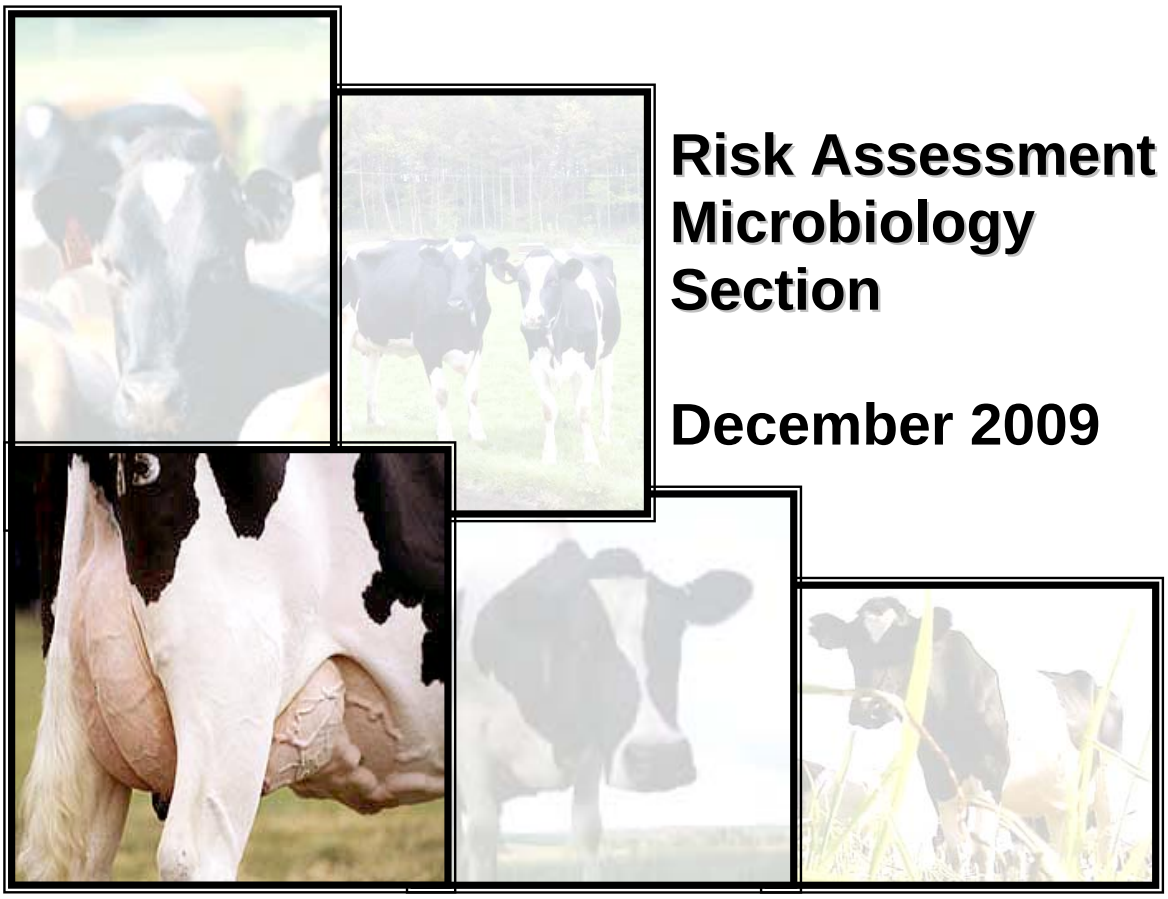
FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Microbiological Risk Assessment of Raw Cow Milk



Risk Assessment Microbiology Section

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Abbreviations

CDC	Centers for Disease Control and Prevention
Codex	Codex Alimentarius Commission
EHEC	Enterohaemorrhagic *Escherichia coli*
FAO	Food and Agriculture Organization of the United Nations
FDA	United States Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
HUS	Haemolytic ureamic syndrome
LP	Lactoperoxidase
MMWR	Morbidity and Mortality Weekly Report
NNS	National Nutrition Survey
OIE	Organisation Mondiale de la Santé Animale
RMSSS	Roy Morgan Single Source Survey
SLT	Shiga-like toxin
STEC	Shiga toxin-producing *Escherichia coli*
SLTEC	Shiga-like toxin-producing *Escherichia coli*
ST	Shiga toxin
The Profile	A Risk Profile of Dairy Products in Australia
VT	Verotoxins
VTEC	Verocytotoxin-producing *Escherichia coli*
WA	Western Australia
WHO	World Health Organization

Executive summary

The risk assessment of raw cow milk brings together information on the public health risks associated with the consumption of raw cow milk, and estimates the resulting burden of illness that may occur under current Australian production and marketing conditions.

The assessment was undertaken to answer the following questions:

(1) What are the risks to public health and safety posed by the consumption, in Australia, of raw cow milk?

(2) What are the factors that would have the greatest impact on public health and safety along the production chain?

The risk assessment considered domestic and international information from published and unpublished sources on: milk production systems, prevalence and levels of pathogens in raw cow milk and in cattle, consumption data, and epidemiological data. This information provided an overall picture of the public health risks associated with consumption of raw cow milk.

In order to estimate the likelihood of illness for Australian consumers following consumption of raw cow milk, quantitative microbiological modelling was undertaken. The modelling predicted the number of illnesses per 100,000 servings of raw milk consumed directly from the bulk milk tank, after farm-gate sale, and retail sale for four identified key milkborne pathogens: *Campylobacter* spp., enterohaemorrhagic *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes*.

The key findings of the risk assessment can be summarised as:

- Raw cow milk is associated with foodborne illness internationally, and has been linked to illnesses in Australia
- Four key pathogens are associated with outbreaks of foodborne illness implicating raw cow milk, these are *Campylobacter* spp., *Salmonella* spp., *Listeria monocytogenes* and pathogenic *Escherichia coli*
- Internationally, raw cow milk has been found to be a significant source of pathogenic microorganisms
- Unpublished research suggests consumption of raw milk is likely to be low among the general population, however, certain groups preferentially consume raw milk
- The burden of illness after retail purchase was predicted to be less than 1 case of campylobacteriosis, 97 cases of EHEC, 153 cases of salmonellosis and up to 170 cases of listeriosis (in a susceptible sub-population). The estimated number of cases are per 100,000 daily serves of a mean daily intake of 540 ml of milk to a child
- The predicted number of illnesses following consumption of raw milk from the bulk milk tank or after farm gate sales per 100,000 daily serves is:
    - 19 cases of campylobacteriosis, 16 cases of EHEC, 17 cases of salmonellosis and less than 1 case of listeriosis (in a susceptible sub-population) when milk is consumed from the farm bulk milk tank.
    - 5 cases of campylobacteriosis, 49 cases of EHEC, 55 cases of salmonellosis and up to 17 cases of listeriosis (in a susceptible sub-population) when milk is consumed after farm gate sale.

Raw milk can often be contaminated with pathogens, either directly through organisms shed as a result of udder infection or indirectly. Indirect contamination may arise from (i) a cow's own faecal matter contaminating the udder and teats, (ii) faecal matter of other cows contaminating the udder (iii) milking clusters contacting surfaces with faecal contamination, and (iv) post-harvest environmental contamination. An intensive search of published and unpublished literature shows that internationally, raw cow milk is often contaminated with pathogens and, whilst data is scarce for Australia, the data which is available confirms that raw cow milk is a source of low levels of pathogenic microorganisms.

Consumption of raw cow milk on school camps, during farm visits or via consumption of products marketed as pet or cosmetic milk, has been implicated in eight outbreaks of illness between 1998 2003 in Australia.

Other than the burden of illness data, the quantitative modelling determined that:

- Increased consumption of raw cow milk corresponds to an increase in the predicted number of illnesses
- Inclusion of spoilage in the model resulted in an overall decrease in the number of predicted illnesses
- Growth of pathogens occurred predominantly during domestic transportation and storage of raw milk
- The occurrence and persistence of infection within herds or individual animals is often intermittent, which proves difficult to detect and routinely monitor
- The ability to detect low levels of pathogens in raw milk is limited unless comprehensive sampling plans are used

Understanding the extent to which cows in the herd carry pathogens in their gut, and shed them in their faeces, provides an important means of eliminating carrier animals and reducing the pathogen load in the farm and milking environment. Effective management of this would require high-level veterinary supervision and ongoing surveillance of individual animals in a herd. Such measures involve significant and often intensive interventions with concomitant enforcement authority oversight.

Pathogen contamination of raw milk may be reduced by exercising enhanced hygienic control throughout the milk harvesting stage. Practices such as teat washing and dipping, foremilk stripping, and good milking hygiene will reduce the number of organisms (pathogenic and spoilage) that may enter the milk from environmental sources. For example, pre-milking udder washing with clean water and drying using hand towels reduces milk contamination by transient bacteria located on the exterior surfaces of the udder. Post-milking teat disinfection reduces the resident teat skin bacterial population, which is the main source of infection for the mammary gland.

Test and hold practices, pending negative test results, is a commonly practised strategy for high risk, perishable foods. Raw milk is highly perishable, with both pathogenic and spoilage organisms expected to proliferate pending the receipt of test results. Furthermore, even low levels of pathogens in raw milk present a risk to consumers, and frequently these are below the levels of detection of current microbiological sampling regime prescribed in the *Australia New Zealand Food Standards Code*. Test and hold regimes may therefore be inadequate to determine with any confidence that contaminated raw milk will not reach consumers. Based on the available data, more than 200 sets of 5 x 25ml samples from every batch would be required to detect low level contamination in raw milk with 95% confidence.

The safety of raw cow milk is influenced by a combination of management and control measures along the entire dairy supply chain. Control of animal health, adherence to good milking practices, and control over milking parlour hygiene are important in reducing the microbial load in raw milk. The modelling undertaken demonstrates that although the pathogen level may be very low in raw milk, there remains a risk of causing illness if consumed. The ability to detect pathogens in raw milk depends on the accuracy of testing, skill of personnel and the limit of detection for specific testing methodologies and pathogens.

1. Background

Food Standards Australia New Zealand (FSANZ) has responsibility for protecting the health and safety of consumers through the development of food standards. A comprehensive evaluation to identify and examine microbiological hazards along the entire dairy supply chain was conducted by FSANZ and is entitled *A Risk Profile of Dairy Products in Australia*[1] (the Profile) (FSANZ, 2006).

A key finding of the Profile was that Australian dairy products have an excellent reputation for food safety, as the majority of dairy products available for sale in Australia are made using pasteurised milk. This finding was supported by the paucity of evidence attributing foodborne illness to dairy products. The Profile did not specifically examine the risks to public health and safety associated with the consumption of raw cow milk; however it did confirm that internationally, unpasteurised dairy products are the most common cause of dairy associated foodborne illness.

This document seeks to assess the risks to public health and safety resulting from consumption of raw cow milk. It utilises available scientific data and addresses uncertainty and variability in the conclusions drawn from the data. For example, the relevance and quality of data, the veracity of its source, and the assumptions made in the quantitative modelling are taken into consideration.

The output of this risk assessment provides an estimate of risk to Australian consumers from specific pathogens following the consumption of raw cow milk. The outputs of the assessment will be used by FSANZ in the consideration of regulatory and/or non-regulatory measures as appropriate.

[1] http://www.foodstandards.gov.au/_srcfiles/DAR_P296_Dairy_PPPS_Attach2%20Parts%20A-B.pdf#search=%22Risk%20Profile%22

2. Introduction

2.1 Purpose

The purpose of this assessment is to provide an objective interpretation of available scientific data on the public health and safety risks associated with the consumption of raw cow milk and to provide advice on strategies which may be employed to impact on any identified risk.

The assessment is undertaken in the context of the existing food safety management practices in the Australian dairy industry, and addresses the risk management questions:

1. What are the risks to public health and safety posed by the consumption of raw cow milk in Australia?
2. What are the factors that would have the greatest impact on public health and safety along the production chain for raw cow milk?

The assessment considers specific microbiological hazards, and evaluates epidemiological and other scientific and technical data to determine whether these hazards have presented, or are likely to present, a public health risk in raw cow milk. The assessment also aims to identify where in the production and supply chain these hazards may be introduced, decreased or amplified.

2.2 Scope

The scope of this risk assessment is to assess the risk to public health and safety from drinking raw cow milk. Assessing the risks resulting from consumption of further processed raw milk products, such as yoghurt and kefir, is outside the scope of this risk assessment.

Although further processed products are outside the scope of this risk assessment, the findings may be used to determine the risks associated with the raw milk intended for processing into other raw milk products. The risk to public health and safety from consumption of selected raw milk cheeses has been undertaken separately[2].

2.3 Definition of raw milk

For the purposes of this assessment, raw milk is milk that has not been heat treated in accordance with the *Australia New Zealand Food Standards Code*[3].

[2] *Microbiological Risk Assessment of Raw Milk Cheese* http://www.foodstandards.gov.au/standardsdevelopment/proposals/proposalp1007primary3953.cfm

[3] The Australia New Zealand Food Standards Code - Standard 4.2.4 – *Primary Production and Processing Standard for Dairy Products*

2.4 Approach

Raw cow milk for human consumption is currently not permitted for sale in Australia; hence specific production and industry information is unavailable. It has therefore been assumed that at a minimum, practices, procedures and regulations pertaining to the existing bovine dairy industry would also apply to the production of raw cow milk.

The assessment draws upon the findings of the Profile (FSANZ, 2006) which identified microbiological hazards in raw milk, primary production practices and other information relevant to raw cow milk. The assessment discusses microbiological hazards associated with raw cow milk, their attribution to raw milk mediated foodborne illness and the primary production and processing factors which impact on raw milk safety.

The assessment is based upon the Codex risk assessment framework and utilises the outputs of quantitative modelling which estimate the risk per random daily serve of raw milk to consumers from enterohaemorrhagic *Escherichia coli* (EHEC), *Listeria monocytogenes*, *Salmonella* spp. and *Campylobacter* spp. present in raw cow milk. The model utilises data encompassing all stages from milking through to consumption.

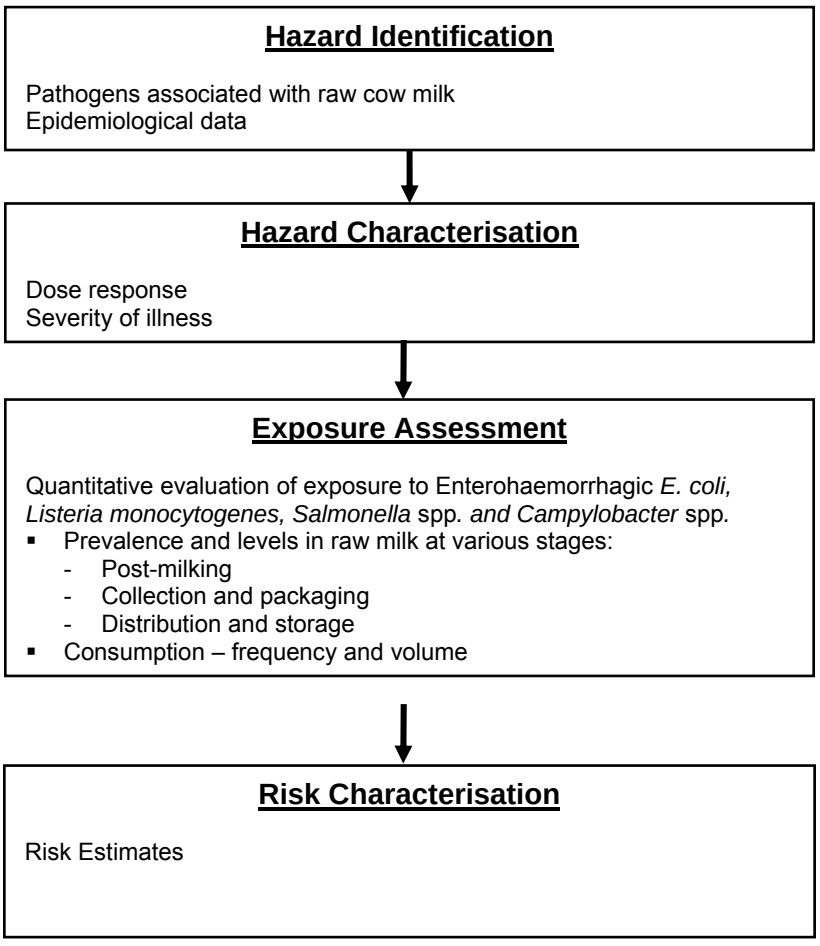

Figure 1: Main components of the microbiological risk assessment

3. Findings

3.1 Production of raw milk

There is no formal marketing of raw cow milk for human consumption in Australia, although ongoing illegal sale of the product occurs in many jurisdictions.

The system for producing raw milk would be expected to be similar to that used for producing cow milk for pasteurisation. However, the scale of operation would more likely be small-scale/boutique. Packaging of raw milk would be expected to occur on farm or at facilities close to the production environment with a capacity for segregating milk intended for raw or pasteurised product.

The assessment identifies and describes the process and risk factors associated with production of cow milk. The main steps in raw milk production are described in Figure 2.

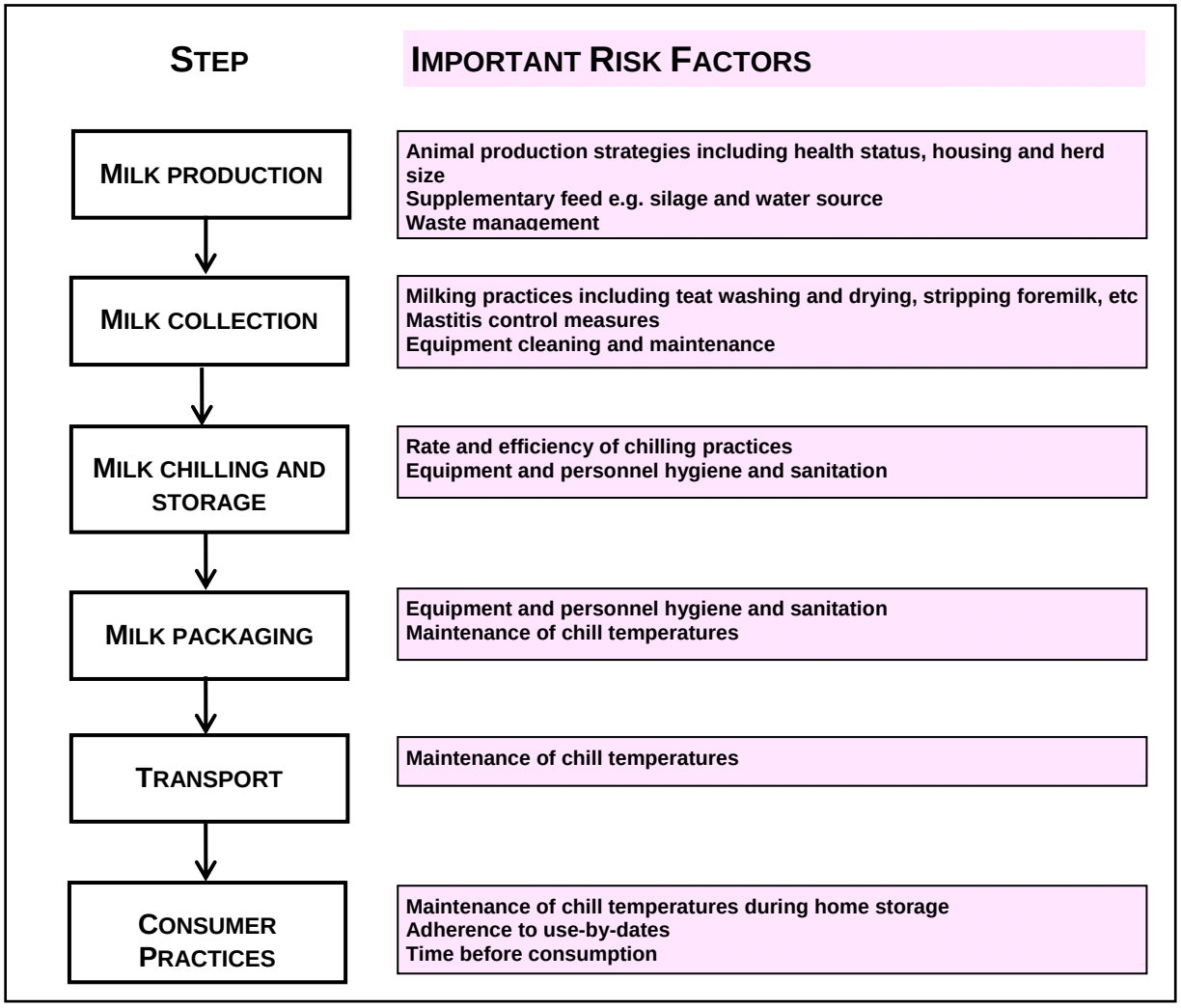

Figure 2: Generic steps in the production of raw cow milk

Measures employed to address the microbiological status of milk destined for pasteurisation would also apply to milk to be sold in the raw form. These include industry best practice regimes such as mastitis control programs, pre and post milking udder treatment practices and milking parlour hygiene programs.

3.2 Consumption of raw milk

There is little data on the level of raw milk consumption in Australia or the potential demand. Generally, consumption data can be calculated from production statistics which provide information on the amount of a food commodity available to the population, or detailed information on the types and amounts of foods consumed by individuals via consumption surveys. For raw cow milk, this type of data is unavailable.

It is probable that raw cow milk consumption is very low among the general population in Australia because it is currently not permitted and access is extremely limited.

Even with limited availability, there are consumers of raw cow milk within Australia who purchase milk in the form of "pet milk" or via "cow share" programs. Unpublished research indicates that these consumers have very strong beliefs regarding the health benefits attributed to raw milk and their right to consume such products, and subsequently raw milk is their milk of choice. During 1994 in a survey of 3,999 respondents in the US, where raw milk sales are legal, only 3.2% reported consuming raw milk in the previous year (Headrick *et al*., 1997). Other studies undertaken internationally indicate generally low consumption of raw milk within the population.

Consumption of raw milk is also practiced by dairy producers who have ready access to raw milk. While Australian data is limited, a study in the US found 42% of dairy producers (105/248 farmers in Pennsylvania) consumed raw milk and cited taste and convenience as their priority reasons (Jayarao *et al*., 2006). This is comparable to the results of Rohrbach *et al.* (1992) who found 34.9% of dairy producers consumed raw bulk milk.

In the absence of reliable data for raw milk consumption within Australia, data on the consumption of pasteurised milk was used to estimate consumption of raw milk. It was not extrapolated to provide a population estimate.

3.3 Consumption of pasteurised milk

Pasteurised milk and milk products are a significant component of the Australian diet. In 2006-07 Australians consumed around 2161 megalitres of market milk per annum (~ 100 litres per person per year), and this is expected to either remain static or decrease slightly (Dairy Australia, 2006). Milk consumption has been decreasing due to perceptions that it contributes to excess fat in the diet and the increased availability of substitute non-dairy products such as soybean-based drinks. Patterns of milk consumption have also been steadily changing from regular whole milk to modified milk types, such as reduced and low-fat milks and fortified specialty milks (Dairy Australia, 2004).

Data obtained from the Australian National Nutrition Survey (NNS) in 1995 indicates that milk and liquid milk products comprise a significant component of the Australian daily diet (see Annex 1). Of 13,858 people surveyed, 11,311 (81.6%) reported consuming milk with an average intake across all consumers of 325g milk per day.

The leading milk consumers were males aged 16 - 18 years, with a mean consumption of 495g/day, with a 95th percentile of 1,553g (even though they comprised only 1.5% of the surveyed population). Mean consumption of milk per day for children aged 2 - 3 years was 447g and 403g with 95th percentiles of 1,037g and 931g for males and females, respectively.

Since 1995, a number of other surveys have been conducted to evaluate food consumption. The Roy Morgan Single Source Survey (RMSSS) is undertaken by an Australian market research company that monitors and provides information about the habits of over 50,000 Australian consumers. The data covers a vast range of consumers and indicates that more than 70 percent of the Australian population over 14 years of age consumes milk each 7 days, typically consuming over six glasses during this period.

More detailed consumption information from both the NNS and RMSSS is contained at Annex 1.

3.4 Microbiological hazards in raw cow milk

A wide range of pathogenic microorganisms have been found in raw milk, and many have been responsible for causing outbreaks of foodborne illness in Australia and internationally.

In 1976 in Whyalla, South Australia, a large outbreak of *Salmonella* Typhimurium PT9 occurred involving over 500 cases. The majority (90%) of patients were under 15 years of age and most were under ten years. Characteristics of the outbreak suggested a common source and 95 percent of patients gave a history of consuming raw milk. Subsequent investigations isolated *S.* Typhimurium PT9 from 78 of the 273 persons investigated and 10 samples of bulk and bottle unpasteurised milk collected early in the outbreak (Seglenloka and Dixon, 1977). Raw milk was permitted for sale in South Australia at the time of the outbreak in 1976. Permissions for the sale of unpasteurised milk in South Australia were rescinded in 2000.

An outbreak of the less common zoonotic pathogen, *Streptococcus zooepidermicus* was reported in Australia in 1992 involving three cases who had reportedly consumed unpasteurised milk from a house cow (Francis *et al*., 1993).

Between 1998 and 2003, OzFoodNet's Outbreak Register identified eight outbreaks comprising 101 cases of illness (and 4 hospitalisations) associated with the consumption of raw cow milk in Australia. *Campylobacter* spp. were the most common aetiological agent (5/8), with *Cryptosporidium* spp. and *S.* Typhimurium PT44 accounting for one outbreak each. There was one outbreak with unknown aetiology.

Four outbreaks occurred on school camps where unpasteurised milk was consumed, while two outbreaks implicated unpasteurised milk consumed on farms. Unpasteurised milk was also consumed and led to outbreaks in a community setting and in a school. The outbreaks identified in the OzFoodNet Outbreak Register were investigated using three point source

studies and one case control study. Data prior to 2001 does not identify how outbreaks were investigated.

Details of outbreak data from OzFoodNet's Outbreak Register are listed in Annex 2.

Internationally, consumption of raw cow milk has commonly been associated with foodborne illness. Between 1992 - 2000, 2% of all foodborne outbreaks in England and Wales were milkborne, with the majority (52%) attributed to the consumption of raw milk (Tayganyilmaz. *et al*., 2009). In the US where permissions for the sale of raw milk currently exist in 22 States, 58 outbreaks were attributed to raw cow milk between 1978 - 2000 (Tayganyilmaz. *et al*., 2009).

More recently, outbreaks of *S.* Typhimurium occurred in the US in 2003 and 2007 involving 62 and 29 cases respectively. In California in 2006, *E. coli* O157:H7 was responsible for an outbreak involving 6 cases of illness with two cases contracting haemolytic ureamic syndrome (HUS). The median age of cases in this outbreak was 8 years and although geographically dispersed throughout California, all had consumed raw milk from the same dairy. *E. coli* O157:H7 was also responsible for an outbreak in 2005 in which 18 people became ill and four cases progressed to HUS. All patients were involved in a herd-share program and had consumed raw milk.

Aetiological agents commonly associated with raw milk mediated illness include *S.* Typhimurium*, E. coli* O157:H7 and *Campylobacter spp*. *Listeria* spp. and other *Salmonella* serovars, including Dublin and Derby have also been reported.

Outbreaks of illness associated with unpasteurised cow milk reported internationally are listed in Annex 2.

A recent systematic review in New Zealand investigated the strength of evidence to support causal links between foodborne illness and consumption of raw milk products. The report findings indicated moderate evidence exists to support a causal link between consumption of raw milk products and *Salmonella* serovars, *E. coli* spp., *Listeria monocytogenes* and *Campylobacter* spp. (Jaros *et al*., 2008).

In outbreak investigations sources of foodborne illness are generally determined through epidemiological and/or microbiological associations. The ability to identify an outbreak through the existing surveillance system is critical to enable an investigation to proceed. Difficulties exist in identifying and attributing illness to a particular food and include:

- Food recall biases when gathering food consumption histories
- Time delays in recognition or notification of an outbreak
- Inability to trace food products to their source
- Reluctance of individuals to participate in investigations, particularly when they have purchased foods that are not permitted to be sold legally
- Long exposure windows for specific pathogens (e.g. *Listeria monocytogenes*)
- Inability to obtain representative food samples for analysis
- A lack of precision in or suitable methods for sample analysis and pathogen identification

It is important to recognise that outbreak data only represents a small proportion of actual cases of foodborne illness, as many outbreaks go unrecognised and/or unreported to health authorities. People do not always seek medical attention for mild forms of gastroenteritis, medical practitioners do not always collect specimens for analysis and not all foodborne illnesses require notification to health authorities.

3.5 Prevalence of pathogens in raw cow milk

While a vast array of microbial pathogens may be encountered in raw milk, there is limited published data on the prevalence and levels of pathogens in raw cow milk in Australia. Milk processors screen incoming raw milk for a range of quality and shelf-life indicators but typically do not perform analyses for pathogens as the current practise of pasteurising milk destroys all pathogens. Where industry does collect such data it is rarely made public or published.

A small survey conducted in Western Australia in 2007 (183 samples) found a high prevalence of organisms such as *E. coli* and coagulase-positive *Staphylococcus aureus,* whilst *Salmonella* spp. was reported at a prevalence of approximately 8%. No *Listeria* spp., *Campylobacter* spp. or EHEC were detected (Figure 3).

Due to the limited number of samples and the restricted spatial and temporal conditions of the survey, insufficient data is available to draw conclusions on contamination levels within the entire Australian raw milk supply. The results do, however, indicate that raw milk produced under existing food safety management systems can contain pathogenic organisms which could result in illness if consumed.

