

Microbiological Risk Assessment of Raw Goat Milk



Risk Assessment Microbiology Section
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Abbreviations

AIDS	Acquired immune deficiency syndrome
CDC	Centres for Disease Control and Prevention
CDT	Cytolethal distending toxin
CJT	<i>Campylobacter jejuni</i> toxin
Codex	Codex Alimentarius Commission
EAEC	Enteraggregative <i>E. Coli</i>
EHEC	Enterohaemorrhagic <i>E. Coli</i>
EIEC	Eenteroinvasive <i>E. Coli</i>
EPEC	Enteropathogenic <i>E. coli</i> (EPEC)
ETEC	Enterotoxigenic <i>E. Coli</i>
FAO	Food and Agriculture Organization of the United Nations
FDA	United States Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service
HACCP	Hazard analysis critical control point
HTST	High-temperature-short-time
HUS	Haemolytic ureamic syndrome
ICMSF	International Commission on Microbiological Specifications for Foods
MAP	<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i>
MMWR	Morbidity and Mortality Weekly Report
NEPPS	National Enteric Pathogen Surveillance Scheme
NSW	New South Wales
OIE	World Organisation for animal health
pers. comm.	Personal communication
PCR	Polymerase chain reaction
QDPI	Queensland Department of Primary Industries
SCC	Somatic cell count
SPC	Standard plate count
STEC	Shiga toxin-producing <i>E. coli</i>
The Profile	A Risk Profile of Dairy Products in Australia
WHO	World Health Organization

1 Executive summary

The risk assessment of raw goat milk describes information on microbiological risks which may be associated with raw goat milk. The purpose of the risk assessment is to provide an objective interpretation of the available scientific data on the public health risks associated with the consumption of raw goat milk. The risk assessment was undertaken within the existing framework of Australian raw goat milk regulations and risk management practices where they exist, and will support the development of regulatory and/or non-regulatory risk management measures as appropriate

The risk assessment was undertaken to address the following overarching questions:

- What are the risks to public health and safety posed by the consumption, in Australia, of raw goat milk?
- What are the factors that would have the greatest impact on public health and safety along the production chain for raw goat milk for direct consumption?

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

- A range of pathogenic microorganisms may contaminate raw goat milk
- Enterohaemorrhagic *Escherichia coli* poses a high risk to the general population
- Enterohaemorrhagic *E. coli*, *Toxoplasma gondii* and *Listeria monocytogenes* pose a high risk and *Salmonella* spp. pose a moderate risk to susceptible populations
- The key risk factors during primary production and processing affecting the microbiological status of raw goat milk are:
 - Disease status of the animal
 - External contamination from the farm and processing environment
- The relative contribution of each risk factor to the overall risk to public health and safety will differ for each pathogen

Raw goat milk has a mixed microflora which is not dissimilar to that found in raw cow milk, with the microbial diversity the result of multiple factors. However, there is little published information available on the incidence and prevalence of pathogens in raw goat milk in Australia.

Where pathogens have been detected in raw goat milk in Australia, they are similar to those reported internationally and reflect those generally found in cow milk. Organisms include *Staphylococcus aureus*, *Campylobacter* spp., *E. coli*, *Salmonella* spp., *Streptococcus* spp., *Bacillus cereus*, *L. monocytogenes* and *Yersinia enterocolitica*. *Brucella* spp. have been reported internationally but have not been reported in Australia. *Coxiella burnetti* and *Mycobacterium avium* subsp. *paratuberculosis* have also been reported internationally in raw milk although foodborne transmission of these agents is the subject of ongoing debate.

The available microbiological data shows a very low level of hazards of public health significance in Australian raw goat milk, however the level and frequency of testing is limited. It is however important to note that shiga-like toxin producing *E. coli* was detected in raw goat milk destined for retail sale during routine sampling in Western Australia. The low level of reported foodborne illness associated with raw goat milk may give the impression this is a safe product, although this may simply reflect the generally low level of consumption in Australia and the overall under-reporting of foodborne illness. The impression of safety must

however, be balanced against the possibly high proportion of the consumers who are within a susceptible population group.

There have been nineteen outbreaks of illness associated with the consumption of raw goat milk reported internationally. This epidemiological evidence supports the finding that the consumption of raw goat milk poses a risk to public health and safety with the degree of risk dependant on various animal production and milk processing factors.

Production of raw goat milk in Australia uses systems and practices similar to the cow dairy industry and sources of microbial contamination are similar. Similarly to cow milk, raw goat milk may be contaminated by two primary means: pathogens shed directly into the milk via the udder, or through external (or environmental) contamination during milk collection or during post harvest handling and storage.

The health and welfare of the goat has a direct impact on the microbiological quality of raw goat milk. Mastitis (contagious and environmental) and other infections or illnesses result in increased levels and diversity of pathogenic microorganisms being shed directly into the raw milk through the udder. Mastitic and ill goats may also experience increased faecal shedding of pathogens which increases the risk of contamination from the environment. Infected animals with no outward signs of disease (asymptomatic carriers) may harbour and shed pathogens either continuously or intermittently into milk, urine and faeces over undefined periods of time.

Environmental contamination of the raw goat milk may occur from a variety of sources including the farm environment *e.g.* housing, feed, water, etc, and the processing environment *e.g.* milking equipment/practices, personnel, cleaning and packaging etc.

The key risk factors affecting the microbiological status of raw goat milk during primary production and processing are summarised in the following table:

Risk factor	Impact on milk safety	Mitigation strategies
Disease	Diseased goats will show increased shedding of pathogens into raw milk or faeces. Infected animals with no signs of disease (carriers) may carry and shed pathogens, continuously or 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 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 ¹ . 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, hairy udders, 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.

¹ Prolonged diarrhoea in animals

Risk factor	Impact on milk safety	Mitigation strategies
Storage	Inappropriate temperature control of raw goat milk after milking can lead to growth of pathogens.	Rapid cooling and holding of milk.
Packaging/ Delivery	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.

The relative contribution that each of these risk factors has on the overall risk will differ for each pathogen and could not be determined without quantitative through chain data. However it should be highlighted that the goat itself is the primary source of contamination on-farm.

Raw goat milk does not undergo any pathogen elimination or reduction step. The safety of raw goat milk is therefore primarily dependent upon the control of risk factors on-farm to minimize the opportunity for microbiological hazards to contaminate raw milk. If raw milk does become contaminated, failure to maintain appropriate temperature control throughout storage, distribution and consumer handling may allow the growth of pathogens and increase risk. Other dairy products are rendered safe principally through the pasteurisation process.

FSANZ employed a qualitative framework based on Codex principles to assess the risk from foodborne hazards associated with the consumption of raw goat milk by Australian consumers. Using the qualitative framework, the principal microbiological risks to public health and safety from the consumption of raw goat milk are:

Organism	Risk rating (Total population unless otherwise stated)
<i>Bacillus cereus</i>	Low
<i>Burkholderia pseudomallei</i>	Negligible (general population) Very Low (susceptible population)
<i>Brucella melitensis</i> *	Moderate (if introduced to Australia)
<i>Campylobacter jejuni/coli</i>	Low
<i>Clostridium perfringens</i>	Low
<i>Cryptosporidium parvum</i>	Low
Enterohaemorrhagic <i>Escherichia coli</i>	High
<i>Leptospira interrogans</i>	Negligible
<i>Listeria monocytogenes</i>	Very low (general population) High (susceptible population)
<i>Salmonella</i> spp.	Low (general population) Moderate (susceptible population)
<i>Staphylococcus aureus</i>	Low
<i>Streptococcus</i> spp.	Low
<i>Toxoplasma gondii</i>	Low (general population) High (susceptible population)
<i>Yersinia enterocolitica</i>	Very low
<i>Yersinia pseudotuberculosis</i>	Very low

* Currently exotic to Australia. Risk rating applies if introduced into Australia either through imported raw goat milk product or its introduction into domestic herds.

Various data gaps were identified during the course of this risk assessment including:

- The prevalence and concentration of pathogens in the domestic raw goat milk supply
- The virulence and infectivity of some pathogens
- The frequency and amount of consumption
- The demographics of the consuming population

Further research in these areas may assist to more accurately estimate the impact and magnitude of any illness resulting from consumption of raw goat milk in Australia, as well as to assess the impact of any control measures put in place.

While the consumption of raw goat milk is considered to be very low among the general population, there is a group of consumers who have very strong beliefs in the health benefits attributed to raw goat milk and subsequently choose this as their milk of choice. Unpublished research conducted in New South Wales indicated that raw goat milk was marketed with a “health food image”, with purchasers of the product including people with serious illnesses such as cancer patients and mothers intending to feed the product to their infants (AgriQ, 2000). Similarly, work undertaken during the South Australian raw goat milk risk assessment identified consumers as including those with allergies to cow milk and children with digestive problems.

This suggests that a higher than normal proportion of raw goat milk consumers may have lowered or less developed immunity and may therefore be more susceptible to foodborne pathogens than the general population.

While the volume of raw goat milk consumed in Australia is very low there are risks for both general and susceptible populations consuming this product. Raw goat milk is frequently provided to members of the population who are more susceptible to infection by *L. monocytogenes*, enterohaemorrhagic *E. coli* and *Salmonella* spp. Raw goat milk is often provided to very young children, children with special dietary needs, older people and people convalescing. These sub-populations are at-risk, and exposure to even low levels of these microbial pathogens may result in serious illness.

2 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 has previously been conducted by FSANZ entitled *A Risk Profile of Dairy Products in Australia* (the Profile) (FSANZ, 2006).².

A key finding of the Profile was that Australian dairy products have an excellent reputation for food safety. This is because dairy products in Australia are pasteurised, and pasteurisation represents the principal process for rendering dairy products safe for consumption. This finding was supported by a lack of evidence attributing foodborne illness to dairy products. The Profile confirmed that unpasteurised dairy products are the most common cause of dairy associated foodborne illness. However, the Profile did not specifically examine the risks to public health and safety from the consumption of raw goat milk.

This document seeks to assess the risks to public health and safety resulting from consumption of raw goat milk. It utilises available scientific data and addresses the uncertainty and variability in the conclusions drawn from the data *e.g.* consideration of the relevance and quality of data and the veracity of its source.

The output of this risk assessment provides an estimate of risk following the consumption of raw goat milk in Australia. It also identifies hazard control measures along the production chain that have the greatest impact on minimising risk, thereby informing risk managers where intervention will be most effective. The outputs of the assessment will be used by FSANZ to develop regulatory and/or non-regulatory measures as appropriate.

²

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

3 Purpose and scope

The purpose of this microbiological risk assessment is to provide an objective analysis of available scientific data and information to identify the public health and safety risks associated with the consumption of raw goat milk, and to identify the factors along the production chain that have the greatest impact on public health and safety for the consumption of raw goat milk.

The assessment of the public health and safety risks posed by consumption of raw goat milk in Australia was undertaken to address the following overarching questions:

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

Specific questions in relation to raw goat milk for human consumption are:

- What are the microbial hazards of public health significance in raw goat milk? What are the prevalence and levels of identified hazards in raw goat milk?
- Do these levels pose a risk if the raw goat milk is directly consumed?
- What are the factors during primary production that impact on the level of these hazards? What practices/controls have the greatest impact on the level of hazard?
- What is the impact of retail and consumer handling on the level of risk to public health and safety on these hazards?

3.1 Definition of raw milk

The Codex definition of **raw milk**³ is “milk⁴ which has not been heated beyond 40°C or undergone any treatment that has an equivalent effect”.

The European Union Directives define raw milk as “milk produced by secretion of the mammary glands of one or more cows, ewes, goats, or buffaloes from a single holding that has not been heated beyond 40°C or undergone any treatment having a similar effect”.

The Food Standards Code⁵ specifies processing temperatures for pasteurisation and thermisation in relation to milk and therefore “raw milk” for the purposes of this assessment, is defined as “milk which has not been heat treated in accordance with the Food Standards Code”.

³ Code of Hygienic Practice for Milk and Milk Products (CAC/RCP 57-2004)

⁴ Defined in Codex General Standard for the Use of Dairy Terms (CODEX STAN 206-1999)

⁵ The Australia New Zealand Food Standards Code - Standard 1.6.2 – *Processing Requirements*

3.2 Approach

The risk assessment qualitatively identifies hazards, epidemiological data and other information to determine whether these hazards have presented, or are likely to present a public health risk, and to identify where in the raw goat milk supply chain these hazards may be introduced. Animal health issues were considered only in the context of those that differ from cow milk production and which specifically impact upon human health via foodborne transmission. The assessment draws upon the Risk Profile of Dairy Products in Australia (FSANZ, 2006) and utilises available information including current scientific and epidemiological data, surveillance data from enforcement agencies and existing published and unpublished Australian risk assessments on the safety of raw goat milk.

The Codex Alimentarius Commission, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have established an internationally recognised framework for undertaking a microbiological risk assessment⁶. The risk assessment process used by FSANZ is consistent with international protocols and involves four distinct steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation.

There is no internationally agreed framework for undertaking a qualitative risk assessment for microbiological hazards. Codex⁷ and FSANZ⁸ have guidelines for conducting microbiological risk assessments but they do not provide actual tools that can be used to objectively assess or rank the risk to public health and safety. In the absence of an internationally agreed method to qualitatively assess the risk of foodborne hazards associated with the consumption of raw goat milk, FSANZ has used a tool developed by Food Science Australia (Appendix 5) for the assessment of microbiological hazards in a raw milk cheese⁹. The approach utilises a qualitative framework based on Codex principles and employs elements of Risk Ranger (Ross and Sumner, 2002), a widely accepted semi-quantitative tool.

3.2.1 A Risk Profile of Dairy Products in Australia

The Profile was undertaken within the framework of existing management and regulations in Australia. It identified and examined hazards along the entire dairy supply chain from milk production through to consumption of dairy products and considered relevant inputs *e.g.* feed, water, etc along the dairy primary production and processing chain.

The primary focus of the Profile was the production of cow milk, however, the report also incorporated information on milk from non-bovine species. Information identified as being relevant to the dairy goat industry has been utilised in this assessment.

⁶ Risk assessment is a scientific process undertaken to characterise the risk to public health and safety posed by foodborne hazards associated with a food commodity. Codex Alimentarius Commission (1999). Principles and guidelines for the conduct of microbiological risk assessment. ALINORM, 99/13, Appendix IV, pp. 58-64

⁷ CODEX (CAC/GL 30, 1999) Principles and Guidelines for the Conduct of Microbiological Risk Assessment http://www.codexalimentarius.net/download/standards/357/CXG_030e.pdf

⁸ FSANZ (2009) The Analysis of Food-Related Health Risks. http://www.foodstandards.gov.au/_srcfiles/Food%20Related%20Health%20Risks%20WEB_FA.pdf

⁹ Application A499 To permit the sale of Roquefort cheese http://www.foodstandards.gov.au/_srcfiles/A499_Roquefort_FAR_FINALv2.pdf#search=%22A499%22

3.2.2 *Australian Risk Assessments*

In undertaking the scientific assessment FSANZ has, with permission, drawn upon the findings of unpublished risk assessments conducted for New South Wales (AgriQ, 2002), South Australia (Pointon *et al.*, 2004) and Queensland (QDPI, 2004).

3.2.3 *Qualitative framework*

The qualitative framework considers the characteristics of identified hazards (hazard identification and characterisation) and an assessment of the likely exposure to these hazards (exposure assessment) to arrive at a final estimate of risk (risk characterisation).

The hazard characterisation module categorises each identified hazard based on the probability of disease (infective dose) and the severity of the disease. The exposure module considers the likelihood of the hazard being present in the raw product and the effect of processing on the hazard. This assumes no change in the hazard over time in the product. The risk characterisation combines the hazard characterisation and exposure modules to give an overall categorisation of risk. Essentially the framework categorises the risk for each hazard by combining information about the hazard (severity and infective dose) with exposure information (prevalence in raw materials and effect of processing).

A detailed example of the risk characterisation for EHEC in the general population is given in Appendix 5. Briefly, the hazard characterisation for EHEC is high due to the low infective dose (conservatively estimated to be <10 organisms) and serious consequence of exposure in the general population. The exposure assessment was rated as low due to the infrequent product contamination combined with no effect of processing. Combining the hazard characterisation and exposure assessment results gives EHEC in raw goat milk a risk characterisation of high for the general population.

Assumptions used for assigning risk categories for all hazards under consideration for both the general and susceptible population groups are given in Appendix 6. Information used to derive these assumptions included scientific data, published literature, professional judgement and expert elicitation.

Susceptible populations have been described as individuals who may be more susceptible to infection from specific microbiological hazards due to an impaired immune system and includes the very young and old, the immunocompromised and pregnant women and their unborn children. This assessment uses the term susceptible populations to include all susceptible individuals.

4 Australian risk assessments

In recent years, authorities in South Australia, Queensland and New South Wales have commissioned risk assessments of the raw goat milk industry. Some differences exist between organisms considered in each state's risk assessment, e.g. *Burkholderia pseudomallei* is an organism limited to tropical regions of Australia such as Queensland and the Northern Territory and was only considered in the Queensland and New South Wales risk assessments. Details of each risk assessment are included in Appendix 7.

The South Australian study was undertaken following a risk profile of the primary industry sector and aimed to identify appropriate food safety risk management options, both policy and regulatory for the dairy goat milk industry. The study adopted a qualitative risk ranking approach, based on International Commission on Microbiological Specifications for Foods principles and considered hazard severity; occurrence of the hazard in foods; potential for growth; effects of production, processing and handling (including a consumer terminal step); and epidemiological data.

Findings from the South Australian study identified *Cryptosporidium parvum*, Enterohaemorrhagic *Escherichia coli* (EHEC), *Listeria monocytogenes*, *Salmonella* spp. and *Toxoplasma gondii* as high risk for susceptible populations, whilst *Campylobacter jejuni/coli*, *Salmonella* and EHEC were rated as medium risk for the general population. *C. parvum*, *L. monocytogenes*, *Staphylococcus aureus* and *T. gondii* were all rated low risk for the general population.

The risk assessment undertaken in Queensland utilised methodology based on Codex Alimentarius Commission principles (CAC/GL-30 1999) to rank food safety hazards identified during an extensive literature search. A semi-quantitative approach assigned risk scores (maximum of 100) to hazards and determined total assessed risk scores for each of four population segments based on exposure and severity of consequence.

Queensland's risk assessment concluded that for the general population where goat milk is typically not consumed, there was an overall low risk. *E. coli* O157:H7 and other pathogenic *E. coli* were a medium risk to babies and infants, and *L. monocytogenes* was a medium risk to babies/infants and the immunocompromised. For the niche market where raw goat milk is consumed as the milk of choice, there was an overall increase in risk compared to the normal market population. *S. aureus* toxins and *B. pseudomallei* were considered a medium risk to all populations segments, while *E. coli* O157:H7 was a medium risk to the general population and immunocompromised and a high risk to babies/infants. *L. monocytogenes*, while considered a low risk to the general population, was a high risk to both babies/infants and the immunocompromised.

The New South Wales (NSW) risk assessment was conducted as two separate parts: a qualitative analysis of risk for each hazard identified in a previous hazard analysis, and a stochastic semi-quantitative model (Excel-@Risk) to scope the public health significance of *Salmonella* spp., *S. aureus* and *L. monocytogenes*.

The NSW risk assessment declined to make any determination, qualitative or quantitative, on the risks associated with microbial hazards identified in the hazard analysis.

Modelling of the public health significance for *Salmonella* spp., *S. aureus* and *L. monocytogenes* indicates that a single contamination event resulting from contamination and subsequent abuse of the product could lead to severe public health consequences.

5 Goat dairy farming in Australia

The main breeds of dairy goats in Australia are the three Swiss breeds (Saanen, British Alpine and Toggenburg), crosses of the Swiss breeds, Anglo-Nubians and crosses of the Swiss breeds with Anglo-Nubians. The Saanen generally produces a greater volume of milk over a longer lactation period than the other dairy goat breeds. Toggenburgs are the second highest producers, with British Alpine and then Anglo-Nubians next in line. The Anglo-Nubians' milk has the highest percentage of butterfat, which is coveted by cheese makers.

Typical lactations last for 300 days or greater and herd production ranges from 2 - 3 litres of milk per doe per day. At the peak of lactation, average production may reach 3.5 - 4 litres of milk per doe per day, with a good doe producing milk for ten years (McGregor, 1997).

The dairy goat industry in Australia has expanded in recent years driven primarily by specialty cheese production (Appendix 1). There are an estimated 65 commercial dairy goat farms in Australia carrying almost 11,000 goats, producing approximately 5.4 million litres of goat milk annually. Currently it is estimated that only 300,000 litres is sold as raw goat milk.

Four states currently permit the sale of raw goat milk: NSW, South Australia, Western Australia and Queensland.

In NSW, raw goat milk is regulated by the NSW Food Authority using Regulations under the Food Production (Safety) Act 1998. In 2000, there were 17 hobby/small permit holding farms and an estimated 50 unlicensed dairy goat units (AgriQ, 2000).

South Australia has experienced rapid development in the dairy goat industry, with five commercial goat milk producers licensed by the Dairy Authority of South Australia in 2004. Three farmers sold raw goat milk direct to the public, and operated under the Authority's Code of Practice for Dairy Food Safety and the Guidelines for Raw or Unpasteurised Goat Milk (Dairy Authority of South Australia, 2005). Recently the number of accredited (previously licensed) goat milk producers selling raw milk has decreased to a single supplier.

In 2004 there were three operating and licensed raw goat milk dairies in Queensland. These operations comprised approximately 700 goats, including 300 milking animals (QDPI, 2004). Safe Food Queensland regulates raw goat milk under the Food Production (Safety) Act 2000, with Part 3 of the Food Production (Safety) Regulation 2002 stating requirements for production, testing and labelling.

At the time of writing, Western Australia had an estimated three unlicensed goat dairies supplying raw goat milk to the public. The Health Department of Western Australia regulates dairy products under the Health Act 1911 and Health (Food Hygiene) Regulations 1993 with regulations specific for raw goat milk defined under Part VIIA, Division 4 of the Health Act. Codes of Practice for the Goat Dairy Industry outline requirements for Building and Facilities (Part 1) and Hygiene (Part 2). These Codes of Practice are currently not enforceable under any legislation but are used by local government enforcement officers who may be required to approve a goat dairy if a development application is received. Regulations applicable to the production of raw goat milk are contained within Appendix 8. State testing specifications for raw goat milk are outlined in Appendix 9.

6 Consumption of raw goat milk in Australia

Food consumption data can be derived from total production statistics or food consumption surveys. Food production statistics provide an estimate of the amount of a specific food commodity that is available to the total population. Consumption surveys (such as national nutrition surveys, independent single source surveys, etc) provide detailed information on the types and amount of food consumed by individuals or households and sometimes the frequency with which these foods are consumed.

6.1 Goat milk production statistics

Total sales of goat milk in Australia are steadily increasing with the majority of supermarket sales occurring in NSW, followed by Queensland, Victoria, Western Australia and South Australia. The majority of the raw goat milk sold in Australia is distributed through health food shops or farm gate sales.

Accurate information on the volumes of goat milk and in particular raw goat milk produced and sold in Australia is difficult to obtain. The most recent production information indicates around 5.4 million litres of goat milk were produced in Australia during 2003/04. It is estimated that approximately 300,000 litres of raw goat milk was marketed in Australia during 2003 (Abud, 2005). However, observations by industry organisations suggest that the amount of raw goat milk entering the market from unlicensed sources could be double the estimated volume from licensed premises (pers. comm. Riches, 2006).

NSW markets around two-thirds of its whole goat milk production as raw milk (approximately 270,000 litres). South Australia had an estimated 32,000 litres of raw milk sales in 2003 (Pointon *et al.*, 2004), however this is expected to decrease in the future as a consequence of a reduction in the number of accredited producers from three to one. Queensland and Western Australia are responsible for small volumes of raw goat milk although no actual figures could be obtained.

6.2 Consumption of raw goat milk

Data from the Australian National Nutrition Survey provides information regarding the types and amounts of dairy foods consumed by Australians. The most recent national survey was conducted during the period February 1995 to March 1996 using the 24-hour recall method. Approximately 13,800 people aged two years or over from urban and rural areas in all States and Territories participated in the survey.

Only 0.08% (11/12,858 respondents) consumed goat milk, with an average consumption of 248 grams per day. Of the eleven people consuming goat milk, one was a child aged 2 - 3 years and two were females aged 65+ years. The data did not permit differentiation between pasteurised or raw goat milk, as there was no specific information available on the consumption of raw goat milk. The very low numbers reported in this survey for consumption of goat milk does not enable an accurate determination of population consumption patterns to be made.

In a recent consumer survey in Australia, less than 1% (7/1000) of those surveyed either consumed or knew of consumers of raw goat milk (Colmar Brunton Social Research, 2008, unpublished). Pointon *et al.* (2004) notes that people owning their own goats or people who

have an allergy to cow milk are the primary consumers of goat milk. There is also a group of consumers who have very strong beliefs regarding the health benefits attributed to raw goat milk and subsequently choose this as their milk of choice.

