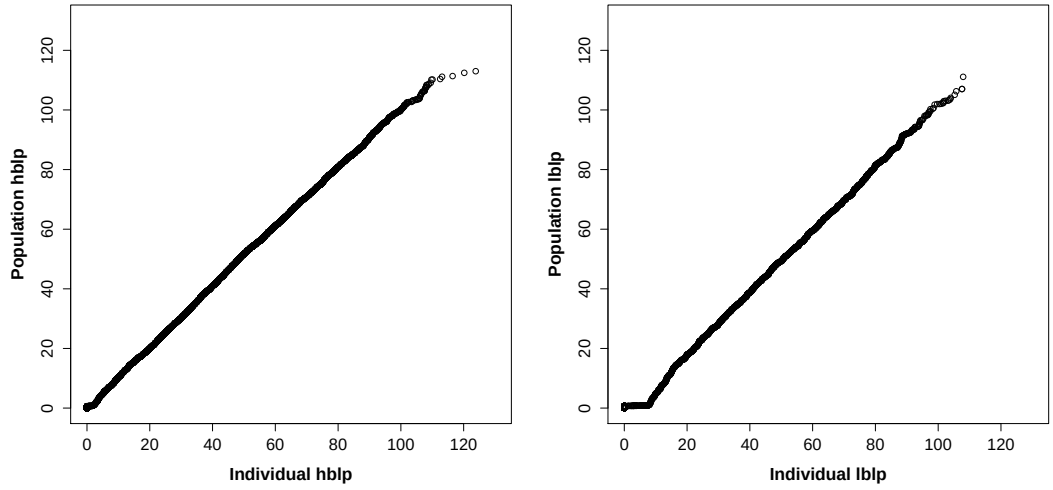
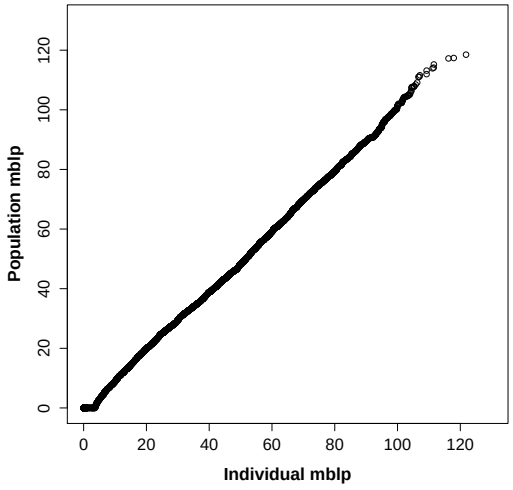


Figure 5.4: Quantile-quantile plots of the predicted distributions of carcass contamination levels output after trimming, from the individual and population based approaches using initial input ZIP distributions of hbhp (a), lbhp (b) and mbhp (c).



(a)

(b)



(c)

Figure 5.5: Quantile-quantile plots of the predicted distributions of carcass contamination levels output after trimming, from the individual and population based approaches using initial input ZIP distributions of hblp (a), lblp (b) and mblp (c).

5.5 Discussion

This study compares the individual and population-based approximation approaches employed in quantitative risk assessment modelling. Understanding the limitations of the latter technique can lead to its more judicious use and therefore minimise model uncertainty. Consequently this will result in determination of more mathematically correct quantitative risk estimates. Further, the inferences gleaned from this paper have implications for model selection particularly when conducting quantitative microbial risk assessments.

The population-based modelling approach has been used by Nauta, van der Fels-Klerx & Havelaar (2005) in a quantitative risk assessment model for *Campylobacter* in chickens processed in the abattoir. These authors defended their choice of employing this methodology by stating that their model simulations predicted the occurrence of an equilibrium in the environment with respect to *Campylobacter* after the passage of no more than ten chickens. In our model, we determined that an environmental equilibrium with respect to *Salmonella* is established after passage of just one pig carcass. The speed with which environmental equilibrium is attained is critical to the validity of the population-based approached as the existence of this equilibrium is one of the underlying assumptions. If the time to reach equilibrium is short, the underlying assumption of environmental equilibrium is quickly satisfied and use of the population-based approach can be more readily justified. This was the situation with respect to this paper. On the other hand, if the time taken to equilibrium is long, or occurs after the passage of a large number of carcasses, then the model assumptions are not quickly satisfied which can result in the prediction of less mathematically correct risk estimates. Therefore, when determining whether the population approximation is an acceptable substitute for the individual-based approach, the time taken for attainment of the environmental equilibrium should be taken into account. The variance of the initial distributions of contamination levels had no effect on the speed of establishment of the environmental equilibrium. The parameter values are possibly more influential in determining the rapidity of establishment of the equilibrium.

The population-based approach is computationally fast and therefore it seems to be the logical and preferred choice, over the tedious, computationally demanding, individual-based approach. However, the former methodology predicts outputs that vary considerably from those predicted by the individual-based approach when the variance of the initial carcass contamination equals and exceeds 10,000. Therefore even though the underly-

ing assumption of stochastic equilibrium is achieved, under circumstances of high degrees of between carcass heterogeneity with respect to contamination levels, the population-based approximation becomes a less reliable alternative to the individual-based approach.

The individual-based modelling approach has been previously used to study the behaviour of pathogens (Prats et al. 2006, Grijspeerdt et al. 2005), animals (Parrott & Kok 2002) and plants (Damgaard 2004). Model development using this technique is time-consuming and model computation time is considerably increased when compared to the population-based approach. However it should be the preferred methodology when the between carcass heterogeneity is large (with levels exceeding 10,000) and environmental equilibrium is not achieved quickly. In the event that carcass heterogeneity is unknown, it is preferable to use the individual-based model approach, as model outputs are more precise irrespective of the distribution of input values.

For models with input distributions described by Zero-inflated Poisson distributions, the population-based method provides a good approximation to the individual-based method as both techniques output similar results. It is possible that the Poisson distributions selected in this paper possessed variances that were sufficiently small for the population-based approach to be a good alternative. However, the number of non-contaminated carcasses impacted on congruity of results output by the two methods. The greater the number of contaminated carcasses, the more congruent the predicted outputs from the two methods.

In this paper we present a simple model, with the environment referring only to the knife and excluding the surrounding air and aerosolised water. We could have expanded our definition of the environment to include these surroundings, but this would have considerably increased model complexity. For the purpose of comparing modelling techniques, we thought it unnecessary to incorporate other factors into the definition of the environment.

Parameter values were derived from the literature. One disadvantage of this technique is that data from different countries with different conditions are used and there may be a lack of consistency. Furthermore, the inactivation rates of *Salmonella* will vary according to the predominant serotype. However, since the parameters were the same for both models, this would be less pertinent for our comparative study of the two approaches. We also considered that parameter estimates for removal of bacteria from the trimmed

region will be dependent on the size and location of this trimmed region, therefore for simplicity we assumed these to be the same for all carcasses. Considering the objective of our study was model comparison, this assumption would not have biased predicted model outputs from either approach.

We used a wide variety of variances for the distributions of initial carcass contamination levels. These numbers have been shown to be very variable, ranging from 0 to 1.9×10^4cfu/cm^2 (Tamplin et al. 2001) depending on the stage of the carcass in the abattoir, initial surface carcass contamination levels, the degree of infection within pigs and the experience of the operators during evisceration (Pearce et al. 2004, Lo Fo Wong et al. 2002, Borch et al. 1996). The initial surface contamination on pigs is proportional to that found in the pig faeces, which has been found to range from 0 to $\log_{10}4.1$ (O'Connor et al. 2006).

We conclude that both population-based and individual-based approaches can be employed to model pig carcass cross-contamination and inactivation in the abattoir. These methods can be implemented in quantitative exposure assessment models. However, despite the assumptions of the the former technique being satisfied, this methodology is limited, as it fails to adequately capture the complex dynamics of cross-contamination in the presence of high between carcass contamination heterogeneity with respect to carcass contamination levels. Therefore, under circumstances where the input distribution has a high variance, the population-based approximation is not a good alternative for the individual-based approach.

5.6 Appendix

Quantitative testing:

For the quantitative testing of carcass contamination levels, 225cm^2 of 130 pig carcasses were swabbed prior to and after completion of abattoir procedural stages; the swabs were placed in 5ml of buffered peptone water and stored on ice. These were then transported to the laboratory and logarithmically plated out in duplicates of 200μl using a spiral plater onto XLD agar for *Salmonella*, mCCDA agar for *Campylobacter* and Eosin Methylene-blue Lactose Sucrose (EMB) plates for *E. coli*. Both *Salmonella* and *E. coli* plates were incubated for 24 hours at 37°C. For growth of *Campylobac-*

ter, all samples were incubated under microaerophilic conditions in a commercial cabinet. The cabinet created and maintained a gaseous atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen. Confirmation of colonies for *Salmonella* was accomplished using biochemical tests (indole, citrate, urease and Methyl Red Voges-Proskauer tests). *Campylobacter* colonies were confirmed using a combination of biochemical and molecular techniques (gram stain, oxidase and genus specific Polymerase chain reaction). The polymerase chain reaction (PCR) amplified the 894 base pair *cadF* gene conserved in *Campylobacter* using the primer sequences of GATCTTTTTGTTTTGTGCTGC and CCTGCTACGGTGAAAGTTTTGC. *E. coli* colonies were confirmed using biochemical tests (indole, citrate, urease and Methyl Red Voges-Proskauer tests). All samples were processed the same day.

Quantitative microbial testing of the surface of carcasses in the abattoir revealed low carcass contamination levels. *Salmonella* was not isolated from any of the carcasses in the abattoir. *E. coli* was isolated in 2% of the carcasses after dehairing, none after singeing, 1% after evisceration and 1% after carcass halving. Counts of *E. coli* on carcasses ranged from 0.11 – 0.3 cfu/cm^2 with three exceptions. Bacteria were isolated from three carcasses at counts equating to 8.6cfu/cm^2, 4.3cfu/cm^2 and 23cfu/cm^2 post-dehairing, post-evisceration and post-halving respectively. *Campylobacter* was isolated in 1% of the sampled carcasses after dehairing, 1% after singeing and 0% after both evisceration and halving. Counts of *Campylobacter* on carcasses ranged from 0.11 – 0.3 cfu/cm^2. Further studies revealed that *Salmonella* was not isolated on the carcasses or in any pig faecal material sampled. This is further detailed in Chapter 3.

Other studies conducted in New Zealand abattoirs

1. In order to determine the amount of leaking faecal material moving onto the knife during evisceration, 30 pieces of gauze were cut approximately 25cm^2 in size, and weighed. These were then individually wrapped and autoclaved. The knife used during carcass evisceration was wiped with one of the weighed, sterilised pieces of gauze, then repackaged in a ziploc bag, transported on ice to the laboratory and re-weighed. The difference in weight was considered to be an estimate of the amount of leaking faecal material moving onto the knife during evisceration.

2. Any faecal material observed that leaked onto the carcass during evisceration at the abattoir was collected, placed in a pre-weighed plastic bag, transported on ice to the

laboratory, where it was weighed. The difference in weight was considered to be an estimate of the amount of leaking faecal material moving onto the carcass surface.

3. The size of trimmed areas resulting from faecal contamination was recorded. These averaged 59cm^2.

For our model, it was assumed that the process of trimming occurred when there was visible faecal leakage onto the carcass. The minimum and maximum weights of faecal material collected from carcasses, corresponded to 6.6 and 19.8g.

Trimming parameters

The mean values of the following described distributions are presented in Table 5.1.

Probability of bacteria (cfu) on carcass skin moving to knife:

Let this equate *P(E)*P(F)* where *E* is the probability of bacteria on the region that is to be trimmed coming into contact with knife and *F* is the probability of transfer of bacteria to the knife. For *E*, we assumed the surface area in the region to be trimmed is a percentage of the total surface area described by: Uniform (0.004,0.006). The average size of the area trimmed as a result of faecal contamination is 59cm^2 (obtained from observational studies), which is approximately 0.004 – 0.006 of the total surface area of the halved carcass. For *F* we used the transfer probability described by Beta (alpha = 7, beta = 25, bounded between 0 and 1) for *Salmonella*. This probability was extrapolated from the percentage transference of *Salmonella* from sponges to a stainless steel surface as described by (Kusumaningrum et al. 2003). Although the sponge has a different consistency of surface from a carcass, in the absence of the ideal probability, it provides an estimate of the possible required probability of transfer between a contaminated surface and the stainless steel portion of the knife.

Probability of bacteria (cfu) on knife moving on carcass skin:

This parameter is calculated from the multiplication of the probability of survival of bacteria in water at 70°C with the probability of transfer of bacteria from the knife to the skin. From Spinks et al. (2006), a minimum of a 1 $\log_{10}$ reduction in pathogen numbers is expected. In terms of probability distributions, this means that the probability that an organism will survive a 1 $\log_{10}$ lethality reduction can be described using a maximum value of $1/10^1$ and an estimated minimum probability value of 0. The probability of transfer from the knife to the skin is described by Uniform $\sim$ (0.9, 1), which was derived from

Kusumaningrum et al. (2003). We used the percentage transfer of bacteria of *Salmonella* from a stainless steel surface to chicken with the application of 500g of pressure.

Probability of inactivation and removal of bacteria (cfu) on carcass skin:
Let the probability of inactivation of bacteria on the carcass skin be *P(I)*. The reduction in pathogen numbers of *Salmonella* between 55°C – 65°C in ground pork as determined by Murphy, Beard, Martin, Duncan & Marcy (2004) ranged from $0.002\log_{10}$ – $0.5\log_{10}$. Assuming the reduction in ground pork to be the same as that on the carcass skin, we use these estimates of the extent of pathogen reduction to determine the probability of inactivation on the carcass skin. The probability that an organism will survive an x $\log_{10}$ lethality reduction is $1/10^x$, the probability of inactivation is calculated as 1 - $1/10^x$. We therefore estimated the probability of inactivation to be described by Uniform $\sim$ (0.05, 0.7). We let the probability of removal of bacteria on the carcass skin be *P(R)*. From (Yeh et al. 2005), we derive the approximate contamination level on carcasses to be 0.12 – 0.24 cfu/cm^2. The average surface area of the pigs was calculated from an equation developed by Kelley et al. (1973): Surface Area = 734(Body Weight in kg)$^{0.656}$. From our observational studies, the weight of pigs ranged from 58.4kg – 86.2kg. Therefore the surface area of pigs ranged from approximately 10,550 cm^2 – 14,000 cm^2. The contamination level of *Salmonella* on carcasses, in the absence of any visible faecal spillage, immediately after evisceration is expected to therefore range from 1266 – 2800 cfu. Since we estimated the total amount of faecal material spilled on the carcass to range from 6.6 to 19.8g and using our knowledge of the range of pathogen numbers that can be contained in that quantity of faecal material, which is 70 – 760,000, we estimated that 0.4% – 98% of the total surface bacteria is located in the contaminated region. Since the bacteria in the contaminated region is removed during trimming, *P(R)* follows the distribution: Uniform (0.04, 0.98). This percentage range was obtained by dividing the bacterial contamination from spilled faecal matter by the average of the total surface contamination on carcasses. The probability of inactivation and removal of bacteria (cfu) on carcass skin which is bounded between 0 and 1 is calculated as follows:

$$P(I) + P(R) - P(I) * P(R) \tag{5.4}$$

Probability of inactivation of bacteria (cfu) on knife:

This parameter is estimated from the probability of survival of bacteria in water at 70°C. From Spinks et al. (2006), a minimum of a 1 $\log_{10}$ reduction in pathogen numbers is expected. In terms of probability distributions, this means that the probability that an organism will survive a 1 $\log_{10}$ lethality reduction (p) will have a maximum value of $1/10^1$ and an estimated minimum probability value of 0. The probability of inactivation of bacteria (cfu) on knife is therefore calculated as 1 - p, resulting in a range of 0.9–1.

CHAPTER 6

Evaluation of pathogen mitigation strategies in pig abattoirs

6.1 Abstract

The control of zoonotic food-borne diseases transmitted by pork is of public health concern. Consequently, pork producers, processors and relevant authorities in New Zealand are interested in the development of measures to control the transmission of pathogens from pork to humans. The aim of this study therefore, was to use a previously developed suite of semi-stochastic, modular process risk mathematical models to evaluate the effects of intervention strategies for the control of *Salmonella*, *E. coli* and *Campylobacter* in pig abattoirs in New Zealand. Sensitivity analyses were performed to identify parameters most influential to the predicted values output by the models. The introduction of a second singeing step was predicted to result in a 100% reduction in the median pathogen levels, as opposed to coverage of the bung with a plastic bag, which was predicted to reduce median pathogen number by 10% to 44%. Different parameters were identified as critical for each of the organisms. However, the length of time that the dressed product spent in blast chilling in storage was found to be a critical parameter for all three pathogens, with extension of this time resulting in reduced carcass contamination levels. We propose the introduction of a second singeing step immediately post-evisceration as a cost effective measure to control pathogen numbers on dressed pork carcasses exiting the abattoir. Further, we also present our models as useful tools to evaluate further proposed measures aimed at alleviating contamination and prevalence levels of *Salmonella, E. coli* and *Campylobacter* in pork at abattoirs in New Zealand.

6.2 Introduction

Zoonotic food-borne diseases contribute substantially to morbidity and mortality rates worldwide. This has raised awareness of food safety which the World Health Organisation is attempting to address. One method employed by this organisation to reduce the deleterious impact of these food-borne diseases on human health is through the use of risk-based techniques (WHO 2007*a*). Three major zoonotic food-borne pathogens of considerable significance are *Salmonella*, *E. coli* and *Campylobacter*, all of which can be transmitted to humans through pork consumption (Alban & Stärk 2005, Bolton et al. 2002). Consequently, there has been an effort to implement systems aimed at controlling illnesses caused by these zoonotic pathogens transmitted through pork products. One such approach is to target control measures in the pig abattoir. Mathematical models have been used in exposure assessments to describe pathogen transmission along the food chain. Sensitivity analyses of mathematical models identify and quantify parameters/variables of greatest important in the prediction of the model output (Cassin et al. 1998, Blower & Dowlatabadi 1994). These analyses allow greater understanding of the investigated system. Prudent manipulation of identified critical parameters can results in the development of effective risk mitigation strategies. To date, little research has been conducted on risk mitigation strategies in the pig abattoir targeted at *Salmonella*, *E. coli* and *Campylobacter* in New Zealand. Although a previous chapter discussed the development of a mathematical model describing zoonotic pathogen propagation in pig abattoirs in New Zealand, no evaluation of possible intervention strategies was conducted. Therefore, in this paper we aim to demonstrate that the previously described suite of mathematical models can be modified and used to predict the efficacy of specific interventions strategies. We also conduct sensitivity analyses on parameters in the models. The results of the sensitivity analysis would further suggest parameters that can be modified to create effective intervention strategies targeted at mitigating pathogen contamination levels in pork exiting New Zealand abattoirs. This information may prove important to risk mangers and policy-makers.

6.3 Model Description

As previously described in Chapter 3, a suite of modular process risk models was developed. These models described the transmission of *Salmonella*, *Escherichia coli* and thermophilic *Campylobacter* throughout consecutive stages of the pig abattoir in New Zealand (Figure 6.1). The abattoir procedures were compartmentalised into six units called modules. Within each module, the processes of cross-contamination, inactivation, partitioning and removal were identified and explicitly modelled. A combination of difference and differential equations was used to execute the model. The criteria governing the selection of the equation type has previously been described (Chapter 3). The models output distributions of both the bacterial concentration and prevalence of the investigated pathogens on carcasses exiting the abattoir. The inputs of the model were the number and prevalence of these pathogens entering the slaughter house. These models are referred to as baseline models. Model parameters were derived from targeted microbial studies and data in the literature as detailed in Chapter 3.

Intervention Strategies

The intervention strategies investigated were based on (i) a control strategy recommended for use in other countries and (ii) a suggestion from the New Zealand Pork Industry Board (NZPIB). Singeing has been found to be effective in reducing carcass pathogen levels (Berends et al. 1997, Borch et al. 1996) and the NZPIB was interested in investigating the effect of contamination levels on dressed pig carcasses leaving the abattoir after introduction of a second singeing procedure immediately post evisceration. This second singeing procedure was modelled in the same fashion as the first singeing procedure with the parameters and methodology used to incorporate this feature into the model was taken from Chapter 3. The rate of pathogen inactivation during singeing was previously calculated to be 11.8/minute and the time for this procedure described by Uniform $\sim$ (0.16, 0.25)/minute.

The effect of the introduction of a plastic bag placed over the pig anus (or bung) to minimise faecal spillage on the carcass during evisceration is also evaluated. To mimic this procedure, we reduce the probability of faecal spillage on each carcass from 0.1 (as described in the baseline models) to 0.01. The later probability of 0.01 is an assumption

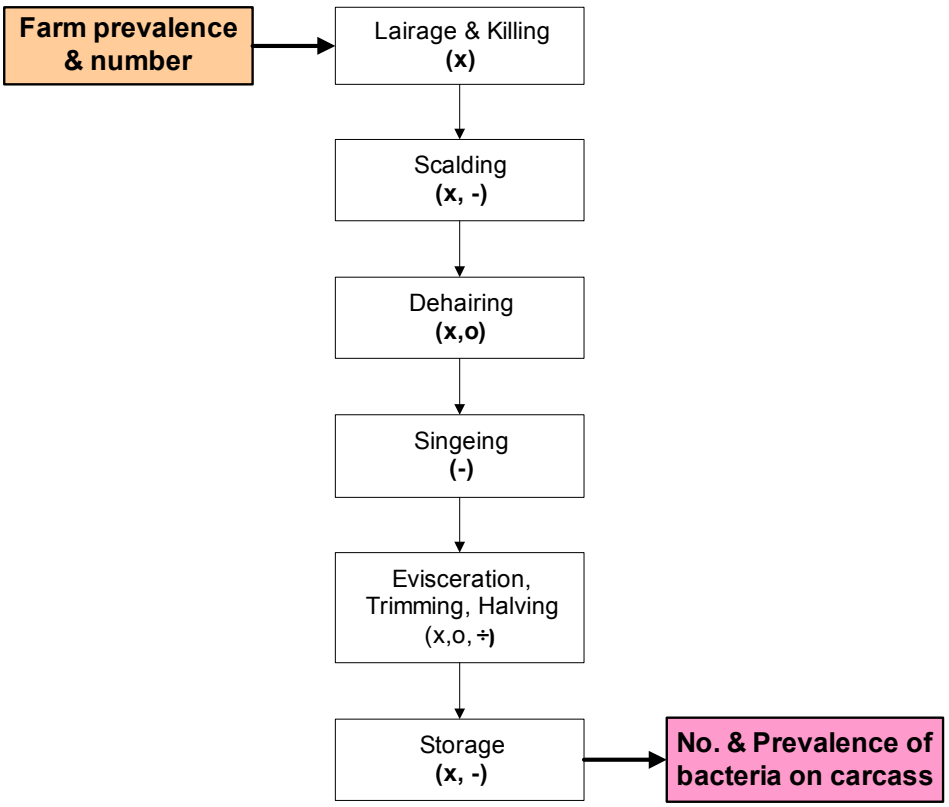


Figure 6.1: Modules and processes in a New Zealand abattoir. Processes are represented as follows: contamination (x), inactivation (-), partitioning (÷), and removal (o). The number of bacteria present at the start of processing is dependent on the prevalence of the pathogen on the farm of origin. Model outputs indicate the prevalence and number of pathogens on carcasses.

and is discussed in a latter section of this chapter. Coverage of the anal region has been reported to result in the reduction of diseases transmitted by porcine intestinal contents (Nesbakken et al. 1994).

Model implementation

The results of the intervention strategies are compared to the baseline model results. When modelling the intervention strategies, numerical simulations were executed in Microsoft Excel with Palisade @Risk 4.5.5 Add-in (Palisade Corporation, 2005) in combination with a Visual Basic for Applications Macro. Five hundred simulations consisting of 10 iterations each are run using Latin hypercube sampling of Monte Carlo simulations, where each simulation describes the events occurring in the abattoir on one day. Each iteration describes the pathogen dynamics on a batch of pigs supplied from a single farm to the

abattoir. The distribution of carcass bacterial numbers was predicted for each module, during every iteration. From the central limit theorem, and using the final model outputs for the baseline model with respect to *E. coli*, a mean output value of 93 pathogens with a confidence interval of 81 – 105 was predicted when the model was executed for 500 simulations. At 1,000 simulations, the model predicted a mean value of 93 pathogens with a confidence interval of 85 – 101. Since the outputs and confidence intervals were so similar at both 500 and 1000 simulation runs, and considering the lengthy computation time required by the model for an additional 500 iterations, interventions strategies were modelled using 500 simulations, instead of 1000 simulations, the latter of which was used in Chapter 3.

Sensitivity Analyses

Sensitivity analyses were undertaken on the baseline models (models with no intervention strategies) and only for parameters described by distributions. Sensitivity analyses using Latin Hypercube sampling in combination with partial rank correlation coefficient (LHS/PRCC) were conducted on each model to identify the parameters most influential in the determination of the model output. These parameters can then be targeted in the development of intervention programmes aimed at mitigating pig carcass contamination with the pathogens of concern. This technique facilitates exploration of the full range of values for each parameter distribution in the model that is being investigated (Blower & Dowlatabadi 1994). The PRCC technique quantifies the correlation between each input parameter and the output variable, while accounting for all other parameters in the model (Turner et al. 2006). For each pathogen, the data used in each sensitivity analysis consisted of 100 parameter sets of 22 parameters. A parameter set was created using Latin hypercube sampling in Palisade @Risk and followed the methodology described by Blower & Dowlatabadi (1994). In summary, one hundred samples from each of the 22 parameter distributions considered in the sensitivity analysis were obtained using Latin hypercube sampling. A matrix of 22×100 was assembled, with each column containing the 100 possible values/samples from each of the parameters. Random sorting within columns was then performed. The numbers present in each row were than appropriately substituted into the model which was executed and the output added to the matrix. The matrix therefore expanded to a dimension of 23×100. This final expanded matrix was

imported into SAS statistical software programme and the PRCC test performed.

6.4 Results

The introduction of a second singeing procedure post-evisceration is predicted to be the more effective intervention strategy resulting in a 100% or approximate 3 log reduction of the median value of all pathogens numbers on completion of storage (Table 6.1). The mean prevalence levels are also reduced by 71%, 81% and 71% for *Salmonella*, *E. coli* and *Campylobacter* respectively, when compared to the baseline models. Coverage of the bung is predicted to be a much less effective mitigation strategy, with very little difference predicted in prevalence values when compared to the baseline models. Our models predict a mere 10% reduction in contamination numbers with respect to *Salmonella* on the dressed pork carcass for this latter intervention strategy. However, *E. coli* and *Campylobacter* are estimated to have greater reductions of 44% and 31.6% respectively. Figures 6.2 to 6.4 demonstrate the predicted effect of the two intervention strategies on different stages of the abattoir procedures. The effect of both intervention strategies are evident post-evisceration.

Sensitivity Analyses Results

For each investigated pathogen, the Partial Rank Correlation Coefficient was calculated between each input parameter described by a distribution and the median pathogen numbers at the end of storage. All significant parameters produced p values < 0.05. The sign of the PRCC describes the nature of the relationship between the parameter and outcome. A positive PRCC sign indicates that an increase in the parameter is associated with increased pathogen levels at storage. On the contrary, a negative PRCC sign means that reduction of the parameter is associated with increased contamination levels on the dressed carcass on completion of storage. The results are presented in Table 6.2. The most critical parameters identified by the sensitivity analysis varied between pathogens. For *Salmonella*, the significant parameters identified were the time spend during singeing, storage and in the lairage; the proportion of pigs shedding the organism in their faecal material in the lairage; the amount and concentration of faecal material shed per pig in the lairage and the amount of faecal material on the pig immediately prior to killing. The

first two parameters were negatively associated with model output.

In the case of *E. coli*, the time spent during dehairing, singeing and storage; the amount of faecal material extruded from the pig during dehairing; the proportion of pigs shedding in lairage and the probability of inactivation of bacteria (cfu) on the carcass skin were identified as the key input parameters having the greatest effect on the predicted model output. The time spent in singeing and storage, as well as the probability of inactivation of bacteria on the carcass skin produced negative PRCC values.