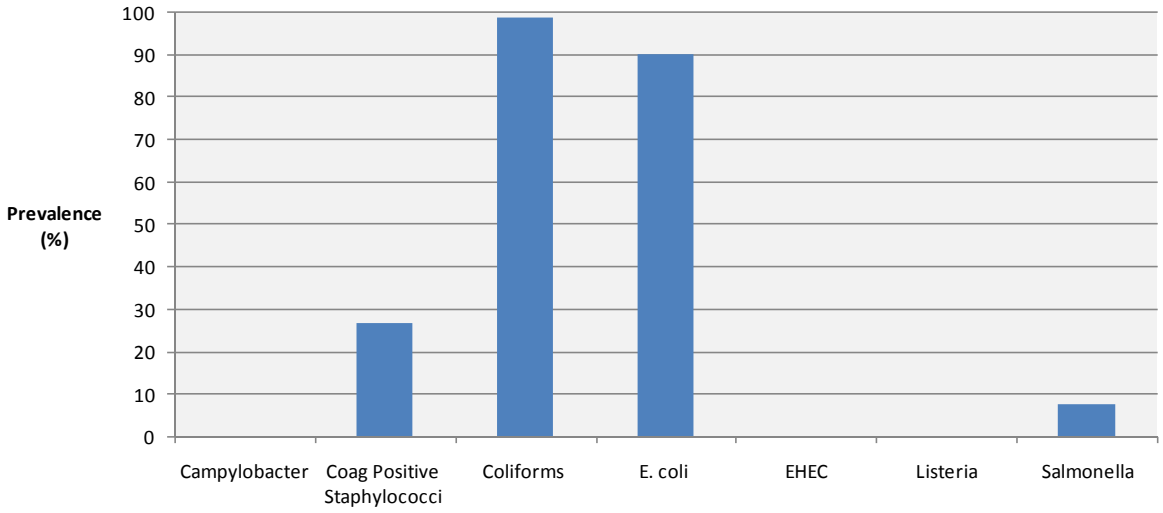

Figure 3: Prevalence of selected organisms in raw cow milk in WA (183 samples), WA unpublished (2007) raw milk data. A total of 28.4% of samples had Coliform concentrations of >100 cfu/ml, while 50.8% of samples had *E. coli* concentrations of >3 cfu/ml. The maximum concentration for both Coliforms (three samples) and *E. coli* (two samples) was greater than 25 000 cfu/ml.

Published international literature indicates various pathogenic microorganisms may be associated with raw milk, including: *Bacillus cereus, Brucella* spp., *Campylobacter* spp., *Coxiella burnetii*, pathogenic *E. coli*, *L. monocytogenes*, *Mycobacterium bovis*, *Mycobacterium avium* subsp. *paratuberculosis*, *Salmonella* spp., *S. aureus* and *Yersinia* spp. (FSANZ, 2006). These include organisms shed by an infected animal (pathogens will be present in milk from mastitis animals) or organisms that enter the milk as a result of poor milking hygiene or from contaminated equipment and personnel.

Table 1 provides a brief summary of the microbiological hazards, possible routes of contamination and the availability of epidemiological data.

Table 1: Summary of microbiological hazards associated with raw cow milk

Organism	Shed directly in milk[#]	Severity of illness[§]	Implicated in foodborne illness
Bacillus cereus	×	Moderate	++
Campylobacter jejuni/coli	✓	Severe^	++
Clostridium perfringens	×	Severe^	+
Coxiella burnetii	✓	-	+
Cryptosporidium parvum	×	Severe^	+
Enterohaemorrhagic *E. coli*	✓	Severe	++
Listeria monocytogenes	✓	Severe^	++
Salmonella spp.	✓	Serious	++
Staphylococcus aureus	✓	Moderate	++
Streptococcus spp.	✓	-	+
Toxoplasma gondii	✓	-	++
Yersinia enterocolitica	✓	Serious	+

Key:
[#] Transmission through udder; mastitis etc
^ Susceptible sub-populations
- No data/unknown
[§] Based on ICMSF (2002)
+ Rare
++ More common

Although *C. burnetii* infection has been associated with consumption of raw milk, ingestion is considered a minor route for human infection (Maurin and Raoult, 1999). Consequently little information exists regarding ingestion mediated illness.

The causative link between Johnes Disease and Crohn's Disease is tenuous. If there were a proven link, then the transmission of *M. avium* subs. *paratuberculosis* through the consumption of raw cow milk would be a risk.

Tuberculosis resulting from milkborne transmission of *Mycobacterium bovis* has been drastically reduced in recent times worldwide by a combination of changing milk consumption habits, mandatory pasteurisation and cattle immunization programs (Ryser, 2001).

Until recently, bovine brucellosis (*Brucella abortus*) was present throughout the world. A number of countries have succeeded in eradicating this disease including: Australia, Canada, Israel, Japan, Austria, Switzerland, Denmark, Finland, Norway, Sweden and New Zealand. *B. melitensis* remains endemic in southern Europe, west and central Asia, Mexico, South America and Africa.

B. abortus in milk producing animals in Australia has been eradicated since 1989 and *B. melitensis* has never been detected in Australian herds. Australia has been recognised as bovine tuberculosis (*M. bovis*) free since 31 December 1997, and continues to conduct screening programs to monitor any *M. bovis* infection in dairy cattle.

The Australian Quarantine and Inspection Service and Biosecurity Australia maintain import requirements focussed on animal health and biosecurity issues. Import conditions are currently being reviewed by Biosecurity Australia for Dairy Products, which includes consideration of *Brucella* spp. and *Mycobacterium bovis*. It should be highlighted that should these organisms be introduced into Australia through importation of contaminated raw milk products, they would pose a risk to consumers from consumption.

While a range of pathogens associated with raw cow milk have been identified in the literature, this risk assessment only considers: *Campylobacter* spp., *L. monocytogenes, E. coli* (EHEC), and *Salmonella* spp. due to their likely occurrence in raw milk, their public health significance and access to suitable data to populate the quantitative model.

M. bovis, *M. avium* subs. *paratuberculosis*, *C. burnetii* and *Brucella* spp. have not been further considered in this assessment.

The frequency of contamination of raw cow milk with the specific pathogens evaluated in this assessment is described in Table 2. This data has been derived from a wide array of literature which is summarised in Annex 3.

Table 2: Summary of prevalence data for pathogens in raw cow milk

Organism	International data
Campylobacter jejuni	0 – 40%
Enterohaemorrhagic Escherichia coli (EHEC)	0 – 33.5%
Listeria monocytogenes	1 – 60%
Salmonella spp.	0 – 11.8%

Importantly, many of these pathogens may occur in milk concurrently. Jayarao *et al.* (2006) found 13% (32/248) of bulk milk samples contained more than one species of bacterial pathogen. Rohrbach (1992) reported a higher percentage (25%) of bulk milk samples contained one or more pathogenic bacteria.

Inappropriate temperature control during the storage of raw milk following milking can lead to the growth of the majority of these pathogens. This may occur on farm, during transport, and packaging, and at various stages during marketing, including during transport, storage and in the home.

3.5.1 Campylobacter spp.

Campylobacter spp. may be shed directly in the milk when the animal has clinical or subclinical mastitis due to *Campylobacter* infection, or indirectly through faecal contamination. Direct excretion of *Campylobacter* spp. into raw milk from mammary infection has been reported (Morgan *et al*., 1985; Orr *et al*., 1995). *Campylobacter* spp. have been isolated from faeces at prevalences up to 83% (Annex 3: Table 3.2).

International data shows the prevalence of *Campylobacter* in raw milk varies between 1 – 40% (Table 2). From a small number of samples (36 samples) taken in South Australia during 1996 - 2000, no *Campylobacter* spp. were detected. Similarly, the Western Australian survey also detected no *Campylobacter* in 183 samples of raw cow milk during 2007.

Campylobacter spp. are notoriously difficult organisms to culture because of the strict requirement for microaerophilic conditions, hence early studies (before the 1980s) frequently reported the absence of *Campylobacter* spp. in milk samples. More recently, the presence of non-culturable but viable *Campylobacter* spp. have been discussed (Sparks, 2009; Cools *et al.,* 2005).

3.5.2 Enterohaemorrhagic E. coli

Pathogenic *E. coli* are characterised into specific groups based on virulence properties, mechanisms of pathogenicity and clinical syndromes (Doyle *et al*., 1997). These groups include enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteroaggregative *E. coli* and enterohaemorrhagic *E. coli* (EHEC).

Many synonyms are used to describe EHEC, including Shiga toxin-producing *E. coli* (STEC), Shiga-like toxin-producing *E. coli* (SLTEC), and verocytotoxin-producing *E. coli* (VTEC). These organisms are often found in the faeces of healthy cattle and as such their presence in raw milk is generally indicative of direct or indirect faecal contamination. However, organisms can be excreted through the udder when systemic infection results in mastitis.

E. coli O157 (a particularly virulent strain of EHEC) has been isolated from raw cow milk both on farm and from bulk raw milk tankers (Desmarchelier and Fegan, 2003; Meng *et al*., 2001; Meng *et al*., 1998). *E. coli* O157 organisms have been found in many raw milk samples from around the world (ranging in prevalence from 1 - 33.5%) (Annex 3: Table 3.3). Contamination of *E. coli* O157 in Australian milk varies from 1 - 3% (Dairy Australia, 2006). The incidence of *E. coli* O157 in raw cow milk in Canada, USA, Europe and France has been reported as 2.3%, 3.2%, 3.6% and 2.4%, respectively (Schlesser *et al*., 2006).

Faecal carriage of pathogenic *E. coli* has been reported at prevalences of up to 39% internationally. In Australia 68 faecal samples obtained in 2005 returned a prevalence of 12% (Annex 3: Table 3.4).

E. coli O157:H7 produce verotoxins (VT) which affect Vero cells, hence they are known as verocytotoxigenic *E. coli* (VTEC) to reflect their biological activity. VTEC are also referred to as Shiga-toxin (ST) producing *E. coli* (STEC) or shiga-like toxin (SLT) producing *E. coli* (SLTEC). STEC strains produce two potent phage-encoded cytotoxins called Shiga toxins (Stx1 and Stx2) or verotoxins (VT1 and VT2). In addition to toxin production, another virulence-associated factor expressed by STEC is a protein called intimin. This protein is responsible for intimate attachment of STEC to the intestinal epithelial cells, causing attaching and effacing lesions in the intestinal mucosas. Intimin is encoded by the chromosomal gene *eae*, which is part of a pathogenicity island termed the locus for enterocyte effacement.

Consequently, SLT, ST and VT have been used interchangeably, resulting in the terms verotoxin-producing *E. coli* (VTEC), shiga-like-toxin producing *E. coli* (SLTEC) and shiga-toxin-producing *E. coli* (STEC) coexisting in the scientific literature.

In Australia, a range of EHEC isolates other than O157:H7 have been typically implicated in cases of HUS, including isolates of *E. coli* O157:H14, the non-motile *E. coli* O157:H-, *E. coli* O26:H11 and *E. coli* O111:H-.

Testing for STEC is complicated due to the difficulty of recognising STEC in a background of commensal *E. coli*. STEC belonging to the O157 clone characteristically do not ferment sorbitol, distinguishing them from the vast majority of *E. coli*, hence colonies can easily be recognised on indicator media containing sorbitol. Culture on sorbitol MacConkey-based medium remains the most commonly employed method to screen for STEC.

3.5.3 Listeria monocytogenes

L. monocytogenes is ubiquitous in the dairy environment; hence it may contaminate raw milk during milking. It can also be a cause of mastitis in milking animals and thus be shed directly into raw milk. Raw cow milk is often tested for *L. monocytogenes* and internationally, prevalence has been recorded up to 60% (Table 2). *L. monocytogenes* has not been reported as detected in either State monitoring programs, targeted surveys or in referenced literature in Australia.

Carriage in the faeces of cattle has been recorded internationally at prevalence up to 50% (Annex 3: Table 3.6).

3.5.4 Salmonella

Salmonella spp. can be found in the intestinal tract of most warm and cold blooded animals. In cattle, the bacterium are carried by both healthy and diseased animals, are shed in the faeces and hence can contaminate raw milk. Faecal carriage prevalence has been reported up to 36.4% in cattle (Annex 3 Table 3.8). International data shows the prevalence of *Salmonella* in raw cow milk ranging between 0 - 11.8% (Table 2).

3.6 Milk production and its impact on milk safety

Primary production factors that impact on contamination and the microbiological status of raw cow milk have been comprehensively reviewed in the Profile (FSANZ, 2006). Pathogenic microorganisms may enter the milk directly via the udder of a diseased[4] or infected[5] animal or indirectly during the milking process due to contamination from the udder and teat canal, from the environment, from contaminated equipment, or from workers.

The microbiological status of raw milk is influenced by animal health[6], exposure to faecal contamination, environmental contamination and temperature control. The key risk factors that may affect the status of raw milk on-farm have been summarised in Table 3, along with strategies employed to manage the risk.

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4 Disease is defined in the OIE Terrestrial Animal Health Code (2007) as the clinical and/or pathological manifestation of infection (http://www.oie.int/eng/normes/mcode/en_chapitre_1.1.1.htm)

5 Infection is defined in the OIE Terristrial Animal Health Code (2007) as the presence of the pathogenic agent in the host (http://www.oie.int/eng/normes/mcode/en_chapitre_1.1.1.htm)

6 Animal health is defined as incorporating both disease (the clinical and/or pathological manifestation of infection), infection and carrier status of the animal.

Table 3: Key on-farm risk factors impacting on raw cow milk

Risk factor	Impact on milk safety	Ways of managing the risk
Disease	Diseased milking animals will show increased shedding of pathogens directly into raw milk or faeces which may contaminate the production and milking environment. Infected animals with no signs of disease (asymptomatic carriers) may also harbour and shed pathogens, often intermittently, into milk and faeces.	Animal health (including mastitis) control programs.
Housing and husbandry	Intensive housing practices may increase the risk of contamination of udders due to high stocking density, concentration of waste, stress and soiled bedding.	Good herd management practices. Attention to animal welfare.
Faeces	Faeces may contaminate the exterior of the udder and introduce pathogens into raw milk.	Reduce scouring. Udder hygiene at milking.
Feed	Contaminated or poorly prepared feed may increase faecal shedding of pathogens. Poor nutritional practices will affect scouring.	Control over preparation, storage and distribution of feed, especially silage.
Water	Contaminated water used for stock drinking, teat washing and cleaning increases risk of environmental contamination.	Ensuring water quality is suitable for purpose.
Milking	Poor milking practices, including dirty, chapped or cracked teats, inadequate cleaning and maintenance of milking equipment, and poor personnel hygiene can lead to contamination of raw milk.	Pre and post milking udder emollients/antiseptics. Effective equipment maintenance, sanitation and cleaning practices.
Storage	Inappropriate temperature control of raw milk after milking can lead to growth of pathogens.	Rapid cooling and holding of milk.
Packaging/ Transport	Packaging and poor hygiene may contribute to cross-contamination of raw milk. Inappropriate temperature control of milk during delivery can lead to proliferation of pathogens.	Correct sanitising and packaging procedures Effective cold chain management.

Defecation during milking may result in contamination of equipment or the generation of aerosols which may contaminate the milking environment (including milk storage vessels). Of the above risk factors, disease and milk have the major effect on milk safety.

3.6.2 Carrier Status

Carriers are animals which are infected with the pathogen without exhibiting clinical signs of disease. These animals may have recovered from clinical disease or never had the disease. Their presence confounds conventional disease diagnosis and herd treatment and may result in the reappearance of a disease in a previously negatively tested group.

Some carriers may be masked and not release organisms unless stressed or immunocompromised. The effects of stress and starvation on shedding of *E. coli* and *Salmonella* spp. in cattle have been known for decades (Grau *et al*., 1968; Grau *et al*., 1969). Detection of carrier animals is often difficult and may not be apparent until the infection re-activates. Destocking and complete replacement with disease free animals may be the only way of removing a disease carrier.

The frequency and amount of pathogen excreted by a carrier varies with the organism, the animal, its husbandry and immune status, and the natural history of the disease in that animal species. In some diseases, carriers continue to be infected for many years while in others it can be a matter of a few months. Good husbandry will reduce stress but will not necessarily relieve certain types of production stresses such as pregnancy, parturition and lactation. These are significant stresses which modulate the immune system and can precipitate the excretion of organisms in a carrier animal.

3.6.2 Pre and post-milking teat cleaning practices

The teat surface is the major avenue of entry for microorganisms into raw milk. It is well recognised that significant opportunity exists for teats to become contaminated by faeces and soil (as dust or mud) (Cook and Sandeman, 2000; Vaerewijck *et al*., 2001). Pre-milking udder hygiene *e.g.* washing with clean water and drying using hand towels reduces milk contamination by transient bacteria located on the udder. Drawing foremilk ejects microorganisms which may have entered the teat canal.

Post-milking teat disinfection reduces the resident teat skin bacterial population, which is the main source of infection for the mammary gland. The majority of Australian dairy farmers rely on post-milking teat disinfection, applied by spray techniques, as an integral part of their mastitis control programs (Lee, 1994). In dairy cattle, the rate of new intramammary infection due to *S. aureus* and *Streptococcus agalactiae* is reduced by approximately 50% when post-milking teat disinfection is practiced (Sheldrake and Hoare, 1980).

3.7 Post-milking factors impacting on milk safety

On-farm cooling and hygiene practices are critical, with any failure adversely impacting the microbial load in raw milk. Correct sanitising procedures for packaging and effective cold chain management practices for the raw milk are important steps for minimising cross-contamination and growth of any microorganism present in the raw milk.

Time and temperature conditions post-milking, *i.e.* through storage and distribution, have an important influence on the concentration of any contaminating pathogens. Even assuming the integrity of the cold chain is maintained, growth of psychrotrophic organisms such as *L. monocytogenes* and *Yersinia enterocolitica* can still occur at refrigeration temperatures if organisms are present in the milk. Other pathogenic microorganisms, if present, will also grow if the temperature increases by only a few degrees, *i.e. E. coli* and *Salmonella* spp. may grow at temperatures between 7 - 8^{o}C (ICMSF, 1996).

3.8 Other factors impacting on milk safety

A number of microbial inhibitors may be found in raw milk, including lactoferrin and the lactoperoxidase system, lysozyme, and specific immunoglobulins. Of these, the lactoperoxidase system (LP) system is the most effective.

Lactoperoxidase is a naturally occurring enzyme in milk and in the presence of hydrogen peroxide and thiocyanate it has a bacteriostatic effect on many microorganisms and a bactericidal effect against some specific Gram-negative bacteria i.e. *Pseudomonas* spp. and *E. coli.*

Natural levels of hydrogen peroxide and thiocyanate in raw milk are insufficient to activate the LP system and require addition. Once activated, its effect has limited duration which is influenced by the initial bacterial load, the species and strains of contaminating bacteria, and the storage temperature of the milk.

An expert consultation on the LP system found it has a role to play as part of an integrated system to improve milk quality and safety. The system, however, is not considered a

replacement of existing well serving technologies, such as cooling and heat treatment. Instead it provides complimentary alternatives, particularly at the primary production stage when the other approaches are not available, feasible or suitable (FAO/WHO, 2006).

4. Quantitative Risk Assessment

4.1 Introduction

The risk assessment has identified four major pathogens in relation to raw cow milk which were considered in this quantitative model: *Campylobacter* spp., enterohaemorrhagic *E. coli* (EHEC), *Salmonella* spp. and *Listeria monocytogenes*. These four pathogens were chosen because of their likely occurrence in raw milk, their public health significance, and access to data to build a quantitative model.

4.2 Model overview

The model is based on an unpublished model developed by the University of Tasmania and a paper by Clough *et al.* (2009).

A modular approach has been taken by splitting the model into several discrete units: Farm level, Farm sales, Retail distribution and storage, Consumer distribution and storage and Consumption.

Three scenarios were modelled:
Scenario 1: A single serve (250 ml) exposure from the bulk milk tank
Scenario 2: Domestic consumption after farm gate purchase
Scenario 3: Domestic consumption after retail purchase[7]

Domestic consumption incorporates transport from the farm to the home and then storage in the domestic refrigerator. The overall structure of the model is illustrated in Figure 4 which depicts the various stages modelled for the production of raw cow milk.

The model simulates farms with relatively small dairy herds that harvest and store raw milk under good temperature control.

For *Campylobacter* spp., EHEC and *Salmonella* spp. the farm module considers a number of factors including with-in herd prevalence (*i.e.* the number of cows that carry any of the pathogens in their faeces), faecal pathogen concentration, degree of teat soiling, efficiency of teat cleaning prior to milking and individual cow milk yield. Information specific to dairy cows was used to develop the quantitative model inputs wherever possible. Instances where data from cattle at slaughter (*e.g.* faecal pathogen concentration) have been used as a surrogate have been indicated.

The approach taken for *L. monocytogenes* is different to the other three pathogens as data on faecal concentration in dairy cows or beef cattle (as a surrogate) could not be found (Rhoades *et al*., 2009). To overcome this limitation, farm bulk milk tank prevalence and concentration data was used.

[7] Retail purchase incorporates packaging, distribution and retail premise storage components.

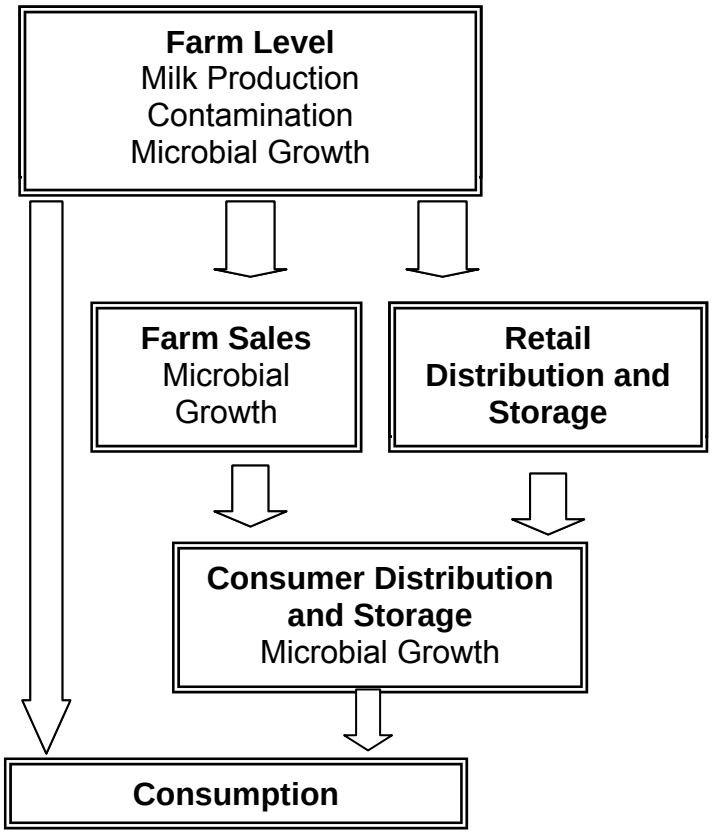

Figure 4: Flowchart of the raw milk model

The potential for growth of any pathogens present in the raw milk is predicted at each stage between milking and consumption. The model also considers the growth of psychrotrophic *Pseudomonas* spp. to assess the effect of spoilage on the likelihood of illness. Secondary cases of illness due to person-to-person transmission are not considered in the estimation of risk.

The quantitative model was built in Microsoft Excel® with simulations run using @Risk version 4.5.3 (Palisade Corporation). Twenty simulations each of 100,000 iterations were performed for each of the four pathogens, with the model outputs presented as predicted illnesses per 100,000 daily servings of raw milk.

4.3 Model inputs

Data used to develop inputs to the quantitative model were obtained from published and unpublished sources and expert opinion. A brief summary of how the data sources were used in the development of the quantitative model is presented below.

4.3.1 Bulk milk tank and with-in herd prevalence

Scientific publications and unpublished sources reporting survey results for pathogen concentrations in bulk milk tank and with-in herd prevalence were evaluated against a number of exclusion criteria, including consideration of survey sample size, geographical location, animal age (weaners, heifers and cows) and microbiological methodology. A summary of the data sources for bulk milk tank and with-in herd prevalences that were accepted after review can be found in Annexes 5 and 6.

A summary of the bulk milk tank prevalence for each of the four pathogens is presented in Figure 5. Each of the plots presents the prevalence of the pathogen (= number of positive samples/total number of samples) with the error bars showing the 95th percentiles around the expected value (Newcombe, 1998).

Statistical analysis of the bulk milk tank prevalence data was performed to investigate differences between published data and those from the most recent (unpublished) Australian survey data of raw cow milk from Western Australia (WA). This survey was limited in scope with five groups of samples taken during the autumn and winter months of 2007. A total of 183 raw milk samples were analysed for a range of indicator and pathogenic bacteria including generic *E. coli*, *Campylobacter* spp., EHEC, *L. monocytogenes* and *Salmonella* spp. A pairwise statistical test was performed between the WA bulk milk tank prevalence results and each of the results for the other studies. For *Campylobacter* spp. and EHEC, only one or two studies were found to have prevalences greater than the WA results. Results found to be statistically different to the WA results are highlighted with an asterisk (*) in Figure 5. The results for *Salmonella* spp. suggested that the WA results (14/183 detections) were greater than six other studies, predominantly from the UK. These six studies had much larger sample numbers (range 456 to 1720) than the WA survey. The UK Food Standards Authority surveys of raw milk in 2000 and 2003 had *L. monocytogenes* prevalences greater than the WA survey. No other results were found to be statistically different.

There are several approaches that can be used to incorporate the bulk milk tank prevalence information of *L. monocytogenes* in the quantitative model. In each case a Beta distribution, Beta(s+1, n-s+1), where s is the number of positive samples and n is the total number of samples, is used. The simplest approach is agglomeration, where all of the positive results and the total number of samples from all surveys are summed. The resulting Beta distribution is Beta(Σs+1, Σn-Σs+1). This distribution does not include any of the structure of the individual studies. The second approach is to include each study in the model together with a weighting. This approach becomes unwieldy when many studies are considered. The third approach is an extension of the second where cumulative distributions for the Beta distributions for each of the studies are averaged (Vose, 2008). This approach captures the uncertainty within each study but the results, as a single cumulative distribution, is more readily included into the @Risk model. The mean bulk milk tank prevalence for *L. monocytogenes* using the cumulative distribution method is 4.1%. Although not used in this model, the prevalences for *Campylobacter* spp., EHEC and *Salmonella* spp. in bulk milk

tanks are 3.1%, 4.6%, 5.5%, respectively. These values do not include the data that were found to be statistically different to the WA survey results.

The average cumulative Beta distribution approach was also used for the with-in herd prevalence results (see Annex 6). The mean with-in herd prevalence for *Campylobacter* spp., *E. coli* (EHEC), *Salmonella* spp. and *L. monocytogenes* was determined to be 25.6%, 6.4%, 8.9% and 5.8%, respectively.

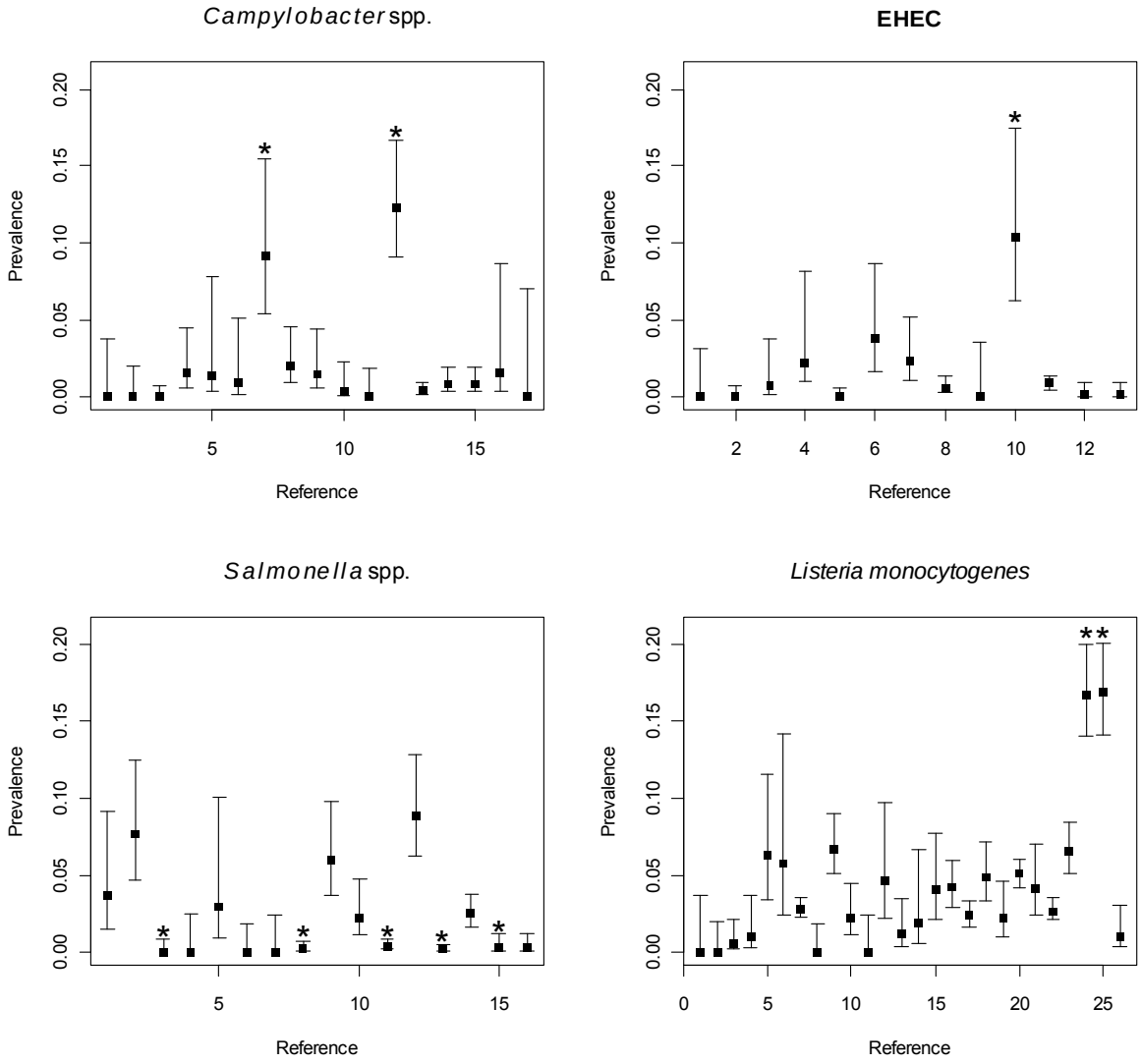

Figure 5: Prevalence of *Campylobacter* spp., EHEC, *Salmonella* spp. and *L. monocytogenes* in bulk milk tanks. Error bars represent the 95% confidence intervals. Results highlighted with an asterisk (*) indicate that the prevalence is significantly different from the WA unpublished (2007) raw milk data. Details of each reference can be found in Annex 5.

4.3.2 Pathogen concentrations in milk

Pathogens can enter raw milk via a number of pathways including faecal contamination, foremilk, udder infection and environmental sources. Amongst these pathways, faecal contamination is often considered to be the most likely and the quantitative model has been developed for this route.