Unpublished research conducted in NSW indicated that raw goat milk was marketed with a “health food image”, with purchasers of the product including people with serious illnesses such as cancer patients and mothers intending to feed the product to their infants (AgriQ, 2002). Goat milk is widely promoted throughout the industry as an infant milk replacement, particularly where infants are intolerant to cow milk (AgriQ, 2000). The population drinking goat milk is therefore assumed to include a high number of children (Pointon *et al.*, 2004).

The consumption of raw goat milk is a contentious issue with the niche market of consumers of this product having strong beliefs in its health benefits. There is also a prevalent belief among its consumers that the product has properties that limit the survival of human pathogens. In many cases these consumers have an equally strong opposition to the commercial processing of foods, in particular pasteurisation and homogenisation (QDPI, 2004).

7 Microbiological hazards associated with raw goat milk

The microbial status of raw goat milk is influenced by numerous factors, with pathogens introduced at various stages along the primary production and processing chain.

Raw goat milk has a mixed microflora that is derived from several sources including the interior of the udder, exterior surfaces of the goat, the environment, milk-handling equipment and personnel. Additionally, the milking procedure, subsequent packaging, storage and delivery of raw milk also carry the risk of further contamination or growth of intrinsic pathogens. Raw goat milk does not undergo any pathogen elimination or reduction step, therefore any pathogenic contamination, regardless of origin, may pose a risk to public health and safety.

A broad range of microbiological hazards were identified from previous risk assessments conducted by NSW, South Australia and Queensland. These microorganisms are representative of those that may be present in raw goat milk, either directly transmitted via the mammary gland or via faecal and environmental contamination. Also considered were microorganisms originating from the milking environment and post-milking contamination.

Table 1 provides a brief summary of the microbiological hazards that may be encountered in raw goat milk and outlines possible routes of contamination and the availability of epidemiological data.

Table 1: Summary of key microbiological hazards associated with raw goat milk

Organism	Shed directly in milk [#]	Contaminant of raw milk ^{##}	Survives pasteurisation	Severity of illness [§]	Implicated in foodborne illness (Reported)
<i>Bacillus cereus</i>	No	Yes	Yes	Mild	++
<i>Brucella melitensis</i>	Yes	Yes	No	Serious	++
<i>Burkholderia pseudomallei</i>	Yes	Yes	-	Severe [^]	++*
<i>Campylobacter jejuni/coli</i>	Yes	Yes	No	Serious [^]	++
<i>Clostridium perfringens</i>	No	Yes	Yes	Mild	+
<i>Coxiella burnetii</i>	Yes	Yes	No	Serious [^]	+
<i>Cryptosporidium parvum</i>	No	Yes	No	Serious [^]	+
Enterohaemorrhagic <i>E. coli</i>	Yes	Yes	No	Serious	++
<i>Leptospira interrogans</i>	Yes	Yes	No	Moderate	-
<i>Listeria monocytogenes</i>	Yes	Yes	No	Severe [^]	++
<i>Mycobacterium avium subs. paratuberculosis</i>	Yes	Yes	No	Moderate [^]	-
<i>Salmonella</i> spp.	No	Yes	No	Serious [^]	++
<i>Staphylococcus aureus</i>	Yes	Yes	No**	Mild	++
<i>Streptococcus</i> spp.	Yes	Yes	No	Mild	+
<i>Toxoplasma gondii</i>	Yes	Yes	Yes	Serious [^]	++
<i>Yersinia enterocolitica</i>	No	Yes	No	Moderate [^]	+
<i>Yersinia pseudotuberculosis</i>	Yes	Yes	-	Moderate [^]	-

[#] Transmission through udder; mastitis etc
^{##} Via faeces, the environment etc

** Enterotoxin is heat stable
 - No data/unknown

[^] susceptible populations

[§] Qualitative Framework (see Appendix 6)

+ Reported, but rare
 ++ Commonly associated with foodborne illness
 * Plausible but not reported

While many of the organisms listed in Table 1 are commonly implicated in foodborne illness, the following organisms are not proven pathogens via ingestion or have not been found in Australian goats:

- *Mycobacterium avium* subsp. *paratuberculosis* is the causative agent for Johne's disease in ruminants. A statistical association between Johne's disease in animals and Crohn's disease in humans has been reported but there is insufficient evidence presently available to either prove or disprove a causal link (Anon, 2004a; Feller *et al.*, 2007).
- Although *Coxiella burnettii* infection has been associated with consumption of raw goat milk (Rampling, 1998), ingestion is considered a minor route for human infection (Maurin and Raoult, 1999). Consequently little information exists regarding ingestion mediated illness.
- *Brucella melitensis* is an important infectious organism within dairy goats although it has not been reported in Australian herds.

These organisms have been included within Table 1 and Appendix 2 as potential pathogens associated with raw goat milk, however the risk characterisation for raw goat milk has been treated separately.

Detailed descriptions of the organisms identified in Table 1 are contained within Appendix 2. Severity rankings are discussed within Appendix 6.

8 Occurrence of microbiological hazards associated with raw goat milk

Raw goat milk has been shown to contain a variety of pathogens including: *Brucella* spp., *C. burnettii*, *Campylobacter* spp., pathogenic *E. coli*, *L. monocytogenes*, *Mycobacterium* spp., *S. aureus*, *Salmonella* serovars, *T. gondii* and *Y. enterocolitica*.

8.1 Australian data

Microbiological data was obtained from a number of sources including the scientific literature, the National Enteric Pathogen Surveillance Scheme, State authorities and FSANZ’s food recall database and is detailed in Appendix 3. Unfortunately, while there is little recently published microbiological data available on raw goat milk in Australia, surveys in the 1980’s and early 1990’s indicate considerable microbiological contamination of raw goat milk in Australia (Arnold and Coble, 1995; Jensen and Hughes, 1980; Ryan and Greenwood, 1990). Recent data from state testing programs indicate an improvement in the overall microbiological quality of Australian raw goat milk (Appendix 3: Table 2 and Table 3).

8.1.1 National Enteric Pathogen Surveillance Scheme data

Data collated by the National Enteric Pathogen Surveillance Scheme from 1983 - 2004 showed that of the 1,156 dairy samples positive for *Salmonella* spp., only 14 (1.2 %) samples were raw goat milk (Appendix 3: Table 1). The data showed that a range of *Salmonella* serovars have been isolated from dairy products in Australia, but the total number of samples is not provided in the dataset so prevalence calculations cannot be made.

8.1.2 State testing data

Microbiological test results were obtained from each State permitting the sale of raw goat milk, covering the period from 1993 to 2006 (Appendix 3: Table 2). Tests were undertaken for coagulase positive *Staphylococcus* spp., coliforms, *E. coli*, *Salmonella* spp., *Listeria* spp. and *Campylobacter* spp. with the overall prevalence displayed graphically in Figure 1.

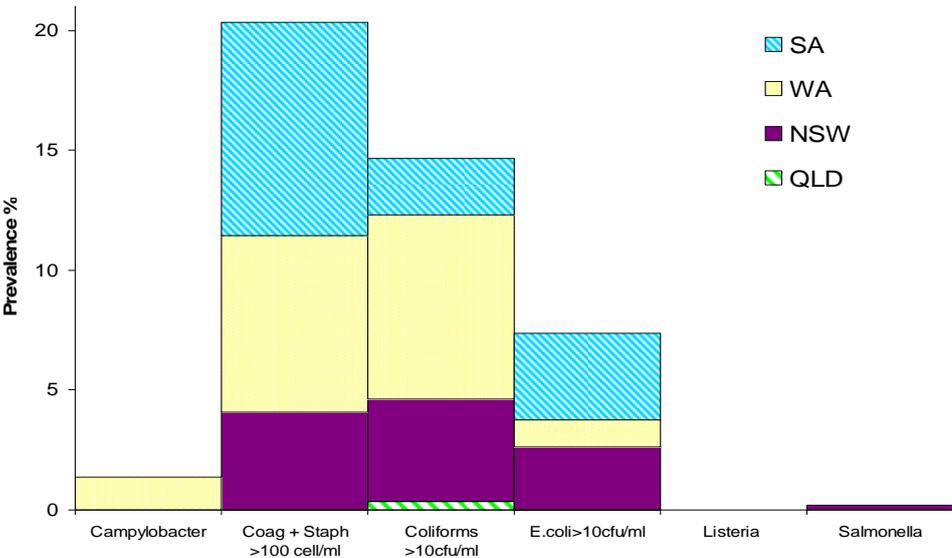


Figure 1: Combined prevalence of organisms from State testing data

Coagulase positive *Staphylococcus* spp., coliforms and *E. coli* were regularly detected, whilst *Campylobacter* spp. had a very low prevalence and *Salmonella* spp. and *Listeria* spp. were generally not detected (*Salmonella* spp. was detected in 1 out of 511 samples) (Appendix 3: Table 2).

A sample of raw goat milk was recently reported as testing positive for Shiga-like toxin producing *E. coli* during routine testing in Western Australia (pers. comm. Calder, 2008).

Direct comparison of results was difficult due to differences between each State in relation to the types of organisms tested for, the frequency of testing and the manner of reporting results e.g. pass/fail or detected/not detected. The effectiveness of State sampling plans to detect pathogens in raw goat milk has been queried. In assessing the South Australian regulations Pointon *et al.* (2004) determined that monthly sampling for indicators of hygiene and quarterly sampling for some pathogens provides minimal confidence that contaminated milk is not entering the marketplace.

8.1.3 Summary of data from other programs

Aside from routine testing programs, raw goat milk has been analysed during pilot studies and during data collection for risk assessments (Appendix 3: Table 3).

There is some overlap between data provided by the South Australia Risk Assessment (Pointon *et al.*, 2004) and the data obtained directly from the Dairy Authority of South Australia. Survey data results cited in the risk assessment undertaken for NSW (AgriQ, 2002) have also been included where not recorded elsewhere in this report.

Generally, coagulase positive *Staphylococcus* spp., coliforms and *E. coli* have been frequently detected whilst *Salmonella* spp., *Campylobacter* spp. and *Yersinia* spp. are rarely detected. *Listeria monocytogenes* was not detected but the non-pathogenic, non-haemolytic *Listeria innocua* was detected at a very low incidence.

8.1.4 Food recalls

There were 43 recalls for dairy products due to microbiological concerns during the period 1990-2005, out of a total of 716 food recalls. Of these 43 dairy recalls, only three were from products made from goat milk (0.42%) and only one was positively identified as being from raw goat milk (Appendix 3: Table 5). Frozen raw goat milk was also recalled in Queensland in early 2008 due to *Salmonella* Zanzibar contamination.

8.2 International data

While limited data is published on Australian raw goat milk, the international literature indicates a range of microorganisms can contaminate raw goat milk (Appendix 3: Section 2). It is difficult to directly compare results between individual studies due to differences in the type and number of samples taken, the point in production from where the sample was taken and the methodology used to isolate and/or enumerate the various organisms. In general, the reported prevalence of microbiological hazards in raw goat milk is highly variable and influenced by local factors.

Pathogens detected in raw goat milk internationally include *Brucella* spp., *C. burnettii*, *Campylobacter* spp., *Listeria* spp., pathogenic *E. coli* including *E. coli* O157:H7, *Mycobacterium* spp., *S. aureus*, *Streptococcus* spp., *T. gondii* and *Y. enterocolitica*.

8.3 Summary

There is little published information available on the incidence and prevalence of pathogens in raw goat milk. Information which is available indicates a variety of pathogens may be isolated from raw goat milk both in Australia and internationally, although greater diversity is reported internationally. This may be an artefact of the level of microbiological testing undertaken in those countries which permit raw goat milk and raw goat milk products.

Similarities exist between the pathogens detected internationally and in Australia. Coagulase positive *Staphylococcus* spp. and *E. coli* are more commonly detected, while *Campylobacter* spp., *Salmonella* spp. and *Yersinia* spp. detections are generally low. Contamination with *Listeria* spp. in Australia is very low whereas it appears to be more problematic internationally (Appendix 3: Table 10).

Particular mention should be made of the prevalence of *Brucella* spp. internationally. Although *Brucella* spp. have been isolated from raw goat milk (Appendix 3: Table 6), it is important to note that Australia has been free from *B. abortus* since 1989 and *B. melitensis* has never been reported in Australian livestock (Australian Quarantine and Inspection Service, 1999).

9 Foodborne illness associated with raw goat milk

Prior to the introduction of pasteurisation, dairy products such as liquid milk were frequently implicated in outbreaks of foodborne illness. In the 19th and early 20th century, milk was a common vehicle for communicable diseases such as scarlet fever, diphtheria and tuberculosis.

Pasteurisation became mandatory for milk products in Australia soon after a major outbreak of typhoid fever in Victoria in 1943. However, provisions exist under the Food Acts for South Australia, Western Australia, NSW and Queensland that negate the requirement for the mandatory pasteurisation of goat milk (Appendix 8).

9.1 Australia

Over the past 20 years, there have been only two incidents of foodborne illness associated with the consumption of raw goat milk reported. In 1990 there were nine cases of Salmonellosis attributed to the consumption of raw goat milk, whilst two cases of illness were attributed to *Cryptosporidium parvum* in 1984 (Appendix 4: Table 1). Since the beginning of OzFoodNet's Outbreak Register in 2000, there have been no reported outbreaks of foodborne illness attributed to the consumption of raw goat milk in Australia (OzFoodNet, 2005).

9.2 International data

A number of outbreaks of illness have reportedly been associated internationally with the consumption of raw goat milk. The literature describes 34 outbreaks between 1973 - 2006 associated with the consumption of raw goat milk and cheese made from raw goat milk. Of the outbreaks, over half (19/34) were attributed to consumption of raw goat milk (Appendix 4: Table 2), with the remainder associated with raw goat milk cheese (Appendix 4: Table 3).

Organisms commonly associated with illness following consumption of raw goat milk include *E. coli* O157, *C. jejuni*, *S. aureus*, *C. burnetti*, *B. melitensis* and *T. gondii* (See Figure 2). In raw goat milk cheese, *B. melitensis* accounted for six outbreaks, *E. coli* and *Salmonella* spp. accounted for three outbreaks each, one outbreak was associated with *Coxiella* spp. and *Listeria* spp. was implicated in a single incident involving an immunocompromised individual.

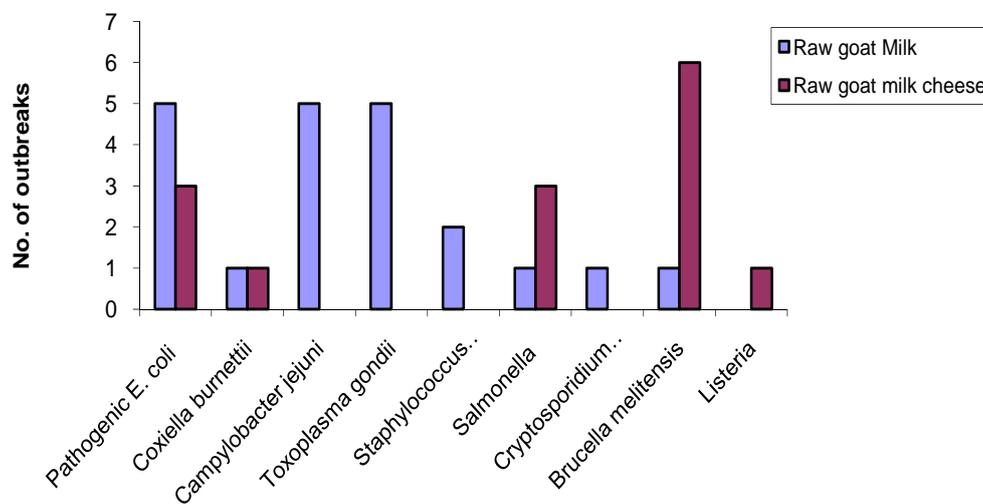


Figure 2: Outbreaks attributed to various pathogens during the period 1973 – 2006

9.3 Attribution of foodborne illness

Over the last 35 years, raw goat milk has only been associated with two reported outbreaks of illness in Australia and 19 reported outbreaks internationally. The extent to which illness can be attributed to raw goat milk does not enable risk assessors to clearly determine the relative risk that consumption of raw goat milk poses to consumers.

Sources of foodborne illness are generally determined through epidemiological and/or microbiological associations in outbreak investigations. Critical in this process is the ability to identify an outbreak through the existing surveillance system to enable an investigation to then 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. *L. 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.

Pointon *et al.* (2004) notes the likelihood of significant under-reporting of illness associated with the consumption of raw goat milk in Australia. A contributing factor is the overall under-reporting of gastrointestinal illness combined with the low frequency of consumption of unpasteurised goat milk among the population (only 32,000 litres were sold in SA in 2002). This means the sensitivity of the surveillance system to detect outbreaks and sporadic illness associated with raw goat milk is low (Pointon *et al.*, 2004). This is evidenced by the fact that only two incidents of illness have been reported in Australia over the last 20 years and none since the inception of OzFoodNet's Outbreak Register in 2000.

10 Primary production factors impacting on raw goat milk safety

Raw goat milk has a mixed microflora which is a result of multiple factors. Contamination may occur when microorganisms are shed directly into the milk from the goat udder, through environmental contamination, and via contamination from the milking environment or personnel. The microflora encountered is not dissimilar to that found in raw cow milk.

Primary production factors that impact on these routes of contamination and the microbiological quality of the raw goat milk include:

- Animal-related factors *e.g.* animal health¹⁰ and husbandry
- Environment-related factors *e.g.* housing, faeces, feed, soil, and water
- Milking related practices *e.g.* milking methods, personnel, equipment, storage, packaging and delivery

In Australia, successful goat dairy farms are operating on systems developed for cow dairying. As indicated in Section 3.2.1 only those factors which differ significantly to those depicted for cow milk production have been discussed.

10.1 Animal health/husbandry

Generally goats are considered clean animals as they produce pelletised faeces and do not like to walk in water or mud (QDPI, 2004). Although goats are generally thought to be naturally healthy animals they succumb quickly when they do become ill, hence veterinary treatment and vaccination of goats may involve the off-label use of veterinary medicines registered for use in other species for other conditions. Therapies developed for dairy cows may be used in goats under veterinary prescription in certain circumstances.

Common diseases of goats include mastitis, toxoplasmosis, leptospirosis, viral infections (including caprine retrovirus) and Johne's disease.

Goat health problems may impact on the microbiological quality of raw milk. Diseased¹¹ goats will show increased shedding of pathogens directly into raw milk through udder infections or into faeces which may contaminate the production and milking environment. Infected¹² animals with no signs of disease (asymptomatic carriers) may harbour and shed pathogens, often intermittently, into milk and faeces.

10.1.1 Carrier status

The retention of a disease agent in a group of animals frequently depends on the presence of an individual animal which carries the organism without showing the disease. These are difficult to detect and frequently require repeated laboratory tests to confirm their carrier status. Carriers may be animals which have recovered from the clinical disease or animals which have never had the disease. Their presence confounds conventional disease diagnosis

¹⁰ Animal health is defined as incorporating both disease (the clinical and/or pathological manifestation of infection), infection and carrier status of the animal.

¹¹ 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)

¹² Infection is defined in the OIE Terrestrial 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)

and herd treatments and may result in the recrudescence of a disease in a previously negatively tested group.

Some carriers may be masked and not release organisms unless stressed or immunocompromised. In these cases the isolation of microorganisms may be negative until the infection re-activates. The specificity and sensitivity of the laboratory testing will also limit the ability to detect carriers. Where detection is difficult, it is often the reappearance of disease in susceptible animals which is the first indication that carrier animals exist in a group. Destocking and complete replacement with disease free animals may be the only way of removing a disease carrier.

Many human pathogens co-exist in their animal host with little or no apparent ill-effect. For example *E. coli* O157:H7 asymptotically colonises the terminal rectum of cattle, and a vaccine is being tested to reduce secretion levels by carrier animals (Peterson *et al.*, 2007). This vaccine may potentially also be used in the treatment of goats. Research by Brownlie and Grau in the 1960's demonstrated the effects that stress and starvation have on the shedding of enteric pathogens such as *E. coli* and *Salmonella* spp. in cattle and sheep (Grau *et al.*, 1968; Grau *et al.*, 1969).

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 do modulate the immune system and can precipitate the excretion of organisms in a carrier animal.

10.1.2 Mastitis

Mastitis, both clinical (actual signs of infection) and subclinical (no outward signs of infection) can be caused by the same organisms which can damage the udder, reduce production and adversely affect the quality and quantity of milk produced. Bacteria which infect the mammary gland are classified into two major categories, contagious or environmental pathogens (Tomita and Hart, 2000).

The most prevalent contagious pathogens associated with mastitis in goats are *Streptococcus agalactiae* and *S. aureus*. Causal pathogens of environmental mastitis¹³ are present in urine, faeces, soil and bedding. Transmission mainly occurs between milking, but can also occur during milking. Environmental pathogens commonly isolated from infected udders are coliform bacteria, *Streptococcus* spp. other than *St. Agalactiae*, and *Staphylococcus* spp. other than *S. aureus* (Tomita and Hart, 2000).

Staphylococci have frequently been reported as the most prevalent organism in clinical and subclinical mastitis in goats. *S. aureus* is the most significant pathogen associated with clinical mastitis and has been reported at prevalences around 13% (Deinhofer and Pernthaner, 1995; Kalogridou-Vassiliadou, 1991; White and Hinckley, 1999), although one study in Norway reported prevalence of 96.2% in bulk tank milk (Jorgensen *et al.*, 2005).

¹³ Environmental mastitis occurs as a result of an ascending infection through the teat canal.

International prevalences of approximately 13% are consistent with those reported in Australia (Appendix 3: Table 2).

Other organisms which have been associated with mastitis in goats include *E. coli*, *Streptococcus* spp., *Pseudomonas* spp., *Corynebacteria* spp. and *Bacillus* spp. (Al-Graibawi *et al.*, 1986; Bergoinier *et al.*, 2003; Deinhofer and Pernthaner, 1995; Jorgensen *et al.*, 2005; Kalogridou-Vassiliadou, 1991; Ryan and Greenwood, 1990; White and Hinckley, 1999).

A case of caprine mastitis associated with *Yersinia pseudotuberculosis* (synonym, *Pasteurella pseudotuberculosis*) was documented in California in 1972. Raw or inadequately pasteurized milk contaminated with *Y. pseudotuberculosis*, regardless from which animal species, may be a possible source of *Yersinia* infections in man (Cappucci *et al.*, 1978).

Somatic cell counts (SCC) are used as a method to determine levels of mastitis infection in individual goats, or in bulk milk samples. Some studies have indicated that an increase in SCC alone is not an accurate indicator of mastitic infection in goats and suggest that the establishment of bacteriological examinations (particularly of mastitis-related pathogens) would help establish a SCC threshold (Wilson *et al.*, 1995; Zeng *et al.*, 1997).

Healthy goat udders can have high SCC levels normally, with stage of lactation influencing the actual counts (Haenlein, 2002). SCC levels in milk from goats are higher than from cows and sheep, with the standard SCC in goat milk at 1×10^6 cells/ml (Olechnowicz and Jaskowski, 2004). This limit is imposed in the USA and France, although levels in the range of 300,000 - 400,000 cells/ml have often been achieved (Stubbs and Abud, 2002).

An acceptable level of SCC in cow milk is less than 400,000 cells/ml with counts above 200,000 indicating that either clinical or subclinical mastitis is present to a significant degree (Stubbs and Abud, 2002). Hence it is inappropriate to attempt to correlate SCC results between cow and goat species. Importantly, goats with mastitis are much more likely than cows to develop lumps, abscesses and fibrosis in the udder.

10.1.3 Other zoonotic diseases/infections

Goat health issues other than mastitis may also influence the microbiological quality of the raw milk. There may be increased shedding of pathogens either directly into the milk or into the faeces or urine from sick and diseased animals. Common zoonotic diseases, other than mastitis, affecting goats are discussed below.

10.1.3.1 Leptospirosis

Leptospirosis, also known as Weil's or Canecutter's Disease, is caused by *Leptospira interrogans* (Appendix 2). *L. interrogans* can be spread by contact directly between infected animals and humans, by ingestion of contaminated water or food, through aerosolised urine particles, animal foetal fluids or through direct contact with skin (Baranton and Postic, 2006). Similarities exist between the serovars of *L. interrogans* found in cows and goats. Serovars Hardjo, Pomona and Grippotyphosa are common to both cattle and goats, while Canicola, Australis and Icterohaemorrhagiae are further associated with cattle (Anon, 2004b).

Humans are susceptible to all pathogenic serovars found in domestic animals with between 100 - 200 human cases of leptospirosis reported each year in the US (CDC, 2005).

Leptospirosis is a notifiable disease in Australia and had an annual notification rate of 1.3 cases per 100,000 population in 2000 (Anon, 2002). Notifications have been declining in recent years from 243 cases reported in 2000 to 177 in 2004. Leptospirosis occurs across Australia, although the majority of cases are reported in Queensland. The most common serovars are generally Zanoni, Hardjo and Australis (QHSS, 2004).

Primarily excreted via the urine of infected animals, shedding of viable leptospires has been recorded in mastitic cow milk (Bolin and Koellner, 1988). Leptospirosis is problematic for cow dairies and can occur in goats but the extent is unknown. Vaccination programs available to control leptospirosis in cows are unavailable for goats. Urine splashing is less common in goat dairies than cow dairies, although the potential for contamination of raw goat milk does exist.