With respect to *Campylobacter*, only the time spent in storage, the quantity of faecal material extruded from pig during dehairing and the probability of bacteria moving from the carcass skin to the knife during evisceration were determined to be significant. Of these three parameters, the time spent in storage and the probability of bacteria moving from the carcass skin to the knife during evisceration produced negative PRCC values.

Table 6.1: Summary of descriptive statistics of the predicted number of, and reduction in *Salmonella*, *E. coli* and *Campylobacter*, on carcasses for mitigation scenarios in the abattoir.

	Salmonella	*E. coli*	*Campylobacter*
Baseline (i.e. no. mitigation)			
Median no. of pathogens at end of storage	30	9	38
10^{th}–90^{th} Decile	1 – 2495	1 – 85	1 – 245
Mean prevalence at end of storage	100	98	94
Mitigation Scenario:bung coverage			
Median no. of pathogens at end of storage	27	5	26
10^{th}–90^{th} Decile	1 – 154	1 – 23	1 – 196
Percent reduction (%) of median	10	44	31.6
Mean prevalence at end of storage	96	94	93
Mitigation Scenario:2^{nd} singeing			
Median no. of pathogens at end of storage	0	0	0
10^{th}–90^{th} Decile	0 – 356	0 – 3	0 – 90
Percent reduction (%) of median	100	100	100
Mean prevalence at end of storage	36	20	30

Table 6.2: Results of the LHS/PRCC sensitivity analyses of the baseline models for *Salmonella*, *E. coli* and *Campylobacter*.

Parameter	*Salmonella*	*E. coli*	*Campylobacter*
Description	PRCC	PRCC	PRCC
Number of pigs in lairage	0.1497	0.698	-0.138
Prevalence of infected pigs in lairage	0.147	-0.158	-0.054
Proportion of pigs shedding in lairage	0.331**	0.262*	0.009
Time spent in lairage	0.518***	0.076	0.180
Amount of faeces shed/pig in lairage	0.423***	0.148	-0.100
Concentration of pathogen in faeces in lairage	0.730***	0.104	-0.036
Proportion of faeces on pig on killing	0.476***	0.299**	0.389
Amount of faeces discharged from pig during dehairing	0.287	0.908***	0.550***
Time spent dehairing	-0.145	0.392***	-0.047
Time spent Singeing	-0.238***	-0.647***	-0.196
Evisceration parameters:			
Probability of bacteria (cfu) on carcass skin moving to knife	0.028	0.109	-0.230*
Probability of bacteria (cfu) in knife moving on carcass skin	0.041	0.110	0.132
Probability of inactivation of bacteria (cfu) on carcass skin	-0.219	-0.872***	0.189
Probability of inactivation of bacteria (cfu) on knife	-0.093	-0.035	0.069
Probability of bacteria (cfu) in leaking faeces moving to knife	0.077	-0.099	-0.018
Amount of faeces (g) leaking from carcass	0.091	0.094	-0.092
Trimming parameters:			
Probability of bacteria (cfu) on carcass skin moving to knife	-0.126	-0.129	0.000
Probability of bacteria (cfu) on knife moving on carcass skin	0.033	0.054	-0.176
Halving parameters:			
Probability of bacteria (cfu) on knife moving on carcass skin	-0.029	0.130	0.024
Probability of inactivation/removal of bacteria (cfu) on carcass skin	-0.014	-0.009	-0.130
Probability of bacteria (cfu) on carcass skin moving to knife	-0.131	-0.168	-0.147
Storage parameters:			
Time spent in storage	-0.332**	-0.647***	-0.388***

The PRCCs are between the input parameters and the output value (median pathogen numbers on dressed carcasses on completion of storage). Results are considered to be significant at the 0.05 level (*), 0.01 level (**) and 0.001 level (***).

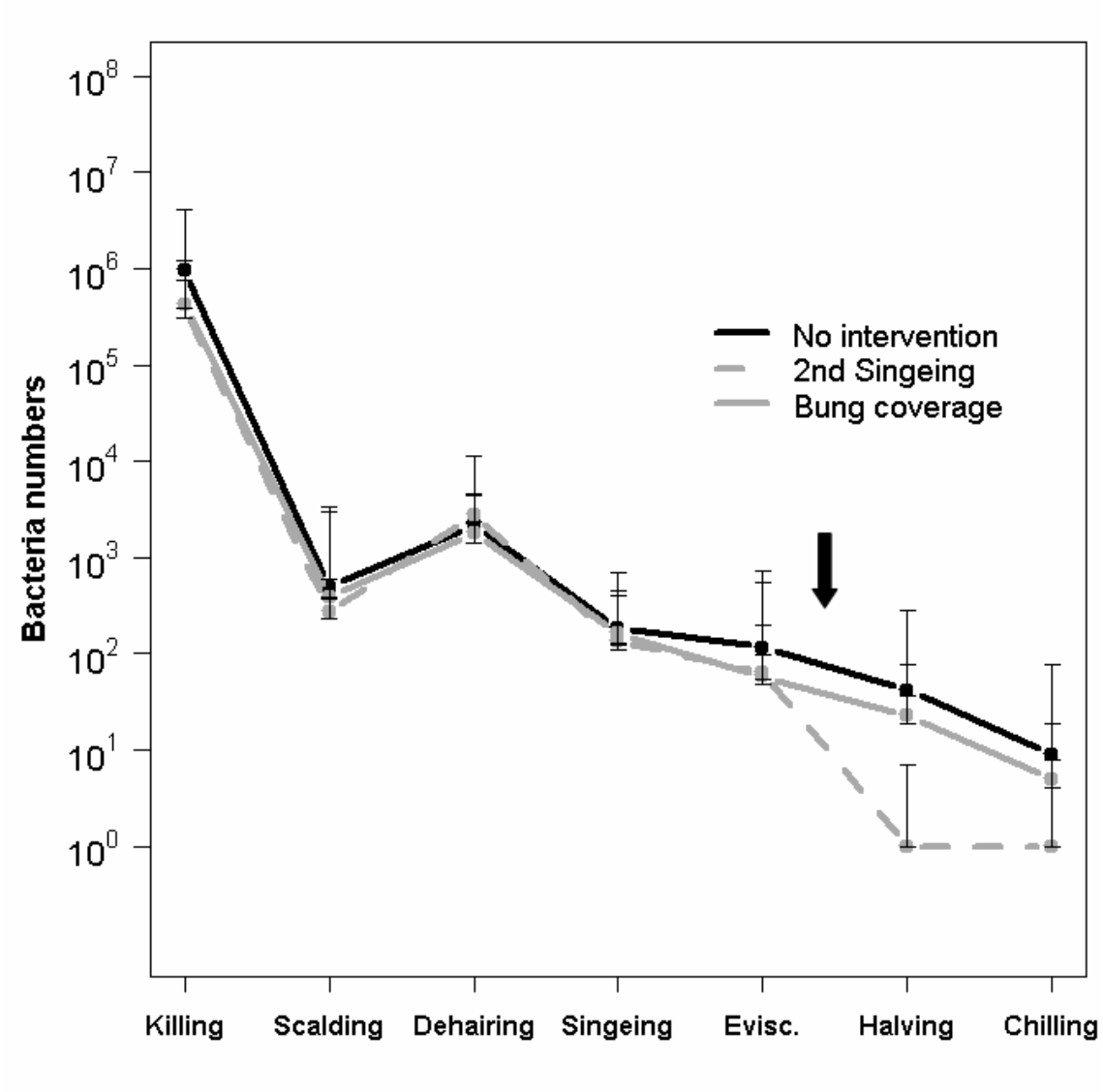


Figure 6.2: Predicted *E. coli* numbers on the surface of pig carcasses at different stages in abattoir with and without mitigation strategies. Arrow indicates application of second singeing step.

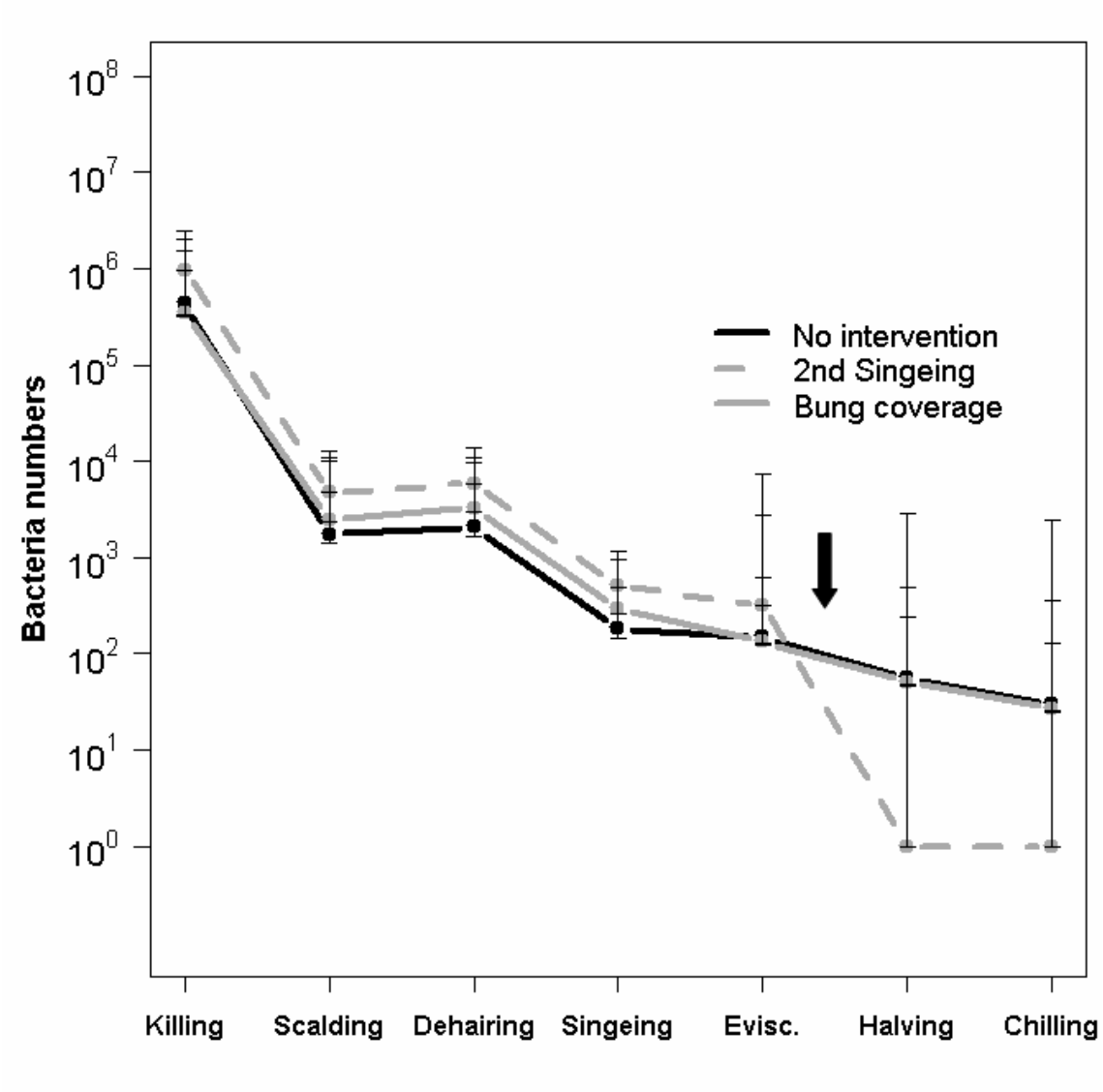


Figure 6.3: Predicted *Salmonella* numbers on the surface of pig carcasses at different stages in abattoir with and without mitigation strategies. Arrow indicates application of second singeing step.

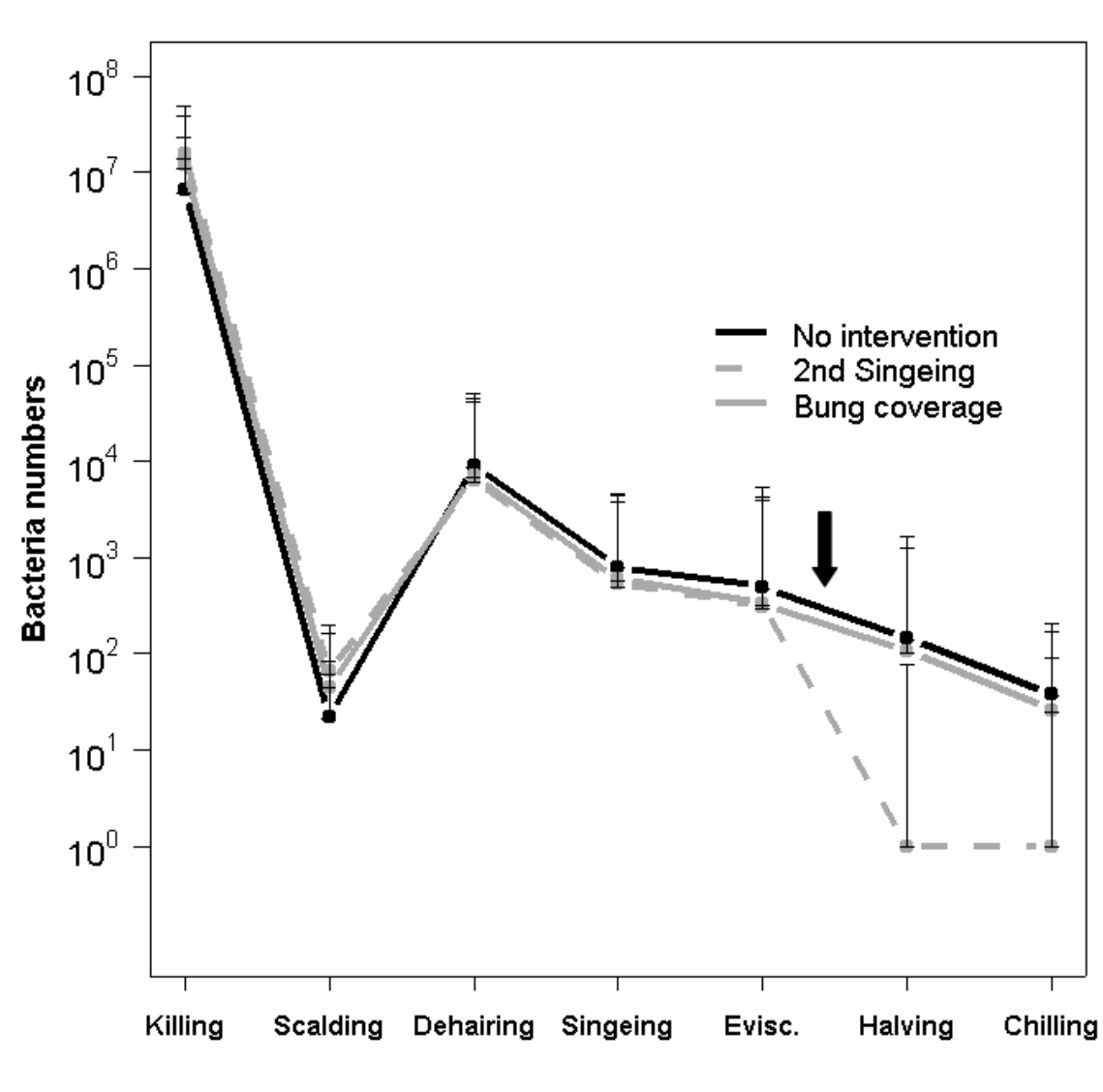


Figure 6.4: Predicted *Campylobacter* numbers on the surface of pig carcasses at different stages in abattoir with and without mitigation strategies. Arrow indicates application of second singeing step.

6.5 Discussion

This article demonstrates that the previously developed suite of semi-stochastic, risk models can be used to evaluate the efficacy of control strategies targeted at minimising pork contamination with *Salmonella*, *E. coli* and *Campylobacter* in New Zealand. We assessed strategies that are cost-effective to implement by abattoirs of all sizes and capacities. While these mitigation techniques are aimed at only three pathogens, its effect may be extrapolated to other zoonotic pathogens on the surface of pigs.

The second singeing procedure was considered to be the more effective control strategy, predicting an approximate 3 log unit reduction in median contamination level and a 71 – 81% reduction in mean prevalence levels for all three investigated pathogens. This result is in accordance with a finding from Alban & Stärk (2005). These authors developed a risk model describing *Salmonella* prevalence from the live pig on the farm to the final dressed carcass at the abattoir, which predicted that singeing has a large impact on pathogen prevalence. Other methods used to control zoonotic pathogens on pork carcasses include hot water decontamination; decontamination by the use of organic acids (van Netten et al. 1995); enclosure of the rectum with a plastic bag and logistic slaughter (Goldbach & Alban 2006, Swanenburg et al. 2001). We did not investigate any control strategy that involved dipping knives in hot water before use on different carcasses, as this is standard procedure in New Zealand and was incorporated into the baseline model.

For hot water decontamination, pig carcasses are showered with hot water (80°C) for 14 to 16 seconds. This has been considered a most effective procedure in reducing *Salmonella* numbers, but the installation of the facilities allowing this procedure, as well as its implementation would be costly. Hot water carcass decontamination is also expected to be as effective in reducing other pathogens such as *Campylobacter*. Goldbach & Alban (2006) stated that this procedure is expected to result in a 2 log unit reduction of *Salmonella* contamination levels. It is therefore comparable to the second singeing technique, the latter technique is however predicted to be more effective in reducing pathogen levels.

Decontamination by the use of organic acids is forbidden in New Zealand and was therefore not investigated in this paper. Logistic slaughter, which is used in Denmark, requires that the serological status or prevalence level of pathogens on farms is known (Chang et al. 2003*b*). Since no such routine testing of pig farms with respect to pathogen

prevalence and has been conducted in New Zealand, execution of this procedure would be time-consuming, and require a considerable amount of resources to implement. It was therefore not considered a feasible option to investigate at this point in time.

It must be considered that the implementation of any intervention strategy comes with a cost. There is a cost to the abattoirs and eventually this will be passed to the consumer via the retail outlets. Consequently, in the development of a control strategy, consideration should be given to efficacy and cost-effectiveness of its implementation, so that pork remains affordable to the consumer. The implementation of a second singeing step in the abattoir, does not only require the purchase of another blow torch, but includes maintenance, increasing the abattoir labour force, possibly lengthening the processing chain and therefore increasing the time to output a single dressed carcass.

The sensitivity analysis, which identified and quantified parameters most influential to the predicted model output, highlighted different parameters for the three organisms. The time spent in storage was a critical factor for all pathogens, therefore, extending the storage time of carcasses was predicted to reduce the pathogen load on carcasses. The time spent in storage is dependent on the storage capacity in abattoirs, the number of carcasses processed and the demand placed by the retailers. As such, considerable extension of storage time may not be possible in some abattoirs. For *E. coli* the time taken for singeing was also found to be an important parameter. It would not however be advisable to greatly extending the time each carcass spends in singeing immediately post-dehairing, as the carcasses are warm as a result of scalding and large increases in scalding times may lead to carcasses incurring burns or cooking, making them friable, unsightly and rendering subsequent processing procedures more difficult to execute. The introduction of another short scalding procedure post-evisceration may be a better option. Other methods to control *E. coli* numbers would be to wash pigs in the lairage just prior to killing. This is sometimes performed in abattoirs in New Zealand. Another strategy to reduce *E. coli* contamination may be to reduce the time each carcass spends in the dehairing machine. However, this action may negatively impact on the hair removal process. The sensitivity analysis also identified the inactivation of bacteria on the carcass skin during evisceration to be a critical parameter that was negatively correlated with the model output. The negative correlation was not unexpected as the we expect pathogen death on the carcass to be inversely proportional to final surface contamination levels.

With respect to *Salmonella*, most of the influential parameters pertained to the lairage section of the model. The number of pigs infected with this pathogen increased during lairage, resulting in the possibility of increased surface contamination. Therefore reduction of the lairage time should reduce the number of this pathogen on dressed pork in the abattoir. Also, as for *E. coli*, the showering of pigs immediately prior to slaughter will further reduce faecal contamination on the carcass surface. Short lairage times however have been sometimes found to be associated with the development of pale soft exudative pork (Warriss 2003). Regular cleaning and disinfection of the lairage is expected to be relatively inefficient as a control measure, as *Salmonella* has been isolated from 70 - 90% of lairage floors and walls after disinfection (Swanenburg et al. 2001). Increased singeing time was also effective in reducing contamination levels on carcasses. As previously stated, an additional singeing step post-evisceration may be an excellent control measure in minimising pathogen numbers on dressed carcasses.

The most effective and simple control measure for *Campylobacter* is the use of blast chilling during storage, which is currently performed. Our sensitivity analysis identified storage time, during which the organism is blast chilled, to be a highly significant parameter ($p < 0.001$). This pathogen is known to be very susceptible to chilling particularly by this method (Chang et al. 2003*b*). Faecal contamination occurring during dehairing was another critical parameter in the model describing pathogen dynamics for *Campylobacter*. This can be explained by the high concentration of this pathogen in faecal material. Therefore even small quantities of faecal spillage can result in large increases in carcass contamination levels. The probability of bacteria moving from the skin to the knife was negatively associated with the model output. This is understandable as less movement of bacteria off the carcass is expected to result in higher contamination levels on the pork surface. Some PRCC recorded negative values which appear to be biologically implausible; however, all of these were non-significant values and these parameters have little influence on the model outcome. Scalding was not included in the sensitivity analysis as fixed point estimates, not distributions were used to describe parameters pertaining to this process.

We assumed that coverage of the bung, with a plastic bag would reduce faecal spillage onto the carcass ten fold, but this is an estimate. Research needs to be conducted to determine the extent of faecal spillage occurring when coverage of the bung is imple-

mented. Unfortunately, due to severely limited resources, model validation was not executed. Also, a general scarcity of data resulted in several assumptions having been made. Nevertheless, this model allows updating and can be executed in the future with accurate New Zealand specific data when it becomes available.

In conclusion, we demonstrate that modification of the baseline model can be performed to evaluate intervention strategies in the abattoir. Additionally, we propose that the introduction of a second singeing step immediately post-evisceration and extension of the time carcasses spend in blast chilling in storage are efficacious methods of achieving reductions in the surface contamination levels of *Salmonella*, *E. coli* and *Campylobacter*. Finally we present our suite of models as a tool for further evaluation of intervention strategies in New Zealand pig abattoirs.

CHAPTER 7

Modelling pathogen dynamics during pork chop production

7.1 Abstract

The consumption of pork products contaminated with food-borne microbial pathogens such as *Salmonella*, *E. coli* and *Campylobacter*, may result in illness in susceptible individuals. This paper describes a suite of semi-stochastic modular process risk models developed to simulate the pathogen dynamics of *Salmonella*, *E. coli* and *Campylobacter*, during the processing of pork chops in New Zealand. Model parameters were estimated from published data. At the point of sale at retail outlets, our models predicted that 2.75% of pork chops will be contaminated with doses of *Campylobacter* associated with probabilities of infection ranging from 0.6 – 0.8. *Salmonella* and *E. coli* are estimated to be present on pork chops in colony forming units associated with probabilities of infection of less than 0.5. The predictions for *Salmonella* are based on the assumption that prevalence values are similar to those in Europe.

Raw pork chops sold at retail in New Zealand therefore present a low risk to the consumer of contracting salmonellosis, colibacillosis and campylobacteriosis. Placing pork chops in a blast chiller for 12 hours prior to display, was predicted to be an effective mitigation measure, resulting in 15 – 61% reduction in the maximum pathogen levels on pork chops, 44 – 100% reduction in the 10^{th} –90^{th} range and 14 – 50% reduction in pathogen prevalence levels.

7.2 Introduction

Modular process risk models (MPRM) have been used effectively for the exposure assessment phase of quantitative microbial risk assessments (Nauta, van der Fels-Klerx & Havelaar 2005, Nauta et al. 2003). This methodology requires compartmentalisation of the food pathway into stages called "modules". Within modules "processes" which include the pathogen dynamics of inactivation and growth, along with food handling dynamics of mixing, cross-contamination, removal and partitioning are identified and explicitly described (Nauta, van der Fels-Klerx & Havelaar 2005). The output of the MPRM is a distribution of the contamination level of the investigated pathogens on the food(s) of interest. Personal communication with the New Zealand Pork Industry Board (NZPIB) indicated that pork chops are one of the most commonly consumed pork products in New Zealand. Pork at retail is known to transmit zoonotic pathogens such as *Salmonella*, *Campylobacter* and *E. coli* (Wong et al. 2007, Conedera et al. 2007, Krumkamp et al. 2007, Gill & Jones 2006), which are known to cause gastroenteritis in humans (Pintar et al. 2007). Despite the fact that pork has been implicated as a source of zoonotic bacteria leading to food-borne diseases, little research has been undertaken in New Zealand to determine the risk posed by locally produced pork products. To address this shortcoming, this paper proposes a novel suite of models employing the MPRM methodology for use in quantitative microbial exposure assessments. These models describe the propagation of *Salmonella*, *E. coli* and thermophilic *Campylobacter* in pork chops made from the longissimus dorsi pig muscle, in New Zealand. These models are referred to as the further processing models. The outputs predicted from a previously developed suite of mathematical models describing the transmission of these pathogens in New Zealand abattoirs (Chapter 3) is used as inputs in the further processing model. We also investigate the effect of an intervention strategy aimed at minimising pathogen contamination levels on pork chops sold at retail.

7.3 Model Description

Definition of scope (pathogen and food type)

In this paper non-typhoidal *Salmonella* refers mainly to *S.* Typhimurium, a serotype responsible for many human salmonellosis cases internationally. We define *Campylobacter* as the thermophilic *Campylobacter* spp. of *C. coli*, *C. jejuni* and to a lesser extent *C. lari*. Initially we considered investigating EHEC *E. coli* O157, however, after very low levels of detection were observed in our data collection studies, we modified our research to incorporate all *E. coli*, both pathogenic and non-pathogenic strains. The presence of this pathogen is considered to be an indicator of faecal contamination. For the purpose of this manuscript, the food under investigation is the halved pork chop at the point of sale at retail outlets in New Zealand.

Model description

The Modular Process Risk Model methodology is used to develop a suite of semi-stochastic models that describes pathogen numbers and prevalence on pork chops in New Zealand. The production of pork chops from the dressed pig carcass is referred to as further processing. Different procedures are used for the production of pork chops in New Zealand. Sometimes the abattoir is responsible for obtaining the pork chops, which are then transported to retail outlets; in other situations, the pork carcass is first transported from the abattoir to the retail outlet, where the pork chops are then produced. For the purposes of this paper, we will model the former scenario. The outputs from the suite of abattoir models are used as inputs for the further processing models. Therefore the further processing models follow from those previously discussed in Chapter 3. The following two modules are therefore identified during pork production:

1. Cutting: the dorsal region of the chilled, halved, pig carcasses is sectioned into pork chops by use of a band saw.
2. Transport/storage/display: the product is transported to retail outlets in refrigerated trucks and displayed for sale at temperatures approximating 4°C.

The processes occurring in this model are partitioning, cross-contamination, inactivation and growth. Figure 7.1 presents a schematic of the modules with their processes. The pork chop extracted from the longissimus dorsi muscle of the pig is the unit of interest.

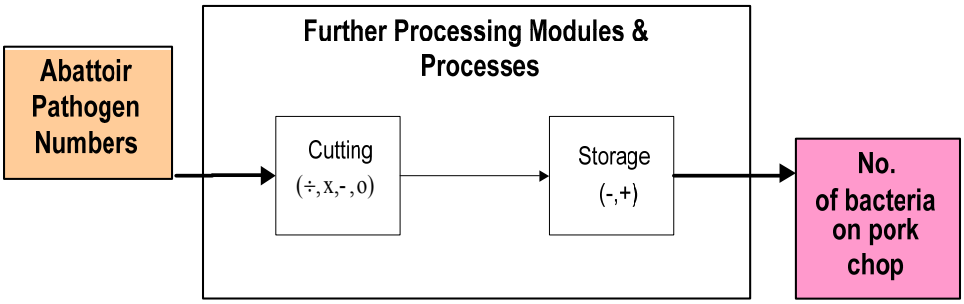


Figure 7.1: Modules and Processes in pork chop production. The processes represented are as follows: Inactivation: (-), Cross-contamination (x), Growth (+), Removal (o) and Partitioning: (÷). The model outputs indicate the number of pathogens on pork chops.

We assume that there is no growth on carcasses unless specifically stated.

