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***Salmonella* Brandenburg in sheep meat
in New Zealand**

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2002

***Salmonella* Brandenburg in sheep meat in New Zealand –
Preliminary studies to support a risk assessment approach**

**A Thesis presented in partial fulfillment of the requirements for the degree
of Masters of Veterinary Sciences in Veterinary Public Health**

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Abstract

Abortion and death of ewes caused by a particular strain of *Salmonella* Brandenburg is an animal disease problem that is unique to the South Island of New Zealand. Like most *Salmonella* serovars, this organism is zoonotic and has caused cases in occupationally exposed people. As *Salmonella* are primarily recognised as agents of foodborne disease, the potential for foodborne transmission must be acknowledged, although human cases attributed to consumption of sheep meat have not yet been reported. *Salmonella* Brandenburg has an additional concern for New Zealand's sheep meat industry owing to the possibility that contamination of sheep meat products could compromise market access. In 1995, the Sanitary Phytosanitary Agreement of the World Trade Organisation specified that scientific risk analysis was required before countries could refuse to import animal or plant materials on the basis of risks to animal, plant, or human health. This thesis presents initial microbiological studies of the prevalence and concentration of *Salmonella* Brandenburg on sheep meat carcasses that were conducted in conjunction with other projects designed to address the *Salmonella* Brandenburg issue using a modern risk assessment approach.

The microbiological studies (Chapters 3 and 4) are preceded by two introductory discussions that provide the context for the project. Chapter 1 presents an overview of national and international regulatory approaches to food safety, foodborne diseases and protection of consumer health relevant to meat and meat products. A selective review of literature on *Salmonella* focuses on *Salmonella* in sheep and on aspects most relevant to food safety. Chapter 2 summarises information on published quantitative microbiological risk assessments (QRA) conducted using the guidelines developed by the Codex Alimentarius Commission to apply QRA to microbiological foodborne hazards. A conceptual framework is presented for developing a QRA for *Salmonella* Brandenburg in sheep meat that covers all sectors of the food supply chain from animal production to the point of consumption. Following the precedent of previous QRA efforts, the food supply chain is divided into a series of five modules: animal production; transport and lairage; slaughter and processing; retail and distribution; and consumer. For each module, key outputs (prevalence and concentration of *Salmonella* in animals or product at various points in the supply chain), and their likely determinants, are identified. The specific objective of the microbiological studies conducted was to estimate the prevalence and

concentration of *Salmonella* on sheep carcasses from animals originating from farms that had experienced *Salmonella* Brandenburg disease and other farms from the same region that had no history of this disease.

Prior to undertaking the field studies, it was necessary to conduct some methodological studies to evaluate the effect of sample handling procedures on the results obtained with quantitative bacteriology. Chapter 3 presents three controlled laboratory experiments with swab samples taken from meat contaminated experimentally with the epidemic strain of *Salmonella* Brandenburg. The Most Probably Number (MPN) method was used to quantify counts of *Salmonella* Brandenburg per 100cm² area of meat swabbed. In each experiment, control samples were processed immediately, and treatment samples were subjected to different periods and conditions of storage. Treatments were chosen to emulate anticipated conditions that would be required for the field studies due to logistic constraints. The three storage protocols evaluated were:

Experiment 1: Storage of swabs diluted in buffered peptone water (BPW) for 48h at 4⁰C

Experiment 2: Storage of swabs diluted in BPW for 5 days at 4⁰C

Experiment 3: Storage of swabs for 24h at 4⁰C before dilution in BPW, followed by storage for a further 48h at 4⁰C.

Differences in counts between control and treatment samples were not tested statistically, owing to the small samples sizes, but were numerically less than one log difference in all experiments. In 2 of the 3 experiments, counts for stored samples were in fact numerically greater than for samples processed immediately. These results suggested that carcass swabs contaminated with *Salmonella* could be stored under the specified conditions without affecting the results of quantitative bacteriology using the MPN method.

Chapter 4 presents a study undertaken to obtain initial qualitative and quantitative estimates of the presence of *Salmonella* organisms on sheep carcasses sampled at 3 points in the processing chain (i.e. slaughter floor, cooler, and boning room). Slaughtered sheep (ewes and lambs) were sourced from six farms in the Central Otago/Southland region of the South Island where *Salmonella* Brandenburg disease is endemic. Three farms (case farms) were selected based on the occurrence of an outbreak of *Salmonella* Brandenburg

disease during the spring of 2000. Three non-case farms from the same region were also sampled. As the disease epidemics are temporally clustered in July and August, well before lambs are sent for slaughter, sampling was replicated after an interval of approximately 2 months to assess likely temporal variation in risk of carcass contamination. For comparative purposes, samples from sheep carcasses were also collected from 6 groups of sheep slaughtered at 2 plants in the North Island where salmonellosis due to *Salmonella* Brandenburg infection in sheep has not been reported. A total of 1417 carcasses were sampled in the study and initially tested by BAX[®] test. Of these, 1214 samples were sourced from the 3 case and 3 non-case farms supplying the South Island plant. The remaining 203 carcasses were sampled at the 2 North Island plants. A total of 138 (11.3%) of the 1214 samples collected in the South Island plant tested positive for the presence of *Salmonella* Brandenburg. No positive findings were obtained from the samples collected in the North Island plants. The vast majority (130 or 94%) of the 138 positive samples was obtained in the first period of sampling, indicating a substantial decline in risk of carcass contamination in the period between the first and second sampling. These findings indicated that the prevalence of carcass contamination with *Salmonella* Brandenburg was markedly elevated in the region where sheep flocks experienced abortion outbreaks caused by the organism. Although clinical *Salmonella* Brandenburg enteric disease has not been reported in lambs, the first sampling revealed that overall prevalence of contamination was higher (33%) for lamb carcasses than ewe carcasses (10%) from the same farms. While the prevalence of lamb carcass contamination was comparable for both case and non-case farms, the prevalence of ewe carcass contamination was strongly clustered and only 2 samples were positive from non-case farms. Estimates of the prevalence of contamination were influenced by the location of sampling carcasses (e.g. slaughter floor, cooler), but estimates of bacterial numbers on positive carcasses were generally similar regardless of class of stock, time of sampling, or sampling location in the plant. No positive samples were obtained from swabs of primary cuts in the boning room. Collectively these findings suggest that the emergence of *Salmonella* Brandenburg infection of sheep in the South Island may have considerable implications for product safety and public health. A strong case can be made for more research to better characterise the potential risks and to explore potential risk mitigation strategies. While the data obtained in this study have provided valuable insights into several important aspects of the issue, due to logistic and other constraints they have

considerable shortcomings with respect to the requirements of the formal QRA. These shortcomings were discussed and evaluated in terms of representativeness and suitability for quantitative risk assessment.

Chapter 5 presents an extension of the conceptual framework for a QRA outlined in Chapter 2, by integrating the data obtained from the bacteriological study, as well as data from other sources. Major data gaps are identified and suggestions are presented with respect to options for ongoing research to advance understanding and management of *Salmonella* Brandenburg in New Zealand sheep meat. More extensive and representative surveys are required to obtain more reliable data on farm, and within-farm, prevalence of infection as well as more extensive and representative longitudinal studies of the prevalence and concentration of the organism during slaughter and processing. It is considered that more systematic surveys at the time of apparent highest risk would be a more reliable means of assessing potential exposure of consumers than predictive microbiology.

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“Never give up, ... there is always hope”

This thesis would have not been possible without the co-operation and enthusiasm from the entire *Salmonella* Brandenburg quantitative risk assessment (QRA) project comprised of professional and dedicated people determined to influence and progress the future. I feel privileged to have had the opportunity to work with this team with the vision and courage to explore the unexplored. I would like to thank the industry (Meat New Zealand) and government (New Zealand Ministry of Agriculture and Food Safety Authority) for providing funding in relation to this thesis.

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List of Abbreviations

ALOP	Appropriate Level Of Protection
BSE	bovine spongiform encephalopathy
CAC	Codex Alimentarius Commission
CCP	Critical Control Point
CCFH	Codex Committee on Food Hygiene
ESR	The New Zealand Institute of Environmental Sciences & Research Limited
EU	European Union
FAO	Food and Agriculture Organisation
FSO	Food Safety Objective
GATT	General Agreement on Tariffs and Trade
GHP	Good Hygiene Practices
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis and Critical Control Point
MPN	Most Probable Number method
NMD	New Zealand National Microbiological Database
NZFSA	New Zealand Food Safety Authority
OIE	Office International des Epizooties (World Organisation for Animal Health)
S. Brandenburg	<i>Salmonella enterica</i> subsp. <i>enterica</i> (Brandenburg)
S. Brandenburg - QRA project	Multisectorial quantitative risk assessment project administered by NZFSA and funded primarily by Meat New Zealand over a 3 year period. Sectors include NZFSA, primary producers, the meat processing industry, field veterinarians, Ministry of Health, local health authorities, science providers (Massey University, AgResearch, ESR, LABNET), animal remedy industry
SPS	Sanitary and Phytosanitary Agreement
QRA	Quantitative Risk Assessment
VCJD	variant Creutzfeldt-Jacob disease
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
USDA-FSIS	United States Department of Agriculture Food Safety and Inspection Services
WHO	World Health Organisation
WTO	World Trade Organisation

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Chapter 1: A Review of the Food Safety Environment

1.1 Introduction

Humans live in constant interaction with the environment through breathing, drinking and eating. Such an intimate interaction always carries a risk of exposure to harmful pathogens or substances that can affect their health (Roberts *et al.*, 1995a). As a part of the evolutionary process, the long-term trend is interaction between microorganisms and the host (Lederberg, 1998), often resulting in mutually beneficial outcomes.

Animals and animal products comprise an integral part of the socio-economic development and well being of human society. Animals, as a source of food, live in close association with environmental sources of microorganisms, which naturally establish themselves on the hide, hair, hooves, skin, feather, feet, and gastrointestinal tract. Although most of them maybe benign to their animal host and produce no clinical signs of infection or disease, some may have pathogenic effects in another susceptible host, including humans (Buchanan and Halbrook, 1995). The food safety environment related to the consumption of protein derived from animals is complex in nature. Despite various control measures along the entire production chain it is almost impossible to absolutely exclude hazardous pathogens that may pose risks to human health. Historically meat, poultry, and eggs are considered as a major source of high quality animal protein. Potentially they may harbour, or become environmentally contaminated with certain pathogenic microorganisms during pre-harvest production or processing throughout the food chain (Forsythe, 1996).

Food is a fundamental requirement for survival. Today's menu of food available for consumption is extensive. In many countries in the world, meat and meat products are high on the list of the most commonly consumed foods. As our ancestors had, modern food producers also consider fresh meat as a highly fragile food product, which unless correctly processed, packaged, stored, and distributed, spoils quickly and becomes hazardous, primarily due to microbial growth.

With increased size and complexity, food production systems have become more vulnerable to a number of potential risk factors. All raw meat can have some level of

microbial contamination present and cannot be expected to be sterile. However, the presence of pathogens in the food supply even in low numbers is undesirable, and within the meat industry, the assurance of meat safety and quality are of paramount importance.

Meat and meat products continue to contribute greatly to New Zealand's economy (approximately NZ \$5.2 bn in export earnings/year). As such, New Zealand is committed to maintain its presence in international markets with products that comply with international requirements and meets high consumer standards. Under the international agreements governed by the World Trade Organisation (WTO), New Zealand is obliged to ensure that existing production systems meet those standards, and have sanitary measures based on sound science and risk assessment techniques.

This thesis addresses a specific microbiological hazard¹ in relation to sheep meat food safety and the development of a pathogen/pathway model to assist application of quantitative risk assessment of *Salmonella* Brandenburg in sheep meat in New Zealand. The thesis does not consider any other hazards (e.g. chemical hazards and toxins) in meat/meat products. These hazards may be mentioned where relevant.

It was considered appropriate to address multiple aspects relevant to trade in meat and meat products as a foundation for logical flow in addressing the specific research of interest. The 1st Chapter considers regulatory and other issues (e.g. food safety, foodborne diseases and protection of consumer health) relevant to meat and meat products, to provide a general basis for further considerations of the management of the specific risks associated with *S. Brandenburg* in sheep meat.

¹ Hazard refers to a biological agent (i.e. microorganism and/or its toxins) that has the potential to cause an adverse health effect (Lammerding and Fazil, 2000)

1.2 Foodborne diseases and pathogens

1.2.1 Sources

Traditionally, foodborne diseases have been associated with bacteria, and to a lesser extent viruses, fungi and protozoa. Although worldwide data on foodborne diseases occurrence are incomplete, some common trends have started to emerge. While some foodborne pathogens (e.g. *Campylobacter*) may have the potential to exceed *Salmonella* in frequency (Buzby, 1995), available information indicates that *Salmonella* is probably still the most important agent causing acute foodborne disease. *S. Enteritidis* and *S. Typhimurium* are the most commonly implicated serovars, while foods of animal origin, particularly meat and eggs seem to be the most common source (Todd, 1997). While there is a huge number of different microorganisms, until recently it was believed that only a few of them (approximately 20) were agents of foodborne disease in humans (Table 1.1 - adopted from Roberts, 1990):

Table 1.1. Principal food sources of the common foodborne pathogens²

<i>Agent</i>	<i>Food Source</i>
<i>Salmonella</i>	Raw meat and poultry, eggs
<i>Clostridium perfringens</i>	Meats, poultry, dried foods, herbs, spices, vegetable
<i>Staphylococcus aureus</i>	Cool foods (much handled during preparation), dairy products, especially if prepared from raw milk
<i>Bacillus cereus</i> and other <i>Bacillus</i> spp.	Cereals, dried foods, dry products, meat and meat products, herbs, spices, vegetables
<i>Escherichia coli</i>	Many raw foods
<i>Vibrio parahaemolyticus</i>	Raw and cooked fish, shellfish, and other seafood
<i>Yersinia enterocolitica</i>	Raw meat and poultry, meat products, milk and milk products, vegetables
<i>Campylobacter jejuni</i>	Raw poultry, meat, raw or inadequately heat-treated milk, untreated water
<i>Listeria monocytogenes</i>	Meat, poultry, dairy products, vegetables, shellfish
Viruses ³	Raw shellfish, cold foods prepared by infected food handlers

* Adopted from Roberts, 1990

During the last decade, improved surveillance of foodborne diseases and new diagnostic techniques resulted in better understanding of foodborne pathogens. It is now considered that more than 200 known diseases are transmitted through food, and that more than half

²(M. Sabirovic - replacement words "foodborne pathogens")

³ For example, small rounded structured viruses, parvovirus, hepatitis virus

of all recognised cases of foodborne illness have unknown causes (Mead *et al.*, 1999; Institute of Food Technologists, 2002). It has been realised that some traditional processes are no longer effective in killing some pathogens (e.g. *Salmonella* in 60-day aged cheese, *E. coli* O157:H7)(Institute of Food Technologists, 2002). Improved reporting systems indicate significant increases in the incidence of *Salmonella*, *Campylobacter jejuni*, enterohaemorrhagic *E. coli*, and the spread of antibiotic resistant *Salmonella* Typhimurium DT104 throughout many countries (World Health Organisation, 1999). While some pathogens may cause a great number of illnesses, the case fatality rate may be small and vice versa. On the other hand, the issue is further complicated in cases where a foodborne pathogen (e.g. *Listeria monocytogenes*, *Toxoplasma gondii*) may not be harmful to healthy individuals, but may cause severe illness and death in immunocompromised individuals. The emergence of pathogens is a concept that is not well defined or understood by general public. While true emergence could be linked to evolution, the concept of “emergence” may also be linked to better diagnostic techniques leading to public perception of a sudden increase in occurrence of a well-known foodborne pathogen (Institute of Food Technologists, 2002).

1.2.2 The role of environment

The wide distribution of foodborne pathogens in animals and food makes control of foodborne diseases very difficult (Johnston, 1990). Foodborne diseases are complex in nature and often characterised by close interaction between the agent, host and environment (Thrusfield, 1995). The main routes by which foodborne pathogens may reach food may vary from environmental contamination of raw foodstuffs and ingredients to food handling (Roberts, 1990). For example, potential sources of foodborne disease may be indirectly attributed to practices such as using human sewage sludge as fertiliser. In some cases the source may occur independently of the commercial circuit (e.g. home kill, hunted wild animals), while others may be independent of meat and meat products (e.g. vegetables or fruit) through contaminated irrigation water or biological fertiliser (European Commission, 2000). Besides contaminated foods, live animals on farms, zoos and animal exhibits might be the source of direct zoonotic infection for humans (World Health Organisation, 2001). The emergence of new pathogens and modes of transmission

require better reporting and tracking to obtain a better insight of foodborne diseases, their incidence, severity and economic burden (Buzby, 1995). Therefore, meat and meat products may not always be the source of foodborne infections and disease, as traditionally believed. The issue of potential cross-contamination also requires further investigation to provide a more balanced perspective on other potential primary sources of foodborne diseases, and enable better consumer education. Thus, any potential consumer confusion that may arise because of misunderstanding of perceived food safety issues may be addressed more appropriately.

1.2.3 Public health

Food safety requires the work of industry, government, international partners, producers and consumers. Consumers must also take an active role in preventing foodborne diseases (Liang *et al.*, 2001). In the past it was considered that foodborne diseases mainly occurred because of poor sanitation, hygiene conditions at slaughter, and inadequate refrigeration and canning practices. While food preparation, storage and distribution conditions have improved, new food safety concerns have arisen (Buzby, 1995). During the early 1960s, public concern focused on the use of antibiotics and their residues in meat creating the demand for increased testing for chemicals, residues and toxins in meat.

1.2.3.1 Surveillance

Surveillance for foodborne diseases, usually viewed as a subset of public health surveillance, is one way to identify foodborne disease trends and emergence. In the US, surveillance systems are passive⁴, active⁵; national or regional in scope; pathogen-specific⁶; focused on molecular subtyping schemes (PulseNet)⁷; or based on a sentinel system of individual sites (FoodNet).⁸ Traditionally, surveillance was aimed to: (1) identify control and prevent outbreaks, (2) determine the causes, and (3) to monitor trends in occurrence of foodborne diseases. It could also be helpful in defining prevention strategies, and supplying information on the effectiveness of control strategies, a rapid

⁴ Passive surveillance – Rely on the ability to recognise foodborne diseases or pathogens and willingness to report the diagnosis. Reports voluntarily submitted to appropriate health authorities,

⁵ Active surveillance – Limited in scope, actively looking for a specific or specified pathogens,

⁶ e.g. PHLIS (USA) – CDC's Public Health laboratory Information System for salmonellosis,

⁷ e.g. PulseNet (USA) – National Molecular Sybtying Network – takes advantage of molecular biology advances and information technology,

outbreak response, or as a basis for qualitative and quantitative risk assessments (Institute of Food Technologists, 2002). The need for better data has prompted recent establishment of various improved surveillance programmes for foodborne diseases in many other countries, regions or internationally (Institute of Environmental Science & Research Limited, 2000; Eurosurveillance Weekly, 2000a; Eurosurveillance Weekly, 2000b; World Health Organisation, 2001). Such programmes may provide for more systematic, integrated and co-ordinated data collection, and the on-going recording of data during larger outbreaks. One report (Roberts *et al.*, 1995b) identified that the major problems related to the availability and quality of data on the incidence of foodborne diseases, in particular, uncertainty about their magnitude and distribution, and lack of data linking foodborne disease to specific foods. Although new methods of communication (internet, e-mail groups) make it possible to quickly share data, the report emphasised the need for an integrated approach to the data collection and analysis, and consensus about how these priorities will be set.

As a part of the epidemiological investigation detailed information on the entire food production chain needs to be collected. While it is important to deal immediately with an outbreak, better understanding of foodborne pathogen transmission would significantly help in the assessment of risks and designing appropriate risk management measures to prevent similar events in the future (Tauxe *et al.*, 1997). Meanwhile foodborne diseases still remain one of the most widespread problems in the contemporary world. While improvements are made, the picture generated by surveillance programmes may not be complete. The programmes often do not capture information on small incidents or individual cases where affected consumers may not seek medical help. These occurrences are difficult to estimate.

⁸ e.g. FoodNet (USA) – Uses active surveillance (public health authorities, clinicians, laboratories),

1.2.3.2 Economic impact

In 1996, the medical costs and value of lives lost from five types of foodborne infections in England and Wales was estimated at GBP 300-700 million annually. In developing countries (excluding China) the morbidity and mortality associated with diarrhoea was

estimated to be in order of 2700 million cases each year, resulting in 2.4 million deaths below the age of five (World Health Organisation, 1999). In New Zealand, the estimated costs of foodborne disease per human case was NZ\$ 200 (Scott *et al.*, 2000), while in Sweden the average cost per illness was SEK 2,164 (Lindquist *et al.*, 2001). These figures also illustrate the potential magnitude of the negative impact of foodborne illnesses on health and development. Mead *et al.*, (1999) consider that approximately 76 million foodborne diseases occur in the USA each year, resulting in 325,000 hospitalisations and 5,000 deaths. It is estimated that around 80% of foodborne illnesses were due to unidentified pathogens. Of the cases where a pathogen was identified (38.6 million foodborne diseases) 5.2 million (13%) were due to bacteria, 2.5 million (7%) due to parasites, and 30.9 million (80%) due to viruses (Mead *et al.*, 1999). A comprehensive estimate of the economic costs to individuals (direct and indirect, e.g. lost work and lost household tasks); employers; and food sellers who may experience decreased sales and reputation is lacking. Another component that may need to be factored in is estimating the industry and the public's willingness to pay for activities aimed at reducing a particular hazard (Kinsey, 1995).

1.2.3.3 Traceability

With global distribution of food, consumers and regulators are demanding stricter safety standards to guarantee safe food delivery. In today's terms, traceability may be defined as the existence of systems that maintain credible identification of animals or animal products through various steps from "farm to retail". Identification may originate at any level and at any step of the process in the food chain and should enable both traceback and traceforward. As food production and marketing have been removed from direct consumer control (McKean, 2001), the importance of traceability of animals and animal products has grown significantly.

Many traceability programmes are initiated at national level (e.g. *Salmonella* programmes in Denmark and Sweden, UK national pork production system, UK response to bovine spongiform encephalopathy (BSE), national brucellosis, tuberculosis, trichinellosis programmes in many countries) or a regional level (e.g. response to bovine spongiform encephalopathy)(McKean, 2001). Apart from increasing standards, retailers have also found that commercial advantage can be gained from certain aspects of source

verification. This led to producer groups developing a multiplicity of assurance schemes⁹ (Pettitt, 2001) for their own purposes to facilitate dealing with production problems or to increase trade opportunities at the national and international level. Whilst various countries have traceability systems in place, there are no internationally accepted standardised systems (Vallat, 2001) relevant to international trade in both live animals and animal products.

1.2.3.4 Changing consumer habits

Modern life in developed countries is now characterised by rapidly changing eating habits, novel foods, cooking processes (e.g. microwave, irradiation), “fast” foods, health awareness, diets, dining out, and buying food in bulk (Waites and Arbuthnott, 1990). It may be speculated that consumer demand has led to globalisation and centralisation of the food supply and thus has resulted in the dispersal and concentration of pathogens. Potentially huge numbers of consumers may be exposed to contaminated foodstuffs in a short period of time.

Additionally, the factors most frequently associated with foodborne infections include improper hygiene or handling practices of food handlers and consumers, increased international travel, and increased reliance on imported produce and other food (Doyle *et al.*, 2000). Common sense and knowledge indicate that use of appropriate hygiene, food handling and proper cooking practices may effectively prevent the vast majority of microbial foodborne diseases. The most common observed unhygienic practices (Jay *et al.*, 1999) were infrequent hand washing; inadequate cleaning of kitchen surfaces; presence of pets in the kitchen; touching the face, mouth, nose, and/or hair during food

⁹ e.g. National farmers' Union British Standards scheme, farm quality assurance schemes in many other countries (Pettitt, 2001)

preparation; and lack of separate hand and dish towels. Consumers play a significant role in preventing foodborne diseases by avoiding consumption of undercooked and uncooked high-risk goods, refrigerating perishable foods and disposing of hazardous foods that have been recalled (Liang *et al.*, 2001).

1.2.3.5 Reactions to foodborne diseases

An individual decides what, when and how much to eat. Consumers like to be informed about the risks they are taking when selecting food, and all food suppliers, governments

and educators should provide that information (van Schothorst, 1997). Reactions to significant foodborne disease outbreaks, either at the national or international level, are often reflected through increased consumer, legal, and political demands on standards in the trade (Hathaway, 1997). Recent events have led to the implementation of various new regulatory models, focused on science-based standards, and a demand on industry to take a more proactive approach and responsibility for food safety.

The media often quickly picks up “sensational” stories that may cover perceived food safety issues and emergence of new diseases, globalisation and lack of confidence in food production and processing industries, including government and regulators. The most common consumer and regulatory responses associated with foodborne disease outbreaks may be summarised as follows (Figure 1.1)¹⁰.

¹⁰ Sabirovic M. (August 2002)

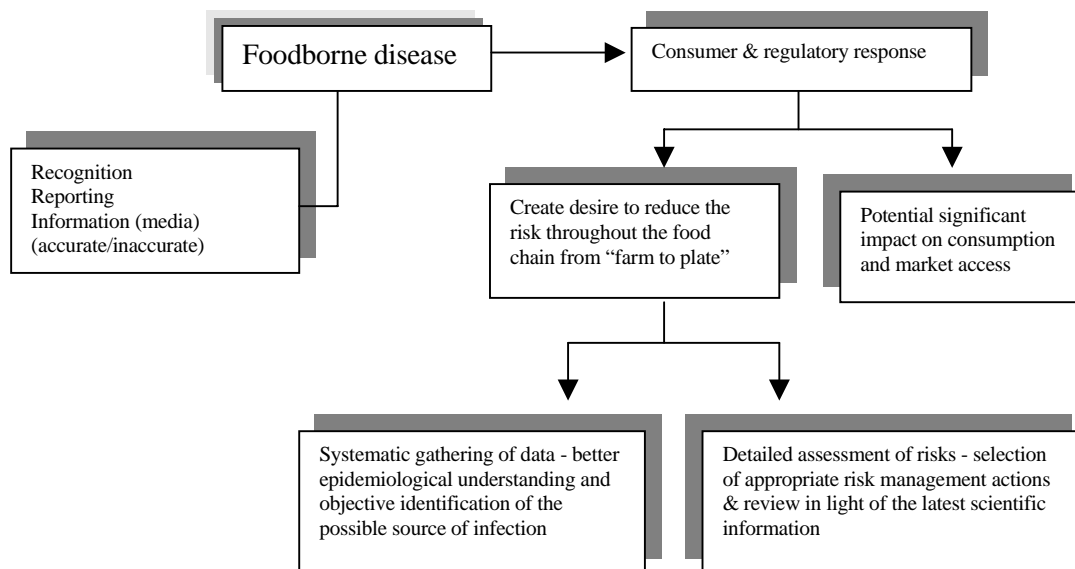


Figure 1.1. Potential reactions to foodborne disease

Thus, it is considered that there is a clear need for co-operation between food safety professionals and experts in the physio-sociological sector to bring professional insight into the parameters that influence the transmission and assimilation of relevant information. People seemingly accord greater weight to risks imposed by others compared to those they face as a result of personal life choice. The latter are not so readily appreciated but are often more serious risks (Mossel *et al.*, 1998). People are more likely to accept risks if they know the risks and their order of magnitude and have a sense that they may be able to control them. While often people take an additional risk for a particular food they like, in some instances governments have made a decision to restrict the sale of certain products to protect consumers regardless of consumer wants. In many instances, industry may take such action for commercial reasons (e.g. brand image, loss of consumers, liability)(van Schothorst, 1997).

1.3 Contemporary meat hygiene

At the end of the 19th century, the “germ theory” of disease caused control measures (e.g. meat inspection) to be dictated by a paradigm of disease causation. Meat inspection, quickly adopted by many countries worldwide, originated at a time of poor animal husbandry, prevalent zoonotic diseases (e.g. tuberculosis, brucellosis) and stock presented for slaughter were often old. Meat inspection was primarily focused on detecting and removing diseased animals and any abnormalities from a carcass (Bell, 1993). Regulators

were concerned about all factors affecting hygiene and safety of meat at all stages of production, processing and distribution. This included, animal health and freedom of specified diseases (ante-mortem inspection), safe removal of all contaminated and diseased carcasses or parts thereof (post-mortem inspection), hygiene conditions of all in-plant procedures and proper identification of carcasses and products (Collins, 1995). Over the past three decades several food safety scares resulted in the rapid decline of public confidence in the role of producers, processors and government in the food supply chain (Pettitt, 2001). Some of the episodes raised the issue of the effectiveness of existing meat inspection practices in detecting the presence of micro-organisms (e.g. *Salmonella*, *Campylobacter*, *Listeria*) on contaminated carcasses (European Commission, 2000). They also highlighted inability of the meat inspection to detect microbial pathogens that do not cause any visible changes to the health of the animal, or the carcass.

Most notably, an outbreak of *E. coli* O157:H7 in the USA in 1982 (Riley *et al.*, 1983) resulted with severe disease (e.g. haemorrhagic colitis, haemolytic uraemic syndrome, and thrombocytopenic purpura) in a number of people (mainly elderly and children). This outbreak was linked to either the consumption of ground beef sandwiches in restaurants or a fast food chain of restaurants that served undercooked hamburgers. Another outbreak of *E. coli* O157:H7 in 1992 resulted in several hundreds of sick people and four child fatalities and prompted North American consumers to began questioning the safety of the food supply (Anonymous, 1994). As a result, public concern and media attention started to shift from residues, particularly pesticides, to microbial contaminants as a greater public health risk (Hueston and Fedorka-Cray, 1995). Huleback and Schlosser (2002) noted that more efficient ways of meat inspection and the establishment of criteria for finished products were recommended to the responsible government agency in 1976. However, the *E. coli* event in 1990s highlighted the need for a change in traditional meat inspection, and accelerated the introduction of significant changes to the entire USA meat processing industries (Anonymous, 1994).