There is a lack of data on raw goat milk mediated foodborne illness from *L. interrogans*.

10.1.3.2 Melioidosis

Burkholderia pseudomallei (previously *Pseudomonas pseudomallei*) (Appendix 2) is the aetiologic agent of melioidosis (also called Whitmore's disease). Melioidosis is generally a disease only seen in tropical and sub-tropical regions; predominately during the wet season. Goats and sheep are particularly susceptible with cases of infection often eventuating in the death of the animal (Choy *et al.*, 2000). *B. pseudomallei* are limited to tropical regions of Australia such as Queensland and the Northern Territory and are hence exotic to southern regions.

It has been suggested that there is a possible public health risk from drinking contaminated milk from an animal infected with the disease melioidosis (Thomas *et al.*, 1988; Choy *et al.*, 2000). *B. pseudomallei* has been isolated from infected goat's udders (Van der Lugt and Henton, 1995), mastitic goat milk (Choy *et al.*, 2000) and is excreted in goat faeces (Dance, 2000). In the Northern Territory, raw goat milk has been banned because of the high incidence of asymptomatic mastitis in dairy goats caused by *B. pseudomallei* (pers. comm. Currie, 2006). A Darwin study undertaken by Choy *et al.*, (2000) found 15/43 (35%) of goats had evidence of mastitis from *B. pseudomallei*, although no information is available on the prevalence of *B. pseudomallei* in raw goat milk.

Melioidosis is known to be a major cause of human morbidity and mortality in the Australian tropics (Dance, 2000). Twelve human deaths out of 33 infections occurred during one outbreak in the Northern Territory during 1990 and 1991 (AgriQ, 2000). *B. pseudomallei* can survive the low pH of the stomach indicating infection by ingestion is possible. A small number of cases in a Darwin prospective study are thought to have resulted from ingestion rather than percutaneous or inhalation routes (Ralph *et al.*, 2004).

There is a lack of data on virulence and infectivity for *B. pseudomallei* obtained via ingestion and no information available on the dose-response relationship for *B. pseudomallei* in human infections. While there is limited information implicating ingestion of *B. pseudomallei* from raw goat milk, it is plausible that foodborne illness could result from consumption of contaminated raw goat milk.

10.1.3.3 Johne's disease

Mycobacterium avium subsp. *paratuberculosis* (MAP) (Appendix 2) is the organism responsible for Johne's disease in many ruminant species, including goats. Goats are susceptible to both cattle and sheep strains of Johne's disease.

MAP is excreted primarily in the faeces of infected animals and is excreted during both the sub-clinical and clinical stages of disease. In dairy animals, MAP can be transmitted both vertically through the placenta to the foetus in advanced infection and also through the young animal ingesting colostrum, milk or faeces from an infected animal. MAP is also transmitted horizontally through the faecal-oral route (Streeter *et al.*, 1995; Sweeney *et al.*, 1992, Scientific Committee on Animal Health and Animal Welfare, 2000; Anon, 2004b).

A statistical association has been reported between MAP and Crohn's disease, a chronic intestinal enteritis in humans. While such an association is reported, whether it is causal is a matter for debate. The debate is characterised by firmly entrenched opinions on either side, and the subject has been comprehensively reviewed several times (Chiodini, 1989; Thompson, 1994; Anon, 1998; Harris and Lammerding, 2001; Lipiec, 2003; Chacon *et al.*, 2004; Feller *et al.*, 2007). Presently there is insufficient evidence to prove or disprove a causal association link between Johne's Disease in ruminants and Crohn's disease in humans (Anon, 2004a; Feller *et al.*, 2007).

10.1.3.4 Q fever

Coxiella burnettii (Appendix 2) causes the zoonotic illness Q fever and is commonly found in cattle, sheep and goats. *C. burnettii* has been associated with consumption of raw goats milk and cheese in Europe, Canada and the USA (Rampling, 1998), although this is considered a minor route for human infection (Vanderlinde, 2004; Maurin and Raoult, 1999).

10.1.3.5 Brucellosis

Brucella spp. (Appendix 2) are pathogenic for both humans and a wide range of animals. *B. melitensis* is a major cause of brucellosis in sheep and goats and is more pathogenic to humans than other *Brucella* spp. *B. melitensis* is widespread in southern Europe, west and central Asia, Mexico, South America and Africa but has never been reported in sheep or goats in Australia.

Zoonotic transmission from infected animals to humans may occur either via direct or indirect transmission (Kasimoglu, 2002). *Brucella* is most commonly transmitted via raw milk or raw milk products, such as cheeses (Kasimoglu, 2002). Ewes and goats milk has been found to be a more significant source of *Brucella* spp. than cow's milk.

10.1.3.6 Enterotoxaemia

Clostridium perfringens (Appendix 2) is a zoonotic organism producing disease in goats which is generically called enterotoxaemia (Uzal, 2004). The infection is characterised by profuse diarrhoea which may last for days or weeks.

10.1.3.7 Toxoplasmosis

The protozoan parasite *Toxoplasma gondii* (Appendix 2) is the cause of the potentially severe disease toxoplasmosis. It can infect a wide range of animals with the primary host belonging to the cat family (Felidae) and secondary hosts including all warm blooded animals (e.g. mammals and birds) (Tenter *et al.*, 2000). *T. gondii* causes great losses in sheep and goats, however the disease is more severe in goats (Hill and Dubey, 2003).

Transmission of *T. gondii* occurs via the faecal-oral route, transplacental transfer between mother and foetus, and through the consumption of infected meat and/or milk containing tachyzoites or other forms of the infective parasite from the secondary host (Chiari and Neves, 1984; Skinner *et al.*, 1990; Smith, 1993b; Tenter *et al.*, 2000). Raw goat milk has been linked as a probable route of infection in outbreaks of *T. gondii* (Smith, 1993b). One study suggests that *T. gondii* has the ability to survive in refrigerated raw goat milk (Walsh *et al.*, 1999). Exposure to 50°C renders tachyzoites non-infectious and therefore pasteurisation will eliminate tachyzoites (Smith, 1993b).

Toxoplasmosis is widespread in humans, being one of the most common parasitic zoonoses worldwide (Tenter *et al.*, 2000). *T. gondii* infection is very serious in cases where the secondary host is pregnant as this organism has the ability to cause spontaneous abortion or severe congenital defects in the off-spring of the host (Tenter *et al.*, 2000).

10.1.3.8 Cryptosporidiosis

Cryptosporidium parvum is a common aetiological agent of diarrhoea in goat kids (de Graaf *et al.*, 1999), with large numbers of oocytes being shed in the faeces during infection. *Cryptosporidium* spp. can also cause illness in humans (Appendix 2).

10.1.3.9 Caprine retrovirus

Caprine retrovirus, formerly known as caprine arthritis encephalitis or “Big Knee” is of major concern for the goat industry. The signs of caprine retrovirus in affected goats are age dependent with kids under 6 months normally developing encephalitis, whilst in older goats the virus primarily affects joints. Chronic progressive pneumonia and the condition known as “hard udder” may also be associated with caprine retrovirus. The disease occurs mainly in dairy goats and has been reported in Australia, New Zealand, UK, USA and other countries (Stubbs and Abud, 2002). Infection with caprine retrovirus usually lasts for the lifetime of the animal with the transmission of the virus primarily being through the colostrum and milk.

The virus does not survive for long in the general environment, such as soil and sheds, and is destroyed by heating such as pasteurisation but not refrigeration (Stubbs and Abud, 2002).

There is no information on the incidence and effect, if any, of caprine retrovirus in humans. Caprine retrovirus, although identified as being of significant concern within dairy goats in Australia, and milk being identified as a transmission vehicle, was not considered in the risk assessment as there is no documented association with human illness.

10.2 Environmental factors

Pathogens may originate from the dairy goat environment such as housing, urine and faeces, feed, soil and water. Environmental contaminants may therefore contaminate raw milk or contribute to mastitic or systemic infection in the animal.

10.2.1 Housing

Goats are kept in situations which vary from extensive grazing to close confinement and housing (Bureau of Animal Welfare, 2001). Mixed housing systems are common in Australia *i.e.* some grazing and some intensive housed herds (Stubbs and Abud, 2002).

Intensive housing has been associated with a higher risk of udder contamination, which may lead to mastitic infection as a result of increased contact with urine and faeces, wet bedding, and contact with other animals, as well as other environmental contaminants.

Pathogens that have been associated with intensive housing for cattle include *L. monocytogenes*, *E. coli*, *B. cereus* and *Salmonella* spp. It is assumed that for goats managed under similar intensive housing systems, the same pathogens may also be of concern.

10.2.2 Faeces

Unlike the moist faeces produced by cows, goats produce drier pelletised faeces. Pelletised faeces may reduce the amount of direct faecal contamination on the udder; however there is a greater risk of dust being formed. The pelletised faeces can become soft and liquid under circumstances of disease, stress and unsuitable feeding regimes. Risk of contamination on the udder is then enhanced. The goat's hairy udder may more readily become contaminated and may be more difficult to clean than that of a cow udder (QDPI, 2004).

Milking animals may shed a variety of enteric pathogens in the faeces as a result of infection or from ingestion of the organisms from feed or water. Pathogens associated with dairy cattle include *Listeria* spp., *Salmonella* spp., *E. coli*, *Bacillus* spp., *Campylobacter* spp. and pathogenic *E. coli*.

There is limited information available regarding the prevalence of pathogens in goat faeces; however a strain of *E. coli* O157:H7 was detected in goat faeces during a study undertaken in Greece. The testing of 351 faecal samples from goat, sheep and cattle found an *E. coli* O157:H7 prevalence of 0.2% in goat faeces, indicating that goats can be a reservoir of *E. coli* O157:H7 and goat milk, dairy products and meat may serve as a vehicle for the transmission of this pathogen to humans (Dontorou *et al.*, 2004).

Faecal carriage of *Campylobacter* spp. is also common in both sheep and goats. *Campylobacter* spp. have been isolated in goat faeces from 0 - 2.7% of healthy goats and 3.7% of diarrheic goats (Kaneene and Potter, 2003).

The clinical manifestations of listeriosis in goats closely resemble those in sheep: encephalitis, septicaemia and abortion. Goats may be asymptomatic carriers, shedding *L. monocytogenes* in the faeces and milk, which may result in environmental contamination (Schukken *et al.*, 2003).

10.2.3 Feeding practices

Feeding regimes for goats vary greatly and include irrigated pasture, scrub, hay, grains, silage, bread and supplements. In Australia, most farms graze goats on some pasture but the degree of pasture management is variable. Some is simply rangeland, much is set stocking and some is highly managed rotational grazing. Hay and grains are commonly used, while silage with minerals and other additives is often fed to goats (Abud and Stubbs, 2005).

Feed can play an important role as a primary vehicle for animal contamination and also an environmental contaminant of raw milk. Contamination of feed may originate from storage of the feedstuff on farm or from the source of the feed (including contamination during feed manufacture). Animal feed can be contaminated with pathogens of faecal, plant and soil origin (Desmarchelier, 2001).

A broad range of pathogens have been associated with dairy cattle feed and feeding practices and may be extrapolated to goats where practices are similar. These pathogens include; *Listeria* spp. and *C. jejuni* in pasture, *Enterobacteriaceae*, *Listeria* spp., *Clostridia*, *Bacillus* spp. and *E. coli* in silage and *Salmonella* spp., *E. coli*, *Campylobacter* spp., *B. cereus* and *Listeria* spp. in feed concentrates.

10.2.4 Soil and water

Soil represents an important source of pathogens for grazing animals with a wide variety of organisms, including pathogens, often found. *B. cereus* spores have been found internationally to vary at levels between <50 - 380,000 cfu/g (Christiansson *et al.*, 1999). Australian studies have found *Bacillus* spores in soil at levels between 5.6×10^2 - 1.8×10^3 cfu/g (Cook and Sandeman, 2000).

Listeria spp., *Salmonella* spp. and enteropathogenic *E. coli* have also been reported as existing and surviving in soil (Desmarchelier, 2001; Fenlon *et al.*, 1996).

Water can be a primary source of contamination and is used extensively on goat dairy farms for cleaning, cooling, stock drinking and irrigation. Various pathogenic bacteria have been reported in water including *E. coli* O157:H7 and other pathogenic *E. coli*, *Campylobacter* spp. and *Salmonella* spp. (Lejeune *et al.*, 2001; Rice and Johnson, 2000; Stanley *et al.*, 1998; Wallace, 1999). *Cryptosporidium parvum* oocysts have been shown to be able to survive up to 176 days in drinking water or river water stored at 4°C (Robertson *et al.*, 1992).

10.3 Milking practices

10.3.1 Milking systems

Goats are milked by hand or by machine (bucket system or pipeline) with milking methods dependent upon the specific dairy's herd management practices.

The most popular milking parlours for goats are herringbone types and side-by-side parlours with two platforms (Billon, 2002). In Australia, most systems are of a herringbone design with rotary systems only used for very large herds. Unlike milking systems for cows, a kick rail is not needed for goats (Stubbs and Abud, 2002). Because goats tend not to kick off cups during milking, the likelihood of faecal contamination being sucked into the milk line is reduced.

The type of milking system employed may influence bacterial contamination of the raw milk. A South African study found that raw milk obtained by the bucket system milking machine had the lowest total bacterial count (16,450 cfu/ml), as compared to that by pipeline milking machine (36,300 cfu/ml) or hand milking (48,000 cfu/ml) (Kyozaire *et al.*, 2005).

Filtration of milk before it enters the bulk milk tank provides a safeguard to ensure sediment or other extraneous matter is removed from the milk prior to storage. Although filtration removes most of the soil and other particles, it does not remove all the bacteria adhering to these particles, nor does it remove any dissolved matter from the milk. Sediment that has been trapped by the filter continues to be washed by the milk flowing through. This dislodges bacteria adhering to the particles and thus contributes to an increased bacterial count in the bulk milk. If filters are not adequately cleaned, this process may be a source of cross-contamination of the milk.

10.3.2 Milking practices

Poor milking practices may lead to contamination of raw milk. The teat surface is the major avenue of entry for microorganisms into raw milk and the goat's hairy udder may be a greater source of contamination than a cow udder. 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. This practice has been advocated for all goat dairies producing liquid milk to be consumed raw (Ryan and Greenwood, 1990).

Post-milking teat disinfection reduces the resident teat skin bacterial population, which is the main source of infection for the mammary gland. In dairy cattle, the rate of new intramammary infection due to *S. aureus* and *St. agalactiae* is reduced by approximately 50% when post-milking teat disinfection is practiced (Sheldrake and Hoare, 1980). A comparable effect on infection rate in goats would also be expected.

10.3.3 Cleaning and sanitation

There are various methods for cleaning dairy goat milking parlours and yards. Cleaning and sanitation procedures applicable to the cow dairy industry may also be applied to the goat dairy industry, however due to goats' drier pelletised faeces; a water washout may not be undertaken after each milking. The milking bays and floors of the milking area may only undergo sweeping out of solids. When a water washout is carried out, a source of cross contamination is the cleaning water contacting the milking equipment and the creation of aerosols.

Wastewater generated by cleaning operations may contaminate pasture and transmit pathogens to grazing animals.

Cleaning of milk handling equipment involves a combination of chemical, thermal and physical processes. The key principles of a good cleaning system involves sufficient hot water (temperature and volume), correct wash solutions (detergent, acid/alkali), adequate contact time and sufficient turbulence to prevent build up of milk residues and bacteria in the equipment.

10.4 Milk storage

The composition of milk makes it an excellent growth medium for many microorganisms unless it is frozen or further processed to kill or prevent their growth. As the temperature of raw milk is over 30°C as it leaves the udder, pathogenic bacteria if present, will grow rapidly. At temperatures between 0 and 5°C, the growth of most pathogenic and spoilage mesophilic bacteria is slowed.

Cooling of milk to less than 5°C in 3½ hours or less from the start of milking minimises the likelihood of bacterial growth. Goat milk requires rapid cooling to lower temperatures to reduce bacterial activity because of infrequent milk pickup/delivery (Stubbs and Abud, 2002).

In Australia, milk is usually stored in cooled vats of sufficient size to cope with up to a week's production. Cooling systems employed range from artisanal to sophisticated, and include pre-cooling in-line, ripple coolers, plate exchangers, direct expansion vat, cool rooms and buckets in ice (Stubbs and Abud, 2003). Any breakdown in the refrigeration system or failure to properly cool milk prior to collection or delivery may adversely impact on the microbial load in raw milk.

10.5 Milk delivery

Most producers of raw goat milk for direct human consumption bottle their own milk on site. Consequently correct sanitising procedures for packaging, aseptic 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.

Because of the size of the industry, the goat farmer will typically need to deliver raw milk to the processor if milk is not sold directly off-farm. An Australia-wide survey undertaken by the Rural Industries Research and Development Corporation indicated that self-delivery in food grade plastic containers, twice a week, was the most common method of milk transport (Stubbs and Abud, 2003). A further study conducted in NSW (Miles and Van Den Hout, 2000) indicated that deliveries do not always occur in refrigerated vehicles, but use alternatives such as Styrofoam boxes and packing products in ice covered with a wet blanket.

Information pertaining to the time and temperature conditions which raw goat milk is subject to post milking is scarce. Furthermore, limited information is available on the integrity of cold chain management throughout distribution, retail storage and consumer handling practices. However it is generally accepted that retail and domestic refrigeration units can sometimes be a weak link in cold chain management.

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 *L. monocytogenes* and *Y. 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*, *Salmonella* and *S. aureus* may all grow at temperatures between 7 - 8°C (ICMSF, 1996).

11 Summary of major primary production risk factors for raw goat milk production in Australia

Raw goat milk may be contaminated by two primary means: pathogens shed directly into the milk via the udder, or through external (environmental) contamination during or post harvest.

The health and welfare of the goat has a direct impact on the microbiological quality of raw goat milk. Mastitis (contagious and environmental) and other infections or illnesses result in increased levels and diversity of pathogenic microorganisms being shed directly into the raw milk through the udder. Mastitic and ill goats may also experience increased faecal shedding of pathogens which increases the risk of contamination from the environment. Infected animals with no outward signs of disease (asymptomatic carriers) may harbour and shed pathogens either continuously or intermittently into milk, urine and faeces over undefined periods of time.

Environmental contamination of the raw goat milk may occur from a variety of sources including the farm environment *e.g.* housing, feed, water, etc, and the processing environment *e.g.* milking equipment/practices, personnel, cleaning and packaging etc.

Raw goat milk is generally packaged on-farm and does not undergo any heat treatment, such as pasteurisation, to reduce or eliminate pathogenic organisms. Consequently, minimising the level of pathogens entering the raw goat milk, combined with strict temperature controls to limit proliferation, are two primary avenues for controlling the safety of raw goat milk.

The key risk factors during primary production and processing affecting the microbiological status of raw goat milk are summarised in Table 2.

Table 2: Key risk factors for raw goat milk

Risk factor	Impact on milk safety	Mitigation strategies
Disease	Diseased goats will show increased shedding of pathogens into raw milk or faeces. Infected animals with no signs of disease (carriers) may carry and shed pathogens, continuously or 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, hairy udders, 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 goat milk after milking can lead to growth of pathogens	Rapid cooling and holding of milk.
Packaging/Delivery	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.

The extent to which one risk factor is more important will be hazard specific, and could not be determined in this risk assessment due to a lack of quantitative through chain data.

12 Assessing the safety of raw goat milk in Australia

The risk assessment process used by FSANZ is consistent with Codex, FAO and WHO protocols and involves four distinct steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation. The previous sections provide a descriptive analysis of the major microbial hazards considered in the risk assessment. A qualitative framework was then utilised to assess the risk selected microbiological hazards pose to public health and safety from the consumption of raw goat milk.

12.1 Qualitative risk rating

The qualitative framework was used to determine risk characterisations for the general and susceptible populations for seventeen microbiological hazards.

There is some uncertainty around the characterisation of two of these microorganisms:

- Although *C. burnettii* infection has been associated with consumption of raw goat milk (Rampling, 1998), 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 Johne's 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 goat milk would be a risk factor.

Risk characterisation results for each microbiological hazard, for the general population, are listed in Table 3.

Table 3: Risk characterisation for the general population

Identified Hazard	Hazard Characterisation Module	Exposure Module	Risk Characterisation
<i>Bacillus cereus</i>	Negligible	Moderate	Low
<i>Brucella melitensis</i> *	Moderate	Low	Moderate (if introduced to Australia)
<i>Burkholderia pseudomallei</i>	Negligible	Very Low	Negligible
<i>Campylobacter jejuni</i>	Very low	Low	Low
<i>Clostridium perfringens</i>	Negligible	Moderate	Low
<i>Coxiella burnettii</i> **	Low	Low	Low
<i>Cryptosporidium parvum</i>	Very Low	Low	Low
Enterohaemorrhagic <i>E. coli</i>	High	Low	High
<i>Leptospira interrogans</i>	Negligible	Very Low	Negligible
<i>Listeria monocytogenes</i>	Negligible	Low	Very Low
<i>Mycobacterium avium</i> subs. <i>paratuberculosis</i> ***	Negligible	Moderate	Low
<i>Salmonella</i> spp.	Low	Low	Low
<i>Staphylococcus aureus</i>	Negligible	Moderate	Low
<i>Streptococcus</i> spp.	Negligible	Moderate	Low
<i>Toxoplasma gondii</i>	Very low	Moderate	Low
<i>Yersinia enterocolitica</i>	Negligible	Low	Very Low
<i>Yersinia pseudotuberculosis</i>	Negligible	Low	Very Low

* Organism not in Australian goat herds

** Foodborne transmission not proven

*** Role in human illness not confirmed

The hazard of most concern to the general population from the consumption of raw goat milk produced in Australia is EHEC. This organism has an overall risk characterisation of **high**. Of the remaining hazards, eight were characterised as being of **low** risk, three were rated as **very low** and two were rated as a **negligible** risk.

The risk characterisation for susceptible populations is outlined in Table 4. A number of hazards pose an overall higher risk to the susceptible population group than to the general population group. EHEC remains in the **high** risk category for susceptible populations, while *T. gondii* and *L. monocytogenes* also become **high** risk for susceptible populations. *Salmonella* spp. becomes a **moderate** risk and the remainder of the hazards fall within the **low** (n=6), **very low** (n=3) and **negligible** (n=1) categories.

Table 4: Risk characterisation for the susceptible population

Identified Hazard	Hazard Characterisation Module	Exposure Module	Risk Characterisation
<i>Bacillus cereus</i>	Negligible	Moderate	Low
<i>Brucella melitensis</i> *	Moderate	Low	Moderate (if introduced to Australia)
<i>Burkholderia pseudomallei</i>	Low	Very Low	Very Low
<i>Campylobacter jejuni</i>	Low	Low	Low
<i>Clostridium perfringens</i>	Negligible	Moderate	Low
<i>Coxiella burnettii</i> **	High	Low	High
<i>Cryptosporidium parvum</i>	Low	Low	Low
Enterohaemorrhagic <i>E. coli</i>	High	Low	High
<i>Leptospira interrogans</i>	Negligible	Very Low	Negligible
<i>Listeria monocytogenes</i>	High	Low	High
<i>Mycobacterium avium subs. paratuberculosis</i> ***	Negligible	Moderate	Low
<i>Salmonella</i> spp.	Moderate	Low	Moderate
<i>Staphylococcus aureus</i>	Negligible	Moderate	Low
<i>Streptococcus</i> spp.	Negligible	Moderate	Low
<i>Toxoplasma gondii</i>	Moderate	Moderate	High
<i>Yersinia enterocolitica</i>	Negligible	Low	Very Low
<i>Yersinia pseudotuberculosis</i>	Negligible	Low	Very Low

* Organism not in Australian goat herds

** Foodborne transmission not proven

*** Role in human illness not confirmed

An assessment of risk utilising this qualitative framework indicates that *Brucella* spp. pose a **moderate** risk to both general and susceptible populations in areas where the organism is present e.g. milk produced in locations where *B. melitensis* is endemic.

B. melitensis is an important infectious organism in dairy goats and a serious zoonoses. Although endemic in some countries the disease has not been reported in Australian herds. The Australian Quarantine and Inspection Service and Biosecurity Australia maintain import requirements for animal health and biosecurity issues. These import conditions are currently being reviewed by Biosecurity Australia for Dairy Products and include consideration of *Brucella* spp. It should be highlighted that were *Brucella* spp. to be imported into Australia in raw goat milk product, it would pose a **moderate** risk from consumption.

Coxiella spp. may pose a **low** risk to the general population and a **high** risk for susceptible populations if a definitive link was established for ingestion as a transmission route. *M. avium* subs. *paratuberculosis* would pose a **low** risk to both the general and susceptible population groups if there was a proven link between Johne's disease and Crohn's disease.

12.2 Comparison with previous risk assessments

As outlined in Section 4 and detailed in Appendix 7, risk assessments on raw goat milk were undertaken in Queensland, NSW and South Australia. These assessments were largely state specific although the South Australian assessment expanded on the other two risk assessments, by evaluating existing controls including the efficacy of microbiological testing programs and provided risk management options. Some differences exist in organisms considered between each risk assessment, e.g. *Burkholderia pseudomallei* is an organism limited to tropical regions of Australia and was only considered in the Queensland and NSW risk assessments.