Model Structure

We assume that good operational procedures are conducted in retail outlets so that there is cleaning of equipment between carcasses. This minimises cross-contamination between carcasses, which was not incorporated into the model. The following sections detail the model structure and equations described in each module. We are concerned with contamination on the skin surface of pig carcasses or portions of the carcasses.

Cutting: During this module, we describe the processes of partitioning, cross-contamination and inactivation/removal. For partitioning, the number of bacteria on the finished product, resulting from partitioning ($N_{x,c}(i)$) is dependent on the number of pathogens on the end of the carcass at the previous stage (storage in the abattoir:$N_{x,storage}(i)$) and the probability that an organism of concern resides on the surface of the skin of the pork chop p_{cut}. This is determined by:

$$N_{x,c}(i) = Binomial(N_{x,storage(i)}, p_{cut}) \tag{7.1}$$

where $p_{cut} = Beta(\alpha_1, \alpha_2)$. The distribution defining p_{cut} is dependent on the skin surface area of each pork chop in proportion to the total surface area of the halved pig carcass and pathogen aggregation. When $N_{x,storage}(i)$ is large, the binomial distribution

approximates a Normal distribution (Vose 2000). Therefore, when $N_{x,storage}(i)$ exceeded 30,000, then $N_{x,c}(i)$ is defined by a Normal distribution with mean = $N_{x,storage}(i)p_{cut}$ and standard deviation = $N_{x,storage}(i)p_{cut}(1-(1-p_{cut}))^{0.5}$.

Cross-contamination and pathogen removal are also modelled during the Cutting module. It is assumed that 24 pork chops are obtained from each halved carcass. Since these processes are considered to occur in discrete time, difference equations are employed to describe the modelled pathogen dynamics. Bacterial numbers present on the skin surface of the i^{th} pork chop after cross contamination ($N_{x,pc}(i)$) are a function of the contamination level on the exterior of the pork chop prior to cutting ($N_{x,pc-1}(i)$) and that on the saw, after use on the previous porkchop ($N_{saw}(i-1)$). Figure 7.2 demonstrates the parameters indicating cross-contamination as a and b respectively, while pathogen inactivation/growth on the carcass or removal from the carcass and saw are represented as c and d, respectively. Further, we assume that the saw is free of pathogens at the beginning of the day and, since it is cleaned between carcasses, it is also pathogen free at the start of cutting each carcass. A full description of parameter values is provided in Table 7.1. Cross-contamination, growth and removal are therefore described by the following two equations which determine pathogen numbers on the saw ($N_{saw}(i)$) and skin surface of the pork chop ($N_{x,pc}(i)$):

$$\begin{aligned} N_{x,pc}(i) &= (1-a)(1-c)N_{x,pc-1}(i) + bN_{saw}(i-1) \\ N_{saw}(i) &= (1-d)(1-b)N_{saw}(i-1) + aN_{x,pc-1}(i) \end{aligned} \tag{7.2}$$

This section is modelled such that the final contamination level on the saw after cutting one pork chop becomes the input value used in the model for $N_{saw}(i)$ when calculating pathogen numbers for the subsequent pork chop from the same carcass.

Transport/storage/display

The transit time between abattoirs and retail outlets varies considerably. For the purpose of these models, we estimate this to vary from 30 minutes to 10 hours. The maximum and minimum values of 0.5 and 10 hours were estimated based on a supermarket chain in New Zealand, where all stores in the North Island receive their meat from a single abattoir in the northern portion of the island (Auckland). Further, it was ascertained from

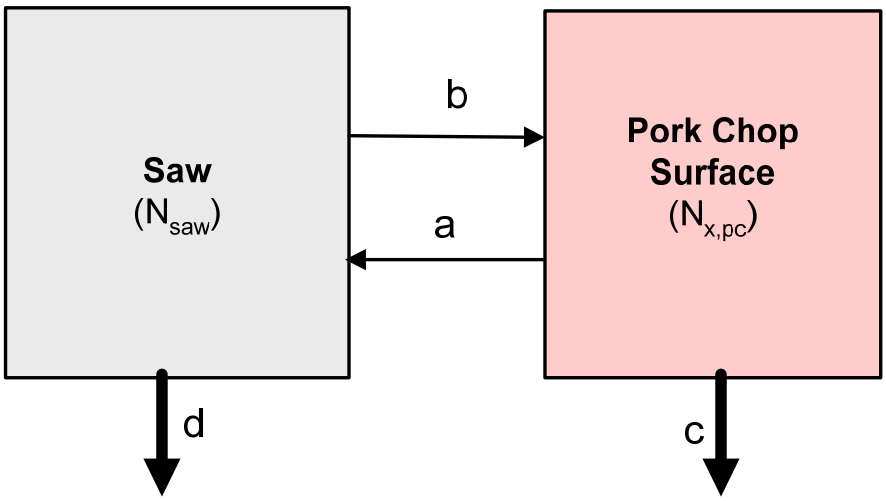


Figure 7.2: Diagram adapted from Nauta, van der Fels-Klerx & Havelaar (2005) showing parameters, variables and compartments modelled using difference equations for the further processing models. Thin arrows indicate the direction of movement of bacteria between compartments, that is, cross-contamination. Thick arrows represent bacterial inactivation/removal or growth. Table 7.1 provides an explanation of the parameters shown.

Table 7.1: Overview of description of model parameters for the Further Processing models. The parameter values are assumed to be constant during the modelling.

Parameter	Description
a	Probability that each cfu of bacteria moves from the carcass exterior to the saw.
b	Probability that each cfu of bacteria moves from the saw to the carcass exterior per cfu.
c	Probability of inactivation/growth and removal from the carcass exterior per cfu of bacteria.
d	Probability of inactivation/growth and removal from the saw per cfu of bacteria.

a questionnaire conducted in the one region of New Zealand, (Palmerston North), that fresh pork has an average turnover time of 2 days from receipt at the supermarket to sale. Consequently, we estimate the duration of this module to range from 1 – 3 days. The questionnaire used to obtain this information is included in the General Appendix.

The temperature at which fresh pork chops are transported to retail and stored for display is reported to be approximately 4°C in New Zealand. The Malthusian growth model, which is also referred to as the simple exponential growth model is used to model growth/inactivation and survival is described by:

$$P_t = P_0 e^{\kappa t} \tag{7.3}$$

where P_t refers to the pathogen numbers on the pork chop at the point of sale, P_0 corresponds to pathogen numbers on the pork chop at the beginning of the module, κ is the inactivation rate or growth rate and *t* represents the time in days. If *r* is negative, there is inactivation. If κ is positive, there is growth.

Model parameters

There is very little data from New Zealand available for the parameters needed for the study. Consequently, parameters values used to inform the model are obtained primarily from published literature applicable to Europe and the United States of America.

Partitioning

The distributions of contamination levels obtained from storage in the abattoir are Lognormal $\sim$ (1090, 40896, truncated (min = 0, max = 99,642)) for *Salmonella*, Loglogistic $\sim$ (0.078146, 8.2793, 1.0377, truncated (min = 0, max = 6164)) for *E. coli* and Inverse Gaussian $\sim$ (14656, 14, truncated (min = 0, max = 351336)) for *Campylobacter* and have been derived from a previous study (Chapter 3). Values designated as limits of the distributions correspond to the minimum and maximum pathogen levels predicted to be on carcasses in the abattoir. These distributions were used as values for $N_{x,storage}(i)$.

In order to determine p_{cut} for the partitioning process, we made the assumption that all pathogens were equally distributed on the halved carcass surface. We then estimated the weight of a pork chop to be 0.15 – 0.36% of the weight of the carcass. The approximate weight of a pork chop is 210g (Sheard et al. 1998), while the range of recorded weights of carcasses in New Zealand abattoirs was 58.4kg – 86.2kg. Using these values, the relative weight of the pork chop was calculated. Assuming that the entire pig carcass is a cylinder, and that the relative weight of the pork chop equates the volume of the pork chop, the surface area of skin corresponding to this volume occupied by the pork chop was estimated to be approximately 0.8 – 2.2% of the surface area of the pig, and twice as much for a half carcass. For this calculation, we used the average length of the carcass (152cm) which was obtained from observational studies conducted in a pig abattoir in New Zealand. (In New Zealand abattoirs the length of the carcasses were recorded to range from 137.7 – 164.5cm, with an average of 152cm.) Although the shape of the pig

does not form a perfect cylinder, our calculated surface area, while not precise, provides a reasonable estimate for use in model calculations. Using the BetaBuster program, a Beta distribution described by Beta $\sim$ (7, 17) is chosen to describe p_{cut} for halved carcasses.

Cross-contamination and Inactivation/Removal

Parameter values used in cross-contamination were all obtained from the literature. In a study by Kusumaningrum et al. (2003), the probability of transfer of *Campylobacter* and *Salmonella* between stainless steel, kitchen utensils and meat are reported. The meat used is chicken and sausages, however, we assumed that the transfer rate to pork is similar. Also, since *Salmonella* and *E. coli* are both flagellated, *Enterobacteriaceae*, we assume similar transfer probabilities for the two pathogens. This study also determined the probability of survival of the two pathogens on stainless steel. Again we assumed *Salmonella* and *E. coli* had the same value for the probability of survival.

The inactivation/removal/growth parameters for the different pathogens are derived from different studies reported in the published literature. Table 7.2 details the parameter values for modelling cross-contamination, inactivation and growth, as well as the referenced literature. The values for the probability of inactivation or growth on the carcass are derived from published literature and are calculated to be 0.0002, (Farrell & Upton 1978) -0.00044 (Bredholt et al. 1999, Ng et al. 1997) and 0 (Solow et al. 2003) for *Salmonella*, *E. coli* and *Campylobacter* respectively (Table 7.2).

Transport/storage/display A growth model equation was used to describe the pathogen dynamics occurring during Transport/storage/display. The values for the rate of inactivation or growth on carcasses are derived from published literature and are calculated to be 0.3453, (Farrell & Upton 1978), -0.063 (Bredholt et al. 1999, Ng et al. 1997) and 0 (Solow et al. 2003) per day for *Salmonella*, *E. coli* and *Campylobacter* respectively.

Second order modelling

The separation of uncertainty and variability is called “second order modelling” and this is accomplished by identifying distributions representing parameter variability and parameter uncertainty and utilising a double-looping technique to determine the contribution of each in the model. The inner loop of the double-looping technique propagates model variability while the outer loop propagates model uncertainty. Latin hypercube sampling of parameters and variables representing uncertainty is conducted once only for each sim-

Table 7.2: Values for model parameters describing cross-contamination in the Further Processing models.

Parameter	Values for *Salmonella*	Values for *Campylobacter*	Values for *E. coli*
a	Normal(0.21, 0.08,Truncate(0,1)) (Kusumaningrum et al. 2003)	Normal(0.28, 0.13, Truncate(0,1)) (Kusumaningrum et al. 2003)	Normal(0.21, 0.08,Truncate(0,1)) (Kusumaningrum et al. 2003)
b	Normal(0.49,0.21, Truncate(0,1) (Kusumaningrum et al. 2003)	Normal(0.66,0.26, Truncate(0,1)) (Kusumaningrum et al. 2003)	Normal(0.49,0.21, Truncate(0,1)) (Kusumaningrum et al. 2003)
c	0.0002 (Farrell & Upton 1978)	0 (Solow et al. 2003)	-0.00044 (Bredholt et al. 1999, Ng et al. 1997)
d	1-(Normal(0.14, 0.07,Truncate(0,1))) (Kusumaningrum et al. 2003)	1-(Normal(0.42, 0.12,Truncate(0,1))) (Kusumaningrum et al. 2003)	1-(Normal(0.14, 0.07,Truncate(0,1))) (Kusumaningrum et al. 2003)

ulation. Therefore, during multiple iterations in a simulation, they are held constant. In contrast, Latin Hypercube sampling of parameters representing variability is executed for each iteration. A non-parametric determination of the distribution of bacteria post-storage is then conducted in the software package MATLAB® 7. Two parameter distributions are classified as representing variability, pathogen numbers at the beginning of the Transport/storage/display module and the number of pathogens on the exterior of the pork carcass destined for pork chop production. The other parameters and variables are considered to describe uncertainty.

Sensitivity analysis

Sensitivity analyses indicate the most influential parameters/variables in the determination of the predicted model output (Anonymous 2004*b*). In this paper we conduct a sensitivity analysis for each organism using 10,000 model iterations with Latin Hypercube sampling of distributions for parameters. A rank order correlation between output values and their associated inputs is then calculated. This procedure is executed in Microsoft Excel with @Risk 4.5.5 Add-in (Palisade Corporation, 2005).

Intervention Strategy

The effect of placing pork chops in a blast chiller for 12 hours to achieve an internal temperature of 2°C prior to display was evaluated. In order to accomplish this, the model was modified to incorporate this control measure. The process of inactivation is modelled

during blast chilling using the following equation:

$$\frac{dN_{p,i}}{dt} = -r_{p,c}N_{p,i} \tag{7.4}$$

where $N_{p,i}$ refers to pathogen numbers on the skin surface of the pork chop during this intervention step; t represents length of time in the blast chiller and $-r_{p,c}$ is the rate of inactivation/hour. Outputs from the model with the intervention strategy were compared to model outputs without the incorporation of an intervention strategy. The latter of which will be referred to as the baseline model in this paper. The parameters and methodology used to incorporate this feature into the model was taken from Chapter 3, where storage in a blast chiller was explicitly described in the model.

Model Implementation

The results presented in this paper have been obtained using Monte Carlo simulations performed in Microsoft Excel with @Risk 4.5.5 Add-in (Palisade Corporation, 2005). One simulation consisted of 10,000 iterations using Latin Hypercube sampling, with each iteration simulating the pathogen dynamics occurring during the production of pork chops from a halved, dressed, carcass, to display at retail. The models output distributions of bacterial numbers present on pork chops at retail. For second order modelling, the model is executed for 100 simulations consisting of 100 iterations.

7.4 Results

The further processing models output the estimated number of pathogens on the surface of the skin of the pork chop. Parametric distributions describing these numbers were obtained using Palisade @Risk software and are presented in Figure 7.3. These distributions are skewed to the right with many pork chops containing low numbers of the three pathogens of interest and a small number of pork chops estimated to be contaminated with high bacterial numbers. Maximum levels of pathogens predicted on pork chops are: 6579, 86 and 13150 cfu, with 10^{th} to 90^{th} values of 0 – 18, 0 – 1 and 0 – 9 cfu for *Salmonella*, *E. coli* and *Campylobacter*, respectively. *E. coli* is predicted to be present on a mere 14% of all pork chops, while considerably more, 56% and 40% of all pork chops are predicted to be contaminated with *Salmonella* and *Campylobacter* respectively.

Generally there is a reduction of pathogen numbers for all three investigated bacteria during the Cutting Module. During the Transport/storage/display module, pathogen numbers decrease in the case of *Campylobacter* and *E. coli,* however increase with respect to *Salmonella*. Table 7.3 shows the change in pathogen numbers for the different modules from partitioning to the point of sale at the retail outlet.

Intervention strategy

The introduction of blast chilling for 12 hours prior to display of the pork chops is predicted to be an effective intervention strategy resulting in 14% – 50% reduction of the prevalence of pathogens at the point of sale, when compared to the baseline model (Table 7.4). The effect of blast chilling on prevalence levels is estimated to be greatest for *E. coli* and least for *Salmonella*. The maximum number of *Campylobacter* on pork chops is predicted to reduce by 60% as a result of the mitigation strategy. *Salmonella* and *E. coli* recorded estimated reductions of 15% and 51% respectively, with respect to the maximum predicted contamination level on the pork chops. For *Salmonella*, *E. coli* and *Campylobacter*, this mitigation scenario is also predicted to reduce the 10^{th}–90^{th} range by 44%, 100% and 66%.

Second order modelling

Results of the second order modelling for *Salmonella*, *E. coli* and *Campylobacter* are shown as “spaghetti-looking” graphs which simultaneously display uncertainty and variability over 100 simulations, as shown in Figure 7.4. The predicted distribution of pathogen numbers on the surface of the skin of pork chops displayed for sale from each simulation is plotted and represented by a single green line in the graphs. Variability is shown by the number of bacteria on the pork chop for sale, which corresponds to the values along the x-axis. This is large for all pathogens. Uncertainty is measured as the vertical difference or span between simulations. Therefore the amount of parameter uncertainty in the model for each pathogen is seen by comparing the uppermost distribution of a single simulation and lowermost distribution of a single simulation. We obtain a median distribution from each of the 100 simulations. The model describing *Campylobacter* contains the largest parameter variability, while the model parameters describing the propagation of *E. coli* contains the smallest. The model pertaining to *Salmonella* displays the greatest extent of parameter uncertainty, while the model for *E. coli* displays the least.

Sensitivity analysis

The sensitivity analysis identified parameters/variables that were most influential in predicting the final pathogen numbers on pork chops, as shown in Figure 7.5. For all pathogens the predicted pork chop contamination level obtained after the cross-contamination module was the single most important factor affecting model output. For *Salmonella* and *Campylobacter*, which were predicted to be present in higher numbers than *E. coli*, the initial pathogen load on the halved carcass, as well as the relative area of the skin of the pork chop with respect to the halved carcass surface area were also identified as being highly influential in the model. For *E. coli*, the highlighted important parameters were the initial pathogen load on the halved carcass, and pathogen numbers predicted to be on the pork chop from the partitioning module. The probability of inactivation/growth and removal from the carcass exterior per cfu "c" was not included in the sensitivity analysis, as it was estimated to be fixed values, not distributions.

Table 7.3: Predicted contamination levels on pork chops on completion of each module within further processing.

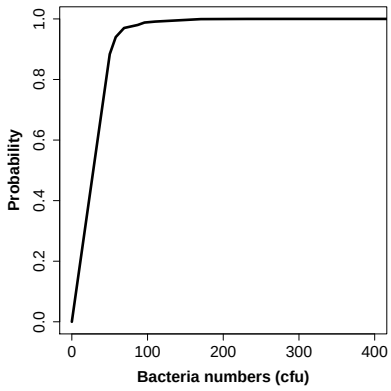
organism	No. of pathogens at Cutting (cfu) (Partitioning)	No. of pathogens at Cutting (cfu) (Cross-contamination)	No. of pathogens at Transport/storage/display (cfu)
Salmonella	1 (0 – 31)[5587]	0 (0 – 8)[4238]	1 (0 – 18)[6579]
E. coli	0 (0 – 2)[195]	0 (0 – 0)[163]	0 (0 – 1)[86]
Campylobacter	1 (0 – 19)[33310]	1 (0 – 3)[17960]	0 (0 – 9) [13150]

Median bacteria numbers given, with 10^{th}–90^{th} values (), as well as maximum values [] reported.

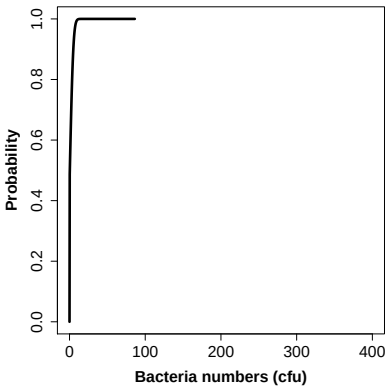
Table 7.4: Predicted numbers of *Salmonella*, *E. coli* and *Campylobacter* on pork chops estimated from the mitigation strategy.

Organism	Scenario	No. of pathogens on pork chop at point of sale (cfu)	Pathogen prevalence (%)
Salmonella	Baseline (i.e. no. mitigation)	1 (0 – 18)[6579]	56
	Additional blast chilling	0 (0 – 10)[5597]	48
E. coli	Baseline (i.e. no. mitigation)	0 (0 – 1)[86]	14
	Additional blast chilling	0 (0 – 0)[42]	7
Campylobacter	Baseline (i.e. no. mitigation)	0 (0 – 9)[13150]	40
	Additional blast chilling	0 (0 – 3)[5112]	26

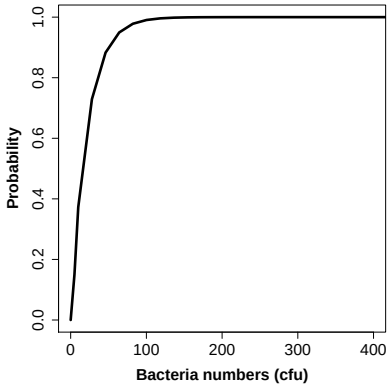
Median bacteria numbers given, with 10^{th}–90^{th} values (), as well as maximum values [] reported.



(a)

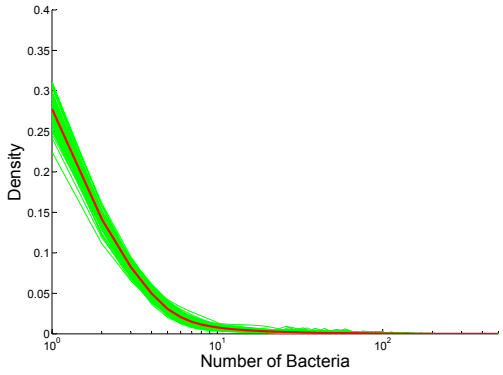


(b)

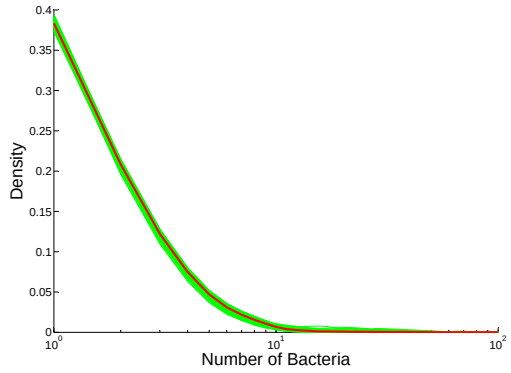


(c)

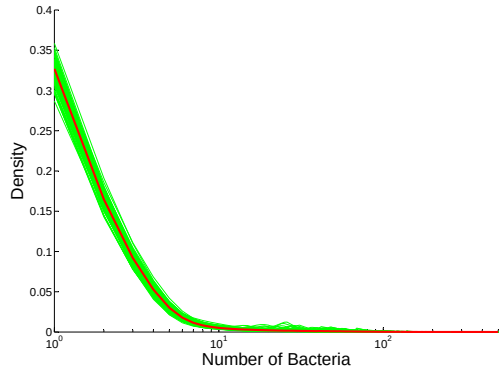
Figure 7.3: Cumulative probability distribution functions of the predicted contamination levels on the skin of pork chops for sale at retail outlets in New Zealand pertaining to *Salmonella* (a), *E. coli* (b) and *Campylobacter* (c).



(a)



(b)



(c)

Figure 7.4: Spaghetti-looking graphs demonstrating the results of the second order modelling for the further processing models. Medians (red, solid line) of the 100 simulations (each simulation is coloured green) for *Salmonella* (a), *E. coli* (b) and *Campylobacter* (c) are plotted. Each green line describes the predicted non-parametric distribution of bacteria numbers on the surface of the skin of the pork chop at the point of sale in retail outlets, from one simulation.

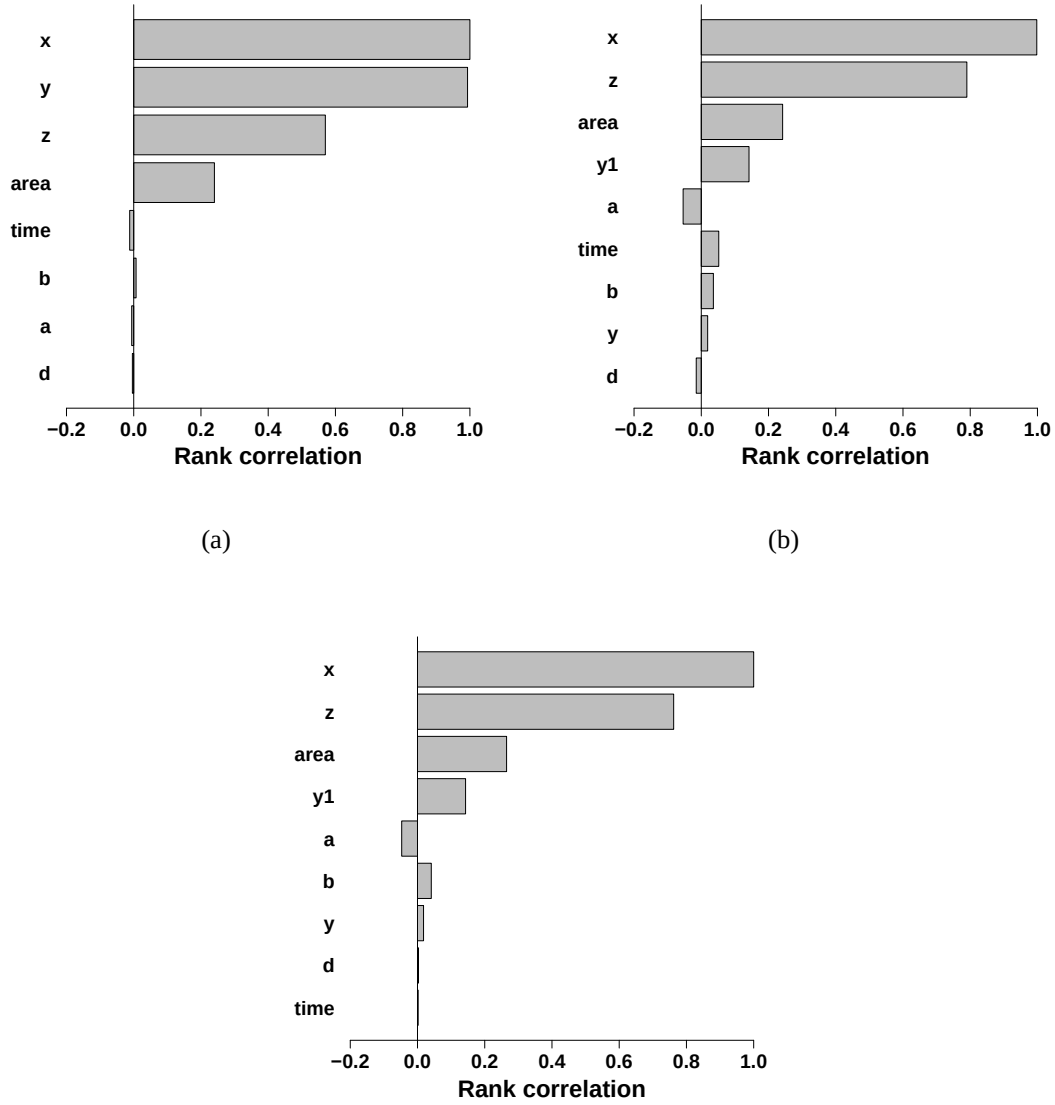


Figure 7.5: Tornado plot of sensitivity analysis results for *E. coli* (a), *Salmonella* (b) and *Campylobacter* (c) showing the relative importance of predictive factors on final contamination levels on the skin of pork chops. The predictive factors are: pork chop contamination level after cross-contamination (x); bacteria numbers on half carcass (z); pathogen numbers on the skin of the pork chop after partitioning when z<30,000 (y); pathogen numbers on the skin of the pork chop after partitioning when z>30,000 (y1); relative proportion of pork chop surface area with respect to the half carcass (area); duration of Transport/storage/display module (time); cross-contamination/inactivation parameters a (a), b (b) and d (d).

7.5 Discussion

To our knowledge this is the first paper to develop a suite of quantitative semi-stochastic risk models to describe the propagation of *Campylobacter*, *Salmonella* and *E. coli* in pork chop production in New Zealand. This paper also quantifies the relative importance of bacterial dynamics influencing pathogen levels on pork chops, as well as provides a framework for examining the quantitative effects of further processing on pathogen dynamics. Our models output the distributions of surface contamination levels on the skin of pork chops, which is one of the most commonly consumed pork products in New Zealand. Pathogen prevalence values were also predicted by the models.