During the past two decades many important changes have occurred in relation to food control and the development of food standards. Controlling authorities were presented with a number of challenges such as the application of updated scientific methods and risk

assessment, the need for structured decision making processes and the application of these to meat hygiene in order to prevent conflict between regulatory and commercial interests. Hathaway and McKenzie, (1989) considered that the future food safety and quality systems should be designed on the basis of formal scientifically validated quantitative assessments of actual public health hazards as a prerequisite for any sound modernisation of the existing meat inspection procedures. HACCP, recommended by the WHO since 1985 was considered as a recognised method of risk management for meat production and processing (Edwards *et al.*, 1997).

1.3.1 Process control

Food safety concerns relate to three categories of hazard: physical, chemical and biological. Some physical hazards (e.g. any extraneous objects such as metal, glass, etc) may cause illness or injury to a person consuming the product. Meat inspection procedures are generally effective in detecting and removing physical hazards. However, sampling and testing programmes are required to monitor for the presence of chemical (e.g. dioxin) and microbial hazards (e.g. microbial agents). Some microbial agents (e.g. bacteria, fungi) may have the ability to multiply on, or in meat. Each group of these hazards, if consumed, may have a major significance for public health. For example, the detection of various bacteria (e.g. *E. coli*, *Salmonella*) on the carcass may suggest faecal contamination of meat, primarily during processing. Water, used during processing may be contaminated either with such bacteria or by human viruses of public health concern (e.g. caliciviruses, rotavirus) – hence the requirement to use clean water.

Measures are required to be taken at all points in the farm to plate continuum to include production, transport, slaughter, processing, storage, retail and food preparation (Hogue, *et al.*, 1998) to ensure the microbiological safety of foods. Systematic gathering of reliable testing data related to the occurrence, elimination, prevention and reduction of foodborne pathogens (Kvenberg and Schwalm, 2000) is seen as an essential element for controlling the microbial hazards of concern (Swanson and Anderson, 2000). However, improving the microbiological quality of foods alone is insufficient since food-processing technologies cannot provide absolute assurance of the absence of pathogens. Given that food can be recontaminated, producers are required to adhere strictly to good hygiene measures by following GHP, GMP, and implementation of HACCP along the whole food

chain (Panisello *et al.*, 2000). The main driving force of the HACCP system is continuous evaluation of the hazards (Berends and van Knappen, 1999) where microbiological testing plays an important role in the verification of the effectiveness of the plan.

The emergence of *E.coli* as a human pathogen of public health concern resulted in the introduction of mandatory microbiological monitoring of meat in the USA. However, it has to be emphasised that, as a part of process control, the meat has been monitored microbiologically in most countries for many years but these programmes were rarely standardised and of no interest to regulators. Containment of microbiological risks is attainable and this goal possibly cannot be achieved by end product testing which is a proven effective strategy when directed towards chemical food safety. Many countries are now developing, or have developed, a range of national standardised programmes to monitor the microbiological status of meat.

Microbiological tests form an integral part of the programme by providing valuable information on critical control points, and trigger actions in the case of non-compliance (Lupien and Kenny, 1998). In the USA, the recently introduced Meat and Poultry Inspection regulations (1996) provide a framework for change (Billy and Wachsmuth, 1997) by improving the safety of meat and poultry products (Schlosser *et al.*, 2000), and establishing pathogen reduction performance standards for *Salmonella* (Sofos *et al.*, 1999). In addition to the large and medium size establishments (plants), the regulations also apply to very small plants (Mossel *et al.*, 1998). The regulations require countries exporting to the USA, including New Zealand, to adopt the same initiative and embark on the development of microbiological standards within regulatory requirements, and microbiological guidelines to be used by manufacturers or regulators to monitor food manufacturing processes (Harris *et al.*, 1995).

In 1995, the EU Council Decisions 95/409 and 95/411 were designed to regulate the sampling regime and testing for *Salmonella*. These requirements now apply to all Member States, including exporting countries. The EU Directives 64/433 and 71/118 have also been amended to regulate the requirements for testing of meat for certification purposes (Akewrberg and Brannstorm, 1997). Since 1997, New Zealand has conducted microbiological monitoring of red meat. The programme, currently known as the

National Microbiological Database (NMD) covers all red meat primary processors. It includes testing in approved laboratories with the aim to provide scientifically valid data and enable the definition of cost-effective regulatory microbiological criteria that are qualitatively and quantitatively linked to stated public health goals. Freshly slaughtered carcasses, chilled carcasses, primal cuts (outside-hind legs) and cartons of bulk meat are now tested according to standardised protocols for generic *E. coli*, aerobic plate count and *Salmonella* where accumulation of data allows (Hathaway *et al.*, 1999) for:

- development of national performance targets,
- on-going monitoring of national performance and individual premises, and
- provision of scientific data to support design of HACCP plans.

In this respect, USDA-FSIS have recognised New Zealand's ability to compare performance of individual establishment (premise) by comparing their data with national norms, when discussing the food safety objectives of the United States Pathogen Reduction/HACCP Rule. In light of this, the NMD programme is deemed by the USA as the equivalent to the *E. coli* testing requirements of the US Rule¹¹. According to the draft policy on the detection of *Salmonella* in Meat (January 2000), New Zealand “*does not accept that testing for Salmonella has any direct value as a control indicator for red meat process in New Zealand for the following reasons: (1) prevalence on carcasses is very low; (2) rare isolations are more likely to reflect farm/transport health status, rather than poor process control; (3) the isolation is more likely to be a chance statistical effect than genuine indicator effect, and (4) the lag time for laboratory analysis means that actions are taken well after the initiating event and their value is really only as a tool for identifying trends, unless there is a specific process failure*”. New Zealand advocated that *E. coli* was a much better process control indicator.

¹¹ Dr Roger L. Cook - Memorandum: New Zealand Meat Hygiene Assurance Programme (17 November 2001), Ministry of Agriculture and Forestry, Food Assurance Authority, PO Box 2526, Wellington

1.3.2 Hazard Analysis and Critical Control Point (HACCP)

Although the food industry bears responsibility for providing safe food for consumption, a framework of laws, regulations and inspection system controls food production. Modern legislative and regulatory requirements are increasingly focusing on performance based standards, while the methods of achieving specified outcomes is left to producers (Liang *et al.*, 2001).

1.3.2.1 Origins

The Pillsbury Company developed HACCP in 1959 for the USA space programme. The primary objective was to provide astronauts with food free of any harmful substances (e.g. pathogens, toxins, chemicals, physical hazards) that may potentially have had catastrophic consequences for the mission. During the development, questions were raised related to the existing methods of quality control in processing industries to identify foodborne hazards (Bauman, 1995). The HACCP system is a form of process control quality assurance, originally targeted at processed products and limited almost exclusively to the manufacturing environment. The basic principle of the system is to identify potential hazards and faulty practices at an early stage of production. These can then controlled in order to prevent them from constituting risks to consumers or an economic burden on the operator from spoilage or recall of marketed items. This is perceived as the key advantage over other reactive approaches such as inspection and end product testing (“test and hold”) which does not prevent the occurrence of the hazards in the first place (Ehiri *et al.*, 1995). End product testing was seen as inefficient because pathogens occur in small numbers and are not evenly distributed within food. The test and hold procedure system is found to be expensive and resources would be better used if focused on the concept of “prevention” rather than trying to “inspect out” the problem (Harris *et al.*, 1995). As a preventative system of food control HACCP allows for identification of the process flow and points that may contribute to a hazard. These are know as Critical Control Points (CCPs), and are defined as “*any point in the chain of food production, from raw materials to finished product, where loss of control could result in unacceptable food safety*”

risk”(Bauman, 1995). While a competent authority is required to define a critical limit (a criterion) that must be met for each CCP, it is the industry responsibility to develop its own HACCP plan, and ensure that each critical limit controls effectively the identified hazard. In most instances critical limits are industry specific (Manis, 1995).

The first comprehensive HACCP document was published in the USA in 1973 and was used for training (Bauman, 1995). In 1973 the HACCP system was successfully introduced to thermally processed ready-to-eat foods such as low-acid canned products (Buchanan and Whiting, 1998; Huleback and Schlosser, 2002). While many industries were interested in establishing their HACCP plans, broad application of HACCP to the entire USA food industry was not considered until 1985 when an authoritative scientific body concluded that end product testing was not adequate in preventing foodborne diseases. In 1987, the USA Congress required that a programme for fish and seafood certification and inspection that was consistent with the HACCP system be designed (Bauman, 1995). Meat and poultry inspection had changed little in decades and various organisations continue to pressure government to move towards a science-based risk inspection system for meat and poultry. In partial response, the government responded with the development of a new slaughter inspection model that has been tested with volunteer plants as a part of the HACCP-based inspection models project (Cates *et al.*, 2001). In 1992, the HACCP was endorsed by scientific bodies in the USA as an effective and rational means of assuring food safety throughout the food chain (Huleback and Schlosser, 2002).

To protect consumers from foodborne diseases and promote public confidence, the EU (Directive 93/43) and the UK introduced legislation in 1993 that require all food businesses to establish a food control system based on HACCP principles (Powel and Attwell, 1998). The same year, the USA Administration mandated safe handling labels for raw meat and poultry products, and declared the presence of *E. coli* O157:H7 on raw ground beef as intolerable. A testing program for *E. coli* was initiated in 1994. The Administration also encouraged the development and use of new technologies in food processing. The Food Safety and Inspection Service (FSIS) of the USDA was asked to design a completely new food safety regulatory system where HACCP supplements, but

not replaces traditional meat inspection with the aim of targeting and reducing harmful bacteria in meat and poultry, and modernising the 90-year-old USDA inspection program (Anonymous, 1998a). The passage of the Pathogen Reduction/Hazard Analysis and Critical Control Point System (HACCP) Rule 1996 (popularly termed “The MegaReg”) introduced radical changes to the regulation and inspection of meat hygiene (Mossel *et al.*, 1998; Hulebak and Schlosser, 2002). The Rule (Anonymous, 1999a):

- a) Requires all meat and poultry plants to develop and implement a system of preventive controls, known as HACCP, to improve the safety of their products,
- b) Sets pathogen reduction performance standards for *Salmonella* that slaughterplants and plants producing ground products must meet,
- c) Requires all meat and poultry plants to develop and implement written standard operating procedures for sanitation,
- d) Requires meat and poultry slaughterplants to conduct microbial testing for generic *E. coli* to verify the adequacy of their process controls for the prevention of faecal contamination.

1.3.2.2 Issues for consideration

Implementation of HACCP as a risk management tool for food safety has helped standardisation of all significant elements related to production and processing practices. It has also highlighted a number of other areas that need to be regulated, which potentially adds pressure to finite regulatory resources. This includes significant requirements for verification and compliance, plus requirements for laboratory approval, accreditation and testing. At the same time, there is also a requirement to develop appropriate education material to enable the diverse production and processing systems to be aligned with HACCP principles, while taking into consideration all the differences that exist between the various industries.

Over time, it became apparent that HACCP is based upon information that is limited, often conflicting, and rapidly outdated. HACCP requires a definitive, reliable source of underpinning information on causal agents, ingredients, and contributing factors that is amenable to constant review and updating (Powel and Attwell, 1998). Experience of HACCP implementation has revealed that many regulatory regimes still contain mixed elements of GMP and HACCP (Hathaway S.C – personal communication, 2002). It has

been quoted that small and medium sized plants may encounter more difficulties in complying with the new requirements than large-scale plants because of the limitations of personnel, financial resources, and structural resources (Upmann *et al.*, 2000).

Implementation of HACCP in developing countries may raise some questions related to their desire to export their food products to developed countries and their overall ability to demonstrate they meet HACCP requirements. This may limit access of products to premium markets, primarily because of biosecurity or consumer concerns, and perceived lack of quality control systems. On the other hand, expansion in worldwide travel provides opportunities for people to visit developing countries and enjoy the diversity, culture and locally produced food. Given this, it seems that two completely different consumer perceptions of food safety risks may exist – the concept of food safety risk acceptable at home, and the concept of food safety risk acceptable when travelling or visiting other countries. However, the risks of unrestricted travel of people abroad who may act as a potential source of infection to their surrounding environment is yet to be determined. The HACCP revolution has also highlighted that as a process control it would require more testing data to be collected at the critical control points to enable an informed decision on whether the process delivers desired food safety outcomes and delivers food acceptable for human consumption. This would probably add significant costs to the industry.

1.3.3 Risk based approach

1.3.3.1 Concept of risk analysis

The Codex concept of risk analysis is primarily focused on food safety and public health (Hathaway, 1999b). It utilises three separate and independent processes: Risk Assessment, Risk Management, and Risk Communication, none of which can function well in the absence of others (Jouve, 2000).

Microbiological risk assessment (MRA) is the primary part of risk analysis. It is used to identify critical gaps in knowledge, characterise the most important risk factors in the food chain, and help identify strategies for risk reduction while providing guidance for

determining research priorities in public health and food safety areas (Lammerding, 1997). With developments in the risk analysis area, the need for development of guidelines for risk communication by agencies such as Codex was emphasised to ensure potential consumer concerns are appropriately addressed when potential hazards in food are detected (Hathaway, 1997).

While risk assessment has historically been applied to physical and chemical hazards, microbiological risk assessment related to foodborne pathogens that may survive on animal products is in its early development stage. Refinements to the microbiological risk assessment model suggested inclusion of the “farm-to-plate” assessment or production/pathogen pathway analysis to enable consideration of a broad range of management options along the food chain (Lammerding and Fazil, 2000). Further conceptual developments indicated the need for quantitative, instead of qualitative risk assessment methods. The reasons for supporting a quantitative approach were (Voysey and Brown, 2000):

- microbial risks are often the result of a single exposure,
- there is variability in population response to infectious pathogens,
- the level of pathogen in food is process-dependant and may either increase or decrease,
- micro-organisms are dynamic and adaptable to change.

As a consequence of the introduction of quantitative assessment, in 1998 Codex published draft general principles of the microbiological risk assessment for food borne hazards. In 1999, Codex outlined the framework for risk assessment for food trade purposes that comprised four steps: Hazard Identification; Hazard Characterisation, Exposure Assessment; and Risk Characterisation (CAC, 1999), and adopted the following approach (Lammerding and Fazil, 2000):

Step 1: Description of food safety problem and context,

Step 2: Hazard Identification: What agents are present in food and capable of causing adverse health effects?

Step 3: Hazard characterisation – what is the nature of adverse effects?

- Dose – response assessment¹²

Step 4: Exposure assessment – What is the likely frequency and level of consumption?

Step 5: Risk characterisation – Integration of Exposure Assessment and Hazard Characterisation,

Step 6: Risk estimate:

- Probability and severity of illness attributable to the food/pathogen source (e.g. no illness per year, or per 100K population),
- Uncertainty – What important data or knowledge is missing?
- Variability: What variable factors influence the magnitude of risk?

Risk assessment outcomes may be used at different levels to help decision-making (e.g. individual, production and processing, national and international)(van Schothorst, 1997). The Codex framework requires a clear statement of the purpose, the nature of the inputs required and descriptions of the assumptions and constraints (Stringer, 2000) to enable industry to incorporate risk assessment with HACCP (Voysey and Brown, 2000). To ensure consumer protection and facilitate trade, the Codex Committee on Food Hygiene (CCFH) is moving towards a broad risk-management based approach. This approach requires microbiological risk assessment and utilises a spectrum of risk management tools (e.g. microbiological risk management guidance documents, codes of hygienic practice, food safety objectives and microbiological criteria (CAC, 2002a).

Quantitative microbiological risk assessment (MRA), a mathematical component of the Risk Analysis, outlines a scientific process that attempts to determine the relationship between exposure to a hazard under specified set of conditions, and the likelihood of disease occurrence in humans (Jouve, 2000). The complete terminology for the quantitative risk assessment has yet to be finalised (Lindquist and Westoo, 2000). Several scientific papers have been published on quantitative risk assessments (Schlundt, 2000).

¹² As the definition of dose response has not been stated in the original text (Lammerding and Fazil, 2000), it was considered that it would be prudent to complement the text by adding Buchanan's (Buchanan, 1997) definition of dose-response: "Estimate of the quantitative relationship between the quantity of the biological agent consumed and the frequency and magnitude of adverse health effects in population".

1.3.3.2 Risk management framework

Hathaway (1997) considered that the food safety environment was in a unique period of re-evaluation and change, both world-wide and in New Zealand. The need for change is primarily due to (1) new trade agreements and new legislative conditions facilitating product liability claims; (2) the increasing need for food hygiene and inspection systems; (3) the inclusion of risk assessment principles in national legislation; (4) more rigorous scientific assessments of traditional national regulatory principles as applied to food hygiene; (5) the increasing need for cost-effective food hygiene and inspection systems; (6) greater public concern over real or perceived food borne hazards to health, and public intolerance of the concept of “risk”.

International trends in food safety are to embrace the food safety risk management framework as a basis for compliance with international standards and trade agreements between trading partners. The framework, an integrated and co-ordinated approach to food safety, potentially includes all affected parties working towards the common goals – health protection of consumers and promotion of fair trade practices. It also provides for monitoring and verification in order to ascertain the effectiveness of selected measures, thus providing a sound basis for potential challenges by trading partners, and trade negotiations. In line with modern international approaches, the New Zealand food safety administration is moving towards a legislative environment based on food safety risk management. In such a risk management environment, selection and implementation of appropriate food safety controls is based on the outcomes of risk assessment, evaluation of other factors relevant to health protection of consumers and the promotion of fair trade practices. In support of the principles of food safety risk management, there is a clear need for a risk-based food control system that involves all stakeholders at all stages of the process that operates “from farm to plate” (Anonymous, 2000a). For example, national legislation in many EU member states relates to the control of notifiable diseases of animals. The EU is focusing on zoonoses and moving towards introducing measures from the consumer point of view. The measures are aimed at ensuring favourable animal

health to combat infections in humans (e.g. *Salmonella*). They would enable elimination of specified pathogens of concern from herds, investigation of all sources with a potential for further specified pathogen introductions, improved biosecurity measures to prevent re-introduction, and developing diagnostic capabilities to identify pathogen carriers before slaughter (Grossklaus, 2001). Within the risk management framework there are several activities, summarised¹³ in Figure 1.2, that demonstrate the close link between animal health, public health and the environment, and the potential application of risk assessment to identify and quantify potential hazards¹⁴.

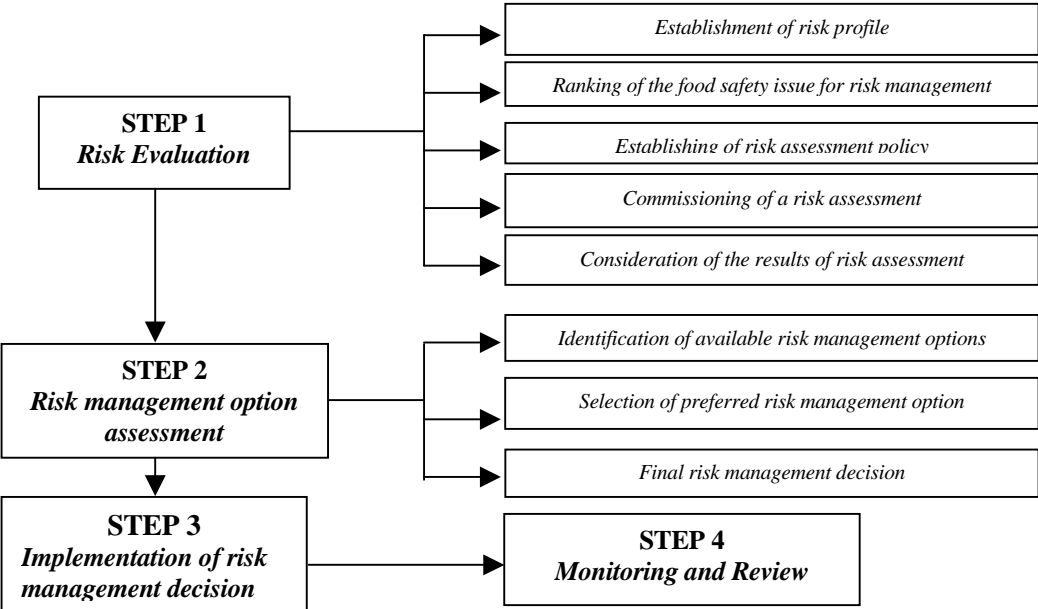


Figure 1.2. Risk management framework steps and activities

Under the SPS Agreement food can freely move across borders as long as it does not pose a public health risk to importing countries. In such cases the entry of food may be blocked. The decision to block the entry of such food should be justified on scientific grounds and use of Codex standards, codes and guidelines where available (van Schothorst, 1997).

¹³ Sabirovic M. (2002)
¹⁴ Sabirovic M. (2002)

1.3.3.3 World Trade Organisation

The modern era witnesses considerable integration and consolidation of the agriculture and food industries. These events have led to more sophisticated wholesale buying techniques including direct sourcing from other countries, involving processors and large retail chains (Garrett *et al.*, 1997). Global trade results in significant amounts of food from a single source being distributed to many and distant countries. Globalisation represents a challenge to food safety authorities as potentially contaminated food in one country may result in an outbreak of foodborne disease elsewhere (World Health Organisation, 1999).

In parallel with the adoption of HACCP principles by a number of governments, the establishment of the World Trade Organisation (WTO) in 1995 made a significant impact on modern food control approaches. In 1974, members of the General Agreement on Tariffs and Trade (GATT) identified the need to define clear rules to deal with sanitary and phytosanitary measures to ensure that there were no unjustified restrictions on trade. The European Commission proposal in 1988 suggested that restrictions be applied to protect public, animal and plant health, while allowing trade based on assessment of risk rather than the theoretical possibility of transmission. The WTO/Sanitary and Phytosanitary Agreement (SPS) enabled harmonisation of sanitary regulations on the basis of standards developed by the:

- Codex Alimentarius Commission (Codex) - related to food,
- Office International des Epizooties (OIE), related to animal health,
- Food and Agricultural Organisation International Plant Protection Convention (IPPC) - related to plant health.

This approach effectively established the link between the SPS Agreement and the three relevant standard setting organisations, particularly in the area of guidelines development and methodologies for risk assessment (World Trade Organisation, 2000). Recent shift in public opinion initiated changes in public food safety policy and highlighted that the responsibility for food safety does not rest with primary or secondary producers only. (Hueston and Fedorka-Cray, 1995). It has also become apparent that different regulatory agencies may differ in approach and in regulatory views on food safety. There is now a growing need to harmonise the regulatory environment related to food safety and bring those agencies together.

1.3.3.4. Codex and HACCP

In 1997, the Codex endorsed HACCP guidelines as the international benchmark relevant to production of food (Anonymous, 1997b). HACCP is based on the following seven principles (NACMCF, 1997; Anonymous, 1997b; Billy and Wachsmuth, 1997) which are summarised (Panisello *et al.*, 2000):

- a) *The first principle* of HACCP refers to the development of a list of hazards, that are likely to cause injury or illness if not controlled,
- b) *The second principle* refers to the identification of Critical Control Points (CCPs). This process involves identification of the steps in the process that should be controlled to prevent or eliminate a food safety hazard, or to reduce it to an acceptable level. A CCP should only include those operations where control can provide a quantifiable reduction in a hazard or its stabilisation and leads to an acceptable, safe food product. That means that CCPs are meaningful only if they can be managed in such a way that the risk is reduced and the reduction can be quantified. Should that not be the case, such control points may not be qualified as critical and are best to be carried out within the framework of GMP,
- c) *The third principle* relates to setting up critical limits in order to relate them to the microbial performance criterion, or reducing the numbers to an acceptable level,
- d) *The fourth principle* relates to the establishment of monitoring procedures that will assess whether an identified CCP is under control,

- e) *The fifth principle* relates to the development and implementation of corrective actions to prevent deviations from established critical limits,
- f) The last two principles – *verification (principle 6)* and *documentation (principle 7)* have to be in place to ensure the effectiveness of a HACCP plan and provide for maintenance of records for future use in verification.

The HACCP principles provide the basis for a well-documented and more narrowly focused programme. It is scientifically justifiable and pragmatic in approach, helps establishment of food safety objectives as a target for achievement of quality goals, and can be validated as achieving food safety objectives (Hathaway, 1999b). Nevertheless, Codex guidelines have several limitations in terms of contemporary food safety. These relate to the non-existent linkage between risk assessment and design of HACCP plans, the absence of specific recognition of HACCP as a risk management tool and specific guidance for validating HACCP plans (Hathaway S.C. – personal communication, 2002).

Over recent years, accelerated by its inclusion in national food safety regulations and guidelines for international trade, the HACCP scope broadened to encompass the entire “farm to table” continuum. It is suggested that HACCP plans with the development of risk-assessment models offer improved consideration of the continuum in a quantitative manner (Buchanan and Whiting, 1998) that would provide for sufficient relationship between food manufacturing operations and public health goals. To implement HACCP through the entire “farm to table” continuum, a monitoring system that records all important hazard throughout the chain, including monitoring of cases of diseases in humans caused by the hazard of concern is required. That would also require legislation that provides for criteria about acceptable and unacceptable health risks for consumers (e.g. appropriate level of protection - ALOP). It determines at what moment which risks should be controlled by the producers. At the same time, the legislation should be flexible to accommodate new or emerging hazards (Berends and van Knappen, 1999). The Annex A of the SPS Agreement recognised that different measures may achieve an

appropriate level of protection (ALOP)¹⁵ as deemed by the importing countries and allow for the establishment of bilateral and multilateral agreements on recognition of equivalence of specified measures. Annex A also equates ALOP with the term of “*the acceptable level*”. The concept of ALOP has been firmly accepted in the context of international trade. However, the notion of ALOP is increasingly the subject of debate because the SPS Agreement does not indicate how to determine the acceptable level of risk (Zepeda *et al.*, 2001) or how ALOP should be expressed (Anonymous 1998b). The determination of ALOP is left to individual countries, which in practical terms has already caused significant trade disputes under the WTO (e.g. Australia vs. Canada regarding import of salmon, EU vs. USA with regard to bovine somatotropin).

The concept of ALOP and the concept of acceptable risk differ to a degree and are subject to open interpretation. A measure developed by one country in a transparent manner and considered appropriate may be perceived by another country as either being restrictive to trade, or poses an unacceptable level of risk. While it would seem appropriate that the level of protection can be considered simply as a difference between the assessed risk and the acceptable risk, it is yet to be seen how the appropriate level of protection is going to be defined officially (Pharo H.J. – personal communication, 2002). Meanwhile, in order to address the issue of determining the ALOP, a microbiological risk assessment was considered as the first step that would have to be completed in estimating the impact of a hazard on public health. The next step would be to develop acceptable sanitary measures based on the assessment of risks, followed by the establishment of a food safety objective (FSO) (Gants, 2000).

The Codex Committee on Food Hygiene defines FSO as “*the maximum frequency and/or concentration of hazard in food at the time of consumption that provides appropriate level of health protection*” (Joint FAO/WHO Food Standards Programme, 1999). FSOs reflect the expected or desired extent of control of foodborne hazards that result from application of a specific sanitary measure(s). When justified by the risk assessment, the FSOs provide a “target” for the control of hazards in food that would enable industry to

¹⁵ Appropriate level of protection is defined as: “The level of protection deemed by the member establishing a sanitary or phytosanitary measure to protect human, animal, or plant life or health within its territory”

objectively evaluate the level of hazard control required to achieve the desired level of consumer protection (Hathaway 1999a). The FSO concept is now embedded in the current international regulatory environment (harmonisation of food standards, mutual recognition for levels of consumer protection, and objective evaluation of equivalence) to facilitate the free trade of food (Gants, 2000).

The New Zealand food industry has accepted food safety objectives as an essential assurance that their HACCP plans are outcome focused, achieving expected food safety goals and have inherent flexibility (Lee and Hathaway, 1999). However, the current definition of a FSO raises a few questions. One of them is to what extent primary producers, primary processors, and in some instances secondary processors may be responsible for the level of hazard that may be present in food at the time of consumption. In some instances chemical (e.g. residues), physical hazards (e.g. metal, glass) or biological hazards (e.g. parasites) control may be gained by better farm management practices. With regard to some other biological hazards (e.g. bacteria that can multiply in a product or at refrigeration temperature) it may be improper to attribute the full responsibility to primary producers. Such products are often subject to further handling and preparation, or repackaging at the retail or home level.

1.3.3.5 Codex and meat hygiene

The new *“contemporary approach to meat hygiene requires that hygiene measures should be applied at those points in the food chain where they will be of greatest value in reducing foodborne risks to consumers”*. *“Greater emphasis”* is placed on prevention and control of unseen microbiological contamination during processing, and a reduced reliance on organoleptic post-mortem meat inspection (Hathaway, 2001).