Information necessary to model faecal transfer of pathogens into raw milk during milking will depend on a number of factors, including the number of lactating cows within a herd, milk yield for each cow, the with-in herd pathogen prevalence, the concentration of pathogens in faeces, the amount of faecal material present on the teat prior to cleaning, the effect of cleaning on reducing faecal material on the teat, and the amount of faecal material that is transferred. Each of these factors is considered below.

Herd size, N

For the quantitative model the herd size is assumed to be Log Normal with a mean of 30 lactating cows and a standard deviation of 10. A minimum herd size is set as 10 cows.

Milk yield, V_i

The daily milk yield is the amount of milk produced by a lactating cow. The yield will depend on a number of factors including the breed, time after calving, parity and health status (including mastitis). The total production of milk from a herd will also be influenced by calving practices. Farms that use seasonal calving will have a greater range in production between seasons than for those that calve throughout the year. An industry average of 20 litres, with a normal distribution and standard deviation of 5 litres, is used to characterise the annual average milk yield for an individual cow.

With-in herd prevalence, p_w

The sources used to develop distributions for the with-in herd prevalence are presented in Annex 6.

Concentration of pathogens in faeces, c_f

In a recent review of pathogens in cattle Rhoades *et al*. (2009) highlighted the limited number of published works reporting faecal pathogen concentration. As a result, survey data for cattle has been used as a surrogate for dairy cattle where necessary. It should be noted that cattle at slaughter will include a proportion of dairy cows. No data for concentration of *L*. *monocytogenes* in dairy cow faeces could be found.

Faecal concentration data were obtained for generic *E*. *coli* and EHEC (Fegan *et al*., 2004c), *Salmonella* spp. (Fegan *et al*., 2004b) and *Campylobacter* spp. (Stanley *et al*., 1998). The logarithms of the faecal concentration were assumed to follow a Normal distribution. Where concentration data were missing a censored regression method was used. The mean ($\log_{10}$ cfu/g) and standard deviation for generic *E*. *coli*, *Campylobacter* spp., EHEC and *Salmonella* spp. are presented in the table below. Figure 6 presents a graphical summary of the concentration of generic *E*. *coli*, *Campylobacter* spp., EHEC and *Salmonella* spp. As might be expected the generic *E*. *coli* concentration is far greater than each of the pathogenic bacteria. The mean (base 10 logarithm) generic *E*. *coli* concentration is 5.9 compared with less than 1 for EHEC. The *Campylobacter* spp. faecal concentration is about 10 times greater than either EHEC or *Salmonella* spp.

Bacteria	Mean (log_{10} CFU/g)	Standard deviation (log_{10} CFU/g)
Generic *E. coli*	5.90	0.98
Campylobacter spp.	1.79	1.01
EHEC	0.87	1.40
Salmonella spp.	0.75	1.39

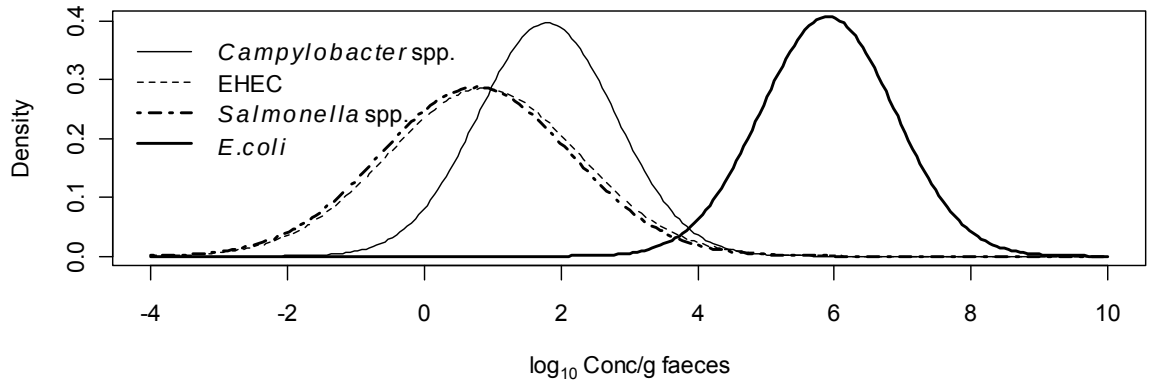

Figure 6: Distributions for faecal concentration of generic *E. coli*, *Campylobacter* spp., EHEC and *Salmonella* spp.

Amount of dirt present on the teat prior to cleaning, m_f

Vissers *et al.* (2006) performed experiments to estimate the amount of dirt (= faecal, soil and bedding material) transferred from a teat during milking. The experiments used spores of butyric acid producing bacteria as a surrogate to estimate the amount of material transferred. Ten cows from each of 11 farms were used in the study. The results suggested that there were large differences between cows both in the amount of soiling of teats and the amount of material transferred from the teat surface into the milk. This variable is taken as the mass of faecal material transferred per litre of milk and not the total mass transferred as used in the model by Clough for EHEC in milk (Clough *et al*., 2009).

Vissers *et al.* (2006) provided a table of the mean and standard errors for the concentration of spores on the udders of cows and the corresponding milk concentration. A preliminary experiment had established that there was no statistical difference in the spore concentration in the dirt on the udders or teats. This result is important as it is not possible to sample the teats prior to milking without influencing the transfer calculations. Using this evidence for the udder and milk concentrations plus the observation of a high correlation between these two concentrations (correlation coefficient = 0.79) it is possible to simulate the mass of dirt transferred per litre of milk. Analysis of this simulation revealed that the amount of dirt transferred was bi-modal; a result that may be interpreted as cows within a herd having either a low or high degree of teat soiling prior to teat cleaning and then milking. The mean amount of dirt transferred were 3.04 mg/l for cows with low teat soiling and 25.1 mg/l for cows with high teat soiling.

Teat cleaning efficiency, ε

Magnusson *et al.* (2006) studied the effect of a range of cleaning techniques for the removal of spores from teats. Alternative cleaning methods evaluated included the type of cleaning material (paper or cotton towels), chemicals, and cleaning duration. Cleaning efficiencies relative to the control ranged from 45 to 93%. Magnusson *et al.* (2006) suggested that the cleaning efficiencies for spores may be less than for bacteria due to their greater hydrophobicity. As such, the experimental cleaning efficiencies for spores may not be directly applicable to vegetative bacteria. A simulation approach is used to predict the cleaning efficiency by anchoring against Australian data on the concentration of generic *E. coli* in raw on-farm bulk milk tanks.

Bulk milk tank concentration, c_{BMT}

(1) *Campylobacter* spp., EHEC and *Salmonella* spp.

The approach taken to calculate the contribution of faecal contamination of teats is similar to that proposed by Clough *et al*. (2009). The Clough model was developed to investigate the effect of pasteurisation failure on the presence of EHEC in milk containers. The model was based on the information on teat cleaning for bacterial spores and not vegetative bacteria and used the total amount of faecal material transferred (Vissers *et al*., 2006) and not the mass per volume method outlined above. The final concentration in the bulk milk tank will depend on the factors outlined above. Each cow within the herd that have pathogens in their faeces may lead to teat contamination and subsequent transfer to the milk. The concentration of these pathogens in the milk will also be diluted by other cows in the herd that do not carry pathogens.

In order to assess the possible teat cleaning efficiency for pathogenic bacteria it would be necessary to perform experiments similar to those performed by Vissers *et al.* (2007) but using vegetative bacteria. In the absence of such data an alternative simulation approach was investigated that incorporated the variables outlined above, together with the use of generic *E. coli* data from the WA raw milk survey. Generic *E. coli* was used as a surrogate as it is present at high concentrations in the faeces of dairy cows, is frequently isolated from raw milk, and quantitative data was available. The outputs of the simulation are presented in Figure 7. The horizontal axis is the logarithm of the generic *E. coli* concentration and the vertical axis is the cumulative proportion of herds tested. The simulation study considered a total of 30 herds.

The solid data points in Figure 7 are the results for the WA survey for each of the five sampling periods. Individual sampling periods are not identified for clarity. The red 'staircase' to the right of the survey data is the simulation outputs assuming that there is no cleaning of teats prior to milking. In all but one case the simulation results are to the right of the survey data up to the 90^{th} percentile. A trial-and-error approach was then used to determine a teat cleaning efficiency that shifted the simulated generic *E. coli* concentration to within the range of the survey data; the green 'staircase' on the left of Figure 7. It is apparent that the simulation does not fully capture the differences between survey periods, but it does shift the 'cleaned' teat results to the left by about one order of magnitude. This simulation study assumes that the only source of generic *E. coli* in raw milk is due to the faecal route. The teat cleaning efficiency was modelled as a Pert distribution with a minimum value of 0.9, a most likely value of 0.93 and a maximum value of 0.99. This teat cleaning efficiency is subsequently used for *Campylobacter* spp., EHEC and *Salmonella* spp.

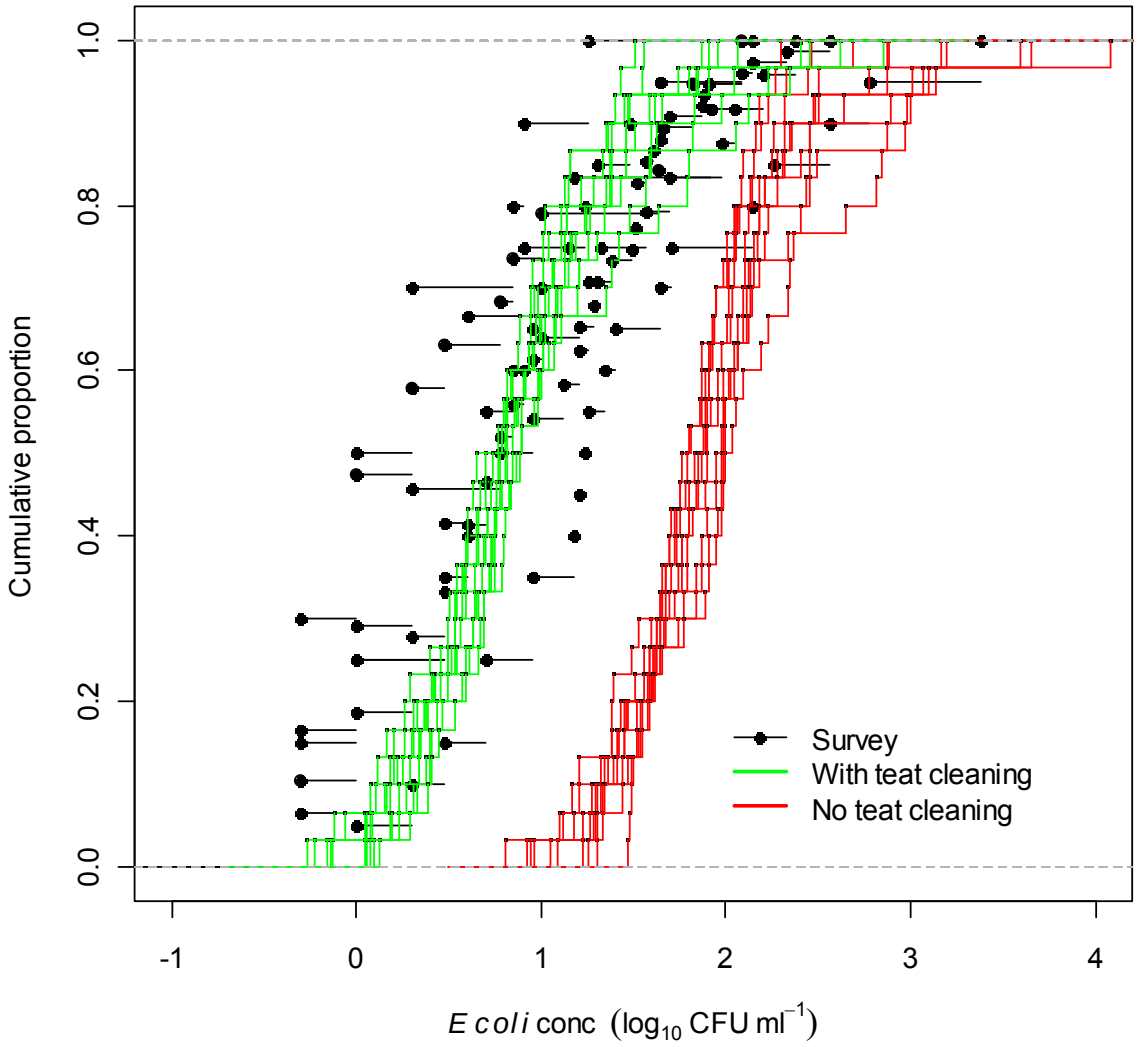

Figure 7: Comparison of the cumulative distributions for generic *E. coli* in raw milk: WA unpublished (2007) survey for raw milk (filled circles) and model predictions with (green lines) and without (red lines) teat cleaning

Mathematically the relationships between each of the variables in determining the bulk milk tank concentration are outlined below:

N herd size, N = Log Normal(30, 10) truncated at 10
V_i volume of milk produced per day by the ith cow (litres)
V_{BMT} total daily herd milk production (litres)

$$V_{BMT} = \sum_{i=1}^{N} V_i$$

p_w with-in herd prevalence (-)
N_c number of dairy cows carrying faecal pathogens, N_c = Binomial(N, p_w)
p_s probability that that the teats are lightly soiled, p_s = 0.337
c_f concentration of pathogens in faeces (cfu/g)
ε teat cleaning efficiency, ε = Pert(0.9, 0.93, 0.99)
m_f mass of faecal material transferred from an unwashed teat per litre of milk (mg/l)

$$\log_{10} m_f = \begin{cases} Normal\,(0.483, 0.315) & \text{with probability } p_s \\ Normal\,(1.399, 0.493) & \text{with probability } 1 - p_s \end{cases}$$

T total number of pathogenic bacteria (as cfu) transferred from j positive cows

$$T = \begin{cases} 0 & j = 0 \\ \sum_{j=1}^{N_c} (1 - \varepsilon) V_j (m_{f,j}/1000)\, c_{f,j} & j \geq 1 \end{cases}$$

c_{BMT} Concentration of pathogenic bacteria in the bulk milk tank (cfu/ml)

$$c_{BMT} = \frac{T}{1000\, V_{BMT}}$$

The method outlined does not explicitly include herd prevalence as a model input. Herd prevalence would be used to determine if a randomly selected herd has at least one cow with faecal carriage of pathogenic bacteria. A search of the literature could not identify suitable data sources for the three pathogenic bacteria, *Campylobacter* spp., *Salmonella* spp and EHEC. The unpublished University of Tasmania quantitative model used bulk milk tank prevalence as a surrogate for dairy herd prevalence. This approach, although appealing, has limitations due to the relatively high limits of detection for standard microbiological methods. As a result, the bulk milk tank prevalence will underestimate true herd prevalence.

Not incorporating a herd prevalence for these three pathogens does have mathematical consequences in the running of the model. The combination of small herd sizes and low with-in herd prevalences would result in a substantial proportion of "positive" herds having no "positive" cows. The resulting risk estimates would therefore underestimate the risk to consumers. There are several approaches that can be taken to deal with this situation. The first is to ensure at least one "positive" cow is present in a "positive" herd. This would result in potentially higher effective with-in herd prevalence. An alternative is to not include the herd prevalence and to determine effective herd prevalence from those farms that do have at

least one "positive" cow with-in the herd. If sources can be found to establish the likely herd prevalence, then the risk estimates can be reviewed against the effective herd prevalence obtained from the quantitative model outputs.

(2) *L. monocytogenes*

Fenlon *et al*. (1995) described a survey of *L. monocytogenes* isolated from bulk milk tanks on Scottish farms over a 12 month period. The survey observed the occurrence and concentration of *L. monocytogenes* contamination of bulk milk tanks. Samples were taken at roughly monthly intervals and analysed using direct plating and enrichment techniques. Samples that were positive by direct plating had the count recorded, while samples positive after enrichment were recorded as presence only. The enrichment method has a limit of detection of 1 cfu/10ml. Censored regression was used to estimate the mean and standard deviation of the base 10 logarithm of the direct plated and enrichment results. The resulting distribution has a mean of 0.196 and a standard deviation of 0.677 and was used as the concentration for *L. monocytogenes* in the bulk milk tank.

4.3.3 Growth/inactivation rates

The growth rate equations used to predict changes in the population of EHEC, *Salmonella* spp. and *L. monocytogenes,* plus the inactivation rates for the survival of *Campylobacter* spp. is provided in Annex 7. The growth rates for EHEC and *Salmonella* spp. were set to zero if the raw milk temperature dropped below 7 °C. Lag times were not explicitly included in the model formulation.

4.3.4 Dose response models

Dose response models are used to link the number of pathogenic bacteria ingested (the dose) to the probability of an individual becoming ill. The dose response model for *Campylobacter spp.* was developed using results of a human clinical trial, while the models for EHEC, *Salmonella* spp. and *L. monocytogenes* were informed by epidemiological evidence. The end-point for the dose response models is illness in all cases.

Beta-Poisson dose response models have been used for predicting illness for *Campylobacter spp.*, EHEC, and *Salmonella* spp. There is no differentiation between the general population and susceptible sub-populations *e.g*. children for these pathogenic bacteria.

The *Campylobacter* spp. dose response model is based on the human feeding trial reported by Black *et al*. (1988) and incorporates two stages. The first stage calculates the probability of infection after an exposure to a dose of *Campylobacter* spp., *p*(infection | dose), while the second stage determines the probability of illness occurring after the person becomes infected, *p*(ill | infected). The probability of illness after exposure is therefore:

$$p(\text{illness} \mid \text{dose}) = p(\text{infection} \mid \text{dose}) \times p(\text{ill} \mid \text{infection})$$

An α-β parameterisation of the Beta-Poisson dose response model is used:

$$p(infection \mid dose) = 1 - \left(1 + \frac{dose}{\beta}\right)^{-\alpha}$$

where $\log_{10}\ \alpha$ = Normal(-0.767, 0.180) and $\log_{10}\ \beta$ = Normal(1.681, 0.742) with a correlation coefficient of 0.6455, and p(illness | infection) = Beta(8.855, 23.254).

The dose response relationship for EHEC uses *Shigella* spp. feeding trial data as a surrogate. The use of *Shigella* spp. has been proposed in other risk assessments (see Marks *et al*. (1998)). The N_{50}-α parameterisation of the Beta-Poisson dose response model was used for the *Shigella* spp. feeding trial data:

$$p(illness \mid dose) = 1 - \left(1 + \frac{dose(2^{1/\alpha} - 1)}{N_{50}}\right)^{-\alpha}$$

where $\log_{10} N_{50}$ is a discrete distribution with three equally weighted values of 2.38, 3.17, 4.48, and a fixed value of α = 0.266. A comparison of the *Shigella* spp. dose response models to the epidemiological evidence from EHEC illnesses from Strachan *et al*. (2005) indicated that the method was appropriate (Figure 8).

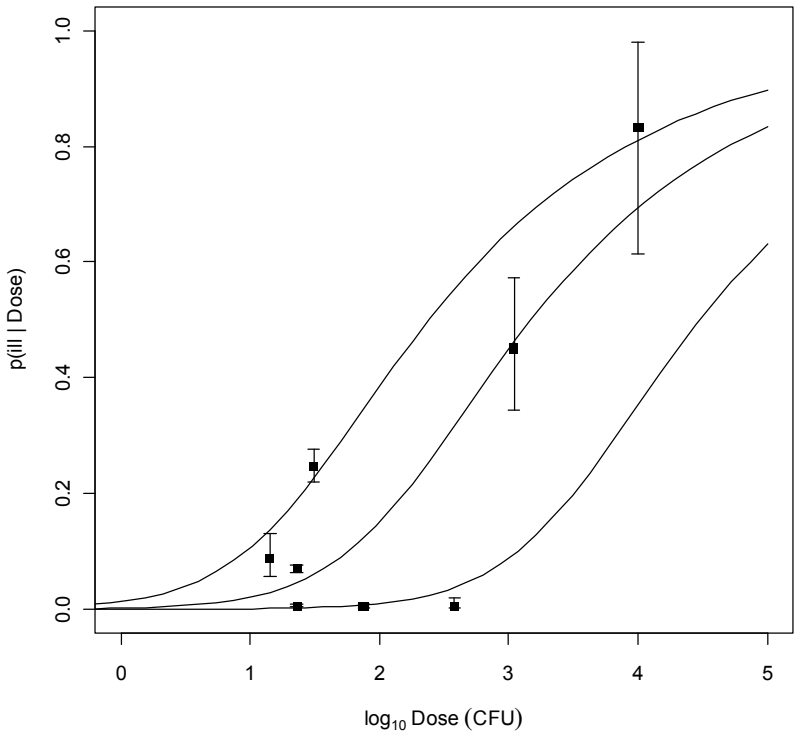

Figure 8: Comparison of human EHEC outbreak data (Strachan *et al*., 2005) and the predicted dose response relationships based on human feeding trials for three strains of *Shigella* spp. The error bars represent the 95% confidence intervals for each outbreak.

The *Salmonella* spp. dose response model is taken from Thomas *et al*. (2006). This model was based on epidemiological data for illnesses caused by a range of *Salmonella* serovars, predominantly *S*. Enteritidis and *S*. Typhimurium. An α–β parameterisation of the Beta-Poisson dose response model was used:

$$p(illness \mid dose) = 1 - \left(1 + \frac{dose}{\beta}\right)^{-\alpha}$$

where $\log_{10} \alpha$ = Normal(-0.871, 0.89) and $\log_{10} \beta$ = Normal(1.727, 0.227) with a correlation coefficient of 0.892.

For *L*. *monocytogenes*, two groups (susceptible and healthy) have been retained based on the epidemiological evidence highlighting the importance of susceptible populations and the occurrence of invasive listeriosis. Additional information on the importance of genetics of *L*. *monocytogenes* lineage has been explicitly included in the model as uncertainty. This uncertainty has been included in the values of the *r*-value used in the Exponential dose response model. The FAO/WHO (2004) estimated an average *r*-value for the susceptible population as 5.85×10^{-12}. A more recent assessment of US epidemiological data which included genetic information regarding different *L*. *monocytogenes* strains found to cause invasive listeriosis, determined average *r*-values of 1.31×10^{-8} for lineage I and 5.01×10^{-11} for lineage II (Chen *et al*., 2006). These results suggest that there are large differences in virulence between *L*. *monocytogenes* strains.

Severity has not been included for the risk estimates.

4.3.5 Consumption data

In the absence of raw milk consumption data, data on pasteurised milk consumption from the National Nutrition Survey (NNS) was used. The NNS data was converted to total daily consumption volume as serving equivalents of 1 cup (250 ml) using a cumulative under-size method (*i.e*. 100 ml is treated as 250 ml) (Figure 9). The mean daily consumption for children and adults is 536 ml and 397 ml, respectively. The range of daily raw milk intakes was 250 - 1750ml.

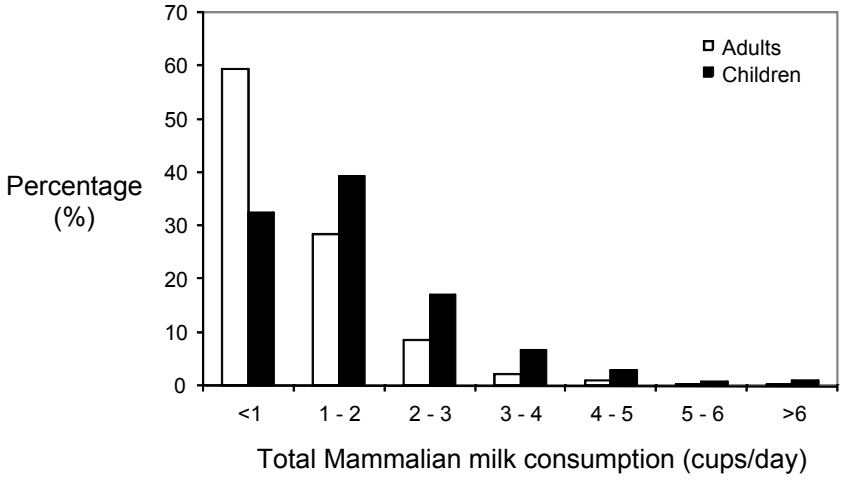

Figure 9: Total mammalian milk consumption based on the NNS (1995)

A summary of the model inputs is presented in Table 4.

Table 4: Summary of quantitative model input distributions

Input	Distribution
Bulk milk tank prevalence	
Campylobacter spp.	Not applicable
EHEC	Not applicable
Salmonella spp.	Not applicable
L. monocytogenes	See Annex 5
Within-herd prevalence	
Campylobacter spp.	See Annex 6
EHEC	See Annex 6
Salmonella spp.	See Annex 6
L. monocytogenes	See Annex 6
Number of lactating cows in a herd	Log Normal(30, 10) truncated at 10
Pathogen faecal concentration (log_{10} cfu/ml)	
Campylobacter spp.	Normal(1.791, 1.007)
EHEC	Normal(0.865, 1.4)
Salmonella spp.	Normal(0.751, 1.39)
Proportion of cows with lightly soiled teats	0.337
Concentration of faecal material transferred during milking from teat surface before cleaning (log_{10} mg/L)	
Lightly soiled	Normal(0.483, 0.315)
Heavily soiled	Normal(1.399, 0.493)
Teat cleaning effectiveness	Pert(0.9, 0.93, 0.99)
***L. monocytogenes* BMT concentration (log_{10} cfu/ml)**	Normal(0.1959, 0.6772)
Maximum population density	
Spoilage bacteria (cfu/ml)	10^9
Pathogens (cfu/ml)	10^8
Spoilage criteria (cfu/ml)	$10^{7.5}$
On-Farm storage	
Bulk milk tank (BMT) time (h)	Triangular(1, 16, 24)
Bulk milk tank (BMT) temperature (°C)	Triangular(2, 4, 10)
Milk collection	
Milk tanker temperature (°C)	Pert(4, 5, 6)
Transportation time (h)	Triangle(1, 3, 6)

Table 4 cont: Summary of quantitative model input distributions

Input	Distribution
Factory storage	
Factory storage time (h)	Triangle(0, 48, 96)
Factory storage temperature (°C)	Pert(1, 4, 5)
Production distribution	
Production distribution time (h)	Triangle(0, 3, 24)
Production distribution temperature (°C)	Pert(0, 4.8, 7)
Retail storage	
Retail storage time (h)	Pert(12, 48, 96)
Retail storage temperature (°C)	Normal(4.9, 2.8) truncated at 0 and 6°C
Domestic transportation	
Domestic transportation time (h)	WeibullAlt(58%, 0.5, 93%, 1.5, "loc", 0)
Domestic transportation temperature (°C)	Pert(7.1, 16.25, 20.4)
Domestic storage	
Domestic storage time (h)	Cumulative distribution
Domestic storage temperature (°C)	Normal(3.88, 2.35) truncated at -2°C
Raw milk consumption (ml/day)	
Children	Empirical distribution
Adults	Empirical distribution
Susceptible (for *L. monocytogenes*)	Same as Adult
Healthy (for *L. monocytogenes*)	Same as Adult

4.4 Simulation results

4.4.1 Consumption scenarios

The model outputs are reported as illness following consumption from the bulk milk tank (Scenario 1); domestic consumption after farm gate purchase (Scenario 2); and domestic consumption after packaging, distribution and retail sale (Scenario 3).

A summary of the predicted illnesses in children per 100,000 daily servings of raw milk for *Campylobacter* spp., EHEC and *Salmonella* spp. is presented in Table 5. A summary of predicted illnesses in adults is presented in Table 6. The mean number of predicted illnesses for children in Scenario 1 (consumption of raw milk from the on-farm bulk milk tank) is 18.8 for *Campylobacter* spp., 16.5 for EHEC and 16.8 for *Salmonella* spp. per 100,000 daily servings.

For *Campylobacter* spp. the mean number of predicted illnesses for children in Scenarios 2 and 3 decreases to 5 and <1 illnesses per 100,000 daily serves. This decrease in predicted cases is a result of the inactivation of *Campylobacter* spp. in chilled raw milk. The decrease in illnesses in Scenario 3 compared with Scenario 2 is due to the longer supply chain for raw milk that has passed through packaging, distribution and retail stages. The variability in predictions is in the order for 20%, as indicated by the 5th and 95th percentiles.

For EHEC and *Salmonella* spp., the increase in the predicted number of illnesses for children between Scenario 1 and Scenarios 2 and 3 is due to the increased consumption and the growth that is possible between milking and consumption in the home. The predicted number of illnesses for children due to EHEC is 48.8 for raw milk purchased at farm gates sales and 96.9 per 100,000 daily servings for packaged raw milk sold at retail premises.

The trend in the cases of illness per 100,000 daily servings for adults is consistent with those for children. However, the absolute values of illnesses for adults are lower than for children for Scenarios 2 and 3. This is as a result of the lower consumption amount recorded in the 1995 NNS. For example the mean number of illnesses due to *Salmonella* spp. for Scenario 3 is 130.2 for adults and 152.6 for children. As the dose response models for EHEC, *Campylobacter* spp. and *Salmonella* spp. do not include sub-populations, the number of illnesses for Scenario 1 is the same for both the children and adults. The small differences in values are due to random variations between simulations for each group.

Table 5: Predicted cases of illness for children from *Campylobacter* spp., EHEC and *Salmonella* spp. per 100,000 daily serves of raw milk

Pathogen	Scenario	Median	Mean	5th percentile	95th percentile
Campylobacter spp.	1	19	18.8	14	23
	2	5	5.2	2	8
	3	0	0.3	0	1
EHEC	1	16	16.5	12	22
	2	49.5	48.8	40	56
	3	96.5	96.9	85	110
Salmonella spp.	1	17.5	16.8	10	21
	2	54	54.9	41	71
	3	150.5	152.6	135	171

Table 6: Predicted cases of illness for adults from *Campylobacter* spp., EHEC and *Salmonella* spp. per 100,000 daily serves of raw milk

Pathogen	Scenario	Median	Mean	5th percentile	95th percentile
Campylobacter spp.	1	19.5	19.9	15	24
	2	4.5	4.7	2	8
	3	0	0.1	0	1
EHEC	1	16	16.5	12	22
	2	38	38.4	34	46
	3	77	77.7	66	89
Salmonella spp.	1	15	14.9	8	21
	2	61	59.4	46	69
	3	128.5	130.2	117	148

The predicted illnesses for *L. monocytogenes* are presented in Table 7. For Scenario 1 the mean predicted number of illnesses is <1 for the three susceptible groups and the healthy sub-population. The highest result is for the susceptible sub-population when using the Chen Lineage I as indicated by the 95th percentile value of only 1 case of invasive listeriosis. The trends between Scenarios 2 and 3 are similar to the results for EHEC and *Salmonella* spp. A greater number of predicted illnesses are observed for Scenario 3 with a mean of 170 illnesses compared with 16.7 illnesses for Scenario 2 when using the Chen Lineage I *r*-value.