The cutting module, which incorporated the pathogen processes of partitioning, cross-contamination and inactivation resulted in a reduction of all three pathogens. On the other hand, the transport/storage/display module had different effects on the three bacteria under investigation. The numbers of *E. coli* were estimated to decrease, while the numbers of *Campylobacter* remained relatively unchanged and *Salmonella* increased. *Salmonella* proliferates at temperatures of 2 – 7°C (D'Aoust 1991) with the fastest growth recorded within the first two days (Farrell & Upton 1978). This explains the increase in pathogen numbers predicted by the model from the Cutting module to the Transport/storage/display module. Therefore, the longer the time taken for transport, storage and display of the pork chop, the more the number of *Salmonella* colonies expected to be present on the contaminated meat. Furthermore, if the meat is not consumed in the same day of purchase, but placed in the refrigerator for days, there may be an even greater number of *Salmonella* colonies present on the contaminated pork chop. Fortunately, the pork chop requires cooking prior to consumption and this will reduce the number of viable organisms. Haas et al. (1999) used a Beta-Poisson dose-response model to predict that ingestion of 2.36×10^4 cfu is expected to produce infection in 50% of the exposed individuals. This dose is more than three times the maximum number of organisms predicted to be on a pork chop from our model.

One of the inputs into the models is the pathogen numbers on the surface of pork carcasses from abattoirs. Results from microbial studies conducted in New Zealand abattoirs (detailed in chapter 3) indicated that the number of *Salmonella* on pig carcasses in New Zealand is very low and/or rare. Wong et al. (2007) found a 0% prevalence level on pork at retail in New Zealand. The authors stated that 231 pork products were purchased from

retail outlets in both North and South Islands and tested for the presence of *Salmonella* using a protocol similar to that used in this thesis. The detection process employs a pre-enrichment step which increases test sensitivity. However, it is still possible that samples containing very low counts of the pathogen are not detected. Despite the recorded absence of the pathogen in pork, given the importance of this pathogen internationally, we chose to describe the propagation of this pathogen in our models. All parameters and data informing the model were derived from Europe and North America. Therefore, the model predicts the prevalence and numbers of *Salmonella* on the skin of pork chops in New Zealand, if the prevalence and numbers of this pathogen in pigs were similar to that currently estimated for Europe and the USA.

The model also predicted that approximately half (56%) of the raw pork chops will be contaminated with this pathogen. Sharma et al. (1987) reported prevalence levels of 42% and 10.3% in pork sausages and fresh pork, respectively. Another study reported the prevalence level of *Salmonella* in pork to be as high as 65% (Angkititrakul et al. 2005). It is estimated that in The Netherlands, pork consumption accounts for approximately 15% of the food-borne salmonellosis cases (Berends et al. 1998). Although salmonellosis has been reputed to cause fewer cases of illnesses in England than *Campylobacter*, it is however responsible for more deaths (Adak et al. 2005). This disease is the second-most frequently reported bacterial gastrointestinal illness in New Zealand (NZFSA 2005).

There was little effect of the transport/storage/display module on *Campylobacter* numbers on pork chops. *Campylobacter* has been found to survive without decreased viability and in the absence of any noticeable growth at 4°C (Pintar et al. 2007, Solow et al. 2003). This is important as pathogen levels of 100 cfu have been associated with moderate to high probabilities of infection (0.6 – 0.8) (Teunis et al. 2005). Results from our model predicted that 2.75% of the pork chops contained this pathogen in numbers greater than or equal to 100 cfu. It is this small percentage of highly contaminated products that pose a risk to the consuming population. Additionally, our model predicted that 40% of raw pork chops are contaminated with *Campylobacter*. This large prevalence value is not in agreement with other studies which reported low prevalence values on pork at retail ranging from 0% – 10.3% (Whyte et al. 2004, Pezzotti et al. 2003, Nesbakken et al. 2003). One possible reason to explain this discrepancy may stem from low sensitivity of isolation tests. In fact both isolation tests and sampling protocols may result in failure to

detect the organism when it is present in low numbers, particularly in situations where the pathogen is not homogenously distributed. Therefore several pork products may go undetected or erroneously be considered to be free of *Campylobacter*. It is also possible that our model over-estimates the estimated prevalence values. More accurate parameters can be obtained by collecting appropriate data.

Campylobacteriosis is of particular importance in New Zealand as the incidence of this disease has increased steadily within the last twenty years (Baker et al. 2007) and is the leading cause of gastro-intestinal illness (Wong et al. 2007). One study conducted in New Zealand found a 9.1% prevalence of this pathogen on pork and most of the serotypes isolated were *C. jejuni* (Wong et al. 2007). In England and Wales, *Campylobacter* infections from contaminated food were estimated to have the greatest burden on the health sector of all food-borne pathogens (Adak et al. 2005).

The predicted prevalence of *E. coli* was the lowest among all three pathogens. The model estimated a mere 18% prevalence level. Zhao et al. (2001) also reported similar prevalence levels of *E. coli* (16.3%) in pork in the Washington, USA. Haas et al. (1999) used a dose-response model informed by data from human feeding studies to estimate that a dose of 2.6×10^6 cfu on *E. coli* is associated with infection of 50% of the exposed population. Since no pork chop was estimated to contain this quantity of *E. coli*, we predict from our model outputs that this food at the point of retail bears a low to negligible risk to the consumer of contracting an illness from *E. coli* as a result of handling.

The difference model equations employed were modified from that used by Nauta, van der Fels-Klerx & Havelaar (2005). Data from the literature were used to determine most parameters values, as New Zealand specific data was not available. In the event that the information was not readily forthcoming from the literature, as was the case with determining the relative skin surface area of a pork chop, approximations with a mathematical basis were employed. In real-life situations, the relative skin surface area of a pork chop is very variable and will depend on the size and breed of the pig among other factors. For simplicity in our model, we estimated a fixed weight for all pork chops.

The pathogen process of cross-contamination is modelled so that the final contamination level on the saw after cutting one pork chop is the input value used in the model when calculating pathogen numbers for subsequent pork chops produced from the same carcass. This approach allows a more accurate estimate of contamination levels to be

predicted. We assumed thorough cleaning of the saw between carcasses. However, observational studies revealed that this hygienic practice was not always exercised in retail outlets in New Zealand. It is this breakdown in sanitation that may increase the risk of food-borne disease occurring in the population.

Any efficient intervention strategy aimed at minimising pathogen levels on pork chops would either arrest growth and/or result in bacterial inactivation and/or removal. Changes in temperature such as heat and cold, and the application of additives to the meat can affect pathogen viability and growth. The application of heat is not an option (since the product is to be sold raw and not cooked) then either exposure to a cold environment or the application of an additive such as salt are other options to consider. From a previous study (Chang et al. 2003*b*) it was determined that blast chilling leads to inactivation of the pathogens of concern. Taking this into consideration, the effect of blast chilling on pork chops was investigated. The time of 12 hours was evaluated as it was thought that this was sufficient to have an effect on pathogen numbers and yet was not too long to impede to a large extent the supply of the product to the consumer. Therefore, if pork chops are produced in the afternoon, they can be displayed the following morning. We assumed that reductions in pathogen numbers due to blast chilling would be evidenced for the entire 12 hour period, that is beyond the cooling period of the pork chop, as a result of an initial temperature reduction of the pork chop followed by desiccation (Chang et al. 2003*b*). The addition of salt and other additives such as nitrites was not investigated for two reasons. Firstly, the introduction of these chemicals may reduce the appeal of the pork product to the public. Secondly, salting of foods and the addition of nitrites has been associated with the formation of carcinogenic compounds (Jakszyn et al. 2006). Freezing was not considered because the fresh product is more desirable and acceptable to the population.

Unfortunately, due to severe time and financial constraints, model validation was not conducted. Therefore the models predictions should be interpreted with caution. The authors assumed that 24 pork chops were obtained from each halved carcass. However, this is an oversimplification to facilitate model computation, as the number of chops produced will vary according to the size of pig carcass. This paper describes the production of pork chops using one protocol, further research can be conducted to develop another suite of models describing pathogen dynamics in pork production for the other protocol.

Second order modelling is instrumental in increasing the accuracy of risk estimates

(Wu & Tsang 2004, Vose 2000). Therefore we separated parameter variability and uncertainty and determined that the relative contribution of parameter variability was greater than parameter uncertainty for all three pathogens.

It is not unexpected that the sensitivity analysis on the suites of models indicated that the initial contamination level of the halved carcass was a critical parameter in determining model outcome, particularly since there is no procedural step resulting in large quantities of pathogen inactivation or removal. Therefore the initial pathogen level will influence to a large extent, the pathogen numbers on the final product. The only process that is expected to have a large impact on bacterial numbers as a result of inactivation and removal from the pork chop (arising from re-distribution of pathogens between saw and pork chop) is the output from the cross-contamination module. This predictor was highlighted to be the single most influential variable in the model for all pathogens. The three most important predictors were the same for *Salmonella* and *Campylobacter*. Both of these pathogens were estimated to have distributions on the halved carcasses that were greater than that for *E. coli* and were modelled using both Binomial and Normal distributions during the partitioning module. This was not the case for *E. coli*, in which only the Binomial distribution was used for modelling of the partitioning process. The third most critical parameter in the model was identified as a component of the partitioning module, highlighting the importance of the pathogen dynamics of partitioning in pork chop production.

In conclusion, we developed a further processing model which comprised a suite of semi-stochastic, quantitative risk models describing the propagation of *Salmonella*, *E. coli* and *Campylobacter* through pork chop production from halved carcasses obtained from abattoirs in New Zealand. These models used the outputs from a previously developed suite of models describing the pathogen dynamics of these three pathogens in pig abattoirs, as input values. They facilitate further understanding of the pathogen dynamics occurring during pork chop production. A combination of the two suites of models (the abattoir and further processing models) describe the propagation of three zoonotic pathogens from the live pig in the abattoir to pork chops, displayed for sale at retail outlets. The further processing models enable insight to be gained into the pathogen dynamics occurring during pork chop production and the effect on contamination levels of pork chops at retail outlets. The models predicted that storage of pork chops during transport

and at retail outlets will have the greatest impact on increased pathogen numbers, but only for *Salmonella*. The same procedure is estimated to lead to reduced levels of *E. coli* and have little to no effect on *Campylobacter* numbers. Partitioning and cross-contamination generally result in reduced pathogen numbers. Our models predict the distributions of pathogen numbers on pork chops on completion of modules or section of the modelled food pathway. Prevalence levels on pork chops can also be obtained. These outputs allow use of our models in quantitative exposure assessments, a component of quantitative microbial risk assessments.

CHAPTER 8

General discussion

8.1 Introduction

This thesis presents novel research in that it is the first to describe a suite of semi-stochastic mathematical risk models describing the propagation of multiple zoonotic enteric *Enterobacteriacea* (*Salmonella*, *E. coli*) and *Campylobacter* in pig abattoirs and during pork chop production in New Zealand. The models are hazard-based and predict the distributions of pathogen numbers and prevalences on dressed pork carcasses leaving the abattoir and on pork chops displayed at retail outlets, thereby making them suitable exposure assessment models. The microbial load on pork chops sold at retail is of great interest to the New Zealand pork industry as it seeks to expand its market locally and regionally — a goal for which consumer confidence in product safety is vital.

The WHO has recently implemented initiatives to curb food-borne illnesses and alleviate the socioeconomic burden caused to countries internationally. Zoonotic diseases comprise a considerable part of food-borne illnesses identified to date, as the WHO estimates that approximately 75% of new communicable diseases that affect humans are zoonotic (WHO 2007*b*). These diseases present as obstacles to international trade, resulting in restricted movement of food. Unfortunately, the economic impact on countries unable to export their product can in some circumstances be devastating. One methodology proposed by the WHO to mitigate food-borne disease is through the use of risk-based techniques, such as quantitative microbial exposure assessments which incorporate the use of mathematical models. With increased accessibility to computers around the globe, as well as enhanced computer capabilities, mathematical models offer a good option for assessing food-borne diseases. It must be remembered however, that inherently, mathematical models are incomplete representations of realistic scenarios.

8.2 Model development and techniques used

In this thesis we proposed a suite of semi-stochastic, quantitative, risk models. The quantitative nature, as opposed to a qualitative model results in less subjectivity being incorporated into the model than that would be expected if the model was developed qualitatively. However, minimisation of subjectivity comes at a cost, since stochastic models are more complex to build/develop. The stochastic aspect of our models allowed the incorporation of randomness or chance in the model producing a range or distribution of values describing pathogen numbers on the food product of concern as the model output. Had deterministic modelling techniques been used, a point estimate would be produced. This would lead to loss of information. If the median value were outputted, the outliers or tail ends of the distribution would not be reported and these values may be most influential in impacting on the risk of disease. Also, reporting only maximum values fails to give an indication of the contamination level on most of the modelled food product. This can result in food erroneous considered to convey a relatively moderate or high risk to the consumer, when in fact it conveys a low risk.

The mechanistic nature of the suite of models increased the complexity of the model development process. Mechanistic models are generally more complex than empirical models. One of the benefits of using this type of model is that it facilitates increased understanding of the system that is being investigated. Citing just two examples, insights were gained into pathogen dynamics during scalding in the pig abattoir and the partitioning module of the pork chop production. The increased understanding of the modelled systems enabled informed mitigation strategies to be investigated. Unlike black box or empirical models, the mechanistic nature of our models enabled evaluation of proposed risk mitigation strategies in a short period of time, which would otherwise be time-consuming and costly to implement and evaluate. We investigated the efficacy of three mitigation strategies targeted at reducing the numbers and prevalence of the pathogens of concern on the food product of interest.

Model building is a critical component of quantitative exposure assessments. The extent of detail incorporated in the model is important as inclusion of excessive detail can produce overly complex models, making model building and execution laborious, tedious, difficult and time-consuming. Conversely, if the model does not incorporate sufficient detail, oversimplification of real-life situations or processes occurs, resulting in the

prediction of less realistic outputs. Model predictions can be useful to risk managers, such as the abattoir and supermarket management, or even governments, providing information which can inform procedural changes or policy-making decisions. Therefore an important objective was to produce the most realistic prediction of the modelled system using the available information.

Mechanistic models usually contain many parameters. Parameter determination is another essential component of model development and this was found to be one of the most challenging aspects of the thesis, particularly since there was very limited information or data available. It is mandatory for the National Microbial Database in New Zealand to collect data on microbial pathogens in abattoirs for all food-producing species except pigs. Therefore there is a dearth of information available pertaining to the microbial contamination levels of pork carcasses processed in abattoirs in New Zealand. However, since 2003, one abattoir in the North Island implemented the quantitative microbial testing of 20 pig carcasses per month, post-evisceration and pre-chilling as part of its quality control programme. Samples collected are tested for the presence and number of *E. coli*, aerobic plate counts and *Salmonella* at three sites — the flank, shoulder and outer side of the hind leg. Unfortunately this was not adequate data for parameter estimation required in our model.

The model development process therefore led to the identification of gaps in the data. Parameter estimation under conditions of data scarcity proved to be a useful exercise, as this situation may not be uncommon in many parts of the world. Modelling techniques appear to be developed faster than relevant data collection, which in many circumstances is a time consuming, financially draining and labour intensive procedure. Consequently, in our model, we resorted to using data from published literature for parameter determination. This method offers the advantage of providing a cost-effective solution. However, there is an underlying assumption that the studies used were conducted in a satisfactory manner and that the data obtained in the studies are externally valid and can be used in the model under development. Another method of dealing with the gaps in the data was to conduct studies which gathered the necessary information. We used this approach also and conducted several microbial and observational studies, as well as retrieved data from questionnaires to inform our model. The microbial studies were laborious, time-consuming and expensive, limiting the quantity of data collected. Had labour, time and

finances not been so restrictive, several quantitative longitudinal studies on carcass and faecal contamination levels in all the major abattoirs in New Zealand would have been undertaken to obtain the necessary data concerning the selected pathogens. If pertinent additional information is collected in future, it can be readily incorporated into the model which is designed to allow updating. This aspect of the model enables the model development process to be iterative, allowing more realistic risk estimates to be generated for New Zealand over time. Assumptions and simplifications were also used in parameter determination when gaps in the data were identified. Yet another method of parameter estimation is through the use of expert opinion. This can introduce bias into the model and it is also a long, detailed process, and under the imposed time restraint was not a feasible option.

The modular process risk model (MPRM) was used to model all aspects of the suite of models. This technique has been designed for use in quantitative exposure assessments (Nauta 2001) particularly in the event of data scarcity, which was relevant to our situation. The compartmental component of this technique simplified the modelling process and facilitated understanding of the processes that were to be explicitly described. Monte Carlo simulations using Latin hypercube sampling were conducted as this sampling technique ensured that the samples selected for use during one simulation reflected the input distribution. This was particularly important since each simulation comprised a small number of iterations. The Monte Carlo sampling technique therefore could not ensure that the samples selected for use in one simulation would reflect the input distribution.

The OIE recommended technique of correlation analysis was used for sensitivity analyses. More specifically, we used partial rank correlation coefficient analyses (PRCC). This technique has the added benefit in that it facilitates exploration of the full range of values for each parameter distribution in a model that is being investigated and quantifies the correlation between each input parameter and the output variable, while accounting for all other parameters in the model (Turner et al. 2006). The Spearman rank correlation technique does not account for the other model parameters. Unfortunately, the PRCC is tedious. Additionally, since the speed of execution of the analysis is dependent on the model computation time, the results for the sensitivity analysis for one pathogen was obtained after at least two days.

8.3 The model

Abattoirs present a viable option for controlling pathogen numbers in pork and therefore this section of the food-pathway was chosen for detailed analysis and modelling. The modular process risk model methodology was used to develop the novel, mechanistic, semi-stochastic, risk model which described the processes of removal, inactivation, cross-contamination, and partitioning occurring during the propagation of *Salmonella*, *E. coli* and *Campylobacter* through a hypothetical abattoir in New Zealand (Chapter 3). This model incorporated both differential and difference equations to describe pathogen dynamics occurring in continuous and discrete time. Difference equations are less mathematically demanding to solve and provide information of the modelled processes only at discrete time points. Differential equations, on the other hand, while being the more mathematically demanding of the two types of equations provide information on the modelled processes over a continuous period of time. The criteria determining the choice of equation type was based on the abattoir procedure being modelled.

The model development process resulted in the model builder gaining an increased understanding of the pathogen processes occurring. As previously stated, the predicted model outputs included distributions of pathogen numbers on carcasses at different stages of the abattoir, thereby showing the predicted effects of abattoir procedures on the pathogen of interest. We were then able to surmise from our model that dressed pork carcasses leaving abattoirs in New Zealand contain relatively low numbers of pathogens which are not associated with high probabilities of infection in humans (probability greater than 0.8). Therefore pork leaving the abattoir may be considered to have a low risk or minimal likelihood of being implicated as a major source of these specific zoonotic microbes of major public health significance — *Salmonella*, *E. coli* and *Campylobacter*. Additionally, our model predicted that of the three pathogens modelled, thermophilic *Campylobacter* was present in the largest numbers on dressed pork carcasses exiting the abattoir. Second order modelling which was conducted for the determination of more mathematically correct risk estimates revealed that parameter variability or the true heterogeneity of contamination levels contributed more to the variance of the output distributions than parameter uncertainty, or imperfect knowledge regarding parameters.

There is room for improvement of the model with respect to reducing model computation time. The long time taken to execute large number of simulations was determined

to result from the large number of calculations required during the scalding section of the model. Possibly changing the technique used to solve the differential equation to one that produces comparable results in a faster time would be beneficial. Additionally, the use of database software packages instead of the spreadsheet application used may have expedited the time taken to execute the model. Alternatively other programming languages such as Visual Basic, C++ and others which facilitate code compilation may also reduce model computation time. Another aspect of the model that can be changed is the reliance on the Palisade @Risk program which needs to be downloaded on the computer to allow model execution. This limits the widespread use of the model. Additional computer programming to incorporate parametric distributions into the Visual Basic for Applications code is needed to eliminate this reliance on a licensed software package.

The scalding procedure was examined in further detail in Chapter 4 as this was the most complex abattoir procedure to model. Here, we detailed the progressive development of the equations used for scalding from a deterministic to the final parallel semi-stochastic model using differential equations. The complexity in modelling this abattoir stage arose as a result of the large extent of cross-contamination occurring. Generally, cross-contamination is a difficult process to explicitly describe in a model, even when it only occurs between two surfaces. However, during scalding, cross-contamination occurs between multiple carcasses and the environment simultaneously, so the incorporation of these features into the model was indeed challenging. Further, the parameter values for the different organisms varied considerably and to accommodate this large variation and maintain mathematical accuracy, the calculations used to solve the differential equations had to be performed with increased frequency, contributing to the long computation time. Exploration of this section of the model revealed the predominant pathogen processes occurring both on the pig surface and in the water at different scalding temperatures and pathogen concentration levels in the scald tank water.

In this thesis, we chose to use a mechanistic individual-based approach in designing our model. However, the population-based approximation methodology has also been used in modelling. In Chapter 5, we undertook a comparative analysis which investigated whether the population-based methodology was an acceptable alternative to the individual-based technique used in our model and under which conditions is this former method limited in its ability to produce results similar to the individual-based approach.

This chapter addressed the issue of model uncertainty. It answers the question — could we not have simply used the population-approximation technique, to expedite model development and model computation time? Although this comparative analysis may appear rather simplistic, the results are important for model building, not only with respect to our model, but other similar quantitative models. We deduced that if the model input distributions are described by large variances, the results from the population-based approach would differ considerably from predictions derived from the individual-based approach. In such situations the individual-based approach is preferred.

Taking into account the findings obtained from Chapter 3, we sought to identify using our model, efficient risk mitigation strategies for the three investigated organisms at the abattoir. Consequently, Chapter 6 focussed on the conduction of a sensitivity analysis and investigated different mitigation strategies. From these analyses it was concluded that the implementation of a second singeing step immediately post-evisceration as well as extending the time spent in blast chillers in storage will result in a reduction of the pathogen levels on dressed carcasses exiting the abattoir. The model predictions can be used to inform management at abattoirs across New Zealand. Extending the time that carcasses spend in storage in abattoirs may affect subsequent portions of the production chain such as supermarkets, which may expect to receive their product at specific dates and times. These intricate details will need to be considered prior to implementation of any mitigation strategy.

We used the outputs from Chapter 3 as inputs for a modular process risk model describing the processes of inactivation, growth, cross-contamination and partitioning during the propagation of pathogens in pork chop production. As for the abattoir model, both difference and differential equations were used. Less than 3% of pork chops produced in New Zealand were predicted to be contaminated with doses of *Salmonella*, *E. coli* and *Campylobacter* associated with moderate to high probabilities of infection (0.6 – 0.8). We concluded from our model predictions that pork chops sold at retail contain low levels of the three pathogens of interest and at concentrations which are generally not associated with high probabilities of infection.

Model validation was not conducted because of financial and time restraints as well as the notable absence of suitable surveillance data. The process of model validation can be considered to incorporate four parts — conceptual validation, validation of algorithms,

software code validation and functional validation. The qualitative process of conceptual validation determines whether the model sufficiently and accurately represents the phenomenon under consideration. Validation of the software code and algorithms are collectively referred to as model verification. This involves verifying that the model code is accurate and that the model performs in the manner expected by the model developer. With respect to algorithms, the model is checked to ensure that the model equations are mathematically correct and represent the concepts attempted to be portrayed in the model. Finally, functional validation refers to checking the model against independent data. Conceptual validation, which is based on the opinion of experts or other risk developers/assessors was not conducted. All mathematical equations in this model were checked by Jonathan Marshall, a mathematician, thereby fulfilling the criteria for algorithm validation. The services of two computer programmers were used to assist in writing the Visual Basic for Application code used in the model and evaluation of the model output was constantly performed. Since relevant independent data was not readily available and time and financial resources hindered its collection, functional validation was not undertaken. If future work is to be performed regarding this model, it is suggested that functional validation be performed as soon as possible.

8.4 Future Applications

We propose our suite of models for use in New Zealand. Also, the models in whole or in part can provide a template for the design of exposure assessment risk models describing the propagation of the zoonotic pathogens modelled in this thesis and others which possess similar epidemiologic patterns (such as *Arcobacter*) on pig carcasses in New Zealand. If other pathogens are modelled, parameter modification is needed. Our model can also be modified for use in other species undergoing similar abattoir processing. Since distributions of pathogens are produced, it can be used by risk assessors in the development of quantitative microbial risk assessments. Although the parameters are developed for New Zealand, modification of the necessary parameters will make the model applicable for use by many countries.

The suite of models eliminates the need for the use of multiple separate models. After validation, these model can potentially be used by government officials, such as risk

assessors to evaluate the safety of pork products delivered to the local market and in the design and optimisation of control programmes pertaining to food-borne disease. Private companies desiring to export their pork products overseas, local abattoirs and supermarket management wishing to monitor and improve the quality of their product can also benefit from the outputs produced by the model. Evaluation of risk mitigation strategies can be undertaken by our model as a cost-effective method of obtaining information that may lead to the reduced incidence of food-borne illnesses. Training required to use the model should be relatively simple and minimal.

The role of the veterinary epidemiologist is not only restricted to determining disease distribution, but also disease determinants and deterrents. Concerning public health, veterinary epidemiologists can play a vital part in providing the basis for developing and evaluating public health programmes. In this vein, the outputs of risk assessments and quantitative risk assessments pertaining to food safety and zoonotic diseases are tools used to accomplish objectives. Mathematical models in exposure assessments can be used to predict the levels of endemic, exotic, emerging and re-emerging pathogens in foods and evaluate control strategies aimed at minimising food-borne illnesses and further our understanding of pathogen dynamics. Exposure models therefore can enable pro-active, cost-efficient methods of optimising pathogen control measures to mitigate the burden caused by food-borne diseases.

Data collection on a national level is required to inform risk models, as model development is an on-going process. This information can be acquired from surveillance programmes. Large databases may therefore be established which governments of the country should be able to access for the purposes of analysis. This may require submission of microbial samples to laboratories on a regular basis from abattoirs, supermarkets and butchers. If possible the developed surveillance system should allow the food product to be traced back to the originating farm.

8.5 Conclusion

This thesis seeks to address the need for determination of the safety of pork products produced in New Zealand. To this end we propose a novel suite of models for use in quantitative exposure assessments of zoonotic pathogens transmitted by pork. The developed

models are mechanistic, semi-stochastic and employ the modular process risk modelling technique to describe the propagation of *Salmonella*, *E. coli* and *Campylobacter* through the abattoir and further processing of pork chop production. Both difference and differential equations are used to aptly describe the complex pathogen dynamics occurring in the food pathway. The model development process and model outputs have enhanced our understanding of the effects of procedural stages on the number and prevalence of bacteria. It is not possible to gather such information from microbial studies. Further, the mechanistic component of the models enables evaluation of the efficacy of intervention strategies on mitigation of the pathogens of interest. Since each of the models output distributions of the numbers and the prevalence of pathogens, they are applicable for use in quantitative microbial risk assessments for select portions of the farm to fork continuum of the pork food chain. Finally we propose that our models can be used as a template for the design and development of future exposure assessment models.