Traditional meat inspection involves examination of the carcass and organs. It includes a requirement to examine certain lymph nodes by incision, and any other additional inspection as required by regulations. These procedures often work reasonably well with septicaemic, generalised or acute conditions and detecting such animals is not perceived as a major problem. However, detection of individual lesion or abnormalities is often less certain (Edwards *et al.*, 1997). Modern views consider existing meat inspection practices

as resource intensive (Hathaway and McKenzie, 1989) while their cost-effectiveness (Hathaway *et al.*, 1987), effectiveness in detecting “aesthetic defects”(Hathaway and Pullen, 1990), or gross abnormalities of public health significance was also questioned (Pointon *et al.*, 2000). Several comparative studies have been carried out to determine the effectiveness of organoleptic and visual¹⁶ meat inspection techniques (Mousing *et al.*, 1997a; Pointon *et al.*, 2000; Hathaway and Pullen, 1990; Kobe *et al.*, 2000). Organoleptic assessment of various conditions (e.g. abscesses, bruising, pleurisy and peritonitis, physical defects, other abnormalities) that would be extremely distasteful to consumers may be of some value (Edwards *et al.*, 1997; Collins, 1995). Implementing visual meat inspection may have the potential for decreased cross-contamination and reduced inspection costs while resources may be reallocated to hygiene and surveillance programmes (Mousing *et al.*, 1997a). However, an alternative to the traditional system would have to be determined by a formal risk assessment (Hathaway and Pullen, 1990).

The USA is moving towards change from an inspection system that requires extensive carcass palpation to a system with no palpation of lamb carcasses. Palpation is considered to be of little value to preventing hazards that may result in meat borne diseases while adding to the spread of pathogens or cross-contamination of carcass (Walker *et al.*, 2000). In assessing the merits and limitations of the meat inspection and meat control procedures, it has been acknowledged that other regulatory measures provide essential support to the industry and protection of consumers. These measures include health certification of the national herd, approval of premises for slaughter for trade, product protection (e.g. through in plant design and operation, control and disposal of contaminated material), controls during all stages of processing, transport hygiene and secure product identification, and health certification of product. As such they illustrate the extent to which the basic principles of HACCP may have been incorporated (Collins, 1995) into the national legislation of many trading nations for a long time. In such a scenario, processing companies remain fully responsible for the safety of their produce and liable in case of any damage to consumer’s health (Berends and van Knappen, 1999) while the regulators specify certain safety levels and verify compliance.

¹⁶ no handling, palpation, incisions

The Codex Committee on Meat and Poultry Hygiene new proposed draft “Code of Hygienic Practice for Fresh Meat” has been on the agenda for discussion at the Codex Committee on Meat and Poultry Hygiene meeting at the beginning of 2002. If adopted, the new Code will replace the existing recommended codes for fresh meat, game, ante-mortem and post mortem inspection of animals, and post-mortem judgement of slaughtered animals and meat. In addition, it includes new material on poultry, farmed game birds and wild game birds. It is comprehensive in nature and includes ante- and post-mortem activities as an integral part of processing operations, thereby facilitating the application of a “farm-to-plate” risk based approach to meat hygiene.

Given that meat hygiene is complex by nature, the proposed draft code reflects the modern scientific approach to risk based design and implementation. Verification is focused on outcome-based performance parameters. There is increasing attention to health monitoring and surveillance, and alternative regulatory models for delivery of post-mortem inspection in an effort to reduce the incidence of food borne illness. Figure 1.3 represents a summary¹⁷ of how the proposed draft general principles of meat hygiene envisage the roles of a competent authority and production plant.

¹⁷ Sabirovic M. (2002)

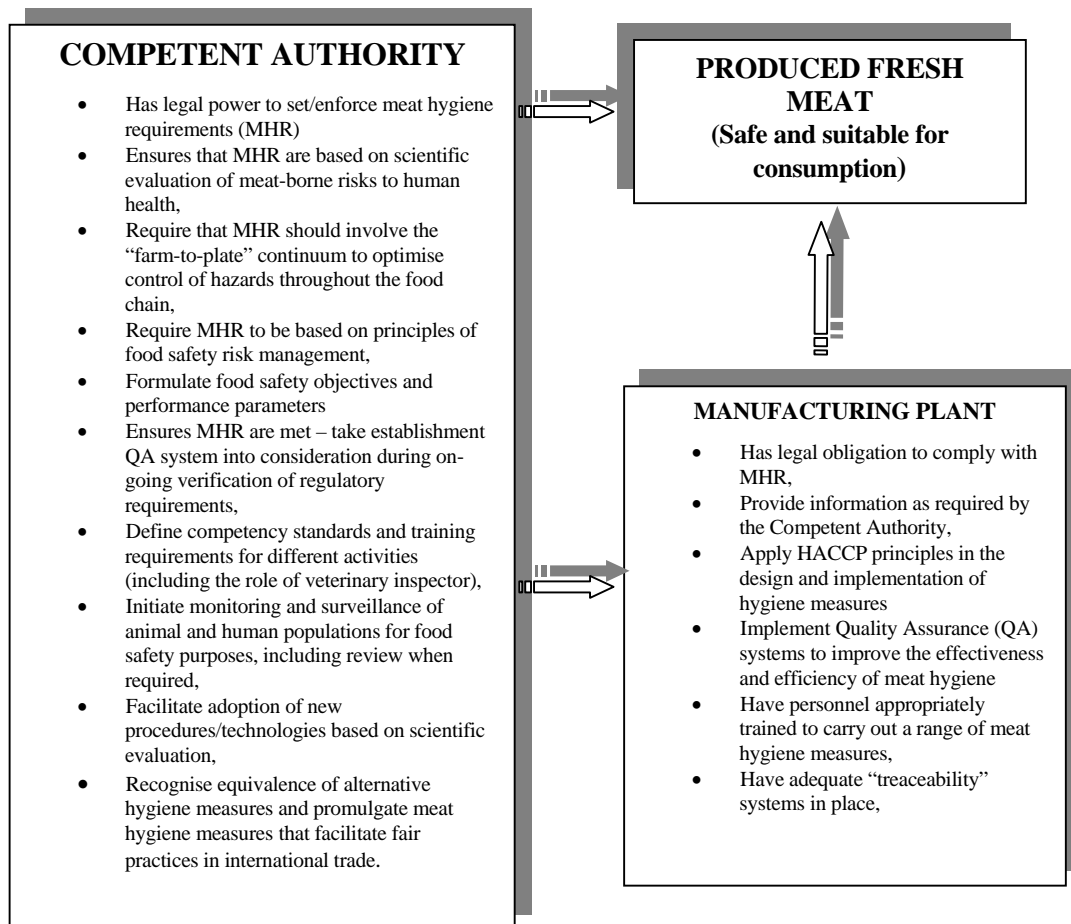


Figure 1.3. The new proposed draft Code – Production of fresh meat

1.4 *Salmonella* – a food safety issue

Salmonella spp. is the one of the most common pathogens causing foodborne illness. It is a small, rod-shaped, straight sided, Gram-negative bacteria, according to Baird-Parker (1990). *Salmonella* spp. are a large group of bacteria that occur worldwide and are ubiquitous among domestic and wild warm-blooded animals. Approximately 2,400 serovars have been identified, yet a relatively small number predominate in animal and human populations at any one time (Preface, 2000). Predominant serovars vary both geographically and temporally.

1.4.1 General considerations

The genus *Salmonella* spp. is the member of the family *Enterobacteriaceae*. For epidemiological purposes, the genus is sub-typed into two species (Wray, 1997): *Salmonella* Bongori (18 serovars) and *Salmonella* Enterica (2300 serovars)¹⁸. Almost all serovars have the potential to cause illness in humans. *Salmonella* are considered to be normal gastrointestinal flora of reptiles, and can occur in invertebrates such as snails. The major reservoir is the gastrointestinal tract of vertebrates, including domestic poultry and farm animals. Infection results primarily from ingestion (although aerosol infection may be also be important), and most infected hosts remain asymptomatic (Preface, 2000) while some may develop clinical signs of varying severity (European Commission, 2000).

The organisms are shed in high numbers in the faeces of infected individuals. Domestic animals are most likely infected following exposure to faeces, contaminated feed, chronic carriers introduced into the population, rodents, or sometimes by contact with infected workers (Giovannacci *et al.*, 2001). *Salmonella* may be found in virtually any phase of the natural and man-made environment. It has often been isolated often from animal feed, rodents, insects, birds, wild animal reservoirs and domestic animal reservoirs. It is considered that *Salmonella* will continue to be a feature of humans, animals and the general environment and that effort should be directed to controlling its introduction and spread into the agricultural and food chains (Murray, 2000). One study found that *Salmonella* may survive in the calf rearing units between four months to two years clearly indicating that many cleaning and disinfection procedures were inadequate (McLaren and Wray, 1991). Based on the degree of host adaptation, *Salmonella* serotypes may generally be categorised as being (European Commission, 2000):

- a) Highly adapted to an animal host (e.g. *S. Abortusovis* in sheep, *S. Dublin* in cattle, *S. Pullorum*, *S. Gallinarum* in poultry, *S. Choleraesuis* in pigs),
- b) Common, non host adapted, that cause food borne illness (e.g. *S. Typhimurium*, *S. Enteritidis*),
- c) Adapted to humans and not usually pathogenic to animals, which cause severe disease with septicaemic-typhoid syndrome (e.g. *S. Typhi*, *S. Paratyphi*).

¹⁸ Further information available from AgResearch Report for Meat New Zealand: 98MZ/FS85 B4 & 00MR/MU11A (May 2002)

Effective epidemiological surveillance and control of *Salmonella* require accurate typing of strains (serotyping, phage-typing, plasmid profiling, restriction enzyme analysis of plasmid and chromosomal DNA, IS200 typing and ribotyping, pulsed-field gel electrophoresis) in order to determine potential pathways of infection (Giovannacci *et al.*, 2001). The primary point of invasion is intestinal mucosa where the organisms multiply in the gut associated lymphoid tissue (GALT). Should the macrophages in the regional lymph nodes limit the organism expansion, the infection will remain localised to the intestine and GALT and clinical disease will be limited to acute gastro-enteritis. However, if the organism is not contained by macrophages, systemic infection occurs. Distinction between *Salmonella* causing localised, or systemic infection is complicated by a number of other determinants, e.g. immune status of the host, age, stress, tissue tropism, husbandry, nutrition, etc., (Baumler *et al.*, 2000). The surface polysaccharides form the outermost components of the bacterial cell and are of great significance in the interaction of the organism and the habitat. They provide protection against the outside world enabling the organism to survive a dry external environment, acidity of stomach, the lumen of the gut, extracellular space of the host tissue and inside macrophages (Rycroft, 2000). *Salmonella* can produce several types of fimbriae, which helps the organism to attach to epithelial cells, colonise tissues, maintain persistent infections, evade the host specific immunological defences, increasing survival in the environment and resistance to changes in temperature, pH, and water availability (Thorns and Woodward, 2000). *Salmonella* can grow both in the presence of oxygen (aerobically) and in its absence (anaerobically) between 5°C and 45°C. The optimum growth occurs at 37°C. The organism is relatively resistant to various environmental factors and can survive for considerable periods in frozen product. *Salmonella* are sensitive to heat, but the degree of sensitivity can vary greatly. They are found to be extremely heat resistant in dried products and may act more like spores in their thermal tolerance. If left in food at room temperature for a time, they are more resistant than those that have been surviving in a refrigerator (Doyle and Mazzotta, 2000).

1.4.2 Diagnosis

Scientific literature contains a tremendous amount of information relating to the media and methods for isolation of *Salmonella*. Direct plating of tissues, except in the case of acute infections is usually non-productive for recovery. Samples that have been dried, heated, irradiated, or otherwise processed (e.g. possibility of injured cells) require incubation of non-selective pre-enrichment media (e.g. buffered peptone water) for 18-24 hours at 35-37°C for optimal recovery of the organism. For the selective enrichment of *Salmonella* from foodstuffs and other materials, selective-enrichment broth (e.g. Rappaport-Vassiliadis) incubated at 40-43°C is recommended because it selectively inhibits other bacteria while allowing *Salmonella* to multiply (D'aoust *et al.*, 1992a). Delayed secondary enrichment, the process where the original selective-enrichment broth is held at room temperature after the initial 24 hours of incubation, seem to enhance the chances of recovery (Waltmann, 2000). An estimation of an indirect detection by most probable number (MPN) only provides a measure of the organism that survive the selection process while it does not provide a real prediction of the actual number that may be present in the sample. There is no gold standard for detection of *Salmonella* (Alvseike and Skjerve, 2000).

In the studies presented in Chapter 3 and 4, the BAX[®] test (BAX[®] (Qualicon L.L.C, Wilmington, Delaware, USA)- Qualicon[™]BAX[®]) was used as a rapid detection test. It is a Polymerase Chain Reaction (PCR) test, an analytical tool based on rapid replication of a targeted DNA fragment. According to Bailey (1998) multiplication must typically result in a target cell concentration of 10⁶ cells ml⁻¹ to achieve a positive result. With the BAX[®] system this process is only required to reach a concentration of 10⁴. Thereafter, the target concentration is increased more rapidly by switching to an alternative target, nucleic acid, which is exponentially amplified 10⁷ fold by the above process in 2-3 hours producing a product detectable by horizontal electrophoresis.

1.4.3 Public health

It is assumed that the real number of human patients with salmonellosis is between five and 20 times higher than the number ascertained by passive surveillance systems (Eurosurveillance Weekly, 2000a). While there are some marked regional variations, the recent increase in infections in Europe has been attributed to *S. Enteritidis*, *S. Typhimurium*, *S. Hadar* and *S. Virchow* (Eurosurveillance Weekly, 2000b) with a similar situation reported in North America (Humphrey, 2000). Of the rest, the highest number of reported cases in Europe were attributed to *S. Infantis* and *S. Brandenburg* (Eurosurveillance Weekly, 2000b). In the UK, for example, *Salmonella* was confirmed in 49.5% of investigated food borne outbreaks (Powel and Attwell, 1998). In 1999, there were 43 outbreaks of salmonellosis reported in New Zealand (Institute of Environmental Science & Research Limited, 2000) which resulted in a total of 2,079 human cases being notified. This number of cases represented the highest number of notifications ever notified in a single year (i.e. 57,5 cases per 100,000 inhabitants). *S. Brandenburg* used to be an infrequent human pathogen in New Zealand, accounting for less than 1% of all salmonella infections. However, in the recent years, the incidence of reported cases due to this organism has increased (Wright *et al.*, 1998).

Most human infections with various types of *Salmonella* are cited to be from eating raw or undercooked foods, including meats, poultry, eggs, and dairy products (Humphrey, 2000, Fach *et al.*, 1999). However there has been growing recognition in some countries of risks associated with a wide range of food products. These include different types of vegetables, spices and seeds, including tahini, fresh and dry spices, banana leaves (Eurosurveillance Weekly, 2001a; Eurosurveillance Weekly, 2001b; Eurosurveillance Weekly, 2001c) and sprouts (Eurosurveillance Weekly, 2000c). *Salmonella* are sensitive to heat and most products may easily be rendered safe to eat by adequate cooking (Rust, 2000).

The infectious doses of *Salmonella* between 10^5 cells to 10^7 were required to cause infection in human volunteers. Human exposure to much lower numbers can result in disease, particularly in the young, elderly or immunocompromised. Foods with high fat content or with good buffering capacity (e.g. chocolate, cheddar cheese, hamburger, potato chips) may protect the organisms during passage through the acid regions of stomach, thus permitting a smaller dose (1-100 cells) to cause infection. *Salmonella* infection may not result only from eating contaminated food, but also represents an occupational hazard for people working with or in contact with infected animals (Humphrey, 2000).

Escalation of antimicrobial resistance as a clinical problem in human medicine during the 1990's has again focused attention on domestic animals as reservoirs of resistant organisms, and the use of antimicrobials in food animal production is under growing scrutiny. Recognition of the emergence of multiple resistant strains, such as *S. Typhimurium* DT 104, has further intensified these concerns. *S. Typhimurium* DT 104 is typically resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines (ACSSuT) (Eurosurveillance Weekly, 2001a). The appearance of *Salmonella* strains resistant to third generation cephalosporins or fluoroquinilones has further raised concerns that transmission of the resistant genes among *Salmonella* and other bacteria may occur commonly and use of antimicrobials in food animals will select for multiresistant pathogens and commensals (Velonakis *et al.*, 2001).

1.4.3.1 National regulatory actions and international impact

Regulatory initiatives to improve the safety of the food supply have frequently been stimulated by outbreaks or foodborne diseases that have initiated consumer and public reactions. The following examples illustrate reactions of some countries to what they have considered to be significant food safety issues and the impact of such reactions on their food animal industries, and international trade.

1.4.3.1.1 Swedish *Salmonella* control programme

During a severe epidemic of salmonellosis in Sweden in 1953-1954, some 9000 people were affected and 90 died. This epidemic demonstrated a need for a more comprehensive control programme (Wierup, 1993) and resulted in the introduction of an active control programme for all *Salmonella* serotypes in domestic animals in 1961 (Wierup *et al.*, 1992). Sweden's main objective for this on-going *Salmonella* programme is to reduce the number of human cases of salmonellosis (Engval *et al.*, 1993). The control programme (Wierup, 1993), supported by government regulation, is based on the following strategies:

- a) Prevent *Salmonella* contamination in all parts of the production chain,
- b) Monitor the production chain at critical points to detect if contamination occurs,
- c) Undertake all necessary actions to fulfil the objective of the control when *Salmonella* contamination is detected.

In 1970, a voluntary control programme for *Salmonella* in poultry was established. Subsequently, compulsory testing of all broiler flocks for *Salmonella* was implemented in 1984. Within the next six years, the prevalence of *Salmonella* infection in broilers sent to slaughter was reduced to less than 1% (Wierup *et al.*, 1992). Testing for *Salmonella* presence in pigs sent for sanitary slaughter¹⁹ during the 1987-1989 period indicated that 3 (0.008%) of 33,899 sows and boars, and 360 (0.7%) of 50,109 fattening pigs tested positive. During the 1992 period, 13 (0.1%) out of 19,999 cattle, and 4 (0.04%) out of 954 calves tested positive (Wierup, 1993). Since the late 1940s, Sweden has had a control and monitoring programme for animal feed. The programme was established primarily to detect anthrax in imported meat-meal, but also has been used to detect *Salmonella*. A voluntary monitoring of commercially available animal feed for the

¹⁹ Sanitary slaughter is defined as follows: "animals that are faecal culture positive may only be slaughtered at sanitary slaughter. All carcasses are either heat treated or condemned. Sanitary slaughter takes place in separate departments isolated from normal slaughter. If not, the sanitary slaughter must take place at the end of the day under special supervision of the official veterinarian. After such slaughter, the premises are thoroughly cleansed and disinfected under supervision of the official veterinarian" (source: Swedish Salmonella Control Programmes for live animals, eggs and meat – National Veterinary Institute, Swedish Board of Agriculture, National Food Administration, 1995-01-16).

presence of *Salmonella* was instituted in 1960. By 1993, more than 90% of commercially produced animal feed was monitored (Haggblom, 1993). During the early 1990s Sweden was in the process of negotiating access to the European Union (EU).

The EU, officially established on 1 November 1993, initially had twelve European countries as member states. Sweden, along with Finland and Austria joined the EU on 1 January 1995. With the EU membership, border controls of all products from other EU countries had to be terminated in Sweden. The *Salmonella* control and monitoring programme in Sweden has been recognised by the European Union (EU) as a part of Sweden's negotiations to join the EU. Given the effectiveness of programme, the positive *Salmonella* findings (6-11%) in samples collected at the border from consignments of meat imported to Sweden between 1990-1993, followed by 4.5% positive findings in 1994, Sweden along with Finland, obtained from the European Commission special *Salmonella* assurances for fresh (chilled and frozen) meat of cattle, pigs and poultry (Akewrberg and Brannstrom, 1997). Subsequent to the relevant EU directives, the Swedish *Salmonella* control and monitoring programme has evolved over the past decade and is currently comprised of the following components (Report to the Commission, National Veterinary Institute, Swedish Board of Agriculture, National Administration, Swedish Institute for Infectious Disease, 1999):

- a) Feedstuff - Surveillance/notification systems which includes testing of:
 - Imported pet food (dog chews),
 - Imported vegetable feed material,
 - Samples taken from feed mills (after heat treatment),
 - Feed for livestock (final product).

- b) Animals – Surveillance/notification systems for:
 - Poultry and eggs,
 - Cattle and pigs,
 - Sheep, goats and other food producing animals.

- c) Epidemiological history
 - Poultry,
 - Cattle and Pigs,
 - Sheep, goats and other food producing animals (e.g. horses),
 - Wildlife/Other (e.g. dogs, reptiles).
- d) Antimicrobial Sensitivity
- e) Salmonella in food – Surveillance/Notification system – testing of samples collected at:
 - Slaughterhouses,
 - Cutting plants,
 - Canteens, shops, restaurants and small to medium size establishments (carried out by municipally).
- f) Salmonella in humans – Surveillance/Notification

Sweden considers its *Salmonella* programme to be a success story and the presence of *Salmonella* is now detected in less than 1% of animal products for human consumption produced in the country. The success of the programme over the past forty years is attributed to strict requirements that apply to the whole process from the farm to sale. However, despite strict control, reported human diseases of *Salmonella* infection has risen in Sweden. Epidemiological investigation of such cases indicates that approximately 85% of reported cases were infected overseas (Gilback, 1999).

The assurances that EU provided to Sweden also prompted issuing EU Council Decisions 95/409 and 95/411, which regulate sampling regimes and testing for *Salmonella* that now apply to all member states, including countries outside the EU. Also, EU Directives 64/433 and 71/118 have been amended to regulate requirements for certification of testing (Akewrborg and Brannstrom, 1997). The EU is currently moving towards the introduction of direct responsibilities on producers for foodborne disease caused by defective products (Grossklaus, 2001). In the same vein, Sweden has already introduced a new product liability law that has the same effect on Swedish producers (Krantz, 1993). The Swedish programme is comprehensive in nature and covers all the relevant aspects from farm to human health. While the programme appears to be very successful, it should be noted that there is no reference to any type of risk analysis conducted in

Sweden to justify the approach. On the other hand, a concern is the ongoing risk of human salmonellosis due to overseas travel. It raises questions of whether the sole focus on animal health will sustain its success on a long-term basis towards reducing cases of human salmonellosis in Sweden.

1.4.3.1.2 United States of America

Following the introduction of the new regulations (“The MegaReg”), which require industry to take primary responsibility for the safety of food (Anonymous, 1997a). FSIS introduced at the beginning of 1997 a mandatory requirement for *Salmonella* testing to verify that the requirement for salmonella pathogen reduction performance standard in red meat is being met (Federal Register – USA, 1998). The standard was based on the prevalence of *Salmonella* as determined by FSIS baseline surveys (Sofos *et al.*, 1999; Food Safety and Inspection Service, 1996) throughout the meat industry. Production based data collected throughout an entire year represented 99% of production in each species category under surveillance (Anonymous, 2000b). Large plants (e.g. those federally inspected, and employing 500 or more employees) became subject to *Salmonella* testing at the beginning of 1998. Testing at smaller establishments (with 10 or more employee, but fewer than 500) was implemented at the beginning of 1999, and at very small establishments (fewer than 10 employees or annual sales of less than US\$2.5 million) at the beginning of 2000 (Anonymous, 1999b; Schlosser *et al.*, 2000). Initial testing results indicated that there was major variation among plants for the presence of *Salmonella* in various carcass sampling sites and that certain plants may have difficulties in meeting the standard (Food Safety and Inspection Service, 1996).

Events surrounding one large plant highlighted a number of issues related to the mandated *Salmonella* testing as a process control indicator. The plant failed several times to comply with the FSIS requirements for *Salmonella*, and the FSIS subsequently stated its intent to cease inspections. In practice, this would mean that the plant had to close operation. However, a US Court of Appeal effectively overturned the FSIS position by ruling that *Salmonella* can be killed by proper cooking, therefore “*the presence of Salmonella in meat products does not render them injurious to health*” (Schlosser, 2002). This case has raised several important issues related to legal FSIS ability to enforce compliance and

deal with non-compliant plants; scientific basis for regulatory standards; quality and standardisation of tests used; and issues related to the responsibility of various parties in the food production chain.

1.4.3.1.3 New Zealand – Draft policy on detection of *Salmonella* in meat

The New Zealand draft policy²⁰ on *Salmonella* has been developed in response to an apparent increase in the *Salmonella* detection rate in sheep and cattle meat, subsequent to the increased rate of testing and the increase of *S. Brandenburg* infections in stock in the South Island. The policy applies to raw meat products from any species which is processed under the Meat Act 1981 or the Animal Products Act 1999 in order to ensure that any regulatory responses to the detection of *Salmonella* in meat are science-based and consistent in the approach. In a case of positive findings, the processor is expected to initiate an investigation according to an industry-agreed standard (e.g. *Salmonella* Performance Standard as set out in the NMD – Bovine and Ovine species). Such industry responses should be fully documented and available for audit.

1.4.4 Production/processing industries

It could be speculated that altered farming practices, coupled with marked changes in food distribution and eating habits of the human population have contributed to increasing *Salmonella* infections of humans (Preface, 2000). Although it is generally accepted that clinical salmonellosis is not a common problem on farms that have good management practises, the development of novel different food products may force the industry to examine its management practices and incorporate new procedures to reduce risks of the problem (Holt, 2000). Nonetheless, agri-food industries accept the responsibility for the supply of safe and wholesome foods. An example of such an approach is the success of the Irish *S. Enteritidis* control programme in poultry flocks. The success is attributed to the high level of co-operation and understanding between poultry producers and processors, private veterinarians, and the Irish Department of Agriculture, Food and Forestry (Collins, 1995). Another example of an industry response is the Danish *S. Typhimurium* control programme in the pig industry initiated on 1995 by the Danish

²⁰ Zohrab Tony, Director Animal Products, MAF Food Assurance Authority: MAF Food Assurance Authority (Animal Products) Draft Policy Relating to the Detection of *Salmonella* in Meat, January 2000.

Ministry of Agriculture, in co-operation with pork producers and processors in 1995. Compared to the Swedish programme, the Danish programme is much narrower in scope. It focuses on reduction of *S. Typhimurium* in the pig industry by control of feedstuff, surveillance and control in breeding, multiplying, and finishing herds, and control measures in abattoirs. Another component of the programme is the adaptation of slaughter routine according to the *Salmonella* status of the herd. In another words, herds with clearly unsatisfactory status (>50% of infection) are slaughtered separately (in time), plucks and gutsets are condemned, and a number of carcasses are tested. If positive, the entire batch is subjected to heat treatment or salted (Mousing *et al.*, 1997b).

1.4.5 Sheep meat and meat products

Salmonella infections in sheep have been recorded in a number of countries of the world. The more common serovars isolated from sheep are *S. Typhimurium*, *S. Arizonae*, *S. Derby*, *S. Dublin*, *S. Montevideo*, *S. Abortusovis* (Wray and Linklater, 2000). Of these, only *S. Abortusovis* is host adapted (Linklater, 1991). *S. Typhimurium* and *S. Dublin* cause general systemic and enteric signs of infection while *S. Abortusovis* and *S. Montevideo* infections are described in many countries in the world as a serious cause of abortion in ewes (Wray and Linklater, 2000).

Salmonella attach to freshly exposed muscle tissue. The mincing of meat will introduce *Salmonella* into the interior of the product (e.g. sausages). Introduction of *Salmonella* onto the carcass is usually by fleece or faecal contamination, the extent of which depends on the degree of carriage in the live animal and the hygiene of the slaughter process. The prevalence is believed to increase as a result of mixing at markets, stress of transportation, lairage prior to slaughter, and the length of time the animals are held before slaughter (Humphrey, 2000). In experimental conditions (using guinea pigs) it is considered that the carcass has a bactericidal effect and that it is unlikely that a small heterogeneous bacterial population would survive. Although low numbers of bacteria may be undetectable by existing tests, potential bactericidal activity of carcasses needs to be taken into account (Gill and Penney, 1979).

In the early 1960s, Nottingham and Urselman (1961) highlighted the potential effect of *Salmonella* spp. infection on the meat industry in New Zealand when they reported that about 15% of cattle and sheep, 13% of calves and 4% of beef cattle sampled tested positive after slaughter. The principal source of infection appeared to be on the farm. A quote has been made (Denmead, 1970) that “*under New Zealand grassland farming conditions there is a very close relationship between the disease in cattle, sheep and pigs, and farm management practices*”. Further studies indicated that over the years the number of sub-clinical infection in calves was significantly reduced (7.3%) compared to the results in previous study (Nottingham and Urselman (1961). The proportion of positive pigs was 9.9%, with the high percentage of isolates from nasal swabs suggesting the possible importance of the upper respiratory tract in spreading of infection among animals (Nottingham *et al.*, 1972).

Data on laboratory testing of *Salmonella* presence are mainly available for raw beef, pork or poultry meat. However, the information on microbiological quality of sheep meat is very limited worldwide. That was one of the reasons why there are no criteria for sheep meat in the USA standard. In the US, one study of chilled lamb carcasses in lamb packing facilities found that 1.9% (autumn/winter collection period), and 1.2% (spring collection period) tested positive for *Salmonella* (Duffy *et al.*, 2001). Data from Australia indicate that 5.7% of sheep carcasses, and 6.5% of frozen sheep meat tested positive for *Salmonella* (Vanderlinde *et al.*, 1999; Adams *et al.*, 1997).

1.4.5.1. New Zealand National Microbiological Database

A routine microbiological monitoring of carcasses and cuts in slaughterhouses for the period 1993-1995 detected *Salmonella* in 0.65% lamb carcasses and 0.3% lamb samples collected in boning rooms. While 754 beef carcasses tested negative for *Salmonella*, 0.09% beef samples collected in boning rooms tested positive (Armitage, 1995). More recent cumulative data [National Meat Industry Microbiological (Pathogen) Profile, 2001] are presented in Table 1.2.