Table 7: Predicted cases of illness from *L. monocytogenes* per 100,000 daily serves of raw milk for the susceptible sub-population

Scenario	Dose response parameter	Median	Mean	5th percentile	95th percentile
1	Chen Lineage I	0*	0.2	0*	1
	Chen Lineage II	0*	0*	0*	0*
	WHO	0*	0*	0*	0*
2	Chen Lineage I	16	16.7	12	23.7
	Chen Lineage II	1	0.9	0*	2
	WHO	0*	0.1	0*	0.85
3	Chen Lineage I	166.5	170	157.2	190.55
	Chen Lineage II	5	5.6	2	10.85
	WHO	1	0.7	0*	2

* Lower bound value (assuming <1 is equal to zero)

There were no predicted illnesses when using the WHO dose response model for the general population. In contrast, using other dose response models, the number of cases of illness in susceptible sub-population scenario is between <1 and 170.

The risk estimates in Tables 5 - 7 are made under the assumption that no spoilage occurs in the raw milk between milking and consumption. The inclusion of spoilage has the effect of eliminating the consumption of raw milk that has elevated levels of bacteria. The criterion for spoilage was exceeding a threshold of $10^{7.5}$cfu/ml of a surrogate spoilage bacterium, *Pseudomonas* spp. If there is no temperature abuse and a long storage time then spoilage will occur with no, or minimal, corresponding increase in pathogen concentration. An alternative may be that spoilage occurs due to a period of temperature abuse that leads to the growth of a pathogen. The inclusion of a spoilage criterion may then remove the iterations where a large amount of pathogen growth has occurred. A summary of predicted illnesses for children incorporating spoilage is presented in Table 8. An example of the inclusion of spoilage is found for *Salmonella* spp. for Scenario 3. Results from the quantitative model determined that the mean number of illness per 100,000 daily servings of raw milk for children is 152.6 without spoilage and 65.9 with the inclusion of spoilage.

Table 8: Predicted cases of illness for children from *Campylobacter* spp., EHEC and *Salmonella* spp. per 100,000 daily serves of raw milk, excluding spoilage

Pathogen	Scenario	Median	Mean	5th percentile	95th percentile
Campylobacter spp.	1	19.5	19.9	15	24
	2	4.1	4.7	3	7
	3	0	0.1	0	1
EHEC	1	16	16.5	12	22
	2	31.4	32.7	25	40
	3	46.6	47.1	40	59
Salmonella spp.	1	15	14.9	8	21
	2	48.6	47.3	36	54
	3	63.9	65.9	54	81

4.5 Sensitivity analysis for on-farm factors

Sensitivity analysis is a systematic evaluation of model inputs to identify the most important factors on the model outputs. A sensitivity analysis, using spider plots, was performed to assess those on-farm factors that have the greatest influence on the concentration of pathogens in the bulk milk tank. In the case of *Campylobacter* spp. four factors were considered: herd size, teat cleaning efficiency, teat soiling and with-in herd prevalence.

Spider plots are constructed by replacing an input distribution by single values based on the cumulative percentiles (*e.g.* 1, 5, 25, 50, 75, 99) of the distribution. For herd size, the input distribution was a Log normal distribution with $\mu = 30$ and $\sigma = 10$, truncated at 10. The corresponding values for the cumulative percentiles were 13, 17, 23, 28, 35, 49 and 61 cows. The 1st and 99th percentile with-in herd prevalence was 0.2% and 70.7%, respectively. The quantitative model was run for a total 10,000 x 7 x 4 iterations in order to construct the spider plot (Figure 10). Each axes of each panel in Figure 10 is the same; the x-axis is the percentile of the distribution and the y-axis is the mean *Campylobacter* spp. concentration calculated from the 10,000 iterations of the quantitative model.

Input factors that have no influence on the model output can be readily identified as those that have horizontal spider plots. In the case of *Campylobacter* spp. concentration, the herd size had no influence, while the teat cleaning efficiency had only a weak influence (Figure 10). The two main factors that influence the mean *Campylobacter* spp. concentration are the degree of teat soiling and the with-in herd prevalence.

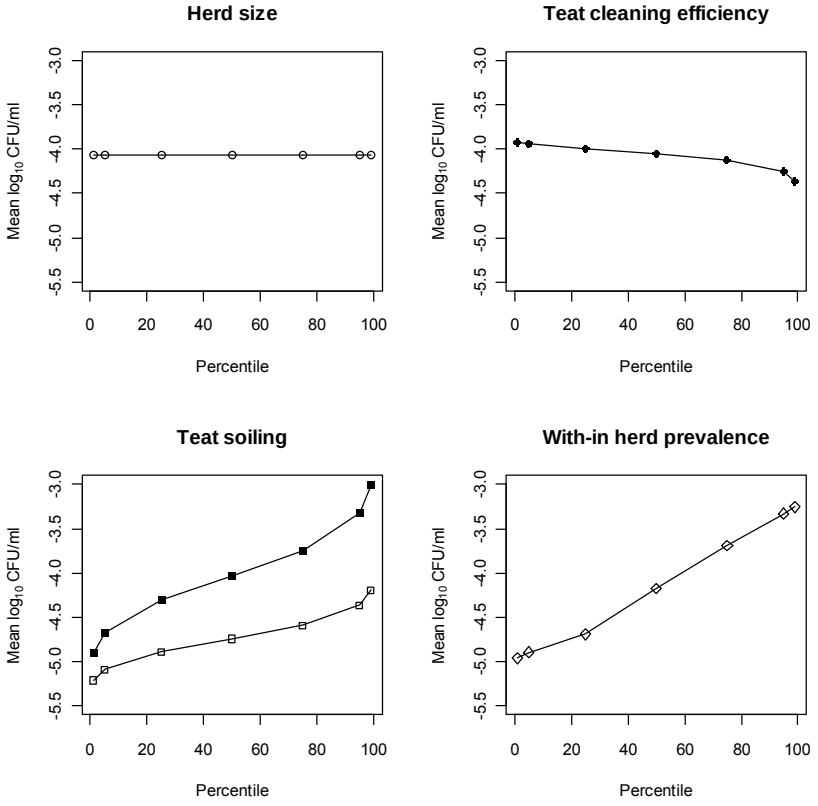

Figure 10: Spider plot for herd size, teat cleaning efficiency, teat soiling (solid squares: high soiling; open squares: low soiling) and with-in herd prevalence on the mean *Campylobacter* spp. concentration, as $\log_{10}$ cfu/ml

4.6 Measures to reduce levels of pathogens in raw milk

The quantitative model has been developed to estimate the risk to consumers from the consumption of raw milk from a randomly selected farm, using three consumption scenarios.

With no major interventions available to destroy pathogens in raw milk, it is essential to minimise the likelihood of pathogens contaminating the raw milk stream and then minimise the growth, if these risk estimates are to be reduced. Two ways in which to minimise contamination are to:

- Minimise shedding of pathogens into milk and the milking environment
- Minimise contamination during the milk harvesting stage

Reducing pathogen shedding requires active surveillance of herds to reduce the numbers of carrier animals or those with sub-clinical infections. Herd prevalence data was used to determine if the milk from this 'random' farm was contaminated with any of the four pathogens. In all cases this prevalence was low, about 5%. Contamination levels in raw milk due to animals with intestinal carriage of pathogens are low, even when considering the variability about the mean concentration. For EHEC the mean concentration was estimated to be around 10^{-4}cfu/ml (-4 log). As the limit of detection is 1cfu/ml, less than 2% of individual samples would be above this level and therefore EHEC would be rarely detectable using culture methods. When this probability is combined with the herd prevalence, the likelihood of successfully using random sampling of farm milk to detect animals shedding this pathogen is low.

However, when considering the case of a single farm, additional factors need to be considered. One important factor is the persistence of pathogens within the animal and the farm environment. Persistence of *L. monocytogenes* infection on-farm has been examined by Fenlon *et al.* (1995). In this Scottish study, bulk milk tanks from 160 dairy farms were sampled at roughly monthly intervals over a 12 month period. All farms were tested four times and if a sample was positive then two additional samples were taken. The raw milk samples were analysed using direct plating (quantifiable) or by enrichment (detection only).

Of the 160 farms tested, 25 farms were found to be positive at least once during the study period. Results for the 25 farms that were positive at least once, is presented in graphical form in Figure 4. The horizontal axis is the sample number and the vertical axis is the farm number. Note that all farms were tested at the start of the study (Sample 1) and again at sampling periods 4, 7 and 10. Samples positive by direct plating are shown as closed circles, those positive after enrichment as crossed circles and the no detects as open circles.

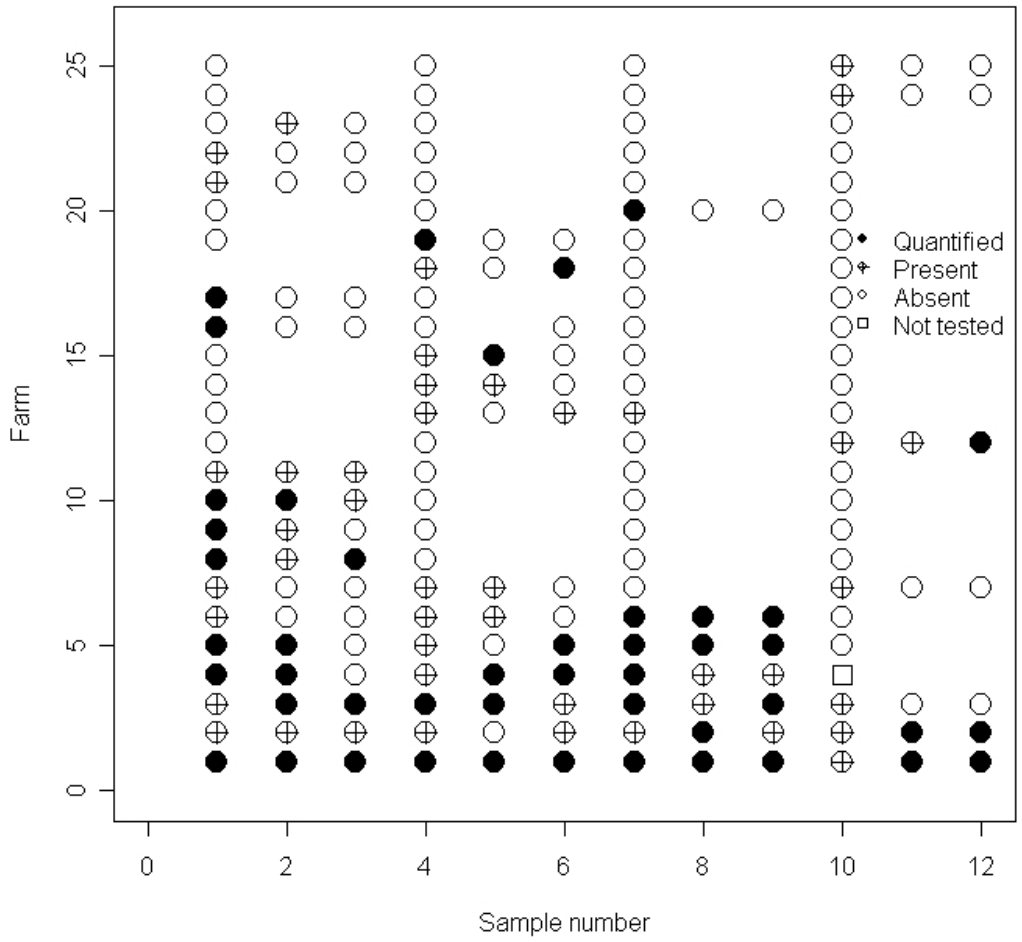

Figure 11: Persistence of *L. monocytogenes* in farm bulk milk tanks

The results of the study highlight large differences in the occurrence of *L. monocytogenes* between farms. For example, farms 1, 2 and 3 had detections in nearly all of the sample periods, while farm 25 only detected *L. monocytogenes* at the final quarterly sampling point and then it was not detected at the two subsequent sampling times. Similar behaviour can be seen with other farms. This suggests there may be important herd management factors that influence the introduction of *L. monocytogenes* into farms and its persistence in bulk milk samples.

The second main means of reducing contamination is control over the milk harvesting stage, including pre and post milking teat cleaning regimes, foremilk stripping and milking hygiene.

As discussed in Section 3.6.2, teat cleaning and disinfection are important for managing the introduction of microorganisms into the milk from the surface of the teat and the teat canal. This practice is also a significant control factor for managing mastitis in milking animals. Hygienic milking practices are firmly entrenched in current industry practices and are designed to reduce the risk of contamination and growth of microorganisms in the milk. Given that these practices are currently widely employed, there is little capacity for significant improvements to be made.

4.7 Variability and uncertainty

Variability and uncertainty in input variables have been included in all stages of the quantitative model development. This is especially the case for inputs that have the largest uncertainty. Other cases, such as uncertainty in the dose response model for *L. monocytogenes,* have also been included.

Other inputs such as the concentration of *Campylobacter* spp., EHEC and *Salmonella* spp. include both variability and uncertainty in the development of the distributions used in the model.

Model uncertainty and regression variability for the growth rate equations was not fully explored in this model, as these factors are generally of little importance as only limited growth of the pathogens was observed. The variability in the inactivation rate for *Campylobacter* spp. in raw milk was included in the quantitative model as this parameter was important in evaluating the risk from this pathogen.

Australian specific data on pathogen prevalence for herds and for individual animals is scarce, as is any concentration data for pathogen levels within Australian raw milk. Consequently, international data has been used for these inputs. In the case of herd prevalence for EHEC, the majority of data has been obtained from surveys of herds in continental Europe and North America. The two Australian studies for the prevalence of EHEC in cows had mean prevalence's of 9.5% and 0% compared with an average of 4.6% for the international studies. The inclusion of the international studies was to maximise the uncertainty for this important model input.

The effective herd prevalence for *Campylobacter* spp., EHEC and *Salmonella* spp. were found to be 83%, 56% and 77%, respectively. As noted earlier, the herd prevalence was not included explicitly in the quantitative model for these pathogens. Clough *et al.* (2009) includes details of two studies that put the EHEC herd prevalence at 33.3% (95% confidence interval 21.7% - 45.0%) and 29.6% (95% confidence interval 15.1% - 49.8%). These results, taken with the observed variability between studies in the with-in herd prevalence data, suggest that for EHEC at least, the effective herd prevalence may not be too far from observed results.

5. Discussion

Prior to the introduction of mandatory pasteurisation in most Australian states in 1943, raw cow milk was implicated in widespread outbreaks of illness in Australia. Following its introduction there was a significant reduction in milkborne illnesses recorded; demonstrating pasteurisation was an effective means of eliminating pathogens from milk. Despite this, consumption of raw cow milk on school camps, during farm visits or via consumption of raw cow milk products has been implicated in eight outbreaks of illness affecting 101 people between 1998 – 2003.

The majority of these incidents have involved milk consumed from a restricted distribution network, such as a single cow whilst on school camps or farm visits. The exception to this was in 1976, when over 500 people became ill after consuming raw milk which had been commercially produced and distributed. *Campylobacter* spp. and *Cryptosporidium* spp. were the most commonly implicated pathogens for the restricted distribution outbreaks, whilst *Salmonella* Typhimurium was involved in both a farm setting and the large retail outbreak.

Internationally, consumption of raw milk continues to be a significant risk factor for foodborne illness, even when the milk is produced under regulatory control. Regular outbreaks of illness have occurred in the US in recent years. *S.* Typhimurium was implicated in outbreaks in 2003 and 2007, involving 62 and 29 cases of illness respectively. In 2005 and 2006, *E. coli* O157:H7 was responsible for outbreaks which lead to the development of six cases of haemolytic ureamic syndrome (HUS) With the 2006 outbreak, the median age was 8 years with the range between 6 and 18 years. There were six cases, two of which contracted HUS.

The foregoing epidemiological evidence suggests the pathogens of most concern in raw cow milk mediated illness are *Campylobacter* spp., *Salmonella* spp. and EHEC. *Listeria* spp. are another commonly implicated pathogen in raw milk.

While these pathogens are frequently implicated in foodborne illness, few studies have actually assessed the weight of evidence to support causal links between these pathogens and consumption of raw milk as a vehicle for foodborne illness. A systematic review undertaken by researchers in New Zealand concluded that moderate evidence existed to support causal links for raw milk mediated illness from *Campylobacter* spp. , *Salmonella* spp. , EHEC and *Listeria* spp.

Consumption of raw cow milk appears to be low among the general population, but in specific groups large amounts are consumed, sometimes in the order of litres per person per day. Prevalent among this group of consumers is the belief that raw milk possesses particular healthy properties or attributes, above the existing nutritional components. As a result of these perceived health benefits, raw milk is often marketed to and consumed by individuals who may have lowered immunity such as the very young, very old or immunocompromised or to people with specific dietary needs.

The very nature of how raw milk is produced and harvested means that raw milk can often be contaminated with pathogens. Contamination can occur either directly through organisms shed as a result of udder infections or indirectly. Indirect contamination may arise from (i) a cow's own faecal matter contaminating the udder and teats, (ii) faecal matter of other cows contaminating the udder (iii) milking clusters contacting surfaces with faecal contamination,

and (iv) post-harvest environmental contamination. An intensive search of published and unpublished literature shows that internationally, raw cow milk is often contaminated with pathogens. Whilst data is scarce for Australia, the data which is available confirms that raw cow milk is a source of low levels of pathogenic microorganisms.

In order to assess the risk to Australian consumers from consumption of raw cow milk, a quantitative microbiological risk assessment was undertaken. The assessment considered the risk associated with four identified key milkborne pathogens: *Campylobacter* spp., EHEC, *Salmonella* spp. and *L. monocytogenes.*

The assessment determined predicted illnesses per 100,000 daily servings of raw milk consumed, at the bulk milk tank, in the home after farm-gate sale and in the home after packaging, distribution and retail sale.

The first scenario of consumption of raw milk from the on-farm bulk milk tank is 19 cases for *Campylobacter* spp., 16 cases for EHEC and 17 cases of illness for *Salmonella* spp. per 100,000 servings for children. This scenario was developed to investigate a point source exposure *e.g.* a farm visit and represents a baseline risk estimate.

In the case of *Campylobacter* spp. the likelihood of illness is lower when raw milk is consumed in the home after purchase from the farm-gate or from retail establishments. The quantitative model predictions show that the mean probability of illness for these two scenarios is 5 and <1 illness per 100,000 daily servings (Table 5). This reduction in illness is due to the finding that *Campylobacter* spp. does not survive well in refrigerated milk or other foods.

The quantitative model is built on the assumption that EHEC, *Salmonella* spp. and *L. monocytogenes* are capable of growth in raw milk at rates determined by the storage temperature and time. The longer supply chain for raw milk sold at retail also results in a greater number of illnesses than for off-farm sales. A greater increase in predicted illnesses was seen for EHEC from Scenario 2 (49) to Scenario 3 (97) than from Scenario 1 to 2. Similarly, *Salmonella* spp. increased from 55 to 153 and *Listeria* spp. increased from 17 to 170 cases, respectively.

The ability to reduce or minimise the risks associated with raw milk is considered to be quite limited. Enhancements in herd health and veterinary supervision, active surveillance to reduce the number of carrier animals, and improvements in milking hygiene could be expected to reduce the risk to consumers. However, they could involve significant and often intensive interventions, with concomitant intensive enforcement authority oversight.

Another strategy would be to test and hold all raw milk before it is sold to consumers. Raw milk is highly perishable, and spoilage organisms would be expected to proliferate pending the receipt of test results. Furthermore, even low levels of pathogens in raw milk present a risk to consumers, and frequently these are below the detection levels for most microbiological screening methods.

6. Data gaps and areas for further research

During the preparation of the risk assessment a number of data gaps were identified along the raw cow milk supply chain. Research into these areas may better assist in describing factors along the farm-to-table continuum which impact on the burden of illness resulting from consumption of raw cow milk in Australia, enhance the modelling outputs, and reduce uncertainty.

Significant data gaps exist hence further research is required in the following areas:

- Spatial and temporal information on the prevalence and levels of pathogens in Australian dairy cows and in raw cow milk using the most sensitive detection methods
- Data on the extent to which external faecal contamination on the udder and flanks etc can contaminate the milking environment and the milk
- Carrier status of lactating cows for enteric pathogens and the effect this may have on the contamination level of raw milk
- Information on the status of sub-clinical mastitis in lactating cows and the contribution this makes to the contamination levels in raw milk
- Dose response models for pathogens
- Pre and post milking teat treatments and their efficacy in removing contamination and improving the microbial status of raw milk
- Raw milk consumption data, especially addressing the frequency and amount of raw cow milk consumed as well as the demographics of the consuming population

7. Conclusion

Raw cow milk has always presented risks to public health because of the potential presence of pathogenic bacteria. The widespread adoption of pasteurisation for liquid milks was precipitated by ongoing outbreaks of illness, and the efficacy of the technique in eliminating most pathogenic microorganisms when combined with hygienic post-pasteurisation packaging and handling of the milk.

This raw cow milk risk assessment sought to gather data on the public health impact of consuming raw cow milk, reviewed milk production and handling practices, and modelled the likelihood of illness associated with four key pathogens.

While raw cow milk for human consumption is not legally available for sale in Australia, it is sold as "pet milk" or as "cosmetic milk" and is also made available to consumers during farm visits and farm stays. Between 1998 - 2003, OzFoodNet reported eight outbreaks comprising 101 cases of illness associated with the consumption of raw cow milk in Australia. Internationally, a large number of outbreaks of foodborne illness have been attributed to raw cow milk.

The quantitative microbiological risk assessment undertaken during this assessment has determined that access to raw cow milk will result in an increased likelihood of foodborne illness in Australia. The burden varies depending on how the raw milk product is handled, with the risk for *Campylobacter* spp. greatest nearer the point where milk is procured from the cow, while the risks from EHEC, *Salmonella* spp. and *L. monocytogenes* increase as the milk is further handled, stored and transported. This reflects storage conditions and subsequent growth as the product passes along the supply chain.

For raw cow milk the probability of illness after retail purchase is less than 1 case of campylobacteriosis, 97 cases of EHEC and 153 cases of salmonellosis in the general population for children, and as high as 170 cases of listeriosis in a susceptible sub-population. This is per 100,000 serves of a mean daily intake of 540 ml of milk to a child.

The risk assessment also sought to identify ways of reducing the burden of illness. Measures available include addressing animal health, animal carrier status and milk procurement activities. Examples of additional controls may include regular testing of animals to determine if they are carriers of pathogens, exclusion of milk from animals with sub-clinical mastitis, advances in milking hygiene, and testing of milk before sale. The assessment determined that the capacity to make a significant impact on the burden of illness is limited. Current animal management structures are based on best practice for controlling mastitis and limiting the contamination of milk with spoilage and pathogenic microorganisms through the hygienic operation of the milking environment. Other measures such as testing of animals for carrier status, or analysis of raw milk are unlikely to detect low levels of pathogens.

Annexes

ANNEX 1: Summary of milk consumption data

Consumption data can be calculated from food production statistics or food consumption surveys. Food production statistics provide an estimate of the amount of specific food commodities available to the total population. This type of data may include national statistics on per-capita food production. Consumption surveys (such as national nutrition surveys) provide detailed information regarding the types and amounts of foods consumed by individuals or households and sometimes the frequency with which the foods are consumed.

1995 Australian National Nutrition Survey
Data from the Australian National Nutrition Survey (NNS) provides detailed information regarding the types and amounts of dairy foods Australian's are consuming. The most recent national survey was conducted during the period February 1995 - March 1996. Approximately 13,800 people aged two years or over from urban and rural areas in all States and Territories participated in the survey.

Two approaches were used in the NNS to collect data on intake. The 24 hour recall method was used as the main indicator. All participants were interviewed by trained nutritionists who sought detailed information on all foods and beverages consumed during the day prior to the interview (from midnight until midnight). A sample of approximately 10% of the NNS participants also provided intake data for a second 24 hour period. A Food Frequency Questionnaire was used to assess usual frequency of intake for those aged 12 years or more.

A summary of milk and liquid milk consumption by gender and age is outlined in Table 1.1.

Table 1.1: Milk consumption by gender and age

Gender	Age (yrs)	No. of Respondents	No. of consumers (% of respondents)	Mean milk consumed (g/day)	95th percentile (g/day)
Male	2 - 3	170	150 (88.2)	447	1037
	4 - 7	416	334 (80.3)	391	922
	8 - 11	385	313 (81.3)	395	903
	12 - 15	349	269 (77.1)	452	1144
	16 - 18	215	160 (74.4)	495	1553
	19 - 24	485	356 (73.4)	341	1033
	25 - 44	2140	1736 (81.1)	272	795
	45 - 64	1554	1289 (82.9)	261	697
	65+	902	772 (85.6)	251	630
Female	2 - 3	213	193 (90.6)	403	931
	4 - 7	383	307 (80.2)	301	764
	8 - 11	354	265 (74.9)	340	873
	12 - 15	304	204 (67.1)	336	780
	16 - 18	218	134 (61.5)	271	692
	19 - 24	575	446 (77.6)	242	648
	25 - 44	2385	2017 (84.6)	217	645
	45 - 64	1752	1486 (84.8)	222	626
	65+	1058	880 (83.2)	217	557
		13858	11311 (		

Roy Morgan Single Source Survey

Roy Morgan data outlines the proportion of population groups who consume milk over a weekly period (weekly consumer) outlined in Table 1.2 below. Due to milk being a fairly staple commodity that can be consumed at various times throughout the day, it is likely that a 'weekly consumer' may indeed also be a 'daily consumer'. 'Milk' was defined in the survey to include low/no fat, regular and packaged/flavoured products. The number of respondents has been extrapolated to represent the total population (in 000's) in that age group, based on Australian Bureau of Statistics data.

Table 1.2: Roy Morgan Single Source Survey data

Age (years)	Gender	Respondents		Respondents Consuming Milk in past 7 days (%)		Mean Glasses Consumed	
		2005	2006	2005	2006	2005	2006
14 - 19	Male	879	899	660 (75%)	660 (73%)	7.5	6.8
	Female	836	804	652 (78%)	618 (77%)	5.9	6.1
20 - 24	Male	671	691	535 (80%)	490 (71%)	6.6	6.8
	Female	671	725	546 (81%)	603 (83%)	6.0	5.9
25 - 44	Male	2760	2766	2213 (80%)	2205 (80%)	6.7	6.8
	Female	2852	2803	2321 (81%)	2274 (81%)	6.2	6.2
45 - 64	Male	2663	2690	2069 (78%)	2065 (77%)	6.1	6.3
	Female	2938	3024	2329 (79%)	2356 (78%)	6.1	6.2
65+	Male	1186	1233	857 (72%)	926 (75%)	6.1	6.0
	Female	1112	1162	836 (75%)	853 (73%)	6.5	6.5

ANNEX 2: Summary of foodborne illness associated with consumption of raw cow milk

Australian data

Outbreaks of foodborne illness attributed to the consumption of raw cow milk listed in OzFoodNet's Outbreak Register are detailed in Table 2.1. Data has been compiled since 1995 with no outbreaks attributed to raw cow milk post 2003.

Table 2.1: Outbreaks associated with milk and milk products in Australia, 1995 - 2004 (OzFoodNet data: 1995-June 2004)

Year	State	Cases (Hosp)	Aetiology	Setting food prepared	Comments
2003	Vic	13	*Campylobacter* spp.	School	The risk of illness was 3.7 times higher among people who had consumed any unpasteurised milk.
2003	SA	14	*Campylobacter* spp.	Camp	Unpasteurised milk was supplied for drinks and cereal.
2001	Vic	12	Unknown	Camp	Relative risk was higher for those that had consumed unpasteurised milk.
2001	Qld	8 (4)	*Cryptosporidium* spp.	Community	Strong epidemiological evidence of association between consumption of unpasteurised milk and cryptosporidium.
2000	SA	12	*Campylobacter* spp.	Farm	Infection due to consumption of unpasteurised milk.
2000	Vic	21	*Campylobacter* spp.	Camp	Infection due to consumption of unpasteurised milk.
1999	SA	12	*Salmonella* Typhimurium 44	Farm	Infection due to consumption of unpasteurised milk.
1998	WA	9	*Campylobacter* spp.	Camp	Consumption of unpasteurised milk on camp.

Four outbreaks occurred on school camps where unpasteurised milk was consumed and two outbreaks were from unpasteurised milk consumed on farms. Unpasteurised milk was also consumed and led to outbreaks in a community setting and in a school. The outbreaks identified in the OzFoodNet Outbreak Register were investigated using three point source studies and one case control study. Data from before 2001 does not identify how outbreaks were investigated.