The South Australian and Queensland risk assessments concluded that consumption of raw goat milk represents a medium to high risk for certain hazards for the general and susceptible populations. *C. parvum*, EHEC, *L. monocytogenes*, *Salmonella* spp. and *T. gondii* were identified in the South Australian risk assessment as posing a high risk for susceptible populations, whilst *C. jejuni/coli*, *Salmonella* spp. and EHEC were all rated as medium risk for the general population. The risk assessment for Queensland determined *E. coli* O157 and *L. monocytogenes* posed a medium risk to members of the general population. *E. coli* O157 and *L. monocytogenes* were a high risk and *S. aureus* toxins and *B. pseudomallei* were a medium risk to certain members of the susceptible population.

These conclusions are consistent with the results of this assessment, although the risk characterisation for EHEC in the general population is higher in this assessment. The lower risk characterisation of *B. pseudomallei* in this assessment would be a result of limited information available on infective dose and product contamination levels which feed into the qualitative framework. The New South Wales risk assessment declined to make any determination, qualitative or quantitative, on the risks associated with microbial hazards, although it did predict the possible magnitude of foodborne illness outbreaks for *Salmonella* spp., *S. aureus* and *L. monocytogenes*.

A direct comparison of the assessments is difficult as approaches varied from qualitative, through semi-quantitative to fully quantitative (Appendix 7).

There is a large degree of uncertainty in all assessments due to assumptions made, particularly in relation to consumption of raw goat milk. The NSW and Queensland assessments quantitatively estimated consumption based on sales/production estimates and population data. This produced a very conservative but definitive outcome which differs to both this assessment and the South Australian assessments.

South Australia and FSANZ characterised the risk of raw goat milk on a "per-serve" basis, whereas New South Wales and Queensland quantified risk as "individual risk" e.g. risk to the consumer.

For all assessments, risk was characterised for both the general and susceptible populations. Some assessments divided susceptible populations into subgroups e.g. infants,

immunocompromised and pregnant. Regardless of the degree of grouping, the overall characterisation of risk is comparable between assessments.

Consistent to all risk assessments was the lack of information available on raw goat milk within Australia. Particularly deficient is information pertaining to the incidence and level of pathogens in Australian goat milk, the amount of goat milk consumed, the identity of the consuming population and the incidence of illness associated with raw goat milk. More data in these areas would reduce the level of uncertainty and produce more accurate risk estimates.

12.3 Uncertainty and variability

In characterising the risk associated with consuming raw goat milk in Australia, the level of confidence in the final estimate of risk depends on the adequacy and quality of the available data. Variability is associated with biological systems, food processing technologies, food preservation methods and human behaviour and is therefore inherent. Uncertainty relates to assumptions which had to be made due to a lack of information. Details of the assumptions used in the qualitative framework are contained in Appendix 6.

There was a degree of uncertainty in each component of the qualitative framework due to limited data being available. Particularly in relation to:

- The prevalence and levels of pathogens detected in Australian raw goat milk
- The levels likely to cause illness in consumers (infective dose) *e.g. B. pseudomallei*
- The severity of illness within certain population groups
- Mode of transmission for some organisms *e.g. Coxiella spp.*
- The effect of processing

Where data was not available, assumptions derived following expert consultations were used to determine inputs to populate the framework. Consultations were primarily sought for infective dose/dose response information, severity of illness in general and susceptible populations and contamination levels for the raw goat milk. The qualitative framework inputs are detailed in Appendix 6: Table 1. Similarly, justifications for assigning raw product contamination levels are outlined in Appendix 6: Table 4.

The level of uncertainty in the model, due to the assumptions used gives an overall conservative estimate of risk. More data, particularly on the incidence and prevalence of pathogens in Australian raw goat milk would reduce this uncertainty and improve the level of confidence.

The exposure assessment module characterises exposure to the hazard based on the likely level of the hazard in the initial raw product and the effect of processing on the hazard.

Variability exists within the processes used for the production of raw goat milk. The type of milking system used *e.g.* hand milking or by various mechanised methods, may influence the level of bacterial contamination in the milk. The efficacy of different types of in-line cooling systems is also not considered in the model. For example, the open design of ripple coolers may not be as efficient at cooling milk as in-line plate heat exchangers and may also allow a greater likelihood of environmental contamination. No data was available on the efficacy of different milking or cooling processes used for Australian goat milk.

Uncertainty exists not only in the degree of contamination of the milk, but also within the effect of processing. Raw goat milk does not undergo any pathogen kill step, either at the packaging stage or the consumer end. For the purposes of the framework, it was assumed that there was no effect of processing. However, this assumes good agricultural, veterinary and processing practices and does not take into account any post-milking contamination, variability in contamination rates due to different milking systems or growth of pathogens which may occur due to poor hygiene practices and ineffective temperature control. Nor does it take into account the growth of any contaminating psychrotrophic pathogens.

Quantitative data was also not available on cold chain integrity from packaging, through storage and transport processes. Failure to maintain appropriate temperature control may allow the growth of pathogens. Even if correct temperature control is maintained, the framework does not take into account the ability of some pathogens to grow at refrigeration temperatures regardless of how and when they enter the raw milk.

The majority of assumptions used in the model introduce conservative estimates of risk to account for worst-case scenarios. The assumptions and variability pertaining to the effect of processing accounts for best-case scenarios and goes some way to offset the conservativeness of the earlier assumptions. More data for all components would assist to reduce the uncertainty and give greater confidence in the estimates of risk.

13 Discussion and summary

The production of raw goat milk in Australia is very small. Only 300,000 litres of an estimated 5.4 million litres of goat milk produced in Australia annually is marketed as raw milk. However, the volume of raw goat milk entering the market from unlicensed sources is unknown.

There is little information available on the consumption of goat milk and in particular raw goat milk. The 1995 National Nutrition Survey indicated 0.08% of the surveyed population consumed goat milk, although it is unknown what proportion of this, if any, was raw goat milk. A recent survey indicated less than 1% of the population consumes raw goat milk. Therefore, it can be assumed that consumption of raw goat milk is generally very low among the general population.

Raw goat milk is sold primarily through health food shops or farm gate sales and it appears there is a niche group of consumers who consume raw goat milk as their milk of choice. This niche group of consumers generally has strong beliefs in the perceived health benefits of raw milk and widely promote it as having restorative powers, especially as a cow milk replacement for babies. It has further been suggested that the niche consumer group has a high proportion of people with a lowered or less developed immunity to infection.

In Australia, illness arising from the consumption of raw goat milk appears to be rare with only two incidents of foodborne illness reported over the past 20 years. Internationally there have been 19 outbreaks of illness associated with the consumption of raw goat milk and 14 with raw goat milk cheese reported. The low level of reported foodborne illness associated with raw goat milk may give the impression this is a safe product, although it may simply reflect the generally low consumption in Australia and the overall underreporting of foodborne illness. The impression of safety must however, be balanced against the possibly high proportion of the consuming population who are within a susceptible population group.

Typical production of raw goat milk in Australia is undertaken using systems and practices similar to the cow dairy industry. Similarly, contamination of raw milk during primary production and along the processing chain is primarily by two means: pathogens shed directly into the milk via the udder, or via external contamination of the milk during or post harvest.

The key risk factors affecting the microbiological quality of raw goat milk are summarised in Table 2. The extent to which one risk factor is more important will be hazard specific, and could not be determined in this assessment due to a lack of quantitative through chain data.

Raw goat milk does not undergo any pathogen elimination or reduction step. Whereas pasteurisation represents the principal processing intervention to render other dairy products safe for consumption, the safety of raw goat milk is primarily dependent upon the control of risk factors to minimize the opportunity for microbiological hazards to contaminate raw milk. If raw milk does become contaminated, failure to maintain appropriate temperature control throughout storage, distribution and consumer handling may allow the growth of pathogens and increase risk. However, it should also be noted that some pathogens, including *L. monocytogenes* and *Y. enterocolitica* are able to grow at refrigeration temperatures.

Australian microbiological survey data show a very low incidence of hazards of public health significance in raw goat milk, although pathogenic bacteria including coagulase positive

S. aureus, *Campylobacter* spp., *E. coli* (including shiga-like toxin producing *E. coli*) and *Salmonella* spp. have been detected. The efficacy of current testing protocols was not specifically considered in this risk assessment. The amount of data received from state testing authorities, in conjunction with analysis made by Pointon *et al.*, (2004) indicate it is unlikely that the current microbiological sampling plans are adequate to detect the presence of pathogens. The lack of a requirement to hold batches of milk (prior to despatch and sale), pending the results of microbiological testing, increases the likelihood that raw milk containing pathogens may enter the marketplace and present a risk to public health.

In addition there is little published information available on the incidence and prevalence of pathogens in raw goat milk. Pathogens detected in raw goat milk in Australia are similar to those reported internationally and reflect those generally found in cow milk. Organisms include *S. aureus*, *Campylobacter* spp., *E. coli*, *Salmonella* spp., *Streptococcus* spp., *B. cereus*, *L. monocytogenes* and *Y. enterocolitica*. Pathogens not normally associated with cow milk, such as *B. pseudomallei*, *L. interrogans* and *T. gondii* may be more of a concern in raw goat milk. *Coxiella* spp. and *M. avium* subsp. *paratuberculosis* have also been reported internationally although the risk these organisms pose to foodborne illness in Australia is minimal. While *Brucella* spp. have been detected in raw goat milk internationally, it is exotic to Australia and therefore domestically it poses no risk of foodborne illness in Australia. Should the organism be introduced into the domestic supply chain, either in a raw goat milk product or through herd infection, the risk would then be substantial.

Table 5 provides a summary of microbiological hazards that have been associated with raw goat milk and their most likely source of contamination, along with the level of risk they pose as assessed using the qualitative framework.

Table 5: Summary of microbiological hazards associated with raw goat milk and risk to public health and safety

Organism	Likely route of contamination	Risk rating* (Total population unless otherwise stated)
<i>Bacillus cereus</i>	Predominantly an environmental contaminant of raw milk but can be associated with environmental mastitis.	Low
<i>Brucella melitensis</i> *	Etiological agent of environmental mastitis. Can be shed in both milk and faeces.	Moderate (if introduced to Australia)
<i>Burkholderia pseudomallei</i>	Environmental contaminant in tropical climates causing melioidosis in both animals and humans. Can be shed in both milk and faeces.	Negligible (general population) Very low (susceptible population)
<i>Campylobacter jejuni/coli</i>	Predominantly an environmental contaminant but can cause mastitis and be excreted in milk and faeces.	Low
<i>Clostridium perfringens</i>	Environmental contaminant. Organism shed in faeces.	Low
<i>Coxiella burnetii</i>	Foodborne transmission not proven.	Low (general population) High (susceptible population)
<i>Cryptosporidium parvum</i>	Environmental contaminant. Shed in faeces of infected animals.	Low
Enterohaemorrhagic <i>Escherichia coli</i>	Etiological agent of environmental mastitis. Can be shed in both milk and faeces. Environmental contaminant.	High
<i>Leptospira interrogans</i>	Primarily shed in urine but can be shed in milk.	Negligible

Table 5 cont: Summary of microbiological hazards associated with raw goat milk and risk to public health and safety

Organism	Likely route of contamination	Risk rating* (Total population unless otherwise stated)
<i>Listeria monocytogenes</i>	Shed directly in milk and via environmental contamination.	Very low (general population) High (susceptible population)
<i>Mycobacterium avium subs. paratuberculosis</i>	Role in human illness is not confirmed.	Low
<i>Salmonella</i> spp.	Predominantly an environmental contaminant of raw milk however can be present in milk during bacteremic phase and before diarrhoea commences.	Low (general population) Moderate (susceptible population)
<i>Staphylococcus aureus</i>	Highly prevalent contagious mastitis agent. Shed directly via udder and faeces. Environmental contamination.	Low
<i>Streptococcus</i> spp.	Contagious mastitis agent and shed directly in milk.	Low
<i>Toxoplasma gondii</i>	Environmental contaminant, however once infected, animals can shed organism in milk.	Low (general population) High (susceptible population)
<i>Yersinia enterocolitica</i>	Contaminant of raw milk.	Very low
<i>Yersinia pseudotuberculosis</i>	Predominantly a contaminant of milk however has been associated with mastitis.	Very low

* Organism is not in Australian goat herds.

For the general population group, this risk assessment indicates the majority of hazards pose a **very low** to **low** risk, although EHEC ranked as a **high** risk. There was an overall higher risk for hazards to the susceptible population group. The increased severity of illness and lower infectious dose required for pregnant women and the elderly resulted in *T. gondii* and *L. monocytogenes* becoming a **high** risk. EHEC remained **high** risk and *Salmonella* spp. became a **moderate** risk for susceptible populations.

14 Data gaps and areas for further research

During the preparation of the risk assessment, a number of data gaps were identified along the raw goat milk supply chain. Research into these data gaps may better assist in describing factors along the farm-to-table continuum that impact on the likelihood and magnitude of any illness resulting from consumption of raw goat milk in Australia.

Significant data gaps exist in the following areas:

- Data on the relative contribution of on-farm risk factors towards contamination of raw goat milk
 - Numerous on-farm factors have been identified as potential contamination sources of raw goat milk; however their relative impact, either singularly or in combination, has not been determined due to a lack of data. Examples of these factors include: animal health and welfare, housing, faeces, feed, water etc.
- Details on animal health and on-farm hygiene control measures
 - There is a lack of qualitative and/or quantitative data on the effect of on-farm control measures on pathogens (either individually or collectively) such as: animal health and mastitis programs, herd management practices, animal welfare, feed preparation, storage and distribution, water quality etc.
- The relative contribution of processing risk factors upon contamination of raw goat milk
 - The extent to which cross-contamination from the farm environment, the milking environment, personnel, storage and packaging etc impacts on the microbial status of raw goat milk.
- Processing control measures
 - Limited data exists on the effect of pre and post milking antiseptics, cleaning and maintenance protocols etc. and the contribution these have on the contamination of raw goat milk.
- The efficacy of different milking or cooling processes used for Australian raw goat milk
- Cold-chain management through packaging, storage and transport processes
- The prevalence of pathogens detected in Australian raw goat milk
 - Minimal surveillance of raw goat milk in Australia, as few States allow its production and sale, and also as a result of the limited testing schedules. Consequently, for those pathogens monitored, there is a low prevalence recorded for raw goat milk in Australia, whilst no domestic data exists for other pathogens.
- The level of pathogens in raw goat milk
 - Very few studies have quantified the contamination level in raw goat milk either domestically or internationally. Under the current testing protocol there is no requirement to quantify any positive detection of pathogens in raw goat milk. Levels of pathogens in raw goat milk will be affected by the source and quality of milk, method of milking, any cross-contamination and the time and temperature conditions during distribution and storage (potential for growth).

- The virulence and infectivity of some organisms as well as the zoonotic potential of certain organisms is not well documented
 - To populate the qualitative framework with input variables for these pathogens, assumptions were made based on expert consultations. Data on the infective dose and severity of illness was either limited or not available for a number of organisms including; *B. pseudomallei*, *L. interrogans*, *M. avium* subs. *paratuberculosis*, *Streptococcus* spp., *Y. pseudotuberculosis* and *T. gondii*. Further information is required on the dose-response information for these organisms. Plus information on the milkborne transmission of *Coxiella* and any causal link association between Johne's disease and Crohn's disease was also not available.
- Limited data currently exists on the frequency and amount of raw goat milk consumed as well as the demographics of the consuming population
 - Discussions with jurisdictional representatives and the industry suggests that raw goat milk is often consumed by individuals with lowered or less-developed immune responses, so further quantitative and/or qualitative data would assist in contextualising the impact of any illness resulting from consumption of raw goat milk.
- Extent and cause of sporadic human cases of raw goat milk associated foodborne illness
 - Outbreak data is not necessarily indicative of the true incidence and causes of sporadic raw goat milk associated foodborne illness. Attribution of sporadic cases is difficult due to factors such as the general under-reporting of foodborne illness, retrospective nature of foodborne illness investigation, the often non-point source nature of exposure and the low frequency of consumption.

Availability of the following data would also assist in more accurately estimating the impact and magnitude of any illness resulting following the consumption of raw goat milk in Australia per year:

- Levels of pathogens present in Australian raw goat milk both at the farm level and along the supply chain, including at retail sale
- Time and temperature conditions which raw goat milk is subject to throughout storage, distribution and retail sale
- The frequency and amount of consumption of raw goat milk, as well as the demographics of the consuming population

15 Conclusion

The assessment of risk associated with raw goat milk demonstrates that raw goat milk may be contaminated with a range of pathogenic microorganisms and pose a public health risk from consumption.

The safety of raw goat milk is primarily dependent upon: ensuring good animal health and welfare; preventing environmental contamination; strict adherence to good milking practices, including cleaning and drying of teats; rapid cooling of milk; strict control over milking parlour hygiene; and correct temperature conditions maintained throughout storage and distribution. This minimizes the opportunity for microbiological hazards to contaminate raw milk and reduces the likelihood that these hazards will proliferate if milk is contaminated. A failure to maintain strict control over these risk factors would be likely to significantly increase the risk to public health and safety from consumption of raw goat milk.

As raw goat milk does not undergo a pathogen elimination step, such as pasteurisation, the microbiological quality of raw goat milk will reflect the stringency of control exercised over these risk factors.

The consumption of raw goat milk in Australia is low, and reflecting this is the low number of reported foodborne outbreaks associated with its consumption. However, raw goat milk has been responsible for a number of outbreaks of foodborne illness overseas.

Using a qualitative framework, the principal risks to public health and safety from the consumption of raw goat milk are:

Organism	Risk rating using qualitative model
Enterohaemorrhagic <i>E. coli</i>	High (all populations)
<i>Salmonella</i> spp.	Moderate (susceptible population only)
<i>Toxoplasma gondii</i>	High (susceptible population only)
<i>Listeria monocytogenes</i>	High (susceptible population only)

The principal identified risk to public health and safety, for both general and susceptible populations from the consumption of raw goat milk is pathogenic *E. coli*. This estimation of risk is supported epidemiologically, as there have been five reported outbreaks of *E. coli* O157:H7 associated with the consumption of raw goat milk overseas. The risk of *Salmonella* spp. is estimated to be moderate for the susceptible population and there has been one outbreak in Australia attributed to the consumption of raw goat milk. For susceptible populations, *T. gondii* and *L. monocytogenes* are estimated to be high risk. Again, *T. gondii* has been epidemiologically linked with foodborne illness in a number of outbreaks overseas. The risk rating for *L. monocytogenes* is still considered conservative as the qualitative framework does not account for possible growth of the organism at refrigeration temperature. Although ranked as a low risk, *C. parvum* has been linked to one foodborne illness outbreak in Australia. It should also be highlighted that although *Brucella* spp. is presently exotic to Australia, were it to be introduced either in raw goat milk product or into domestic herds, it would pose a moderate risk following the consumption of raw goat milk.

While the volume of raw goat milk consumed in Australia is very low, raw goat milk poses a risk to both general and susceptible population groups who consume this product. The information available suggests raw goat milk is often provided to very young children,

children with special dietary needs, older people and people convalescing. These sub-populations are at-risk, and exposure to even low levels of microbial pathogens could result in serious illness.

APPENDICES

Appendix 1: Dairy Goat Industry

In 2003/04, around 5.4 million litres of goat milk was produced in Australia, with about 2.1 million litres (38%) entering the liquid whole milk sector, as well as some yoghurt production. Approximately 60% of the total volume of goat milk produced in Australia in 2003/04 (~3.4 million litres) went into the specialty cheese sector (Abud, 2005). This is an increase from the estimated 2.5 million litres of goat milk used for cheese in 2000/01 (Stubbs and Abud, 2002). Approximately 300,000 litres of raw goat milk was marketed in Australia during 2003.

The estimated retail market for specialty cheese and whole milk production in Australia is valued at approximately \$20 million and \$7 million dollars respectively. These products are increasingly becoming available in supermarket chains and specialty food outlets as well as the hospitality sector.

1. Production statistics

Approximately 65 commercial dairy goat farms are currently in operation across Australia, carrying close to 11,000 goats. The main goat milk producing States are Queensland, Victoria and Tasmania, with unpasteurised milk permitted for sale in South Australia, Queensland, New South Wales and Western Australia.

New South Wales has the majority of farms followed by Victoria and Queensland; however production is highest in Queensland at 1.47 million litres, then Victoria, Tasmania, South Australia, New South Wales and Western Australia with approximately 1.12 million, 1.1 million, 0.77 million, 0.6 million and 0.4 million litres, respectively.

Figure 1 illustrates the proportion of goat milk used for whole milk, cheese and yoghurt production in each state.

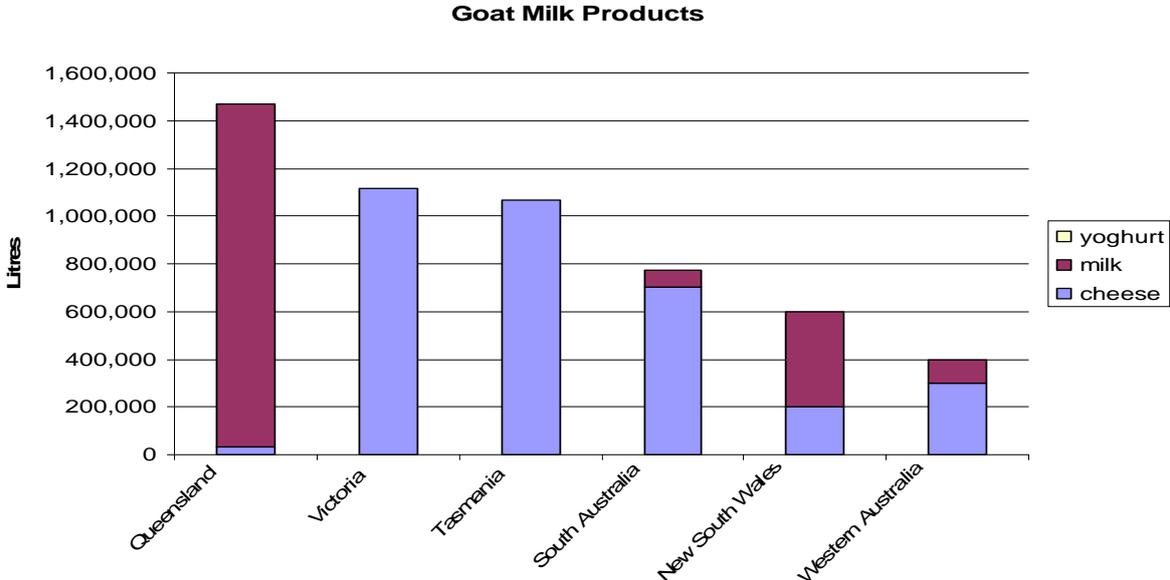


Figure 1: Proportion of goat milk products by state

Queensland produces around 27% of the total volume which is predominantly for whole milk sales (1.44 million litres). Victoria and Tasmania have similar product distribution with the bulk of milk used for cheese and a very small amount for yoghurt. The main usage of goat milk in South Australia and Western Australia is for cheese (0.7 and 0.3 million litres respectively), then whole milk with a little for yoghurt. Whole milk is the predominant product in New South Wales (0.4 million litres) followed by cheese and then yoghurt. In all States, the contribution of yoghurt to production volume is minimal (Abud and Stubbs, 2005)

The growth in supermarket sales of all goat milk (raw and pasteurised) over the last four years is depicted in Figure 2 (Herd Improvement and Producers Association, 2006).

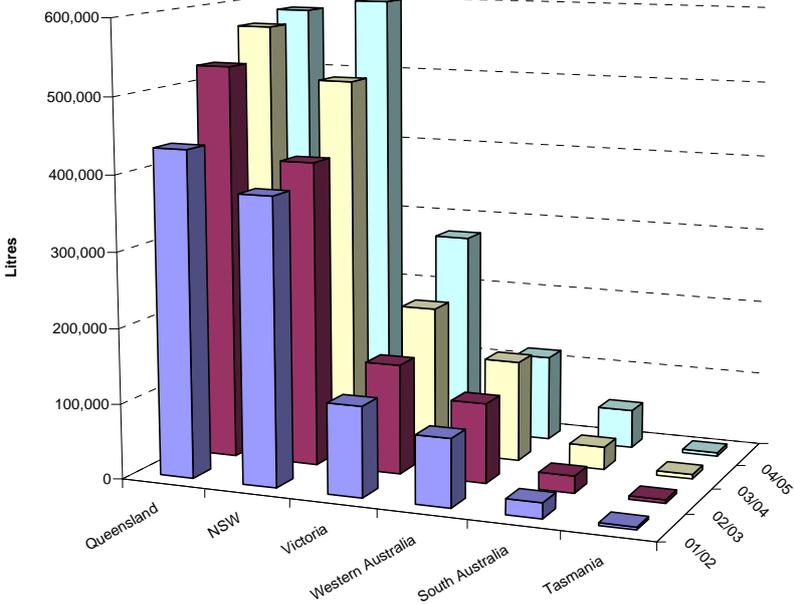


Figure 2: Goat milk supermarket sales by State

Appendix 2: Hazard identification / hazard characterisation of pathogens

1. *Bacillus cereus*

The genus *Bacillus* encompasses a great diversity of species and strains. *Bacillus cereus* are Gram-positive, facultatively aerobic spore-forming bacterium shaped as large rods and are motile by means of peritrichous flagella. *B. cereus* is widely distributed in the environment and is readily isolated from soil, dust, cereal crops, vegetation, animal hair, fresh water and sediments (ICMSF, 1996).