Table 1.2. *Salmonella*: New Zealand Meat Industry Microbiological (Pathogen) Profile, 2001(NMD)

<i>Salmonella</i>	No of samples	A single sample positive	All five samples positive
NZ Baseline survey			
Ovine – fresh carcass	500	0.40%	-
National Microbiological Database – Ovine			
Fresh carcasses	15866	0.132%	0.662%
Chilled carcasses	2012	ND	ND
Primal cuts	17980	ND	ND
Bulk meat	16504	0.024%	0.121%

NMD surveillance of *Salmonella* for the period September 1999-April 2002 showed that *S. Brandenburg* contributed approximately to 50% of all detection on ovine carcasses after slaughter (Armitage, 2002).

1.5 Objective of this thesis

The primary objectives of this thesis are to:

- a) Determine the prevalence and the numbers of *S. Brandenburg* on sheep carcasses during various stages of processing (Chapter 3 and 4), and
- b) Provide an original contribution to the development of a pathogen/pathway model (presented in Chapter 5) that could be used as a core element of a QRA.

To achieve this objective, an attempt is made in Chapter 5 to integrate relevant available literature data in New Zealand and the data generated by studies carried out under or outside the *S. Brandenburg* quantitative risk assessment (QRA) model. This approach is undertaken to help in identifying data gaps that would have to be considered to complete the QRA. It would enable the co-ordination of farm and processing module for monitoring *Salmonella* status of sheep flocks and their potential linkage with the storage/distribution modules and retail/consumer module.

The author of this thesis had considerable interaction with the *S. Brandenburg* QRA project, particularly the teams from AgResearch (Table 2.2), and the sheep meat processors (Plants A). He has contributed to the specific field studies under the auspices of New Zealand Food Safety Authority (formerly MAF Food Assurance Authority) which are described in Chapter 3 and 4.

Chapter 2: Quantitative microbiological risk assessment – practical application in New Zealand

2.1 Background

When considering food safety from “farm to plate”, it is recognised that traditional food science is forward-focused on food products and their potential to cause adverse effects on public health. Conversely, animal disease epidemiologists tend to focus retrospectively on factors that contribute to potential contamination of animals and meat.

Integration of these complementary orientations should lead to a more holistic approach to management of foodborne risks, and risk analysis is promoted as a tool for achieving this (Zwietering and van Gerwen, 2000; Lammerding and Fazil, 2000; McNab, 1998).

Risk analysis, including quantitative microbiological risk assessment (QRA), is currently preferred approach for assigning and managing risks associated with microbial food hazards. Key outcomes of QRA are:

- Assessment of the risks of exposure and illness due to the agent and food of concern (risk estimates),
- Evaluation of expected changes in risks likely to occur if certain interventions are implemented at various points in the chain of production, processing and food handling,
- Identification of priority areas for further research.

QRA is in its infancy as a discipline, and only a small number of analyses have been undertaken on high profile issues, such as *Listeria monocytogenes* in soft cheese (Bemrah *et al.*, 1998), *Escherichia coli* O157:H7 in hamburgers (Cassin *et al.*, 1998) and *Salmonella* Enteritidis in pasteurised liquid eggs (Whiting and Buchanan, 1997). Protocols for performing QRA are yet to be standardised or validated, yet these pioneering studies provide the foundation for embarking on QRA for any pathogen-food combination.

2.2 Sheep meat as a source of foodborne salmonellosis

An outbreak of salmonellosis among farm workers occurred in the South Island (i.e. Otago, Southland and Canterbury) between August and October, 1998. Seventy-five human cases coincided with an outbreak of *S. Brandenburg* infection in sheep on over 100 farms (Peacock *et al.*, 1999). Most of the human cases have been traced to contact with infected sheep (NZFSA Fact Sheet, 2002). Human foodborne salmonellosis linked to sheep meat consumption has not been perceived as an important problem. There are no published reports of programs to control *Salmonella* infection of sheep for purpose of food safety, or to conduct QRA for any microbial hazard in sheep meat. Unlike the pathogen-food combinations listed above, to which numerous foodborne illnesses or deaths have been attributed, as yet there is no concrete evidence of foodborne illness linked to *S. Brandenburg* in sheep meat and minimal data demonstrating that the organism occurred on sheep meat products. The existence of human risk is inferred by analogy with the well documented occurrence of foodborne salmonellosis (including other strains of *S. Brandenburg*) linked to many other foods, and due to the occurrence of human *S. Brandenburg* cases in sheep farmers following occupational exposure. The dose-response characteristics for *Salmonella* spp. are relatively well characterised (Lammerding *et al.*, 2000). This Chapter reviews approaches taken to apply QRA to microbial foodborne hazards in other species and outline framework for undertaking this approach for *S. Brandenburg* in New Zealand sheep meat.

2.3 Quantitative microbiological risk assessments

Ideally, a full QRA should span the entire food production and consumption continuum and include:

- Hazard identification,
- Hazard characterisation – determining the severity of foodborne disease and ideally including a dose-response relationship,
- Exposure assessment – determining patterns of exposure of consumers to the hazard in the food product,

- Risk characterisation – estimating the “risk estimate”, or burden of illness (e.g. incidence rates and severity of disease) due to the exposure (integration of hazard characterisation and exposure assessment).

As yet, no single published analysis has completely met this challenge. Individual assessments have tended to focus primarily on either exposure assessment or hazard characterisation, or have been limited to certain sectors of food production. The relative merit of focusing on specific sectors in food production (i.e. scope of assessment) depends on the purpose of the assessment, which needs to be clearly articulated before an analysis is commenced. For logistic reasons, it is desirable to split the overall “farm to plate” continuum into several distinct modules, each of which will represent a particular stage in the continuum (Kelly *et al.*, 2000).

It is obvious that a QRA requires intensive resource input, supported by substantial inputs of data and expert knowledge from diverse sources (Lammerding and Fazil, 2000). While the ultimate outcome of concern is human health risk, from an industry perspective exposure assessment is of major importance. QRA attempts to integrate existing knowledge about a hazard and product through a sequence of diverse environmental scenarios (e.g. farm, plant, distribution, retail, consumption). In comparison with toxicology, from which the framework for QRA was derived, there are additional difficulties when addressing living hazards in biological systems. Considerable data are required in both circumstances, but both biological variability in terms of model inputs and uncertainty (lack of precision in data due to sampling issues and measurement errors) are arguably greater with microbiological hazards. Judgement is required in deciding whether to invest resources to obtain more definitive data on model inputs, or to estimate uncertainty using modelling approaches. It is desirable in risk assessment to separate uncertainty and variability as sources of variation in model parameters (Nauta, 2000). The predicted risk might be overstated or understated, without proper accounting of uncertainty due to measurement or sampling error (Marks and Coleman, 1998).

Approaches to evaluate the impact of changes in variables included sensitivity analysis in deterministic models and use of stochastic models. Another alternative that may be applicable in some circumstances is analysis of the “worst-case” scenario. Zwietering and

van Gerwen (2000) suggested that deterministic sensitivity analysis, including analysis of the “worst-case” scenario, should proceed stochastic modelling.

2.3.1 Stochastic modelling

Ability to deal with uncertainty or variability has been enhanced by the availability of computers and software for simulation modelling. Consequently, complex models that link together food ingredients, batch processing, cross-contamination, microbial growth, cooking, recontamination, consumption, human exposure to pathogens, the dose-response relationship and the biological and economic impact components of the identified risks are conceivable (McNab, 1998; van Gerwen *et al.*, 2000). Where data are unavailable or uncertain, probability density functions can be used to represent the known, most likely, or expected values for a parameter. Input parameters may include the prevalence of infected animals in herds, prevalence of contaminated carcasses at slaughter, or factors (e.g. temperature, water activity) that influence microbial multiplication on products during transportation or storage. Multiple iterations of models are generated, with each iteration sampling at random from the distributions specified for each model parameter. The distribution of model outputs reflects the pattern of expected results given the variability specified in the input parameters. Three somewhat distinct and complimentary applications of modelling that can be considered in QRA are:

1. Exposure assessment related to animal production and slaughter (defining the prevalence and concentration of hazard on product),
2. Predictive microbiology – modelling the predicted growth of pathogens under various environmental conditions (time, temperature, water activity, pH, etc) to estimate numbers following processing steps or at the point of consumption,
3. Dose-response modelling (hazard characterisation)

2.3.1.1 Exposure assessment in production and slaughter

Simulation approaches have been applied to model pathogen transmission in the farm or slaughter environments (Jordan *et al.*, 1999; Hartnett *et al.*, 2001). Key parameters include the prevalence of infected farms in a region, and of animals within farms, and ideally would include quantitative estimates of the concentration of organisms in infected animals. The diversity among farms of animal population dynamics, management systems and environmental conditions present considerable challenges for data collection

and modelling. The most comprehensive attempt to incorporate a production module in a QRA has been for *Campylobacter* in broiler production (Hartnett *et al.*, 2001) which attempted to estimate the probability that a random bird from the Great Britain poultry flock would be *Campylobacter* positive at the point of slaughter. This was modelled simply as the product flock prevalence and within flock prevalence of infected birds. Broiler production is arguably the most uniform form of animal production in developed countries, and a considerable body of literature exists on the epidemiology of the organism in broilers. However, data on flock prevalence was identified an area where data were sufficient. Similarly, parameters for estimating within flock prevalence were largely based on averages of expert opinion incorporated in a triangular distribution (indicating the lowest, highest and most probable values). The authors indicated a lack of data or high level of uncertainty related to many elements of the model.

2.3.1.2 Predictive microbiology

Predictive microbiology can be used to contribute to calculation of the likely number of organisms at the time when food is consumed (Walls and Scott, 1997). Such modelling may provide an estimate of the effect of processing steps on microbial growth and product safety in food production and distribution (Zwietering and Hasting, 1997). It is known that growth and multiplication of microorganisms depends on a variety of factors, which do not act independently from each other under normal circumstances. Predictive microbiology is based on the body of knowledge about the combined and complex effects of the diverse factors and their respective interactive influence. These factors relate to growth, survival and death responses of microbes of concern in food that should be modelled with respect to main controlling factors, initially the combined effect of temperature, pH and water activity (Roberts, 1997), and fitting data to a mathematical equation. The models may be either probabilistic or kinetic. Probabilistic models are useful in obtaining an indication of the wisdom of change in product formulation or product storage, while kinetic models are focused on establishing a quantitative relationship between growth, including duration of the lag phase and controlling factors. Kinetic modelling is achieved in two stages: (1) deriving parameters by fitting a sigmoid curve to growth data, (2) using the function, commonly quadratic, to describe how the derived parameters of each curve were affected by the controlling factors (Roberts, 1997).

2.3.1.3 Dose response modelling

The infectious dose of a pathogen is not a fixed value, but depends on the susceptibility of the host and other factors. Thus, the probability of disease following exposure to a microbial hazard is particularly dependant on the numbers of the pathogen present (dose)(Stringer, 2000) in the product at the time of consumption. The “dose response” relationship can be empirically modelled by using beta-Poisson, Weibull-gamma and Gompertz models (Stringer, 2000). A sigmoid curve relationship is seen when the log of the number of organisms ingested is plotted against the percentage of the population that becomes infected (Voysey and Brown, 2000).

Lammerding *et al.* (2000) described the relationship between the numbers of the *Salmonella* organisms ingested if/when present in food (i.e. regardless of the food) and the public health outcome (illness). To provide a background and rationale for the use of three different dose-response models, the authors reviewed sets of data provided by various countries, including published data on *Salmonella*. The dose-response curve was generated by each of the models. The first model used was a beta-Poisson function. This model was developed by the USDA/FSIS for *S. Enteritidis* in eggs where data from a surrogate microorganism (i.e. *Shigella dysenteriae*) was used to model the *Salmonella* dose-response. The second model used was the Weibull function. It was developed by Health Canada and based on volunteer studies for several pathogens, including data from two *Salmonella* outbreaks. The third model used a beta-Poisson distribution based on data generated by volunteer studies using faecal shedding (infection) as the dependant variable. All three models were then compared with the actual data collected during an outbreak in a country that has a good record on *Salmonella* outbreaks. The conclusion was that none of the models had advantages over others and that all three models generated reasonable estimates.

2.4 Selected QRA models

Subsequent to the FAO/WHO Consultation group (World Health Organisation, 1995), in 1999 the Codex Committee on Food Hygiene (CCFH) identified a list of pathogen-commodity combinations for which an expert risk assessment was required. In response, FAO and WHO jointly embarked on the programme of developing recommendations for

the use of risk assessment and with the objective of providing expert advice to the CCFH Member states and to the Codex Alimentarius Commission. The work included an evaluation of existing risk assessments (working documents) focused on the pathogen-commodity combinations. Some examples included (CAC, 2001; CAC, 2002a):

- a) Exposure assessment of *Salmonella* spp. in broilers (Kelly *et al.*, 2000),
- b) Hazard identification and Hazard Characterisation of *Salmonella* in broilers and eggs (Lammerding *et al.*, 2000),
- c) Exposure assessment of *Listeria monocytogenes* (Ross *et al.*, 2000),
- d) Exposure Assessment for *Salmonella* Enteritidis in eggs (Ebel *et al.*, 2000).

Information available to date indicates that there has been no complete quantitative risk assessment (i.e. full exposure assessment through all stages of a “farm to plate” continuum) for *Salmonella* in products of any species.

2.4.1 *E.coli* O157:H7 in ground beef

The study on *Escherichia coli* O157:H7 in hamburgers (Cassin *et al.*, 1998) was considered to be very close to a formal QRA (Stringer, 2000). The authors (Cassin *et al.*, 1998) based the model on the document by the FAO/WHO Consultation group (World Health Organisation, 1995). It used a hypothetical scenario, simulates probability distributions of uncertainty using Monte-Carlo simulation, and introduced the term “Process Risk Model” (PRM) which incorporated two mathematical sub-models. The first model was divided into four modules (i.e. production, processing and grinding, post-processing, consumption). The pathogen/pathway process was outlined from production through processing to post processing (handling and consumption) to predict human exposure. The second model was focused on the dose-response and attempted to integrate the data from the first model into the dose-response model to calculate the risk to public health associated with consumption of the product.

As a starting point in the first model, major characteristics of *E. coli* O157:H7 as a hazard associated with the consumption of hamburgers were identified and outlined. The aim of the exposure assessment was to estimate the potential of human exposure to the organism in a single-meal serving. That is, both the probability of exposure and the dose-response

of the organism formed the outputs for the mathematical model. Each of the models is briefly summarised in the following paragraphs.

During the production module, the authors estimated the extent of the carcass contamination by taking into consideration the prevalence and number of organisms in the faeces. However, the authors acknowledged that seasonality, geographical factors and feeding practices were not considered. Based on the experimental data, the authors estimated distribution of the numbers of the organism in the faeces of infected cattle. Recognising between-herd variance, the authors used beta distribution to estimate prevalence data under the assumption that the outcome was a binomial random variable. The data set used to estimate prevalence was limited to the studies that involved classes of cattle most likely to be used for manufacturing of hamburgers.

The processing module was divided into three main components – skinning, evisceration, and trimming. In this module, the risk factors such as cross-contamination, log dilution between faecal and carcass surface contamination, log reduction due to decontamination measures and microbial growth during processing were taken into consideration. However, uncertainty associated with non-homogeneous spread of the organism over the carcass surface and the effect of excessive carcass handling and evisceration was not modelled. The authors estimated the probability of the organism being present in fresh ground beef and modelled the likelihood of the prevalence in retail ground beef.

The post-processing module focused on estimating the changes in the numbers of the organism between retail and consumption. It is recognised that the number of organisms in the product was a function of the probability of contaminated packages and the probability that the organism survived the growth and inactivation stages of post processing. The microbial growth, thermal inactivation and consumption component were considered. Recognising that microbial growth is dependent on many conditions of the food matrix (i.e. pH, NaCl, water activity), the log increase was estimated by using the modified Gompertz equation, a commonly used mathematical model, which predicts the growth at constant temperature. The growth curve is sigmoid, has two shoulders and a period of rapid growth. For thermal inactivation (cooking), the internal temperature of the product is modelled using a normal distribution with standard deviation of 2⁰C.

In the consumption module, the authors estimated the ingested dose of the organism as a function of the number of the organism at the time of consumption (i.e. an output of the post-processing stage) and the quantity consumed by using lognormal distribution for both adults and children.

The dose-response model was based on the Beta-Poisson model for infection to estimate the probability of the disease in humans due to a certain level of exposure. In this model, the effect of three intervention strategies on the risk reduction was evaluated. The strategies under study were: (1) storage temperature control; (2) pre-slaughter screening; and (3) consumer information program. Storage temperature control has been estimated to be the most effective in reducing the risk.

The authors (Cassin *et al.*, 1998) recognised that their study was based on the data from limited studies based on small sample sizes, and that further work will have to include a separation of uncertainty and variability in model inputs. It has been noted (Schlundt, 2000) that a final risk estimate including uncertainty was not included in the study.

2.4.2 *Salmonella* in broilers

The model by Kelly *et al.*, (2000) describes the changes in prevalence and concentration of the organism during production, transport and processing, retail, distribution and storage and preparation for consumption. It commences with the production module that is aimed to estimate the number of contaminated broilers at the time of leaving the farm for processing and the number of *Salmonellae* per contaminated bird. The objective was to estimate the probability of a randomly selected broiler being positive at the time of leaving farm for slaughter. The model recognises that the prevalence of positive birds will vary from producer to producer, from region to region, and from season to season. Given this, the authors used so-called “second order modelling” to explicitly separate uncertainty and variability. Uncertainty distributions for within the flock prevalence during the two seasons (i.e. “cold and warm” months) were estimated using the Beta distribution. Recognising that no information was available on the sensitivity and

specificity of the test used, the authors estimated the cumulative probability (with 90% confidence) of a randomly selected broiler being infected with *Salmonella* to be between 0.12 – 0.32. Due to lack of data, the authors have not attempted to model the number of organisms per bird.

In the second module (transport and processing) the aim was to estimate the prevalence and concentration of *Salmonella* at the end of processing. In this module, changes in the prevalence of contaminated carcasses and the number of the organism on the carcasses were not been modelled as the authors considered that the data available was old and only a few references provided an estimate of the numbers of the organism. Nevertheless, following a HACCP flow chart for raw poultry meat, the module discussed several stages where an increase or decrease of the risk of *Salmonella* contamination may occur. The main stages considered were transport, stun and kill, scalding, de-feathering (plucking), evisceration and chilling. During the transport stage, a particular consideration was given to the issue of cross-contamination in relation to transporting crates (their position and cleanness), and the stress associated with the length of transportation, truck conditions, temperature and road conditions that favours *Salmonella* excretion. The stun and kill stage as this stage was not considered to be of importance to cross-contamination of broilers. The different scalding stage systems used, is however recognised as important for cross-contamination due to the potential effect of lower temperature (soft-scald), and higher temperature (hard-scald) on *Salmonella* survival. While recognising that the use of chemicals at this stage may reduce potential for cross-contamination, it has been highlighted that this practice may have an effect on the residues on carcass, which is against regulations in some countries (e.g. EU). The potential impact of different scalding systems on cross-contamination is noted. De-feathering stage (plucking) has been classified as the main site for contamination, particularly due to aerosol generation and the possibility of that *Salmonella* accumulate in the cracks in the rubber fingers and grow due to favourable conditions (i.e. warm and moist). Evisceration, washing, different types of chilling, and portioning/packaging were also considered to pose varying level of risk of cross-contamination and contamination, thus having the effect on change in numbers of the organism and prevalence of contaminated carcasses throughout the processing.

The authors recognised that growth and survival would have to be considered during the time between processing and preparation. Prediction of growth would have to take into account temperature, pH, water activity and previous growth conditions. The authors noted that it is not a simple exercise to predict the consequences of temperature abuse during retail/distribution and outlined several mathematical models that may be used. During the preparation stage several consumer handling and preparation steps (i.e. thawing, raw preparation, cooking, cooling/re-heating, meal preparation, general hygiene) and availability of data and appropriate mathematical models were outlined.

As the work on *Salmonella* in broiler chickens progressed, it became evident that the lack of qualitative and quantitative information from all sources did not allow for the construction of a full model. There was a need to increase the understanding of cross-contamination processes in a “farm-to-plate” continuum, improve the survival and growth modules to include the whole continuum, and carry out sensitivity analysis to identify the factors that may have the most impact on human health (FAO/WHO, 2001). Additionally, the data related to the importance of various routes for introduction of *Salmonella* into flocks (e.g. feed, replacement birds, vectors and hygiene) were inconclusive (CAC, 2002b).

2.4.3 *Listeria monocytogenes* in ready-to-eat foods

More progress appears to have been made regarding risk assessment of *Listeria monocytogenes* in ready-to-eat foods (FAO/WHO, 2001). In considering the exposure assessment of *Listeria monocytogenes* in ready-to-eat foods, the authors (Ross *et al.*, 2000) started with very broad objectives related to microbiological safety of food. For the purpose of assessing public health priorities, the first objective was quantification of risk that consumption posed to a defined population group by consumption of a specified product. The second objective was to consider all aspects and stages of food production throughout the “farm to plate” continuum (i.e. production, processing, handling) that creates potential for hazard introduction, including consumption patterns (Figure 2.1)

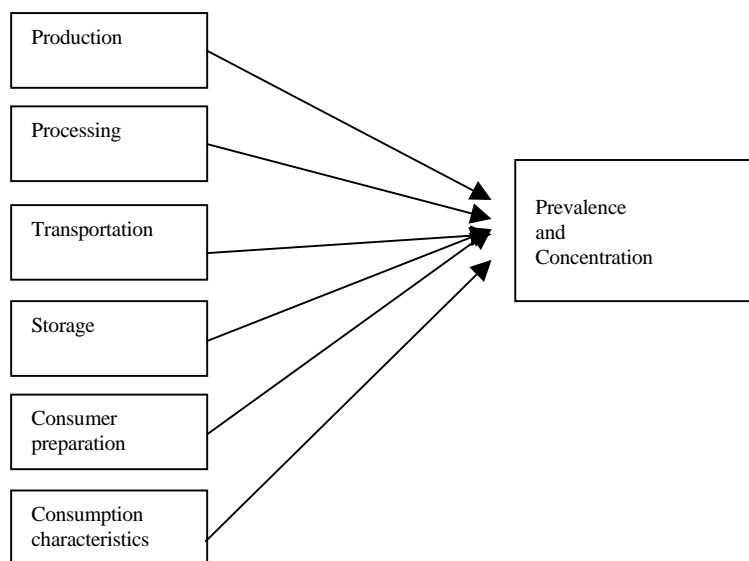


Figure 2.1. Influence diagram showing steps in food production process that contribute to level of hazard experienced at the point of consumption²¹

The authors reviewed eight existing published and unpublished models. Each of the selected models covered a specific subject with regard to growth and inactivation of the organism. For example, the subjects covered included a variety of foods, different types of processing for ready-to-eat food, inactivation potential, process decontamination, and post process contamination and consumption rates. To help construction of a mathematical model, the authors presented a generic conceptual exposure assessment model (Figure 2.2) that, in qualitative manner covers the relationship between identified risk factors.

²¹ Figure 2 - adopted from Ross *et al.*, (2000)

Point in food continuum	Variables Affecting Dose		
	<i>Consumption</i>	<i>Concentration in contaminated units</i>	<i>Prevalence of Contaminated unites</i>
Consumption	Frequency, amount consumed affected by: season, wealth, age, sex, culture/region	Heating; mixing with other components; breakdown to smaller units	Breakdown to smaller units/serving portions
Home/Food Service		Time, temperature, product composition	Cross-contamination, combination with other foods
Retail sale		Time, temperature, product composition; breakdown to smaller units	Packaging and cross-contamination, portioning, breakdown to smaller units
Transport and storage		Time, temperature, product composition	
Processing		<i>Volunetric changes: ...</i> <i>Growth and inactivation changes ...</i>	Cross-contamination, mixing with other ..., Splitting into smaller units for retail...
Raw ingredients		Environmental sources affecting concentration ...	Season, harvest area, fodder and feeding regimes, irrigation water...

Figure 2.2. A generic exposure assessment model for pathogens in foods²²

The authors identified several data gaps related to prevalence, concentration data for environmental sources, the concentration and potential contamination and decontamination sources from production through processing, effect of other spoilage organisms on *Listera monocytogenes* survival and growth in ready-to-eat foods, storage time and temperatures, consumer handling, meal servings, and consumption patterns, particularly of “at risk” population, including data on the disease and its subsequent effects on the exposed population (FAO/WHO, 2001).

2.4.4 *Salmonella* Enteritidis in eggs

To explain existing practices and techniques in constructing an exposure assessment model of *Salmonella* Enteritidis in eggs, Ebel *et al.*, (2000), used data from previously completed studies, and data provided by participating countries. The authors considered four components of the “farm to plate” continuum (i.e. production, distribution/storage, processing of eggs, and preparation and consumption patterns of consumers).

²² Figure 3 – adopted from Ross *et al.*, 2000, and slightly modified by the author of this thesis

In the production model, the authors modelled flock prevalence, egg-contamination frequency and the number of organisms per egg per lay. An output of this model “*was frequency distributions for contaminated eggs at varying levels of contamination*”. In the distribution and storage model, the authors modelled marketing fractions (i.e. home, food service outlets), time and temperature and microbial growth dynamics. An output of this model was “*a frequency distribution for the contamination levels in eggs just prior to preparations, cooking and consumption*”. In the egg products processing model, the authors modelled the contamination sources and the effect of pasteurisation. In this model an output “*would describe a frequency distribution of the number*” of the organism “*surviving the pasteurisation process*”. Another output that may be modelled would be “*prevalence of contaminated containers ...sold for preparation and consumption*”. In the preparation and consumption module, the authors modelled egg pooling and serving size, pathway probabilities and cooking. They emphasised that in this model particular consideration should be given to the location where end-user consumed eggs (i.e. home, restaurants, food outlets) and highlighted the lack of data on the effect on handling eggs from farm to consumption on the organism numbers. The authors concluded that different pathway probabilities exist and that “*the consequence of the servings consumed at a particular pathway endpoint is a distribution of number of contaminated servings at different dose levels*”.

2.5 QRA of *S. Brandenburg* in sheep meat in New Zealand

The recent emergence of *S. Brandenburg* in sheep in New Zealand (i.e. South Island) as an animal health problem with potential implications for food safety provides an opportunity to apply current principles of risk analysis to assess risks and develop risk mitigation strategies if warranted. Two important features distinguish the *S. Brandenburg* scenario from other organisms for which QRA has been undertaken:

1. An almost complete void of epidemiological information about the organism in animal populations and all subsequent stages of food production,
2. No confirmed human cases infected with the epidemic strain of the organism that have been attributable to foodborne infection.

The following comments relate to the key assessment components with respect to *S. Brandenburg*:

1. Hazard identification: The epidemic strain in sheep has been shown to infect people (including healthy adults) following occupational exposure. Focused surveillance and investigation of human cases of *S. Brandenburg* infection is warranted to gather evidence on whether foodborne cases are occurring,
2. Hazard characterisation: The disease in humans is clinically manifested as diarrhoea. No specific data on dose-response are available for the strain of *S. Brandenburg*. However, the information on dose-response for other *Salmonella* serovars can be viewed as useful surrogate data. Efforts to better define the dose-response relationship are less urgent than for exposure assessment,
3. Exposure assessment: based on reports of clinical disease the organism is expected to be widespread in Otago and Southland sheep farms, but the status of other areas of New Zealand is uncertain. At the commencement of this study no data existed on the prevalence of contamination of any sheep products with *S. Brandenburg*. The proposal to undertake a QRA has been strongly supported by industry in New Zealand, and estimation of the prevalence and contamination of sheep meat products with *S. Brandenburg* is a high priority,
4. Risk characterisation: currently impossible, primarily due to the void of data on exposure assessment.

Efforts to initiate a QRA approach to this problem began in May 2000. A meeting involving industry, government and other stakeholders was held in Gore in July 2000 to review existing information and draft potential research priorities. Several general goals and potential benefits of this process were articulated (Hathaway *et al.*, 2000a; Hathaway *et al.*, 2000b):

- a) Establish an estimate of occupational risk and identify optimal risk reduction measures,
- b) Establish an estimate of foodborne risk, and where appropriate, identify specific risk reduction measures that ensure an agreed level of consumer protection,

- c) Provide strategies and interventions to reduce the potential for adverse market access events arising from the presence of *Salmonella* in fresh meat and meat products,
- d) Provide a model for investigating other foodborne pathogens,
- e) Utilise data generated from the on-farm module to prevent and reduce animal health problems in sheep and associated livestock groups,
- f) Develop a case for equivalence of sanitary measures applied to meat and meat products exported from New Zealand; investigate any animal welfare issues that may be associated with *Salmonella* infection in slaughter population,
- g) Contribute to a risk profile for establishing broad safety priorities for New Zealand consumers,
- h) Ensure optimal market access for sheep meat exported from New Zealand and develop a case for “equivalence” of sanitary measures; utilise the QRA model to optimise existing generic HACCP plans for controlling faecal contamination of fresh meat (all slaughter species); contribute to sustainable agricultural production systems in New Zealand,
- i) Develop cost-effective and efficient controls at the farm level, including vaccines,
- j) Provide an effective scientific contribution to achieving all risk management goals and develop a generic risk assessment capability.

Subsequently, a 3 year research program titled “A quantitative risk assessment of *Salmonella* in sheep meat produced in New Zealand” has been funded from industry and government sources with the aim of generating both short-term and medium-term risk management strategies for *Salmonella* in sheep meat, with particular emphasis on *S. Brandenburg*.

The work reported in this thesis was conducted under the umbrella of this research program, and thus constitutes only one component of a broader co-ordinated research effort. General objectives for the work of this thesis are:

1. Development of a conceptual pathogen/pathway model as a contribution to the QRA (Chapter 2),
2. Undertaking microbiological studies to fill data gaps essential for completing exposure assessment (Chapters 3 and 4),
3. Integration of data obtained from the studies in Chapter 3 and 4, and in complementary studies in the farm module to identify major data gaps and priorities for further research (Chapter 5).