International data

Table 2.2: Outbreaks of illness associated with unpasteurised cow milk internationally

Year	Country	Cases (deaths)	Causative Agent	Cause	Reference
2007	USA	29	*Salmonella* Typhimirium	Laboratory confirmed cased. Isolated from raw milk	(MMWR, 2007b)
2006	USA	6 (2 HUS)	*E. coli O157:H7*	Laboratory confirmed cased. Median age was 8 years (range 6 – 18). Geographically dispersed throughout California, all had consumed raw milk products from same dairy.	(MMWR, 2008)
2005	USA	18 4 HUS	*E. coli* O157:H7	Cow herd share program. 8/18 laboratory confirmed. Isolated from raw milk, floor of milking parlour. Risk increased with increased consumption amount.	(MMWR, 2007a)
2003	USA	62	*Salmonella* Typhimurium	Unpasteurised milk at dairy/petting zoo	(Mazurek *et al*., 2004)
2003	USA	13	*C. jejuni*	Unpasteurised milk	(Peterson, 2003)
2001	USA	75	*C. jejuni*	Unpasteurised milk obtained at a local dairy farm	(Harrington *et al*., 2001)
2001	Austria	2	*E. coli* O157	isolated from dairy cow and goat, raw milk	(Allerberger *et al*., 2001)
2000	Austria	38	*C. jejuni*	Unpasteurised milk distributed by a local dairy	(Lehner *et al*., 2000)
2000	Germany	31	*C. jejuni*	Consuming raw milk farm visit	(Thurm *et al*., 2000)
1998	Hungary	52	*C. jejuni* and *C. coli*	Unpasteurised milk	(Kalman *et al*., 2000)
1996	UK	33	*C. jejuni* resistotype 02	Educational farm visit, exposure to raw milk	(Evans *et al*., 1996)
1995	USA	3	*S*. Typhimurium, variate Copenhagen	Consumed in private home	(CDC, 2002)
1993	USA	4	*E. coli* O157:H7	Consumed in a nursing home	(CDC 2002)
1993	USA	6	*E. coli* O157:H7	Commercially distributed Unpasteurised milk	(Keene *et al*., 1997)
1992	USA	50	*C. jejuni*	Consumed at church	(CDC 2002)
1992	USA	11	*Campylobacter* spp.	Consumed in private home	(CDC 2002)
1990	USA	13	*Campylobacter* spp.	Consumed at school	(CDC 2002)
1990	USA	5	Unknown	Consumed in private home	(CDC 2002)
1990	USA	42	*C. jejuni*	Consumed at a dairy	(CDC 2002)
1986	Austria	28 (5)	*L. monocytogenes*	Consumption of raw milk and biologically grown vegetables as possible source of infection	(Allerberger and Guggenbichler, 1989)
1985	USA	25	*C. jejuni*	Unpasteurised milk	(Korlath *et al*., 1985)
1984	USA	23	not identified	Associated with drinking raw milk from local dairy	(MMWR, 1984a)
1984	Canada	9	*C. jejuni*	A raw milk dairy	(Anon., 1984)
1983	USA x 2 OB	31 26	*C. jejuni*	A raw milk dairy	(MMWR, 1983)
1983	USA	122	not identifed	Associated with consumption of raw milk from a single dairy	(Osterholm *et al*., 1986)
1983	USA	? (1)	*S*. Typhimurium	Unpasteurised milk	(Tacket *et al*., 1985)
1983	UK	130	Streptobacillus moniliformis	Unpasteurised milk	(Shanson *et al*., 1983)
1982	USA	38	*C. jejuni* + thermo-tolerant strain (*C. fetus* subsp *fetus*)	Unpasteurised milk	(Klein *et al*., 1986)
1981-1983	USA	46 70 123(32)	*S*. Dublin		(MMWR, 1984b)

Table 2.2 cont: Outbreaks of illness associated with unpasteurised cow milk internationally

Year	Country	Cases (deaths)	Causative Agent	Cause	Reference
1981	USA	8	*S.* Derby	Unpasteurised milk from a single dairy	(Vogt *et al.* 1981)
1981	USA	250	*C. jejuni*	Unpasteurised milk	(Kornblatt *et al*., 1985)
1979	UK	700	*S*. Dublin	Milk which had not been subjected to heat treatment	(Small and Sharp, 1979)
1979	USA	3	*C. jejuni*	All three patients had been consuming unpasteurised milk from a cow whose faeces were infected with *C. fetus ss. jejuni*	(Blaser *et al*., 1979)
1973 - 1992	USA	40 out-breaks	Various	In states with legal raw milk	(Headrick *et al*., 1998)

ANNEX 3: Summary of key microbiological hazards associated with raw cow milk

Organism	Shed directly in milk[#]	Severity of illness[§]	Implicated in foodborne illness
Campylobacter jejuni/coli	✓	Severe^	++
Enterohaemorrhagic *E. coli*	✓	Severe	++
Listeria monocytogenes	✓	Severe^	++
Salmonella spp.	✓	Serious	++

Key:
[#] Transmission through udder; mastitis etc
^ Susceptible sub-populations
- No data/unknown
[§] Based on ICMSF (2002)
+ Rare
++ More common

Table 3.1: *C. jejuni* in raw milk

Year	Country	Samples	Prevalence (%)	Count	Description	Reference
1982	USA	195	1.5	-	105 individual farm bulk tank milk.	(Lovett *et al.,* 1983)
1982	USA	108	0.9	-	Bulk tanks of 9 Grade A farms.	(Doyle and Roman, 1982)
1982	Netherlands	200	0		Farm milk tanks.	(Oosterom *et al*., 1982)
1982	USA	50	0	-	Milk from University dairy herd	(Wyatt and Timm, 1982)
1983	UK	11 - F 5 - R 40 - C	18.2 - F 40 - R 5 - C	-	F = bulk milk samples, R = pooled retail samples, C = individual cow samples.	(Hutchinson *et al*., 1985)
1984	UK	985 - R 153 - F	5.9 5.9	-	R = retail samples, F = samples from 12 farm bulk milk tanks.	(Humphrey and Hart, 1988)
1987	USA	237	0.42	-	Bulk tank trucks from 16 sites.	(McManus and Lanier, 1987)
1987	UK	111	8.1	1 – 100 MPN per 100ml	500ml farm bulk milk tanks – 111 samples from five positive farms, 30 samples from two negative farms.	(Humphrey and Beckett, 1987)
1988	Netherlands	904	4.5	-	Freshly drawn milk from individual cows, LP system inactivated.	(Beumer *et al.,* 1988)
1989	Canada	192 - F 64 - D	1.56 - F 0 - D	-	F = 48 farms, D = 4 dairies. Samples collected over 4 x 1 month periods. Dairy samples from balance tanks, farm bulk milk tank coolers – enrichment procedure.	(Davidson *et al*., 1989)
1990	Switzerland	496	0	-	Herd milk samples tested by Federal Swiss Dairy Inspection and Advisory service.	(Bachmann and Spahr, 1995)
1992	USA	292	12.3	-	Individual farm bulk tanks – direct plate.	(Rohrbach *et al.,* 1992)
1993	Switzerland	3/83	3.6	-	-	(Wegmuller *et al*., 1993)
1996	UK	1097	1.7		28 Retail outlets – samples "green top" milk.	(De Louvois and Rampling, 1998)
1997	USA	131	9.2	-	Bulk tank milk.	(Jayarao and Henning, 2001)
1997	Canada	1720	0.5	-	Samples from individual farm bulk tanks, prior to tanker pick-up.	(Steele *et al.,* 1997)
1997	France	69	1.45	-	27 different farms bulk tank milk over 6 months, enrichment procedure	(Desmasures, 1997)
1999	UK	610	0.8	-	From 255 participating dairies, bulk tank milk.	(Food Standards Agency, 2003)
1999	Turkey	211	8.1	-	Dairy plants.	(Uraz and Yucel, 1999)
2001	Ireland	62	1.6	-	On-farm bulk tank milk.	(Whyte *et al.,* 2004)

Table 3.1 cont: *C. jejuni* in raw milk

Year	Country	Samples	Prevalence (%)	Count	Description	Reference
2001	EU	1403	0.21	-	-	(European Commission, 2003)
2001	US	248	2.2		Bulk tank milk from 248 dairy herds – enrichment procedure	(Jayarao *et al*., 2006)
2002	Pakistan	127	10.2	-	On farm bulk tank, enrichment, incubated 42 for 48h (92.4% C. jejuni, 7.6% C. coli)	(Hussain *et al*., 2007)
2002	EU	1431	0.35	-	-	(European Commission, 2004)
2004	US	265	0	-	Bulk tank milk samples	(Murinda *et al*., 2004)

Table: 3.2: *C. jejuni* in cattle

Year	Country	Samples	Positive	Prevalence (%)	Description	Reference
1981	USA	42	20	48	Rectal Faecal swabs from individual cows.	(Taylor *et al*., 1982)
1982	Netherlands	200	11	5.5	Caecal samples obtained from slaughtered cattle.	(Oosterom *et al*., 1982)
1982	USA	50	0	0	Rectal swabs from University dairy herd.	(Wyatt and Timm, 1982)
1982	USA	78	50	64	Rectal swabs.	(Doyle and Roman, 1982)
1982	USA	15	2	13.3	Faecal samples taken from cows implicated in outbreaks.	(Vogt *et al*., 1984)
1983	UK	40	18	45	In-milk samples.	(Hutchinson *et al*., 1985)
1986	UK	12	10	83	12 herds, rectal swabs – prevalence of cows within herds ranged between 10 – 72%.	(Humphrey and Beckett, 1987)
1988	Netherlands	904	197	21.8	13 farms, rectal swabs from individual cows.	(Beumer *et al.,* 1988)
2000	USA	2085	786	37.7	From 31 farms – individual cow direct faecal retrieval.	(Wesley *et al*., 2000)
2002	USA	686	48	7	Faecal samples from dairy cattle sent to slaughter.	(Dodson and LeJeune, 2005)
2002	USA	1450	745	51.4	Faecal cultures.	(USDA/APHIS, 2003a)
2002	USA	311	97	31.2	Dairy cattle – rectal or free faecal samples.	(Bae *et al*., 2005)
2003	Australia	150	64	42.6	6 dairies - rectum retrieval.	(Bailey *et al*., 2003)
2003	Finland	952	186	19.5	Rectal faecal samples, direct culture and enrichment procedure.	(Hakkinen *et al*., 2007)
2004	USA	411	5	1.2	Faecal samples.	(Murinda *et al.,* 2004)
2004	USA	720	20	2.8	Lactating dairy cows, rectal faecal sample, 9 farms across the US.	(Harvey *et al*., 2004)
2004	USA	1191	234	19.6	Cattle faecal samples from 60 farms.	(Sato *et al*., 2004)

Table 3.3: *E. coli* in raw milk

Year	Country	Samples	Prevalence (%)	Count per ml	Isolate	Description	Reference
1984	UK	985 – R 153 - F	63.4 – R 61.4 - F	R = 11% >100cfu F = 2.6% >100cfu	*E. coli*	3 year sampling period. R = retail samples, F = bulk milk tanks from 12 farms.	(Humphrey and Hart, 1988)
1991	USA	23	4.3	-	O157:H7	Raw milk samples collected from 2 famrs during an outbreak investigation.	(Wells et al., 1991)
1991	USA	115	10		O157:H7	Bulk raw milk samples from 69 different farms.	(Padhye and Doyle, 1991)
1993	UK	329	0	-	O157:H7	Study conducted over 15 months. 329 bulk milk tank samples. Samples were also taken from individual milk jars, milk filters and individual cows (fore and midstream). O157:H7 was isolated from one foremilk sample.	(Mechie et al., 1997)
1994	USA	77,172	4.4	-	*E.coli*	Milk samples submitted for routine testing.	(Makovec and Ruegg, 2003)
1994	Trinidad	287	75.6	4.2×10^4 - 1.6×10^6cfu	*E.coli*	13 (6.9%) of 188 strains of *E. coli* agglutinated with O157 antiserum	(Adesiyun et al., 1995)
1994	Trinidad	507	4.9	-	VTEC	Pre-processed pooled bulk raw milk from 16 milk collection centres.	(Adesiyun, 1994)
1994	USA	603	0	-	O157:H7	Bulk milk tank samples obtained from routine testing laboratory.	(Hancock et al., 1994)
1995	Trinidad	188	6.9	-	O157	Raw milk at 8 collection centres in Trinidad.	(Adesiyun et al., 1995)
1997	France	69	89.8	-	*E. coli*	27 farms bulk milk tanks.	(Desmasures, 1997)
1997	Scotland	500	0	-	O157	Samples collected over 2 year period from farm bulk milk tanks.	(Coia et al., 2001)
1997	USA	131	3.8	-	STEC	131 farm bulk tank milk samples. 4 of 5 isolates of *E. coli* encoded for the Shiga toxin 2 gene, while 1 strain encoded for the Shiga toxin 1 gene. *E. coli* O157:H7 was not isolated.	(Jayarao and Henning, 2001)
1997	Netherlands	1,011	0	-	O157:H7	Bulk storage tanks originating from 1011 different dairy herds located throughout the Netherlands	(Heuvelink et al., 1998)
1997	Canada	1,720	0.87	-	VTEC	Pick-ups (loads of raw milk from a single farm bulk tank) from Ontario farm bulk tanks. Serotypes included O121, O26, O113, O163	(Steele et al., 1997)
1997	Trinidad	175	9.7	-	VTEC	Samples from collection centres.	(Adesiyun et al., 1997)
1997	USA	42	0	-	O157:H7	Samples obtained from 15 dairy processing plants.	(Ansay and Kaspar, 1997)
1999	Northern Ireland	420	2.14	-	VTEC	Samples collected from 2 dairies.	(McKee et al., 2003)
1999	Italy	100	0	-	O157:H7	Bulk tanks of eight dairy farms.	(Massa et al., 1999)
1999	UK	610	0.2	-	O157:H7	From 255 participating dairies, bulk tank milk.	(Food Standards Agency 2003)
1999	UK	610	52	18% = 10^2 - 10^5cfu	*E. coli*	From 255 participating dairies, bulk tank milk.	(Food Standards Agency 2003)

Table 3.3 cont: *E. coli* in raw milk

Year	Country	Samples	Prevalence (%)	Count per ml	Isolate	Description	Reference
2000	Italy	811	0	-	O157	Samples obtained from dairy plant.	(Conedera et al., 2004)
2000	USA	268	0.75	-	O157:H7	O157:H7 - 30 dairy farms over one year period.	(Murinda *et al*., 2002b)
2000	Zimbabwe	6	33.3	10^3cfu	ETEC	Raw milk from three small-scale dairies. ETEC positive samples produced heat-stable enterotoxin (ST1).	(Gran et al., 2003)
2001	EU and Norway	1629	3.4	-	VTEC	Summary zoonoses report.	(European Commission, 2003)
2001	USA	248	2.4	-	STEC	Bulk tank milk from the 248 participating dairy herds.	(Jayarao et al., 2006)
2002	Costa Rica	100	2	-	O157:H7	Samples obtained from the principal producing zones of the country.	(Reuben et al., 2003)
2002	EU and Norway	2968	0.7	-	VTEC	Summary zoonoses report.	(European Commission, 2004)
2002	USA	859	0.6	-	O157:H7	Bulk raw milk samples collected during NAHMS Dairy 2002 study. Samples from 859 farms in 21 states analysed for EHEC virulence factors.	(Karns et al., 2007)
2003	UK	-	-	0.35 +/- 0.735logcfu	*E. coli*	24 farms bulk tank milk.	(Hutchison et al., 2005)
2004	Belgium	143	0.7	-	O157:H7	Farm bulk tank milk. Positive sample confirmed to be verotoxigenic, positive for VT2, eaeA and hlyA.	(De Rue et al., 2004)
2004	Malaysia	930	65 – E 33.5 - O	6.8 x 10^3cfu	E = *E. coli* O = O157:H7	Raw milk from 360 dairy farms sampled from 40 collection centres in 4 regions.	(Chye et al., 2004)
2004	Brazil	210	36.8	-	*E. coli*	Raw milk produced in 210 small and medium farms located in four important milk-producing Brazilian states.	(Nero et al., 2004)
2004	Turkey	100	1	-	O157	Raw milk samples collected randomly from villages. Presumptive colonies confirmed serologically using antibodies to O157 antigen.	(Oksuz *et al*., 2004)

Table: 3.4: *E. coli* in cattle

Year	Country	Samples	Positive	Prevalence (%)	Description	Reference
1993	USA	662	19	2.9	**O157:H7** – Rectum retrieval of faecal samples from 50 control herds in 14 states. Counts ranged from $<10^2$ cfu/g to $> 10^5$ cfu/g.	(Zhao *et al*., 1995)
1993	Canada	291	49	16.8	**VTEC** – Rectal swab faecal samples from cows on 8 dairy farms testing positive in a previous study.	(Rahn *et al*., 1997)
1993	UK	3593	153	4.3	**O157:H7** – Study conducted over 15 months. Rectal swabs taken from lactating and non-lactating cows.	(Mechie *et al.,* 1997)
1994	USA	3570	10	0.28	**O157:H7** – Faecal samples from dairy cattle in 5 of 60 herds. Samples were rectal swabs and fresh faecal pats.	(Hancock *et al*., 1994)
1995	USA	965	31	3.2	**O157:H7** - Study conducted as follow-up to the National Dairy Heifer Evaluation Project study. Direct rectum faecal retrieval of samples.	(Garber *et al*., 1995)
1995	US	2395	105	4.4	**O157:H7** - 14 month study on 4 dairy farms. Counts ranged from 2.0×10^2 to 8.7×10^4cfu/g. Samples obtained by digital rectal retrieval.	(Shere *et al*., 1998)
1996	USA	4361	52	1.2	**O157** – Rectal retrieval of faecal samples collected from 91 dairy operations.	(Garber *et al*., 1999)
1996	Germany	726	131	18.0	**STEC** – 103 farms surveyed with 49.5% farms positive for STEC	(Zschock *et al*., 2000)
1997	Australia	1802	171	9.5	**STEC** - Survey of Australian cattle.	(Desmarchelier, 1997)
1997	USA	308	11	3.6	**O157** – Samples from 19 dairy herds across three states obtained via faecal swabs.	(Rice *et al*., 1997)
1997	Trinidad	313	68	21.7	**VTEC** – Faecal or rectal samples taken from dairy cattle.	(Adesiyun *et al*., 1997)
1997	France	471	330	70	**STEC** – Faecal samples collected at slaughterhouse. Positive for *stx* gene.	(Pradel *et al*., 2000)
1997	China	176	3	1.7	**O157 –** Faecal swab samples collected from two farms and two slaughterhouses.	(Zhou *et al*., 2002)
1998	Australia	505	1	0.2	**O157** – Faecal samples obtained from intestine post slaughter.	(Hallaran and Sumner, 2001)
1999	India	206	37	18	**STEC** – Healthy domestic cow stool samples.	(Khan *et al*., 2002)
1999	Australia	588	128	21.8	**STEC –** Samples collected on three occasions over 12 month period. Rectal swabs.	(Cobbold and Desmarchelier, 2000)
2000	Korea	990	66	6.6	**O157:H7** – Study conducted over 2 year period. Faecal samples obtained from beef and dairy cattle.	(Jo *et al*., 2004)
2000	US	415	8	1.93	**O157:H7** - 30 dairy farms over 1 year period.	(Murinda *et al*., 2002b)
2000	Australia	128	12 - O157:H7 10 - O26:H11	9.4 – O157:H7 7.8 – O26:H11	**O157:H7, O26:H11** – Faecal samples collected during a previous study and testing positive for STEC were examined to determine virulence and serotype.	(Cobbold and Desmarchelier, 2001)
2000	Australia	310	39	12.6	**O157:H7** - Faecal samples collected from cattle at slaughter. Three samples had counts between $10^3 – 10^5$ MPN/g.	(Fegan *et al*., 2004c)
2000	Canada	240	2	0.8	**O157:H7** – Fresh faecal samples from slaughterhouse pens, over a 1 year period	(Van Donkersgoed *et al*., 2001)
2000	Denmark	1570	103	6.6	**O157** – Eight dairy herds sampled four times over a 1 year period. Samples obtained by digital rectal retrieval.	(Rugbjerg *et al*., 2003)

Table: 3.4 cont: *E. coli* in cattle

Year	Country	Samples	Positive	Prevalence (%)	**Description**	Reference
2001	USA	305	16	5.2	**O157** - Samples from 19 farms collected via rectal retrieval. Positive samples obtained from 7 out of 19 (36.8%) farms.	(Cho *et al*., 2006)
2001	USA	720	52	7.2	**O157:H7** - Faecal samples were obtained from lactating Holstein dairy cattle on four commercial farms in the south-western US. Samples obtained via rectal palpation.	(Edrington *et al*., 2004)
2001	US	50	4	8	**STEC** – 4 farms sampled twice. Tested for *stx* gene. 50 freshly passed faecal samples.	(Cobbold *et al*., 2004)
2002	USA	1026	21	2.1	**O157:H7** - Isolated from cull dairy cattle at 2 livestock auctions in north-eastern Ohio. 1,026 faecal samples were collected.	(Dodson and LeJeune, 2005)
2002	USA	750	5	0.66	**O157** - Comparison of mature dairy cattle in 50 dairy farms in Ohio and Norwegian using identical methodology. Samples collected via recto-anal swabs.	(Lejeune *et al*., 2006)
2002	Norway	680	0	0	**O157** - Comparison of mature dairy cattle in 50 dairy farms in Ohio and Norwegian using identical methodology. Samples collected via recto-anal swabs.	(Lejeune *et al*., 2006)
2002	USA	132	6	4.5	**O157** - Samples from 17 farms collected via rectal retreival. Positive samples obtained from 4 out of 17 (23.5%) farms.	(Cho *et al*., 2006)
2003	USA	574	118	20.6	**O157:H7** – 144 cattle sampled four times by direct rectal retrieval over 7 month period. For each sampling period prevalence was 1.4, 6.9, 53.1 and 22.4%, respectively.	(Khaitsa *et al*., 2006)
2005	USA	2043	527	25.8	**STEC** – 11 month period. 49 O serogroups were identified.	(Renter *et al*., 2005)
2005	USA	408	16	3.9	**O157:H7** - Samples collected over 24 month period from four different farms in four states. Rectal faecal swabs.	(Doane *et al*., 2007)
2005	Brazil	100	39	39	**STEC** – Rectal swab faecal samples collected at slaughterhouses.	(Timm *et al*., 2007)
2003	Australia	22	-	-	**O157 –** Faeces of naturally infected cattle enumerated for O157 by a combination of MPN and AIMS. Counts of O157 between <3 MPN/g of faeces to 2.4 x 10^4 MPN/g. Comparison of *E. coli* and O157 counts.	(Fegan *et al*., 2004a)
2005	Australia	108	13	12	**O157 –** 68 Faecal samples collected post evisceration and 40 samples collected from pen floors. Counts ranged from pen: <3 MPN/g to 4.6 x 10^4 MPN/g and from post evisceration: 3.6 MPN/g to 7.5 x 10^5MPN/g	(Fegan *et al*., 2005a)

Table 3.5: *L. monocytogenes* in raw milk

Year	Country	Samples	Prevalence (%)	Count per ml	Description	Reference
1972	Denmark	36199	1.2	-	23 year period testing bulk tank milk for herd prevalence in Danish mastitis control programme. 1132958 individual cows (quarter milk samples).	(Jensen *et al*., 1996)
1983	USA	121 - M 14 - S	12 – M 14 - S	-	M = Bulk tanks and milk collection trucks. S = milk filter socks at processing plant prior to pasteurising.	(Hayes *et al*., 1986)
1985	USA	650	4.2	-	Farm bulk tanks in three areas of the USA.	(Lovett *et al*., 1987)
1985	Spain	95	45.3	-	Over a period of 16 months.	(Rodriguez *et al*., 1985)
1985 1992	Switzerland	4046 340	0.4 0.6	-	Herd milk samples tested by Federal Swiss Dairy Inspection and Advisory service.	(Bachmann and Spahr, 1995)
1986	USA	200	4	-	Bulk storage tanks from 100 dairy farms at sampling times.	(Liewen and Plautz, 1988)
1987	Finland	314	4.1	-	Farm bulk tank milk from 80 farms.	(Husu, 1990)
1987	USA	2511	2.9	-	19 dairy processing facilities tested bulk milk tankers.	(Doores and Amelang, 1988)
1988	Canada	315	5.4	-	Routine testing conducted over a 1 year period.	(Slade *et al*., 1988)
1988	Canada	445	1.3	-	Farm bulk tanks in three regions of Ontario.	(Farber *et al.,* 1988)
1988	Ireland	113	5.3	-	Bulk tank milk from 8 farms tested over 12 months.	(Harvey and Gilmour, 1992)
1988	Australia	600	0	-	Samples tested by NSW Dairy Corporation Laboratory.	(Anon, 2003)
1989	Scotland	180	1.0 – 3.8	-	180 farm bulk milk tanks collected on 3 occasions.	(Fenlon and Wilson, 1989)
1989	Germany	201	0	-	Bulk milk tanks sampled during winter.	(Eliskases-Lechner and Ginzinger, 1999)
1989	Canada	192 - F 64 - D	1.04 3.13	-	4 dairies and 48 farms collected over 4 x 1 month periods. Dairy samples from balance tanks, farm bulk milk tank coolers – enrichment procedure.	(Davidson *et al*., 1989)
1989	France	2000	3.2	-	Bulk tank milk used for producing raw milk cheese.	(Sanaa *et al*., 1993)
1989	USA	300	3	-	Bulk tank milk obtained from 12 farms over 13 month period.	(Lund *et al*., 1991)
1989	Canada	-	-	4.6 x 10^3 cfu	Identified during routine bulk milk testing, 1 animal with subclinical mastitis in 1 quarter. No visible difference in milk.	(Fedio *et al*., 1990)
1990	Canada	36 - T 36 – T 426 - B	2.8 - T 11.1 - T 1.9 - B	-	T = milk tankers, B = Bulk milk tank samples.	(Fedio and Jackson, 1990)
1990	Australia	150	0	-	Bulk milk tank samples from Queensland United Foods.	(Ibrahim and Macrae, 1991)
1990	USA	292	4.1	-	Bulk tank milk sampled from 292 farms	(Rohrbach *et al.,* 1992)
1990 1991	Japan	943 - B 504 - F	0.32 - B 28.6 - F	-	B = Farm bulk tanks from individual farms. Serotypes 1/2a and 4b.F = 1991 samples collected 9 times from 56 farms.	(Yoshida *et al*., 1998b)
1991	Japan	51	50.9	-	3 farms bulk tank milk samples over 17 months.	(Yoshida *et al*., 1998a)
1992	Canada	20 – B 401 - C	60 – B 5.2 - C	-	B = bulk tank milk, C = individual cow. Bulk tank milk, individual cow milk and aseptic quarter milk from 4 farms.	(Fedio and Jackson, 1992)
1992	England and Wales	2009	5.1	62 cfu	Farm bulk tanks sampled over 15 month period.	(O'Donnell, 1995)

Table 3.5 cont: *L. monocytogenes* in raw milk

Year	Country	Samples	Prevalence (%)	Count per ml	Description	Reference
1995	Scotland	640	6.6	35 cfu	Bulk tank milk from 160 producers tested at 3 monthly intervals over 1 year. Prevalence over the intervals ranged from 4.4% – 9.4%.	(Fenlon *et al.,* 1995)
1997	Sweden	294 – B 295 - S	1.0 - B 19.6 - S	60 cfu	B = Farm bulk tanks from 153 farms, sampled twice. (12 excluded from second sample set) S = Factory silos over 18 months.	(Waak *et al.,* 2002)
1997	USA	131	4.6	-	131 individual farm bulk milk tanks, all isolates contained O antigen (serotypes 1/2a, 1/2b and 1/2c).	(Jayarao and Henning, 2001)
1997	Canada	1,720	2.7	-	3 day old samples from farm bulk tanks, collected prior to tanker pick-up.	(Steele *et al.,* 1997)
1997	France	69	5.8	-	27 individual farm bulk milk tanks.	(Desmasures *et al*., 1997)
1997	France	1459	2.4	105 cfu	25 farm bulk tanks over 50 month period.	(Meyer-Broseta *et al*., 2003)
1998	Spain	774	3.62	-	114 farms collected bulk tank milk twice per season over 1 year period.	(Gaya *et al.,* 1998)
1998	USA	404	12.6	-	In-line milk filters from 404 dairy farms.	(Hassan *et al*., 2000)
1998	Turkey	100	4	-	-	(Vardar-Unlu *et al*., 1998)
1998	Mexico	1300	13	-	1300 samples of raw milk from 201 bulk milk tanks from 4 different dairies over a 1 year period.	(Carlos *et al*., 2001)
1999	UK	610	17	≤ $2\log_{10}$ cfu	From 255 participating dairies, bulk tank milk.	(Food Standards Agency 2003)
1999	Brazil	12	8.3	-	6 samples of raw milk from two different factory receival areas. Serotype 4b.	(Silva *et al*., 2003)
1999	Turkey	2/211	0.94	-	Dairy plant.s	(Uraz and Yucel, 1999)
2000 2001 2002	USA	474 474 25	4.9 7.0 68	-	3 States of Pacific Northwest. 474 herds samples over 3 time periods. 25 of 33 herds which tested positive in 2001 were tested in 2002.	(Muraoka *et al.,* 2003)
2000	Czech Republic	278	2.1	-	18 farms bulk tanks.	(Navratilova *et al*., 2004)
2001	EU and Norway	1377	1.3	-	Summary zoonoses report.	(European Commission, 2003)
2001	USA	248	1.2	-	Bulk tank milk from 248 dairy herds, enrichment procedure.	(Jayarao *et al*., 2006)
2002	USA	861	6.5		Bulk tank milk samples from 21 different states collected during NAHMS Dairy 2002 study. 5 serotypes isolated - 1/2a, 1/2b, 3b, 4b and 4c.	(van Kessel *et al.,* 2004)
2002	Slovak Republic	25	20	-	Raw milk samples.	(Holko *et al*., 2002)
2002	Belgium	143	6.3	-	143 farm bulk tank milk. Samples taken from cooled bulk milk.	(De Rue *et al*., 2004)
2002	US	860	6.5	-	NAHMS Dairy 2002 study – bulk tank milk of dairy operations with at least 30 milk cows over 21 states.	(USDA/APHIS, 2003b)
2002	Brazil	210	0	-	210 farms across Brazil using different milking systems.	(Nero *et al*., 2004)
2002	Portugal	105	1.9	-	Samples obtained once a month from 7 processing plants over 1 year.	(Kongo *et al*., 2006)

Table 3.5 cont: *L. monocytogenes* in raw milk

Year	Country	Samples	Prevalence (%)	Count per ml	Description	Reference
2003	Costa Rica	100	3.0	-	Raw milk from principle producing zones of the country.	(Reuben *et al*., 2003)
2004	Malaysia	930	1.9	-	360 dairy farms, milk collected at 40 collection centres.	(Chye *et al*., 2004)
2006	Turkey	47	0	-	Samples obtained from dairy plants.	(Aygun and Pehlivanlar, 2006)

Table: 3.6: *L. monocytogenes* in cattle

Year	Country	Samples	Positive	Prevalence	Description	Reference
1992	Canada	69	14.5	21%	Direct rectal retrieval of feacel samples	(Fedio and Jackson, 1992)
1986	Finland	3878	373	9.6%	Rectal faecal samples from 249 farms over two years	(Husu, 1990)
1991	Japan	38	19	50%	Three farms sampled over 17 months. Dropped faecal samples collected.	(Yoshida *et al.,* 1998a)