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APPENDIX A

General appendix

Visual Basic Code for Applications: Model describing the propagation of zoonotic pig pathogens in an abattoir in New Zealand

EpiCentre, Massey University, 2005 – 2007

API Functions

Private Declare Function apiLoadLibrary Lib “kernel32” Alias “LoadLibraryA” (ByVal lpLibFileName As String) As Long Private Declare Function apiFreeLibrary Lib “kernel32” Alias “FreeLibrary” (ByVal libHandle As Long) As Long

Declaring @Risk Functions Methods. These Distribution Functions can be used in the program module

Declare Function RDK_Beta Lib “RSKLIB32.DLL” (ByVal a1#, ByVal a2#, ByVal label As String) As Double
Declare Function RDK_BetaSubj Lib “RSKLIB32.DLL” (ByVal min#, ByVal Mode#, ByVal Mean#, ByVal Max#, ByVal label As String) As Double
Declare Function RDK_Binomial Lib “RSKLIB32.DLL” (ByVal n%, ByVal p#, ByVal label As String) As Double
Declare Function RDK_Chisq Lib “RSKLIB32.DLL” (ByVal V%, ByVal label As String) As Double
Declare Function RDK_Cumul Lib “RSKLIB32.DLL” (ByVal min#, ByVal Max#, ByVal n%, ByRef X#, ByRef p#, ByVal label As String) As Double
Declare Function RDK_Discrete Lib “RSKLIB32.DLL” (ByVal n%, ByRef X#, ByRef p#, ByVal label As String) As Double
Declare Function RDK_Duniform Lib “RSKLIB32.DLL” (ByVal n%, ByRef X#, ByVal label As String) As Double
Declare Function RDK_Erf Lib “RSKLIB32.DLL” (ByVal h#, ByVal label As String) As Double Declare Function RDK_Erlang Lib “RSKLIB32.DLL” (ByVal M%, ByVal beta#, ByVal label As String) As Double
Declare Function RDK_Expon Lib “RSKLIB32.DLL” (ByVal beta#, ByVal label As String) As Double
Declare Function RDK_Extvalue Lib “RSKLIB32.DLL” (ByVal alpha#, ByVal beta#, ByVal label As String) As Double Declare Function RDK_Gamma Lib “RSKLIB32.DLL” (ByVal alpha#, ByVal beta#, ByVal label As String) As Double
Declare Function RDK_General Lib “RSKLIB32.DLL” (ByVal min#, ByVal Max#, ByVal n%, ByRef X#, ByRef p#, ByVal label As String) As Double
Declare Function RDK_Geomet Lib “RSKLIB32.DLL” (ByVal p#, ByVal label As String) As Double
Declare Function RDK_Histogrm Lib “RSKLIB32.DLL” (ByVal min#, ByVal Max#, ByVal n%, ByRef p#, ByVal label As String) As Double
Declare Function RDK_Hypergeo Lib “RSKLIB32.DLL” (ByVal n%, ByVal d%, ByVal M%, ByVal label As String) As Double
Declare Function RDK_Invgauss Lib “RSKLIB32.DLL” (ByVal mu#, ByVal lambda#, ByVal label As String) As Double
Declare Function RDK_Logistic Lib “RSKLIB32.DLL” (ByVal alpha#, ByVal beta#, ByVal label As String) As Double
Declare Function RDK_Loglogistic Lib “RSKLIB32.DLL” (ByVal Gamma#, ByVal beta#, ByVal alpha#, ByVal label As String) As Double
Declare Function RDK_LogNorm Lib “RSKLIB32.DLL” Alias ”RDK_Lognorm” (ByVal Mean#, ByVal SD#, ByVal label As String)

```
As Double
Declare Function RDK_Lognorm2 Lib “RSKLIB32.DLL” (ByVal Mean#, ByVal SD#, ByVal label As String) As Double
Declare Function RDK_Negbin Lib “RSKLIB32.DLL” (ByVal s%, ByVal p#, ByVal label As String) As Double
Declare Function RDK_Normal Lib “RSKLIB32.DLL” (ByVal Mean As Double, ByVal SD As Double, ByVal label As String) As Double
Declare Function RDK_Pareto Lib “RSKLIB32.DLL” (ByVal theta#, ByVal A#, ByVal label As String) As Double
Declare Function RDK_Pearson5 Lib “RSKLIB32.DLL” (ByVal alpha#, ByVal beta#, ByVal label As String) As Double
Declare Function RDK_Pearson6 Lib “RSKLIB32.DLL” (ByVal alpha1#, ByVal alpha2, ByVal beta#, ByVal label As String) As Double
Declare Function RDK_Pert Lib “RSKLIB32.DLL” (ByVal min#, ByVal Mode#, ByVal Max#, ByVal label As String) As Double
Declare Function RDK_Poisson Lib “RSKLIB32.DLL” (ByVal lambda#, ByVal label As String) As Double
Declare Function RDK_Rayleigh Lib “RSKLIB32.DLL” (ByVal beta#, ByVal label As String) As Double
Declare Function RDK_Simtable Lib “RSKLIB32.DLL” (ByVal n%, ByRef TABLE#, ByVal label As String) As Double
Declare Function RDK_Student Lib “RSKLIB32.DLL” (ByVal V#, ByVal label As String) As Double
Declare Function RDK_Texpon Lib “RSKLIB32.DLL” (ByVal beta#, ByVal min#, ByVal Max#, ByVal label As String) As Double
Declare Function RDK_Tlognorm Lib “RSKLIB32.DLL” (ByVal Mean#, ByVal SD#, ByVal min#, ByVal Max#, ByVal label As String) As Double
Declare Function RDK_Tnormal Lib “RSKLIB32.DLL” (ByVal Mean#, ByVal SD#, ByVal min#, ByVal Max#, ByVal label As String) As Double
Declare Function RDK_Uniform Lib “RSKLIB32.DLL” (ByVal min As Double, ByVal Max As Double, ByVal label As String) As Double
Declare Function RDK_Triang Lib “RSKLIB32.DLL” (ByVal MinVal#, ByVal MidVal#, ByVal MaxVal#, ByVal label As String) As Double
Declare Function RDK_Trigen Lib “RSKLIB32.DLL” (ByVal LeftVal#, ByVal MidVal#, ByVal RightVal#, ByVal LeftArea#, ByVal RightArea#, ByVal label As String) As Double
Declare Function RDK_Weibull Lib “RSKLIB32.DLL” (ByVal alpha#, ByVal beta#, ByVal label As String) As Double

    ’ Global Region
Dim NumberPigsInLairage As Integer
Dim Lairage_TotalAmtFeacesInEnviron As Double
Dim Lairage_TotalamtInfectedFaecalMaterial As Double
Dim Lairage_TotalamtSalmonellaEnvt As Double

    ’ Statistics
Dim NumberOfIterations As Integer
Dim NumberSimulatons As Integer
Dim CurrentIteration As Long
Dim CurrentItraRunRow As Long
Dim CONST_NUMBEROFSTATISTICS As Integer
’Dim InterIterationValues() As Double
Dim InterIterationValues() As Variant
Dim InterSimulationValues() As Double
Dim NumberSimulations As Double
Dim CurrentSimulation As Integer
Dim InterSimulationPrevalenceMatrix() As Double
Dim NameOfColumnsToSummarise() As String
```

```
    ' Holds values that are only calculated once per iteration.
Dim CONST_NUMBEROFSINGLEITERATIONVALUESTOSUMMARISE As Integer

    Dim PrevalenceMatrix() As Double

    ' Independent Iterations flag
Dim IndependentIteration As Boolean
Dim PreviousIterationsScaldingW As Double
Dim PreviousIterationsDehairingM As Double
Dim PreviousIterationsEviscerationK As Double
Dim PreviousIterationsTrimmingK As Double
Dim PreviousIterationsHalvingK As Double
Dim Inputs As String

    ' RDK Initialisation
Declare Function RDK_Init Lib "RSKLIB32.DLL" (LibId As Long) As Integer

    ' RDK Simulation Settings
Declare Sub RDK_Settings Lib "RSKLIB32.DLL" (ByVal SetFlag As Boolean, Sampling As Integer, Seed As Integer, NumSim As Integer, NumIter As Long, SaveSamples As Boolean)
Declare Function RDK_MaxLabelledFunctions Lib "RSKLIB32.DLL" (ByVal MaxLabelledFuncs As Integer) As Integer
Const VB_BASE_INDEX = 1 Declare Function RDK_BaseIndex Lib "RSKLIB32.DLL" (ByVal index As Integer) As Integer
```

Lairage Module

```
Declaring Variables And Types used in the Lairage Worksheet
Private Sub LairageModule()

Dim Lairage_Prevalenceofinfection As Double
Dim Lairage_ProportionofInfectedShedding As Double
Dim Lairage_NumberSheddingPigsinlairage As Integer
Dim Lairage_NumberInfectedNotshedding As Integer
Dim Lairage_Timeinlairage As Double
Dim Lairage_Transmissioninfectionparameter As Double
Dim Lairage_ProbabilityofInfectionfromPigs As Double
Dim i As Integer
Dim Lairage_AmtofFaecesShedPPig As Double
Dim Lairage_TotalAmtFeacesInEnviron As Double
Dim Lairage_ProportionAllPigsShedding As Double
Dim Lairage_TotalamtInfectedFaecalMaterial As Double
Dim Lairage_ConcSalmonellaInInfectedFaeces As Double
Dim Lairage_TotalamtSalmonellaEnvt As Double

    ' Lairage Outputs
This section Calculates the Outputs for the Lairage Worksheet

    'Number Of Pigs In Lairage
' =Round(RiskNormal(Inputs B6, Inputs C6),0)
```

```
NumberPigsInLairage = Round(RDK_Normal(Worksheets(Inputs).Cells(6, 2).value, Worksheets(Inputs).Cells(6, 3).value, “”), 0)
’Output in B8 in Lairage Worksheet
Worksheets(“Lairage”).Cells(8, 2).value = NumberPigsInLairage

    ’PrevalenceInputs B7,Sheet C7)
Lairage_Prevalenceofinfection = RDK_Beta(Worksheets(Inputs).Cells(7, 2).value, Worksheets(Inputs).Cells(7, 3).value, “”)
’Output in B9 in Lairage Worksheet
Worksheets(“Lairage”).Cells(9, 2).value = Lairage_Prevalenceofinfection

    ’ProportionofInfectedShedding
’=RiskBeta(Inputs B8,Inputs C8)
Lairage_ProportionofInfectedShedding = RDK_Beta(Worksheets(Inputs).Cells(8, 2).value, Worksheets(Inputs).Cells(8, 3).value, “”)
’Output in B10 in Lairage Worksheet
Worksheets(“Lairage”).Cells(10, 2).value = Lairage_ProportionofInfectedShedding

    ’NumberSheddingPigsinlairage
’=RiskBinomial(Lairage B8,Lairage B9*Lairage B10)
Lairage_NumberSheddingPigsinlairage = RDK_Binomial(Worksheets(“Lairage”).Cells(8, 2).value, Worksheets(“Lairage”).Cells(9,
2).value * Worksheets(“Lairage”).Cells(10, 2).value, “”)
’Output in B11 in Lairage Worksheet
Worksheets(“Lairage”).Cells(11, 2).value = Lairage_NumberSheddingPigsinlairage

    ’NumberInfectedNotshedding
’=RiskBinomial(Lairage B8,Lairage B9)-Lairage B11)
Lairage_NumberInfectedNotshedding = RDK_Binomial(Worksheets(“Lairage”).Cells(8, 2).value, Worksheets(“Lairage”).Cells(9, 2).value,
“”) - Worksheets(“Lairage”).Cells(11, 2).value
’Output in B12 in Lairage Worksheet
Worksheets(“Lairage”).Cells(12, 2).value = Lairage_NumberInfectedNotshedding

    ’Timeinlairage
’=RiskUniform(B9,C9)
Lairage_Timeinlairage = RDK_Uniform(Worksheets(Inputs).Cells(9, 2).value, Worksheets(Inputs).Cells(9, 3).value, “”)
’Output in B13, B21, B28, B45 in Lairage Worksheet
Worksheets(“Lairage”).Cells(13, 2).value = Lairage_Timeinlairage
Worksheets(“Lairage”).Cells(18, 2).value = Lairage_Timeinlairage

    ’Transmissioninfectionparameter
’=Inputs, B10
Lairage_Transmissioninfectionparameter = Worksheets(Inputs).Cells(10, 2).value
’Output in B14 in Lairage Worksheet
Worksheets(“Lairage”).Cells(14, 2).value = Lairage_Transmissioninfectionparameter

    ’ProbabilityofInfectionfromPigs
’=Binomial(1, 1-exp(-LairageB14*LairageB11*lairageB13))
If Worksheets(Inputs).Cells(10, 2).value = 0 Then
Lairage_ProbabilityofInfectionfromPigs = RDK_Binomial(1, Worksheets(“Lairage”).Cells(10, 2).value, “”)
Else
Lairage_ProbabilityofInfectionfromPigs = RDK_Binomial(1, 1 - Exp(-Worksheets(“Lairage”).Cells(14, 2).value * Worksheets(“Lairage”).Cells(11,
2).value * Worksheets(“Lairage”).Cells(13, 2).value), “”)
```

```
End If
'Output in B15 in Lairage Worksheet
Worksheets("Lairage").Cells(15, 2).value = Lairage_ProbabilityofInfectionfromPigs

    'AmtofFaecesShedPPig
'=RiskUniform(Inputs B13, Inputs C13)* Lairage B18
Lairage_AmtofFaecesShedPPig = Worksheets("Lairage").Cells(18, 2).value * RDK_Uniform(Worksheets(Inputs).Cells(13, 2).value,
Worksheets(Inputs).Cells(13, 3).value, "")
'Output in B19 in Lairage Worksheet
Worksheets("Lairage").Cells(19, 2).value = Lairage_AmtofFaecesShedPPig

    'TotalAmtFeacesInEnviron
'= LairageB19*LairageB8
Lairage_TotalAmtFeacesInEnviron = Worksheets("Lairage").Cells(19, 2).value * Worksheets("Lairage").Cells(8, 2).value
'Output in B20 in Lairage Worksheet
Worksheets("Lairage").Cells(20, 2).value = Lairage_TotalAmtFeacesInEnviron
Worksheets("Killing").Cells(9, 3).value = Lairage_TotalAmtFeacesInEnviron

    'Proportion of pigs in lairage that are shedders
'=LairageB9*LairageB10
Lairage_ProportionAllPigsShedding = Worksheets("Lairage").Cells(9, 2).value * Worksheets("Lairage").Cells(10, 2).value
'Output in B21 in Lairage Worksheet
Worksheets("Lairage").Cells(21, 2).value = Lairage_ProportionAllPigsShedding
Worksheets("Killing").Cells(8, 3).value = Lairage_ProportionAllPigsShedding

    'Total amount of Infected Faecal Material in Lairage
'=LairageB20*LairageB21
Lairage_TotalamtInfectedFaecalMaterial = Worksheets("Lairage").Cells(20, 2).value * Worksheets("Lairage").Cells(21, 2).value
'Output in B22 in Lairage Worksheet
Worksheets("Lairage").Cells(22, 2).value = Lairage_TotalamtInfectedFaecalMaterial
Worksheets("Killing").Cells(10, 3).value = Lairage_TotalamtInfectedFaecalMaterial

    'ConcSalmonellaInInfectedFaeces
'Round(RiskPert(Inputs B14,Inputs C14,Inputs D14),0)
'Round(RiskUniform(Inputs B14,Inputs C14),0)
Lairage_ConcSalmonellaInInfectedFaeces = Round(RDK_Uniform(Worksheets(Inputs).Cells(14, 2).value, Worksheets(Inputs).Cells(14,
3).value, ""), 0)

    'Output in B23 in Lairage Worksheet
Worksheets("Lairage").Cells(23, 2).value = Lairage_ConcSalmonellaInInfectedFaeces

    'Total amount of Salmonella (cfu) in environment
'=B22*B23
Lairage_TotalamtSalmonellaEnvt = Worksheets("Lairage").Cells(22, 2).value * Worksheets("Lairage").Cells(23, 2).value
'Output in B24 in Lairage Worksheet
Worksheets("Lairage").Cells(24, 2).value = Lairage_TotalamtSalmonellaEnvt
Worksheets("Killing").Cells(11, 3).value = Lairage_TotalamtSalmonellaEnvt

    End Sub
```

Killing Module

```
    ' Helper method to calcualte the Columns in excel using @Risk functions

    'Declaring variables and types of the latter worksheets

Private Function KillingModule() As Boolean
Dim Killing_ProportionFaecalContaminationPPig() As Double
Dim Killing_ProportionContaminatedFeaces() As Double
Dim Killing_NoSalmonellaContaminationPPig() As Double
Dim i As Integer

ReDim Killing_ProportionFaecalContaminationPPig(NumberPigsInLairage)
ReDim Killing_ProportionContaminatedFeaces(NumberPigsInLairage)
ReDim Killing_NoSalmonellaContaminationPPig(NumberPigsInLairage)

    'Killing Worksheet Calcluations and Outputs

    ' ProportionFaecalContaminationPPig
' =RiskBeta(InputsB17,C17)
For i = 0 To NumberPigsInLairage - 1 Killing_ProportionFaecalContaminationPPig(i) = RDK_Beta(Worksheets(Inputs).Cells(17, 2).value, Worksheets(Inputs).Cells(17, 3).value, "")
Next

    Call FillColumnFromArray(2, 8, 8 + NumberPigsInLairage, "Killing", Killing_ProportionFaecalContaminationPPig)
Call FillColumn(2, 8 + NumberPigsInLairage, 8 + NumberPigsInLairage + 1000, "Killing", "")

    ' Killing_NoSalmonellaContaminationPPig
For i = 0 To NumberPigsInLairage - 1
Killing_NoSalmonellaContaminationPPig(i) = (Worksheets("Killing").Cells(i + 8, 2).value * Worksheets("Killing").Cells(8, 3).value * Worksheets("Killing").Cells(9, 3).value * Worksheets("Killing").Cells(11, 3).value) / Worksheets("Killing").Cells(10, 3).value
Next

    Call FillColumnFromArray(4, 8, 8 + NumberPigsInLairage, "Killing", Killing_NoSalmonellaContaminationPPig)
Call FillColumn(4, 8 + NumberPigsInLairage, 8 + NumberPigsInLairage + 1000, "Killing", "")

    End Function
```

Public Sub

```
    Declaring Variables & Data Types used in Scalding Calculations, Calling Modules

    'Declaring Variables & Data Types used in Scalding Calculations

    Public Sub RKInitalValueSolutionMultipleScaldingPigs()
```

```
    Dim TimeStart As Date: TimeStart = Now
Dim Time0 As Double 'Initial time period
Dim Timef As Double 'Final time period
Dim stepSize As Double

    Dim W0 As Double 'Initial infectivity of the water
Dim Mo As Double
Dim Ko As Double
Dim Ka As Double
Dim So As Double

    Dim alpha As Double
Dim beta As Double
Dim tau1 As Double
Dim tau2 As Double

    Dim PInit() As Double 'Array of the initial infectivitys of the pigs
Dim Pt0() As Double 'Time pig enters the bath
Dim Ptspan() As Double 'Duration of bath

    Dim Result() As Double
Dim NumberPigsInLairageToOuput As Integer
Dim timeDifferanceBetweenPigs As Double 'currently assuming these two values are constant for all pigs
Dim timeEachPigIsInBath As Double
Dim n As Integer
Dim i As Integer 'Loop variable
Dim j As Integer

    If CBool(Worksheets("model map").Cells(5, 5)) Then
Inputs = "Inputs Ecoli"
End If

    If CBool(Worksheets("model map").Cells(6, 5)) Then
Inputs = "InputsSal"
End If

    If CBool(Worksheets("model map").Cells(7, 5)) Then
Inputs = "InputsCampy"
End If

    'Clean intra run page from previous runs
CleanWorkSheet ("Intra run Statistics")

    'Clean calculations from previous runs
CleanWorkSheet ("Calculations")

    'Clean intra run page from previous runs
CleanWorkSheet ("Inter run Statistics")
```

```
    'Clean Simulation statistics page from previous runs
'CleanWorkSheet ("Simulation statistics")

    If Not Load_AtRiskLibrary Then
Exit Sub
End If

    CurrentItraRunRow = 2

    NumberOfIterations = Worksheets(Inputs).Cells(3, 2).value

    ReDim PrevalenceMatrix(NumberOfIterations)

    NumberSimulations = CInt(Worksheets(Inputs).Cells(3, 6))
ReDim InterSimulationPrevalenceMatrix(NumberSimulations)

' NOTE: the following two constant variables are important because that are used to
'determine summary statistics specifically and the size of the inter iteration matrix.

    'The number of summary statistic functions.
CONST_NUMBEROFSTATISTICS = 16

    'The number of columns that will be summarised
CONST_NUMBEROFCOLUMNSTOSUMMARISE = 9

    'The number of single iteration values to summarise
CONST_NUMBEROFSINGLEITERATIONVALUESTOSUMMARISE = 1

    ReDim NameOfColumnsToSummarise(CONST_NUMBEROFCOLUMNSTOSUMMARISE)

    ReDim InterIterationValues(0 To CONST_NUMBEROFSTATISTICS, 1 To CONST_NUMBEROFCOLUMNSTOSUMMARISE,
0 To NumberOfIterations)

    'ReDim SingleIterationInterIterationValues(0 To CONST_NUMBEROFSINGLEITERATIONVALUESTOSUMMARISE, 0 To
NumberOfIterations)
'ReDim NameOfSingeIterationValuesToSummarise(CONST_NUMBEROFSINGLEITERATIONVALUESTOSUMMARISE)
    'Don't update screen
Application.EnableEvents = False
Application.UserControl = False

IndependentIteration = CBool(Worksheets(Inputs).Cells(2, 2))
PreviousIterationsScaldingW = 0
PreviousIterationsDehairingM = 0
PreviousIterationsEviscerationK = 0
PreviousIterationsTrimmingK = 0
PreviousIterationsHalvingK = 0
ReDim InterSimulationValues(0 To CONST_NUMBEROFSTATISTICS, 0 To CONST_NUMBEROFCOLUMNSTOSUMMARISE,
0 To NumberSimulations)
```

```
    ' start of simulation loop
For CurrentSimulation = 1 To NumberSimulations

    'Start of iteration loop
For CurrentIteration = 1 To NumberOfIterations

    DoEvents
    ' calculate and display status and time remaining If CurrentSimulation = NumberSimulations Then dblSim = ((CurrentSimulation) / NumberSimulations) * 100 Else dblSim = ((CurrentSimulation - 1) / NumberSimulations) * 100 End If dblItr = (CurrentIteration / NumberOfIterations) * 100 lngMinutes = (DateDiff("n", TimeStart, Now) * (100 / ((dblSim + dblItr) / NumberSimulations))) - DateDiff("n", TimeStart, Now) If NumberSimulations = 1 Then Application.StatusBar = "Sim: " & CurrentSimulation & "/" & NumberSimulations & " Itr: " & CurrentIteration & "/" & NumberOfIterations & " (" & (CurrentIteration / NumberOfIterations) * 100 & "% complete)" Else Application.StatusBar = "Sim: " & CurrentSimulation & "/" & NumberSimulations & " Itr: " & CurrentIteration & "/" & NumberOfIterations & " (" & (dblSim + dblItr) / NumberSimulations & "% complete)" End If If lngMinutes > 60 Then Application.StatusBar = Application.StatusBar & " est finish: " & lngMinutes / 60 & " hrs" ElseIf lngMinutes > 0 Then Application.StatusBar = Application.StatusBar & " est finish: " & lngMinutes & " mins"
End If Call LairageModule



    Corrected version of Scald Tank Calacualtions Model

    'Load Users Data
NumberPigsInLairage = CInt(Worksheets("Lairage").Cells(8, 2))

    'ReDim TimePigDischargesRectumBacteria(NumberPigsInLairage - 1)

    Scalding Module
alpha = RDK_Uniform(CDbl(Worksheets(Inputs).Cells(21, 2)), CDbl(Worksheets(Inputs).Cells(21, 3)), "")
beta = RDK_Uniform(CDbl(Worksheets(Inputs).Cells(22, 2)), CDbl(Worksheets(Inputs).Cells(22, 3)), "")
tau1 = CDbl(Worksheets(Inputs).Cells(23, 2))
tau2 = CDbl(Worksheets(Inputs).Cells(24, 2))

    'Check that none of the input values are zero
If (NumberPigsInLairage = 0) Then
CustomError ("number of Pigs == 0")
ElseIf (alpha = 0) Then
CustomError ("Alpha == 0")
ElseIf (beta = 0) Then
CustomError ("Beta == 0")
ElseIf (tau1 = 0) Then
CustomError ("tau1 == 0")
ElseIf (tau2 = 0) Then
CustomError ("tau2 == 0")
End If

    Dim AtRiskSamplingType As Integer

    'Setup @Risk
'AtRiskSamplingType = 2 ' Monte Carlo
```

```
AtRiskSamplingType = 3 ’ Latin Hypercube
’Call RDK_Settings(True, AtRiskSamplingType, Worksheets(Inputs).Cells(4, 2).value, Worksheets(Inputs).Cells(3, 6).value, 1, True)
’Declare Sub RDK_Settings Lib “RSKLIB32.DLL” (ByVal SetFlag As Boolean, Sampling As Integer, Seed As Integer, NumSim As Integer, NumIter As Long, SaveSamples As Boolean)
’Seed As Integer = Math.Round(Math.Rnd * 1000)

    Call KillingModule

    ’ the time difference between when a pig enters the bath and the time the next pig will enter
timeDifferanceBetweenPigs = RDK_Uniform(1, 1.1, “”)

    ’ the amount of time each pig is in the bath
timeEachPigIsInBath = RDK_Uniform(6, 8, “”)

    If (IndependentIteration = True) Then
W0 = 0 ’assume zero initial infectivity of the water
Else
W0 = PreviousIterationsScaldingW
End If

    If (IndependentIteration = True) Then
Mo = 0 ’assume zero initial infectivity on dehairing machine
Else
Mo = PreviousIterationsDehairingM
End If

    If (IndependentIteration = True) Then
Ko = 0 ’assume zero initial infectivity of the water
Else
Ko = PreviousIterationsEviscerationK
End If

    If (IndependentIteration = True) Then
Ka = 0 ’assume zero initial infectivity of the water
Else
Ka = PreviousIterationsTrimmingK
End If

    If (IndependentIteration = True) Then
So = 0 ’assume zero initial infectivity of the water
Else
So = PreviousIterationsHalvingK
End If

    ’Load array variables
PInit = LoadArrayFromColumns(4, 8, NumberPigsInLairage + 8, “Killing”)

    ’Time variables
Pt0 = ConstructIncreasingArray(0, timeDifferanceBetweenPigs, NumberPigsInLairage) ’time pig enters water
```

```
Ptspan = ConstructArrayIntegers(timeEachPigIsInBath, NumberPigsInLairage) ’time pigs in water
stepSize = 0.15 ’(or 0.01 or 0.15 for speed)’CDbl(Worksheets(“InputData”).Cells(17, 2))
Time0 = 0
Timef = Pt0(NumberPigsInLairage - 1) + timeEachPigIsInBath

    ’Number of time steps for RK
n = CInt((Timef - Time0) / stepSize)

    ReDim Result(0 To (n + 1), 0 To NumberPigsInLairage + 2)

Call RungeKutta4(Time0, Timef, PInit, W0, stepSize, alpha, beta, tau1, tau2, Pt0, Ptspan, Result)
Dim currentTime As Integer
Dim timeStepsPerMinute As Double
timeStepsPerMinute = 1 / stepSize

    ’Output infectivity of the pig and the water as it leaves the bath
For i = 0 To NumberPigsInLairage - 1
currentTime = (Pt0(i) + timeEachPigIsInBath) * timeStepsPerMinute
Worksheets(“Scalding”).Cells(i + 27, 2) = Result(currentTime, i + 2) ’pig
Worksheets(”dehairing”).Cells(i + 18, 6) = Result(currentTime, i + 2) ’pig

    Worksheets(“Scalding”).Cells(i + 27, 3) = Result(currentTime, NumberPigsInLairage + 2) ’ water
Worksheets(“Scalding”).Cells(i + 27, 4) = Result(currentTime, 1) ’time
Next
Call FillColumn(2, 27 + NumberPigsInLairage, 4 + NumberPigsInLairage + 1000, “Scalding”, “”)
Call FillColumn(6, 18 + NumberPigsInLairage, 18 + NumberPigsInLairage + 1000, “dehairing”, “”)
Call FillColumn(3, 27 + NumberPigsInLairage, 4 + NumberPigsInLairage + 1000, “Scalding”, “”)
Call FillColumn(4, 27 + NumberPigsInLairage, 4 + NumberPigsInLairage + 1000, “Scalding”, “”)

    Dim k As Long
For k = 2 To 11
Call FillColumn(k, 28, 28 + NumberPigsInLairage + 1000, “Evisceration+ ”, “”)
Next k

    Call DehairingModule
Call SingeingModule
Call EviscerationModule
Call TrimmingModule
Call HalvingModule
Call StorageModule

    ’NumberPigsInLairageToOuput = 30
’to
If (NumberPigsInLairage < 30) Then
NumberPigsInLairageToOuput = NumberPigsInLairage
Else
NumberPigsInLairageToOuput = 30
End If
```

```
        Worksheets("Calculations").Cells(1, 1).value = "Time Period"
For i = 2 To NumberPigsInLairageToOuput + 1

        Worksheets("Calculations").Cells(1, i).value = "dp[" & CStr(i - 1) & "]"
Next

        Worksheets("Calculations").Cells(1, NumberPigsInLairageToOuput + 2).value = "dw"

        ' Output time and infection information for the first 30 pigs
For i = 0 To (Pt0(NumberPigsInLairageToOuput - 1) + timeEachPigIsInBath) * timeStepsPerMinute
For j = 1 To NumberPigsInLairageToOuput + 1
Worksheets("Calculations").Cells(i + 2, j) = Result(i, j)
Next
Next

        For i = 0 To (Pt0(NumberPigsInLairageToOuput - 1) + timeEachPigIsInBath) * timeStepsPerMinute
Worksheets("Calculations").Cells(i + 2, NumberPigsInLairageToOuput + 2) = Result(i, NumberPigsInLairage + 2)
Next

        'Ouput the infectivty of each pig and the water as it leaves the bath
'Label columns
Worksheets("Scalding").Cells(26, 2).value = "Pig"
Worksheets("Scalding").Cells(26, 3).value = "Water"
Worksheets("Scalding").Cells(26, 4).value = "Scald tank"

        'Output this runs summary statistics
Call IntraIterationStatistics(NumberPigsInLairage)
'OutputSummaryStatistics (NumberPigsInLairage)

        ' End of iteration loop
Next
```