2.5.1 Outline of pathogen pathway model

The principal stimulus to undertake a QRA for *S. Brandenburg* is the occurrence of an epidemic of animal disease that poses a theoretical risk for product contamination that could lead to either human illness or disruption of commerce. In contrast to *Campylobacter* in broilers, the amount of data available on any aspect of *S. Brandenburg* in sheep production is negligible, and systems of production much more diverse. Although no specific data on dose response are available for *S. Brandenburg*, the existing models summarised by Lammerding *et al.*, (2000) are arguably applicable for this agent. Consequently, for QRA of *S. Brandenburg* in sheep meat in New Zealand, a case can be made for initially focusing on the exposure assessment component, where there are no surrogate data available. Ideally, the ultimate scope of the analysis will be from farm to table, including a risk estimate regarding public health impact. Due to complexity of the task, in line with previous QRA attempts it was generally seen to be essential to divide the task into related modules. A schematic outline depicting proposed modules and other major elements to be considered in developing a pathogen/pathway model is presented in Figure 2.3 (Hathaway *et al.*, 2000b). A description of key outputs for each module, and of likely key determinants of those outputs is presented in Figure 2.4

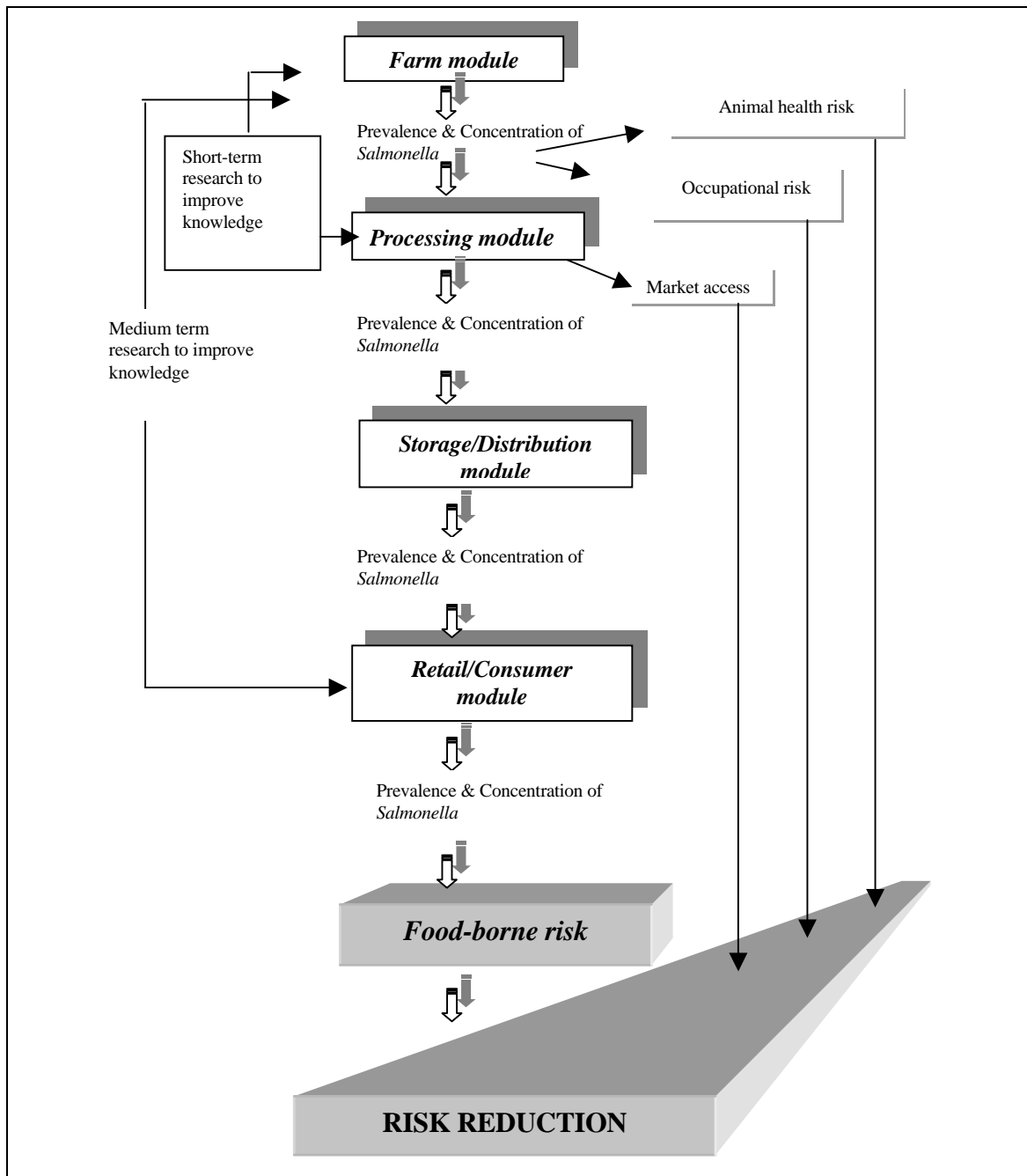


Figure 2.3. Project development for the management of risks associated with *Salmonella* in sheep

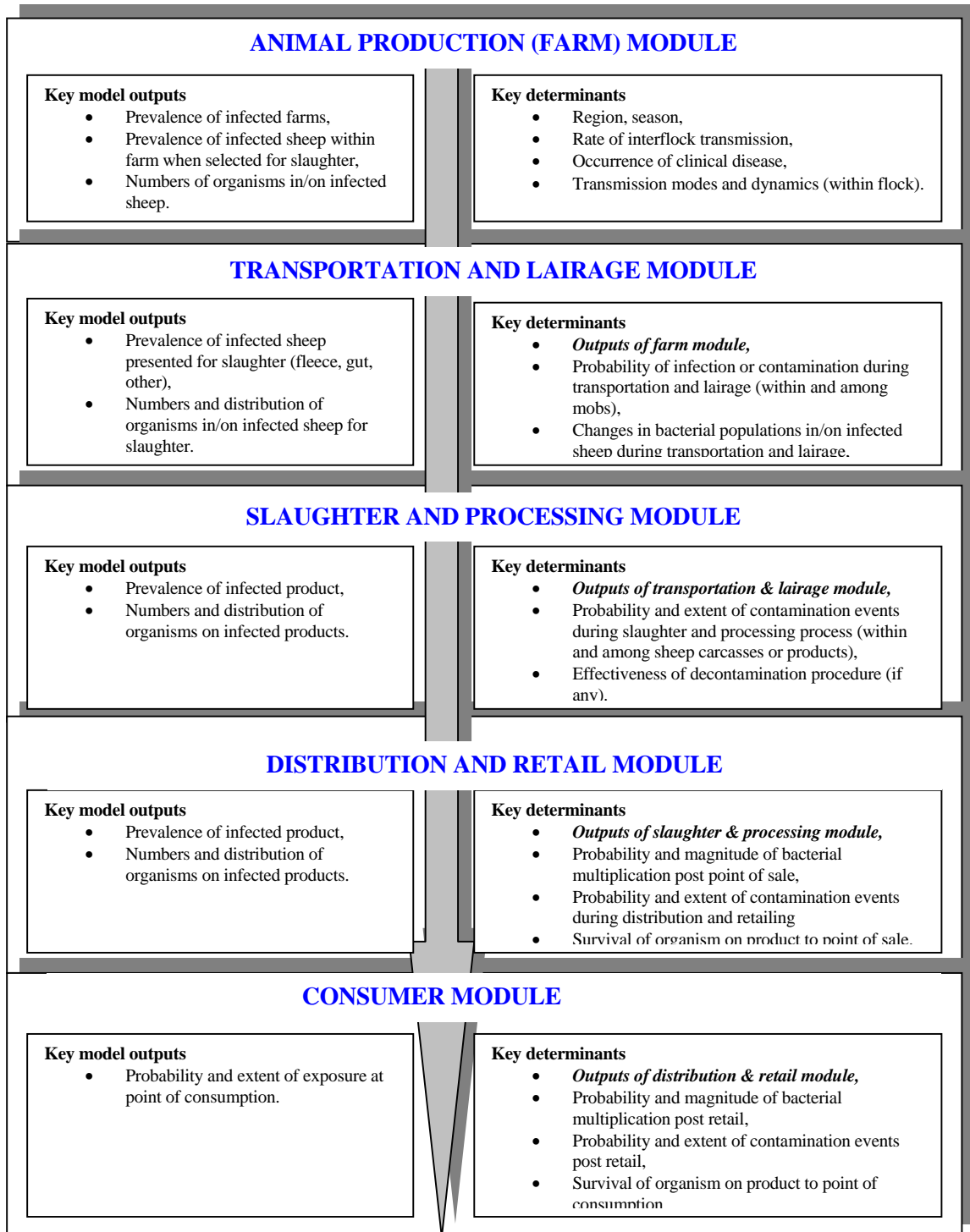


Figure 2.4. Description of key model outputs and their likely determinants in respective modules of the pathogen pathway

Existing sources of data, and expected outputs of initial studies proposed as a part of the QRA research project are summarised for the respective modules in Tables 2.1 to 2.4. Apart from the farm module, no data were available from any sources other than the initial research to be undertaken as a part of the QRA project.

a) **Farm module** (Table 2.1)

Table 2.1. Farm module: data sources and research initiatives

Module	Pilot study – title	Agency
1. Farm module*	<i>Salmonella</i> prevalence in faecal and caecal samples from lambs and ewes (case and non-case farms)	Massey University
	Retrospective case-control study of Otago/Southland farms	
1.1 Farm module – Other related projects	Faecal excretion rate in ewes and the carrier state of black-backed gulls (<i>Larus dominicanus</i>)	LABNET Invermay
	Spread of <i>Salmonella</i> Brandenburg organisms in sheep yards	
	Spread of <i>Salmonella</i> organisms in sheep yards and effect on lambs and ewes at meat plants	
	<i>Salmonella</i> Brandenburg – a new molecular pattern its occurrence on the farm of origin and a study on possible spread	
	Survival of <i>Salmonella</i> Brandenburg in yard dust	Avivet Ltd, Christchurch
	The use of an attenuated <i>Salmonella</i> Typhimurium vaccine in sheep to prevent abortion due to <i>Salmonella</i> Brandenburg: challenge and field trials	
Experimental infection of pregnant sheep with attenuated <i>Salmonella</i> Typhimurium	Lincoln University	

* QRA initiated projects

The key objectives of the farm module were:

- To evaluate the association between occurrence of disease outbreaks and prevalence of *S. Brandenburg* in sheep sent for slaughter (exposure assessment),
- To determine management factors associated with occurrence of disease epidemics (animal health and potential risk mitigation).

b) Processing module (Table 2.2)

Table 2.2. Processing module: data sources and research initiatives

Module	Pilot study – title	Agency
1.2. Processing module	Food safety environment – literature review (Chapter 1)	NZFSA**
	Effect of sample storage on detection of <i>Salmonella</i> Brandenburg in swabs of experimentally contaminated meat (Chapter 3)	NZFSA*/ AgResearch
	Validation BAX/other methods	AgResearch
	Prevalence and concentration of <i>Salmonella</i> on pelleted carcasses (Chapter 4)	NZFSA*/ AgResearch
	Qualitative development of pathogen/pathway model and identification of data gaps (Chapter 2 and 5)	NZFSA**
	Prevalence and concentration of <i>Salmonella</i> on freshly pelleted case-farm carcasses and boned cuts	AgResearch
	Prevalence and concentration of <i>Salmonella</i> on randomly pelleted carcasses	AgResearch

Legend: *Combined effort; **The author's original contribution

The key objective of the processing module was to:

- Obtain data on *S. Brandenburg* prevalence and numbers during slaughter and processing to enable completion of exposure assessment.

c) Storage and distribution module (Table 2.3)

Table 2.3. Storage and distribution module – studies

QRA Module	Pilot study – title	Agency
1.3 Storage & Distribution module	Retail sampling of sheep meat	AgResearch

The key objective of the storage and distribution module was:

- Obtain data on *S. Brandenburg* prevalence and numbers to enable completion of exposure assessment.

d) Consumer module (Table 2.4)

Table 2.4. Consumer module: data sources and research initiatives

QRA Module	Pilot study – title	Agency
1.4 Consumer module	Human case-control study	ESR

The key objective of the consumer module is to examine risk factors associated with human cases of *S. Brandenburg*, and to determine the level of risk posed by the consumption of sheep meat.

Chapter 3: Effect of sample storage on detection of *Salmonella* Brandenburg in swabs of experimentally contaminated meat

3.1 Introduction

The microbiological status of a meat product (carcass or cut) can be described qualitatively (contaminated or not) or quantitatively (number of organisms per unit area or weight of product). In the context of risk assessment for a given pathogen, the most valuable information is the numbers of organism that are present on a product. Both variability (true biological variation) and uncertainty (measurement errors) contribute to variation in detection of bacteria on meat products, and methodological factors relating to both sampling and bacteriological procedures may impact observed counts.

An essential requirement for developing a quantitative risk assessment model for *S. Brandenburg* in sheep meat is to estimate the numbers of *Salmonella* present on carcasses or product at some points during processing. In preparation for conducting the principal field study for this purpose (Chapter 4), an experimental study was required to evaluate the impact of sample handling procedures on detection of *S. Brandenburg*. For logistic reasons (distance from slaughter facility to laboratory), it was unavoidable that, during the field study, delays in transporting samples to the laboratory, and therefore in sample processing, would be incurred. Delays of at least 24 hours, and in some cases up to several days, were anticipated. Prior to undertaking the field study, it was necessary to determine what impact, if any, these logistic constraints would have on the results observed. Three experiments were conducted at the AgResearch microbiology laboratory in Hamilton during November 2000 using meat samples experimentally contaminated with *S. Brandenburg*. Using the most probable number (MPN) method, the numbers of *Salmonella* detected following the respective storage treatments were compared with the numbers recorded following immediate processing without storage. The findings of the experiments provided a basis for the sample handling procedures subsequently used for the field studies described in Chapter 4.

3.2 Material and Methods

3.2.1 Experimental contamination of meat samples

Unpublished data²³ from recent studies at AgResearch indicate that bacteria in suspension readily attach to freshly cut meat surfaces. For the proposed field study, it was envisaged that relatively large areas of carcasses or prime cuts would be swabbed to detect the presence of *Salmonella*. For the pilot experiments described here, it was preferable that the samples used would be sufficiently large to enable the swabbing process to be emulated, and provide estimates of bacterial counts per unit area of meat swabbed. Therefore, hot-boned beef cube roll (*m. longissimus dorsi*) was obtained from a commercial meat plant. It was recognised that physical properties of beef meat may differ from sheep meat. However, given that the primary objective of this study was to study effect of prolonged storage on detection of *Salmonella*, it was considered that such differences would be unlikely to have deleterious effect on survival of *Salmonella*. Thirty steaks of approximately 1 cm thickness were cut aseptically such that a cut surface area of greater than 100cm² was easily accessible for swabbing.

A culture of *Salmonella* Brandenburg (laboratory designation - Y24) was used for seeding the meat. This strain, originally isolated from clinically affected sheep in the South Island, was obtained from Massey University, Palmerston North, and had been confirmed by Pulsed Field Gel Electrophoresis (PFGE) to possess the PFGE profile consistent with the epidemic strain. The isolate had been maintained on Protect Bacterial Preservers medium (TSC Ltd., Heywood, UK) at -85°C, and was resuscitated by three serial incubations at 37°C for 24 hours in Tryptic Soy Broth (TSB) (Difco, Detroit, USA). A Brain Heart Infusion (BHI) agar (Difco, Detroit, USA) slant was inoculated over the entire surface with *S.* Brandenburg and incubated at 35°C for 22 hours. The cells were washed from the slant with 10 ml 0.1% peptone/0.85% sodium chloride, and further washed 3 times by centrifugation and resuspension in 0.1% peptone/0.85% sodium chloride solution. The cells were diluted a further 10⁵ times to yield a suspension containing approximately 10³ cfu/ml of *S.* Brandenburg.

²³ Bell & Narendran (AgResearch-MIRINZ Centre, private Bag 3123, Hamilton, New Zealand), personal communication

The prepared suspension of *S. Brandenburg* was poured into a beaker. One side of each steak was dipped into the beaker then removed and allowed to stand, dipped side upright, on a disinfected stainless surface for 30 minutes before sample collection. Swab samples from the upper surface of the steaks were collected using a sponge tipped swab (“Flexiswab” – B/N05800, Biolab, Auckland). The swab was rubbed vertically, horizontally and diagonally across the entire surface delineated by a sterilised 100cm² template. Three experiments were conducted to evaluate effects of different sample storage treatments.

3.2.2 Sample storage treatments

In each of the 3 experiments, 5 samples were designated as control samples (CS), which were processed by the most probable number (MPN) method immediately after collection. The remaining 5 samples were treatment samples (TS), with treatments varying among the 3 experiments. In Experiments A and B, swabs were immediately placed in buffered peptone water (BPW; Difco, Detroit, US) and the storage treatments consisted of storage of the BPW suspensions at 4°C for 24 hours or 5 days respectively. For Experiment C, swabs were stored for 24 hours before placement in BPW, then stomached, and the BPW suspensions were subsequently stored for a further 48 hours before conducting the MPN procedure. Storage treatments for the respective experiments are summarised below:

- **Experiment A:** Swab suspensions in 300ml of BPW were held at 4°C for 48 hours before preparing MPN dilutions,
- **Experiment B.** Swab suspensions in 300ml of BPW were held at 4°C for 5 days before preparing MPN dilutions,
- **Experiment C.** Swabs were stored at 4°C/24 hours prior to being added to 300ml of BPW and stomached. The 300ml sample swab suspension was then stored for a further 48 hours/4°C before preparing MPN dilutions.

3.2.3 Detection of *Salmonella*

For the proposed field study (Chapter 4), a two step procedure was to be implemented. Initial screening of cultures would employ a PCR based method (BAX[®], Qualicon L.L.C, Wilmington, Delaware, USA) to identify those field samples that were positive for *Salmonella* (qualitative result). Subsequently, *Salmonella* present in the BAX[®] PCR positive samples would be enumerated using the MPN method, and the organisms isolated for identification, and forwarded for further serotyping (section 4.2.3). For the pilot experiments described in this chapter, the BAX[®] PCR method was performed as the detection system for the MPN, (AgResearch, unpublished data), but isolates were not confirmed by serotyping.

3.2.3.1 Most Probable Number method

The MPN method for quantifying *Salmonella* organisms following enrichment culture is based on enumerating colonies in serial dilutions of substrate. The MPN method enumerates only viable organisms. Samples are prepared to obtain a random distribution of bacteria, and diluted to the extent that substrate will sometimes, but not always, contain viable organisms. Conditions of incubation have been selected so that every inoculum that contains even one viable organism should produce detectable growth. Therefore, the essence of the MPN method is the dilution of a sample to such a degree that inocula will sometimes, but not always contain viable organisms (Garthright, 1998). For these studies a 3-tube MPN procedure was employed. The MPN index from dilution tubes was referred to 3-tube table²⁴ to obtain the MPN count per 100ml of the original samples (Garthright, 1998). The tables also provide estimates of 95% confidence intervals, reflecting a 95% probability that the true number of organisms in the original sample is within this range.

The following dilution procedures were performed for all control and treatment samples:

- a) The sponge tip from each swab was inserted into an individual stomacher bag, to which 300 ml of sterile BPW was added. Bags were stomached (Stomacher 400, Seward, London, UK) on high speed for 2 minutes.

²⁴ Table 1, FDA Bacteriological Analytical Manual, 8th Ed (Revision A)/1998 (page App. 2.07)

- b) Aliquots were then taken as follows (Figure 3.1)
- 100ml into a sterile 200ml bottle (undiluted sample),
 - 10ml into each of 3 sterile 20ml bottles (undiluted sample),
 - 1ml into each of 3 sterile 20ml bottles, each containing 9ml of Buffered Peptone Water (Dilution 10^{-1}),
 - 0.1ml into each of 3 sterile 20ml bottles, each containing 9.9ml of Buffered Peptone Water Dilution (10^{-2})

According to the 3-tube table, these dilutions enable the baseline MPN estimation of numbers of organisms within the range of 3-1000. The number of cells/cm² is calculated further based on the surface area sampled (100cm²) and the dilution of the sample.

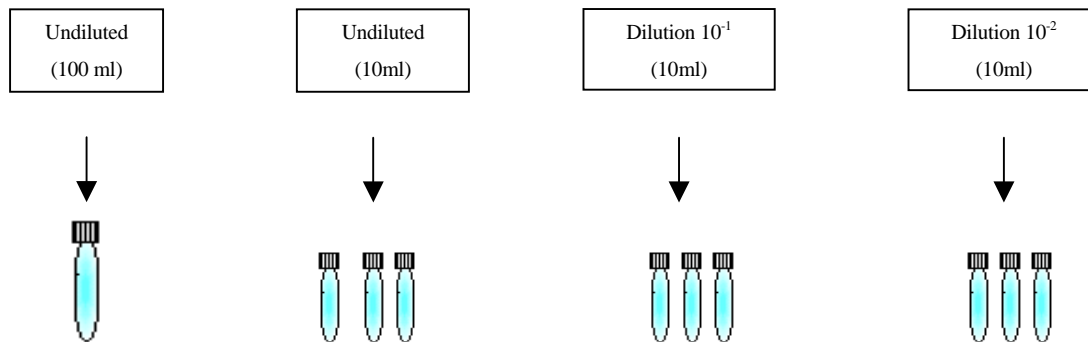


Figure 3.1. Dilution scheme used for MPN method

All tubes were incubated at 37°C for 16 to 20 hours. After incubation, the number of tubes showing growth was recorded. The results of positive (for illustrative purposes presented by red colour in Fig. 3.2) and negative dilutions (for illustrative purposes presented by blue colour in Fig. 3.2) were collated, and enumerated with MPN tables using the Bacteriological Analytical Manual (BAM) criteria (Garthright, 1998) as illustrated in Figure 3.2.

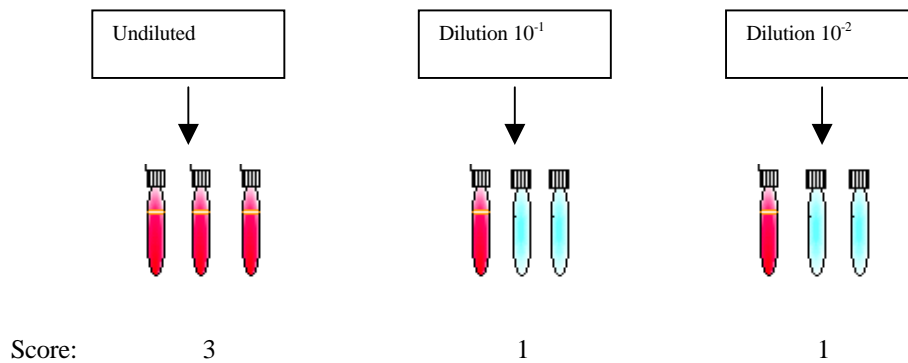


Figure 3.2. Example of MPN scoring procedure*

*Note: This example test (Figure 3.2) is scored 3.1.1 for these dilutions, which corresponds with MPN score of 75 (with confidence limits 17-200).

The base MPN counts were then multiplied by 3 and expressed as counts/100cm² of swabbed surface to provide standardised estimates of numbers of viable bacteria per unit area of swabbed surface.

Owing to the small sample size for each group (5), and likely non-normality in the distribution of counts (and particularly MPN estimates), statistical analysis was not appropriate due to lack of power. The purpose of this pilot study was to examine whether obvious reduction in detection of *Salmonella* would occur, rather than to test for statistical significance for relatively small differences.

3.3 Results

3.3.1 Experiment A

The actual observed results for the control samples was 0.2 log units per 100cm² greater than for the stored samples. MPN counts did not appear to differ markedly between samples stored for 48 hours in BPW (mean Log₁₀MPN/100cm² of 1.87) and samples processed immediately (mean Log₁₀MPN/100cm² of 2.03) (Table 3.1).

Table 3.1. MPN results for samples processed immediately after collection (control) and samples stored for 48hours in BPW solution before processing (treatment)

	Sample No	Base MPN	MPN/100cm ² (Swab)	Log ₁₀ MPN/100cm ²
Control	1	75	225	2.35
	2	23	69	1.84
	3	15	45	1.65
	4	93	279	2.45
	5	23	69	1.84
Mean			137	2.03
Treatment (48h)	1	43	129	2.11
	2	23	69	1.84
	3	9	27	1.43
	4	43	129	2.11
	5	23	69	1.84
Mean			85	1.87

3.3.2 Experiment B

The actual observed results for the control samples was 0.2 log units per 100cm² less than for the stored samples. MPN counts did not appear to differ significantly between samples stored for 5 days in BPW (mean Log₁₀MPN/100cm² of 1.97) and samples processed immediately (mean Log₁₀MPN/100cm² of 1.76) (Table 3.2).

Table 3.2. MPN results for samples processed immediately after collection (control) and samples stored for 5 days in BPW solution before processing (treatment)

	Sample	Base MPN	MPN /100cm ² (Swab)	Log ₁₀ MPN /100cm ²
Control	1	9	27	1.43
	2	43	129	2.11
	3	4	12	1.08
	4	75	225	2.35
	5	23	69	1.84
Mean			92	1.76
Treatment samples	1	23	69	1.84
	2	240	720	2.86
	3	11	33	1.52
	4	20	60	1.78
	5	23	69	1.84
Mean			190	1.97

3.3.3 Experiment C

The actual observed mean for the control samples was 0.3 log units per 100cm² less than for the stored samples. MPN counts did not appear to differ significantly between samples stored 24 hours before dilution, then for 48 hours in BPW (mean Log₁₀MPN/100cm² of 2.25), and samples processed immediately (mean Log₁₀MPN/100cm² of 1.99) (Table 3.3).

Table 3.3. MPN results for samples processed immediately after collection (control) and swabs stored 24 hours before dilution in BPW, then a further 48 hours in BPW before processing (treatment)

	Sample	Base MPN	MPN /100cm ² (Swab)	Log ₁₀ /MPN/ 100cm ²
Control	1	4	12	1.08
	2	23	69	1.84
	3	240	720	2.86
	4	75	225	2.35
	5	23	69	1.84
Mean			219	1.99
Treatment	1	9	27	1.43
	2	23	69	1.84
	3	75	225	2.35
	4	1100	3300	3.52
	5	43	129	2.11
Mean			750	2.25

3.4 Discussion

Methodological factors can markedly affect the recovery of *Salmonella* organisms from various substrates (D'aoust *et al.*, 1992b). Prior to undertaking field studies to estimate the prevalence and concentration of *Salmonella* on sheep carcasses in commercial abattoirs, it was desirable to evaluate the possible impact of delays in sample processing on ability to detect and enumerate *Salmonella* in swabs of contaminated meat. The storage treatments were selected to emulate unavoidable delays in sample handling due to transportation and also to consider options for sample storage during transport (storage of swab sample alone or following dilution in BPW).

The small sample size in all experiments was insufficient for a meaningful statistical analysis to be carried out. For example, the Mann-Whitney test, as a non-parametric test, may be used to compare two unpaired groups, but has little power if total sample size is seven or less. Evaluation of the pooled data from all experiments for control and treatment samples suggested that storage of samples under the conditions evaluated in this study had no deleterious effect on the ability to detect *S. Brandenburg* in swabs of contaminated meat samples. Overall, it means that the treatments evaluated had minimal effect on results obtained by the MPN method. In all cases, differences in mean counts were less than 1 log, and in 2 of the 3 experiments values for stored samples were numerically greater than for samples processed immediately. These results suggest that carcass swabs contaminated with *Salmonella* can be stored for up to five days at 4°C without diminishing the ability to detect the organisms with the methods employed. In addition, storage of the swab for 24 hours before addition to BPW, and subsequently for a further 48 hours before processing by the MPN method also appeared to have minimal effect on the results obtained. *Salmonella* are considered to be relatively resistant organisms, and storage of samples for several days at room temperature (delayed secondary enrichment) has been used to enhance detection of *Salmonella* (Hammack, 1999).

It should be noted that ability to detect *Salmonella* in contaminated samples depends on both the concentrations of *Salmonella* and of competing contaminant bacteria in the samples (Jameson, 1962). Other factors that may affect the growth and recovery of *Salmonella* (e.g. inhibitory metabolites produced by other bacteria, the depletion of available nutrients, lowered redox potential, pH changes, the build up of reactive oxygen species) have also been referenced (Baylis, 2000). Arguably, the competing flora on the surface of sheep carcasses following commercial slaughter would differ from the competing flora on the surfaces of the experimentally contaminated cuts of beef used in this study. However, for practical purposes in undertaking the field pilot studies of sheep carcasses described in Chapter 4, the following sample handling protocol was proposed:

- a) Suspend a field sponge swab sample into 235 ml of BPW,
- b) Aliquot 100ml into a sterile bottle and refrigerate remaining sample at 4°C,
- c) Incubate aliquot portion of 100ml at 37°C for 16-20 hours,
- d) Perform BAX[®] PCR *Salmonella* test (refer to Chapter 4, sections 4.2.3.1),

- e) Negative samples - declare samples below the detection limit of the BAX[®] PCR *Salmonella* test (i.e. less than 10^4 *Salmonella* cells/ml of pre-enriched sample). Note: the detection limit is based on the ability of the primary enrichment to bring a single cell to detectable levels and this is a value achievable from a 1.0.0.0 score,
- f) Positive samples – should be further tested inside 48 hours, and must be tested within 5 days. The remaining refrigerated sample (3.4.b) is to be brought to room temperature, then apportioned in a 3 tube MPN dilution series (Chapter 3, section 3.2.3.1.b), and followed by further testing (Chapter 4, section 4.2.3.2).