Table 3.7: *Salmonella* spp. in raw milk

Year	Country	Samples	Prevalence (%)	Count per ml	Description	Reference
1984	UK	2/985 0/153	0.2 0	-	985 retail samples, 153 samples from 12 farm bulk milk tanks. Serotypes were *S.* agama and *S.* Agona.	(Humphrey and Hart, 1988)
1985	Canada	1140	2.5	-	Collection of in-line milk filters. Serotypes included *S.* muenster, *S.* Newington and *S.* Montevideo.	(McEwen et al., 1988)
1987	USA	678	4.7	-	Bulk tank trucks from 16 sites. Serotypes included *S.* Montevideo (9), *S.* Bredeney, (5) *S.* Cerro (4) *S.* Infantis (3).	(McManus and Lanier, 1987)
1989	Germany	201	0	-	Bulk milk tanks sampled during winter.	(Eliskases-Lechner and Ginzinger, 1999)
1992	England and Wales	1,673	0.36	-	Farm bulk tanks sampled over 15 month period. Serotypes included *S.* Dublin, *S. enteritidis*, *S.* Typhimurium and *S.* Newport.	(O'Donnell, 1995)
1992	USA	292	8.9	-	Bulk tank milk sampled from 292 farms.	(Rohrbach et al., 1992)
1993	Switzerland	456	0	-	Herd milk samples tested by Federal Swiss Dairy Inspection and Advisory service.	(Bachmann and Spahr, 1995)
1995	Canada	1,720	0.17	-	Samples from individual farm bulk tanks, prior to tanker pick-up. Serovars isolated were *S.* muenster, *S.* thompson and S. Typhimurium.	(Steele et al., 1997)
1997	USA	131	6.1	-	131 individual farm bulk milk tanks, Isolates of Salmonella belonged to group D (n = 4), B (n = 2), C (n = 1), and E (n = 1) "O" serogroups.	(Jayarao and Henning, 2001)
1997	France	69	2.9	-	Bulk tank milk from 27 different farms sampled over a six month period. *S.* Indiana isolated.	(Desmasures, 1997)
1998	USA	404	1.5	-	In-line milk filters from 404 dairy farms. 1 isolate was *S.* Typhimurium DT 104.	(Hassan et al., 2000)

Table 3.7 cont: *Salmonella* spp. in raw milk

Year	Country	Samples	Prevalence (%)	Count per ml	Description	Reference
1999	UK	610	0.3	-	From 255 participating dairies, bulk tank milk. One serotype was *S.* Goldcoast, the other unknown.	(Food Standards Agency 2003)
2000	Ireland	29	3.4	-	Compulsory monitoring programme.	(Food Safety Authority of Ireland, 2004)
2001	USA	248	6.0	-	Bulk tank milk from 248 dairy herds – enrichment procedure. *Salmonella* isolates were identified as *S.* Typhimurium (n = 10) and *S.* Newport (n = 5).	(Jayarao *et al*., 2006)
2001	USA	268	2.2	-	Bulk tank milk samples from 7 of 30 (25.3%) dairy farms were *Salmonella*-positive	(Murinda *et al*., 2002a)
2002	USA	861	2.6	-	Bulk tank milk samples from 21 different states collected during NAHMS Dairy 2002 study. Of the 22 positive samples, 9 serotypes were identified. Monetevideo (7), Newport (4) Muenster (2) Meleagridis (2) Cerro (2) Dublin (1) Anatum (1) and two others.	(van Kessel et al., 2004)
2002	USA	854	11.8	-	Samples collected as part of National Animal Health Monitoring System Dairy 2002 survey.	(Karns et al., 2005)
2002	USA	860	2.7	-	NAHMS Dairy 2002 study - bulk tank milk of dairy operations with at least 30 milk cows over 21 states. Most common serovars were Montevideo, Newport, Muenster, Meleagridis and Cerro.	(USDA/APHIS, 2003b)
2002	Brazil	210	0	-	210 farms across Brazil using different milking systems	(Nero et al., 2004)
2004	Belgium	143	0	-	143 farm bulk tank milk. Samples taken from cooled bulk milk.	(De Rue et al., 2004)
2004	Malaysia	930	1.4	-	360 dairy farms, milk collected at 40 collection centres. Thirteen *Salmonella* serotypes were identified, including *S*. Muenchen, *S.* Anatum and *S.* Agona.	(Chye et al., 2004)
1976	Australia	-	10		Associated with an outbreak of S. Typhimurium phage type 9 in Whyalla. *Salmonella* isolated from bulk and bottled raw milk.	(MMWR, 1977)

Table: 3.8: *Salmonella* in cattle

Year	Country	Samples	Positive	Prevalence (%)	Description	Reference
1991	USA	6862	145	2.1	Conducted as part of the USDA National Dairy Heifer Evaluation Project, across 28 states. *S.* Typhimurium, *S.* Dublin most common serovars isolated.	(USDA/APHIS, 1994)
2002	USA	415	9	2.17	Culled dairy cow samples. Faecal samples from 7 of 30 (25.3%) dairy farms were *Salmonella*-positive.	(Murinda *et al*., 2002a)
2002	USA	585	39	6.7	Isolated from cull dairy cattle at 2 livestock auctions in north-eastern Ohio. 1,026 faecal samples were collected.	(Dodson and LeJeune, 2005)
2001	Europe	25661	584	2.3	Summary zoonoses reports.	(European Commission, 2003)
2000	Europe	27494	753	2.7	Summary zoonoses reports. Faecal samples obtained at slaughterhouse.	(European Commission, 2004)
2002	USA	3669	267	7.3	USDA's National Animal Health Monitoring System (NAHMS) conducted Dairy 2002, faecal samples were collected via rectal retrieval from approximately 5 operations in each of the 21 States participating in the study.	(USDA/APHIS, 2003a)
2001	USA	720	262	36.4	Faecal samples were obtained from lactating Holstein dairy cattle on 4 commercial farms in the south-western US. Samples obtained via rectal palpation.	(Edrington *et al*., 2004)
1991	USA	6861	145	2.1	Preweaned dairy heifers on 1063 operations tested. Faecal specimen obtained via rectal retrieval.	(Losinger *et al*., 1995)
1996	US	91	25	27.5	4299 faecal samples from 91 herds. Faecal samples obtained via rectal retrieval.	(Kabagambe *et al*., 2000)
2002	Australia	310	21	6.8	Faecal samples from cattle presented for slaughter. Counts ranged from <3 MPN/gof faeces to 2.8 x 10^3 MPN/g.	(Fegan *et al*., 2004b)
2005	Australia	606	157	26	Counts obtained from cattle presented for slaughter. Maximum count in faeces was 93 MPN/g.	(Fegan *et al*., 2005b)
1976	Australia	193	2	1	Detection associated with an outbreak of *S.* Typhimurium phage type 9 in Whyalla.	(MMWR, 1977)

ANNEX 4: Hazard identification and characterisation of modelled pathogens

1. *Campylobacter* spp.

Campylobacter spp. are Gram-negative non-spore forming bacteria. Their cells are 0.2 - 0.8 μm wide and 0.5 - 5 μm long. They are mostly slender, spiral, curved rods, with a single polar flagellum at one or both ends of the cell. They are typically motile with a characteristic rapid darting corkscrew-like mobility (Smibert, 1984; Vandamme, 2000).

Campylobacter spp. are classified under *Campylobacteraceae*, a bacterial family comprised of genera *Campylobacter*, *Arcobacter* and *Sulfurospirillum*. Among the 16 species and six subspecies of *Campylobacter*, two are most commonly isolated from stool samples of human gastroenteritis (Vandamme, 2000). They are *Campylobacter jejuni* subspecies *jejuni* and *Campylobacter coli*. *C. jejuni* accounts for approximately 95% of *Campylobacter* spp. caused human gastroenteritis, and *C. coli* are responsible for approximately 3 - 4% of the human illness.

Campylobacter spp. are often a normal part of the intestinal flora of young cattle, sheep, goats, dogs, rabbits, monkeys, cats, chickens, turkeys, ducks, seagulls, pigeons, blackbirds, starlings and sparrows pigs (Smibert, 1984; Nielsen *et al.*, 1997), and in blood and faecal material from humans with *Campylobacter* enteritis. They have also been found in the reproductive organs and oral cavity of humans and animals. Healthy puppies and kittens, rodents, beetles and houseflies have also been shown to carry *Campylobacter* spp. (Hartnett *et al.*, 2002).

Growth characteristics

Campylobacter spp. require microaerophilic conditions for growth and have varying degrees of oxygen tolerance (3 - 5%) between species (Forsythe, 2000). Optimal growth occurs under conditions of 5% oxygen and 2 - 10% carbon dioxide (Park, 2002). Most strains do not grow in the presence of air, other than a few that may grow slightly under aerobic conditions. Some species can grow under anaerobic conditions with fumarate, formate and fumarate, or fumarate and hydrogen in the medium (Smibert, 1984; Vandamme, 2000).

Campylobacter spp. grow optimally at 42 - 43^{o}C. *C. jejuni* can grow in the temperature range of 30 - 45^{o}C, pH of 4.9 - 9.5 and water activity above 0.99. At 32^{o}C, *C. jejuni* may double its biomass in approximately 6 hours (Forsythe, 2000). *Campylobacter* spp. do not multiply at temperatures below 30^{o}C, which means that the numbers of *Campylobacter* in foods will not increase at normal room temperatures (20 – 25^{o}C). Although unable to grow below 30^{o}C, *Campylobacter* remain metabolically active, are able to generate ATP, and are motile at temperatures as low as 4^{o}C (Park 2002).

Although *Campylobacter* spp. are considered thermotolerant, they are sensitive to heat and are readily inactivated by pasteurisation treatment or domestic cooking processes. Cooking at 55 - 60^{o}C for several minutes readily destroys *Campylobacter* spp. The D value for *C. jejuni* at 50^{o}C is 0.88 - 1.63 minutes (Forsythe, 2000). *Campylobacter* spp. are also sensitive to freezing and/or freeze thawing (Chan *et al.*, 2001).

Other than temperature, a range of other environmental factors including desiccation, oxidation and osmotic stress influences the survival of *Campylobacter* spp. They. are highly sensitive to desiccation and do not survive well on dry surfaces (Fernandez, 1985).

The microaerophilic nature of *Campylobacter* spp. means that these organisms are inherently sensitive to oxygen and its reduction substances (Park 2002). *Campylobacter* spp. are much less tolerant to osmotic stress than a number of other foodborne pathogenic bacteria. For example, they are not capable of multiplication in an environment where sodium chloride concentration is 2% or higher (Doyle and Roman, 1982)

Due to its sensitivity to environmental conditions and inability of growth at temperatures below 30^{o}C or under aerobic conditions, the ability of *Campylobacter* spp. to multiply outside of an animal host is severely restricted. Although not capable of multiplication in food during processing or storage, *Campylobacter* spp. may have the ability to survive outside their optimal growth conditions (Park 2002).

Pathology of illness

C. jejuni causes fever and enteritis in human, resulting in acute inflammatory diarrhoea with clinical signs similar to those of other acute bacterial infections of the intestinal tract, such as salmonellosis. Principal symptoms are diarrhoea, nausea, abdominal pain, fever, myalgia, headache, vomiting and blood in faeces (Lastovica and Skirrow, 2000).

The onset of symptoms is often abrupt with cramping abdominal pains quickly followed by diarrhoea. The mean incubation period is approximately 3 days with a range of 18 hours to 8 days. A particular feature of infection is abdominal pain, which may become continuous and sufficiently intense to mimic acute appendicitis. This is the most frequent reason for admission of *Campylobacter* enteritis patients to hospital (Skirrow and Blaser, 2000).

Although incidents are rare, *Campylobacter* spp. have been implicated in causing a range of extra-intestinal infections including appendicitis, haemolytic ureamic syndrome, abortion, hepatitis, cholecystitis, pancreatitis, nephritis and others (Skirrow and Blaser, 2000). *C. jejuni* may cause septicaemia, meningitis and serious neurological disorders such as Guillain-Barré syndrome, an acute neuromuscular paralysis, and reactive arthritis such as Reiter syndrome (Lastovica and Skirrow, 2000).

Mode of transmission

Friedmann *et al.* (2000) examined data from 111 food and waterborne outbreaks of campylobacteriosis reported in the US between 1978 - 1996. Other than unknown foods, milk and water were the most common food vehicles associated with transmission of *Campylobacter* spp. Raw (unpasteurised) milk is largely responsible for dairy-related transmission. Of four milk-borne outbreaks in the period of 1990 - 1992, three were linked to raw cow's milk and raw goat's milk (CDC, 2003). Surveys in other developed countries, including the United Kingdom, Sweden, Germany, New Zealand, Denmark, US and Norway, indicate milk is the most frequent cause of foodborne *Campylobacter* spp. infection (Friedman *et al.*, 2000). Outbreak data of foodborne campylobacteriosis recorded in Australia between 1992 - 2001 present a similar picture to the above, where approximately 42% of recorded outbreaks were the result of consumption of milk, and among this, raw milk accounted for approximately 80% of milk-borne *Campylobacter* spp. outbreaks.

Published information by Eberhart-Phillips *et al.* (1997), Friedman *et al.* (2000), WHO (2000) and Vellinga and Loock, (2002) suggests that major routes of *Campylobacter* spp. transmission to humans are:

- Consumption of food contaminated with *Campylobacter* spp., including consumption of raw and unpasteurised milk and milk products, consumption of undercooked meat such as poultry meat, and consumption of raw seafood
- Consumption of water contaminated with *Campylobacter* spp.
- Bathing or swimming in a *Campylobacter* spp. contaminated lake or pool
- Direct contact with infected farm animals, such as cattle, sheep, chicken, etc
- Contact with infected domestic animals, such as pet dogs, cattle and bird

Incidence of illness

C. jejuni is one of the most commonly reported aetiological agents of foodborne illness in developed countries, including Australia, NZ, UK and US (Mead *et al.*, 1999; Park 2002). In the US, approximately 80% of all the cases of human campylobacteriosis are foodborne (Mead *et al.,* 1999). In the period of 1998 – 2004, the notification rate of campylobacteriosis in Australia has been 100 – 120 cases per 100,000 population per annum. Notification rates were highest in the 0 – 4 year age group (Anon 2005).

Occurrence in foods

Foods potentially contaminated with *Campylobacter* spp. include raw and unpasteurised milk and milk products, raw poultry, raw beef, raw pork and raw shellfish, as well as foods that may have been exposed to water contaminated with *Campylobacter* spp. (Institute of Food Technologists, 2002).

Virulence and infectivity of campylobacter

Although not fully understood, *Campylobacter* spp. virulence is thought to involve production of microbial toxins. An enterotoxin Wassenaar (1997) abbreviated as CJT for *C. jejuni* toxin, is immunologically similar to the *Vibrio cholerae* toxin and the *E. coli* heat-liable toxin. At least six cytotoxins have been observed in *Campylobacter* spp., these being a 70-kDa cytotoxin, a Vero/HeLa cell cytotoxin, a cytolethal distending toxin (CDT), a shiga-like toxin, a haemolytic cytotoxin and a hepatotoxin. The CDT toxin has been shown to cause dramatic distension of human tumour epithelial cells, which leads to cell disintegration (Pickett *et al.*, 1996). Active CDT toxin has been found in roughly 40% of the over 700 *Campylobacter* strains tested (Johnson and Lior, 1988). However, the role of enterotoxin and the cytotoxins in *Campylobacter* pathogenesis has not been fully identified.

Dose response

Dose-response relationships have been developed based on results from human feeding studies, whereby human volunteers were fed known numbers of *Campylobacter* spp. cells and then monitored for their response (Black *et al.*, 1988). These models make the assumption that (1) a single cell has the ability to initiate an infection and (2) the probability of causing infection increases as the level of the pathogen increases. Data from human trial experiments indicates that *Campylobacter* spp. infection correlates proportionally to the dose ingested and

gradually reaches saturation. Despite a direct dose-response relationship being observed for the probability of infection, the probability of illness following from infection was independent of the dose ingested. The FAO/WHO Joint Expert Meetings on Microbiological Risk Assessment proposed a conditional probability of illness based on the probability of infection. Beta distribution of this conditional probability Hartnett *et al.* (2002) suggests the probability of illness is 20 - 50% after the establishment of a *Campylobacter* infection.

For the human feeding trials 50% of individuals who ingested the minimum dose of 800 cells became infected (Black *et al.*, 1988). Taking into consideration the limited size of the study, it has been proposed that the lowest infective dose would be somewhere close to 100 cells, which is comparable with epidemiological data (Prendergast *et al.*, 2004)

Immune status

People with existing diseases are considered to have a higher susceptibility to campylobacteriosis than the general population (Pigrau *et al.*, 1997). The incidence of *Campylobacter* spp. infection in patients with acquired immune deficiency syndrome has been calculated to be 40-fold higher than that in the general population (Sorvillo *et al.*, 1991). In addition, 16% of *Campylobacter* spp. infections resulted in bacteraemia in these immunocompromised patients, a rate much higher than those occurring in the general population.

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2. *Escherichia coli* (pathogenic)

Escherichia coli are members of the family Enterobacteriaceae and are a common part of the normal intestinal flora of humans and other warm-blooded animals. The organisms are described as gram-negative, facultative anaerobic rod shaped bacteria (Desmarchelier and Fegan, 2003). Although most strains of *E. coli* are considered harmless, the species does contain certain strains that can cause severe illness in humans (Bell and Kyriakides, 1998). Strains of *E. coli* are differentiated serologically, based on O (somatic) and H (flagella) antigens (Lake *et al.*, 2003).

Pathogenic *E. coli* are characterised into specific groups based on virulence properties, mechanisms of pathogenicity and clinical syndromes (Doyle *et al.*, 1997). These groups include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and enterohaemorrhagic *E. coli* (EHEC). Many synonyms are used to describe EHEC, including Shiga toxin-producing *E. coli* (STEC), Shiga-like toxin-producing *E. coli*, and verocytotoxin-producing *E. coli*.

E. coli O157:H7 is the best known and most widely studied serotype of *E. coli.* One of its natural habitats is the intestines of cattle, which creates the potential for contamination of milk and dairy products. In spite of this risk, milk and dairy products have only occasionally been implicated in outbreaks of *E. coli* O157:H7 food poisoning, and even more rarely does an outbreak involve a pasteurised product (Kirk and Rowe, 1999).

Growth characteristics

Growth and survival of pathogenic *E. coli* is dependent on the simultaneous effect of a number of environmental factors such as temperature, pH and water activity. In general, pathogenic *E. coli* strains behave similarly to non-pathogenic strains, however certain EHEC strains have been found to have a higher tolerance to acidic conditions than other groups of *E. coli* (Desmarchelier and Fegan, 2003).

The optimum temperature for growth of *E. coli* is 37°C, and it can grow within the range of 7 - 8°C to 46°C (ICMSF, 1996). Heat sensitivity of pathogenic *E. coli* is similar to that of other Gram-negative bacteria and is dependent on the pH, water activity and composition of the food (Bell and Kyriakides, 1998). Due largely to its importance as a cause of foodborne illness in the US, most studies on the growth and/or survival of pathogenic *E. coli* have been undertaken with *E. coli* O157:H7 (an EHEC organism). Studies on the thermal sensitivity of *E. coli* O157:H7 have revealed that it is no more heat sensitive than *Salmonella* (Doyle and Schoeni, 1984). Therefore, heating a product to kill typical strains of *Salmonella* will also kill *E. coli* O157:H7.

Studies have demonstrated that some EHEC strains are acid-tolerant and can survive for at least five hours at pH 3.0 - 2.5 at 37°C (Benjamin and Datta, 1995). Stationary phase and starved pathogenic *E. coli* have been found to have an increased acid tolerance compared with exponential growth phase organisms (Arnold and Kaspar, 1995). Pathogenic *E. coli* may therefore be able to survive and/or grow in food products previously considered too acidic to support the survival of other foodborne pathogens. The effect of pH on *E. coli* survival is, however, dependent on the type of acid present. For example, *E. coli* O157:H7 can survive in a medium adjusted to pH 4.5 with hydrochloric acid but not when adjusted to the same pH with lactic acid (ICMSF, 1996).

The minimum water activity required for growth of pathogenic *E. coli* is 0.95, or approximately 8% sodium chloride (ICMSF, 1996). In sub-optimal temperature or pH conditions, the water activity required for growth increases (Desmarchelier and Fegan, 2003).

Pathology of illness

EPEC causes illness primarily in infants and young children in developing countries. Symptoms include watery diarrhoea, with fever, vomiting and abdominal pain. The diarrhoea is usually self-limiting and of short duration, but can become chronic (more than 14 days). EPEC is also recognised as a foodborne and waterborne pathogen of adults, where it causes severe watery diarrhoea (with mucus, but no blood) along with nausea, vomiting, abdominal cramps, fever, headache and chills. Duration of illness is typically less than three days (Doyle and Padhye, 1989; Dalton *et al.*, 2004)

ETEC is another major cause of diarrhoea in infants and children in developing countries, as well as being recognised as the main cause of 'travellers diarrhoea' (Doyle and Padhye, 1989). Symptoms include watery diarrhoea, low-grade fever, abdominal cramps, malaise and nausea. In severe cases the illness resembles cholera, with severe 'rice-water' diarrhoea and associated dehydration. Duration of illness is 3 - 21 days (Doyle and Padhye, 1989).

EIEC cause a dysenteric illness similar to shigellosis. Along with profuse diarrhoea, symptoms include chills, fever, headache, muscle pain and abdominal cramps. Onset of symptoms is usually rapid (<24 hours) and may last several weeks (Doyle and Padhye, 1989).

EHEC infection normally results in diarrhoea-like symptoms. Haemorrhagic colitis, an acute illness caused by EHEC organisms, is characterised by severe abdominal pain and diarrhoea. This diarrhoea is initially watery but becomes grossly bloody. Symptoms such as vomiting and low-grade fever may be experienced. The illness is usually self-limiting and lasts for an average of 8 days. The duration of the excretion of EHEC is about one week or less in adults, but it can be longer in children (ICMSF, 1996).

Complications resulting from EHEC infections vary. About 5% of haemorrhagic colitis victims may develop haemolytic ureamic syndrome (HUS) (European Commission, 2000). This involves the rupture of red blood cells (haemolysis), subsequent anaemia, low platelet count and kidney failure. The case-fatality rate of HUS has been reported to be 3 – 7% (Codex, 2002). Shiga toxins produced by EHEC attack the lining of the blood vessels throughout the body, predominantly affecting the kidney. However other organs such as the brain, pancreas, gut, liver and heart are also affected and may result in further complications such as thrombotic thrombocytopenic purpura.

Table 1: Clinical, pathological and epidemiological characteristics of disease caused by the five principal pathotypes of *E. coli* (Robins-Brown, 1987)

Pathotype	Clinical symptoms	Intestinal pathology	Susceptible population
ETEC	Watery, cholera-like diarrhoea	No notable change	Children in developing countries; travellers to those countries
EIEC	Bacillary dysentery	Inflammation and disruption of the mucosa, mostly of the large intestine	All ages; more common in developing countries
EPEC	Non-specific gastroenteritis	Attaching-effacing lesions throughout the intestine	Children under 2 years of age in developing countries
EHEC	Bloody diarrhoea	"Haemorrhagic colitis"; attaching-effacing lesions confined to the large intestine; necrosis in severe cases	Children and the elderly in developed countries.
EAEC	Persistent diarrhoea	Inflammation, cytotoxic changes in enterocytes (data from experimental studies)	Children in developing countries; travellers to those countries

Mode of transmission

Pathogenic *E. coli* are transmitted by the faecal-oral route. Sources of transmission include person-to-person, foodborne, waterborne (drinking water and direct contact with faecal contaminated water) and direct contact with infected animals (ICMSF, 1996).

Incidence and outbreak data

Infection with pathogenic *E. coli* is a cause of significant morbidity and mortality worldwide. Outbreaks caused by EPEC, ETEC and EIEC occur infrequently in developed countries (ICMSF, 1996). In contrast, outbreaks caused by EHEC are more common, with a number of large foodborne outbreaks being reported in many countries, including Australia (Goldwater and Bettelheim, 1998). In developing countries, the incidence of EHEC infection is reported to be much lower than that of ETEC and EPEC infection (Nataro and Kaper, 1998).

EIEC stains have been isolated with low frequency from diarrhoeal cases in both industrialised and less developed countries (Nataro and Levine, 1994). Outbreaks have occurred in hospitals, on a cruise ship, and from contaminated water (Desmarchelier and Fegan, 2003).

ETEC stains are a major cause of diarrhoea in infants and young children in developing countries, particularly in the tropics, and are a leading cause of travellers' diarrhoea (Gross and Rowe, 1985; Doyle and Padhye, 1989; Nataro and Levine, 1994). Although uncommon, a number of foodborne outbreaks due to ETEC have occurred internationally (Olsvik *et al.*, 1991). Mead *et al.* (1999) estimated that ETEC infection is responsible for approximately 0.4% of foodborne illnesses in the US. In 1983 a multi-state ETEC outbreak occurred in the US that was associated with consumption of imported Brie and Camembert cheese (Anon, 1984; MacDonald *et al.*, 1985).

EPEC stains have caused infantile diarrhoea in hospitals and nurseries in the UK and the US (Robins-Brown 1987; Nataro and Levine, 1994). In developing countries, EPEC stains are still responsible for a high incidence of sporadic infant diarrhoea. Limited information is available on foodborne outbreaks associated with EPEC. An outbreak of EPEC (serotype O111) occurred amongst people on a coach trip to France, although no specific food was identified. The infection was believed to have been the result of consuming food at a restaurant in northern France (Wight *et al.*, 1997).

In the US, consumption of undercooked hamburger meat has been an important cause of EHEC outbreaks (Nataro and Kaper 1998). Since its identification as a human pathogen in 1982, and implication in a number of outbreaks in the US, *E. coli* O157:H7 has become identified as the most predominant cause of EHEC related disease (FAO/WHO, 2000). It is estimated that 85% of EHEC infections in the US are foodborne (Mead *et al.,* 1999). A large multi-state *E. coli* O157:H7 outbreak involving consumption of contaminated hamburgers occurred in December 1992 – January 1993 with 732 cases identified, of which 195 were hospitalised and 4 died (Nataro and Kaper 1998). Foodborne outbreaks of *E. coli* O157:H7 have also been associated with consumption of contaminated fresh produce. In the US, outbreaks occurred in 1995 and 1996 (70 and 49 cases respectively), which were traced to consumption of lettuce (Tauxe, 1997). Studies have shown that *E. coli* O157:H7 can be transmitted to lettuce plant tissue from soil contaminated with manure and contaminated irrigation water (Solomon *et al.*, 2002). Another large *E. coli* O157:H7 outbreak occurred in the US in 1996 which was linked to apple juice. Although the low pH of fruit juices will generally not allow the survival and growth of many Enterobacteriaceae, some strains of *E. coli* O157:H7 may survive due to their high acid-tolerance. In 2002, an outbreak of *E. coli* O157:H7 in Canada was attributed to the consumption of unpasteurised Gouda cheese (Honish *et al.*, 2005).

Over 200 non-O157 STEC serotypes have been isolated from humans, with the World Health Organisation identifying O26, O103, O111 and O145 as the most important foodborne non-O157 serogroups worldwide (WHO, 1998). STEC has been a notifiable disease in most Australia States and Territories since August 1998 (Roche *et al.*, 2001). During the period of 2001 – 2005, the notification rate for STEC (excluding HUS cases) in Australia has been 0.2 – 0.3 cases per 100,000 population per annum (Ashbolt *et al.*, 2002; OzFoodNet, 2003; OzFoodNet, 2004; OzFoodNet, 2005). *E. coli* O157 has been the most commonly reported serotype. Significant variations in notifications exist between states and territories, and part of this variation is likely to be a result of different practices employed by pathology laboratories when screening faecal samples for toxin producing *E. coli* (OzFoodNet, 2003).

A large EHEC outbreak occurred in South Australia during 1995, which resulted in approximately 200 cases of illness. Twenty-two people aged between 4 months and 12 years developed HUS and were hospitalised and a 4 year old child died. Investigations of the outbreak identified EHEC strain O111:NM (or strain O111:H-, NM for non-motile) as the principal cause of the outbreak. A locally produced uncooked, fermented mettwurst was identified as the vehicle for the pathogen. The product was found to contain a variety of EHEC strains in addition to O111 (Paton and Paton, 1998).

Occurrence in food

Humans appear to be the primary reservoir of EIEC, ETEC and EPEC organisms (Desmarchelier and Fegan, 2003). Therefore, contamination of food with these organisms is often due to human faecal contamination, either directly from an infected food handler or indirectly via contaminated water. Very little information is available on the occurrence of these organisms in food. The detection of these organisms in food is difficult, requiring sophisticated methodology and therefore food is not routinely screened for these organisms.

In general, EPEC and ETEC organisms are more commonly isolated in foods from developing countries and their presence is associated with poor hygiene (Desmarchelier and Fegan, 2003). EPEC has been isolated from milk products in Iraq as well as from a variety of raw and cooked food in Malaysia (Abbar and Kaddar, 1991; Norazah *et al.*, 1998). In Brazil, EPEC has been isolated from 21.1% of soft cheeses sampled (n=45) and has frequently been isolated from pasteurised milk (da Silva *et al.*, 2001; Araújo *et al.*, 2002). EIEC has only sporadically been isolated from foods (Olsvik *et al.,* 1991).

In addition to being a major cause of infantile diarrhoea in developing countries, ETEC organisms are a leading cause of traveller's diarrhoea, which has been linked to the consumption of contaminated food and water (Nataro and Kaper 1998). ETEC has been isolated from Brazilian fish and shrimp which were harvested from waters contaminated with raw sewage (Teophilo *et al.*, 2002). ETEC has also been detected in sauces at Mexican-style restaurants, and in chilli sauce sold by street vendors in Mexico (Adachi *et al.*, 2002; Estrada-Garcia *et al.*, 2002). In general, these sauces had been prepared and handled under poor hygienic conditions. The major reservoir of EHEC organisms appears to be the intestinal tract of ruminants, in particular cattle and sheep (Desmarchelier and Fegan, 2003).
E. coli O157:H7 and other EHEC species have been isolated from both healthy and diarrhoeic animals, and individual animals can carry more than one serotype (Anon, 1998). Foods derived from these animals may become contaminated via exposure to faecal material during processing.

Prevalence of STEC in raw milk has been determined in a limited number of studies. Caution must be exercised when comparing results between independent studies due to differences in sample size, stage of production where the samples were taken and different methodologies used to isolate the organisms. *E. coli* O157:H7 is the most widely studied EHEC serovar due to it being associated with a large number of outbreaks worldwide. In general, prevalence of STEC in raw milk is low. Adequate pasteurisation will ensure that STEC is inactivated. Very little information is available of the prevalence of EHEC organisms in food in Australia. Of the limited studies undertaken, the prevalence of *E. coli* O157:H7 in beef and sheep meat appears to be low, however, the prevalence of non-O157:H7 EHEC serotypes is unknown (Vanderlinde *et al.*, 1998; Vanderlinde *et al.*, 1999; Phillips *et al.*, 2001a; Phillips *et al.*, 2001b).