Summary Statistics

```
        Worksheets("Intra run Statistics").Cells(1, 2).value = "min"
Worksheets("Intra run Statistics").Cells(1, 3).value = "max"
Worksheets("Intra run Statistics").Cells(1, 4).value = "mean"
Worksheets("Intra run Statistics").Cells(1, 5).value = "stdev"
Worksheets("Intra run Statistics").Cells(1, 6).value = "var"
Worksheets("Intra run Statistics").Cells(1, 7).value = "lQ"
Worksheets("Intra run Statistics").Cells(1, 8).value = "uQ"
Worksheets("Intra run Statistics").Cells(1, 9).value = "10th decile"
Worksheets("Intra run Statistics").Cells(1, 10).value = "20th decile"
Worksheets("Intra run Statistics").Cells(1, 11).value = "30th decile"
Worksheets("Intra run Statistics").Cells(1, 12).value = "40th decile"
Worksheets("Intra run Statistics").Cells(1, 13).value = "median"
Worksheets("Intra run Statistics").Cells(1, 14).value = "60th decile"
Worksheets("Intra run Statistics").Cells(1, 15).value = "70th decile"
Worksheets("Intra run Statistics").Cells(1, 16).value = "80th decile"
```

Worksheets(“Intra run Statistics”).Cells(1, 17).value = “90th decile”

’ Inter run statistics
Call InterIterationStatistics

’ end of simulation loop
Next

Unload_RSK

Beep
MsgBox ”Finished” & vbCrLf & vbCrLf & “Simulation time: ” & DateDiff(“n”, Now, TimeStart) & “ minutes”

End Sub

Calculated summary statistics of various columns

Private Sub IntraIterationStatistics(numberOfPigs As Integer)
NameOfColumnsToSummarise(1) = “Killing - Amount of contamination/pig”
Call ColumnToSummarise(“Killing”, CurrentItraRunRow, 1, 4, NameOfColumnsToSummarise(1), 8, (8 + numberOfPigs))
NameOfColumnsToSummarise(2) = “Scalding - Bacteria Counts on carcass after scalding”
Call ColumnToSummarise(“Scalding”, CurrentItraRunRow, 2, 2, NameOfColumnsToSummarise(2), 27, (27 + numberOfPigs))
NameOfColumnsToSummarise(3) = “Dehairing - Bacteria Counts on dehairer after use”
Call ColumnToSummarise(”dehairing”, CurrentItraRunRow, 3, 9, NameOfColumnsToSummarise(3), 18, (18 + numberOfPigs))
NameOfColumnsToSummarise(4) = “Dehairing - Bacteria Counts on carcass after dehairing”
Call ColumnToSummarise(”dehairing”, CurrentItraRunRow, 4, 8, NameOfColumnsToSummarise(4), 18, (18 + numberOfPigs))
NameOfColumnsToSummarise(5) = “Singeing - Bacteria Counts on carcass after singeing”
Call ColumnToSummarise(”Singeing”, CurrentItraRunRow, 5, 3, NameOfColumnsToSummarise(5), 11, (11 + numberOfPigs))
NameOfColumnsToSummarise(6) = “Evisceration - Bacteria Counts on carcass after evisceration”
Call ColumnToSummarise(“Evisceration+ ”, CurrentItraRunRow, 6, 6, NameOfColumnsToSummarise(6), 28, (28 + numberOfPigs))
NameOfColumnsToSummarise(7) = “Evisceration - Bacteria Counts on carcass after trimming”
Call ColumnToSummarise(“Evisceration+ ”, CurrentItraRunRow, 7, 8, NameOfColumnsToSummarise(7), 28, (28 + numberOfPigs))
NameOfColumnsToSummarise(8) = “Evisceration - Bacteria Counts on half carcass after halving”
Call ColumnToSummarise(“Evisceration+ ”, CurrentItraRunRow, 8, 11, NameOfColumnsToSummarise(8), 28, (28 + numberOfPigs))
NameOfColumnsToSummarise(9) = “Storage - Bacteria Counts on half carcass after chilling”
Call ColumnToSummarise(”Storage”, CurrentItraRunRow, 9, 4, NameOfColumnsToSummarise(9), 19, (19 + numberOfPigs))

CurrentItraRunRow = CurrentItraRunRow + 1
End Sub

Private Sub ColumnToSummarise(Worksheet As String, rowToWrite As Long, SummarisedColumnCounter As Integer, column As Integer, columnName As String, startRow As Integer, endRow As Integer)
Dim colValues() As Double
Dim min As Double
Dim Max As Double
Dim Mean As Double
Dim stdev As Double
Dim var As Double
Dim lQ As Double

```
Dim median As Double
Dim uQ As Double
Dim decile10 As Double
Dim decile20 As Double
Dim decile30 As Double
Dim decile40 As Double
Dim decile60 As Double
Dim decile70 As Double
Dim decile80 As Double
Dim decile90 As Double

        ' load the values for this iteration out of specific column
colValues = Module1.LoadArrayFromColumns(column, startRow, endRow, Worksheet)
min = Module2.min(colValues)
Max = Module2.Max(colValues)
Mean = Module2.Mean(colValues)
stdev = Module2.StdDev(colValues)
var = Module2.Variance(colValues)
lQ = Module2.LQ2(colValues)
'median = Module2.median(colValues)
median = Module2.DecileCL(colValues, 50)
uQ = Module2.UQ2(colValues)
decile10 = Module2.DecileCL(colValues, 10)
decile20 = Module2.DecileCL(colValues, 20)
decile30 = Module2.DecileCL(colValues, 30)
decile40 = Module2.DecileCL(colValues, 40)
decile60 = Module2.DecileCL(colValues, 60)
decile70 = Module2.DecileCL(colValues, 70)
decile80 = Module2.DecileCL(colValues, 80)
decile90 = Module2.DecileCL(colValues, 90)

        'Write this iterations statistics to the intra-statitics worksheet.
'Worksheets("Intra run Statistics").Cells(rowToWrite, 1).value = columnName
'Worksheets("Intra run Statistics").Cells(rowToWrite, 2).value = min
'Worksheets("Intra run Statistics").Cells(rowToWrite, 3).value = Max
'Worksheets("Intra run Statistics").Cells(rowToWrite, 4).value = Mean
'Worksheets("Intra run Statistics").Cells(rowToWrite, 5).value = stdev
'Worksheets("Intra run Statistics").Cells(rowToWrite, 6).value = var
'Worksheets("Intra run Statistics").Cells(rowToWrite, 7).value = lQ
'Worksheets("Intra run Statistics").Cells(rowToWrite, 8).value = uQ
'Worksheets("Intra run Statistics").Cells(rowToWrite, 9).value = decile10
'Worksheets("Intra run Statistics").Cells(rowToWrite, 10).value = decile20
'Worksheets("Intra run Statistics").Cells(rowToWrite, 11).value = decile30
'Worksheets("Intra run Statistics").Cells(rowToWrite, 12).value = decile40
'Worksheets("Intra run Statistics").Cells(rowToWrite, 13).value = median
'Worksheets("Intra run Statistics").Cells(rowToWrite, 14).value = decile60
'Worksheets("Intra run Statistics").Cells(rowToWrite, 15).value = decile70
'Worksheets("Intra run Statistics").Cells(rowToWrite, 16).value = decile80
'Worksheets("Intra run Statistics").Cells(rowToWrite, 17).value = decile90
```

```
        CurrentItraRunRow = CurrentItraRunRow + 1

        ' write this iterations + statistic values to the inter iteration array.
InterIterationValues(0, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'min
InterIterationValues(1, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'Max
InterIterationValues(2, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'Mean
InterIterationValues(3, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'stdev
InterIterationValues(4, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'var
InterIterationValues(5, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'lQ
InterIterationValues(6, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'uQ
InterIterationValues(7, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'decile10
InterIterationValues(8, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'decile20
InterIterationValues(9, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'decile30
InterIterationValues(10, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'decile40
InterIterationValues(11, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'median
InterIterationValues(12, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'decile60
InterIterationValues(13, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'decile70
InterIterationValues(14, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'decile80
InterIterationValues(15, SummarisedColumnCounter, CurrentIteration - 1) = colValues 'decile90

        End Sub

        Private Sub InterIterationStatistics()
Dim intraRunValues() As Double
Dim i As Integer
Dim j As Integer

        'ReDim intraRunValues(NumberOfIterations)

        ' write column names
Worksheets("Inter run statistics").Cells(1, 2).value = "min"
Worksheets("Inter run statistics").Cells(1, 3).value = "max"
Worksheets("Inter run statistics").Cells(1, 4).value = "mean"
Worksheets("Inter run statistics").Cells(1, 5).value = "stdev"
Worksheets("Inter run statistics").Cells(1, 6).value = "var"
Worksheets("Inter run statistics").Cells(1, 7).value = "lQ"
Worksheets("Inter run statistics").Cells(1, 8).value = "uQ"
Worksheets("Inter run statistics").Cells(1, 9).value = "10th decile"
Worksheets("Inter run statistics").Cells(1, 10).value = "20th decile"
Worksheets("Inter run statistics").Cells(1, 11).value = "30th decile"
Worksheets("Inter run statistics").Cells(1, 12).value = "40th decile"
Worksheets("Inter run statistics").Cells(1, 13).value = "median"
Worksheets("Inter run statistics").Cells(1, 14).value = "60th decile"
Worksheets("Inter run statistics").Cells(1, 15).value = "70th decile"
Worksheets("Inter run statistics").Cells(1, 16).value = "80th decile"
Worksheets("Inter run statistics").Cells(1, 17).value = "90th decile"
Worksheets("Inter run statistics").Cells(5, 18).value = "Scalding"
Worksheets("Inter run statistics").Cells(6, 18).value = "Dehairing"
```

```
Worksheets(“Inter run statistics”).Cells(7, 18).value = “Singeing”
Worksheets(“Inter run statistics”).Cells(8, 18).value = “Evisceration”
Worksheets(“Inter run statistics”).Cells(9, 18).value = “Halving”
Worksheets(“Inter run statistics”).Cells(10, 18).value = “Chilling”

    For i = 1 To CONST_NUMBEROFCOLUMNSTOSUMMARISE
’ Output column name
Worksheets(“Inter run statistics”).Cells(i + 2, 1) = NameOfColumnsToSummarise(i)

    ’ Calcualte summary of intra run values
’ Min
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(0, i, j)
ArrayAppend InterIterationValues(0, i, j), j, intraRunValues
Next

    InterSimulationValues(0, i, CurrentSimulation - 1) = Module2.min(intraRunValues)
Worksheets(“Inter run statistics”).Cells(i + 2, 2) = Module2.min(intraRunValues)

    ’Max
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(1, i, j)
ArrayAppend InterIterationValues(1, i, j), j, intraRunValues
Next
InterSimulationValues(1, i, CurrentSimulation - 1) = Module2.Max(intraRunValues)
Worksheets(“Inter run statistics”).Cells(i + 2, 3) = Module2.Max(intraRunValues)

    ’ Mean
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(2, i, j)
ArrayAppend InterIterationValues(2, i, j), j, intraRunValues
Next
InterSimulationValues(2, i, CurrentSimulation - 1) = Module2.Mean(intraRunValues)
Worksheets(“Inter run statistics”).Cells(i + 2, 4) = Module2.Mean(intraRunValues)

    ’ StDev
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(3, i, j)
ArrayAppend InterIterationValues(3, i, j), j, intraRunValues
Next
InterSimulationValues(3, i, CurrentSimulation - 1) = Module2.StdDev(intraRunValues)
Worksheets(“Inter run statistics”).Cells(i + 2, 5) = Module2.StdDev(intraRunValues)

    ’ Var
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(4, i, j)
ArrayAppend InterIterationValues(4, i, j), j, intraRunValues
Next
InterSimulationValues(4, i, CurrentSimulation - 1) = Module2.Variance(intraRunValues)
```

```
Worksheets(“Inter run statistics”).Cells(i + 2, 6) = Module2.Variance(intraRunValues)

    ’ Lcl
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(5, i, j)
ArrayAppend InterIterationValues(5, i, j), j, intraRunValues
Next
InterSimulationValues(5, i, CurrentSimulation - 1) = Module2.LQ2(intraRunValues)
Worksheets(“Inter run statistics”).Cells(i + 2, 7) = Module2.LQ2(intraRunValues)

    ’ Ucl
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(6, i, j)
ArrayAppend InterIterationValues(6, i, j), j, intraRunValues
Next
InterSimulationValues(6, i, CurrentSimulation - 1) = Module2.UQ2(intraRunValues)
Worksheets(“Inter run statistics”).Cells(i + 2, 8) = Module2.UQ2(intraRunValues)

    ’ 10th decile
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(7, i, j)
ArrayAppend InterIterationValues(7, i, j), j, intraRunValues
Next
InterSimulationValues(7, i, CurrentSimulation - 1) = Module2.DecileCL(intraRunValues, 10)
Worksheets(“Inter run statistics”).Cells(i + 2, 9) = Module2.DecileCL(intraRunValues, 10)

    ’ 20th decile
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(8, i, j)
ArrayAppend InterIterationValues(8, i, j), j, intraRunValues
Next
InterSimulationValues(8, i, CurrentSimulation - 1) = Module2.DecileCL(intraRunValues, 20)
Worksheets(“Inter run statistics”).Cells(i + 2, 10) = Module2.DecileCL(intraRunValues, 20)

    ’ 30th decile
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(9, i, j)
ArrayAppend InterIterationValues(9, i, j), j, intraRunValues
Next
InterSimulationValues(9, i, CurrentSimulation - 1) = Module2.DecileCL(intraRunValues, 30)
Worksheets(“Inter run statistics”).Cells(i + 2, 11) = Module2.DecileCL(intraRunValues, 30)

    ’ 40th decile
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(10, i, j)
ArrayAppend InterIterationValues(10, i, j), j, intraRunValues
Next
InterSimulationValues(10, i, CurrentSimulation - 1) = Module2.DecileCL(intraRunValues, 40)
Worksheets(“Inter run statistics”).Cells(i + 2, 12) = Module2.DecileCL(intraRunValues, 40)
```

```
        ’ Median
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(11, i, j)
ArrayAppend InterIterationValues(11, i, j), j, intraRunValues
Next
InterSimulationValues(11, i, CurrentSimulation - 1) = Module2.median(intraRunValues)
’Worksheets(“Inter run statistics”).Cells(i + 2, 13) = Module2.median(intraRunValues)
Worksheets(“Inter run statistics”).Cells(i + 2, 13) = Module2.median(intraRunValues)

        ’ 60th decile
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(12, i, j)
ArrayAppend InterIterationValues(12, i, j), j, intraRunValues
Next
InterSimulationValues(12, i, CurrentSimulation - 1) = Module2.DecileCL(intraRunValues, 60)
Worksheets(“Inter run statistics”).Cells(i + 2, 14) = Module2.DecileCL(intraRunValues, 60)

        ’ 70th decile
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(13, i, j)
ArrayAppend InterIterationValues(13, i, j), j, intraRunValues
Next
InterSimulationValues(13, i, CurrentSimulation - 1) = Module2.DecileCL(intraRunValues, 70)
Worksheets(“Inter run statistics”).Cells(i + 2, 15) = Module2.DecileCL(intraRunValues, 70)

        ’ 80th decile
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(14, i, j)
ArrayAppend InterIterationValues(14, i, j), j, intraRunValues
Next
InterSimulationValues(14, i, CurrentSimulation - 1) = Module2.DecileCL(intraRunValues, 80)
Worksheets(“Inter run statistics”).Cells(i + 2, 16) = Module2.DecileCL(intraRunValues, 80)

        ’ 90th decile
For j = 0 To NumberOfIterations - 1
”intraRunValues(j) = InterIterationValues(15, i, j)
ArrayAppend InterIterationValues(15, i, j), j, intraRunValues
Next
InterSimulationValues(15, i, CurrentSimulation - 1) = Module2.DecileCL(intraRunValues, 90)
Worksheets(“Inter run statistics”).Cells(i + 2, 17) = Module2.DecileCL(intraRunValues, 90)

        Next

        Worksheets(“Inter run statistics”).Cells(12, 1).value = “Prevalence (%) of bacteria in Storage”
Worksheets(“Inter run statistics”).Cells(12, 2) = Module2.min(PrevalenceMatrix)
Worksheets(“Inter run statistics”).Cells(12, 3) = Module2.Max(PrevalenceMatrix)
Worksheets(“Inter run statistics”).Cells(12, 4) = Module2.Mean(PrevalenceMatrix)
Worksheets(“Inter run statistics”).Cells(12, 5) = Module2.StdDev(PrevalenceMatrix)
```

```
Worksheets("Inter run statistics").Cells(12, 6) = Module2.Variance(PrevalenceMatrix)
Worksheets("Inter run statistics").Cells(12, 7) = Module2.LQ2(PrevalenceMatrix)
Worksheets("Inter run statistics").Cells(12, 8) = Module2.UQ2(PrevalenceMatrix)
Worksheets("Inter run statistics").Cells(12, 9) = Module2.DecileCL(PrevalenceMatrix, 10)
Worksheets("Inter run statistics").Cells(12, 10) = Module2.DecileCL(PrevalenceMatrix, 20)
Worksheets("Inter run statistics").Cells(12, 11) = Module2.DecileCL(PrevalenceMatrix, 30)
Worksheets("Inter run statistics").Cells(12, 12) = Module2.DecileCL(PrevalenceMatrix, 40)
Worksheets("Inter run statistics").Cells(12, 13) = Module2.DecileCL(PrevalenceMatrix, 50)
Worksheets("Inter run statistics").Cells(12, 14) = Module2.DecileCL(PrevalenceMatrix, 60)
Worksheets("Inter run statistics").Cells(12, 15) = Module2.DecileCL(PrevalenceMatrix, 70)
Worksheets("Inter run statistics").Cells(12, 16) = Module2.DecileCL(PrevalenceMatrix, 80)
Worksheets("Inter run statistics").Cells(12, 17) = Module2.DecileCL(PrevalenceMatrix, 90)

    ' We recorded the mean of the PrevalenceMatrix to represent this simulation
InterSimulationPrevalenceMatrix(CurrentSimulation - 1) = Module2.Mean(PrevalenceMatrix)

    Worksheets("Inter run statistics").Cells(5, 19).value = Worksheets("Inter run statistics").Cells(5, 4).value - Worksheets("Inter run statistics").Cells(5, 9).value
Worksheets("Inter run statistics").Cells(6, 19).value = Worksheets("Inter run statistics").Cells(6, 4).value - Worksheets("Inter run statistics").Cells(6, 9).value
Worksheets("Inter run statistics").Cells(7, 19).value = Worksheets("Inter run statistics").Cells(7, 4).value - Worksheets("Inter run statistics").Cells(7, 9).value
Worksheets("Inter run statistics").Cells(8, 19).value = Worksheets("Inter run statistics").Cells(8, 4).value - Worksheets("Inter run statistics").Cells(8, 9).value
Worksheets("Inter run statistics").Cells(9, 19).value = Worksheets("Inter run statistics").Cells(9, 4).value - Worksheets("Inter run statistics").Cells(9, 9).value
Worksheets("Inter run statistics").Cells(10, 19).value = Worksheets("Inter run statistics").Cells(10, 4).value - Worksheets("Inter run statistics").Cells(10, 9).value
Worksheets("Inter run statistics").Cells(5, 20).value = Worksheets("Inter run statistics").Cells(5, 17).value - Worksheets("Inter run statistics").Cells(5, 4).value
Worksheets("Inter run statistics").Cells(6, 20).value = Worksheets("Inter run statistics").Cells(6, 17).value - Worksheets("Inter run statistics").Cells(6, 4).value
Worksheets("Inter run statistics").Cells(7, 20).value = Worksheets("Inter run statistics").Cells(7, 17).value - Worksheets("Inter run statistics").Cells(7, 4).value
Worksheets("Inter run statistics").Cells(8, 20).value = Worksheets("Inter run statistics").Cells(8, 17).value - Worksheets("Inter run statistics").Cells(8, 4).value
Worksheets("Inter run statistics").Cells(9, 20).value = Worksheets("Inter run statistics").Cells(9, 17).value - Worksheets("Inter run statistics").Cells(9, 4).value
Worksheets("Inter run statistics").Cells(10, 20).value = Worksheets("Inter run statistics").Cells(10, 17).value - Worksheets("Inter run statistics").Cells(10, 4).value
Worksheets("Inter run statistics").Cells(11, 20).value = Worksheets("Inter run statistics").Cells(11, 17).value - Worksheets("Inter run statistics").Cells(11, 4).value

    End Sub
```

Scald Tank - fourth order Runge-Kutta

```
’ ’ Private Sub RungeKutta4(Time0 As Double, Timef As Double, PInit() As Double, W0 As Double, stepSize As Double, alpha As Double, beta As Double, tau1 As Double, tau2 As Double, Pt0() As Double, Ptspan() As Double, returnValue() As Double)

Dim n As Long
Dim t As Double
Dim p() As Double
Dim w As Double
Dim i As Long ’local loop variable
Dim j As Integer

Dim RKFirstStep() As Double
Dim RKSecondStep() As Double
Dim RKThirdStep() As Double
Dim RKFourthStep() As Double

    ’ used to break down complex step
Dim tmpArray1() As Double
Dim tmpArray2() As Double
Dim tmpArray3() As Double
Dim tmpArray4() As Double
Dim tmpArray5() As Double
Dim tmpArray6() As Double

ReDim p(NumberPigsInLairage) As Double
ReDim RKFirstStep(NumberPigsInLairage) As Double
ReDim RKSecondStep(NumberPigsInLairage) As Double
ReDim RKThirdStep(NumberPigsInLairage) As Double
ReDim RKFourthStep(NumberPigsInLairage) As Double

    n = CInt((Timef - Time0) / stepSize)

    ReDim returnValue(0 To (n + 1), 0 To NumberPigsInLairage + 2)

    t = Time0

    ’Set initial infectivity of each pig
p = PInit
w = W0

    returnValue(1, 1) = t

    For j = 1 To NumberPigsInLairage
returnValue(i, j + 1) = PInit(j - 1)
Next

    returnValue(1, NumberPigsInLairage + 2) = W0
```

```
        For i = 1 To n
' Individual Pig Infectivity

RKFirstStep = ArrayMultiplicationdouble(MultiplePigsScalding(t, p, w, alpha, beta, tau1, tau2, Pt0, Ptspan), stepSize)
RKSecondStep = ArrayMultiplicationdouble(MultiplePigsScalding(t, ArrayAddition(p, ArrayDivisionInt(RKFirstStep, 2)), w + RK-
FirstStep(NumberPigsInLairage) / 2, alpha, beta, tau1, tau2, Pt0, Ptspan), stepSize)
RKThirdStep = ArrayMultiplicationdouble(MultiplePigsScalding(t, ArrayAddition(p, ArrayDivisionInt(RKSecondStep, 2)), w + RK-
SecondStep(NumberPigsInLairage) / 2, alpha, beta, tau1, tau2, Pt0, Ptspan), stepSize)
RKFourthStep = ArrayMultiplicationdouble(MultiplePigsScalding(t, ArrayAddition(p, RKThirdStep), w + RKThirdStep(NumberPigsInLairage),
alpha, beta, tau1, tau2, Pt0, Ptspan), stepSize)

        ' break down step five: p = p + (RKFirstStep + 2 * RKSecondStep + 2 * RKThirdStep + RKFourthStep) / 6
tmpArray1 = ArrayMultiplicationdouble(RKSecondStep, 2)
tmpArray2 = ArrayMultiplicationdouble(RKThirdStep, 2)
tmpArray3 = ArrayAddition(RKFirstStep, tmpArray1)
tmpArray4 = ArrayAddition(tmpArray3, tmpArray2)
tmpArray5 = ArrayAddition(tmpArray4, RKFourthStep)
tmpArray6 = ArrayDivisionInt(tmpArray5, 6)

p = ArrayAddition(p, tmpArray6)

        ' Infectivity of the Water
w = w + (RKFirstStep(NumberPigsInLairage) + 2 * RKSecondStep(NumberPigsInLairage) + 2 * RKThirdStep(NumberPigsInLairage)
+ RKFourthStep(NumberPigsInLairage)) / 6

PreviousIterationsScaldingW = w

t = Time0 + i * stepSize

        returnValue(i, 1) = t

For j = 1 To NumberPigsInLairage
returnValue(i, j + 1) = p(j - 1)
Next

returnValue(i, NumberPigsInLairage + 2) = w

        Next
End Sub
```

Derivatives method of Multiple Pigs Scalding Simultaneously

```
Private Function MultiplePigsScalding(t As Double, PInit() As Double, W0 As Double, alpha As Double, beta As Double, tau1 As
Double, tau2 As Double, Pt0() As Double, Ptspan() As Double) As Double()

Dim p() As Double
Dim dp() As Double
Dim InBath() As Double
```

```
Dim i As Integer
Dim returnValue() As Double
Dim NumberPigsInLairageToOuput As Integer
Dim w As Double
Dim ps As Double
Dim dw As Double
Dim ss As Double
Dim ws As Double

    Dim currentNumberPigsInLairage As Long
ReDim p(NumberPigsInLairage) As Double
ReDim InBath(NumberPigsInLairage)
ReDim dp(NumberPigsInLairage) As Double
ReDim returnValue(0 To NumberPigsInLairage + 1)

currentNumberPigsInLairage = 0

p = PInit
w = W0

    'Get the number of pigs in the bath at the current time period
For i = 0 To (NumberPigsInLairage - 1)
If Not ((Pt0(i) > t) Or ((Pt0(i) + Ptspan(i)) < t)) Then
currentNumberPigsInLairage = currentNumberPigsInLairage + 1
End If
Next

    For i = 0 To (NumberPigsInLairage - 1)
If ((Pt0(i) > t) Or ((Pt0(i) + Ptspan(i)) < t)) Then
dp(i) = 0
InBath(i) = 0
Else
dp(i) = (alpha / currentNumberPigsInLairage) * w - (tau1 + beta) * p(i)
InBath(i) = 1
End If
Worksheets("Calculations").Cells(1, NumberPigsInLairageToOuput + 3).value = "InBath"

    Next

ps = 0
ws = 0
For i = 0 To (NumberPigsInLairage - 1)
ws = ws + InBath(i) * (-(alpha + tau2) * w)
ps = ps + (InBath(i) * p(i))
Next

ss = beta * ps
dw = ss + ws
```

```
    For i = 0 To (NumberPigsInLairage - 1)
returnValue(i) = dp(i)
Next

returnValue(NumberPigsInLairage) = dw
MultiplePigsScalding = returnValue

    End Function
```