Chapter 4: Prevalence and numbers of *Salmonella* on sheep and lamb carcasses during processing

4.1 Introduction

S. Brandenburg recently emerged as the cause of an important clinical disease of ewes in the most intensive sheep producing areas of New Zealand. Reported cases of human salmonellosis caused by this serovar have increased during the same period, and most human cases have been linked by occupational or other exposure to sheep. Given the virulence of this strain of *S. Brandenburg* in humans, the potential for *Salmonella* to cause foodborne infection, and the large proportion of the New Zealand sheep flock reared in the affected area, it is desirable to quantify the potential foodborne risks due to *S. Brandenburg* in sheep meat.

At the commencement of this study, there were no existing data to indicate possible foodborne risks associated with *S. Brandenburg* contamination of sheep meat. The study was undertaken to obtain initial qualitative and quantitative estimates of the presence of *Salmonella* organisms on sheep meat at 3 points in the processing chain. The study was coordinated with a farm-based study that evaluated (qualitatively) the prevalence of *Salmonella* in faecal (on-farm) and caecal (post-slaughter) samples from the same groups of animals.

In this study, samples were obtained from carcasses of slaughtered sheep (ewes and lambs) sourced from six farms in the Central Otago/Southland region of the South Island of New Zealand where *S. Brandenburg* disease is prevalent. Three farms (case farms) were selected based on the occurrence of an epidemic of *S. Brandenburg* disease during the spring of 2000. Three non-case farms from the same region were also sampled. As the disease epidemics are temporally clustered in July and August, well before lambs are sent for slaughter, sampling was replicated after an interval of approximately 2 months to assess likely temporal variation in risk of carcass contamination. For comparative purposes, samples from sheep carcasses were also collected from 6 groups of sheep slaughtered at two slaughter plants in the North Island, where salmonellosis due to *S. Brandenburg* infection in sheep has not been reported.

The principal objective of the study described here was to obtain preliminary estimates of the prevalence and numbers of *Salmonella* on sheep carcasses in the affected region of Southland and Otago. Such data are essential to support a risk assessment approach to evaluate the existence of any incremental foodborne risks attributable to the emergence of *S. Brandenburg* disease in sheep in this region.

4.2 Material and methods

4.2.1 Selection of sheep farms and animals

4.2.1.1 South Island

Initially, eight flocks were purposively selected by Dr. Gary Clark (LABNET Invermay) based on the occurrence and laboratory confirmation of clinical outbreaks of *S. Brandenburg* in the 2000 lambing season. Only flocks that had not been vaccinated against *Salmonella* were eligible. Selection of case flocks was deliberately biased to include flocks that had experienced significant clinical outbreaks, and thus reflected a ‘worst-case scenario’ with respect to risk of infection at slaughter and carcass contamination. All farms were located in the Southland/Otago region, South Island.

The study was conducted in conjunction with a companion study designed to compare the prevalence of *S. Brandenburg* in faecal (on-farm) and caecal (post-slaughter) samples from the same groups of animals. The specific objective of the farm-based study was to compare the on-farm prevalence of *S. Brandenburg* in flocks that had (case farms), or had not (non-case farms), experienced outbreaks of abortion and ewe death due to *S. Brandenburg* infection. For the companion study, a target sample of approximately 50 cull ewes and 50 lambs was chosen to enable reasonable estimation of point prevalence (at least 90% confidence, 10% accuracy) at the mob level. Sheep sampled on-farm were not individually identified, and the animals sampled at the slaughter plant were from the same mob, but were not necessarily the same individuals sampled on farm. For carcass sampling of the same mobs, the target sample size was 51 carcasses, with each carcass sampled at one of the 3 chosen points (slaughter floor, cooling floor, boning room) in the processing chain (17 carcasses per sampling point for each mob). The entire sampling protocol was replicated following an interval of approximately 2 months (Table 4.1).

Table 4.1. Sampling dates for ewes and lambs sourced from case (C) and non-case (NC) farms

<i>Farm</i>	<i>First sampling (~2 months after lambing)*</i>	<i>Second sampling (~4 months after lambing)</i>
C1	27/11/00	7/02/01
C2	11/12/00	14/02/01
C3	13/12/00	20/02/01
NC-B	30/11/00	8/02/01
NC-C	6/12/00	13/02/01
NC-D	6/12/00	21/01/01

*First week of processing season

4.2.1.2 North Island

Ovine salmonellosis due to *S. Brandenburg* has not been reported in the North Island of New Zealand. In addition to comparison of case and non-case flocks within the affected region, sampling of carcasses in two North Island plants was also conducted. The two plants selected were of equivalent size and throughput as the collaborating plant in the South Island. However, sampling at the North Island plants differed in several respects from that conducted in the South Island. Sampling was only conducted on one occasion per plant, and carcass swabs were only collected at one location in the processing line (slaughter floor) compared with three locations in the South Island. Carcass swabbing procedures were identical. In each plant, a convenience sample of 50 ewes and 50 lamb carcasses was targeted, with all samples collected on a single day. Due to commitments to the South Island sampling, the North Island sampling was scheduled soon after completion of the South Island project at a time of high plant throughput (peak of season).

The sampling scheduled is detailed below:

a) Plant A (6 March 2001)

- 51 sampled from ewes (sourced from 3 different mobs; non-equal numbers),
- 51 sampled from lambs (sourced from 3 different mobs; non-equal numbers)

b) Plant B (9 March 2001)

- 51 sampled from ewes (sourced from 3 different mobs; non-equal numbers),
- 50 sampled from lambs (sourced from 3 different mobs; non-equal numbers).

4.2.2 Sample collection

4.2.2.1 Preparation

In preparation for the sampling, two training and practice sessions were carried out to ensure effective co-ordination of on-line plant staff and the team during the sampling of designated mobs. The supplier (BioMerieux, New Zealand Ltd, Auckland) provided each sponge in an individually sealed plastic bag (Microsponge MDK-MS). At the AgResearch laboratory, 10 ml of sterile BPW was added aseptically to each bag, which was then sealed manually using ready-made seal available on the bag.

Each sponge kit batch was provided in a sealed plastic bag marked with corresponding stickers and contained 17 individual sponge kits for sampling in one sealed plastic bag, plus 3 individual sponge kits in a separate sealed plastic bag. Sponge kit batches and ice block containers were packed into chilly bins, sealed, and despatched to the sampling team by courier 24-48 hours before collection. To avoid any potential for confusion during sample collection and laboratory testing, separate chilly bins were used for samples from each mob. The outside of each individual plastic bag was identified with two stickers. To facilitate sample collection in the plant, kits were clearly identified for specific sampling events:

- Ewes: green and white for slaughter floor, green and silver for cooler, green and red for boning room,
- Lambs: yellow and white for slaughter floor, yellow and silver for cooler, yellow and red for boning room.

Samples were collected from carcass sites considered to have a high probability of contamination (Bell and Hathaway, 1996) at three points (i.e. slaughter floor, ageing floor, boning room) in the processing chain:

- a) Slaughter floor point of samples collection (Figure 4.1) immediately after removal of hides from carcasses. The forequarter and ventral samples were collected prior to the pelt being removed from the hindquarters (between “shoulder pull” and automatic depelting – “final puller”). Anal (“bung”) surround samples were collected immediately after the pelt was fully removed (the area between the automatic hide removal and the trim area)



Figure 4.1. Slaughter floor point of samples collection

- b) Ageing floor (cooler) point of samples collection. The sampling took place immediately after arrival of the marked carcasses at the refrigerated ageing floor and its separation to a designated chain,
- c) Boning room point of samples collection. The sampling took place immediately after cutting and deboning and before bagging.

To prevent cross-contamination of samples, sterile disposable gloves were used and changed between each carcass swabbed.

4.2.2.2 Sampling of carcasses or primal cuts

The co-operating slaughter plant has a line speed of approximately 420 ewes/hour and 540 lambs/hour. The process on the slaughter floor is a typical inverted sheep chain

process²⁵. The sampling procedure for both ewes and lambs was the same. The first and the last sheep slaughtered in the mob destined for sampling was marked with a visible mark on the line by a foreman. From each line of 51 ewes and 51 lambs the samples are collected from the carcass surfaces as follows.

4.2.2.2.1 Slaughter floor

Seventeen carcasses were to be swabbed at the site most likely to be most contaminated during pelt removal (i.e. opening cut lines). The first carcass in a mob, followed by every third subsequent carcass, was swabbed. The final swab sample was a composite sample from three carcass locations taken in three steps. The first step was mark the carcass with a yellow sticker, and take a 5-stroke sponge swab from the sternum and abdomen along the incision line (Figure 4.2). The second step was to take a 5-stroke sponge swab with the same side of the sponge from the forelegs on the line of the opening cut lines (Figure 4.3) where skin incisions were made during the dressing (commonly known as a Y-cut because of the shape), including the area between the forelegs (Figure 4.4). The third step involved taking a 5-stroke sponge circular swab of the bung area using the other side of the same sponge (Figure 4.5). At this stage:

- The second incoming carcass, and every third subsequent carcass, was marked with a visible metal marker for sampling in the ageing floor (section 4.2.2.2.2),
- The third incoming carcass, and every third subsequent carcass, was marked with a visible metal marker for sampling in the boning room (section 4.2.2.2.3).

²⁵ Inverted dressing is the removal of the pelt from the animal whilst it is suspended by the forelegs. Conventional dressing of ovine and other small animals is regarded as the removal of the pelt from the animal whilst it is suspended from the hind legs (Armitage N. – personal communication, 2002).



Figure 4.2. Slaughter floor - first step (sternum/abdomen)



Figure 4.3. Slaughter floor - second step (Y-cut)



Figure 4.4. Slaughter floor - second step (forelegs)



Figure 4.5. Slaughter floor - third step (bung area)

4.2.2.2.2 Cooling floor (ageing floor)

For each mob, seventeen carcasses were to be swabbed on the opening cut lines. The incoming carcasses designated for the cooling floor (cooler) sampling were separated on a single chain and the swabbing took place immediately. The same technique for obtaining a composite sample was used for the sampling areas as described in section 4.2.2.2.1.

In some instances, the number of carcasses destined for the cooler sampling was less than 17 due to some of the carcasses being condemned during post mortem inspection due to various defects that required carcass condemnation. These were mainly case carcasses from old ewes.

4.2.2.2.3 Boning room

The remaining marked seventeen carcasses from each mob were separated at the cooling floor, subjected to an ageing process (up to 8 hour at 10⁰C) and then railed to the chiller. In the chiller, the carcasses were subjected to the normal refrigeration regime, which includes reducing the deep meat temperature +7⁰C within the first 24 hours while the chiller temperature is not reduced to more than +1⁰C. Carcasses were refrigerated for 16-24 hours before boning.

In the boning room, one prime cut per carcass was swabbed. A hind limb was selected because of its anatomical closeness to the anal rim as the most probable site of contamination. The upper surface of each cut was swabbed by rubbing a sponge vertically, horizontally and diagonally across the entire surface delineated by a sterilised 100cm² template. The template was located on the surface of the cut at the site nearest the anal cavity (presumed greatest risk of contamination).

Based on the results obtained in the boning room during the first sampling round (December/November 2000), the swabbed area for each carcass was increased during the second sampling round (February/March 2001). In addition to the leg, other sites swabbed were the shoulder (close to line of Y-cut area) and available flank area (as close to the incision lines as possible) with an area of 1258 cm² in lambs and 1720cm² in ewes.

4.2.2.2.4 Estimation of the swabbed area of carcasses

On a sample of ewe and lamb carcasses, swabbing area was measured at the 3 sites sampled. Areas were calculated by multiplying swab width (5 cm) by the measured length of the sites sampled. These were the 1) the length of forelegs along the line of the opening cut lines where skin incisions were made during the dressing (Y-cut) including the area between the forelegs; 2) the length along the line from the sternum and abdomen along the incision line; 3) the area around the bung.

4.2.2.3 Sample handling and transport

Swab samples were packed into individual plastic bags and labelled with the identities of the farm and owner, point of sampling, and date of collection. The bags were placed on ice in sealed insulated containers and transported overnight by courier to the microbiology laboratory (AgResearch-MIRINZ Centre) in Hamilton for analysis within 24 hours.

4.2.3 Detection of *Salmonella*

A two step procedure was performed at the AgResearch laboratory for qualitative and quantitative assessment of the presence of *Salmonella* in swab samples. Initial screening of samples was employed using a PCR based method (BAX[®], Qualicon L.L.C., Wilmington, Delaware, USA) to identify those cultures that were positive for *Salmonella* (qualitative results). This method had been previously been evaluated by AgResearch and found to give reliable results compared with the standard culture method used in the laboratory. All samples that were BAX[®] PCR positive were then submitted for the MPN procedure (described in section 3.2.3.1) to enumerate the organisms present in the samples (quantitative results) (Mills and Clemens, 2002).

4.2.3.1 BAX[®] PCR detection of *S. Brandenburg* in field samples (qualitative analysis)

4.2.3.1.1 Culture and DNA extraction

The sponge sample was inserted into an individual stomacher bag, to which 235 ml of sterile BPW was added. Bags were stomached (Stomacher 400, Seward, London, UK) on

high speed for 2 minutes. One hundred millilitres of the sample was incubated for 16-20 hours at 37°C while the remaining portion was stored at +4°C to be used for further testing (i.e. MPN procedure – Chapter 3, section 3.2.3.1.b) if BAX[®] PCR test result was positive (Chapter 3, section 3.4.f). For BAX[®] PCR test, 1ml of each pre-enriched sample was inoculated into 9 ml of Brain-Heart Infusion (BHI) broth (Difco, Detroit USA) and incubated for 3 hours at 37°C. BAX[®] lysis buffer was prepared by adding 62.5µl protease to 5 ml of BAX[®] buffer (Appendix 1). Aliquots of 200µl of lysis buffer were transferred to lysis tubes, and an aliquot of 5µl of a sample incubated BHI broth was added to each lysis tubes. Samples were processed in lots of 48 samples (one BAX[®] kit). A blank control tube was included in each lot of 48 (i.e.1 blank control plus 47 samples). Lysis of bacterial DNA was performed by incubating the tubes for 20 minutes in a waterbath at 37°C, followed by heating on a heating block for 10 minutes at 98°C to complete the lysis and inactivate the protease. Samples were then allowed to cool for 5 minutes. From each sample, a 50µl aliquot was transferred to a PCR sample tube, and another 50µl to a PCR control tube (provided in kit). The sample tubes contain a lyophilised tablet that contains all the reagents necessary for PCR (primers, enzymes and deoxyribonucleosides), while the positive control tube contains target nucleic acid as an internal quality check for PCR.

4.2.3.1.2 DNA amplification and detection area

The PCR was performed in a DNA thermal cycler (PTC-100, MJ Research, Massachusetts, USA) under the following conditions:

- hold period of 2 minutes and 10 seconds at 93°C (one cycle),
- hold period of 25 seconds at 93°C followed by hold period of 3 minutes and 10 seconds at 71°C (35 cycles),
- hold period of 7 minutes and 1 seconds at 71°C (one cycle), hold at 4°C.

Gels in the electrophoresis unit were than covered with 0.5xTBE running buffer (Appendix 1), and loading dye was added to PCR tubes by multi-channel pipette and mixed. PCR mix was than transferred to gel wells. Molecular weight marker DNA Mass Ladder (15µl) was added to the last well in each row. Amplified product was detected by agarose gel electrophoresis for 30 minutes at 180 volts using 2% Seakem[®] Gold Reliant[®] Agarose gels pre-stained with ethidium bromide (BMA, Rockland, USA). After that, gel was placed on transilluminator (Spectroline, New York, USA).

4.2.3.1.3 Reading test results

A positive result was indicated by a fluorescent band at the 752 base pair level (corresponds to the third band of six that arise in a line on the gel loaded with DNA Mass Loader), visualised by photographing (Polaroid DS34 camera/Polaroid Polaplan 665 film) the gel over a 312 nm ultra-violet transilluminator Gel (Aperture: f/4.5 – 8.0; Shutter speed: ¼-1 second) (Figure 4.6).

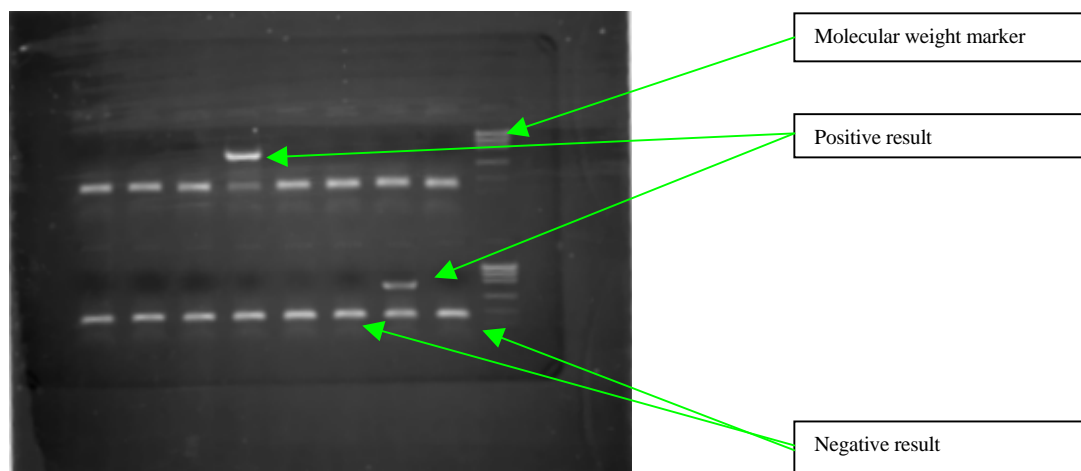


Figure 4.6: Result of BAX[®] PCR *Salmonella* test analysis on electrophoresis gel

4.2.3.1.4 Confirmation of BAX[®] PCR positive samples

For each field sample, a 1ml aliquot was collected from the incubated MPN culture, and transferred to a sterile 1.8ml centrifuge and stored at -85⁰C pending BAX[®] PCR test results. Aliquots from BAX[®] PCR positive samples were thawed and mixed by vortex mixer (IKA, Malaysia) and further processed along the lines as outlined in Chapter 3 (section 3.2.1). *Salmonella* organisms were isolated from the PCR-positive MPN cultures using conventional enrichment methods. Suspect colonies were confirmed to be *Salmonella* using a latex agglutination test with polyclonal *Salmonella* antisera (Serobact, MedVet Diagnostics, South Australia), and isolates were forwarded for serotyping at the Environmental Science & Research Institute, Kenepuru. All *Salmonella* were confirmed as *S. Brandenburg*.

4.2.3.2 Enumeration of *Salmonella* by MPN (quantitative analysis)

According to the protocol (Chapter 3, Section 3.4.), residual field samples were kept at +4⁰C pending BAX[®] PCR test results (Chapter 3, Section 3.4.b). The MPN method was performed on positive samples as per the procedure outlines in Chapter 3 (sections 3.4.f, 3.2.3.1.b and 4.2.3.2). Adding 235ml of sterile BPW to the sample before the sample was stomached modified the section 3.2.3.1(a). Thus, the base MPN score was multiplied by 2.35 to obtain the base MPN score for the sample. The base MPN was selected from the 3-tube MPN table²⁶.

For example, if the MPN score was 1.3.1, according to the 3-tube table, the base MPN will be 43. If the estimated total swabbed surface area for a lamb was 842cm², and for an ewe 1150cm², to obtain the MPN/cm² of the carcass swab sample the following formula was used:

$$\text{MPNcm}^2 = \text{base MPN} * 2.35 / (\text{total area swabbed} / 100)$$

Therefore, if the above hypothesised base MPN was:

- For lambs: $\text{MPN/cm}^2 = 43 \times 2.35 / (842 / 100) = 12.0$
- For ewes: $\text{MPN/cm}^2 = 43 \times 2.35 / (1150 / 100) = 8.8$

4.2.3 Analysis of data

The study was a pilot study and in-depth statistical analysis could not be applied to data from this study because of the process for farm selection ('worst-case scenario'). Descriptive analysis was used because of this lack of independence of the samples, and the small number of farms involved in the study.

²⁶ Table 1, FDA Bacteriological Analytical Manual, 8th Ed (Revision A)/1998 (page App. 2.07)

4.3 Results

4.3.1 Summary of overall results

Of the 8 selected farms, one case farm did not have cull ewes available for slaughter in the period that sampling was scheduled. Consequently, the study was reduced to ewes and lambs from 6 farms (3 case, 3 non-case). In some mobs, the target sample size was not achieved due to lack of availability of stock. A total of 1417 carcasses were sampled in the study. Of these, 1214 carcasses were of animals sourced from the 3 case and 3 non-case properties in the affected region of the South Island. The remaining 203 carcasses were sampled at the 2 North Island plants. A total of 138 (11.3%) of the 1214 samples obtained from the South Island plant were positive for *S. Brandenburg* by BAX[®] PCR, while *Salmonella* were not detected by in any of the samples obtained from the North Island plants. The vast majority (130 or 94%) of the 138 BAX[®] PCR positive samples was obtained in the first period of sampling, indicating a substantial decline in risk of carcass contamination in the period between the first and second samplings.

4.3.2 First sampling – (November/December 2000)

4.3.2.1 BAX[®] PCR detection of *Salmonella* (Qualitative results)

During the first sampling period, a total of 602 samples were tested (i.e. 301 samples from case, and 301 samples from non-case farms). Qualitative results indicated that the proportion of BAX[®] PCR *Salmonella* positive carcasses sourced from case farms was being approximately twice that detected on carcasses from non-case farms (Table 4.2).

Table 4.2. Proportion of *Salmonella* positive carcasses by BAX[®] PCR from case and non-case farms at first sampling

<i>Farm Status</i>	<i>First sampling (November/December 2000)</i>		
	<i>n Carcasses</i>	<i>n positive</i>	<i>% positive</i>
Case	301	84	28
Non-Case	301	46	15

In total, the proportion of lamb carcasses that were positive for *Salmonella* organisms was approximately threefold that observed for ewe carcasses (Table 4.3).

Table 4.3. Proportion of *Salmonella* positive lamb and ewe carcasses by BAX[®] PCR at first sampling

<i>Class of stock</i>	<i>First sampling (November/December 2000)</i>		
	<i>n Carcasses</i>	<i>n positive</i>	<i>% positive</i>
Lambs	307	101	33
Ewes	295	29	10

4.3.2.1.1 Qualitative results by individual farms of origin

The proportion of *S. Brandenburg* positive carcasses varied dramatically among individual mobs from case and non-case farms (Tables 4.4 and 4.5). Most notably, PCR-positive carcasses were detected among all mobs of lambs, and overall prevalence was comparable for lamb mobs from both case and non-case farms. However, positive carcasses were detected in only 2 of the 6 ewe mobs (both case farms), and the large majority (26 of 29) of positive ewe carcasses were clustered within a single case mob.

Table 4.4. Proportions of *Salmonella* positive carcasses by BAX[®] PCR from case farms at first sampling

<i>Case Farm</i>	<i>Lambs</i>			<i>Ewes</i>		
	<i>n Carcass</i>	<i>n positive</i>	<i>% positive</i>	<i>n Carcass</i>	<i>n positive</i>	<i>% positive</i>
C1	52	8	15	54	3	5.5
C2	51	21	41	43	26	60
C3	51	26	51	50	0	0
Total	154	55	36	147	29	20

Table 4.5: Proportions of *Salmonella* positive carcasses by BAX[®] PCR from non-case farms at first sampling

<i>Non-Case Farm</i>	<i>Lambs</i>			<i>Ewes</i>		
	<i>n Carcass</i>	<i>n positive</i>	<i>% positive</i>	<i>n Carcass</i>	<i>n positive</i>	<i>% positive</i>
NC1	51	27	53	51	0	0
NC2	51	15	29	51	0	0
NC3	51	4	8	46	0	0
Total	153	46	30	148	0	0

4.3.2.1.2 Qualitative results by point of sampling during processing

For each lamb mob sampled, approximately one third of carcasses were sampled at each of 3 points during processing (slaughter floor, cooler, boning room). The proportion of PCR-positive results tended to decline from the first (slaughter floor) to the final (boning room) sampling, and no positive *Salmonella* results were detected for any samples collected in the boning room (Tables 4.6 and 4.7).

Table 4.6. Proportion of *Salmonella* positive test samples by BAX[®] PCR from lambs from case farms at first sampling

<i>Farm</i>	<i>Lambs – Period A</i>								
	<i>Slaughter floor</i>			<i>Cooler</i>			<i>Boning room</i>		
	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>
C1	18	6	33	17	2	12	17	0	0
C2	17	13	76	17	8	47	17	0	0
C3	17	15	88	17	11	65	17	0	0
Total	52	34	66	51	21	41	51	0	0

Table 4.7. Proportion of BAX[®] PCR *Salmonella* positive test samples from lambs from non-case farms at first sampling

<i>Farm</i>	<i>Lambs – Period A</i>								
	<i>Slaughter floor</i>			<i>Cooler</i>			<i>Boning room</i>		
	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>
NC1	17	17	100	17	10	58	17	0	0
NC2	17	11	65	17	4	24	17	0	0
NC3	17	4	24	17	0	0	17	0	0
Total	51	32	63	51	14	28	51	0	0

In contrast, for ewe mobs positive BAX[®] PCR results were obtained only for samples from case farms and all samples from non-case farms were negative (Tables 4.8 and 4.9). Similar to the trend seen with lambs, the proportion of PCR-positive results was highest on the slaughter floor, and no positive results were obtained for samples collected in the boning room.

Table 4.8. Proportion of *Salmonella* positive test samples by BAX[®] PCR from ewes from case farms at first sampling

<i>Farm</i>	<i>Ewes – Period A</i>								
	<i>Slaughter floor</i>			<i>Cooler</i>			<i>Boning room</i>		
	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>	<i>n</i> <i>Carcass</i>	<i>n</i> <i>Positive</i>	<i>%</i> <i>positive</i>
C1	20	1	5	17	2	12	17	0	0
C2	17	16	94	12	10	83	14	0	0
C3	17	0	0	16	0	0	17	0	0
Total	54	17	31.5	45	12	26.7	48	0	0

Table 4.9. Proportion of *Salmonella* positive test samples by BAX[®] PCR from ewes from non case farms at first sampling

<i>Farm</i>	<i>Ewes – Period A</i>								
	<i>Slaughter floor</i>			<i>Cooler</i>			<i>Boning room</i>		
	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>	<i>n</i> <i>Carcass</i>	<i>n</i> <i>positive</i>	<i>%</i> <i>positive</i>
NC1	17	0	0	17	0	0	17	0	0
NC2	17	0	0	17	0	0	17	0	0
NC3	17	0	0	13	0	0	16	0	0
Total	51	0	0	47	0	0	50	0	0

4.3.2.2 Enumeration of *Salmonella* by MPN (quantitative results)

The mean area of 17 carcass surface swabbed on lambs was 842 square centimetres (SD 62), comprised of mean areas of 375, 251, and 215 cm² for the ventral cut, Y-cut, and bung respectively (Table 4.10). Variability in area swabbed was greatest for the bung (CV 22%), being considerably higher than for the other sites (5%, 9%).

Table 4.10. Areas (cm²) of sites swabbed on lamb carcasses at first sampling

<i>Lambs</i>	<i>Ventral</i>	<i>Y-cut</i>	<i>Bung</i>	<i>Total Swab</i>
Mean	375	251	215	842
SD	18.0	22.4	48.7	61.9
CV	4.8	8.9	22.7	7.4

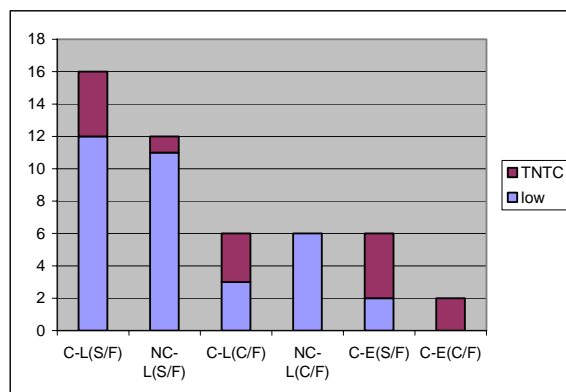
For 17 ewe carcasses, the mean area of carcass surface swabbed on lambs was 1150 cm² (SD 30), comprised of mean areas of 520, 307, and 323 cm² for the ventral cut, Y-cut, and bung respectively.

The MPN procedure was limited to the first 48 positive carcasses owing to financial constraints, because the number of PCR-positive cultures was much higher than anticipated when planning the project. Thus these observations cannot be considered representative of all positive carcasses in the study because they were not randomly selected. However, because of the nature of the study the farms themselves also were not selected at random, and therefore random selection within an already biased sample would arguably be of little advantage. Regardless, the observations in this pilot study do indicate a potential range of expected values, and point to the need to include higher dilutions when enumerating *Salmonella* on carcass swabs using these methods. Of the 48 MPN tests, fourteen carcasses were arbitrarily assigned the maximum MPN base number of 240 according to the 3-tube table²⁷ used. These were characterised as TNTC (too numerous to count) and their proportion compared to those with the low MPN base number (MPN <240) is presented in Figure 4.7. Of the 14 TNTC carcasses, 10 were from a case farm (C2) with 6 being from ewes and four from lambs. There was a little difference in the proportion of positive lamb and ewe carcasses at the slaughter floor and the cooling floor. A further three were from another case farm (C3) with all being from lambs. Again there was a little difference in the proportion of positive lamb and ewe carcasses at the slaughter floor and the cooling floor. The remaining TNTC carcass was from a non-case farm (NC1) from a lamb sampled at the slaughter floor.