Virulence and infectivity

Clinical, pathological and epidemiological characteristics of disease caused by pathogenic *E. coli* vary between pathotypes and are discussed below.

EPEC have technically been defined as "diarrhoeagenic *E. coli* belonging to serogroups epidemiologically incriminated as pathogens but whose pathogenic mechanisms have not been proven to be related either to heat-labile enterotoxins or heat-stable enterotoxins or to

Shigella-like invasiveness" (Edelman and Levine, 1983). EPEC cause characteristic attaching and effacing lesions in the intestine, similar to those produced by EHEC, but do not produce Shiga toxins. Attachment to the intestinal wall is mediated by a plasmid-encoded outer membrane protein called the EPEC Adherence Factor in type I EPEC. However, pathogenicity is not strictly correlated to the presence of the EPEC Adherence Factor, indicating that other virulence factors are involved (ICMSF, 1996).

ETEC that survive passage through the stomach adhere to mucosal cells of the proximal small intestine and produce a heat-labile toxin and/or a heat-stable toxin. The heat-labile toxins are similar in structure and mode of action to cholera toxin, interfering with water and electrolyte movement across the intestinal epithelium (Desmarchelier and Fegan, 2003). If the volume of accumulated fluid exceeds the normal absorptive capacity of the large intestine, the excess is evacuated as watery diarrhoea.

EAEC strains are defined as *E. coli* strains that do not secrete heat-labile or heat-stable toxins. These strains adhere to cultured human epithelial cells in a characteristic aggregative or "stacked-brick" pattern (Yatsuyanagi *et al.*, 2002). The mechanisms causing enteric disease are not fully understood, however EAEC have been associated with persistent diarrhoea, primarily in infants and children (Desmarchelier and Fegan, 2003).

Following ingestion, EIEC invade epithelial cells of the distal ileum and colon. The bacteria multiply within the cytoplasm of the cells, causing cell destruction and ulceration. Pathogenicity is associated with a plasmid-encoded type III secretory apparatus and other plasmid-encoded virulence factors (Desmarchelier and Fegan, 2003).

The Shiga toxins (Stx1 and Stx2) of EHEC are closely related, or identical, to the toxins produced by *Shigella dysenteriae.* Additional virulence factors allow the organism to attach tightly to intestinal epithelial cells, causing what is commonly referred to as attaching-and-effacing lesions.

Dose response

EPEC: It is thought that only a few EPEC cells are necessary to cause illness in children (FDA 2003). Volunteer studies in adults demonstrated that illness could be caused by ingesting $10^6 – 10^{10}$ cells with sodium bicarbonate to neutralise stomach acidity (Doyle and Padhye, 1989).

ETEC: Volunteer studies have shown that $10^8 – 10^{10}$ cells of ETEC are necessary for illness in adults (DuPont *et al.*, 1971) although the infective dose is probably less for infants and children (FDA, 2003).

EIEC: Volunteer studies have shown that 10^8 EIEC cells are necessary to cause illness in adults, with the infectious dose reduced to 10^6 when ingested with sodium bicarbonate (DuPont *et al.,* 1971). However, the US Food and Drug Administration suggest that as few as 10 cells may be needed to cause illness in adults, based on the organisms similarity with *Shigella* (FDA 2003).

The dose-response relationship for EHEC is complicated by the large number of serotypes and the association of EHEC with a variety of foods. Haas *et al.* (2000) developed a dose-response relationship for *E. coli* O157:H7 based on data from a prior animal study undertaken

by Pai *et al.* (1997) which involved oral administration of bacterial suspension to infant rabbits. The model was validated by comparison with two well-documented human outbreaks, one foodborne and the other waterborne. The model estimated that the dose required to result in 50% of the exposed population to become ill was 5×10^5 organisms. The corresponding probability of illness for the ingestion of 100 organisms was 2.6×10^{-4}.

Dose-response relationships for *E. coli* O111 and O55 have been developed from human feeding trial data (Haas *et al.,* 2000). The relationship estimated a dose required for 50% of the exposed population to become ill was 2.55×10^6 and the probability of illness for ingestion of 100 organisms was 3.5×10^{-4}. Investigations of other known outbreaks of foodborne illness due to *E. coli* O157:H7 and systematic studies aimed at quantifying the dose–response relationship suggest as few as 1 – 700 EHEC organisms can cause human illness (FDA 2003).

Host susceptibility

A variety of host factors may be important in the pathogenesis of specific *E. coli* serotypes. In general, the young and the elderly appear to be more susceptible to pathogenic *E. coli* infection. Epidemiological studies have identified that children are at higher risk of developing post-diarrhoeal HUS than other age groups (Cummings *et al.*, 2002).

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3. *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, non-spore forming rod-shaped bacteria that may be isolated from a variety of sources including soil, silage, sewage, food-processing environments, raw meats and the faeces of healthy humans and animals (FDA, 2003). *L. monocytogenes* belongs to the genus *Listeria* along with *L. innocua, L. welshimeri, L. selligeri, L. ivanovii and L. grayi*. Thirteen serotypes are associated with *L. monocytogenes* (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7).

Growth characteristics

Growth of *L. monocytogenes* in foods is influenced by a variety of factors, including the nature and concentration of essential nutrients, pH, temperature, water activity, the presence of food additives that could enhance or inhibit growth and presence of other microbial flora (Lovett *et al.*, 1987). Under conditions outside the growth range, the bacteria may survive and growth may recommence once suitable conditions are encountered. Temperatures of >50ºC are lethal to *L. monocytogenes*. When in a suitable medium, *L. monocytogenes* can grow between ~0 - 45°C. Although *L. monocytogenes* does not grow below –1.5ºC, it can readily survive at much lower temperatures. Nonetheless, freezing and frozen storage will cause a limited reduction in the viable population of *L. monocytogenes*. Optimal conditions for growth are between 30 - 37ºC (Ryser and Marth, 1999).

L. monocytogenes will grow in a broad pH range with the upper limit being approximately 9.3 and the lower limit being 4.6-5.0 (ICMSF, 1996). Although growth at pH <4.3 has not yet been documented, *L. monocytogenes* appears to be relatively acid tolerant. It has been suggested that food fermentations, which involve a gradual lowering of pH, could lead to acid adaptation of *L. monocytogenes*.

Like many bacterial species, *L. monocytogenes* grows optimally at a of approximately 0.97. However, when compared with most foodborne pathogens, the bacterium has the unique ability to multiply at water activity values as low as 0.90. While it does not appear to be able to grow below 0.90, the bacterium can survive for extended periods at lower values (Ryser and Marth, 1999).

L. monocytogenes is reasonably tolerant to salt and can grow in NaCl concentrations up to 10%. Extended survival occurs at a wide range of salt concentrations and *L. monocytogenes* has survived for up to eight weeks in a concentration of 20% NaCl (Sutherland *et al.*, 2003). Survival in the presence of salt varies with storage temperature and studies have indicated that survival of *L. monocytogenes* in concentrated salt solutions can be increased dramatically by lowering the incubation temperature (Ryser and Marth, 1999). *L. monocytogenes* grows well under both aerobic and anaerobic conditions (Ryser and Marth, 1999; Sutherland *et al.*, 2003).

The listericidal effect of preservatives is strongly influenced by the interactive effects of temperature, pH, type of acidulant, salt content, water activity, and type and concentration of food additives present in the food. For example, the ability of potassium sorbate to prevent growth of *L. monocytogenes* is related to temperature and pH. The lower the storage temperature and pH of the medium, the greater the effectiveness of sorbates against *L. monocytogenes*. Sodium benzoate is more inhibitory to *L. monocytogenes* than is either potassium sorbate or sodium propionate. Inhibition and inactivation of *L. monocytogenes* in the presence of sodium benzoate is affected by temperature (more rapid at higher than lower

incubation temperatures), concentration of benzoic acid (more rapid at higher than lower concentrations) and pH (more rapid at lower rather than higher pH values) as well as the type of acid used to adjust the growth medium (Ryser and Marth, 1999).

Pathology of illness

There are two main forms of illness associated with *L. monocytogenes* infection: listerial gastroenteritis, where usually only mild symptoms are reported, and invasive listeriosis, where the bacteria penetrate the gastrointestinal tract and invade normally sterile sites within the body (FDA 2003).

Symptoms of the mild form of *L. monocytogenes* infection are primarily those generally associated with gastrointestinal illness: chills, diarrhoea, headache, abdominal pain and cramps, nausea, vomiting, fatigue, and myalgia (FDA, 2003). The onset of illness is usually greater than 12 hours.

Invasive listeriosis is clinically defined when the organism is isolated from blood, cerebrospinal fluid or an otherwise normally sterile site (*e.g.* placenta, foetus). The manifestations include septicaemia, meningitis (or meningoencephalitis), encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion in the second or third trimester, or stillbirth (FDA, 2003). The onset of these manifestations is usually preceded by influenza-like symptoms including persistent fever. Gastrointestinal symptoms such as nausea, vomiting and diarrhoea may also precede the serious forms of listeriosis. Listeriosis typically has a 2 - 3 week incubation time, but onset time may extend to 3 months (FDA/Centre for Food Safety and Applied Nutrition, 2003).

It is estimated that approximately 2 – 6% of the healthy human population harbour *L. monocytogenes* in their intestinal tract, which suggests that people are frequently exposed to *L. monocytogenes* (Farber and Peterkin, 1991; Rocourt and Bille, 1997). This may also suggest that most people have a tolerance to infection by *L. monocytogenes*, and given the relatively low number of reported cases, exposure rarely leads to serious illness in healthy individuals (Hitchins, 1996; Marth, 1988).

Mode of transmission

Foodborne exposure is the primary route of transmission for listeriosis, however listeriosis can be transmitted vertically (*i.e.* mother to child), zoonotically and through hospital acquired infections (Ryser and Marth, 1999; Bell and Kyriakides, 2005).

Incidence of illness

Most cases of listeriosis are sporadic. The number of reported cases of invasive listeriosis in Australia between 2001 - 2004 varied between 61 – 72 cases (Ashbolt *et al.*, 2002; Anon, 2002; Anon, 2003; Anon, 2004b), which equates to approximately 3 – 4 cases per million population per annum. In Australia, the exact mortality rate is not known, although the data available would suggest a rate of approximately 23%. The case fatality rate in New Zealand is approximately 17% (Anon, 2004a).

The estimated incidence of invasive listeriosis in European countries has been reported to between 0.3 - 7.5 cases per million of the general population per annum (European Commission, 2003). In France, the estimated incidence is sixteen cases per million (general population) per annum (Bille, 1990; ICMSF, 1996). The annual incidence of listeriosis in the United States has been estimated to range from 3.4 per million (CDC, 2002) to 4.4 per million (Tappero *et al.*, 1995). Of all foodborne pathogens, *L. monocytogenes* results in the highest hospitalisation rate in the US, with fatality rates of 20 - 30% being common (WHO/FAO, 2004).

Outbreaks of invasive listeriosis have been linked to Hispanic-style soft cheeses; soft, semi-soft and mould-ripened cheeses; hot dogs; pork tongue jelly; processed meats; pate; salami; pasteurised chocolate flavoured milk; pasteurised and unpasteurised milk; butter; cooked shrimp; smoked salmon; maize and rice salad; maize and tuna salad; potato salad; raw vegetables; and coleslaw (FDA 2003). In addition, sporadic cases have been linked to the consumption of raw milk; unpasteurised ice cream; ricotta cheese; goat, sheep and feta cheeses; soft, semi-soft and mould-ripened cheeses; Hispanic-style cheese; salami; hot dogs; salted mushrooms; smoked cod roe; smoked mussels; undercooked fish; pickled olives; raw vegetables; and coleslaw (WHO/FAO, 2004).

Occurrence in foods

L. monocytogenes has been found in foods such as milk, dairy products (particularly soft-ripened cheeses), meat, poultry, seafood and vegetables. The worldwide prevalence of *L. monocytogenes* in raw milk is estimated to be around 3-4% (Hayes *et al.*, 1986; Lovett *et al.,* 1987; Doores and Amelang, 1988). In Australian surveys on soft and surface ripened cheeses and ice-cream, *L. monocytogenes* has been isolated from 2% of locally produced cheese samples and 6% of ice-cream samples (Sutherland *et al.*, 2003). For imported cheeses, camembert and blue vein, 7% were positive for *L. monocytogenes* (Sutherland *et al.*, 2003). For European soft and surface-ripened cheeses, 25% have been found to be positive for *L. monocytogenes* (Terplan, 1988).

Meat products from which *L. monocytogenes* has been isolated include beef, lamb, pork, minced meat products, sausages, salami, ham, mettwurst, pate, frankfurters and vacuumed packed meat, chicken products, and processed seafood (Farber and Peterkin 1991; Cox *et al.*, 1999; Ojeniyi *et al.*, 2000). Additionally vegetable products have also been shown to be contaminated (Heisick *et al.*, 1989; Brackett, 1999).

Virulence and infectivity of L. monocytogenes

When ingested, *L. monocytogenes* penetrates the intestinal tissue and is taken up by macrophages and non-phagocytic cells in the host. *L. monocytogenes* is disseminated throughout the host via blood or lymphatic circulation to various tissues. Its presence intra-cellularly in phagocytic cells permits access to the brain and probably transplacental migration to the foetus in pregnant women. The pathogenesis of *L. monocytogenes* relies on its ability to survive and multiply in phagocytic host cells. Not all strains appear to be equally virulent. The 4b and occasionally 1/2a and 1/2b serovars account for most cases of human listeriosis (ICMSF, 1996). The virulence of *L. monocytogenes* is increased when the bacterium is grown at low rather than high temperatures. The possibility exists that cold storage may enhance the virulence of some *L. monocytogenes* strains isolated from refrigerated foods (Ryser and Marth, 1999).

Dose response

Cases of non-invasive listeriosis (also referred to as febrile listerial gastroenteritis) have been observed during outbreaks, involving symptoms such as diarrhoea, fever, headache and myalgia, generally following a short incubation period (WHO/FAO, 2004). Insufficient quantitative data is available to develop a dose-response model for this milder form of listeriosis, however, outbreak situations have generally involved the ingestion of high doses of *L. monocytogenes*.

The dose-response relationship for invasive listeriosis is highly dependent on a number of factors, such as the virulence characteristics of the organism, the number of cells ingested, the general health and immune status of the host, and the attributes of the food matrix that may alter the microbial or host status. WHO/FAO (2004) and FDA/FSIS (2003) developed separate dose-response models for both healthy and susceptible populations by combining data from surrogate animal models with epidemiological data. The Exponential dose-response model was used for both populations. This dose-response model has a single parameter, the *r*-value. The *r*-value is the probability that a person will become ill from the consumption of a single *L. monocytogenes* cell. For the healthy population (classified as "intermediate-age") the median r-value was estimated to be 2.37×10^{-14}. For more susceptible populations the median *r*-value was estimated to be 1.06×10^{-12}. A more recent assessment of US epidemiological data on invasive Listeriosis in susceptible sub-populations which included genetic information regarding different *L. monocytogenes* strains (lineages), determined average *r*-values of 1.31×10^{-8} for lineage I and 5.01×10^{-11} for lineage II (Chen *et al*., 2006). Further analysis of the epidemiological data by the *L. monocytogenes* ribotype found *r*-values as small as 6.29×10^{-3}. These results suggest that there are large differences in virulence between *L. monocytogenes* strains.

The infectious dose is unknown but it is believed to vary depending on the strain and susceptibility of the individual. There is a lack of information concerning the minimal infectious dose, although it is generally thought to be relatively high (>100 viable cells) (ICMSF, 1996). From cases contracted via raw or inadequately pasteurised milk, it is assumed that for susceptible individuals, ingestion of fewer than 1,000 organisms may cause disease (FDA/FSIS, 2003). It is thought the consumption of food with exceptionally high levels of *L. monocytogenes* ($>10^7$/g) is required to cause the mild gastrointestinal form of illness in healthy persons (Sutherland *et al.*, 2003).

Host factors

Specific sub-populations at risk for invasive listeriosis include pregnant women and their foetuses, neonates, the elderly and persons with a compromised immune system, whose resistance to infection is lowered (*e.g.* transplant patients, patients on corticosteroid treatments, AIDS patients and alcoholics). Less frequently reported diabetic, cirrhotic, asthmatic and ulcerative colitis patients are also at a higher risk (FDA 2003). Another physiological parameter thought to be relevant to susceptibility is a reduced level of gastric acidity (WHO/FAO, 2004).

Food matrix

To date, the properties of the food vehicle have been viewed as having little effect on the infective dose of *L. monocytogenes*. However, it is possible that food vehicles with high buffering capacity may protect the bacteria from inactivation by the pH of gastric acids in the stomach. In general, there are insufficient data available as to whether the food matrix affects the dose-response curve for *L. monocytogenes* (WHO/FAO, 2004).

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4. *Salmonella* spp.

Salmonellosis is a leading cause of enteric illness, with symptoms ranging from mild gastroenteritis to systemic illness such as septicaemia and other longer-term conditions. A wide range of foods have been implicated in foodborne salmonellosis. However, as the disease is primarily zoonotic, foods of animal origin have been consistently implicated as the main sources of human salmonellosis (FAO/WHO, 2002). The genus *Salmonella* is currently divided into two species: *Salmonella enterica* (comprising six subspecies) and *Salmonella bongori* (Brenner *et al.*, 2000). The subspecies of most concern in relation to food safety is *S. enterica* subsp. *enterica,* as over 99% of human pathogens belong to this subspecies (Bell and Kyriakides, 2002).

Over 1,400 *S. enterica* subsp. *enterica* serotypes are currently recognised, and all are regarded as capable of causing illness in humans (Brenner *et al.,* 2000). The formal names to describe *Salmonella* serotypes are rather cumbersome, for example *S. enterica* subsp. *enterica* serotype Typhimurium (formerly *Salmonella typhimurium*). For practical reasons, the shortened versions of these names are commonly used, such as *Salmonella* Typhimurium. Some *Salmonella* serotypes are host-adapted to individual animal species. For example *S.* Typhi and *S.* Paratyphi are specifically associated with infections leading to severe illness in humans (Bell and Kyriakides, 2002).

Growth characteristics

Salmonellae have relatively simple nutritional requirements and can survive for long periods of time in foods and other substrates. The rate of growth and extent of survival of the organism in a particular environment is influenced by the simultaneous effect of a number of factors such as temperature, pH, and water activity. Being facultative anaerobic, salmonellae also have the ability to grow in the absence of oxygen. Growth and survival is also influenced by the presence of inhibitors such as nitrite and short-chain fatty acids (Jay *et al.*, 2003).

The growth of most salmonellae is substantially reduced at temperatures <15°C and prevented at <7°C. Growth generally does not occur at temperatures >46.2°C. The optimum temperature for growth is 35 – 43°C. Freezing can be detrimental to *Salmonella* spp. survival, although it does not guarantee destruction of the organism (ICMSF, 1996). There is an initial rapid decrease in the number of viable organisms at temperatures close to freezing point as a result of freezing damage. However, at lower temperatures (-17 to -20°C) there is a significantly less rapid decline in the number of viable organisms. *Salmonella* spp. have the ability to survive long periods of time at storage temperatures < -20°C (Jay *et al.*, 2003). Heat resistance of *Salmonella* spp. in foods is dependant on the composition, nature of solutes, pH, and water activity of the food (Jay *et al.*, 2003). In general, heat resistance increases as the water activity of the food decreases. A reduction in pH results in a reduction of heat resistance (ICMSF, 1996).

The minimum pH at which *Salmonella* spp. can grow is dependent on the temperature of incubation, the presence of salt and nitrite and the type of acid present. However, growth can usually occur between pH 3.8 – 9.5 (Jay *et al.*, 2003). The optimum pH range for growth is 7.0 – 7.5. Volatile fatty acids are more bactericidal than acids such as lactic and citric acid.

Water activity has a significant effect on the growth of *Salmonella* spp., with the lower limit for growth being 0.94 (ICMSF, 1996). *Salmonella* can survive for long periods of time in foods with a low water activity (such as black pepper, chocolate, gelatine). Exposure to low water activity environments can greatly increase the heat resistance of *Salmonella* spp.

Pathology of illness

Outcomes of exposure to *Salmonella* spp. can range from having no effect, to colonisation of the gastrointestinal tract without symptoms of illness (asymptomatic), or colonisation with the typical symptoms of acute gastroenteritis (FAO/WHO, 2002). Gastroenteritis symptoms may include abdominal pain, nausea, diarrhoea, mild fever, vomiting, headache and/or prostration, with clinical symptoms lasting 2 – 5 days. Most symptoms of salmonellosis are mild, and only a low proportion of cases within the community are reported to public health agencies (Mead *et al.*, 1999). In a small number of cases, *Salmonella* spp. infection can lead to more severe invasive diseases characterised by septicaemia and sometimes death. In a study of 48,857 patients with gastroenteritis (of which 26,974 were salmonellosis), Helms *et al.* (2003) found an association with increased short-term (mortality within 30 days of infection) and long-term (mortality within a year of infection) risk of death compared with controls.

In cases of acute gastroenteritis, the incubation period is usually 12 - 72 hours (commonly 12 - 36 hours) and is largely dependant on the sensitivity of the host and size of the dose ingested (Hohmann, 2001; FAO/WHO, 2002). Illness is usually self-limiting, with patients fully recovering within one week, although in some severe cases of diarrhoea, significant dehydration can ensue which may require medical intervention such as intravenous fluid replacement. Septicaemia is caused when *Salmonella* spp. enters the bloodstream, with symptoms including high fever, pain in the thorax, chills, malaise and anorexia (FAO/WHO, 2002). Although uncommon, long-term effects or sequelae may occur including arthritis, appendicitis, cholecystitis, endocarditis, local abscesses, meningitis, osteomyelitis, osteoarthritis, pericarditis, peritonitis, pleurisy, pneumonia and urinary tract infection (ICMSF, 1996). At the onset of illness large numbers of *Salmonella* spp. are excreted in the faeces. Numbers decrease with time, but the median duration of excretion after acute non-typhoid salmonellosis has been estimated at five weeks, and approximately 1% of patients become chronic carriers (Jay *et al.*, 2003).

Due to the general self-limiting nature of the disease, antibiotics are not usually recommended for healthy individuals suffering from mild to moderate *Salmonella* spp. gastroenteritis (Hohmann 2001). Antibiotics should be used, however, for those who are severely ill and for patients with risk factors for extra intestinal spread of infection, after appropriate blood and faecal cultures are obtained.

Of recent concern worldwide is the emergence of multiple antibiotic resistant strains of *Salmonella* spp., an example being *S.* Typhimurium definitive phage type 104 (DT104). Multi-resistant *S.* Typhimurium DT104 is a significant human and animal pathogen, with high morbidity observed in cattle and poultry (Crerar *et al.*, 1999). To date, this organism is not endemic in Australia, although it is a significant health problem in European countries, North America, the Middle East, South Africa and South-East Asia (Jay *et al.*, 2003).
S. Typhimurium DT104 constitutes 8 – 9% of human *Salmonella* spp. isolates in the US. Sporadic human cases are reported in Australia, although these are commonly acquired overseas (Blumer *et al.*, 2003). During 2001 an outbreak of *S.* Typhimurium DT104 occurred in Victoria and was linked to contaminated imported halva (a sesame seed product).

Mode of transmission
Salmonellae are transmitted by the faecal-oral route. Sources of transmission include person-to-person, foodborne, waterborne (drinking water and direct contact with faecally contaminated water) and direct contact with infected animals.

Incidence and outbreak data
Salmonellosis is one of the most commonly reported enteric illnesses worldwide (FAO/WHO, 2002). Approximately 7,000 - 8,000 cases of salmonellosis per annum are formally notified to health authorities in Australia. Taking into account under-reporting it has been estimated (based on published rates of under-reporting) that 80,000 cases of foodborne salmonellosis occur annually (Hall, 2003). The salmonellosis notification rate in Australia for 2002 was 40.3 cases per 100,000 population. This varied from 24.8 cases per 100,000 population in Victoria to 166.7 cases per 100,000 population in the Northern Territory (Anon, 2003). Children less than five years of age have by far the highest notification rate, with a rate of 210.6 cases per 100,000 population reported for 2002 (Yohannes *et al.,* 2004). The higher rate of notified salmonellosis cases in this age group may reflect an increased susceptibility upon first exposure, but may also be a result of other factors such as an increased likelihood of exposure and increased likelihood to seek medical care and be tested.

Of the total number of *Salmonella* serovars reported to Australian health authorities during 2002, *S.* Typhimurium 135 was the most commonly reported. Distribution of *Salmonella* serovars varies geographically, with the most commonly reported serovars in Queensland, Tasmania and the Northern Territory being *S.* Virchow (10%), *S.* Mississippi (48%) and *S.* Ball (15%) respectively. Of the other States and Territories, *S.* Typhimurium was the most commonly reported serovar, representing 34% of cases in the Australian Capital Territory, 28% in New South Wales, 60% in South Australia, 66% in Victoria and 15% in Western Australia. Salmonellosis notifications in Australia fluctuate seasonally, from a low in August - September to a peak in January - March, with 36% of salmonellosis cases notified during this period (Yohannes *et al.,* 2004).

It has been estimated that in the US (Mead *et al.,* 1999) and England and Wales (Adak *et al.*, 2002), 95% and 91.6%, respectively of salmonellosis cases are foodborne. Other sources of infection may be via contaminated water, person-to-person transmission and direct contact with infected animals. Based on results from national and international epidemiological data (primarily outbreak investigations) a wide range of foods have been implicated in human salmonellosis. Foods of animal origin (*e.g.* meat, eggs, and dairy) are important sources of human salmonellosis.

Following notifications of salmonellosis to Australian health authorities, over 50 epidemiological investigations are initiated each year in an attempt to identify a common source of infection (Anon 2003). It is often difficult, however, to confirm a single food commodity as a source due to the difficulty of investigating commonly consumed foods, conducting trace-back, and lack of systematically collected microbiological data from foods.

In a review of reported foodborne disease outbreaks in Australia during 1995 – 2000, meats (in particular poultry meat) were associated with 33% of identified salmonellosis outbreaks (Dalton *et al.*, 2004). A large outbreak (consisting of 502 cases) of *S.* Typhimurium 135a occurred in 1999 and was associated with consumption of unpasteurised commercial orange juice (Roche *et al.*, 2001). In 2001 a community-wide outbreak of *S.* Typhimurium 126

occurred in South Australia (Ashbolt *et al.*, 2002). A subsequent case-control study associated illness with the consumption of chicken meat. This link was corroborated with microbiological testing of raw poultry, and the likely source of contaminated products was traced to a single poultry processing facility.

Occurrence in food

The primary reservoir of *Salmonella* spp. is the intestinal tract of warm and cold-blooded vertebrates. Infected animals shed large numbers in their faeces, and this leads to contamination of the surrounding environment including soil, pasture, streams and lakes. *Salmonella* spp. have been isolated from a wide range of foods, particularly those of animal origin and those foods that have been subject to faecal contamination (ICMSF, 1996). Raw meat products (in particular poultry) have frequently been associated with the presence of *Salmonella* spp. *Salmonella* spp. positive animals at the time of slaughter may have high numbers of organisms in their intestines as well as on external surfaces (faecal contamination of hides, fleece, skin or feathers). Cross contamination during processing may also lead to increased prevalence of *Salmonella* spp. in finished products (Bryan and Doyle 1995). Pasteurisation of dairy products effectively inactivates *Salmonella* spp., however contamination of milk has occurred due to improper pasteurisation and/or post-processing contamination (Jay *et al.*, 2003).

Virulence and infectivity

Once ingested, *Salmonella* spp. must be able to overcome the low pH of the stomach, adhere to the small intestine epithelial cells and overcome host defence mechanisms to enable infection (Jay *et al.*, 2003). *Salmonella* spp. possess a number of structural and physiological virulence factors enabling them to cause acute and chronic disease in humans.

Virulence of *Salmonella* spp. varies with the length and structure of the O side chains of lipopolysaccharide molecules at the surface of the cell. Resistance of *Salmonella* spp. to the lytic action of complement is directly related to the length of the O side chain (Jay *et al.*, 2003). The presence of virulence plasmids has been associated with the ability to spread rapidly after colonisation and overwhelm the host immune response (D'Aoust, 1997). These virulence plasmids are large cytoplasmic DNA structures that replicate independently of the chromosomal DNA. Virulence plasmids are present in a limited number of *Salmonella* serovars and have been confirmed in *S.* Typhimurium, *S.* Dublin, *S.* Gallinarum, *S.* Pullorum, *S.* Enteritidis, *S.* Choleraesuis and *S.* Abortusovis. It is notable, however, that virulence plasmids are absent from *S.* Typhi, which is host-adapted and highly infectious.

Once attached to small intestine epithelial cells, the organism is drawn into the host cell in a vesicle (endosome) where it can multiply in the mildly acidic environment. Heat labile enterotoxin may be released during *Salmonella* spp. growth, resulting in the loss of intestinal fluids. This enterotoxin is closely related functionally, immunologically and genetically to cholera toxin and the heat labile toxin of pathogenic *E. coli* (Jay *et al.*, 2003). Most *Salmonella* strains also produce heat labile cytotoxin which may cause damage of the intestinal mucosal surface and general enteric symptoms and inflammation. For non-typhoidal *Salmonella* spp., infection is generally limited to a localised intestinal event.

Dose response

Human feeding trials for a range of *Salmonella* serovars were undertaken during the 1950's to determine the relationship between the dose of pathogen ingested and the response of the individual (McCullough and Eisele.C.W, 1951a; McCullough and Eisele.C.W, 1951b; McCullough and Eisele.C.W, 1951c; McCullough and Eisele.C.W, 1951d). The study population consisted of healthy males confined in an institutional setting who were fed known doses of an individual *Salmonella* serovar. Infection was confirmed by recovering the administered *Salmonella* serovar from faecal samples.

Fazil (1996) combined all the data from the feeding trials and found that a single Beta-Poisson relationship could adequately describe the dose-response for all serovars. However, a number of limitations exist on the use of such feeding trial data. Firstly the use of healthy adult male volunteers could underestimate the pathogenicity to the overall population. In addition, volunteers were exposed to high doses of *Salmonella* spp., with the minimum dose being 10^4 cells.