Dehairing Module

```
    ' Declaring Variables and Types

Private Function DehairingModule() As Boolean
Dim Dehairing_ProbabilityPigShedding() As Double
Dim Dehairing_AmountFeacesExtruded() As Double
Dim Dehairing_ConcentrationBAFaeces() As Double
Dim Dehairing_AmtFaecalContamination() As Double
Dim Dehairing_BaNoOnPigBeforedehairing() As Double
Dim Dehairing_BaOnPigAfterDehairing() As Double
Dim Dehairing_BaOnDehairieratEnd() As Double
Dim Dehairing_BaOnKnifeBeforeUse() As Double
Dim i As Integer

ReDim Dehairing_ProbabilityPigShedding(NumberPigsInLairage)
ReDim Dehairing_AmountFeacesExtruded(NumberPigsInLairage)
ReDim Dehairing_ConcentrationBAFaeces(NumberPigsInLairage)
ReDim Dehairing_AmtFaecalContamination(NumberPigsInLairage)
ReDim Dehairing_BaNoOnPigBeforedehairing(NumberPigsInLairage)
ReDim Dehairing_BaOnPigAfterDehairing(NumberPigsInLairage)
ReDim Dehairing_BaOnDehairieratEnd(NumberPigsInLairage)
ReDim Dehairing_BaOnKnifeBeforeUse(NumberPigsInLairage)

    ' Dehairing worksheet calculations

    ' Probability of Pig shedding
' =IF('Killing'!B8=1,RiskBinomial('Killing'!1,B9*B10),0)
'Dehairing_ProbabilityPigShedding
For i = 0 To NumberPigsInLairage - 1
Dehairing_ProbabilityPigShedding(i) = RDK_Binomial(1, Worksheets("Lairage").Cells(9, 2).value * Worksheets("Lairage").Cells(10,
2).value, "")
Next
Call FillColumnFromArray(2, 18, 18 + NumberPigsInLairage, "Dehairing", Dehairing_ProbabilityPigShedding)
Call FillColumn(2, 18 + NumberPigsInLairage, 18 + NumberPigsInLairage + 1000, "Dehairing", "")

    'Amount of feaces extruded
'=RiskGamma(0.5, 0.5)
' Dehairing_AmountFeacesExtruded
```

```
For i = 0 To NumberPigsInLairage - 1
Dehairing_AmountFeacesExtruded(i) = RDK_Gamma(Worksheets(Inputs).Cells(27, 2).value, Worksheets(Inputs).Cells(27, 3).value, “”)
Next
Call FillColumnFromArray(3, 18, 18 + NumberPigsInLairage, “Dehairing”, Dehairing_AmountFeacesExtruded)
Call FillColumn(3, 18 + NumberPigsInLairage, 18 + NumberPigsInLairage + 1000, “Dehairing”, “”)

    ’ Concentration of b’a in Faeces
’ =RiskPert(Inputs B14, Inputs C14 Inputs D14)*Dehairing column B
’=RiskUniform(Inputs B14, Inputs C14,)*Dehairing column B
’ Dehairing_ConcentrationBAFaeces
For i = 0 To NumberPigsInLairage - 1
If (Worksheets(“Dehairing”).Cells(i + 18, 2).value = 1) Then
’Dehairing_ConcentrationBAFaeces(i) = (RDK_Pert(Worksheets(Inputs).Cells(14, 2).value, Worksheets(Inputs).Cells(14, 3).value, Worksheets(Inputs).Cells(14, 4).value, “”))
Dehairing_ConcentrationBAFaeces(i) = (RDK_Uniform(Worksheets(Inputs).Cells(14, 2).value, Worksheets(Inputs).Cells(14, 3).value, “”))

    Else
Dehairing_ConcentrationBAFaeces(i) = 0
End If
Next
Call FillColumnFromArray(4, 18, 18 + NumberPigsInLairage, “Dehairing”, Dehairing_ConcentrationBAFaeces)
Call FillColumn(4, 18 + NumberPigsInLairage, 18 + NumberPigsInLairage + 1000, “Dehairing”, “”)

    ’Amount of Faecal Contamination
’=columns in Dehairing:B*C*D
For i = 0 To NumberPigsInLairage - 1
Dehairing_AmtFaecalContamination(i) = Worksheets(“Dehairing”).Cells(i + 18, 2).value * Worksheets(“Dehairing”).Cells(i + 18, 3).value * Worksheets(“Dehairing”).Cells(i + 18, 4).value
Next
Call FillColumnFromArray(5, 18, 18 + NumberPigsInLairage, “Dehairing”, Dehairing_AmtFaecalContamination)
Call FillColumn(5, 18 + NumberPigsInLairage, 18 + NumberPigsInLairage + 1000, “Dehairing”, “”)

Dim temp3 As Double ’time
temp3 = RDK_Uniform(Worksheets(Inputs).Cells(31, 2).value, Worksheets(Inputs).Cells(31, 3).value, “”)
Dim temp4 As Double
temp4 = Exp(-temp3 * (Worksheets(Inputs).Cells(29, 2).value + Worksheets(Inputs).Cells(30, 2).value))

Dim delta As Double
Dim beta As Double
Dim Po As Double
Dim Mo As Double
Dim C As Double
Dim mu As Double

delta = Worksheets(Inputs).Cells(30, 2).value
mu = Worksheets(Inputs).Cells(29, 2).value
```

```
    For i = 0 To NumberPigsInLairage - 1
'Dehairing_BaNoOnMachineAtStart
'Initial value =0; other subsequent values = dehairing (i+19,9)

    If (i = 0) Then
Worksheets("Dehairing").Cells(i + 18, 7) = 0
Else
Worksheets("Dehairing").Cells(i + 18, 7) = Dehairing_BaOnDehairieratEnd(i - 1)
End If

    Po = Worksheets("Dehairing").Cells(i + 18, 6).value
C = Worksheets("Dehairing").Cells(i + 18, 5).value
Mo = Worksheets("Dehairing").Cells(i + 18, 7).value

Dehairing_BaOnPigAfterDehairing(i) = (Po * delta * mu + Po * delta ^ 2 + Po * mu ^ 2 * temp4 + Po * mu * temp4 * delta - delta * Mo
* temp4 _
* mu - delta ^ 2 * Mo * temp4 + delta * Mo * mu + delta ^ 2 * Mo + 2 * C * delta ^ 2 * temp3 + 2 * C * delta * mu * temp3 + C * delta *
temp4 - C * delta - C * mu * temp4 + C * mu) / (mu + delta) ^ 2

Dehairing_BaOnDehairieratEnd(i) = (-Po * mu ^ 2 * temp4 - Po * mu * temp4 * delta + Po * mu ^ 2 + Po _
* delta * mu + Mo * mu ^ 2 + delta * Mo * mu + delta * Mo * temp4 * mu + delta ^ 2 * Mo * temp4 + 2 * C _
* delta * temp3 * mu + C * delta - C * delta * temp4 + 2 * C * mu ^ 2 * temp3 - C * mu + C * mu * temp4) / (mu + delta) ^ 2

PreviousIterationsDehairingM = Dehairing_BaOnDehairieratEnd(NumberPigsInLairage - 1)

    Next
Call FillColumn(7, 18 + NumberPigsInLairage, 18 + NumberPigsInLairage + 1000, "Dehairing", "")

    Call FillColumnFromArray(8, 18, 18 + NumberPigsInLairage, "Dehairing", Dehairing_BaOnPigAfterDehairing)
Call FillColumnFromArray(2, 11, 11 + NumberPigsInLairage, "Singeing", Dehairing_BaOnPigAfterDehairing)
Call FillColumn(8, 18 + NumberPigsInLairage, 18 + NumberPigsInLairage + 1000, "Dehairing", "")
Call FillColumn(2, 11 + NumberPigsInLairage, 11 + NumberPigsInLairage + 1000, "Singeing", "")

    Call FillColumnFromArray(9, 18, 18 + NumberPigsInLairage, "Dehairing", Dehairing_BaOnDehairieratEnd)
Call FillColumn(9, 18 + NumberPigsInLairage, 18 + NumberPigsInLairage + 1000, "Dehairing", "")

    End Function
```

Singeing

```
    'Singeing - Calling Variables and Types

Private Function SingeingModule() As Boolean
Dim Singeing_BaNoOnPigAfterSingeing() As Double
ReDim Singeing_BaNoOnPigAfterSingeing(NumberPigsInLairage)
Dim i As Integer
```

```
    'Singeing worksheet calculations

    'Singeing_BaNoOnPigAfterSingeing
'=Singeing B10*exp(-Inputs B47* RiskUniform(Inputs B48,Inputs C48))
For i = 0 To NumberPigsInLairage - 1
Dim temp As Double
temp = RDK_Uniform(Worksheets(Inputs).Cells(35, 2).value, Worksheets(Inputs).Cells(35, 3).value, "")
Dim temp1 As Double
temp1 = Exp(-Worksheets(Inputs).Cells(34, 2).value * temp)
Singeing_BaNoOnPigAfterSingeing(i) = temp1 * Worksheets("Singeing").Cells(i + 11, 2).value
Next
Call FillColumnFromArray(3, 11, 11 + NumberPigsInLairage, "Singeing", Singeing_BaNoOnPigAfterSingeing)
Call FillColumn(3, 11 + NumberPigsInLairage, 11 + NumberPigsInLairage + 1000, "Singeing", "")

    Call FillColumnFromArray(4, 28, 28 + NumberPigsInLairage, "Evisceration+ ", Singeing_BaNoOnPigAfterSingeing)
Call FillColumn(4, 28 + NumberPigsInLairage, 28 + NumberPigsInLairage + 1000, "Evisceration+ ", "")

    End Function
```

Evisceration+ Module

```
    'Evisceration+ - Calling Variables and Types

Private Function EviscerationModule() As Boolean
Dim Evisceration_ProbabilityofInfectionPPig() As Double
Dim Evisceration_BacteriaInLungs() As Double
Dim Evisceration_BacterialNosOnKnifeBeforeUse() As Double
Dim Evisceration_BacterialNosOnPigAfterEvisceration() As Double

ReDim Evisceration_ProbabilityofInfectionPPig(NumberPigsInLairage)
ReDim Evisceration_BacteriaInLungs(NumberPigsInLairage)
ReDim Evisceration_BacterialNosOnKnifeBeforeUse(NumberPigsInLairage)
ReDim Evisceration_BacterialNosOnPigAfterEvisceration(NumberPigsInLairage)

Dim Ko As Double
Dim A As Double
Dim B As Double
Dim s As Double
Dim d As Double
Dim e As Double
Dim f As Double
Dim M As Double
Dim w As Double
Dim g As Double
Dim i As Integer

A = RDK_Uniform(0.001, 0.002, "") * RDK_Beta(Worksheets(Inputs).Cells(38, 2).value, Worksheets(Inputs).Cells(38, 3).value, "")
If (A < 0) Then
```

```
A = 0
ElseIf (A > 1) Then
A = 1
End If
B = RDK_Uniform(0.9, 1, “”) * RDK_Uniform(Worksheets(Inputs).Cells(39, 2).value, Worksheets(Inputs).Cells(39, 3).value, “”)
s = RDK_Uniform(Worksheets(Inputs).Cells(40, 2).value, Worksheets(Inputs).Cells(41, 2).value, “”)
d = RDK_Uniform(Worksheets(Inputs).Cells(41, 2).value, Worksheets(Inputs).Cells(41, 2).value, “”)
e = RDK_Uniform(Worksheets(Inputs).Cells(42, 2).value, Worksheets(Inputs).Cells(42, 3).value, “”) / RDK_Uniform(0.5, 20.5, “ ”)
If (e < 0) Then e = 0 ElseIf (e > 1) Then e = 1 End If w = RDK_Uniform(Worksheets(Inputs).Cells(45, 2).value, Worksheets(Inputs).Cells(45,
3).value, “”)

    ’Evisceration worksheet calculations

    ’Evisceration_ProbabilityofInfectionPPig
’RDK_Binomial(1, 1-exp(-lairageB14*B11*B13))
For i = 0 To NumberPigsInLairage - 1
If (Worksheets(“Dehairing”).Cells(i + 18, 2).value = 1) Then
Evisceration_ProbabilityofInfectionPPig(i) = 1
Else
Evisceration_ProbabilityofInfectionPPig(i) = RDK_Binomial(1, 1 - Exp(-Worksheets(“Lairage”).Cells(14, 2).value * Worksheets(“Lairage”).Cells(11,
2).value * Worksheets(“Lairage”).Cells(13, 2).value), “”)
End If

    ’Evisceration_ProbabilityofInfectionPPig(i) = RDK_Binomial(1, 1 - Exp(-Worksheets(“Lairage”).Cells(14, 2).value * Work-
sheets(“Lairage”).Cells(11, 2).value * Worksheets(“Lairage”).Cells(13, 2).value), “”)
Next
Call FillColumnFromArray(2, 28, 28 + NumberPigsInLairage, “Evisceration+ ”, Evisceration_ProbabilityofInfectionPPig)

    ’BacterialNosOnKnifeBeforeUse
’Initial value =0; other subsequent values = calculated
For i = 0 To NumberPigsInLairage - 1
If (i = 0) Then
Worksheets(“Evisceration+ ”).Cells(i + 28, 5) = 0
Else
Worksheets(“Evisceration+ ”).Cells(i + 28, 5) = Evisceration_BacterialNosOnKnifeBeforeUse(i - 1)
End If

Ko = Worksheets(“Evisceration+ ”).Cells(i + 28, 5).value

If (Worksheets(“Evisceration+ ”).Cells(i + 28, 2).value = 0) Then
g = 0
Else
g = RDK_Binomial(1, 0.1, “”) * Round(RDK_Uniform(Worksheets(Inputs).Cells(46, 2).value, Worksheets(Inputs).Cells(46, 3).value,
“”), 0)
End If

Evisceration_BacterialNosOnKnifeBeforeUse(i) = A * Worksheets(“Evisceration+ ”).Cells(i + 28, 4).value _
+ (1 - B) * (1 - d) * Worksheets(“Evisceration+ ”).Cells(i + 28, 5).value + e * w * g
```

```
Evisceration_BacterialNosOnPigAfterEvisceration(i) = (1 - A) * (1 - s) * Worksheets("Evisceration+ ").Cells(i + 28, 4).value _
+ B * Worksheets("Evisceration+ ").Cells(i + 28, 5).value + (1 - e) * w * g

PreviousIterationsEviscerationK = Evisceration_BacterialNosOnKnifeBeforeUse(NumberPigsInLairage - 1)
Next
Call FillColumnFromArray(6, 28, 28 + NumberPigsInLairage, "Evisceration+ ", Evisceration_BacterialNosOnPigAfterEvisceration)
```

End Function

Trimming and Halving Module

```
Private Function TrimmingModule() As Boolean

Dim Evisceration_BaOnKnifeBeforeTrimming() As Double
Dim Evisceration_BaOnPigAfterTrimming() As Double
Dim Evisceration_BaNosOnSawBeforeHalving() As Double
Dim Evisceration_BaNosOn1PigCarcassAfterHalving() As Double
Dim Evisceration_BaNosPerHalfCarcass() As Double
Dim i As Integer

ReDim Evisceration_BaOnKnifeBeforeTrimming(NumberPigsInLairage)
ReDim Evisceration_BaOnPigAfterTrimming(NumberPigsInLairage)
ReDim Evisceration_BaNosOnSawBeforeHalving(NumberPigsInLairage)
ReDim Evisceration_BaNosOn1PigCarcassAfterHalving(NumberPigsInLairage)
ReDim Evisceration_BaNosPerHalfCarcass(NumberPigsInLairage)

Dim Ka As Double
Dim Sa As Double
Dim h As Double
Dim l As Double
Dim j As Double
Dim k As Double

h = RDK_Uniform(0.004, 0.006, "") * ((RDK_Beta(Worksheets(Inputs).Cells(49, 2).value, Worksheets(Inputs).Cells(49, 3).value, ""))
2̂) 0̂.5
If (h < 0) Then
h = 0
ElseIf (h > 1) Then
h = 1
End If
l = RDK_Uniform(0.9, 1, "") * RDK_Uniform(Worksheets(Inputs).Cells(50, 2).value, Worksheets(Inputs).Cells(50, 3).value, "")

' Line for inputs = Sal If CBool(Worksheets("model map").Cells(6, 5)) Then j = RDK_Uniform(Worksheets(Inputs).Cells(51,
2).value, Worksheets(Inputs).Cells(51, 3).value, "") + RDK_Uniform(0.04, 0.98, "") - (RDK_Uniform(0.04, 0.98, "") * RDK_Uniform(Worksheets(Inputs).Cells
2).value, Worksheets(Inputs).Cells(51, 3).value, "")) End If
'campy, If CBool(Worksheets("model map").Cells(7, 5)) Then j = RDK_Uniform(Worksheets(Inputs).Cells(51, 2).value, Work-
sheets(Inputs).Cells(51, 3).value, "") + RDK_Uniform(0.16, 1, "") - (RDK_Uniform(0.04, 0.98, "") * RDK_Uniform(Worksheets(Inputs).Cells(51,
2).value, Worksheets(Inputs).Cells(51, 3).value, "")) End If
'E. coli If CBool(Worksheets("model map").Cells(5, 5)) Then j = RDK_Uniform(Worksheets(Inputs).Cells(51, 2).value, Work-
```

```
sheets(Inputs).Cells(51, 3).value, “”) + RDK_Uniform(0, 0.08, “”) - (RDK_Uniform(0.04, 0.98, “”) * RDK_Uniform(Worksheets(Inputs).Cells(51,
2).value, Worksheets(Inputs).Cells(51, 3).value, “”)) End If
If (j < 0) Then j = 0 ElseIf (j > 1) Then j = 1 End If
    k = RDK_Uniform(Worksheets(Inputs).Cells(52, 2).value, Worksheets(Inputs).Cells(52, 3).value, “”)

Dim n As Double
Dim o As Double
Dim p As Double
Dim q As Double

n = 0.001 * ((RDK_Beta(Worksheets(Inputs).Cells(55, 2).value, Worksheets(Inputs).Cells(55, 3).value, “”)) ^ 2) ^ 0.5
If (n < 0) Then
n = 0
ElseIf (n > 1) Then
n = 1
End If
o = RDK_Uniform(0.9, 1, “”) * RDK_Uniform(Worksheets(Inputs).Cells(56, 2).value, Worksheets(Inputs).Cells(56, 3).value, “”)
p = RDK_Uniform(Worksheets(Inputs).Cells(57, 2).value, Worksheets(Inputs).Cells(57, 3).value, “”)
q = RDK_Uniform(Worksheets(Inputs).Cells(58, 2).value, Worksheets(Inputs).Cells(58, 3).value, “”)

    ’Trimmimg & Halving

Ka = Worksheets(“Evisceration+ ”).Cells(i + 28, 7).value

For i = 0 To NumberPigsInLairage - 1
If (i = 0) Then
Worksheets(“Evisceration+ ”).Cells(i + 28, 7) = 0
Else
Worksheets(“Evisceration+ ”).Cells(i + 28, 7) = Evisceration_BaOnKnifeBeforeTrimming(i - 1)
End If

    If (Worksheets(“Evisceration+ ”).Cells(i + 28, 6).value > Worksheets(Inputs).Cells(47, 2).value) Then

Evisceration_BaOnPigAfterTrimming(i) = (1 - h) * (1 - j) * Worksheets(“Evisceration+ ”).Cells(i + 28, 6).value _
+ l * Worksheets(“Evisceration+ ”).Cells(i + 28, 7).value

Evisceration_BaOnKnifeBeforeTrimming(i) = h * Worksheets(“Evisceration+ ”).Cells(i + 28, 6).value _
+ Worksheets(“Evisceration+ ”).Cells(i + 28, 7).value * (1 - l) * (1 - k)
Else
Evisceration_BaOnPigAfterTrimming(i) = Worksheets(“Evisceration+ ”).Cells(i + 28, 6).value
End If

PreviousIterationsTrimmingK = Evisceration_BaOnKnifeBeforeTrimming(NumberPigsInLairage - 1)

    Next
Call FillColumnFromArray(8, 28, 28 + NumberPigsInLairage, “Evisceration+ ”, Evisceration_BaOnPigAfterTrimming)

    ’ Halving after trimming
```

```
    For i = 0 To NumberPigsInLairage - 1

    Sa = Worksheets("Evisceration+ ").Cells(i + 28, 9).value

    If (i = 0) Then
Worksheets("Evisceration+ ").Cells(i + 28, 9) = 0
Else
Worksheets("Evisceration+ ").Cells(i + 28, 9) = Evisceration_BaNosOnSawBeforeHalving(i - 1)
End If

Evisceration_BaNosOnSawBeforeHalving(i) = n * Worksheets("Evisceration+ ").Cells(i + 28, 8).value _
+ Worksheets("Evisceration+ ").Cells(i + 28, 9).value * (1 - o) * (1 - q)

Evisceration_BaNosOn1PigCarcassAfterHalving(i) = (1 - n) * (1 - p) * Worksheets("Evisceration+ ").Cells(i + 28, 8).value _
+ o * Worksheets("Evisceration+ ").Cells(i + 28, 9).value

    Next
Call FillColumnFromArray(10, 28, 28 + NumberPigsInLairage, "Evisceration+ ", Evisceration_BaNosOn1PigCarcassAfterHalving)

    PreviousIterationsHalvingK = Evisceration_BaNosOnSawBeforeHalving(NumberPigsInLairage - 1)

    'Evisceration_BaNosPerHalfCarcass
For i = 0 To NumberPigsInLairage - 1
Evisceration_BaNosPerHalfCarcass(i) = 0.5 * Worksheets("Evisceration+ ").Cells(i + 28, 10).value
Next
Call FillColumnFromArray(11, 28, 28 + NumberPigsInLairage, "Evisceration+ ", Evisceration_BaNosPerHalfCarcass)
Call FillColumn(11, 28 + NumberPigsInLairage, 28 + NumberPigsInLairage + 1000, "Evisceration+ ", "")
```

End Function

,

Halving Module

```
Private Function HalvingModule() As Boolean
Dim Evisceration_BaNosOnSawBeforeHalving() As Double
Dim Evisceration_BaNosOn1PigCarcassAfterHalving() As Double
Dim Evisceration_BaNosPerHalfCarcass() As Double
Dim i As Integer
Dim n As Double
Dim o As Double
Dim p As Double
Dim q As Double
Dim Sa As Double

ReDim Evisceration_BaNosOnSawBeforeHalving(NumberPigsInLairage)
ReDim Evisceration_BaNosOn1PigCarcassAfterHalving(NumberPigsInLairage)
ReDim Evisceration_BaNosPerHalfCarcass(NumberPigsInLairage)
```

```
n = 0.001 * ((RDK_Beta(Worksheets(Inputs).Cells(55, 2).value, Worksheets(Inputs).Cells(55, 3).value, “”)) ^ 2) ^ 0.5
If (n < 0) Then
n = 0
ElseIf (n > 1) Then
n = 1
End If
o = RDK_Uniform(0.9, 1, “”) * RDK_Uniform(Worksheets(Inputs).Cells(56, 2).value, Worksheets(Inputs).Cells(56, 3).value, “”)
p = RDK_Uniform(Worksheets(Inputs).Cells(57, 2).value, Worksheets(Inputs).Cells(57, 2).value, “”)
q = RDK_Uniform(Worksheets(Inputs).Cells(58, 2).value, Worksheets(Inputs).Cells(58, 2).value, “”)

    ’Halving with no trimming

    Sa = Worksheets(“Evisceration+ ”).Cells(i + 28, 9).value

    For i = 0 To NumberPigsInLairage - 1
If (i = 0) Then
Worksheets(“Evisceration+ ”).Cells(i + 28, 9) = 0
Else
Worksheets(“Evisceration+ ”).Cells(i + 28, 9) = Evisceration_BaNosOnSawBeforeHalving(i - 1)
End If

Evisceration_BaNosOnSawBeforeHalving(i) = n * Worksheets(“Evisceration+ ”).Cells(i + 28, 6).value _
+ Worksheets(“Evisceration+ ”).Cells(i + 28, 9).value * (1 - o) * (1 - q)

Evisceration_BaNosOn1PigCarcassAfterHalving(i) = (1 - n) * (1 - p) * Worksheets(“Evisceration+ ”).Cells(i + 28, 6).value _
+ o * Worksheets(“Evisceration+ ”).Cells(i + 28, 9).value
Next
Call FillColumn(9, 28 + NumberPigsInLairage, 28 + NumberPigsInLairage + 1000, “Evisceration+ ”, “”)
Call FillColumnFromArray(10, 28, 28 + NumberPigsInLairage, “Evisceration+ ”, Evisceration_BaNosOn1PigCarcassAfterHalving)
Call FillColumn(10, 28 + NumberPigsInLairage, 28 + NumberPigsInLairage + 1000, “Evisceration+ ”, “”)

    PreviousIterationsHalvingK = Evisceration_BaNosOnSawBeforeHalving(NumberPigsInLairage - 1)

    ’Evisceration_BaNosPerHalfCarcass

    For i = 0 To NumberPigsInLairage - 1
Evisceration_BaNosPerHalfCarcass(i) = 0.5 * Worksheets(“Evisceration+ ”).Cells(i + 28, 10).value
Next
Call FillColumnFromArray(11, 28, 28 + NumberPigsInLairage, “Evisceration+ ”, Evisceration_BaNosPerHalfCarcass)
Call FillColumn(11, 28 + NumberPigsInLairage, 28 + NumberPigsInLairage + 1000, “Evisceration+ ”, “”)

    End Function
```

Storage Module

```
Private Function StorageModule() As Boolean
Dim Storage_baNobeforeContamination() As Double
Dim Storage_baNoAfterContamination() As Double
Dim Storage_baNob4GrwthInactivation() As Double
Dim Storage_baNoAfterChilling() As Double
Dim i As Integer
Dim R1 As Double
Dim R2 As Double
Dim Z As Double

ReDim Storage_baNobeforeContamination(NumberPigsInLairage)
ReDim Storage_baNoAfterContamination(NumberPigsInLairage)
ReDim Storage_baNob4GrwthInactivation(NumberPigsInLairage)
ReDim Storage_baNoAfterChilling(NumberPigsInLairage)

    'Storage_baNobeforeContamination
'= evisceration column 10
For i = 0 To NumberPigsInLairage - 1
Storage_baNobeforeContamination(i) = Worksheets("Evisceration+ ").Cells(i + 28, 11).value
Next
Call FillColumnFromArray(2, 19, 19 + NumberPigsInLairage, "Storage", Storage_baNobeforeContamination)
Call FillColumn(2, 19 + NumberPigsInLairage, 19 + NumberPigsInLairage + 1000, "Storage", "")

    'Storage_baNoAfterContamination
R1 = Worksheets(Inputs).Cells(62, 2).value
R2 = Worksheets(Inputs).Cells(64, 2).value
Z = Exp(-(Worksheets(Inputs).Cells(62, 2).value + Worksheets(Inputs).Cells(64, 2).value) * RDK_Uniform(Worksheets(Inputs).Cells(63,
2).value, Worksheets(Inputs).Cells(63, 3).value, ""))

Dim Run() As Double 'to determine whether cross contamination occurs or not
ReDim Run(NumberPigsInLairage)
Dim Direction() As Double 'direction in which cross-contamination occurs
ReDim Direction(NumberPigsInLairage)

    For i = 0 To NumberPigsInLairage - 1

If (i = 0) Then
Direction(i) = 1
Else
Direction(i) = Round(RDK_Binomial(1, 0.5, ""), 0)
End If

    Next

    For i = 0 To NumberPigsInLairage - 1
Run(i) = RDK_Uniform(0, 1, "")
Next
```

```
    For i = 0 To NumberPigsInLairage - 2
If (Run(i) < 0.31 And Direction(i) = 1) Then

Storage_baNoAfterContamination(i) = (-Worksheets("Storage").Cells(i + 19 + 1, 2).value * R2 * Z _
+ R2 * Worksheets("Storage").Cells(i + 19 + 1, 2).value + Worksheets("Storage").Cells(i + 19, 2).value * R2 _
+ R1 * Z * Worksheets("Storage").Cells(i + 19, 2).value) / (R1 + R2)

Storage_baNoAfterContamination(i + 1) = (Worksheets("Storage").Cells(i + 19 + 1, 2).value * R1 _
+ Worksheets("Storage").Cells(i + 19 + 1, 2).value * R2 * Z - R1 * Worksheets("Storage").Cells(i + 19, 2).value * Z _
+ R1 * Worksheets("Storage").Cells(i + 19, 2).value) / (R1 + R2)

Else
Storage_baNoAfterContamination(i) = Worksheets("Storage").Cells(i + 19, 2).value
End If
Next

    For i = 1 To NumberPigsInLairage - 1
If (Run(i) < 0.31 And Direction(i) = 0) Then

Storage_baNoAfterContamination(i - 1) = (Worksheets("Storage").Cells(i + 19 - 1, 2).value * R1 _
+ Worksheets("Storage").Cells(i + 19 - 1, 2).value * R2 * Z - R1 * Worksheets("Storage").Cells(i + 19, 2).value * Z _
+ R1 * Worksheets("Storage").Cells(i + 19, 2).value) / (R1 + R2)

Storage_baNoAfterContamination(i) = (-Worksheets("Storage").Cells(i + 19 - 1, 2).value * R2 * Z _
+ R2 * Worksheets("Storage").Cells(i + 19 - 1, 2).value + Worksheets("Storage").Cells(i + 19, 2).value * R2 _
+ R1 * Z * Worksheets("Storage").Cells(i + 19, 2).value) / (R1 + R2)

Else
Storage_baNoAfterContamination(i) = Worksheets("Storage").Cells(i + 19, 2).value
End If

    Next
Call FillColumnFromArray(3, 19, 19 + NumberPigsInLairage, "Storage", Storage_baNoAfterContamination)
Call FillColumn(3, 19 + NumberPigsInLairage, 19 + NumberPigsInLairage + 1000, "Storage", "")

    For i = 0 To NumberPigsInLairage - 1
If Worksheets("Storage").Cells(i + 19, 3).value < 1000 Then
Storage_baNoAfterChilling(i) = Worksheets("Storage").Cells(i + 19, 3).value * Exp(-Worksheets(Inputs).Cells(66, 2).value * RDK_Uniform(Worksheets(Inputs
2).value, Worksheets(Inputs).Cells(63, 3).value, ""))
Else
Storage_baNoAfterChilling(i) = Worksheets("Storage").Cells(i + 19, 3).value * Exp(-Worksheets(Inputs).Cells(66, 3).value * RDK_Uniform(Worksheets(Inputs
2).value, Worksheets(Inputs).Cells(63, 3).value, ""))
End If

    Next
Call FillColumnFromArray(4, 19, 19 + NumberPigsInLairage, "Storage", Storage_baNoAfterChilling)
Call FillColumn(4, 19 + NumberPigsInLairage, 19 + NumberPigsInLairage + 1000, "Storage", "")
```

```
    ’ sum the number of columns greater than 0 in storage...
Dim count As Double
count = 0
Dim theArray() As Double
theArray = LoadArrayFromColumns(4, 19, NumberPigsInLairage + 19, “Storage”)
For i = 0 To NumberPigsInLairage - 1
If theArray(i) > 0.5 Then
count = count + 1
End If
Next
count = count / NumberPigsInLairage

    ’ write the value
PrevalenceMatrix(CurrentIteration - 1) = count

    End Function
```