Growth characteristics

Strains of *B. cereus* vary widely in their growth and survival characteristics. Psychrotrophic strains are able to grow at 4 – 5°C but not at 30 – 35°C, whilst mesophilic strains grow between 15 - 55°C. The optimum growth temperature ranges from 30 – 40°C. The pH range at which growth will occur is 5.0 - 8.8 with an optimum of 6.0 - 7.0. The minimum water activity for survival and growth for *B. cereus* is 0.93 (ICMSF, 1996). The maximum salt concentration tolerated by *B. cereus* is 7% at pH 6 - 7 and 30 - 35°C (Jenson and Moir, 2003). Growth is optimal in the presence of oxygen but can occur under anaerobic conditions. Toxin production is reduced under anaerobic conditions (ESR, 2001).

Vegetative cells are relatively sensitive to environmental stress such as heat, chemicals, preservatives and radiation. However, *B. cereus* spores are more resistant due to their metabolic dormancy and tough physical nature (Jenson and Moir, 2003). Spores are more resistant to dry heat than moist heat. Spores can survive for long periods in dried foods. The heat resistance of *B. cereus* spores has been reported as $D_{85^{\circ}\text{C}} = 33.8 - 106$ minutes in phosphate buffer; $D_{95^{\circ}\text{C}} = 1.5 - 36.2$ minutes in distilled water and 1.8 - 19.1 minutes in milk. Thus, there is considerable strain variability, with D-values for spores of some *B. cereus* strains up to 15 to 20 times greater than for the more heat sensitive strains (ICMSF, 1996). Preservatives such as 0.26% sorbic acid at pH 5.5 and 0.39% potassium sorbate at pH 6.6 can inhibit growth. Nisin is inhibitory to *B. cereus*. Other antimicrobials which have an effect on *B. cereus* include benzoate, ethylenediaminetetraacetic acid and polyphosphates (Jenson and Moir, 2003). Spores are also more resistant to radiation than vegetative cells (Farkas, 1994).

Pathology of illness

There are two types of *B. cereus*-mediated intoxications. The two forms of illness are caused by significantly different toxins; diarrhoeal toxins (enterotoxins) and emetic toxins.

Diarrhoeagenic enterotoxins are formed in the small intestine following consumption of a large number of cells, which then results in illness. These toxins are heat labile, being inactivated in 5 minutes at 56°C (but not 45°C for 30 minutes). Four enterotoxins have been identified and characterised: two three-component enterotoxins (haemolysin BL and non-haemolytic); enterotoxin T; and a cytotoxin. The toxins are unstable at pH values outside the range 4 to 11 and sensitive to proteolytic enzymes (Jenson and Moir, 2003). Toxin activity is reduced after 1 to 2 days at 32°C, one week at 4°C and several weeks at –20°C (Andersson *et al.*, 1995). The incubation period for the diarrhoeal type of food poisoning is usually 10 - 13 hours post ingestion, although incubation periods from 8 – 16 hours have been reported. Gastroenteritis is usually mild, with abdominal cramps, profuse watery diarrhoea,

rectal spasms and moderate nausea, usually without vomiting. Recovery typically occurs within 24 hours.

The emetic toxin is preformed during *B. cereus* growth in foods, survives the gut environment and causes illness. It has been identified as a small ring form peptide of 1.2 kDa, called cereulide (Hui *et al.*, 2001), and is thought to be an enzymatically synthesised peptide (Granum and Lund, 1997). The emetic toxin is extremely resistant to heat and can survive 90 minutes at 126°C (ESR 2001). It is also very resistant to pH and proteolysis, but is not antigenic. Illness caused by the ingestion of emetic toxin generally has a short incubation period. Acute nausea and vomiting often occurs 1 - 5 hours post ingestion, with recovery within 12 - 24 hours. Diarrhoeal symptoms are not normally associated with the emetic illness.

Neither form of illness is considered life-threatening to normal healthy individuals; with very few fatal cases have being reported (Jenson and Moir, 2003). Humans may vary in their susceptibility to *B. cereus* illness. Since most strains of *B. cereus* have the potential to produce toxins, the severity of illness is dependent on the quantity and type of toxins produced (Notermans and Batt, 1998). In a small number of cases, both types of symptoms (diarrhoeal and vomiting) have been recorded, and this is probably due to the production of both types of toxin. Characteristics of the two types of illness caused by *B. cereus* are summarised in Table 1.

Table 1: Characteristics of the two types of illness caused by *B. cereus* (Granum and Lund 1997)

	Diarrhoeal syndrome	Emetic syndrome
Infective dose	10 ⁵ - 10 ⁷ (total)	10 ⁵ - 10 ⁸ (cells/g)
Toxin produced	In the small intestine of the host	Preformed in foods
Type of toxin	Protein	Cyclic peptide
Incubation period	8 - 16 h (occasionally >24 h)	0.5 - 5 h
Duration of illness	12 - 24 h (occasionally several days)	6 - 24 h
Symptoms	Abdominal pain, watery diarrhoea and occasionally nausea	Nausea, vomiting and malaise (sometimes followed by diarrhoea, potentially due to additional enterotoxin production)
Foods most frequently implicated	Meat products, soups, vegetables, puddings/sauces and milk/milk products	Fried and cooked rice, pasta, pastry and noodles

B. cereus has also been associated with non-foodborne non-gastrointestinal infections such as ocular and wound infections, bacteraemia, central nervous system infections, respiratory tract infections and endocarditis. Individuals who are immunocompromised, either by illness or medication, are more susceptible to all infections and illnesses caused by this organism (Hui *et al.*, 2001).

Mode of transmission

The enterotoxin (diarrhoeal syndrome) form of *B. cereus* poisonings is caused by the ingestion of a large number of cells and the subsequent production of the toxin in the small intestine. The emetic syndrome of *B. cereus* food poisoning occurs after the ingestion of food in which the organism has grown and formed toxin(s). Most documented reports of *B. cereus*

intoxication from this toxin have involved a cereal, or cereal- or spice-containing product as the food vehicle (ICMSF, 1996).

Incidence of illness

B. cereus food poisoning is not considered a reportable illness in most countries and therefore incidence data is limited (Granum and Lund 1997). However, France, Germany and the USA report less than 0.1 cases per 10,000,000 population per annum whereas Finland, Scotland, England/Wales, Hungary and Cuba all report more than 4.0 cases per 10,000,000 per annum (Jenson and Moir, 2003).

Within Australia, during the years 1977-1984, *B. cereus* was associated with 39% of foodborne illness incidents investigated in New South Wales, and this was mostly associated with fried rice (Jenson and Moir, 2003). In the period 1995 – 2000 there were 2 identified foodborne outbreaks (total of 28 cases) due to *B. cereus* in Australia (Dalton *et al.*, 2004). In 2002 there were two outbreaks, one involving 37 people and the other (in combination with *S. aureus*) involving approximately 270 people (Anon, 2003). A further outbreak was identified during 2004 involving 6 cases (Anon, 2005). It is recognised however, there may be significant under reporting of *B. cereus* illness due to the generally mild, short duration, self-limiting symptoms, in addition to it being infrequently tested for in routine laboratory analyses of stool samples.

Outbreaks of emetic-type illness have resulted from consumption of rice products or starchy foods (such as potato or pasta) that have been cooled slowly and stored incorrectly. Fried or cooked rice has been implicated in approximately 95% of cases with emetic symptoms and only a small proportion of cases have been attributed to the consumption of other foods such as crumpets, vanilla slices, cream and pasta (Kramer and Gilbert, 1989; Lee, 1988). A wide range of foods have been associated with the diarrhoeal syndrome, including meat-based dishes, soups, vegetables, puddings and sauces (Kramer and Gilbert, 1989).

Powdered milk used in the preparation of vanilla slices, a milk-gelatine dessert and macaroni cheese was indicated as the source of the *B. cereus* contamination contributing to outbreaks involving these foods (Holmes *et al.*, 1981; Pinegar and Buxton, 1977; Anon, 1977). A foodborne outbreak involving 35 neonates was linked to *B. cereus* in powdered milk in Chile (Cohen *et al.*, 1984). Levels of *B. cereus* detected in the powder ranged between 50 - 200 spores/g. However, analysis of preparation methods revealed a certain degree of time and temperature abuse. No further cases were detected following changes to preparation systems of infant formula.

Occurrence in foods

B. cereus is distributed widely in the environment and hence foods are often contaminated, particularly raw foods of plant origin. Cereal products are often a source, but numbers are rarely high. Rice is a well recognised source, with most samples containing low levels of the organism. Spices may also frequently be contaminated with *B. cereus* (Jenson and Moir, 2003).

A survey by Nygren (1962) of the incidence of *B. cereus* in food materials revealed that 52% of 1546 food ingredients, 44% of 1911 cream and dessert dishes, and 52% of 431 meat and vegetable products were contaminated, illustrating the widespread distribution of *B. cereus*.

A study of milk and dairy products showed contamination rates of 9 - 48% and UHT-treated milk was contaminated in approximately 50% of samples (ICMSF, 1996). The available data indicates that under normal circumstances, *B. cereus* is found in food at concentrations $<10^3$ cfu/g and mostly $<10^2$ cfu/g (ICMSF, 1996). The presence of *B. cereus* in processed foods results from contamination of raw materials and the subsequent resistance of spores to heat treatment processes undergone during manufacture.

Virulence and infectivity of B. cereus

The pathogenic mechanism for the emetic toxin has been elucidated. The emetic toxin is a dodecadepsipeptide named cereulide, and causes vacuole formation in Hep-2 cells and emesis.

The pathogenic mechanism for the diarrhoeal form of illness has not been clearly elucidated, although it is known that at least four different enterotoxins are involved (Jenson and Moir, 2003). One of these enterotoxins, Haemolysin BL, consists of three protein components (L2, L1, and B), and causes the destruction of red blood cells. The second enterotoxin, non-haemolytic enterotoxin, also consists of 3 protein moieties (B, L1 and L2) and all components are needed for maximum cytotoxicity. Both of these enterotoxins have been responsible for outbreaks of diarrhoeal food poisoning. The third enterotoxin, Enterotoxin T, consists of a single protein that is cytotoxin positive in the mouse ileal loop assay and possesses vascular permeability activity but does not appear to be involved in food poisoning (Hui *et al.*, 2001). The role of enterotoxin T is unclear (Jenson and Moir, 2003). Lund *et al.* (2000) identified the fourth enterotoxin which is a single cytotoxin protein (CytK). CytK is necrotic and haemolytic. This toxin was implicated in a severe food poisoning outbreak in France resulting in three deaths (Lund *et al.*, 2000).

Since diarrhoeal enterotoxins are unstable and are inactivated by low pH and digestive enzymes, any preformed toxins should be destroyed during passage through the stomach and are not likely to cause illness (Notermans and Batt 1998; Granum and Lund 1997).

Other potential virulence factors associated with diarrhoeal illness that have been identified include sphingomyelinase, phosphatidylinositol- and phosphatidylcholine-specific phospholipases and haemolysins I and II (Jenson and Moir, 2003).

The involvement of intestinal receptor site(s) for the tripartite enterotoxins in diarrhoeal symptoms has not been fully elucidated. It has been postulated that the enterotoxins disrupt the membrane of epithelial cells (Notermans and Batt 1998). The mechanisms for cereulide synthesis are also unclear, but data suggest the peptide is enzymatically produced (Hui *et al.*, 2001).

Dose response

Kramer and Gilbert (1989) have summarised a large number of outbreaks caused by *B. cereus*. The concentration of *B. cereus* in foods implicated in diarrhoeal illness ranged from $1.2 \times 10^3 - 10^8$ cfu/g. It has also been reported that 10% of outbreaks have been associated with foods containing less than 10^5 cfu/g (Kramer and Gilbert, 1989). It has been found that concentrations of *B. cereus* of $10^3 - 10^5$ cfu/g can result in illness in infants or aged and infirm individuals, although such illness was rare (Becker *et al.*, 1994).

A study reported that concentrations ranging from 200 to 10^9 cfu/g (or /ml) of *B. cereus* have been reported in foods implicated in food poisoning, giving total infective doses ranging from about 5×10^4 to 10^{11} organisms (Granum and Lund 1997). Partly due to the large differences in the amount and type of enterotoxin produced by different strains, the total infective dose seems to vary between about 10^5 and 10^8 viable cells or spores. Thus it was suggested that an average serving of food containing more than 10^3 *B. cereus*/g cannot be considered completely safe for consumption. It has also been suggested that the infectious dose for *B. cereus* may vary from about 1×10^5 to 1×10^8 viable cells or spores (Rowan *et al.*, 1997; Notermans and Batt 1998). Notermans and Batt (1998) also suggest food servings containing greater than 1×10^4 *B. cereus*/g may not be safe for consumption. From the available data it is estimated that the minimum total infectious dose is 10^5 viable cells or spores.

Immune status

All people are believed to be susceptible to *B. cereus* food poisoning. *B. cereus* has the potential to cause mild food-poisoning which does not, as a rule, last more than 12 - 24 hours. However, some individuals, especially young children, are particularly susceptible and may be more severely affected (ICMSF, 1996). Infants, therefore, may be susceptible to illness from a lower infectious dose, but there is no available data to support this.

Food matrix

The impact of the food matrix on the heat resistance of spores has been investigated. *B. cereus* spores are moderately heat resistant, however resistance is increased in high-fat and oily foods (e.g. for soybean oil, $D_{121^\circ\text{C}} = 30$ minutes) and in foods with lower water activity (Jenson and Moir, 2003).

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2. *Brucella melitensis*

Brucella spp. are non-motile, short, Gram-negative coccoid to short rod-shaped cells which grow aerobically. They are catalase-negative and usually oxidase-negative (ICMSF, 1996). *Brucella* spp. are pathogenic for both humans and a wide range of animals and are often located intracellularly in infected animals (Tantillo *et al.*, 2001). Until recently bovine brucellosis was present throughout the world with areas of high incidence including the Mediterranean, Middle East, South America and some areas of Asia (Corbel, 1997). However, a number of countries have now succeeded in eradicating this disease. Australia declared freedom from bovine brucellosis (*Brucella abortus*) in 1989 and there have been no recurrences of the disease since that time (Whittem, 1978; Mylrea, 1991; Cousins and Roberts, 2001).

Brucella melitensis (along with *Brucella abortus*, *Brucella suis* and *Brucella ovis*) is a major cause of brucellosis in sheep and goats. The disease affects mainly adult female animals, causing abortion and udder infections. It is a serious zoonosis and is more pathogenic to human than other *Brucella* spp. *B. melitensis* infection has never been reported in sheep or goats in Australia (Animal Health Australia, 2005). However, overseas travellers occasionally arrive in Australia suffering from *B. melitensis* infection and since the organism is excreted in the urine of infected humans, infection of sheep and goats from this source is a possibility, although highly unlikely.

Growth and survival characteristics

Whilst the optimum temperature for growth on artificial media is 37°C, *Brucella* spp. can grow at temperatures between 20 - 42°C (ICMSF, 1996). There is some discrepancy regarding what time and temperatures are adequate to kill these bacteria. A time/temperature combination of 75 minutes at 85°C was necessary to kill all 40 tested strains of *B. abortus* (Swann *et al.*, 1981).

Survival of *Brucella* spp. in milk and milk products declines with increasing storage temperatures. Brucellae at a concentration of 8×10^9 cfu/ml survived for 800 days at -40°C, but were eliminated within 2 days at 25°C (Kuzdas and Morse, 1954). An increase in storage temperature from 2 - 4°C to 18 - 22°C reduced survival time by approximately 50% for *Brucella* spp. in Egyptian white cheese of the Domiati and Tallaga variety (Salem *et al.*, 1977). In addition, the high fat content of products may have a protective effect.

A sodium chloride content of 4% will prevent growth of *B. melitensis* on liver agar (Lerche *et al.*, 1960). The survival rate of *Brucella* spp. appears to decrease with increased sodium chloride in milk products. A survival time of 6 months was reported for salted butter (2.3% NaCl), whereas in unsalted butter *Brucella* spp. remained viable for 13 months (ICMSF, 1996). However, *Brucella* spp. may resist high salt concentrations at lower temperatures. A survival time of 45 days was reported for *Brucella* spp. in a sheep cheese brine containing 27% salt and stored at a temperature of between 11 - 14°C (ICMSF, 1996).

There is a positive correlation between the survival of *Brucella* spp. in different cheeses and the water content of the cheeses. *Brucella* spp. survived six days in hard cheese (Emmentaler and Gruyer: water content of 35 - 36%), 15 days in Tilsit cheese (water content of 39 - 41%), 20 days in 'quarterfat' round cheese (water content 41 - 45%) and 57 days in soft cheeses (Munster and Camembert: water content 50%).

The optimum pH for growth in artificial media for all *Brucella* spp. is between 6.6 - 7.4 at 37°C (Gerhardt, 1958; Corbel and Morgan, 1982). The upper growth limit is between pH 8.4 (Zobell and Meyer, 1932) and 8.7. Huddleson(1954) reported a lower growth limit of between 5.8 - 6.8, whilst Lerche and Entel(1959) reported a lower growth limit of between pH 4.1 - 4.5.

Pathology of illness

Brucellosis is a significant public health problem in endemic areas such as the Mediterranean region, western Asia, parts of Africa, the Indian subcontinent and Latin America (Kasimoglu, 2002). The signs and symptoms of foodborne illness associated with *B. melitensis* include fever, chills, sweating, weakness, headache, muscle and joint pain, diarrhoea and bloody stools during the acute phase (CDC, 2003; CDC, 2004). The incubation period ranges from 7 - 21 days, with the duration of illness being in the order of weeks. Treatment is usually with a combination of antibiotics such as tetracyclines, streptomycin and sulphonamides/trimethoprim (ICMSF, 1996).

Mode of transmission

Zoonotic transmission from infected animals to humans may be either via direct or indirect transmission (Kasimoglu, 2002). Direct transmission occurs via close contact with infected animals and involves the respiratory, conjunctival and cutaneous routes. Airborne transmission of *Brucella* spp. is often associated with occupational exposure to infected animals. Indirect transmission to humans is generally foodborne, and is often associated with consumption of raw milk and raw milk products (Lin *et al.*, 2002).

Incidence of illness

There have been several outbreaks of brucellosis in humans in various parts of the world, these were often caused by the consumption of contaminated raw milk or raw milk products (Eckman, 1975; Young and Suvannoparrat, 1975; Acedo *et al.*, 1997; Wallach *et al.*, 1997; Altekruise *et al.*, 1998; Mendez *et al.*, 2003; Arimi *et al.*, 2005).

During the years 2000 - 2004 there were 139 cases of human brucellosis notified in Australia. The primary source of these infections, in Australia, is thought to be due to overseas infections (often from the consumption of unpasteurised dairy products) or may be related to the feral pig population in northern Queensland (*B. suis*) (Lin *et al.*, 2002; Blumer *et al.*, 2003; Yohannes *et al.*, 2004; Miller *et al.*, 2005; Yohannes *et al.*, 2006).

Occurrence in foods

Brucella spp. are most commonly transmitted via raw milk or raw milk products, such as cheeses (Kasimoglu, 2002). Ewes and goats milk has been found to be a more significant source of *Brucella* spp. than cow's milk. Raw milk, goat cheese made from unpasteurised milk, and contaminated meats are the foods most commonly associated with foodborne transmission.

Virulence and infectivity

Brucella spp. can infect and multiply in both phagocytic and non-phagocytic cells (Sarinan and Chitkara, 2003). The exact mechanism of *Brucella* spp. pathogenesis is not fully understood, as no cell components specifically promoting cell adhesion and invasion have been characterised (Corbel and Morgan, 1982).

Dose response

There is no quantitative data on the infective dose (ICMSF, 1996). Precise information is lacking on the minimal effective oral dose of *Brucella* spp., but it is estimated that inhalation of 10 - 100 bacteria is sufficient to cause disease in humans (Kasimoglu, 2002).

Host factors

Individuals who are generally at greater risk of infection and/or risk of developing more severe outcomes from exposure to *Brucella* spp. include the very young, the elderly, pregnant women and the immunocompromised (organ transplant patients, cancer patients, AIDS patients).

Food matrix

Brucella spp. are unlikely to multiply in food kept under hygienic conditions, and are controlled most effectively by eliminating infected subjects from the animal stock. Pasteurisation or sterilisation of milk pre-market is sufficient to prevent milk-borne brucellosis (ICMSF, 1996). The combined effect of reduced water activity and pH has been found to reduce and/or eliminate *Brucella* spp. during the production of hard cheeses; however, *Brucella* spp. may survive conditions during the production of other types of cheeses such as soft cheeses.

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3. *Burkholderia pseudomallei*

Burkholderia pseudomallei (previously named *Pseudomonas pseudomallei*) are Gram-negative bacteria often present in soil and surface water of tropical regions. This organism is the aetiologic agent of melioidosis (also called Whitmore's disease); an animal and human disease predominantly found in tropical climates. It has been identified as being endemic in northern Australia, the South Pacific and South East Asia. It is however generally only found within 20° either side of the equator (Munckhof *et al.*, 2001; Howe *et al.*, 1971). Infection occurs in a wide range of animal species, with sheep and goats being particularly susceptible (Choy *et al.*, 2000).

Growth characteristics

B. pseudomallei is an environmental saprophyte which is a oxidase-positive, bacillus. It is a strict aerobe, motile, and can break down arginine. It is easily cultured on blood or nutrient agar incubated at a temperature of 37 - 42°C and shows corrugated, wrinkled, and dry looking colonies in around 1 - 2 days. It may also develop an orange pigmentation on prolonged incubation. *B. pseudomallei* are resistant to aminoglycosides and polymyxins (Thomas *et al.*, 1988a). *B. pseudomallei* is unable to grow at temperatures below 21°C and requires high moisture levels, however it has been known to survive in soil and water samples for up to 36 months (Dance, 2000).

Pathology of illness

B. pseudomallei have the ability to cause disease, melioidosis, in humans as well as in a wide range of animal species. Melioidosis is generally a disease only seen in tropical and sub-tropical regions, predominately during the wet season. The most commonly affected animals in Australia include goats, sheep, pigs, alpacas, camels, horse and deer, with goats and sheep being particularly susceptible with cases of infection often eventuating in the death of the animal (Choy *et al.*, 2000). Wildlife can also be affected with species such as birds, crocodiles and kangaroos having presented with signs of melioidosis disease previously (Choy *et al.*, 2000).

Infections can manifest in a wide variety of clinical symptoms and may involve a latency period. Depending on the mode of transmission melioidosis may manifest as mastitis (especially in goats), pulmonary infection or lung lesions, septicaemia, chronic suppurative infection or localised skin infections. These manifestations may range from acute to chronic and may be also associated with sub-clinical infections in both animals and humans (Choy *et al.*, 2000).

Mode of transmission

Infection may occur through inhalation or ingestion of the organism, or through contamination of penetrating injuries or skin wounds with dust particles or water containing viable *B. pseudomallei* organisms (Choy *et al.*, 2000).

It is assumed that the majority of infections occur through direct contact of skin wounds and abrasions to contaminated soil and/or water (Dance 2000). Zoonotic transmission is also believed to be a possible means of transmitting *B. pseudomallei*, with evidence that viable

organisms are found in infected individual's (animal and human) pus, sputum, urine and faeces, possibly leading to contamination of the individual's surroundings.

It has been suggested that there is a possible public health risk from drinking contaminated milk from an animal infected with the disease melioidosis (Thomas *et al.*, 1988a; Thomas *et al.*, 1988b; Choy *et al.*, 2000). Rarely has person to person transmission (Dance 2000) or transmission in human breast milk been shown to occur (Ralph *et al.*, 2004).

Incidence of illness

The average annual human incidence of melioidosis in Australia is estimated at 5.8 cases per 100,000 population per annum depending on the rainfall during the year (Cheng *et al.*, 2003), however in the Northern Territory the incidence is estimated at around 16.5 cases per 100,000 population per annum, with one particularly prolonged rainy season in 1997-1998 seeing 34.5 cases per 100,000 (Currie *et al.*, 2000). These cases are thought to be caused by skin contact with contaminated soil and/or water with the risk of infection greatly increasing with rainfall. Melioidosis is a notifiable disease in the Northern Territory.

Two outbreaks in Northern Australia have been attributed to ingestion of contaminated drinking water sourced from the community water supply (Inglis *et al.*, 1999; Cheng *et al.*, 2003).

Only three possible zoonotic cases (direct transmission from animal to human) of melioidosis have been thought to have occurred in Australia, however these manifested as skin infections and were not due to ingestion of the organism (Choy *et al.*, 2000).

Occurrence in foods

B. pseudomallei can be an environmental contaminant and thus may be present on the surface of water-contaminated fruit and vegetables. *B. pseudomallei* has also been isolated from infected goat's udders (Van der Lugt and Henton, 1995) and is excreted in goat faeces (Dance 2000); which suggests that contamination of raw goat's milk is a possibility. However no information is available on the occurrence of *Burkholderia pseudomallei* in raw goat's milk or in any other food source.