In dose-response analysis, the critical region is the lower-dose region, as these are the doses that are most likely to exist in real food contamination events. This requires extrapolation of the model to doses much lower than those used in the human feeding trials. It must also be noted that the dose-response models are based on the risk of infection as an endpoint rather than illness, and therefore may introduce a level of conservatism into the dose-response relationship.

It has been shown through salmonellosis outbreak investigations, that doses resulting in illnesses (gastroenteritis) were often several orders of magnitude lower than the doses reported in the feeding trials (D'Aoust, 1994). Using a reasonably large data set, the FAO/WHO in 2002 developed a dose-response model based on actual outbreak data. Although not subject to some of the inherent flaws associated with using purely experimental data, the data used in this model have a certain degree of uncertainty, which required assumptions to be made. This uncertainty is primarily due to the uncontrolled settings under which the information and data were collected. It is often difficult to determine the actual dose ingested (based on the level of the organism in the food at the time of consumption and the amount of food consumed), as well as determining the actual number of people exposed or ill during the outbreak.

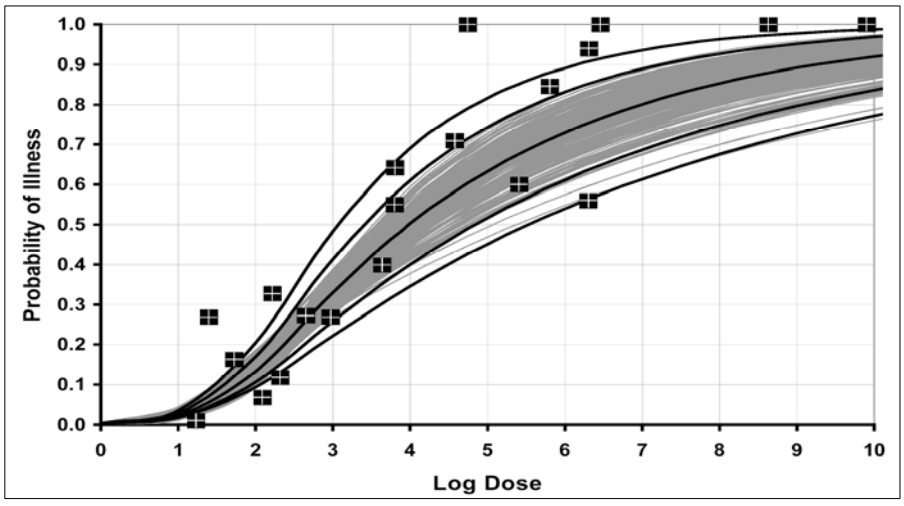

Figure 1: Uncertainty bounds for dose-response curves compared with expected value for the outbreak data (FAO/WHO, 2002).

Host factors

Individual susceptibility to *Salmonella* spp. infection and/or disease can vary significantly, depending on host factors such as pre-existing immunity, nutrition, age, ability to elicit an immune response, structural and functional anomalies of the intestinal tract, or pre-existing disease (Gerba *et al.*, 1996; Jay *et al.*, 2003). Individuals who are generally at greater risk of infection and/or risk of developing more severe outcomes from exposure to *Salmonella* spp. include the very young, the elderly, pregnant women and the immunucompromised (organ transplant patients, cancer patients and AIDS patients) (Gerba *et al.,* 1996).

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ANNEX 5: Bulk milk tank prevalence

This annex presents a summary of the literature sources used to establish the prevalence of pathogenic bacteria in on-farm bulk milk tanks. Exclusion criteria included geographical considerations, the age profile of the herds, sample size and analytical methodology.

The geographical location of the studies that were accepted was limited to those countries that were considered to have production systems similar to those found in Australia. Five geographical regions were considered: Australia and New Zealand, North America, UK/Ireland, continental Europe and Scandinavia. Within these, countries consideration was also made about the production systems. For example, results from Scandinavian countries where cows were housed during the winter months were generally excluded.

For the four pathogens considered, *Campylobacter* spp., enterohaemorrhagic *Escherichia coli*, *Salmonella* spp. and *L*isteria *monocytogenes*, there is a separate summary table. This includes information on the source of the data, the country, the total number of samples, the number of samples where pathogens were detected and the prevalence. In addition, there is a plot of the cumulative probability distribution of the herd prevalence based on the data presented in each table.

The method used for developing a combined cumulative distribution for the herd prevalence was by using weighted average of the cumulative percentiles (Vose, 2008). In this case all studies are given equal weighting. The method is briefly outlined below:

- For each study the number of detections (positives) and the total number of samples are found
- The parameters of a beta distribution, Beta(α_1, α_2) with parameters, $\alpha_1 = s + 1$ and $\alpha_2 = n - s + 1$ (assuming a uniform prior) are calculated
- In the statistical program, R (www.r-project.org) the cumulative percentiles for the corresponding Beta distribution is determined using the `pbeta` command
- The cumulative distributions for each Beta distribution are then average to give an overall cumulative distribution
- The resulting distribution is entered into @Risk as a cumulative distribution with a lower limit of zero, and an upper limit judged on the cumulative distribution values

Campylobacter spp.

Author/Publication	Reference	Country	Samples (n)	Positive (s)	Prevalence
SA unpublished (1995-2002)	1	Australia	95	0	0
WA unpublished (2007)	2	Australia	183	0	0
Bachmann and Spahr (1995)	3	Switzerland	496	0	0
Davidson *et al*. (1989)	4	Canada	192	3	0.016
Desmasures *et al*. (1997)	5	France	69	1	0.014
Doyle and Roman (1982)	6	USA	108	1	0.009
Jayarao and Henning (2001)*	7	USA	131	12	0.092
Jayarao *et al*. (2006)	8	USA	248	5	0.02
Lovett *et al.*(1983)	9	USA	195	3	0.015
McManus and Lanier (1987)	10	USA	237	1	0.004
Oosterom *et al*. (1982)	11	Netherlands	200	0	0
Rohrbach *et al*. (1992)*	12	USA	292	36	0.123
Steele *et al.* (1997)	13	Canada	1720	8	0.005
Food Standards Agency (2002)	14	UK	602	5	0.008
Food Standards Agency (2003)	15	UK	610	5	0.008
Whyte *et al*. (2004)	16	Ireland	62	1	0.016
Wyatt and Timm (1982)	17	USA	50	0	0

* Statistically different to the WA unpublished (2007) prevalence

E. coli (EHEC)

Author/Publication	Reference	Country	Samples	Positive	Prevalence
WA unpublished (2007)	1	Australia	118	0	0
Coia *et al*. (2001)	2	Scotland	500	0	0
de Rue *et al*. (2004)	3	Belgium	143	1	0.007
Desmarchelier (1997)	4	Australia	1802	171	0.095
Hancock *et al*. (1994)	5	USA	603	0	0
Jayarao and Henning (2001)	6	USA	131	5	0.038
Jayarao *et al*. (2006)	7	USA	248	6	0.024
Karns *et al*. (2007)	8	USA	859	36	0.042
Massa *et al*. (1999)	9	Italy	100	0	0
Padhye and Doyle (1991)*	10	USA	115	11	0.096
Steele *et al*. (1997)	11	Canada	1720	15	0.009
Food Standards Agency (2002)	12	UK	602	1	0.002
Food Standards Agency (2003)	13	UK	610	1	0.002

* Statistically different to the WA unpublished (2007) prevalence

Salmonella spp.

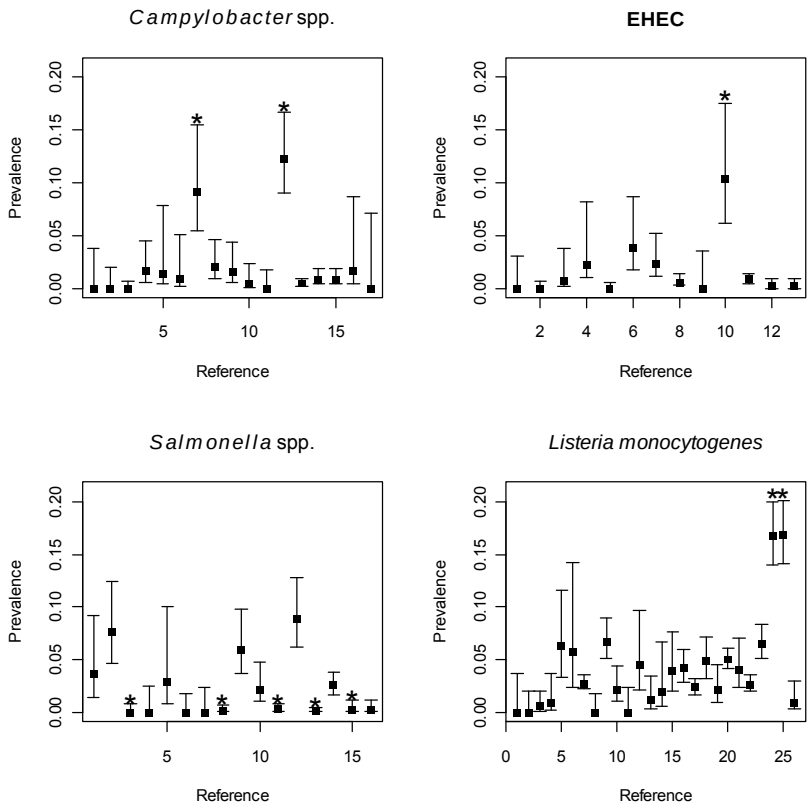
Author/Publication	Reference	Country	Samples	Positive	Prevalence
SA unpublished (1995-2002)	1	Australia	108	4	0.037
WA unpublished (2007)	2	Australia	183	14	0.077
Bachmann and Spahr (1995)*	3	Switzerland	456	0	0
de Rue *et al*. (2004)	4	Belgium	143	0	0
Desmasures *et al*. (1997)	5	France	69	2	0.029
Eliskases-Lechner and Ginzinger (1999)	6	Austria	201	0	0
Humphrey and Hart (1988)	7	UK	153	0	0
Humphrey and Hart (1988)*	8	UK	985	2	0.002
Jayarao *et al*. (2006)	9	USA	248	15	0.06
Murinda *et al*. (2002a)	10	USA	268	6	0.022
O'Donnell (1995)*	11	England and Wales	1673	6	0.004
Rohrbach *et al*. (1992)	12	USA	292	26	0.089
Steele *et al*. (1997)*	13	Canada	1720	3	0.002
van Kessel *et al*. (2004)	14	USA	861	22	0.026
Food Standards Agency (2002)*	15	UK	602	2	0.003
Food Standards Agency (2000)*	16	UK	610	2	0.003

* Statistically different to the WA unpublished (2007) prevalence

Listeria monocytogenes

Author/Publication	Reference	Country	Samples	Positive	Prevalence
SA unpublished (1995-2002)	1	Australia	97	0	0
WA unpublished (2007)	2	Australia	183	0	0
Bachmann and Sphar (1995)	3	Switzerland	340	2	0.006
Davidson *et al*. (1989)	4	Canada	192	2	0.01
de Rue *et al*. (2004)	5	Belgium	143	9	0.063
Desmasures *et al*. (1997)	6	France	69	4	0.058
Doores and Amelang (1988)	7	USA	2511	71	0.028
Eliskases-Lechner and Ginzinger (1989)	8	Austria	201	0	0
Fenlon *et al*. (1995)	9	Scotland	638	43	0.067
Husu (1990)	10	Finland	314	7	0.022
Ibrahim and MacRae (1991)	11	Australia	150	0	0
Jayarao and Henning (2001)	12	USA	131	6	0.046
Jayarao *et al*. (2006)	13	USA	248	3	0.012
Kongo *et al*. (2006)	14	Portugal	105	2	0.019
Liewen and Platz (1988)	15	USA	200	8	0.04
Lovett *et al.*(1987)	16	USA	650	27	0.042
Meyer-Brosetta *et al*. (2003)	17	France	1459	35	0.024
Muraoka *et al*. (2003)	18	USA	474	23	0.049
Navratilova *et al*. (2004)	19	Czech Republic	278	6	0.022
O'Donnell (1995)	20	England and Wales	2009	102	0.051
Rohrbach *et al*. (1992)	21	USA	292	12	0.041
Steele *et al*. (1997)	22	Canada	1720	47	0.027
van Kessel *et al*. (2004)	23	USA	861	56	0.065
Food Standards Agencu (2002)*	24	UK	602	101	0.168
Food Standards Agency (2003)*	25	UK	610	103	0.169
Waak *et al*. (2002)	26	Sweden	294	3	0.01

* Statistically significant different to the WA unpublished (2007) prevalence



Prevalence of *Campylobacter* spp., EHEC, *Salmonella* spp. and *L. monocytogenes* in bulk milk tanks. Error bars represent the 95% confidence intervals. Results highlighted with an asterisk (*) indicate that the prevalence is significantly different from the WA unpublished (2007) raw milk data. Details of each numbered reference can be found in the corresponding tables in this annex.

Annex 6: With-in herd prevalence

This annex presents a summary of the literature sources used for with-in herd prevalence that were accepted after a preliminary review. With-in herd prevalence was largely based on the detection of pathogens in faecal samples. Exclusion criteria included geographical considerations, the age profile of the herds, sample size and analytical methodology.

The geographical location of the studies that were accepted was limited to those countries that were considered to have production systems similar to those found in Australia. Five geographical regions were considered: Australia and New Zealand, North America, UK/Ireland, continental Europe and Scandinavia. Within these, countries consideration was also made about the production systems. For example, results from Scandinavian countries where cows were housed during the winter months were generally excluded.

For the four pathogens considered, *Campylobacter* spp., enterohaemorrhagic *Escherichia coli,* , *Salmonella* spp. and *Listeria monocytogenes*, there is a separate summary table. This includes information on the source of the data, the country, the total number of samples, the number of samples where pathogens were detected and the prevalence. In addition, there is a plot of the cumulative probability distribution of the herd prevalence based on the data presented in each table.

The method used for developing a combined cumulative distribution for the herd prevalence was by using weighted average of the cumulative percentiles (Vose, 2008). In this case all studies are given equal weighting. The method is briefly outlined below:

- For each study the number of detections (positives) and the total number of samples are found
- The parameters of a beta distribution, Beta(α_1, α_2) with parameters, $\alpha_1 = s + 1$ and $\alpha_2 = n - s + 1$ (assuming a uniform prior) are calculated
- In the statistical program, R (www.r-project.org) the cumulative percentiles for the corresponding Beta distribution is determined using the `pbeta` command
- The cumulative distributions for each Beta distribution are then average to give an overall cumulative distribution
- The resulting distribution is entered into @Risk as an cumulative distribution with a lower limit of zero, and an upper limit judged on the cumulative distribution values

Campylobacter spp.

Author	Reference	Country	Samples	Positive	Prevalence
(Bae *et al*., 2005)	1	USA	311	97	0.312
(Bailey *et al*., 2003)	2	Australia	150	64	0.427
(Beumer *et al*., 1988)	3	Netherlands	904	41	0.045
(Dodson and LeJeune, 2005)	4	USA	686	48	0.07
(Doyle and Roman, 1982)	5	USA	78	50	0.641
(Hakkinen *et al*., 2007)	6	Finland	952	186	0.195
(Harvey *et al*., 2004)	7	USA	720	20	0.028
(Murinda *et al*., 2004)	8	USA	411	5	0.012
(Oosterom *et al*., 1982)	9	Netherlands	200	11	0.055
(Sato *et al*., 2004)	10	USA	1191	234	0.196
(Wesley *et al*., 2000)	11	USA	2085	786	0.377

E. coli (EHEC)

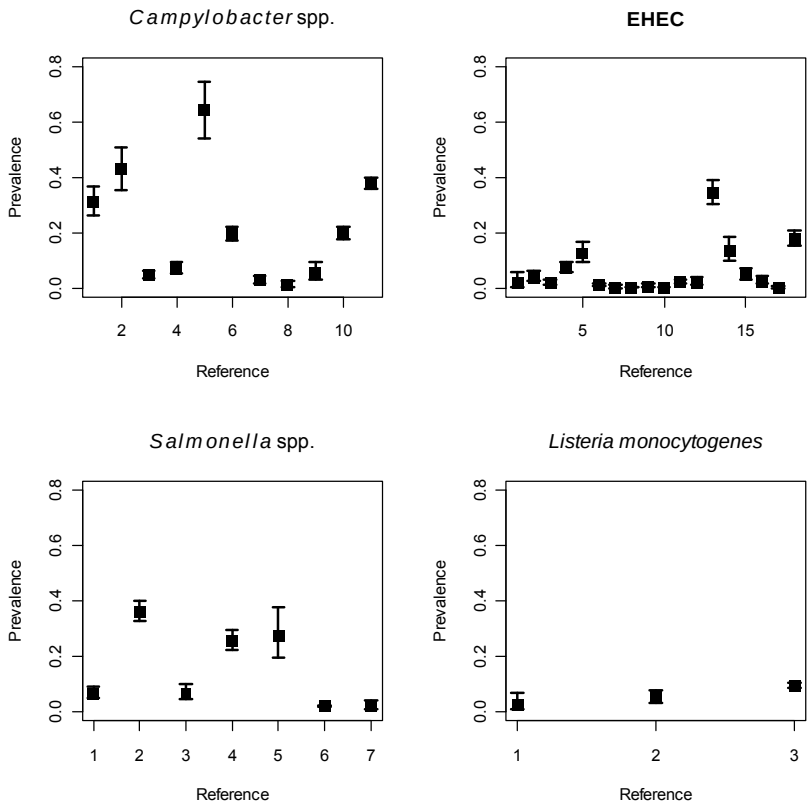
Author	Reference	Country	Samples	Positive	Prevalence
(Cobbold and Desmarchelier, 2000)	1	Australia	120	2	0.017
(Doane *et al*., 2007)	2	USA	408	16	0.039
(Dodson and LeJeune, 2005)	3	USA	1026	21	0.02
(Edrington *et al*., 2004)	4	USA	720	52	0.072
(Fegan *et al*., 2004c)	5	Australia	310	39	0.126
(Garber *et al*., 1999)	6	USA	4361	52	0.012
(Hallaran and Sumner, 2001)	7	Australia	505	1	0.002
(Hancock *et al*., 1994)	8	USA	3570	10	0.003
(Lejeune *et al*., 2006)	9	USA	750	5	0.007
(Lejeune *et al*., 2006)	10	Norway	680	0	0
(Mechie *et al*., 1997)	11	UK	2786	63	0.023
(Murinda *et al*., 2002b)	12	USA	415	8	0.019
(Pradel *et al*., 2000)	13	France	471	162	0.344
(Renter *et al*., 2005)	14	USA	246	33	0.134
(Rice *et al*., 1997)	15	USA	397	18	0.045
(Rugbjerg *et al*., 2003)	16	Denmark	560	14	0.025
(Wells *et al*., 1991)	17	USA	662	1	0.002
(Zschock *et al*., 2000)	18	Germany	726	131	0.18

Salmonella spp.

Author	Reference	Country	Samples	Positive	Prevalence
(Dodson and LeJeune, 2005)	1	USA	580	39	0.067
(Edrington *et al*., 2004)	2	USA	720	262	0.364
(Fegan *et al*., 2004b)	3	Australia	310	21	0.068
(Fegan *et al*., 2005b)	4	Australia	606	157	0.259
(Kabagambe *et al*., 2000)	5	USA	91	25	0.275
(Losinger *et al*., 1995)	6	USA	6861	145	0.021
(Murinda *et al*., 2002a)	7	USA	415	9	0.022

L. monocytogenes

Author	Reference	Country	Samples	Positive	Prevalence
(Bailey *et al*., 2003)	1	Australia	150	4	0.027
(Fedio and Jackson, 1992)	2	Canada	401	21	0.052
(Husu, 1990)	3	Finland	3878	373	0.096



With-in herd prevalence of *Campylobacter* spp., EHEC, *Salmonella* spp. and *L. monocytogenes* in dairy cows. Error bars represent the 95% confidence intervals. Results highlighted with an asterisk (*) indicate that the prevalence is significantly different from the WA unpublished (2007) raw milk data. Details of each numbered reference can be found in the corresponding table in this appendix.

Annex 7: Growth and inactivation rates equations

Enterohaemorragic *Escherichia coli* (EHEC)

The growth rate of EHEC is considered to be identical as that of non-pathogenic *E. coli* based on the experimental results from Salter (1998). Ross *et al*. (2003) proposed an extension of the Square-root model to include factors such as temperature, water activity(a_w), pH and lactic acid concentration [LAC] on the relative growth rate, μ (Equation 1):

$$\sqrt{\mu} = c(T - T_{min}) \cdot \left(1 - exp\left(d(T - T_{min})\right)\right) \cdot \sqrt{a_w - a_{w,min}} \cdot \sqrt{1 - 10^{(pH_{min} - pH)}} \cdot \sqrt{1 - 10^{(pH - pH_{max})}} \cdot \sqrt{1 - [LAC]/\left(U_{min}\left(1 + 10^{(pH - pK_a)}\right)\right)} \cdot \sqrt{1 - [LAC]/\left(D_{min}\left(1 + 10^{(pK_a - pH)}\right)\right)} + error$$

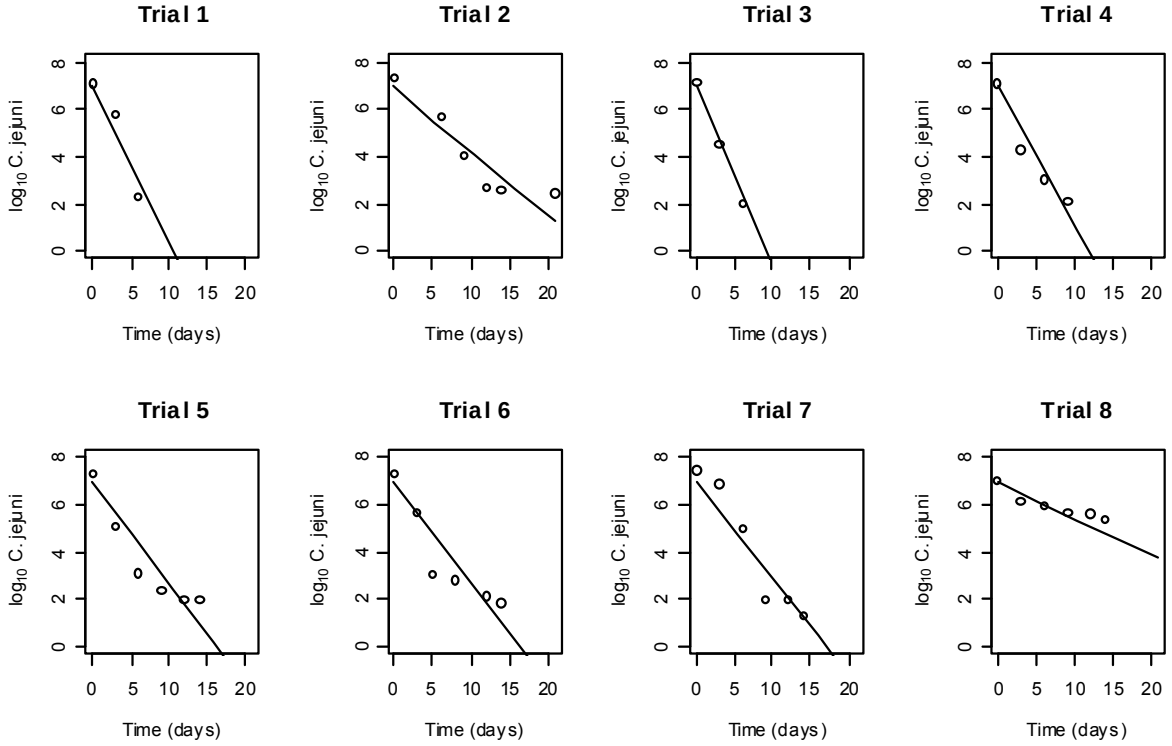
Equation 1

Table 1: Growth model equation parameters for *E. coli* (Ross *et al.*, 2003)

Parameter	Value	Parameter	Value
c	0.2345	U_{min}	10.43
T_{min}	4.14	D_{min}	995.508
T_{max}	49.55	a_{wmin}	0.9508
pH_{min}	3.909	d	0.2636
pH_{max}	8.86	pK_a	3.86

Campylobacter spp.

Campylobacter spp. including *C. jejuni* are microaerotolerant and are not capable of growth in raw milk. Doyle and Roman (1982) performed experimental studies into the survival of eight different strains of *C. jejuni* in unpasteurised milk stored at 4 °C. Initial inoculum levels of $>10^7$cfu/ml were used. Results of this study showed that there are considerable differences in survival between strains of *C. Jejuni*. Some strains were not detected after 9 days (6 log decline), while the concentrations of other strains declined by only 1 log in the same time. A graphical summary of the experimental trials is presented below:



In order to capture the between-strain variability a non-linear mixed effects model was fitted to the experimental data:

$$log_{10}N \sim (\beta_0 + a_i) - exp(\beta_1 + b_i)\ time$$

where β_0 is the common intercept, β_1 is a common slope and a_i and b_i are the random effects for the intercept and slope, respectively. The slope parameter, β_1 is the logarithm of the first-order inactivation rate.

Parameter	Value (Standard error)
β_0	6.898379 (0.2101275)
β_1	-0.878915 (0.1985563)
Standard deviation of a_i	3.575302×10^{-5}
Standard deviation of b_i	0.5040929

The first-order rate constant for the inactivation of *Campylobacter* spp. in raw milk was coded into @Risk as: EXP(RiskNormal(-0.8789,0.5041)), using the expected value of β_1 and the standard deviation of the random effect b_i.

***Salmonella* spp.**

The growth rate model for *Salmonella* spp. is based on the mixed cocktail study by Gibson *et al*. (1988) in broth culture. The original growth curve data was obtained from the Combase predictive microbiology database (www.combase.cc). A modified form the Baranyi model: was fitted to the experimental growth curves using the statistical program R Version 2.6.2 (R Development Core Team, 2007) to determine the maximum specific growth rate, μ_m. A quadratic surface model including temperature, pH and added salt concentration was fitted to the specific growth rate data:

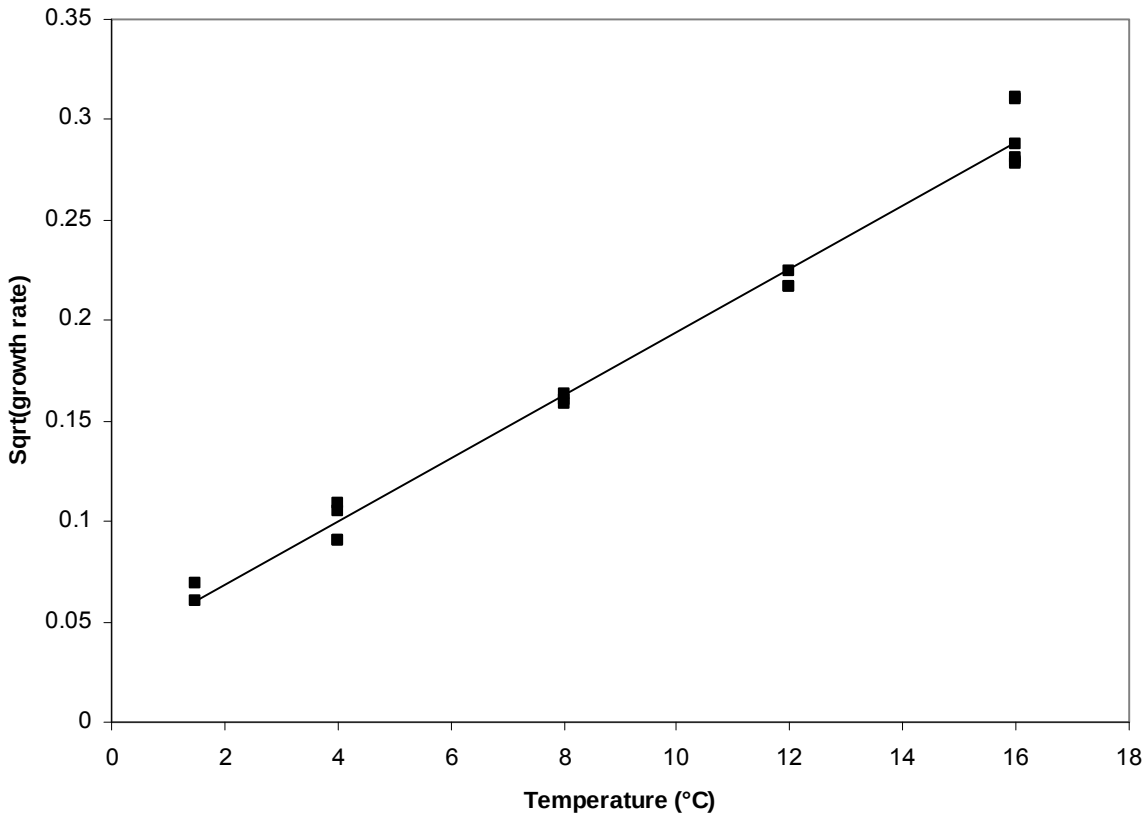
$$ln\ \mu_m = -10.24 + 0.5587T - 0.006397T^2 + 0.6105pH + 0.1178NaCl - 0.0489NaCl^2 - 0.02308T \cdot pH$$

Adjusted R^2 = 0.97, Residual standard error = 0.1915 on 58 degrees of freedom

Listeria monocytogenes

The growth rate for *L. monocytogenes* was derived from data presented in Xanthiakos *et al*. (2006). As there is only sub-optimal temperature data the Square root model was used to predict the effect of temperature of growth rates:

$$\sqrt{\mu_m} = 0.0549 + 0.0239T$$



ANNEX 8: Risk management questions

1. What are the risks to public health and safety posed by the consumption, in Australia, of raw cow milk?

2. What are the factors that would have the greatest impact on public health and safety along the production chain for raw cow milk for direct consumption?

Specific questions posed by the project team in relation to raw cow milk for human consumption are:

1) What are the microbial hazards of public health significance in raw milk? Are there hazards specific to particular species, *i.e.* cow milk?
2) What are the prevalence and levels of identified hazards in raw cow milk?
3) Do these levels pose a risk if the raw cow milk is used for direct consumption?
4) What are the factors during primary production that impact on the level of these hazards?
5) What practices/controls have the greatest impact on the level of hazard?
6) What is the impact of retail and consumer handling on the level of risk to public health and safety on these hazards?

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