Quantitative results (log₁₀ MPN/100cm²) were obtained for the remaining 34 carcasses. In these, *Salmonella* counts per 100cm² ranged from -0.25 to 0.85 logs, and were of similar order for positive samples of lambs from both case and non-case farms and between positive samples of lambs and ewes from case farms. Counts were also similar for swabs collected on the slaughter floor or in the cooler (Table 4.11).

²⁷ Table 1, FDA Bacteriological Analytical Manual, 8th Ed (Revision A)/1998 (page App. 2.07)

Figure 4.7: Total number of *Salmonella* positive carcasses of lambs or ewes collected on the slaughter floor (SF) or cooling floor (CF) where MPN number was higher than 240 (TNTC), or <240 (low)



Legend: C-L(S/F – slaughter floor) – case farms lamb carcasses; NC-L (S/F – slaughter floor) – non-case farms lamb carcasses; C-L (C/F – cooling floor) – case farm lambs; NC-L (C/F – cooling floor) – non-case farms lambs; C-E (S/F – slaughter floor) - case farms ewes; C-E (C/F – cooling floor) – case farms ewes.

Table 4.11. MPN counts (\log_{10} MPN/100cm²) of 34 BAX[®] PCR *Salmonella* positive test samples at the first sampling period (MPN number less than < 240)**

<i>Farm</i>	<i>Lambs</i>				<i>Ewes</i>	
	<i>S/floor</i>		<i>Cooler</i>		<i>S/floor</i>	
	<i>n</i> <i>Carcass</i>	<i>Mean</i> <i>Log₁₀/100cm²</i>	<i>n</i> <i>Carcass</i>	<i>Mean</i> <i>Log₁₀/100cm²</i>	<i>n</i> <i>Carcass</i>	<i>Mean</i> <i>Log₁₀/100cm²</i>
C1	6	-0.10	2	-0.25	1	-0.39
C2	2	0.71	1	-0.25	1	0.94
C3	4	0.75	0	-	0	-
Mean*	-	0.45	-	-0.25	-	0.27
C1 to C3 - Total No. of positive carcasses	12	-	3	-	2	-
NC1	5	0.63	2	1.11	0	-
NC2	4	0.64	4	0.12	0	-
NC3	2	1.29	0	-	0	-
Mean*	-	0.85	-	0.62	-	-
NC1 to NC3 - Total No. of positive carcasses	11	-	6	-	0	-

Legend: S/floor – slaughter floor

*Mean of the means

**Note: All samples collected from lamb and ewe carcasses in the boning room were negative for *Salmonella*

4.3.3 Second sampling – Period B

When the sampling protocol was repeated on mobs of lambs and ewes from the same farms during February 2001, only 8 (1.3%) of 612 carcass swabs were positive for *Salmonella*. The positive results were equally distributed among case and non-case farms (4 each). Six positive results were from lamb carcasses, and 2 from ewe carcasses. Four of the positive lamb carcasses were sourced from a single case farm (C1), and single positive lamb carcasses were sourced from two non-case farms (NC1 and NC3). Both positive ewe carcasses were sourced from NC3. Positive swabs were all collected on either the slaughter floor (6) or the cooler (2) (Table 4.12), and all boning room samples were negative. However, among the positive carcasses, numbers of *Salmonella* detected were of the same order of magnitude as that observed on positive carcasses during the first sampling period.

Table 4.12. Numbers of *Salmonella* detected by the MPN method in swabs of 8 BAX[®] PCR *Salmonella* positive sheep carcasses at the second sampling

<i>Animal</i>	<i>Farm</i>	<i>Site</i>	<i>MPN/100cm²</i>	<i>Log₁₀MPN/100cm²</i>
Lamb	C1	SF	11.17	1.05
Lamb	C1	SF	<1	-0.25
Lamb	C1	CF	42	1.62
Lamb	C1	CF	6.4	0.81
Lamb	NC1	SF	4.19	0.62
Lamb	NC3	SF	<1	-0.25
Ewe	NC3	SF	224	2.35
Ewe	NC3	SF	<1	-0.25
Mean				0.71

Legend: SF – slaughter floor; CF – cooling floor

4.3.4 Discussion

Apart from anecdotal reports of sporadic isolations of *S. Brandenburg* on sheep meat sampled by processors, at the commencement of the study there were no data available to indicate the scale of contamination of sheep meat with this organism.

The key findings from the study were:

1. Prevalence of carcass contamination was considerably higher among South Island samples (11.3%) compared with North Island samples (0%) and baseline national

data from the National Microbiological Database (Table 1.2)(NMD, 2001). Therefore, the risk of carcass contamination with *Salmonella* Brandenburg is markedly elevated in the region where sheep flocks experienced abortion outbreaks due to this organism. All PCR *Salmonella* positive samples were also sent for further investigation as a part of a companion study, and all were subsequently confirmed as *Salmonella* Brandenburg. These observations indicate that risk of carcass contamination with *Salmonella* is markedly elevated in the region where sheep flocks experienced abortion outbreaks due to this organism.

2. Risk of carcass contamination was greatly reduced by the second sampling, indicating marked temporal variation in risk of carcass contamination. Peak risk is likely to be when animals are first shipped for slaughter following the outbreak, that is in the initial weeks of operation of plants in late spring.
3. Although clinical *S.* Brandenburg enteric disease has not been reported in lambs, overall risk of contamination was higher (33%) for lamb carcasses than ewe carcasses (10%) from the same farms. In addition to domestic public health concerns, this has important implications for industry given the importance of lamb exports to New Zealand.
4. For lambs, the risk of carcass contamination was comparable for lambs sourced from case or non-case farms in the affected region. In contrast, the risk of ewe carcass contamination was strongly clustered to case farms during the first sampling period, and only 2 samples were positive from non-case farms (both at the second sampling). The majority of positive ewe carcasses were sourced from a single case property (C2). The observation that swabs from all carcasses from one case farm (C3) were culture negative at the first sampling, compared with 5.5% (C1) and 60% (C2) positive carcasses from the other two case farms suggests broad variability in risk among mobs of animals that have experienced the disease. During the second sampling, two ewe samples were positive from non-case farms while no positive ewe carcasses were detected.

5. The location of sampling carcasses (e.g. slaughter floor, cooler) influenced estimates of prevalence of contamination, but estimates of bacterial numbers on positive carcasses were generally similar regardless of class of stock, time of sampling, or sampling location in the plant.

Collectively these findings indicate that the emergence *S. Brandenburg* infection of sheep in the South Island has considerable implications for product safety and public health. Consequently, a strong case can be made for more definitive research to develop risk management strategies for this problem. The currently preferred approach for foodborne hazards of this nature is to undertake a quantitative risk assessment to estimate the risk to consumers and to assess potential risk management interventions.

For purposes of risk assessment and predictive modelling, ideal data would describe the distribution of levels of *Salmonella* contamination on sheep carcasses (or specific derived meat products from these carcasses) among the population of animals slaughtered at a plant (or region) over time. Both true biological variability (e.g. variation among flocks, classes of stock, temporal changes) and measurement uncertainty (including sampling error and methodological limitations) impact on the validity of the estimates that have been obtained for application to quantitative risk assessment (Nauta, 2000). The data obtained in this study have provided valuable insights into several important aspects of the issue, but due to logistic and other constraints have considerable shortcomings with respect to the requirements of formal quantitative risk assessment. The following discussion evaluates these data obtained in terms of representativeness and suitability for risk assessment purposes.

Sampling factors that could contribute uncertainty to the estimates include the process of selection of flocks for study; the selection of carcasses within flocks to be sampled, and the sampling procedures for swabbing and enumeration of *Salmonella*. Formal random sampling was not conducted because of logistic difficulties and the absence of any data on expected prevalence to guide sampling procedures. Given the time lag of around 3 months between occurrence of clinical disease and the time when lambs (or cull ewes) were sent for slaughter, there was a possibility that prevalence of carcass contamination

could be very low. Data from the National Microbiological Database (NMD, 2001), which are broadly representative of carcasses processed at export plants across New Zealand, indicate a base-line prevalence of the order of 0.4% for fresh carcasses. If risk of carcass contamination was only modestly elevated, or only on severely affected farms, random sampling of all carcasses or flocks in the region (which would give a valid indication of regional risk) may not have identified elevated risks associated with outbreaks. Also, the low number of farms that could be included was inadequate to achieve representative estimates even if formal random selection had been used.

This combination of factors underpinned the decision to purposively include case flocks that had experienced severe outbreaks, together with an equivalent number of unaffected farms in the same region. The deliberate bias to sample case flocks with more severe outbreaks, reflecting a 'worst-case scenario', should have maximised the likelihood that any increase in risk of carcass contamination due to disease outbreaks in flocks would be detected.

Within farms, the convenience sampling of culled ewes and lambs for slaughter was preferred, so that selection of animals for the study was determined by normal marketing practices of the farms. That is, the groups slaughtered represented a valid sample in time of the ewes and lambs leaving the farm for slaughter. The selection of times for sampling farms considered several factors.

Firstly, risk was expected to be greatest for animals slaughtered first following an outbreak. Therefore the initial sampling round was scheduled as early in the slaughter season as possible. Secondly, due to logistic constraints imposed by fixed laboratory resources, only two farms could be processed within a week. Therefore each 'point' sampling of the 6 farms was spread over several weeks. Thirdly, the interval between sampling rounds, approximately 2 months led to the second sampling being around the seasonal peak for lamb slaughter. Finally, studies of *Salmonella* in several species suggest that experimentally infected animals will become culture negative over a period of about 2 months (Berends *et al.*, 1996).

For this study, and the companion (synchronised) study of faecal and caecal prevalence, it was considered important to sample mobs of cull ewes and lambs. Foodborne risks are likely to differ between classes of stock due to many factors, the most obvious being the preceding occurrence of disease outbreaks (clinical disease not reported in lambs) and different destinations of subsequent meat products. Given the absence of data on expected prevalence, the target sample size of 50 sheep per mob was considered adequate to give reasonable estimation of prevalence of carcass contamination under the worst case assumptions (infinite population, 50% prevalence). However, stratification of carcasses across three points of processing (n = 17 per mob per sampling location) leads to broad confidence limits for prevalence estimates at each respective point of processing. As such, the sampling could only indicate very marked differences between sampling locations (e.g. slaughter floor and boning room).

Similar to the strategy for selecting farms, sampling of carcasses was also biased to sites where probability of contamination was higher (Biss and Hathaway, 1996a). The selected swabbing areas for composite swabs during different stages of processing (slaughter floor, cooler) was guided by previous research indicating that the areas of forequarter region where skin incisions are made during dressing (Y-cut) are the areas of highest contamination, and that little increase in contamination occurred after pelt removal (Bell and Hathaway, 1996). The abdominal and bung areas were selected based on the likelihood of fleece or faecal contamination. The unavoidable differences in sampling procedures for the boning room (prime cuts) compared to the other locations (e.g. high-risk carcass locations) also could account for differences observed.

Thus neither the qualitative estimates for prevalence of contaminated carcasses, nor the quantitative estimates of bacterial populations can be considered to reflect average population values. However, if interpreted as ‘worst case scenarios’, the estimates could arguably be used to specify upper limits of distributions for purposes of quantitative modelling.

A somewhat unexpected observation was that the prevalence of contaminated lamb carcasses was high (33%) and very similar for case and non-case farms during the first sampling period. This could be equally be explained by either:

1. Widespread exposure and infection of lambs with *S. Brandenburg* throughout the affected region (with clinical outbreaks determined by management or other factors unrelated to the existence of the agent); or
2. Cross-contamination of lambs or carcasses during transport, lairage, or processing.

Some insight into these possibilities can be gained from the results of the companion study of faecal and caecal prevalence of *Salmonella* in the same mobs of sheep carried out synchronously with this study during the first sampling. Key findings of that study (Motsamai, 2002; Motsamai *et al.*, 2002), discussed in the context of the results for carcasses in this study were:

1. *S. Brandenburg* was isolated from all 8 farms, and in faecal samples collected from sheep on seven of the farms prior to transport. This supports the contention that *S. Brandenburg* has become widely established in the affected region, and that *Salmonella* contamination of sheep with other serovars was of negligible concern in this region at the time of the study;
2. The proportion of positive samples declined markedly in the period from between the first and second samplings, indicating that the risk of carcass contamination observed in this study reflects the prevalence of infected animals arriving at plants observed in the companion study;
3. There was minimal difference in faecal or caecal isolation rates between lambs and cull ewes, indicating that elevated risk occurs in both classes of stock. The higher risk of carcass contamination observed in lambs cannot be readily explained by higher proportion of infected animals arriving at the plant. This suggests that cross-contamination of lamb carcasses may be more common than for ewe carcasses;
4. Results for faecal and caecal samples were very similar, suggesting that transport and lairage had minimal impact on the enteric *Salmonella* status of animals;

5. *Salmonella* were more frequently detected on case farms than non-case farms, although positive results were strongly clustered within farms. For the first sampling period, the prevalence ratios (case versus non-case) for all lamb samples (fecal and caecal) was 3.0, and for all ewe samples was 5.5. The corresponding ratios for carcass contamination in the first sampling were 1.18 for lambs (indicating little difference in risk for lambs from case or non-case farms), while that for ewes was undefined (as no non-case ewe carcasses were positive). These differences among the two studies again point to cross-contamination being a greater problem for lamb carcasses rather than ewe carcasses. It is also notable that high MPN counts (TNTC) (Figure 4.2) were much more common among samples from case farms compared with non-case farms. For future studies, higher 10-fold dilutions should be included in the MPN procedures.

From a public health perspective, an important finding was that no PCR-positive prime cut samples were detected in the boning room (after carcasses storage for 24 hours/7⁰C). This finding correlates with previous research which suggested that the level of visible contamination on the hind leg area (primal cut) site was relatively low compared to other sites that are more prone to fleece or faecal contamination (Biss and Hathaway, 1996a). However, failure to detect *Salmonella* at this location should not be interpreted to indicate complete absence of viable *Salmonella*, but that the numbers of organisms on the sites swabbed were below thresholds of detection with the methods employed. For example, sampling by excision rather than swabbing could result in more sensitive detection. Such techniques can vary widely and be of equivalent value for recovery of bacteria from fresh carcasses (Gill and Jones, 2000). However, it has been reported that in experimental conditions recovery of bacteria from carcass tissue stored for 24 hours at 7⁰C was more successful by using incision technique than sponge swabbing (Ware *et al.*, 1999). Ability to detect *Salmonella* by enrichment culture of contaminated samples is affected by the numbers of both *Salmonella* and competing organisms (Jameson, 1962), and whether the *Salmonella* may be sub-lethally injured. It has been found that selection of appropriate pre-enrichment media that enable controlled release of selective agents significantly improves the rate of detection (Baylis *et al.*, 2000). Qualitative and quantitative estimates of contamination need to be considered in the light of the methods employed to derive them, all of which have limitations.

CHAPTER 5: Analysis of available microbiological data in the context of risk assessment, and identification of future research needs

5.1 Introduction

S. Brandenburg disease in ewes in the South Island of New Zealand is a novel animal disease. It has been associated with occupational human infections, but as yet no confirmed foodborne cases have been identified. The studies reported in Chapter 4 indicate that significant risk of contamination of sheep carcasses with *S. Brandenburg* exists in the affected region, particularly during the early part of the slaughter season (November/December). These findings support the need for an active and objective response to this issue, and a quantitative risk assessment approach has been proposed (Hathaway *et al.*, 2000a). The purpose of this Chapter is to further develop the 'farm to table' framework presented in Chapter 2 (Figure 2.4) by integrating the data obtained in Chapter 4 and from other sources. The discussion is focused on the animal production, transport-lairage, and slaughter-processing modules, where some data are now available from studies conducted under the umbrella of the QRA project, and directed towards identifying future research priorities.

5.2 Animal production module

With respect to modeling risk of foodborne disease due to *S. Brandenburg*, the key parameters from the animal production module are the:

- Prevalence of infected farms,
- Prevalence of infected sheep within farms when selected for slaughter,
- Numbers of organisms in/on infected sheep when selected for slaughter.

For purposes of this discussion, the term disease refers to the occurrence of clinical disease (abortion or death) due to *S. Brandenburg* in ewes. Infection describes the state in which an animal harbours *S. Brandenburg* in its gastrointestinal tract or other organs; and contamination refers to the presence of the organism on the external surface of an animal (e.g. fleece) or on any other object. However, the term infection is also applied to farms

(or mobs) to indicate that the organism is present in the farm environment (including sheep) or mobs of animals. For a given slaughter facility, the probability of contamination of a single carcass is presumed to be a function of the proportion of supplying farms that are infected with *S. Brandenburg*, the distribution of within-mob prevalence of colonisation (or contamination), and numbers of organisms in mobs of animals supplied from those farms (Figure 5.1).

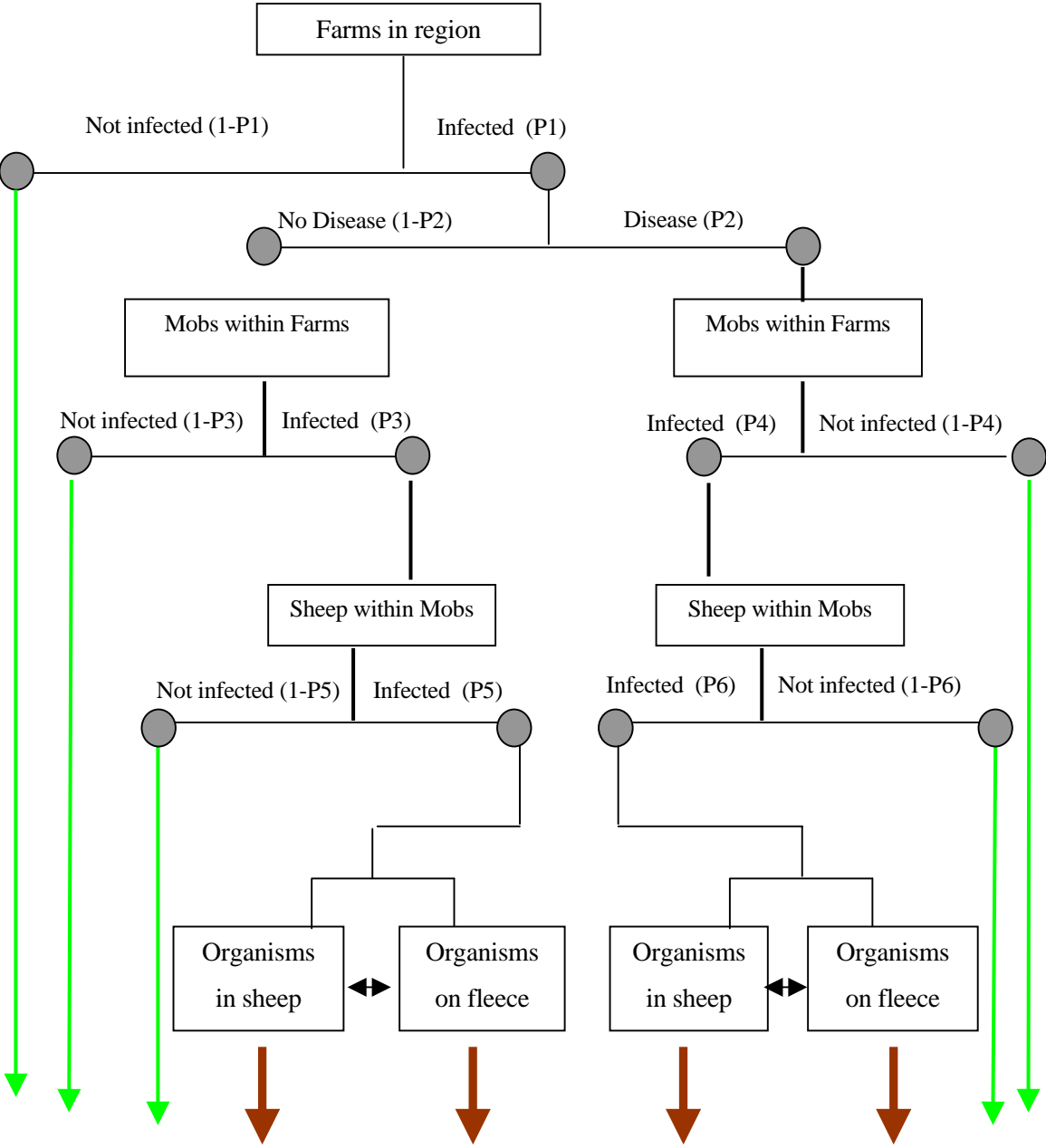


Figure 5.1: Probability scenario tree for Salmonella *Brandenburg* infection or fleece contamination of sheep on farms

5.2.1 Prevalence of infected farms in the region

At the regional (or supplier base) level, the unit of interest is the sheep farm. There are approximately 13,500 sheep farms in the South Island. Based on farms registered in the national farm database (AgriBase), approximately 5000 of these are located in the Southland/Otago region regions where this disease problem is of greatest concern. The number of sheep per farm is highly variable, but the majority of the farms appear to manage between 500-7000 sheep (New Zealand census, 1999).

There have been no surveys to determine what proportion of sheep farms may be infected (P1 in Figure 5.1), nor the proportion of infected farms that experience outbreaks of clinical disease (P2). Thus the actual proportions of farms in these categories are unknown, but data from various sources allow inferences of the likely range in prevalence of infected farms.

The number of flocks in which *S. Brandenburg* disease is diagnosed is a conservative estimate of the population of properties experiencing disease. Since the diagnosis on the index case farm in Canterbury in 1996 (Bailey, 1997), Figure 5.2 shows the numbers of laboratory confirmed farms from 1997 to 2000 (Clark, 2001). Subsequently, the total numbers of laboratory diagnosed farms in Otago and Southland has declined from the peak of 295 in 2000, to 208 and 106 respectively in 2001 and 2002 respectively (Clark, G. – personal communication, 2002). The apparent reduction in reported cases since 2000 could be due to a decline in disease incidence, or reporting bias as farmers and veterinarians become accustomed to the disease. The rapid spread of this epidemic over a few years also has implications for estimating risk, as flock prevalence is likely to be both temporally and spatially heterogeneous. Hence for purposes of modeling, it is important to define the regional scope of any analysis.

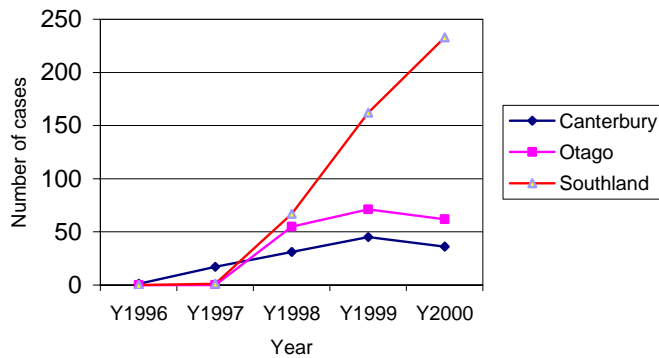


Figure 5.2. Sheep farms with laboratory confirmed cases of *S. Brandenburg* infection in affected regions in the South Island (1996 to 2000)

In a mail survey of over 400 Otago and Southland farms not thought (based on veterinary practice records) to have experienced *S. Brandenburg* disease in 2000, approximately 20% of respondents indicated that *S. Brandenburg* disease had occurred in their flocks (Davies *et al.*, 2002). Although the veracity of these responses is uncertain, they do confirm that many farms that experienced disease events consistent with *S. Brandenburg* did not seek veterinary or laboratory confirmation, and therefore laboratory data are likely to greatly underestimate the number of farms experiencing outbreaks.

Furthermore, Motsamai (2002), in a companion study to the work reported in Chapter 4, isolated *S. Brandenburg* from sheep faeces on 3 of 4 farms that had no history of disease, confirming that infection may occur commonly in the absence of clinical disease. The negative result on one of the four farms must be considered in the context of the sampling protocol. The methods used were chosen to obtain preliminary estimates of animal prevalence in the case (worst-case scenario) versus control groups. Given the imperfect sensitivity of faecal culture (possibly of the order of 50%), the number of animals tested on farm (approximately 100 animals) would be insufficient to reliably detect the organism in flocks with low prevalence. For example, using the FreeCalc software (AusVet Animal Health Services, Australia), and assuming test sensitivity and specificity of 0.5 and 1.0 respectively, the probability of all 100 samples testing negative from a flock of 2500 sheep with a within herd prevalence of 6% is of the order of 5%. A sample of 195 animals would be required to be 95% confident of detecting one or more infected animals if within flock prevalence was 3%.

Although there are limitations to these sources of data, both studies point to the likelihood that a majority of farms in the Southland/Otago region may harbour the organism, with or

without observation of clinical disease. Based on reports in other species, efforts to eradicate *Salmonella* organisms from previously affected properties are likely to be impractical, and it is probable that few infected farms are likely to revert to a 'non-infected' state, at least within a few years. At least in the epidemic region, evidence points to a high prevalence (50% to 100%) of infected farms.

There are no data to indicate rates of interfarm spread of *S. Brandenburg*, and no data on what mechanisms may be important. Mechanisms of long distance spread are likely to differ from mechanisms of spread among neighbouring properties. Potential routes based on published data in other species or from first principles of disease transmission (Figure 5.3) include:

- Stock movements – including buying and selling of stock (sheep and other species), off-farm grazing of sheep and grazing of stock from other farms.
- Movements of wild or feral animals, including seagulls, dogs and cats, rodents, ferrets, stoats, possums, rabbits, reptiles, insects,
- Vehicles – stock and fertilizer trucks,
- People – field visits, shearers, veterinarians, scanners etc, with dogs,
- Water,
- Feed – hay, silage, root grazing, concentrates, grain, feed for other species on farms,
- Wind/dust.

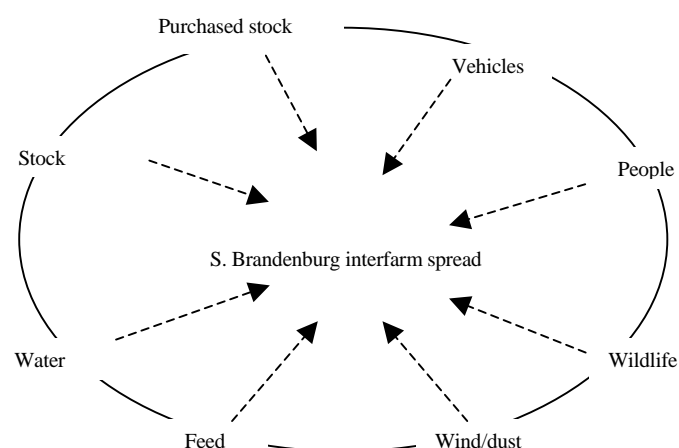


Figure 5.3. Potential routes for regional interfarm spread of *S. Brandenburg*

Although knowledge of the relative importance of these potential routes of interfarm transmission is almost negligible, it is questionable whether future research is warranted to reduce this uncertainty. The current evidence suggests that *S. Brandenburg* infection may already be widespread among sheep farms and other locations in the affected regions. Furthermore, published literature is consistent with the view that all the routes listed above constitute potential routes for introduction, and that their relative importance is likely to vary among farms and over time. Research to define their relative importance would be expensive, and some of these routes may not be amenable to management changes. Any efforts to manage risk by preventing further interfarm spread should be focused at the regional rather than a local level, to reduce the probability of other regions of New Zealand becoming affected. Given the frequency of stock transport in New Zealand, it is likely that *S. Brandenburg* has been disseminated into other regions, but disease has yet to be reported. As the disease is yet to occur in most regions of New Zealand, application of basic biosecurity principles for flocks purchasing stock is obviously prudent. However, within the currently affected regions, if one assumes that the organism is widely disseminated in the ecosystem, risk mitigation efforts should be focused on reduction of the prevalence of the organism on affected farms (if practicable) or in the post farm sectors.

5.2.2 Within-farm prevalence of *S. Brandenburg*

Sheep on farms are not a homogeneous population, as animals of different ages (and sex) are typically managed in separate mobs. However, animals within mobs tend to be relatively homogeneous (e.g. two tooth, mixed age ewes, lambs), and potential variability in prevalence between mobs needs to be acknowledged. Animals sent for slaughter comprises culled breeding stock and lambs. Some lambs may be sent for slaughter before weaning (i.e. still in contact with breeding females), but most will be marketed some time after weaning (i.e. from mobs of lambs assembled at weaning). Most infections of animals with *Salmonella* organisms are asymptomatic. The occurrence of clinical disease may increase exposure of unaffected animals on a farm and hence the probability that infected or contaminated animals enter the food chain (Figure 5.1). The dynamics of transmission of the agent and occurrence of clinical disease must be considered in the context of their ultimate impact on the prevalence of infected and contaminated stock leaving farms for slaughter. The pilot study of Motsamai (2002)

provides estimates of the point prevalence of faecal shedding at this time for both case (P6 in Figure 5.1) and control (P5 in Figure 5.1) farms. The following points are important for consideration in developing a quantitative model:

- Prevalence estimates (mean 15%) at the first sampling in infected mobs ranged from 2% (single animal positive) to 56%, indicating considerable clustering of positive results within farms. As expected with a binomial variable and small sample size, the distribution of within farm prevalence was not normal (SD 17%). Hence, a lognormal or beta distribution would be more appropriate for modelling the distribution of within farm (or mob) prevalence,
- Some heterogeneity was observed between mobs within farms, which may be attributable to true variability or to uncertainty due to measurement error.
- Overall, at the first (November) sampling, the pooled prevalence (noting clustering in particular mobs) for 5 infected mobs from case farms (20%) was twofold that in 3 infected mobs from non-case farms (10%),
- The estimates do not consider the sensitivity of faecal culture and are therefore likely to be conservative,
- Marked temporal variability (approximately 5 fold reduction) occurred over a 2 month period between samplings,
- No data are available on fleece contamination.