Virulence and infectivity

Melioidosis is known to be a major cause of human morbidity and mortality in the Australian tropics (Dance 2000). There were 12 human deaths out of 33 infections during one outbreak in the Northern Territory during 1990 and 1991. It is however unknown how these infections were acquired and are not thought to be via ingestion (AgriQ, 2000). There is a complete lack of data on virulence and infectivity for *B. pseudomallei* obtained via ingestion.

Dose response

There is no information available on the dose-response relationship for *B. pseudomallei* in human or animal infections.

Host factors

Risk factors for human melioidosis include diabetes, high alcohol intake and renal disease (Inglis *et al.*, 1999; Cheng *et al.*, 2003).

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4. *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°C. *C. jejuni* can grow in the temperature range of 30 - 45°C, pH of 4.9 - 9.5 and water activity above 0.99. At 32°C, *C. jejuni* may double its biomass in approximately 6 hours (Forsythe, 2000). *Campylobacter* spp. do not multiply at temperatures below 30°C, which means that the numbers of *Campylobacter* in foods will not increase at normal room temperatures (20 - 25°C). Although unable to grow below 30°C, *Campylobacter* remain metabolically active, are able to generate ATP, and are motile at temperatures as low as 4°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°C for several minutes readily destroys *Campylobacter* spp. The D value for *C. jejuni* at 50°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. *Campylobacter* spp. 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°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-labile 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 an infection by *Campylobacter* spp.

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 AIDS 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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5. *Clostridium perfringens*

Clostridia are generally anaerobic, Gram-positive, spore forming bacteria that are considered to be saprophytes. They are widely distributed in the environment and only a few species are known to be pathogenic to humans. *Clostridium perfringens* and *Clostridium botulinum* are thought to be the principal species likely to be transmitted via the foodborne route.

C. perfringens is considered to be microaerophilic, widely distributed in soils and vegetation and is part of the normal intestinal flora of humans and animals. *C. perfringens* are grouped into five types (A - E) according to the particular soluble antigens (exotoxins) produced (Labbe, 1989). Only types A, C and D are pathogenic to humans, and only types A and C have been associated with foodborne illness (Bates and Bodnaruk, 2003). *C. perfringens* types A and C also produce an enterotoxin (*Clostridium perfringens* enterotoxin) which is associated with the acute abdominal pain, nausea and diarrhoea of *C. perfringens* foodborne illness.

Growth characteristics

C. perfringens requires anaerobic or microaerophilic conditions for growth. Cells of *C. perfringens* will grow between 12 - 50°C, with an optimum temperature of 43 - 45°C (Solberg and Elkind, 1970; Labbe, 1989). This organism is capable of rapid growth. Generation times as short as 7.1 minutes at 41°C were reported in a study of a number of strains, with an average generation time of 13 minutes at 40°C (Willardsen *et al.*, 1978). Vegetative cells die rapidly below 10°C. In experiments in laboratory media it has been shown that the thermal resistance of vegetative cells increases as the growth temperature increases (Roy *et al.*, 1981). It has also been suggested that temperature stability is enhanced in foods, perhaps due to a protective effect of fats (Bradshaw *et al.*, 1977; Labbe, 1989). Optimum pH for growth is in the range 6.0 - 7.0, with growth inhibited below pH 5.5 and cell death occurring slowly below pH 5.0. Growth is also inhibited below a water activity of 0.93 (Bates and Bodnaruk, 2003).

In general, conditions for sporulation are more limited than for growth. The optimal temperature range is between 35 - 40°C, and a pH between 6.0 - 8.0 (Labbe and Duncan, 1974). The water activity must be above 0.98 in order for sporulation to occur (Labbe, 1989). A large amount of enterotoxin formation accompanies sporulation, so the optimal conditions for sporulation and enterotoxin formation are similar. In foodborne outbreaks, sporulation occurs primarily in the small intestine (Labbe, 1989). There is a wide range of thermal resistance in spores of *C. perfringens* strains. In water, D_{90°C} can be as long as 27.5 minutes (Adams, 1973), and thermal stability is greater in cooked meats than in water (Collee *et al.*, 1961). Germination in some strains of *C. perfringens* is improved by a moderate heat shock, in the range of 65 - 80°C, usually for up to ten minutes (Labbe, 1989). Strains implicated in food poisoning are more likely to require heat-activation of germination.

Pathology of illness

Symptoms of *C. perfringens* food poisoning include diarrhoea and abdominal cramps (sometimes severe), typically without fever being present. There is normally no vomiting, shivering, headache or nausea. Onset of symptoms is usually within 8 - 24 hours after ingestion, and full recovery occurs within 24 - 48 hours. Unlike other toxin-mediated

foodborne pathogens, toxin production occurs after the organism has been ingested, and is excreted during the process of sporulation (Bates and Bodnaruk, 2003).

Symptoms of enteritis necroticans, caused by *C. perfringens*, include abdominal pain and swelling, vomiting, profuse and often bloody diarrhoea, and patchy necrosis of the upper small intestine that can lead to obstruction requiring surgical intervention. This illness can be fatal (Millar, 1989).

Mode of transmission

C. perfringens is transmitted by the faecal-oral route and by contamination of food from the environment. *C. perfringens* produces spores which vary in their heat resistance. Those spores which are highly heat resistant will be more likely to cause food poisoning due to survival and subsequent outgrowth during and after cooking. The food vehicles are usually cooked meat and poultry dishes stored for long periods of time at ambient temperature after cooking (Millar *et al.*, 1985; Millar 1989).

Spores may survive normal cooking procedures, with germination being triggered by the heat shock received during cooking. Slow cooling and non-refrigerated storage can permit growth of vegetative cells to high numbers, particularly in anaerobic environments within food such as in cooked meat and poultry dishes. The high number of vegetative cells produced under these conditions allows some to survive through the acidic environment of the stomach to reach the intestine, where sporulation is accompanied by production of the enterotoxin (Foster, 1978; Brynestad and Granum, 2002).

Incidence of illness

Outbreaks of *C. perfringens* food poisoning are usually associated with inadequately heated or reheated meats, pot pies, stews or gravies. Spores become activated by the temperature shock of cooking, and if the food is not cooled to below 15°C rapidly enough, vegetative cells are able to rapidly multiply to high levels as competing bacteria are greatly reduced in numbers by the cooking process (Brynestad and Granum, 2002).

A summary of the epidemiology of foodborne disease outbreaks in Australia from 1995 - 2000 reported that *C. perfringens* was the responsible agent in 30 outbreaks (14% of 214 identified outbreaks) involving 787 cases (10% of the total reported foodborne illness cases) and 1 death (Dalton *et al.*, 2004). The median number of cases per outbreak was 25, with a range from 2 - 171. Meats were the food vehicles in 60% (18/30) of those outbreaks. The outbreak settings were approximately equally split between restaurants, commercial caterers, institutional and 'other' settings. Dairy products were implicated in one outbreak (27 cases). In 2001 - 2005 OzFoodNet, Australia's enhanced foodborne disease surveillance network, catalogued a further 20 outbreaks of *C. perfringens* food poisoning involving 424 cases. Dairy products were not implicated in any of these outbreaks (Anon, 2002; Anon, 2003; Anon, 2004b; Anon, 2005).

The US Centres for Disease Control and Prevention (CDC) listings of foodborne disease outbreaks for 1990 - 2002 (CDC, 2003), as reported to CDC through the Foodborne Disease Outbreak Surveillance System, demonstrate that *C. perfringens* was responsible for about 6% of outbreaks (10% of cases) of foodborne illness of confirmed aetiology during that period. The number of outbreaks due to *C. perfringens* ranged from 10 - 30 each year.

Approximately 70% of the *C. perfringens* outbreaks were attributable to meat products or dishes. One outbreak (1995: 9 cases) was due to hard cheese and one was due to white sauce (1997: 7 cases). Vegetable dishes are only rarely implicated in outbreaks of *C. perfringens* poisoning. In an analysis of several databases, only one outbreak due to *C. perfringens* relating to a vegetable product was identified in the period 1969 - 1998 (Roach and Sienko, 1992; Carlin *et al.*, 2000).

Outbreaks are often in institutional or mass-catering settings, where the large volumes of food prepared and/or inherent difficulties in maintaining appropriate standards of hygiene and sanitation may lead to improper cooking, cooling, holding and handling of potentially hazardous food. Because of the specific conditions leading to sporulation and growth of *C. perfringens* to high levels, it is believed that relatively few sporadic cases occur.

There is some data on the incidence of enteritis necroticans (also known as pigbel or darmbrand) due to *C. perfringens*. The disease is most commonly encountered in developing countries and is associated with poor nutrition and protein-poor and/or trypsin-inhibitor rich diets. These conditions allow for survival of the β -toxin of type C strains, a protein which is usually rapidly proteolysed in healthy and well-nourished individuals (Millar 1989).

Occurrence in foods

C. perfringens spores and vegetative cells are likely to be present in uncooked foods of animal origin, vegetables exposed to soil, dust or faecal material, and in some dried spices (ICMSF, 1996). During the mid-1990s, the Food Safety and Inspection Service of the US Department of Agriculture conducted a number of surveys of the microbiological status of raw meat products. The results for *C. perfringens* showed a high prevalence of contamination in poultry meat products, at relatively low levels, while for pork and beef the prevalence was lower but the level of contamination was generally higher (Anon, 2004a).

C. perfringens contamination has been found at relatively high prevalence, but usually at low levels, in some dried spices (ICMSF, 1998; Banerjee and Sarkar, 2003). A review of the scientific literature on the incidence of pathogenic spore-forming bacteria (including *C. perfringens*) in vegetables, spices and foods containing vegetables found that of 4040 samples, 3998 had $<2 \log \text{ cfu/g } C. perfringens$, and the remaining 42 samples had less than $5 \log \text{ cfu/g}$ (Carlin *et al.*, 2000).

Virulence and infectivity

There are four major *C. perfringens* exotoxins, α , β , ϵ and ι (iota), and eight minor ones. All strains produce the α -toxin, a phospholipase C (lecithinase C) which causes enzymatic degradation of bilayer phospholipids (Bernheimer and Rudy, 1986) leading to disruption of cell membranes and cell lysis of erythrocytes, leukocytes, platelets, fibroblasts, and muscle cells (Titball, 1993). Several of the other toxins possess enzymatic activities, including a protease (λ -toxin), a deoxyribonuclease (ν -toxin) and a collagenase (κ -toxin). The β -toxin is implicated as the necrotic factor in enteritis necroticans ('pigbel').

Dose response

Ingestion of a large number of vegetative cells is required to cause *C. perfringens* food poisoning. From outbreak investigations, it has been estimated that levels of around

10^6 to 10^8 cfu/g in implicated foods will cause illness (Bates and Bodnaruk, 2003). Volunteer feeding studies have suggested a total dose of 5×10^9 cells is required to cause illness (Hauschild and Thatcher, 1967). Ingestion of 8 - 10 mg of purified enterotoxin induces symptoms of gastroenteritis (Skjelkvale and Uemura, 1977a; Skjelkvale and Uemura, 1977b). However, food poisoning usually occurs from production of the enterotoxin in the gut, rather than ingestion of preformed toxin, so those levels may not represent a toxic dose under normal conditions of food poisoning.

Host factors

C. perfringens food poisoning may be more serious in the elderly and debilitated, but fatal cases are rare (Bates and Bodnaruk, 2003).

Food matrix

Germination and outgrowth of *C. perfringens* is enabled by the generation of microaerophilic environments in foods cooked for long periods of time with poor heat penetration and inadequate aeration, and/or prolonged holding of food at insufficient temperatures to prevent growth and/or toxin production (Bates and Bodnaruk, 2003).

It has been suggested that the temperature stability of *C. perfringens* vegetative cells is enhanced in foods, perhaps due to a protective effect of fats (Bradshaw *et al.*, 1977; Labbe, 1989).

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6. *Coxiella burnettii*

Coxiella burnettii is a Gram-negative-like (non-staining) species of rickettsia. The organisms are variously described as coccobacillus or rod-like and are of the size 0.2 - 0.4µm by 0.4 - 1.0µm (Weiss and Moulder, 1984). *C. burnettii* is distributed globally and is the causative agent of the zoonotic illness 'Q fever' (Vanderlinde, 2004a). The usual animal reservoirs of *C. burnettii* are cattle, sheep and goats. *Coxiella burnettii* is also carried by ticks (Weiss and Moulder, 1984), with transmission to animal hosts occurring through contact, blood sucking and contaminated tick faeces (Hilbink *et al.*, 1993). Infection in animals is usually subclinical but infected animals can shed large quantities of bacteria into the environment. Infected females can shed very large quantities during parturition and the bacteria can survive harsh environmental conditions.

Growth characteristics

C. burnettii is an obligate intracellular microorganism - it will not grow in foods or outside host cells. It is however able to survive in a desiccated form in soil and the environment for several months (Hilbink *et al.*, 1993). This may be due its ability to form spore-like structures (Marrie, 2003). *C. burnettii* has a high resistance to drying, elevated temperatures and chemical agents including many common disinfectants. Complete inactivation may not be attained at 63°C for 30 minutes, or at 85 - 90°C for a few seconds (Weiss and Moulder, 1984; Vanderlinde, 2004a).

Studies conducted with milk containing 100,000 guinea pig units (10 times that considered the maximum possible in cow's milk) became non-infectious when held at 62.7°C for 30 minutes, but holding milk at 61.6°C for the same period of time was insufficient to inactivate the organism. It is strongly recommended that products undergo pasteurisation at 72°C for 15 seconds to be sure of complete elimination of viable *C. burnettii* from whole raw milk (Enright *et al.*, 1957). *C. burnettii* is also able to retain viability at 4°C for one or more years in dried fomites such as tick faeces or in wool.

Pathology of illness

Of those people infected with *C. burnettii*, only about half develop clinical signs of illness (Kazar, 2005; Parker *et al.*, 2006). Symptoms of acute infection may include the sudden onset of one or more of the following: high fever, severe headache, general malaise, myalgia, confusions, sore throat, chills, sweats, non-productive cough, nausea, vomiting, diarrhoea, abdominal pain and chest pain (Vanderlinde, 2004a). If a fever is present, it may last 1 - 2 weeks. Longer term symptoms include persistent weight loss, pneumonia (30 - 50% of cases), abnormal liver function tests and hepatitis. The majority of patients will make a full recovery without any treatment. Tetracycline compounds are the antibiotics of choice for treatment if required (Weiss and Moulder, 1984). The mortality rate in patients with acute Q fever is 1 - 2%. Although uncommon, Q fever infection may persist beyond the acute phase of six months and develop into the more serious situation of a chronic illness. This may develop as soon as a year after initial infection, or may occur as long as 20 years later. The chronic form may manifest as endocarditis. Those at risk of developing chronic Q fever are those with a pre-existing valvular heart disease, vascular graft, other transplant patients, patients with cancer and those with chronic kidney disease. The mortality rate for patients with chronic Q fever is as high as 65% (Vanderlinde, 2004a).

Mode of transmission

Infection in humans usually occurs via inhalation of the organisms from air containing dust contaminated by dried biological fluids from infected herd animals. Ingestion of contaminated raw milk or raw milk products is also suggested as a route of transmission although this is considered a minor route for human infection (Maurin and Raoult 1999; Vanderlinde, 2004a).

Incidence of illness

Reliable estimates of the number of cases of Q fever worldwide are unavailable. This is due to the illness being rare and possibly under reported, with many human infections being subclinical (Vanderlinde, 2004a). Infected herd animals do not usually exhibit clinical disease. Abortion in goats and sheep may occur. Organisms are excreted in milk, urine and faeces. Additionally, high numbers of the organism are present in amniotic fluids and placenta during birthing (Maurin and Raoult 1999).

The incidence rate of Q fever in France is estimated at 50 cases per 100,000 population per annum (Maurin and Raoult 1999). The number of clinical cases of disease increased from one reported case in France in 1982, to 107 reported in 1990 (Tissot *et al.*, 1992). The majority (61%) of these cases presented with hepatitis, which is linked with oral exposure rather than aerosol exposure (Vanderlinde, 2004b). In 1985, five cases of hepatitis were reported from workers at a meat packing plant in California. Further investigation of the workforce found that 31 of the 42 persons tested were positive via serological testing for Q fever rickettsiae, with eight of these having recently experienced clinical symptoms of Q fever (MMWR, 1983). Exposure was concluded to be due to the handling of sheep carcasses.

The notification rate for Q fever in Australia 1999 - 2002 was between 2.7 - 3.9 cases per 100,000 population (Australian Institute of Health and Welfare, 2004). In Australia, the incidence rate was estimated to be between 3.11 - 4.99 cases per 100,000 inhabitants for the period 1991 - 1994, whilst the hospital morbidity data for 2001 - 2002 indicates a case rate of 1.3 cases per 100,000 (Australian Institute of Health and Welfare, 2002). Despite the close proximity with Australia, New Zealand is generally believed to be free of Q fever, with the disease not being established in the ruminant population (Hilbink *et al.*, 1993).

Occurrence in foods

C. burnettii has been associated with consumption of unpasteurised goats milk and cheese in Europe, Canada and the USA (Rampling, 1998). On average, 5% of sheep in France tested positive for *C. burnettii* in seroprevalence studies (Rousset *et al.*, 2001), with *C. burnettii* recovered from 50% of milk samples collected from infected ewes (Berri *et al.*, 2000). Infected animals may not show overt signs of clinical infection (Vanderlinde, 2004b). Of 147 goats within a cooperative of eight goat farms in Newfoundland, 82 (55.8%) tested seropositive with antibody titers ranging from 1:8 to > 1:4,096 (Hatchette *et al.*, 2001).

Virulence and infectivity

The incubation period for Q fever is dependent upon the number of organisms that initially infected the patient, with greater numbers of organisms resulting in a shorter incubation period (Maurin and Raoult 1999). On average, most patients will exhibit symptoms within 2 - 3 weeks of exposure. Lifelong immunity against re-infection may be attained should a person fully recover from the infection (Vanderlinde, 2004a).

Dose response

As humans are often very susceptible to the disease, very few organisms may be required to cause infections. Vanderlinde (2004a) reports that inhalation of as few as 10 organisms may result in disease in humans. MMWR (1983) and MMWR(1986) indicate a single inhaled organism is sufficient to initiate infection. No information is available on the number of organisms required to cause infection via ingestion.

Host factors

Persons at greatest risk of exposure to *C. burnettii* fever include those occupationally exposed such as farmers, veterinarians, livestock transport workers, abattoir workers, those in contact with dairy products, laboratory personnel performing *C. burnettii* culture and others working with *C. burnettii*-infected animals.

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7. *Cryptosporidium* spp.

Cryptosporidium spp. are an intestinal protozoan parasite that induce gastrointestinal symptoms when ingested by humans. Being an obligate parasite, the organism requires a host to reproduce, and is transmitted to humans via ingestion of the environmental stage of its life cycle, the oocyst. The oocysts are approximately 4 – 6 µm in diameter and are shed in the faeces of infected hosts in large numbers. *Cryptosporidium* spp. were discovered in 1907 but the first recognised case of human *Cryptosporidium* spp. infection was in 1976 (Berkelman, 1994).

Many species of *Cryptosporidium* have been identified. Some strains appear to be adapted to certain hosts but cross-strain infectivity occurs and may or may not be associated with illness. The most important species in relation to human illness is *Cryptosporidium parvum*; however this species also infects and causes disease in a range of mammals, particularly cattle and sheep (Dawson, 2005).

Growth and survival characteristics

Cryptosporidium spp. will not grow outside an animal host. *Cryptosporidium* oocysts appear to be sensitive to heat, losing infectivity rapidly at >60°C (Rose and Slifko, 1999). Standard high-temperature-short-time (HTST; 72°C/15 seconds) pasteurisation has been demonstrated to be sufficient to destroy the infectivity of *C. parvum* in milk and water (Harp *et al.*, 1996). Low temperatures have also been shown to reduce oocyst infectivity. Fayer and Nerad (1996) investigated the infectivity of *C. parvum* oocysts stored at low temperatures (suspended in deionised water) in mice. Oocysts stored at 5°C and -10°C remained infective for seven days (the duration of study). At temperatures below -15°C, infectivity reduced after 1 day and no infection was noted by 7 days.

Oocysts will survive and remain infective in moist conditions for long periods of time. *C. parvum* oocysts have been shown to be able to survive up to 176 days in drinking water or river water stored at 4°C, with inactivation between 89-99% of the population (Robertson *et al.*, 1992). Desiccation is detrimental to oocyst survival and low water activity has been reported to result in reduced viability (Rose and Slifko, 1999). A study by Robertson *et al.* (1992) showed air-drying at room temperature resulted in 97% inactivation within 2 hours and 100% inactivation within 4 hours. Studies have demonstrated survival of *C. parvum* oocysts in different medias (such as yoghurt) down to an acidity level of pH 4.0 (Deng and Cliver, 1999; Dawson *et al.*, 2004).

Pathology of illness

Symptomatic cryptosporidiosis is usually characterised by profuse watery diarrhoea, often leading to rapid weight loss and dehydration. Other symptoms can include abdominal cramping, nausea, vomiting, low grade fever and headache (Smith, 1993). The disease is usually self-limiting, with symptoms normally lasting from 2 - 4 days (FDA, 2003). Severity and duration of symptoms is considerably greater for immunocompromised individuals. In these susceptible populations, infection may extend to other organs including the lungs and the bile duct and may be considered life threatening (Dawson, 2005).

Mode of transmission

Cryptosporidium spp. are transmitted via the faecal-oral route. Person-to-person contact with oocysts is of particular concern in settings such as childcare centres (Berkelman, 1994). The majority of documented cryptosporidiosis outbreaks have been associated with waterborne transmission.

Epidemiological data

Cryptosporidiosis became a notifiable disease in Australia in 2001. A total of 3,255 cases (16.6 cases per 100,000 population) were notified to health authorities during 2002 (Yohannes *et al.*, 2004). Children under the age of four have the highest cryptosporidiosis notification rate (129 cases per 100,000 population per annum). This may reflect an increased susceptibility of children to *Cryptosporidium* spp. and/or increased likelihood of exposure.

The most prominent waterborne outbreak occurred in Milwaukee in 1993 and resulted in an estimated 403,000 cases of illness (Mac Kenzie *et al.*, 1994). *Cryptosporidium* spp. oocysts are resistant to many disinfection techniques (Korich *et al.*, 1990). It is for this reason that conventional water treatment plants are not always effective in removing the oocysts.

Although the majority of reported cryptosporidiosis outbreaks are waterborne, a number of foodborne outbreaks have occurred. For example an outbreak was observed in Maine, US that was associated with consumption of fresh-pressed apple cider (Millard *et al.*, 1994).

Cryptosporidium spp. oocysts were detected in the apple cider, on the cider press and in the stool specimen of a calf on the farm that supplied the apples. The secondary transmission rate to other household members was 15%. Outbreaks have also been linked to consumption of unwashed green onions (MMWR, 1998).

Two outbreaks of cryptosporidiosis occurred in Australia during 2001 which were associated with the consumption of unpasteurised cow's milk (Ashbolt *et al.*, 2002). One outbreak consisted of 8 children developing cryptosporidiosis following consumption of milk labelled as "unpasteurised pet milk" (Harper *et al.*, 2002). For the other outbreak, it was suspected that consumption of unpasteurised milk during school camp was the cause of infection. A cryptosporidiosis outbreak (48 cases) occurred at a school in the UK during 1995 that was associated with consumption of pasteurised milk (Gelletlie *et al.*, 1997). It was suggested that the milk may have been inadequately pasteurised to inactivate the *Cryptosporidium* spp. oocysts.

Occurrence in foods

Food may be contaminated via a number of sources such as direct contact with faecal material during production (*e.g.* slaughtering or during milking), exposure to contaminated water or exposure via infected food handlers. Once contaminated, *C. parvum* oocysts may survive in wet/moist foods, however they are not able to grow. Very few studies have been undertaken to determine the prevalence of *C. parvum* oocysts in food. Of the data that is available, it is hampered by the lack of consistent methodologies used to isolate oocysts from samples, methods of detection and viability assays.

Virulence and infectivity

Cryptosporidium spp. are considered highly infective. Once ingested oocysts excyst in the small intestine and release sporozoites that attach to the gut epithelium. The sporozoites undergo several asexual and sexual reproduction cycles within the epithelium, resulting in the formation of both thick- and thin-walled oocysts. Thin-walled oocysts re-infect the same host and start a new life cycle, which can lead to severe tissue damage and changes to the absorptive properties of the small intestine. Thick-walled oocysts are excreted in the faeces.

Dose-response

DuPont *et al.* (1995) developed an exponential dose-response relationship for *Cryptosporidium* infection based on data from a feeding study using healthy adult volunteers. The median infectious dose was determined mathematically to be 132 oocysts. At the lowest dose of 30 oocysts, a probability of infection of 20% was observed.

Host factors

Severity and duration of cryptosporidiosis is generally more severe in immunocompromised individuals, including children aged under five years. It is estimated that approximately 1% of the immunocompetent population may be hospitalised with very little risk of mortality, *Cryptosporidium* spp. infections are associated with high rates of mortality, however, in the immunocompromised population (Rose and Slifko, 1999).