Region Helpers

```
    ’ construct an array of integers
Private Function ConstructArrayIntegers(value As Double, length As Integer) As Double()
Dim i As Integer
Dim arrayTemp() As Double
ReDim arrayTemp(length)
For i = 0 To length - 1
arrayTemp(i) = value
Next
ConstructArrayIntegers = arrayTemp
End Function

    ’ construct an array of integers with constant incrementing value
Private Function ConstructIncreasingArray(start As Double, step As Double, length As Integer) As Double()
Dim i As Integer
Dim arrayTemp() As Double
ReDim arrayTemp(length)
arrayTemp(0) = start
For i = 1 To length - 1
arrayTemp(i) = arrayTemp(i - 1) + step
Next
ConstructIncreasingArray = arrayTemp
End Function

    ’ Array initialization routine
Private Function ArrayInteger(ParamArray values() As Variant) As Integer()
Dim i As Long
Dim res() As Integer
ReDim res(UBound(values)) As Integer
For i = 0 To UBound(values)
```

```
res(i) = values(i)
Next
ArrayInteger = res() End Function

    ' Array initialization routine
Private Function Arraydouble(ParamArray values() As Variant) As Double()
Dim i As Long
Dim res() As Double
ReDim res(UBound(values)) As Double
For i = 0 To UBound(values)
res(i) = values(i)
Next
Arraydouble = res()
End Function

    ' Array initialization routine
Private Function ArrayAppend(FromArray As Variant, IterationNumber As Integer, ToArray() As Double)
Dim i As Long
Dim j As Long
For i = 0 To UBound(FromArray)
If (IterationNumber + i) = 0 Then
ReDim ToArray(0)
Else
ReDim Preserve ToArray(UBound(ToArray) + 1)
End If
ToArray(UBound(ToArray)) = FromArray(j)
j = j + 1
Next
End Function

    Private Function ArrayDivisionInt(values() As Double, divisor As Double) As Double()
Dim tmpArray() As Double
ReDim tmpArray(UBound(values)) As Double
Dim i As Integer
For i = 0 To UBound(values)
tmpArray(i) = values(i) / divisor
Next
ArrayDivisionInt = tmpArray()
End Function

    Private Function ArrayMultiplication(array1() As Double, array2() As Double) As Double()
Dim tmpArray() As Double
Dim i As Integer
ReDim tmpArray(UBound(array1)) As Double
For i = 0 To UBound(array1)
tmpArray(i) = array1(i) * array2(i)
Next
ArrayMultiplication = tmpArray()
End Function
```

```
    Private Function ArrayMultiplicationdouble(values() As Double, Factor As Double) As Double()
Dim tmpArray() As Double
ReDim tmpArray(UBound(values)) As Double
Dim i As Long
For i = 0 To UBound(values)
tmpArray(i) = values(i) * Factor
Next
ArrayMultiplicationdouble = tmpArray()
End Function

    Private Function ArrayAddition(array1() As Double, array2() As Double) As Double()
Dim i As Long
Dim tmpArray() As Double
ReDim tmpArray(UBound(array1)) As Double
For i = 0 To UBound(array1)
tmpArray(i) = array1(i) + array2(i)
Next
ArrayAddition = tmpArray()
End Function

    Private Function ArrayAdditionInt(theInteger As Integer, array1() As Double) As Double()
Dim tmpArray() As Double
Dim i As Integer
ReDim tmpArray(UBound(array1)) As Double
For i = 0 To UBound(array1)
tmpArray(i) = array1(i) + theInteger
Next
ArrayAdditionInt = tmpArray()
End Function

    Private Function LoadArrayFromRows(row As Integer, NumberPigsInLairage As Long) As Double()
Dim i As Integer
Dim difference As Integer
Dim tmpArray() As Double
ReDim tmpArray(NumberPigsInLairage) As Double
For i = 0 To NumberPigsInLairage - 1
tmpArray(i) = CDbl(Worksheets("InputData").Cells(row, i + 2))
Next
LoadArrayFromRows = tmpArray()
End Function

    ' load a column of doubles into an array
Private Function LoadArrayFromColumns(column As Integer, rowStart As Integer, rowEnd As Integer, Worksheet As String) As Dou-
ble()
Dim i As Integer
Dim tmpArray() As Double
ReDim tmpArray(rowEnd - rowStart - 1) As Double
For i = 0 To (rowEnd - rowStart) - 1
```

```
tmpArray(i) = CDbl(Worksheets(Worksheet).Cells(i + rowStart, column))
Next
LoadArrayFromColumns = tmpArray()
End Function

    'Fill a Column from array
Private Sub FillColumnFromArray(column As Integer, rowStart As Integer, rowEnd As Integer, Worksheet As String, theArray() As Double)
Dim i As Integer
For i = 0 To (rowEnd - rowStart) - 1
Worksheets(Worksheet).Cells(i + rowStart, column) = theArray(i)
Next
End Sub

    'Fill a Column
Private Sub FillColumn(ByVal column As Integer, rowStart As Integer, rowEnd As Integer, Worksheet As String, ByValue As String)
Dim i As Integer
For i = rowStart To rowEnd
Worksheets(Worksheet).Cells(i, column).Clear '.value = " "
Next
End Sub

    'Fill a Cell
Private Sub FillCellFromArray(column As Integer, row As Integer, Worksheet As String, valuefromtheArray As Double)
Dim i As Integer
For i = 0 To NumberPigsInLairage
Worksheets(Worksheet).Cells(row, column).value = valuefromtheArray
Next

    End Sub

    'Clear a Column
Private Sub Clearcolumn(column As Integer, rowStart As Integer, rowEnd As Integer, Worksheet As String)
Dim i As Integer
For i = rowStart To rowEnd
Worksheets(Worksheet).Cells(i, column).value = "0"
Next
End Sub

    Private Sub CustomError(message As String)
MsgBox ("Error: " & message)
Close
End Sub

    Private Function Load_AtRiskLibrary() As Boolean
Dim mstrDToolsSystemDirectory As String
Dim RSK_LIBRARY As String
Dim mlngRSKHandle As String
RSK_LIBRARY = "RSKLIB32.DLL"
```

```
mstrDToolsSystemDirectory = Worksheets(Inputs).Cells(4, 6).value
mlngRSKHandle = apiLoadLibrary(mstrDToolsSystemDirectory & “& RSK_LIBRARY)
Load_AtRiskLibrary = CBool(mlngRSKHandle <> 0)
If Not Load_AtRiskLibrary Then
MsgBox “@RISK Library NOT Found at ” + mstrDToolsSystemDirectory, vbOKOnly Or vbExclamation
End If

    End Function

    Private Sub Unload_RSK()
Dim mlngRSKHandle As Double

    If mlngRSKHandle <> 0 Then
Call apiFreeLibrary(mlngRSKHandle)
mlngRSKHandle = 0
End If
End Sub

    Private Sub CleanWorkSheet(nameWorkSheet As String)
Worksheets(nameWorkSheet).Cells.Clear
End Sub
```

Module 2 of Visual Basic Code

```
    Public Sub TestLowerQuartile()
Dim evenArray() As Double
ReDim evenArray(7) As Double

    Dim evenArray2() As Double
ReDim evenArray2(9) As Double

    Dim oddArray() As Double
ReDim oddArray(8) As Double

    Dim smallArray() As Double
ReDim smallArray(0) As Double

    evenArray(0) = 1
evenArray(1) = 2
evenArray(2) = 3
evenArray(3) = 4
evenArray(4) = 5
evenArray(5) = 6
evenArray(6) = 7
evenArray(7) = 8

    smallArray(0) = 1
```

```
        oddArray(0) = 1
oddArray(1) = 2
oddArray(2) = 3
oddArray(3) = 4
oddArray(4) = 5
oddArray(5) = 6
oddArray(6) = 7
oddArray(7) = 8
oddArray(8) = 9

        evenArray2(0) = 1
evenArray2(1) = 2
evenArray2(2) = 3
evenArray2(3) = 4
evenArray2(4) = 5
evenArray2(5) = 6
evenArray2(6) = 7
evenArray2(7) = 8
evenArray2(8) = 9
evenArray2(9) = 10

        Dim lQEven As Double

        lQEven = LQ2(evenArray)

        Dim lQSmall As Double
'= 1
lQSmall = LQ2(smallArray)

        Dim lQOdd As Double

        lQOdd = LQ2(oddArray)

        Dim lQEven2 As Double

        lQEven2 = LQ2(evenArray2)

        Dim test As Integer
test = 5
End Sub

        Public Sub TestDecile()

        Dim theArray() As Double
ReDim theArray(19) As Double
Dim i As Integer

        For i = 0 To 20 - 1
theArray(i) = i + 1
```

```
Next
Dim lQ As Double
Dim uQ As Double
Dim median As Double

    median = DecileCL(theArray, 50)
lQ = LQ2(theArray)
uQ = UQ2(theArray)

    End Sub

    Public Function Ceiling(value As Double) As Integer
Dim low As Double
Dim high As Double

    If (Math.Round(value) - value > 0) Then
Ceiling = CInt(value)
Else
Ceiling = CInt(value + 0.5)
End If
End Function

    Public Function Floor(value As Double) As Integer
Dim low As Double
Dim high As Double

    If (Math.Round(value) - value > 0) Then
Floor = CInt(value - 0.5)
Else
Floor = CInt(value)
End If
End Function

    ' load a column of doubles into an array
Public Function LoadArrayFromColumns(column As Integer, rowStart As Integer, rowEnd As Integer, Worksheet As String) As Double()
Dim i As Integer

    Dim tmpArray() As Double
ReDim tmpArray(rowEnd - rowStart) As Double

    For i = 0 To (rowEnd - rowStart)
tmpArray(i) = CDbl(Worksheets(Worksheet).Cells(i + rowStart, column))
Next

    LoadArrayFromColumns = tmpArray()
End Function

    Private Function BubbleSort(ToSort() As Double) As Double()
```

```
Dim AnyChanges As Boolean
Dim value As Long
Dim SwapFH As Double
Do
AnyChanges = False
For value = LBound(ToSort) To UBound(ToSort) - 1
If (ToSort(value) > ToSort(value + 1)) Then
SwapFH = ToSort(value)
ToSort(value) = ToSort(value + 1)
ToSort(value + 1) = SwapFH
AnyChanges = True
End If
Next value
Loop Until Not AnyChanges
BubbleSort = ToSort
End Function

    ' STATISTICS FUNCTIONS

    Public Function Mean(ByRef theArray() As Double) As Double
Dim length As Integer
length = UBound(theArray)
Dim i As Integer

    Dim tmp As Double

    tmp = 0
For i = 0 To length
tmp = tmp + theArray(i)
Next

    If length = 0 Then
Mean = tmp + theArray(i)
Else
Mean = tmp / (length + 1)
End If

    End Function

    Public Function median(theArray() As Double) As Double
theArray = BubbleSort(theArray)
Dim length As Double
length = CDbl(UBound(theArray))
Dim tmp As Double
Dim pos1 As Integer
Dim pos2 As Integer

    If (length + 1) Mod 2 = 0 Then
pos1 = AsymArith((length - 1) / 2)
```

```
pos2 = AsymArith(length / 2)

    tmp = (theArray(pos1) + theArray(pos2)) / 2
Else
If (length = 0) Then
tmp = theArray(0)
Else
pos1 = AsymArith(length / 2)
tmp = theArray(pos1)
End If
End If
median = tmp
End Function

    ' Asymmetric arithmetic rounding - rounds .5 up always.
Function AsymArith(ByVal X As Double, Optional ByVal Factor As Double = 1) As Double
AsymArith = Int(X * Factor + 0.5) / Factor
End Function

    Public Function min(ByRef theArray() As Double)
Dim length As Integer
length = UBound(theArray)
Dim tmp As Double
Dim i As Integer

    tmp = theArray(0)
For i = 0 To length
If (theArray(i) < tmp) Then
tmp = theArray(i)
End If
Next

    min = tmp
End Function

    Public Function Max(ByRef theArray() As Double)
Dim length As Integer
length = UBound(theArray)
Dim tmp As Double
Dim i As Integer
tmp = theArray(0)
'For i = 1 To length - 1
For i = 0 To length
If (theArray(i) > tmp) Then
tmp = theArray(i)
End If
Next

    Max = tmp
```

```
End Function

    Function StdDev(ByRef theArray() As Double) As Double
Dim i As Integer
Dim avg As Double
Dim length As Integer
length = UBound(theArray)
Dim SumSq As Double

    avg = Mean(theArray)
For i = 0 To length
SumSq = SumSq + (theArray(i) - avg)^2
Next i

    If length = 0 Then
StdDev = 0
Else
StdDev = Math.Sqr(SumSq / (length))
End If
End Function

    Public Function Variance(ByRef theArray() As Double) As Double
Dim i As Integer
Dim avg As Double
Dim length As Integer
length = UBound(theArray)
Dim SumSq As Double

    avg = Mean(theArray)
For i = 0 To length
SumSq = SumSq + (theArray(i) - avg)^2
Next i

    Dim std As Double
std = StdDev(theArray)

    If (length = 1) Then
Variance = (Math.Sqr(SumSq / (length)))^2
Else
Variance = (Math.Sqr(SumSq / (length - 1)))^2
End If

    End Function

    Public Function DecileCL(theArray() As Double, decile As Double) As Double
theArray = BubbleSort(theArray)
Dim length As Double
length = CDbl(UBound(theArray))
Dim tmp As Double
```

```
Dim pos1 As Integer
Dim pos2 As Integer

    decile = decile / 100

    If (length + 1) Mod 2 = 0 Then
pos1 = AsymArith((length - 1) * decile)
pos2 = AsymArith(length * decile)

    tmp = (theArray(pos1) + theArray(pos2)) / 2
Else
If (length = 0) Then
tmp = theArray(0)
Else
pos1 = AsymArith(length * decile)
tmp = theArray(pos1)
End If
End If
DecileCL = tmp
End Function

    Public Function LQ2(theArray() As Double) As Double
theArray = BubbleSort(theArray)
Dim length As Double
length = CDbl(UBound(theArray))
Dim tmp As Double
Dim pos1 As Integer
Dim pos2 As Integer

    If (length + 1) Mod 2 = 0 Then
pos1 = AsymArith((length - 1) / 4)
pos2 = AsymArith(length / 4)

    tmp = (theArray(pos1) + theArray(pos2)) / 2
Else
If (length = 0) Then
tmp = theArray(0)
Else
pos1 = AsymArith(length / 4)
tmp = theArray(pos1)
End If
End If
LQ2 = tmp
End Function

    Public Function UQ2(theArray() As Double) As Double
theArray = BubbleSort(theArray)
Dim length As Double
length = CDbl(UBound(theArray))
```

```
Dim tmp As Double
Dim pos1 As Integer
Dim pos2 As Integer

    If (length + 1) Mod 2 = 0 Then
pos1 = AsymArith((length - 1) * 3 / 4)
pos2 = AsymArith(length * 3 / 4)

    tmp = (theArray(pos1) + theArray(pos2)) / 2
Else
If (length = 0) Then
tmp = theArray(0)
Else
pos1 = AsymArith(length * 3 / 4)
tmp = theArray(pos1)
End If
End If
UQ2 = tmp
End Function
```

Palmerston North Meat Retailer study

This study is part of a much larger project investigating microbial contamination in the food chain. The aim of this study is 1) to gain a better understanding of the range of meat products on sale for human consumption in the Palmerston North area and 2) to assess the possibility of cross-contamination between products from different species. Some parts of this questionnaire were developed to determine the variety of management practises conducted in the processing of specific pork products sold at retail.

The information obtained will be used only for research purposes and all information given will be treated confidentially and anonymously.

Contacts for all enquiries are P. Mullner[1] and S. Titus[2]

Thanks very much for your cooperation. Your contribution is greatly appreciated.

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Part 1: General Information

Name and location of premise:

Person interviewed (include position):

Interviewer:

Date and time of interview:

Status of business

☐ operating under NZFSA programme

☐ registered with Palmerston North City Council

Comments

Part 2: Questions

- Please note: This survey does not look at seafood -

1 Range of raw meat products for sale

1.1 What types of raw meat were handled in your business over the last 12 months?

___ Chicken

___ Other Poultry (Specify):

___ Beef

___ Veal

___ Pork

___ Lamb / Mutton

___ Others (e.g. deer, game):

1.2 If you handle raw CHICKEN,

1.2.1 What raw chicken products did you sell over the last 12 months?

___ Whole carcasses

___ Cuts (Specify):

___ Minced product

___ Offal (Specify):

___ Others (Specify):

1.2.2 Who supplied the products to your business?

_____
from_____

_____
from_____

_____
from_____

1.2.3 Which of these products were sold frozen, which were sold fresh?

Frozen:
_____

_____

Fresh:
_____

_____

Comments 1.2

1.3 If you handle raw Other Poultry.

1.3.1 What raw products did you sell over the last 12 months (include species)?

___ Whole carcasses

___ Cuts

___ Offal (Specify):

___ Others (Specify):

1.3.2 Who supplied the products to your business?

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

1.3.3 Which of these products were sold frozen, which were sold fresh?

Frozen:
_____

_____

Fresh:
_____

_____

Comments 1.3:

1.4 If you handle raw Beef,

1.4.1 What raw products did you sell over the last 12 months?

___ Whole carcasses

___ Cuts

___ Minced Product

___ Offal (Specify):

___ Others (Specify):

1.4.2 Who supplied the products to your business?

from_____

_____
from_____

_____
from_____

1.4.3 Which of these products were sold frozen, which were sold fresh?

Frozen:
_____

_____

Fresh:
_____

_____

Comments 1.4:

1.5 If you handle raw Veal,

1.5.1 What raw products did you sell over the last 12 months?

___ Whole carcasses

___ Cuts

___ Offal (Specify):

___ Others (Specify):

1.5.2 Who supplied the products to your business?

_____
from_____

from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

1.5.3 Which of these products were sold frozen, which were sold fresh?

Frozen:
_____

_____

Fresh:
_____

_____

Comments 1.5:

1.6 If you handle raw Lamb and Mutton,

1.6.1 What raw products did you handle over the last 12 months?

___ Whole carcasses

___ Cuts

___ Minced product

___ Offal

___ Others (Specify):

1.6.2 Who supplied the products to your business?

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

1.6.3 Which of these products were frozen, which were fresh?

Frozen:
_____

_____

Fresh:
_____

_____

Comments 1.6:

1.7 If you handle raw Other Meats,

1.7.1 What raw products did you sell over the last 12 months (include species)?

___ Whole carcasses

___ Pieces

___ Offal

___ Others (Specify):

1.7.2 Who supplied the products to your business?

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

1.7.3 Which of these products were sold frozen, which were sold fresh?

Frozen:

_____

___ _____

Fresh:

_____

___ _____

Comments 1.7:

1.8 If you handle Pork,

1.8.1 What raw products did you sell over the last 12 months?

___ Whole carcasses

___ Cuts

___ Offal (Specify):

___ Others (Specify):

Please indicate which of the following are sold in your store? More than one item can be selected.

Shoulder roast	1
Pork chops	2
Shaved ham	3
Pre-packed bacon	4
None	5

Only complete Section **A** if **pork chops** and or **shoulder roast** have been selected.

Only complete Section **B** if **shaved ham** has been selected.

1.8.2 Who supplied the products to your business? (Please list all pork products)

from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

_____
from_____

1.8.3 Which of these products were sold frozen, which were sold fresh?

Frozen:
_____

_____
___

Fresh:
_____

_____
___

A. Pork Chops & Shoulder Roast

All questions in this section refer to pork chops and shoulder roasts only

1.8.4 What carcass/ carcass sections is/are purchased for the preparation of Pork Chops & Shoulder Roasts?

(a) Pork Chops

	Frozen	Fresh
Whole carcasses	1	5

Halved carcasses	2	6
Pork pieces	3	7
Other	4	8

.9. If other, specify_____

(b) Shoulder Roast

	Frozen	Fresh
Whole carcasses	10	14
Halved carcasses	11	15
Pork pieces	12	16
Other	13	17

.18. If other, specify_____

1.8.5. At what temperature range are these pork products **stored** before being displayed?

Less than 2°C	1
3 - 5°C	2
Greater than 5°C	3

1.8.6 At what temperature range are these pork products kept during **display** to customers?

Less than 2°C	1
3 - 8°C	2
8 – 13°C	3
Greater than 13°C	4

1.8.7 Please fill the relevant parts of the Table [below], indicating how long takes from the delivery of pork to your premise, to the display of pork meat for pork chops and shoulder roasts.

	Time in Days	Pork Chops	Shoulder Roasts
Fresh	average		
	Minimum		
	maximum		
Chilled	average		
	Minimum		
	maximum		
Frozen	average		
	Minimum		
	maximum		

Specify number of hours in a day please_____

B. Shaved Ham/Thinly Sliced Ham

All questions in this section refer to shaved ham only.

1.8.8 Please fill the relevant parts of the Table [below], indicating how long takes from the delivery of ham to your premise, to the display of shaved ham.

	Time in Hours/Days
Average	1
Minimum	2
Maximum	3

Specify number of hours in a day please_____

1.8.9 Are separate utensils used in the delicatessen for each meat product?

Yes	1
No	2

If you wish to comment on any of the topics raised, please write in the space below.

_____

_____

_____

_____

_____

2 Risk of cross-contamination of products from different species

2.1 When do you and your staff wash your hands?

Between handling raw meat of different species?	
After handling ready to eat products and before handling raw meat?	
After handling ready to eat products and before handling raw meat?	
Other times (please specify)	

1.8.12 What raw meats are sliced or cut in your establishment?

Chicken	1
Other poultry	2
Beef	3
Veal	4
Lamb/mutton	5
Other	6

.7. If other, specify_____

1.8.13 What cooked meats are sliced in your establishment?

Chicken	1
Other poultry	2
Beef	3
Veal	4
Lamb/mutton	5
Other	6

.7. If other, specify_____

2.1 Do you handle raw meat of different species at the same time?

☐ Yes

☐ No

If yes, please specify:

	Chicken	Beef	Veal	Lamb/Mutton	Pork	Pork Shoulder Roast	Pork Chops	Other Meat
Chicken	X							
Beef		X						
Veal			X					
Lamb/Mutton				X				
Pork					X			
Pork Shoulder Roast						X		
Pork Chops							X	
Other Meat								X

Comments

1. Do you use the same equipment for preparing raw meat from different species?

☐ Yes

☐ No

If yes, please specify:

	Chicken	Beef	Veal	Lamb/Mutton	Pork	Pork Shoulder Roast	Pork Chops	Other Meat
Chicken	X							
Beef		X						
Veal			X					
Lamb/Mutton				X				
Pork					X			
Pork Shoulder Roast						X		
Pork Chops							X	
Other Meat								X

2. 2. Do you use the same equipment for preparing raw meat and ready to eat products such as ham?

☐ Yes

☐ No

If yes, please specify:

Products/raw meats	Ham	Other "Ready to eat products"
Chicken		
Beef		
Veal		
Lamb/Mutton		
Pork		
Other Meat		

If yes to any of these questions, how often do you clean this equipment?

Equipment/ Cleaning Regime	1X daily	2X daily	3X daily	After every use	Between raw meats from different species	After ready to eat products and before raw meat	After raw meat and before ready to eat products	Other
Work Bench								
Cutting board(s)								
Chopping block(s)								
Knives								
Mincer								
Bandsaw								
Bandsaw esp. for pork products								
Mechanical slicer								
Mechanical slicer especially for pork products								
Other equipment (please specify)								

3. What do you do to clean equipment at these times:

Equipment/ Cleaning Process	Water only	Water & Sanitiser	Water, Detergent & Sanitiser	Visible meat removal only	Other (please specify
Work Bench					
Cutting board(s)					
Chopping block(s)					
Knives					
Mincer					
Bandsaw					
Bandsaw esp. for pork products					
Mechanical slicer					
Mechanical					

Figure A.1:

Mechanical slicer especially for pork products					
Other equipment (please specify)					

2.7 Do you store **raw** unwrapped meat of different species in close proximity so the surfaces contact each other?

☐ Yes

☐ No

If yes, specify meat combinations:

	Chicken	Beef	Veal	Lamb/Mutton	Pork	Pork Shoulder Roast	Pork Chops	Other Meat
Chicken	X							
Beef		X						
Veal			X					
Lamb/Mutton				X				
Pork					X			
Pork Shoulder Roast						X		
Pork Chops							X	
Other Meat								X

2.7.2 What **cooked** meats from other animals may touch **pork** (destined for chops and shoulder roasts), during storage?

Chicken	1
Beef	2
Veal	3
Lamb/Mutton	4
Other	5
None	6

.7 If other, specify _____

2.7.3 What raw and cooked meats from other animals may touch **ham** during storage?

Figure A.2:

2.7.4

	Raw	Cooked
Chicken	1	7
Beef	2	8
Veal	3	9
Lamb/Mutton	4	10
Other	5	11
None	6	12

.13 If other, specify (raw) _____

.14 (cooked) _____

Comments:

2.8 Is unwrapped raw meat (e.g. carcasses) from different species transported to you in the same vehicle?

☐ Yes

☐ No

Comments:

2.9.1 If yes, which unwrapped raw products from which species are transported with each other?

	Chicken	Beef	Veal	Lamb/Mutton	Pork	Other Meat
Chicken	X					
Beef		X				
Veal			X			
Lamb/Mutton				X		
Pork					X	
Other Meat						X

If you chose other meat, please specify type:

2.9 Is there contact between unwrapped raw meats of different species on any of these transports?

Yes

☐ No

2.9.1 If yes, which raw products from which species contact each other?

	Chicken	Beef	Veal	Lamb/Mutton	Pork	Other Meat
Chicken	X					
Beef		X				
Veal			X			
Lamb/Mutton				X		
Pork					X	
Other Meat						X

3. Manufacturing of own products

3.1 Do you manufacture your own meat products?

Product	Y/N
Ham	
Corned Beef	
Uncooked fermented products ie salami	
Other (please state)	

3.2 Do you use the same equipment to pickle different products?

Equipment	Y/N
Brine Injector	
Brine tanks	
Other equipment (please state)	

3.3. If you prepare fermented products such as Salami do you:

Use a commercially prepared pre-mix	
Use a commercially prepared pre-mix provided with clear manufacturers instructions for use	
Use ingredients specifically sourced for the product	
Use ingredients derived from other aspects of your business (ie 'offcuts')	
Introduce material used in previous batches of the product	

3.3.1 Do you monitor the development of the product? Please specify what you check for and how you know when the product is ready for sale:

THANK YOU!

End of questionnaire