5.2.2.1 Epidemiology of *S. Brandenburg* infection in sheep

The epidemiology of *S. Brandenburg* infection in sheep is still poorly understood. The relative importance of the different transmission pathways between and within sheep flocks has not been established, particularly in relation to the establishment and the spread of infection within a flock. Most often, the presence of the infection is diagnosed when flocks experience clinical signs of the disease manifested by an abortion storm in pregnant ewes. Sheep, in common with cattle and poultry, appear to be more susceptible to *Salmonella* infection by inhalation of the organism than by ingestion (Robinson, 1967). Under natural conditions, the infectious dose of *S. Brandenburg* for sheep is not known.

The duration of shedding of the organism is also unknown, but likely to be highly variable among animals and dose dependent. In experimental studies in several species, faecal shedding tends to decline to very low levels over a period of approximately 2 months

(Berends *et al.*, 1996). Existing information on shedding of *S. Brandenburg* by sheep is largely anecdotal. In experimental conditions, the organism was detected in the faeces of infected pregnant sheep within 2 days of exposure (Christensen *et al.*, 2002). Ewes may shed the organism for up to 6 months, and *S. Brandenburg* has been detected in healthy ewes during scanning for pregnancy (Clark G. – personal communication, 2002).

Following the onset of the disease, the spread within a flock may be due to licking aborted foetuses and placenta and from ingestion of contaminated faeces (Clark *et al.*, 1999b). The average duration of the abortion outbreaks on affected farms was 29 days, and abortions occurred primarily in ewes with multiple lambs (Boxall *et al.*, 1999). Following an abortion, large numbers of the organism are shed from aborted foetuses and placentae resulting in heavy contamination of the environment, and sometimes waterways (Clark, 2001). On one occasion only has *S. Brandenburg* been linked to disease in non-pregnant sheep, when the organism was isolated from an 8-month-old sheep with gastroenteritis (Clark *et al.*, 1999a). However, there are no data on the survivability of *S. Brandenburg* in different environmental conditions that give insight into the predominant sources and modes of transmission of the organism.

Kerslake *et al.*, (2002) considered that management and environmental factors may play an important role in explaining the recurrent outbreaks on farms that have previously experienced the disease. Information on some management practices, gathered from randomly selected farms suggest that the grazing management and feeding practices to be the most important determinants of the occurrence of *S. Brandenburg* disease. Most strikingly, the practice of strip grazing, particularly in combination with back fencing was strongly associated with the risk of disease outbreaks (Davies *et al.*, 2002). Both the incidence and severity of disease may be greater in mixed age mobs compared with hogget and two-tooth mobs. Of 176 affected farms in 2000, only 8 farms (4.5%) reported *S. Brandenburg* disease in hoggets, with an average risk of 5.2% for abortions and 1.0% for ewe deaths. In contrast, 78 (44.3%) and 172 (97.7%) of affected farms reported outbreaks in two-tooth and mixed-aged ewes respectively. Within the affected farms that managed hogget and two-tooth ewe flocks in 1999, the cumulative abortion incidence (i.e. attack rate) ranged from 0.4%-30%. The cumulative abortion incidence for mixed-age ewe flocks ranged from 0.3% to 17.3% (Perkins *et al.*, 2000). The wide variability in

severity of disease among mobs may reflect highly variable levels of flock exposure and consequently within mob prevalence of infection and contamination at time of slaughter.

The potential role of various environmental factors on the disease occurrence is not well understood. Davies *et al.* (2002) indicated that the disease is more likely to occur in mobs grazed on flat, rather than hilly terrain. Bailey (1997) indicated that the worst affected mob was grazed in a very muddy paddock, visited by a large number of ducks some months before the disease outbreak. It appears that there is no rapid substantial increase in flock immunity following an outbreak and that farms may continue to experience outbreaks in the future (Kerslake *et al.*, 2002). It has been suggested that mass medication of aborting mobs of ewes may be clinically and economically justifiable (Hicks, 2002). Vaccination has shown some promise in reducing the severity of outbreaks on affected farms, but the implications for food safety are unknown. It is plausible that a reduction of abortion cases in a flock would lead to less exposure to the organism. The efficacy of vaccination in reducing shedding of the organism remains unknown.

No data are available on the dynamics of *S. Brandenburg* infection between or within mobs of sheep on infected farms in the South Island. Extrapolating from general knowledge on *Salmonella* infection on other species and limited information obtained so far in sheep in the South Island, numerous means of transmission are likely to exist and their relative importance will vary among farms and over time. Research to define routes of transmission on farms will be very expensive and difficult to generalise across properties. Efforts to understand risk factors for disease outbreaks, as opposed to infection, on infected farms may prove more beneficial, and reduction of disease can be expected to have some beneficial effect on food safety. For purposes of risk assessment, further research to estimate (qualitatively or quantitatively) the presence of *S. Brandenburg* infection and contamination among stock selected for slaughter may be warranted.

From a food safety perspective, *S. Brandenburg* disease is significant to the extent that it influences the prevalence of infection and contamination of animals within a mob at the

time of slaughter. Reports of clinical disease have been limited to the period from July to September, and stock cannot legally be sent for slaughter within 28 days of an outbreak of salmonellosis. In the study reported in Chapter 4 and the companion study of Motsamai (2002), the first groups of animals from case or control farms were not sent for slaughter until late November following the commencement of the slaughter season at the plant involved. Hence a theoretical minimum interval of 28 days exists between disease in flocks and slaughter, but in practice this interval will usually be much longer. This temporal factor is particularly important, given the marked decline in prevalence of positive animals and carcass contamination observed between December and February (Motsamai, 2002; Chapter 4). At the time when animals are selected for market, no disease should be evident in any mobs, but disease that occurred some months previously on a farm is likely to affect the probability of infection or contamination of animals. Overall, it is logical that control of *S. Brandenburg* on farms could be a useful mitigation strategy for foodborne risks. However, the collective information about the epidemiology of this organism, and therefore potential intervention tactics, remains minimal.

5.2.3 Future data needs - animal production module

Considerable research will be required to develop and validate interventions for reducing the infection in the animal production sector. The data from Motsamai (2002) provide initial estimates of prevalence of infection among mobs sent for slaughter. However, these were a deliberately biased sample of farms chosen to include worst affected farms. Acknowledging the limited amount of data available, the detection of *S. Brandenburg* on 3 of 4 control unaffected farms (and at a mean prevalence of 10% among infected mobs on those farms in November/ December) indicates considerable risk of infected animals being sent for slaughter, irrespective of disease history. More extensive data on mob prevalence, and within mob prevalence, is desirable to reduce uncertainty in these parameters for purposes on risk assessment.

5.3 Transport and lairage module

The key outputs of the transport and lairage module are the:

- Prevalence of infected sheep presented for slaughter (fleece, gut, other),
- Numbers and distribution of organism in/on infected sheep presented for slaughter.

The key determinants of these parameters will be the prevalence and concentration of organisms in animals selected for slaughter (outputs of animal production module), and the extent of cross contamination that occurs within and among mobs of sheep during transport and lairage (Figure 2.4). This module spans 3 separate environments in which diverse factors may impact the probability of infection or cross-contamination events:

- a) Sheep yarding and loading on farm,
- b) Transportation,
- c) Lairage and preslaughter handling at abattoirs.

Excretion of enteric organisms and cross-contamination of stock is known to occur during the interval between leaving the farm and the arrival at the slaughter plant (Bryan & Doyle, 1995), and continues during the lairage. As is the case of some other infectious diseases, direct transmission may be considered as the driving force in the dynamics of *S. Brandenburg* contamination of wool during transportation during which sheep free of contamination are mixed with ewes of known or unknown contamination status. The force of contamination will depend on the rate of contact, and the probability that contact between “clean” and contaminated sheep results in a cross-contamination of clean sheep. Usually, trucks are washed after each load of sheep, however, there are no data available on the numbers of organisms before and after washing.

During lairage, there is further potential for wool contamination. The plant where the studies described in Chapter 4, and by Motsamai (2002), were conducted uses spray washing of sheep where shower nozzles are directed over the sheeps' backs for two to five minutes. After washing, sheep are rested in pens (with an elevated steel-mesh grating floor) for another 10-12 hours before slaughter. Pens are well ventilated, and designed to prevent contamination from dirty floors. Animals are subjected to fasting to further reduce the volume of faeces in the guts, thus reducing the potential for carcass contamination during dressing. Sheep prepared for slaughter are moved onto the crush conveyor and positioned by operating a crush conveyor pedal, and stunned. There is no data to indicate the role of the crush conveyer in sheep fleece (wool) contamination.

The fleece is generally recognised as the most important source of contamination of sheep carcasses (Biss and Hathaway, 1995). Furthermore, pre-slaughter washing of sheep,

adopted by New Zealand as an effective method of reducing visible contamination on carcass on the slaughter floor, was found to have a detrimental effect on microbiological loads on ovine carcasses. Some authors consider that the assumption that dirty sheep that are visually clean following pre-slaughter wash are microbiologically less contaminated is incorrect (Biss and Hathaway, 1996b). In addition, increased wooliness has been considered as a significant pre-slaughter risk factor that contributes to increased microbial contamination of sheep carcasses (Biss and Hathaway, 1995; Biss and Hathaway, 1996a).

The importance of transport and lairage with respect to the risk of cross-contamination with *Salmonella* has been found to be variable in other species (Hurd *et al.*, 2002). The diversity of facilities and practices involved in transport and lairage of sheep can also be expected to impact the probabilities of transmission of *Salmonella*. In the companion study of Motsamai (2002), prevalence of positive caecal samples after slaughter was comparable to prevalence of positive faecal samples prior to slaughter, and both declined

markedly from the first to second sampling. One interpretation of these data, which did not include quantitative microbiology, was that the prevalence of enteric infection did not change appreciably during transport and lairage of sheep. However, no data were obtained on occurrence of fleece contamination.

5.3.1 Future data needs - transport and lairage module

The current absence of data on prevalence and numbers of *S. Brandenburg* on fleeces is a significant deficiency with respect to developing a farm to table risk assessment. As with within-farm prevalence of infection, more extensive and representative data (e.g. based on random sampling) of prevalence of enteric infection and fleece contamination of mobs sent for slaughter in this region is desirable.

5.4 Slaughter and processing module

The key outputs of the slaughter and processing module are the:

- Prevalence of contaminated products (carcasses other),
- Numbers and distribution of organisms on contaminated products.

The key determinants of these parameters will be the prevalence and concentration of organisms in and on animals at the point of slaughter (outputs of transport lairage module), and the extent of contamination that occurs from these or other sources during slaughter and processing. It is assumed that the risk of *S. Brandenburg* being present within muscle at the time of stunning is negligible, and that presence of the organism in swabs taken from the surface of meat results from contamination occurring during slaughter and processing. At the outset of this project (and other companion projects), there was little information to indicate whether *S. Brandenburg* epidemics in sheep were linked to increased risks of contamination of sheep meat products, and consequently the implications for food safety and consumers. The results presented in Chapter 4 indicate that at certain times of the year the risks of contamination with *S. Brandenburg* of sheep and lamb carcasses sourced from the affected region are greatly elevated compared with historic data, and current data from the North Island. Clearly, any attempt at risk assessment or management requires a clear description of the environment and procedures involved during slaughter and processing.

5.4.1 Sheep slaughter

The general processes discussed in this section are considered to be common among many sheep slaughter establishments in New Zealand, although specific details of the procedures will differ among plants. This is the sector in which the sheep meat industry has the greatest responsibility and ability to implement risk mitigation measures, and was the focus of the bacteriological studies described in the preceding chapters. The discussion does not extend to specific sheep meat products or by products, as no data are currently available at those levels.

Several procedures in sheep meat processing may pose a risk of *S. Brandenburg* contamination of sheep carcasses or meat products. During some of these operations surface contamination, cross-contamination (i.e. carcass to carcass), and contamination redistribution (i.e. from one part of the carcass to another) can affect carcass hygiene. In contrast, some operations (decontamination measures) are designed to reduce the level of contamination. Figure 5.4 outlines sequential steps in sheep meat production process that entail potential risks for surface carcass contamination and decontamination.

5.4.2 Slaughter and dressing

The results reported in Chapter 4 indicate that slaughter and processing entail significant risks for contamination of carcasses. In Chapter 4 we sampled specific sites based on previous work (Bell and Hathaway, 1996) that indicated a high probability of contamination (e.g. Y-cut, flanks, anus). For purposes of risk management, it is desirable to understand which specific operations are responsible for contamination. For purposes of risk assessment, it is important to quantify the levels of contamination and to understand the net impact on these bacterial populations that are likely to ensue during downstream processing, distribution and consumption.

Plant A operates “Halal slaughter” where, after stunning, sheep are slaughtered by a slash cut towards the atlas joint as soon as possible. This is a single movement to sever all major blood vessels, the trachea and oesophagus, after which the sheep are hoisted onto a moving rail and electrically stunned. At this point potential risks include wound contamination from the knife and semi-digested food that may be regurgitated (this is less likely to happen as the oesophagus is tied off after skinning the neck). The relative risks of contamination by *S. Brandenburg* posed by ingesta from different levels of the gastrointestinal tract (e.g. rumen vs. caecum vs. colon) are not known.

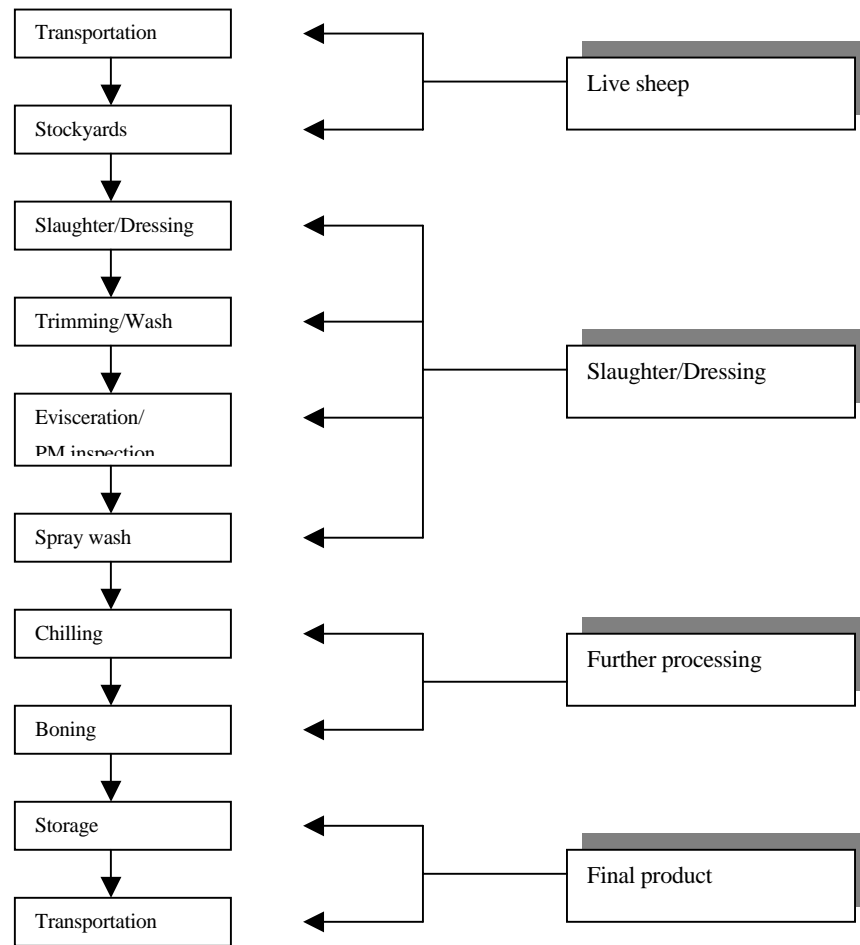


Figure 5.4: Flow chart of slaughter plant operations for sheep meat production at Plant A (site of studies reported in Chapter 4)

Plant A uses inverted dressing at chain speeds of 7 ewes/minute and 9 lambs/minute. The inverted system is considered to minimise the direct contact of the large part of the carcass with the pelt (“roll-back”) and workers hands (Biss and Hathaway, 1996a). Broadly, during this process, initial opening cuts are made on the forequarters (Y-cut) followed by cuts on the medial side of the hindlegs and the ventral mid-line. The pelt is removed automatically by the mechanical puller. In the study reported in Chapter 4, carcasses were sampled on the slaughter floor before pelt removal and before trimming for visible contamination. However, the study did not evaluate individual operations or sources that may have been responsible for the contamination, such as the effect of bung tying, knife blades, sharpening steels, scabbard and belt for holding knives, operator’s hands or aerosols. Other authors have indicated that “bung tying” of cattle has the potential to decrease carcass contamination (Sheridan, 1998), while the rinsing of operator’s hands was found to remove 90% of the hide-derived microflora.

Contamination of knife blades was found to be an order of magnitude lower than was found on operator's hands (Bell, 1997).

An important observation in Chapter 4 was that contamination was more prevalent in lambs than in ewes. This could be attributable to the difference in the processing speeds or other physical factors during dressing of lambs and ewes. In addition, lambs from both case and non-case farms returned similar prevalence of positive carcasses despite an approximate five-fold difference in the prevalence of faecal and caecal positive results between the two groups (Motsamai, 2002). Also, positive carcass results occurred in lambs from all non-case farms (Table 4.5), but no positive carcass contamination was detected for ewes from these farms. These observations imply significant cross contamination occurred among lambs but not ewes, and suggests that the cross contamination is related to slaughter and dressing rather than transport and lairage (though the latter cannot be excluded). While the majority of cell counts on individual carcasses were low, a small proportion of lamb carcasses (Figure 4.7) from two case and one non-case farm had high counts of the organism (too numerous to count). These low frequency events resulting in relatively high levels of contamination may have significant implications for food safety but the specific causes may be difficult to investigate.

5.4.3 Trimming and washing

At Plant A, after automatic depelting, carcasses were trimmed to remove any visible contamination before entering into a tunnel with a pre-evisceration cold water wash of forequarters to reduce contamination that was not visible during trimming. Cold water carcass washes are considered effective in reducing macroscopic contamination and to a lesser extent digesta and faeces. However, this step is recognised as having the potential for redistribution of the microbiological load on the carcass surface (Bell, 1997). There are currently no data to show the effectiveness of these risk reduction measures on reducing the prevalence and the load of *S. Brandenburg* concentration on sheep carcasses, nor on the effect of pre-evisceration washing on the risk of the organism redistribution on the carcass.

5.4.4 Evisceration/post-mortem inspection

Following the pre-evisceration wash, a small cut is made in the abdominal cavity in the area of cod fat or udder. The fingers of the operator's other hand are inserted to lift the abdominal wall away from the viscera. The cut is made towards the brisket, omentum is removed, the rectum is enclosed and the paunch freed and pulled out. The brisket is cut, and the lungs along with the oesophagus are removed. Following removal of kidneys, skirts and pizzle, the carcasses are passed onto the floor for post-mortem inspection. This part of the process also carries potential for carcass contamination as a number of further processing steps are carried out. Microbiological contamination may occur during enclosing the anus, and by ingesta or direct faecal contamination due to perforation during evisceration (Bell, 1997). It is considered that the activities of post-mortem inspectors may have a deleterious effect on contamination of the carcass, as they are required to touch the carcass (Biss and Hathaway, 1998). The potential for contamination of carcasses and the pros and cons of traditional versus visual meat inspection has been outlined in Chapter 1. However, there are no data for *S. Brandenburg* that would provide insight into potential risks of these activities for carcass contamination, redistribution of the contamination, or the concentration of the organism on the carcass surface.

5.4.5 Spray washing

Sheep carcasses that pass post-mortem inspection are subject to grading and the final cold water wash, before being marshalled to the cooler. This is another decontamination measure designed to reduce potential carcass contamination and the microbiological load on the carcass surface.

5.4.6 Cooling floor and chillers

With regard to results obtained from the slaughter floor and cooler (Table 4.8), the data indicated a slightly lower prevalence of *S. Brandenburg* positive carcasses from case farms on the cooling floor (26.7%) compared to the slaughter floor (31.5%). This observation has implications for risk assessment and its validity and biological mechanisms warrant further investigation. The following factors may contribute to the observed changes in prevalence of *Salmonella* positive carcasses from the slaughter floor to the cooler. As the carcasses are railed to the cooling floor and chillers, there is further handling by operators, and possible direct contact between carcasses. The implications of

these events are unknown. Again, majority of bacterial counts on positive carcasses in the cooler were relatively low, but a small number of carcasses yielded higher counts (too numerous to count with the methods used).

Before carcasses are subjected to boning, the conditions at the cooling floor and chillers are briefly explained in Chapter 4 under section 4.2.2.2.3. During the cooling and chilling, carcasses will undergo some drying and this reduced water activity may also have an impact on reducing the number of viable *Salmonella* cells. Another potential factor is temperature, although the storage of swabs at 4°C did not reduce counts (Chapter 3). Although there is a wealth of literature describing the post-mortem changes of meat pH, it may be that pH on the meat surface is more important than the pH of the meat. Another theoretical aspect to consider is whether the *Salmonella* are indeed dying or entering a filamentous growth phase at refrigeration temperatures, with a possibility of rapid septation upon warming (Mattick et al., 2000). This would mean that the DNA of *Salmonella* is replicated but the cell does not undergo 'normal' division. Each newly formed genome has the ability to generate a new cell, and under favourable conditions (when warmed) filamentous cells will divide to yield multiple new cells rather than the two. Should that be the case, there would be clear public health implications.

5.4.7 Boning room

Carcasses arrive in a boning room either frozen or chilled. Further potential for contamination between carcasses and handling exists similar to that in the cooling floor and chillers. Carcasses are again subjected to trimming before being cut or boned, providing a means for decontamination if visible faecal material has passed through the system without removal. The next stage is where carcasses are placed on a table and cut.

These surfaces are likely to become contaminated and become a vehicle for spread of *Salmonella* between carcasses. The temperature in the boning room (10°C) is such that growth of salmonellae is not likely to occur, or be very slow. Cutting tables are cleaned infrequently during a shift, creating potential for a carcass with a high *Salmonella* cell count to result in multiple carcasses becoming contaminated at a low level. The observed prevalence of *S. Brandenburg* on carcasses early in the slaughter season meant that introduction of the organism into the boning room was likely to be a common event.

However, we were unable to detect the organism in a total of 200 samples collected in the boning room during the period of apparently highest risk. It is generally considered that bactericidal activity of the carcass is comparable to that observed *in vitro* with whole blood. In such conditions, a small heterogeneous population of bacteria is unlikely to survive on carcass tissue (Gill and Penney, 1979). Regardless, the increased temperatures in the boning rooms when the boning does not occur do make it important to thoroughly clean and sanitise the boning room so that the resumption of boning during the new shift occurs “with a clean slate”. There are no data to indicate what would be the levels of *S. Brandenburg*, if any, on various equipment in the boning room, nor are there data on the efficiency of sanitation procedures for reducing the levels of the organism, if present.

The observation of uniformly negative culture results for all samples in the boning room, despite a high prevalence of culture positive carcasses from the same mobs on the slaughter floor and in the cooler needs to be validated. In particular, methodological explanations need to be eliminated and the underlying mechanisms need to be determined. With respect to modeling of risk, this example illustrates the importance of field data when conducting risk assessments. If predictive microbiology were used to model levels of contamination of product based on the data from carcasses, the outputs would differ markedly from those based on data from the boning room (all samples negative).

5.4.8 Storage and transportation of the product

Subsequent to boning, the product is held in cold storage in either a chilled or frozen form. In neither case, the growth of *Salmonella* in, or on the product, should occur provided adequate temperature control is maintained. However, one experimental study showed that *S. Brandenburg* has the ability to survive on beef meat following standard preservative packaging conditions and frozen stage without injury for 9 months (Dykes and Moorhead, 2000). The product will leave the slaughterhouse either for export or for domestic consumption. It is important that the cold chain is maintained during the transport. There are no data to indicate what the impact of storage and transport might be on the levels of the organism on the product, if present.

5.4.9. Future data needs - slaughter and processing module

For development of a quantitative model, more extensive and representative studies of the prevalence and concentration of *S. Brandenburg* on carcasses in the on the slaughter floor and cooler. In particular, more extensive research is required to verify the apparent absence of culturable *Salmonella* in boning room samples on a larger and more representative population, and thereby obtain more reliable data on prevalence and numbers of organisms at this point in the process. It would be prudent to replicate sampling in several slaughter facilities to determine whether the data are generalisable to the wider industry. For purposes of trade and access to international markets, accurate determination of prevalence and concentration of *Salmonella* on sheep meat products eligible for export is a reasonable priority for the industry. Such estimations would best be conducted longitudinally, commencing early in the slaughter season, in order to refine understanding of the apparent temporal variability in risk of contamination.

5.5 Retail distribution and consumer modules

There are no data available of the occurrence of *S. Brandenburg* on sheep meat products beyond the boning room of slaughter plants. For modeling purposes, broad options are to use predictive microbiology or to conduct systematic surveys of product in these downstream sectors of the meat supply chain. This module will only be addressed superficially owing to the absence of data.

Product may be transported to wholesale packinghouses or retail butcher shops where further handling will occur. There is less supervision by regulatory authorities of these premises and the potential for the temperature abuse is much higher. Product may also be converted into minced product, or undergo other processing into final products. Ideally, risk assessment from farm to table should incorporate the complete range of processing steps employed, with priority given to processes that account for the majority of product sold to consumers (constituting the bulk of exposure) or processes thought to be associated with higher risk (e.g. products such as minced meat, ground meat). At all stages until ultimate food preparation, product may be subjected to external contamination or to temperature abuse. While a complete risk assessment will incorporate these factors, it is arguably more urgent, in the absence of unequivocal cases of foodborne *S.*

Brandenburg disease, to focus research efforts on exposure assessment up to the end of processing. Longitudinal studies of the prevalence and concentration of *Salmonella* in retail sheep meat products is also a priority to obtain data on potential exposure of consumers.

5.6 Conclusion

A full farm-to-table quantitative microbiological risk assessment following Codex guidelines requires considerable resources in personnel and research. The framework outlined in Chapter 2 was developed to place the sparse data available on *S. Brandenburg* in sheep into a risk assessment context. As stated in Chapter 2, key outcomes envisaged from QRA are:

- Assessment of the risks of exposure and illness due to the agent and food of concern (risk estimates),
- Evaluation of expected changes in risks likely to occur if certain interventions are implemented at various points in the chain of production, processing and food handling,
- Identification of priority areas for further research.

The pilot studies, including those reported in Chapters 3 and 4, were designed to fill obvious data gaps and provide direction for subsequent research investment. Major points that have arisen from this process are:

1. Although foodborne cases remain to be documented, the prevalence of *Salmonella* contamination of carcasses was sufficiently high to warrant further investigation of this issue due to the potential public health and market access implications,
2. Data from the pilot studies of case and control farms point to a likely high prevalence of infected farms in Otago and Southland. More extensive and representative surveys will be required to obtain more reliable data on farm prevalence and within-farm prevalence of infection,
3. The marked drop in positive culture results between the slaughter floor and boning room has enormous implications for assessing risk, and needs to be verified and

investigated in more detail. More extensive and representative longitudinal studies of the prevalence and concentration of *S. Brandenburg* during slaughter and processing are suggested to obtain more certain data and to quantify the temporal variability evident in both the on-farm and slaughter studies,

4. Systematic surveys (preferably longitudinal commencing at the time of apparent highest risk) of the prevalence and concentration of *S. Brandenburg* in sheep meat is likely to be a more reliable means of assessing exposure of consumers than predictive microbiology.

Appendices

Appendix 1. Reagent Preparation for BAX[®] test

a) Preparation of 0.5m EDTA:

- 186.1 gram of EDTA was added to 800 ml of ultra pure water. The mixture was stirred vigorously on stirrer. pH to 8.0 was adjusted with 20 grams of NaOH pellets.
- Volume was adjusted to 1000 ml by adding ultra pure water and prepared solution was autoclaved.

b) Preparation of SxTBE running buffer (stock and working dilution):

- 108 grams of TRIS base was put into glass container,
- 55 grams of Boric Acid was added,
- 40 ml of 0.5m EDTA (pH = 8.0) was added,
- Up to 2 litres of ultra pure water was added and the stock solution was autoclaved and stored on room temperature,
- Working solution of 0.5 SxTBE was prepared by adding 200 ml of stock SxTBE buffer to 1800 ml of ultra pure water.

Where positive samples were detected by BAX[®] test, an MPN calculation method was used to quantify the microbial load. The microbial load quantified based on the following calculation:

- Standard 3-tube MPN table was considered,
- If 100cm² swab was inoculated into 100ml (i.e. 1cm²/ml), than a tube series of 3 of 10cm² (10 ml), 3 tubes of 1cm² (1ml) and tubes of 0.1cm²(0.1ml) will give a detection range of 3-1100MPN/100cm²,
- If sample is suspended in 300ml, result have to be multiplied by 3; if suspended in 235ml, the result has to be multiplied by 2.35, etc,
- For lambs, surface carcass swab²⁸ is estimated to be 842cm², therefore, the number of estimated colony-forming unites (cfu) in MPN has to be divided by 8.42,
- For ewes, surface carcass swab²⁹ is estimated to be 1150cm², therefore, the number of estimated colony-forming unites (cfu) in MPN has to be divided by 11.50.

²⁸ Measured at the Plant A during Period A

²⁹ Measured at the plant A during Period A

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