Food matrix

Survival data for *Cryptosporidium* spp. in different food and beverages is limited. Water activity and temperature appear to be major factors that determine oocyst survival (Rose and Slifko, 1999). Studies have shown that *Cryptosporidium* spp. oocysts are not able to survive the ice-cream making processes, largely due to sensitivity to low temperature. Oocysts inoculated into milk have been found to survive the yoghurt-making process (Deng and Cliver, 1999).

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8. *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* spp. (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 uremic 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 (Handysides and Cowden, 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).

EPEC 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).

EPEC: Volunteer studies have shown that $10^8 - 10^{10}$ cells of EPEC 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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9. *Leptospira interrogans*

Leptospira are aerobic spirochetes whose cells are flexuous, motile, tightly coiled and have a single axial filament. They are generally about 0.1 µm in diameter and 5 - 25µm in length. They are Gram-negative, although they share features of both Gram-positive and Gram-negative cellular walls (Bharti *et al.*, 2003).

The genus *Leptospira* is an incredibly varied group of organisms, containing hundreds of serovars and genetic types. There are over 200 known serovars with pathogenic bacteria almost entirely within the *Leptospira interrogans* genomospecies. Approximately 24 serovars are currently identified within the *L. interrogans* genomospecies with *L. interrogans* serovar Icterohaemorrhagiae best known for human infection (Anon, 2004).

Leptospira spp. are ubiquitous environmental bacteria found around the world (Zhou *et al.*, 2004). Infections due to the organism *L. interrogans* occur in both animals and humans, however they are more prevalent in developing and tropical areas (Baranton and Postic, 2006).

Serovars Hardjo, Pomona and Grippotyphosa are common in cattle, sheep and goats, while Canicola and Icterohaemorrhagiae are further associated with cattle and Ballum associated with sheep and goats.

Growth characteristics

This organism requires high humidity for survival and is killed by dehydration or temperatures greater than 50°C, however they can remain viable for many weeks or months in contaminated soil and several weeks in cattle slurry. This organism is also easily destroyed by pasteurisation (Anon, 2005). Optimum growth temperatures are between 28 - 30°C with pH levels between 6.8 - 7.4 being required for growth (Bharti *et al.*, 2003).

Pathology of illness

Leptospirosis (also called “Weil’s syndrome” or “Cane cutters disease - in its more extreme forms of infection) is the disease caused by the bacterium *L. interrogans* and other species of the genus *Leptospira* including *L. noguchii*, *L. santarosai*, *L. meyeri*, *L. borgpetersenii*, *L. kirschneri*, *L. weilii*, *L. inadai*, *L. fainei* and *L. alexanderi* (Anon, 2005).

The symptoms of the infection may include fever, weakness, rash and headaches and possibly lead to more severe complications such as liver and kidney malfunction, febrile illness and jaundice (Brito *et al.*, 2006). Leptospirosis can occur as a biphasic illness where sudden onset of febrile illness is reported with a typical duration of one week. This may then be followed by aseptic meningitis which occurs in up to 25% of all cases. Alternatively, Weil’s syndrome will progress and is distinguished by development of renal failure and jaundice. It is only in the most severe cases of Leptospirosis that Weil’s disease will develop without prior onset of febrile illness (Bharti *et al.*, 2003).

Infection of this organism in cattle, sheep or goats can lead to decreased milk productivity and cause abortions (Faine, 2000; Anon, 2000).

Mode of transmission

L. interrogans is a very common zoonotic pathogen of cattle, sheep, goats, domestic and feral pigs, dogs, cats, rabbits, horses, deer, possums, and various rodents including rats and mice (Anon, 2000). *L. interrogans* can be spread by contact directly between infected animals and humans; by ingestion or absorption of contaminated water, soil or food; through aerosolized urine particles, animal foetal fluids or through direct contact with skin (Anon 2000; Baranton and Postic, 2006). This organism is excreted via the urine and other body fluids of acutely infected animals and can enter its host through mucosa or broken skin (Zhou *et al.*, 2004). This bacterium is frequently found as the cause of complications following surgical operations, recreational water exposure and rural occupational injuries (Anon 2000; Zhou *et al.*, 2004).

Incidence of illness

It has been estimated that in the US between 100 - 200 human cases of leptospirosis are reported each year (CDC, 2005). The incidence of infection seems to increase during warmer periods (*e.g.* summer) and during the rainy season (Baranton and Postic, 2006). In Australia prevalence of Leptospirosis is much higher in the tropical and wetter areas of the country, such as Far North Queensland and the Northern Territory (Anon, 2000). Incidence of *L. interrogans* due to foodborne contamination is unknown due to a lack of available data.

Cases of infection often go unreported if symptoms are only mildly exhibited. This was illustrated in 1995 when an outbreak of Leptospirosis was recorded in Nicaragua. Investigations found that of the tested population, only 25 of a possible 85 serotype positive inhabitants reported febrile illness in the 2 months prior to the study. It has been shown in other studies that symptom-less infections are common in endemic areas (Bharti *et al.*, 2003).

In the case of acute infections, fatality rates are estimated at between 5 - 15%. Acute renal failure is reported in 16 - 40% of cases whilst pulmonary involvement is estimated to occur within 20 - 70% of cases (Bharti *et al.*, 2003).

Occurrence in foods

No evidence in the literature can be identified that indicates this organism is shed in the milk of infected animals, or if it occurs naturally in any food source, other than undercooked infected kidney (Anon 2000). However cross-contamination of foods with infected urine or water is a possible mechanism for foodstuffs to be contaminated with *L. interrogans* and thus have the ability to cause infection upon ingestion (Bharti *et al.*, 2003; Baranton and Postic, 2006).

Virulence and infectivity

Motility is thought to be one of the most important virulence factors for this organism. Additionally, studies have shown evidence of other virulence factors including phospholipase, sphingomyelinase and haemolytic activities *in vitro* and pore forming proteins have also been identified when involved with mammalian cells. Some virulent serovars have also been found to express fibronectin-binding proteins on the cellular surface and this is thought to aid in adhesion and invasion of host organisms (Bharti *et al.*, 2003).

Dose response

There is a lack of information on the dose-response relationship for *L. interrogans* for humans, however it has been suggested that invasion of some highly susceptible animals with less than 10 leptospire is sufficient to cause infection (Anon, 2000).

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10. *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 >50°C 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 -1.5°C, 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 - 37°C (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 be 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 intracellularly 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* (>10⁷/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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11. *Mycobacterium avium subsp. paratuberculosis*

The genus *Mycobacterium* comprises approximately 95 species, of which over 30 have been associated with disease in humans (Katoch, 2004). *Mycobacterium* spp. are also pathogens of food producing animals such as cattle, sheep, other ruminants and fish. Some *Mycobacterium* spp. have zoonotic potential in humans (Sutherland, 2003). *Mycobacteria* spp. are aerobic, non-sporeforming, Gram-positive (though difficult to stain) acid-fast rod-shaped bacilli without flagellae. They are slow growing and difficult to culture, having fastidious and nutritionally-exacting growth requirements (Anon, 1998).

Mycobacterium spp. are widely distributed in the environment, being found in soil and water. They readily form biofilms in drinking water distribution systems (Falkinham, 2002; Sutherland, 2003). *Mycobacterium* spp. have particularly hydrophobic cell walls, giving them a propensity to form aerosols, to clump together in liquid media and to form biofilms (Sattar *et al.*, 1995; Anon 1998; Woelk *et al.*, 2003).

The position of *M. avium subsp. paratuberculosis* (hereafter *M. avium subsp. paratuberculosis* or MAP) as a human pathogen is still unclear. Debate centres on the possible role of MAP in Crohn's disease, a chronic intestinal enteritis in humans. Similarities have been observed between Crohn's disease and Johne's disease in cattle and sheep, a disease which is known to be caused by MAP (Anon, 1998). The debate is characterised by firmly entrenched opinions on either side, and the subject has been comprehensively reviewed several times (Chiodini, 1989; Thompson, 1994; Anon 1998; Harris and Lammerding, 2001; Lipiec, 2003; Chacon *et al.*, 2004).

Growth characteristics

MAP is an obligate parasite and is absolutely dependent on mycobactin, an iron-chelating siderophore, for *in vitro* growth (Anon 1998; Motiwala *et al.*, 2004). The temperature range for growth of MAP is 25 - 45°C with an optimum of around 39°C (Anon 1998). Batch (63°C for 30 minutes) and HTST (72°C for 15 seconds) pasteurisation are sufficient to inactivate high levels of pathogenic *Mycobacteria* in milk, although they will survive thermisation (treatment at 62°C for 15 seconds for cheese production) (Stabel and Lambertz, 2004). MAP does not grow in the presence of 5% sodium chloride but is able to grow in microbiological media at pH 5.5. The organism is resistant to drying and may survive in faeces on pasture land for approximately one year (Anon 1998).

Pathology of illness

Although there is ongoing disagreement regarding the role of MAP in human Crohn's disease, the following brief description of the disease is included for information. Crohn's disease is a chronic, granulomatous inflammatory disease of humans, which primarily affects the terminal ileum and colon (Anon, 2000; Rubery, 2002). The disease is characterised by periods of activity interspersed with periods of remission. The clinical signs of Crohn's disease include weight loss, abdominal pain, diarrhoea, reduced appetite and fatigue. Crohn's disease has also been associated with arthritis, skin lesions, anaemia and, in the younger age group, reduced growth rate (Anon, 2004). It has also been observed that mycobacterial illnesses can reactivate many years after recovery from overt illness (Rutala *et al.*, 1991; Gregory *et al.*, 1999; Kubica *et al.*, 2003; Gibson *et al.*, 2004).

Mode of transmission

MAP is excreted primarily in the faeces of infected animals and is excreted during both the subclinical and clinical stages of disease. In dairy animals, MAP can be transmitted both vertically through the placenta to the foetus in advanced infection and also through the calf ingesting colostrum, milk or faeces from an infected animal. MAP is also transmitted horizontally through the faecal-oral route (Sweeney *et al.*, 1992; Streeter *et al.*, 1995; Scientific Committee on Animal Health and Animal Welfare, 2000; Anon 2004). Young animals are most susceptible to MAP infection (Morgan, 1987).

Although there has been concern that MAP could survive the time and temperature combinations routinely used for batch and HTST milk pasteurisation, recent studies have confirmed the efficacy of these processes (Pearce *et al.*, 2001; Pearce *et al.*, 2004; Stabel and Lambertz 2004). However, the potential for its presence and survival in unpasteurised dairy products still exists. Other potential sources of human infection include water supplies, raw vegetables and undercooked meat, although there are no definitive studies on these routes of exposure (Anon 1998). Pickup *et al.* (2005) demonstrated the survival of MAP in river water and inferred a link to clusters of Crohn's disease. DNA fingerprinting studies have indicated that water was the source of *Mycobacterium avium* infection in AIDS patients (von Reyn *et al.*, 1994).

Goat's milk, which is often drunk unpasteurised, may also contain MAP and may therefore pose a potential source of human exposure (Anon 1998; Muehlherr *et al.*, 2003). Human to human transmission occurs rarely, mainly among immuno-compromised patients suffering pulmonary symptoms (Kubica *et al.*, 2003; Gibson *et al.*, 2004).

Incidence of illness

There is ongoing uncertainty regarding any role of MAP in human Crohn's disease. The current estimated prevalence of Crohn's disease in Australia is 50 cases per 100,000 population (estimated 1 per 1,000 in western countries world-wide: (Selby, 2003; Anon 2004). The incidence of Crohn's disease is highest in the 15 - 35 year age group, followed by the 55-65 year age group. Crohn's disease incidence appears to be increasing worldwide. However, this may be due to more sensitive diagnostic measures and an increased awareness of the disease. There is currently no cure for Crohn's disease (Rubery 2002).

Occurrence in foods

Infected cattle may shed MAP in their faeces at levels up to 108 cfu/g. MAP has been cultured from the milk of 35% of infected cattle and 11.6% of asymptomatic carriers, the latter having been found to contain 2 - 8 cfu/50ml of milk (Sweeney *et al.*, 1992; Anon 1998).

Concern regarding the ability of MAP to survive pasteurisation has been prompted by a number of surveys for the organism in pasteurised milk. Interpretation of the results of these surveys is complicated because of large discrepancies between results of polymerase chain reaction (PCR) methods (detecting the presence of DNA) and culture methods (detecting viable organisms). For example, 15% (110/710) of retail milk samples collected in southwest Ontario, Canada, tested positive for the presence of MAP DNA by PCR, although broth and agar culture of 44 of those positives failed to demonstrate any survivors (Gao *et al.*, 2002).

A survey for MAP in milk in England and Wales conducted by (Millar *et al.*, 1996) raised significant concern regarding the possible survival of MAP during pasteurisation. 7% (22/312) of samples tested positive by PCR, and the authors concluded that since the positive PCR signal segregated to either (or both) the pellet and/or cream fractions, the results were indicative of the presence of intact mycobacterial cells. 50% of PCR positive samples and 16% of PCR negative samples yielded MAP-positive cultures. However, other researchers questioned the conclusion drawn that MAP could survive pasteurisation (Stabel, 2000).

A survey of 104 samples of raw sheep and goat's milk from bulk tanks on farms throughout England, Wales and Northern Ireland identified 1 goat milk sample positive by PCR and no positive MAP culture results (Grant *et al.*, 2001). Grant *et al.* (2002) tested a total of 814 cow's milk samples, 244 bulk raw and 567 commercially pasteurised (228 whole, 179 semi-skim, and 160 skim), over a 17-month period to July 2000. MAP DNA was detected by PCR in 19 (7.8%) and 67 (11.8%) of the raw and pasteurised milk samples, respectively. Confirmed MAP isolates were cultured from 4 (1.6%) and 10 (1.8%) of the raw and pasteurised milk samples, respectively. The authors noted that pasteurisation conditions complied with the legal requirement for the HTST process, and considered that post-process or laboratory contamination was unlikely to have occurred, leading them to conclude that viable MAP is occasionally present at low levels in commercially pasteurised cow's milk in the UK.

A similar 13-month study of 389 bulk raw and 357 commercially pasteurised liquid milk supplies was conducted in Ireland (O'Reilly *et al.*, 2004). MAP DNA was detected by PCR in 50 (12.9%) of raw milk samples and 35 (9.8%) of pasteurised milk samples. Confirmed MAP was cultured from one raw milk sample and no pasteurised milk samples. It was concluded that MAP DNA is occasionally present at low levels in both raw and commercially pasteurised cow's milk but, since no viable MAP was isolated from pasteurised milk samples, current pasteurisation procedures were considered to be effective.

Virulence and infectivity

Virulence factors of MAP remain largely unknown (Collins *et al.*, 1995; Collins, 1996). MAP is an intracellular pathogen, able to grow and multiply inside macrophage cells, thus effectively avoiding attack by the host's immune system. A major distinguishing feature of MAP is its requirement of exogenous mycobactin for growth. Mycobactin is an iron-chelating agent produced by all other mycobacteria, which MAP does not produce, or only produces an insufficient amount. The other main virulence factor identified is a catalase-peroxidase which appears to protect the cells from destruction by macrophages (Collins 1996).

Dose response

As described earlier, there is ongoing debate around the role of MAP in human Crohn's disease. There is no data available on a likely dose-response relationship.

Host factors

It has been well documented that there is a genetic component associated with developing Crohn's disease (Rubery 2002). It has been linked to mutations in the NOD2 gene (chromosome 16) which regulates the activity of macrophages against bacterial pathogens.

Food matrix

Limited studies have investigated the survival of MAP in foods, with most research being undertaken on dairy products. For cheddar cheese, Donaghy *et al.* (2004) observed an increased concentration of MAP in one day old cheese compared to the original concentration inoculated into the milk, then a gradual decrease during the ripening period. When numbers of MAP in one day old cheese was high ($>3.6 \log_{10}$), the organism was able to be cultured after a 27 week ripening period. D-values for a different MAP strains ranged from 90 – 107 days.

Spahr and Schafroth (2001) studied the survival of MAP in Swiss Emmentaler (hard) and Swiss Tisliter (semi-hard) cheeses. For both cheeses, MAP numbers decreased steadily, although slowly, during ripening. Calculated D-values for the hard and semi-hard cheese were 27.8 and 45.5 days, respectively. Based on ripening periods of between 90 – 120 days, the estimated reduction during the cheese making process would be between 3 – 4 \log_{10} . Factors that were identified as having the greatest impact on MAP survival were the temperatures applied during the cheese making process and the low pH at the early stages of ripening.

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12. *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* spp. 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.

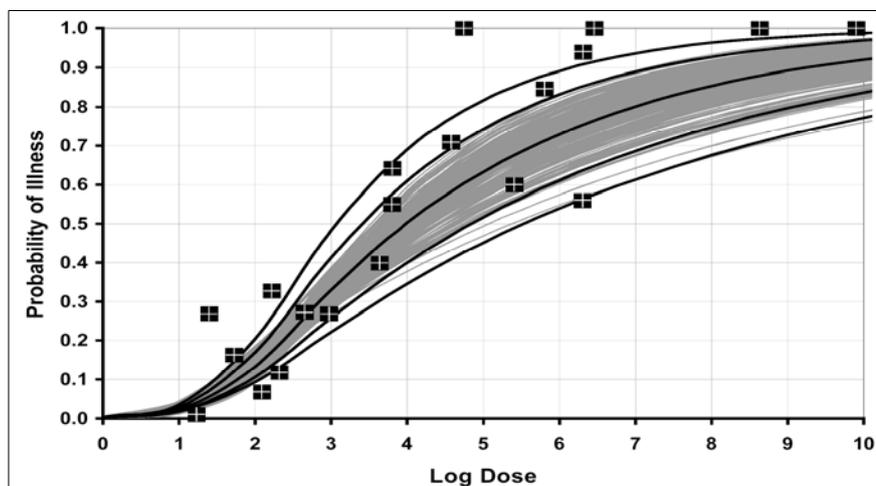


Figure 1: Uncertainty bounds for dose-response curves compared with expected value for the outbreak data (FAO/WHO, 2002).

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 immunocompromised (organ transplant patients, cancer patients and AIDS patients) (Gerba *et al.*, 1996).

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13. *Staphylococcus aureus*

The genus *Staphylococcus* is subdivided into 28 species and 8 subspecies.

Staphylococcus aureus is a non-motile, Gram-positive, non-spore forming spherical bacterium. On microscopic examination, *S. aureus* appears in pairs, short chains, or bunched, grape-like clusters (Stewart, 2003). *S. aureus* is ubiquitous and inhabits the mucous membranes and skin of most warm-blooded animals, including all food animals and humans. Up to 50% of humans may carry this organism in their nasal passages and throats and on their hair and skin (FDA, 2003).

S. aureus counts are often estimated by detecting coagulase-positive staphylococci, with further confirmatory tests required to specifically identify *S. aureus*. Nevertheless, the identification of coagulase-positive staphylococci or *S. aureus* is essentially an indicator test for the likelihood of enterotoxin production, as not all of these organisms have the ability to produce toxin, in addition, some strains of enterotoxin-producing staphylococci do not possess the coagulase enzyme (Stewart, 2003).

Growth characteristics

The temperature range for growth of *S. aureus* is 7 - 48°C with optimum growth occurring at 35 - 40°C. The temperature range for toxin production is 10 - 48°C with the optimum temperature being from 40 - 45°C. *S. aureus* grows over a wide water activity range (0.83 - 0.99) with an optimum water activity of >0.99. The pH range for growth is 4.0 - 10 and the pH range for toxin production is 4.5 - 9.6 (ICMSF, 1996). *S. aureus* is tolerable to salt up to 25% NaCl (water activity of 0.85). *S. aureus* grows under both aerobic and anaerobic conditions; however growth is better in the presence of oxygen. Toxins are also produced under both aerobic and anaerobic conditions with greatest toxin production in the presence of oxygen (Bergdoll, 1989). *S. aureus* is generally considered a poor competitor with other bacteria.

S. aureus is readily killed at cooking and pasteurisation temperatures, however heat resistance is increased in dry, high-fat and high-salt foods. In contrast, *S. aureus* enterotoxins are extremely resistant to heat. Heat resistance for enterotoxin B has been reported at D₁₄₉ = 100 minutes (water activity of 0.99) (ESR, 2001). Heat resistances for *S. aureus* vegetative cells have been reported at D₆₀ = 0.43 - 8.0 minutes whereas a time/temperature equivalent for enterotoxin is 121°C for 3 - 8 minutes (Baird-Parker, 1990; ICMSF, 1996). The enterotoxin is not affected by frozen storage.

Preservatives such as sorbate and benzoate are inhibitory to *S. aureus*, with their effectiveness increasing with a reduction in pH. Methyl and propyl parabens also have an effect on *S. aureus*, and high concentrations of carbon dioxide cause a substantial reduction in growth rates of *S. aureus* (Molin, 1985).

Most chemical sanitisers used routinely in the food industry such as chlorine, other halogens and quaternary ammonium compounds destroy *S. aureus* on surfaces. However some strains, for example those that become established on poultry processing equipment, have increased resistance (Bolton *et al.*, 1988).

Pathology of illness

Staphylococcal foodborne illness is caused by the ingestion of food that contains preformed toxins produced by *S. aureus*. Usually this occurs when *S. aureus* is introduced into a food that will support growth of the organism, and that food is stored under conditions allowing the organism to grow and produce sufficient quantities of enterotoxin (Ash, 1997).

Symptoms generally appear around 3 hours after ingestion but can occur in as little as 1 hour (range 1 - 6 hours) and are self-limiting (Ash, 1997; Stewart, 2003). Symptoms include nausea, vomiting, abdominal cramps of varying severity and diarrhoea. Some individuals may not demonstrate all of the symptoms associated with the illness. In severe cases, blood and mucus may be observed in stools and vomitus. Marked prostration, headaches and sweating accompany severe attacks and there may be fever or shock with subnormal temperatures and lowered blood pressure. Recovery is usually between 1 - 3 days requiring no medical treatment. Fatalities are rare, but are occasionally reported in young children and the elderly (Ash, 1997). All people are susceptible to staphylococcal food poisoning; however the intensity/severity may vary, depending on individual sensitivities.

S. aureus is also an opportunistic pathogen that causes infections via open wounds. *S. aureus* causes several types of infection including skin eruptions and inflammations (boils, acne, sties, etc) and wounds (Ash, 1997). *S. aureus* can also cause respiratory infections or may become established in the gut causing enteritis. *S. aureus* is an important bacterial cause of mastitis (an inflammatory disease of the mammary gland) in cows (Akineden *et al.*, 2001). Mastitis in dairy cattle is characterised by changes in the udder tissue, clots and changes in milk quality, and is sometimes accompanied by heat and pain in the udder.

Mode of transmission

Staphylococcal food poisoning is caused by the consumption of food containing enterotoxins produced by certain strains of *S. aureus*. Despite wide-spread association of *S. aureus* with animals, humans tend to be the main reservoir for *S. aureus* infections in humans. Hand contact by food handlers with ready-to-eat foods is an important means by which *S. aureus* may enter the food supply. Foods that present the greatest risk of causing illness are those in which the normal flora has been destroyed (*e.g.* cooked meats) or inhibited (*e.g.* cured meats containing high salt content) (Stewart, 2003).

Incidence of illness

Food poisoning caused by *S. aureus* is one of the most common types of foodborne diseases world-wide (ICMSF, 1996). The incidence of staphylococcal food poisoning is often under-reported due largely to the self-limiting nature of illness, with most people recovering within 1 - 2 days without requiring medical attention. Foods commonly associated with staphylococcal food poisoning are meat and poultry, dairy products (particularly cheese and cream due to inappropriate handling, as well as contaminated raw milk), salads, cream filled bakery products and processed meat. Improper storage/temperature abuse of food is the greatest factor attributing to outbreaks (Homberg and Blake, 1984).

In July 2000, an extremely large outbreak of staphylococcal food poisoning occurred in Japan, with an estimate 13,420 people being affected (Asao *et al.*, 2003). The source of the outbreak was traced to powdered low-fat milk produced at a single factory in Osaka and was used as an ingredient in a number of dairy products. Staphylococcal enterotoxin was detected

in the implicated milk powder; however, viable *S. aureus* was not isolated. This suggests that staphylococci were able to produce enterotoxin in the milk prior to pasteurisation, and remained immunologically and biologically active despite being pasteurised three times at 130°C for 2 – 4 seconds.

Despite *S. aureus* not being a notifiable illness in Australia, in 2002, three outbreaks of food poisoning attributed to *S. aureus* were reported. In one outbreak, a meal of lamb, rice and potatoes was implicated, in which *Bacillus cereus* was also identified. Other outbreaks implicated rice served in a childcare centre and pizza as the causative agent (Ashbolt *et al.*, 2002; Anon, 2003). An outbreak was also reported in 2001 from consumption of barbequed chicken strongly suggesting an enterotoxin-producing bacterium as the causative agent, possibly *S. aureus* (Armstrong *et al.*, 2002). In 2003, *S. aureus* was also implicated in foodborne illness after the consumption of a rice, beef and black bean sauce meal (Anon 2003).

Mead *et al.* (1999) stated that sporadic illness from *S. aureus* is not reportable in the US through either passive or active systems. The authors estimated 185,060 illnesses, 1753 hospitalisations and 2 deaths per year are attributed to *S. aureus* illness via contaminated food (Mead *et al.*, 1999). Between 1975 and 1982, 36% of all reported *S. aureus* illness in the US were attributed to red meat, 12.3% to salads, 11.3% to poultry, 5.1% to pastries and 1.4% attributed to milk products and seafoods. In 17.1% of cases the food involved was unknown (Genigeorgis, 1989).

In Canada, the average number of cases of illness from *S. aureus* for the years 1975 - 1984 was 232 cases per year (Todd, 1992). Foods implicated included pork (ham), turkey, chicken, cheese, pasta, salads and sandwiches. In France, *S. aureus* was attributed to 16 of 530 foodborne disease outbreaks recorded between 1999 - 2000 (Le Loir *et al.*, 2003). Of these outbreaks, 32% were attributed to milk products (especially cheeses), 22% were attributed to meats, 15% were attributed to sausages and pies, 11% were attributed to fish and seafood, 11% were attributed to eggs and egg products and 9.5% were attributed to poultry 95% (Haeghebaert *et al.*, 2002). In the UK for the years 1969 - 1981, 1 - 6% of all cases of bacterial food poisoning were attributed to *S. aureus*. From 1982 - 1990, 0.5 - 1% of all cases of bacterial food poisoning was attributed to staphylococcal food poisoning. For the years 1969 - 1990 a study of 359 incidents of staphylococcal food poisoning was investigated. Poultry and poultry products accounted for 22% of incidents, most attributed to cold cooked chicken and in nine incidents turkey was the food vehicle (Wieneke *et al.*, 1993; Bertolatti *et al.*, 1996).

Occurrence in foods

Animals carry *S. aureus* on various parts of their bodies. Cow's udders and teats, and the tonsils and skin of pigs, chickens and turkeys are also known sources. Occurrence of staphylococci is common in raw milk. *S. aureus* in milk is related to the health status of the herd in respect to mastitis, and organisms numbers can range from <10 to several thousands per ml of milk with occasional counts of 10⁵ cfu/ml (Asperger and Zangerl, 2002).

The prevalence of coagulase-positive staphylococci (which can include *S. aureus*, *S. intermedius* and some *S. hyicus*) in Australian beef and sheep carcasses and boneless beef and sheep surveyed in 1998 were 24.3% (beef carcasses), 24.1% (sheep carcasses), 17.5%

(boneless beef) and 38.6% (boneless sheep), respectively (Phillips *et al.*, 2001a; Phillips *et al.*, 2001b; Phillips *et al.*, 2005).

Virulence and infectivity

S. aureus forms a wide range of substances associated with infectivity and illness, including the heat stable enterotoxins that cause food poisoning (Ash, 1997). Eleven antigenic types of staphylococcal enterotoxins are currently recognised, with types A and D being most commonly involved in food poisoning outbreaks. To date, staphylococcal enterotoxins A, B, C1, C2, C3, D, E, G, H, I and J toxins have been identified (Balaban and Rasooly, 2000). These enterotoxins are single-chain proteins comprising a polypeptide chain containing relatively large amounts of lysine, tyrosine and aspartic and glutamic acids and characterised by containing only two residues of half cystine and one or two residues of tryptophan. Most of them possess a cystine loop required for proper conformation and which is probably involved in the emetic activity. They are highly stable, resist most proteolytic enzymes, such as pepsin or trypsin, and thus keep their activity in the digestive tract after ingestion. They also resist chymotrypsine, rennin and papain (Bergdoll, 1989).

The production of enterotoxins is dependent on *de novo* synthesis within the cell. The quantity of toxin produced is variable and can be categorised by the type of toxin produced. Although weakly antigenic, enterotoxin antibodies have been produced in a variety of animal hosts. The mode of action of the toxin causing illness is not fully understood. However, it is thought that vomiting in response to ingestion of preformed toxin occurs due to the stimulation of local neuroreceptors in the intestinal tract which transmit the stimuli to the vomiting centre of the brain via the vagus nerve and other parts of the sympathetic nervous system (ICMSF, 1996). A number of studies have identified toxin genes present in *S. aureus* isolates from the milk of cows with mastitis (Akineden *et al.*, 2001; Cenci-Goga *et al.*, 2003; Lim *et al.*, 2004; Zschock *et al.*, 2004; Loncarevic *et al.*, 2005). The rate of enterotoxigenic *S. aureus* isolates from dairy cattle is highly variable and demonstrates the diversity of *S. aureus* strains (Cenci-Goga *et al.*, 2003).

Dose response

The amount of enterotoxin that must be ingested to cause illness is not known exactly, but it is generally believed to be in the range 0.1 - 1.0 µg/kg (ICMSF, 1996). Toxin levels within this range are typically reached when *S. aureus* populations exceed 100,000/g (Ash, 1997).

Immune status

All people are believed to be susceptible to staphylococcal intoxication, but the severity of symptoms may vary depending on the amount of food ingested and the susceptibility of the individual to the toxin.

Food matrix

The range of conditions that allow growth of staphylococci and the production of toxin vary with food type. The amount of starch and protein present in the food may enhance toxin production (Frazier and Westhoff, 1988).

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14. *Streptococcus* spp.

The term, ‘*Streptococcus*’, was first used to describe the chain-forming, coccoid bacteria that had been observed in wounds and discharges of animals (ICMSF, 1996). Streptococci are Gram-positive, spherical or ovoid, non-motile bacteria. The classification of the genus *Streptococcus* has long been in a state of flux (Jones, 1978), however, current information groups them into pyogenic streptococci and enterococci. Pyogenic streptococci include *Streptococcus pyogenes* and *Streptococcus agalactiae*. Enterococci include *Enterococcus faecalis* and *Enterococcus faecium*.

The genus is sorted into Groups A, B, C, D, F and G on the basis of antigenic, haemolytic and physiological characteristics. Streptococci from Groups A and D can be transmitted to humans via food (FDA, 2003). *Streptococcus zooepidemicus* (Group C) has been implicated in several episodes of human illness, including death (Barrett, 1986). *S. agalactiae* is a major cause of bovine mastitis (ICMSF, 1996) and is a highly contagious obligate parasite of the mammary gland (Martinez *et al.*, 2000).

Growth characteristics

The temperature range for growth of *S. pyogenes* is 10 - 45°C with optimum growth occurring at 37°C. The pH range for growth is 4.8 - 9.3 (ICMSF, 1996). *S. pyogenes* is tolerable to salt between 4 - 6.5%. *S. pyogenes* have the ability to grow aerobically or microaerophilically.

Experiments conducted by Obiger (1976) found that *S. pyogenes* would not survive exposure to 66°C for 20 – 40 seconds resulting in a calculated D-value at 66°C of 0.1 - 0.2 minutes. Heat resistance figures reported by (Stumbo, 1973) included a D-value at 65.6°C of 0.2 - 2.0 and a z value of 4.4 - 6.7°C. Based on these figures, ICMSF (1996) conclude that pasteurisation at 62°C for 30 minutes and 70°C for 30 seconds would ensure only a 1.6 - 2.3 decimal reduction of *S. pyogenes*. However, using the D-value at 66°C of 0.2 as per Obiger (1976), pasteurisation would result in a 20 decimal reduction of *S. pyogenes* in milk.

Group A streptococci grow poorly in raw milk, but there is some evidence that pyogenic streptococci may multiply in raw meat held at ambient temperature (Fraser *et al.*, 1977).

Pathology of illness

The symptoms of group A streptococcal infection include sore and red throat, pain on swallowing, tonsillitis, high fever, headache, nausea, vomiting, malaise and rhinorrhea. A rash may occur within the first few days. Group A streptococci may also cause acute rheumatoid fever following infection of the upper respiratory tract, and acute glomerulonephritis after skin infection (ICMSF, 1996).

Although rare, complications may occur when the bacteria enter the blood, muscles or lungs. These infections are termed “invasive group A streptococcal disease”. Two of the least common but most severe forms of this disease are necrotising fasciitis and Streptococcal Toxic Shock Syndrome. Necrotising fasciitis destroys the muscles, fat and skin tissue. Streptococcal Toxic Shock Syndrome causes blood pressure to drop rapidly and organs, such as the kidneys, liver and lungs, to fail. About 20% of patients with necrotising fasciitis and more than 50% with STSS die.

Group D streptococci infections may result in a clinical syndrome similar to staphylococcal intoxication. The symptoms commence within 2 - 36 hours of infection and include diarrhoea, abdominal cramps, nausea, vomiting, fever, chills and dizziness (FDA 2003).

Mode of transmission

Humans are usually the source of contamination of pyogenic streptococcal infections. Transmission occurs from infected hosts to foods. The bacteria are generally spread via direct contact with mucus from the nose or throat of infected persons, or through contact with infected wounds or sores on the skin (FDA 2003). Group A streptococci may be carried in the throat or on the skin of people with no symptoms of illness (CDC, 2005).

Group C streptococci (*e.g. S. zooepidemicus*) is found in the nasopharynx, tonsils, respiratory tract and genital mucous of cattle and horses and has been associated with mastitis in goats, sheep and cows along with other mammals. Human infection with these organisms is most often due to direct contact with animals or the ingestion of unpasteurised milk and milk products (Kuusi *et al.*, 2006).

Incidence of illness

Outbreaks of septic sore throat and scarlet fever were numerous prior to the introduction of milk pasteurisation. Most current outbreaks have involved foods such as salads, with the source of infection being an infected food handler. Outbreaks of Group D streptococcal infections are not common and have usually been the result of unsanitary preparation, storing or handling of food (FDA 2003).

In 2006 an outbreak occurred involving 7 cases of *S. zooepidemicus* caused by the consumption of unpasteurised goats milk cheese in Finland (Kuusi *et al.*, 2006). A further outbreak of foodborne illness due to *S. zooepidemicus* (Group C) involving at least 11 cases occurred in the UK in 1984, with 7 deaths occurring during the outbreak. Unpasteurised milk from a dairy herd that had experienced intermittent mastitis was implicated as the source of infection (Edwards *et al.*, 1988).

Sixteen cases of invasive group C streptococcal infection were identified in northern Mexico between July 25 - September 9 1983. The organism was isolated from the blood of 15 patients and from the pericardial fluid of one patient. A homemade white cheese produced from raw cow's milk at a small family dairy in northern Mexico was indicated as the food source of the infection, with samples testing positive for streptococci. The cows at the dairy were found to have mammary infections due to *S. zooepidemicus* (MMWR, 1983).

In 1984, there was one outbreak of *S. zooepidemicus* associated with the consumption of raw milk in England. Twelve people were admitted to hospital with meningitis or endocarditis. Eight of the twelve died, although the infection was not necessarily the primary cause of death. Ten of the patients were aged over 70 years, and one was a one-day-old infant. Cows at a local dairy that had supplied the milk were subsequently found to be excreting *S. zooepidemicus* in their milk (Barrett 1986).

Occurrence in foods

Food associated with streptococcus Group A foodborne illness include milk, ice cream, eggs, steamed lobster, ground ham, potato salad, egg salad, custard, rice pudding and shrimp salad. Foodstuffs were allowed to stand at room temperature for several hours between preparation and consumption in almost all cases. Poor hygiene, ill food handlers or the use of unpasteurised milk were the main routes for streptococcus Group A into food (FDA 2003). Food sources for streptococcus Group D foodborne illness include sausage, evaporated milk, cheese, meat croquettes, meat pie, pudding, raw milk and pasteurised milk. Under processing and/or poor food preparation is the usual mechanisms for entrance into the food chain (FDA 2003).

200 samples of raw milk from Zulia State, Venezuela were examined, with 19 samples testing positive for the presence of *Streptococcus* spp. Seventeen samples were positive for Enterococcus (Faria-Reyes *et al.*, 2002). Results from the microbiological testing of 77,172 milk samples submitted to the Wisconsin Veterinary Diagnostic laboratory from January 1994 - June 2001 were analysed. Milk samples obtained included cases of clinical and subclinical mastitis as well as samples obtained from mastitis surveillance programmes. The proportion of samples from which *Streptococcus* spp. were isolated decreased from 8.1% in 1994 to 3.0% in 2001 (Makovec and Ruegg, 2003).

Raw bulk tank milk samples from 48 dairy farms in New York State were tested over a five month testing period. Streptococci accounted for 69% of the total bacterial counts. The most commonly identified streptococcal species were *Streptococcus uberis* (found in 81% of the bulk milk samples), *Aerococcus viridans* (found in 50% of the bulk milk samples) and *S. agalactiae* (found in 31% of the bulk milk samples) (Zadoks *et al.*, 2004).

Virulence and infectivity

Pyogenic streptococci possess specific virulence proteins which enable the organism to adhere to epithelial cells and protect the streptococci from phagocytosis (ICMSF, 1996).

Dose response

The infectious dose for streptococcus Group A is likely to be quite low, with less than 1,000 organisms required for infection (FDA 2003). In contrast, it is estimated that foodborne streptococcus Group D has a high infectious dose of greater than 10^7 organisms.

Host factors

All individuals in a population are equally susceptible to streptococcal illness (FDA 2003). People with chronic illnesses such as cancer, diabetes and kidney dialysis and those using medications such as steroids have a higher risk of getting invasive group A streptococcal disease (CDC, 2005).

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15. *Toxoplasma gondii*

The protozoan parasite *Toxoplasma gondii* is the cause of the potentially severe disease toxoplasmosis. *T. gondii* is a ubiquitous parasite that occurs in all areas of the world. It can infect a wide range of animals with the primary host belonging to the cat family (Felidae) and secondary hosts including all warm blooded animals (such as mammals and birds) (Tenter *et al.*, 2000). *T. gondii* causes great losses in sheep and goats; however the disease is more severe in goats (Hill and Dubey, 2003). *T. gondii* also has the ability to survive in faeces, soil and in other environments.

Growth characteristics

T. gondii is an obligate intracellular coccidian protozoan which has a complex lifecycle involving sexual reproduction and oocyst production in a primary carnivorous host (cats) and asexual reproductions phase in the secondary host (generally mammals and birds, including livestock and humans). The life cycle is completed when the primary host consumes the secondary host and is infected with cysts, which have been formed previously in its tissues and the cycle continues (Jones *et al.*, 2000). There are three identified infectious stages of *T. gondii* during its lifecycle; these are tachyzoites and bradyzoites, which are found within the tissue cysts of infected animals, and oocysts (containing sporozoites) (Tenter *et al.*, 2000).

Pathology of illness

The majority of infections in humans tend to be asymptomatic. Additionally, initial infection usually results in lifelong immunity against further infection. It is generally only in immunocompromised individuals and in pregnancies where clinical manifestations of *T. gondii* arise (Tenter *et al.*, 2000). However, immunocompetent individuals may exhibit self-limiting mild flu-like symptoms such as swollen lymph glands, malaise, fatigue, joint and muscle pain (Dawson, 2005).

In immunocompromised individuals *T. gondii* may cause severe encephalitis (especially in patients with AIDS), eye infections, damage to the central nervous system and pulmonary infections. *T. gondii* infection is very serious in cases where the secondary host is pregnant as this organism has the ability to cause spontaneous abortion or severe congenital defects in the off-spring of the host (Tenter *et al.*, 2000). If a pregnant woman, who has not been previously infected with *T. gondii*, becomes infected during her pregnancy the parasite can pass through the placenta to the foetus and cause congenital defects in the unborn child. These may include loss of the child's vision, damage to the child's central nervous system, mental retardation, abnormalities and possible neonatal death/abortion (Goldsmid *et al.*, 2003).

Mode of transmission

Transmission of *T. gondii* occurs via the faecal-oral route, transplacental transfer between mother and foetus, and through the consumption of infected meat and/or milk containing tachyzoites or other forms of the infective parasite from the secondary host (Chiari and Neves, 1984; Skinner *et al.*, 1990; Tenter *et al.*, 2000; Smith, 2006).

Incidence of illness

Toxoplasmosis is widespread in humans, being one of the most common parasitic zoonoses worldwide (Tenter *et al.*, 2000). It is estimated that up to one third of the human population may have been infected with *T. gondii*, the majority of cases being asymptomatic (Montoya and Liesenfeld, 2004). Clinical manifestations of toxoplasmosis, caused by *T. gondii*, are generally confined to immunocompromised individuals and during pregnancies.

Occurrence in foods

Infectious oocysts tend to be very resistant particles and hence have the ability to survive well in many different environments. Contaminated drinking water and other foods exposed to water containing the organism, such as horticultural produce, may be a method of transmission of the organism to humans (Dawson 2005). One study suggests that *T. gondii* has the ability to survive in refrigerated unpasteurised goat's milk (Walsh *et al.*, 1999).

Food sources that have been linked as the probable route of infection in outbreaks of *T. gondii* include raw goat's milk, raw or rare lamb, beef and venison (Smith 2006). Viable infectious tissue cysts may be present in raw or undercooked meats and consumption can lead to infection. Additionally, the organism may be transmitted through the raw milk of an infected mammal such as goat or sheep's milk (Sacks *et al.*, 1982; Skinner *et al.*, 1990; Tenter *et al.*, 2000; Goldsmid *et al.*, 2003). Consumer hygiene may be an important factor in toxoplasmosis, especially after contact with domestic cats, their faeces or through gardening and before handling and consuming foods. Cross contamination of raw meat and ready-to-eat products may also be a possible mechanism of transmission (Jones *et al.*, 2000).

Virulence and infectivity

T. gondii has a high level of virulence with the ability to survive well in a wide range of environments and to infect a large variety of animals either as primary or secondary hosts (Tenter *et al.*, 2000).

Dose response

There is a lack of information on the dose-response relationship for *T. gondii*. However, it is assumed that consumption of a single oocysts or tissue cysts is able to initiate infection in humans (Tenter *et al.*, 2000; Hill and Dubey, 2003; Montoya and Liesenfeld 2004).

Host factors

Epidemiologic studies have identified risk factors such as immunocompromised individuals and those who are pregnant. Additionally, factors such as owning a cat and having poor food hygiene skills, may also place a consumer at additional risk of infection (Jones *et al.*, 2000).

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16. *Yersinia enterocolitica*

Yersinia is a facultative anaerobic organism, a member of the *Enterobacteriaceae* family (Farmer, 1995). Among 11 named species in the genus *Yersinia*, 3 are considered important human pathogens. *Yersinia pestis* is the cause of the plague. *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* are enteropathogenic strains. *Y. pestis* and *Y. pseudotuberculosis* do not frequently infect humans. *Y. enterocolitica* is more commonly found in human clinical specimens.

Y. enterocolitica are Gram-negative, small rods with dimension in the range of 0.5 - 0.81 µm by 1 - 3 µm. Young cells of *Y. enterocolitica* are oval or coccoid shape. The organism produces peritrichous flagella and is actively motile when it is grown at 25°C but not at 35°C (Forsythe, 2000). *Y. enterocolitica* is often isolated from faeces but also from wounds, sputum and mesenteric lymph nodes of patients and sick animals. *Y. enterocolitica* is found in cows, pigs, cats, dogs and birds; and in water, soil and a variety of food. However, they are not part of the normal human flora (FDA, 2003).

Growth characteristics

Optimal growth temperature for *Y. enterocolitica* is at approximately 22 - 28°C, however the organism can grow at refrigerated temperatures and up to 44 °C. The ability to grow at -5°C has also been reported (Barton and Ribins-Browne, 2003). Its optimum pH for growth is between 7 - 8, with growth occurring between 4.6 - 10. Minimum water activity for growth is 0.945. *Y. enterocolitica* is able to grow in the presence or absence of oxygen, but growth in the absence of oxygen is retarded at refrigerated temperatures.

It has been reported that *Y. enterocolitica* can survive in spring water stored at 4°C for up to 64 weeks. Survival of *Y. enterocolitica* is enhanced at low temperatures when the environment pH is below the minimum allowing for its growth. The D values for *Y. enterocolitica* are approximately 2 minutes at 55°C, 0.5 minutes at 60°C and 2 seconds at 65°C (Forsythe, 2000). The D value for *Y. enterocolitica* in milk at 62.8°C is 0.24 – 0.96 minutes (Lovett *et al.*, 1982). As such, cells of *Y. enterocolitica* in milk are readily inactivated by pasteurisation.

Pathology of illness

Yersiniosis refers to the illness caused by *Y. enterocolitica*. Yersiniosis is characterised by gastroenteritis with diarrhoea and/or vomiting, fever and abdominal pain. Many patients seek medical attention for persistent fever, night sweats, or secondary features of the disease. Self-limiting enterocolitis is the most usual syndrome and often seen in young children (Barton and Ribins-Browne, 2003). Mesenteric lymphadenitis caused by *Y. enterocolitica* shows symptoms similar to appendicitis, and can be seen in older children or adolescents. Long-term sequelae as a result of infection by *Y. enterocolitica* include reactive arthritis, erythema nodosum, uveitis and others.

Incubation period for enterocolitis is 24 - 36 hours or longer and the illness lasts usually 1 - 3 days. Duration of excretion of the organisms in the stool of infected patients ranges from 14 - 97 days (Cover and Aber, 1989).

Mode of transmission

Cells of pathogenic *Y. enterocolitica* that are ingested and travelled through the gastrointestinal tract can bind to the epithelial cells of the ileum, penetrate the intestinal mucosa and colonise the Peyer's Patches. Cells that multiply may spread to the mesenteric lymph nodes via the lymphatics and in rare situations may spread to the bloodstream, liver and spleen (Barton and Ribins-Browne, 2003). Pigs are the primary source of human infections of yersiniosis. *Y. enterocolitica* is carried in healthy pigs worldwide. Tonsils and oral cavities of pigs are generally heavily contaminated. Consumption and handling of raw pork meat are a primary source of human infection by *Y. enterocolitica* (Barton and Ribins-Browne, 2003).

Incidence of illness

Since its peak in the early 1990s, there has been a continuing decline in the number of yersiniosis in Australia, as reported by the National Notifiable Disease Surveillance Systems. As such, yersiniosis is no longer a notifiable disease since 2001 (Lin *et al.*, 2002). The OzFoodNet recorded 117 cases of yersiniosis in 2002, representing 1.7 cases per 100,000 of the population.

Most cases of foodborne yersiniosis are sporadic, but some outbreaks have been reported. In September - October of 1976, an outbreak of illness due to consumption of *Y. enterocolitica* contaminated chocolate milk in the US affected 218 people including 36 hospitalisation and 16 appendectomies. Investigations found that pasteurised milk was contaminated during the mixing by hand of chocolate syrup (Black *et al.*, 1978). In October 1995, another outbreak in the US reported 10 cases of yersiniosis associated with consumption of pasteurised milk with 3 hospitalisations and 1 appendectomy. The research found that the pasteurised milk was possibly contaminated post-pasteurisation by unchlorinated rinsing water and dairy pigs were identified as the most likely source of *Y. enterocolitica* (Ackers *et al.*, 2000). An investigation of an Australian outbreak of yersiniosis associated with consumption of pasteurised milk in 1987 - 1988 reported 11 cases of *Y. enterocolitica* enteritis among which three were presented as appendicitis (Butt *et al.*, 1991b). Other than milk, tofu (Tacket *et al.*, 1985), pig meat products and bean sprouts have been implicated as vehicles of outbreaks of yersiniosis.

Yersiniosis caused by *Y. enterocolitica* appears to be a particular health problem in northern Europe, Scandinavia, parts of North America, Japan and New Zealand (Barton and Ribins-Browne, 2003). The number of reported yersiniosis is high in New Zealand where the incidence of yersiniosis is 15.1 cases per 100,000 population in 1998 and 13.9 cases in 1999 (ESR, 2001). In Finland, the reported varied from 11.7 - 17.5 cases per 100,000 population.

Occurrence in foods

Y. enterocolitica is ubiquitous; frequently found in soil, water, animals and can grow in a variety of foods even at refrigeration temperatures. It has been found in many food sources such as raw milk and cream, meat and meat products, oysters, vegetables, fish, and poultry (Barton and Ribins-Browne, 2003). It has also been isolated from well water, streams, lakes, and soil.

Virulence and infectivity

There are 5 biotypes (described as biotype 1A, 1B, 2, 3, 4 and 5) and at least 60 O-antigen¹⁴ serological groups. Human infections are mainly caused by a small number of pathogenic bioserotypes that carry a plasmid encoding a number of virulence factors (Barton and Ribins-Browne, 2003). Bioserotype 4,O:3 is the most common pathogenic *Y. enterocolitica* found in humans worldwide. In addition, bioserotype 2,O:9, 2,O:5,27 and 3,O:5,27 are important human pathogens reported in Northern Europe, and Bioserotype 1B,O:8; 1B,O:13a,13b, 1B,O:20, 1B,O:21 are important pathogens in North America. The North American biotypes are more virulent than those of the Northern Europe (Barton and Ribins-Browne, 2003). The genes encoding for invasion of mammalian cells are located on the chromosome, and other virulence factors are associated with a 70-kb virulence plasmid in pathogenic bioserotypes (Forsythe, 2000). The North American biotype 1B strains carry a high pathogenicity island on their chromosome, which enhances their virulence (Barton and Ribins-Browne, 2003). In Australia, biotype O:3 and O:6,30 have been reported in outbreak investigations (Butt *et al.*, 1991a).

Dose response

Although the minimum infectious dose of *Y. enterocolitica* is not known (Forsythe, 2000), there is estimation that the infective dose is around 10^6 (Health Canada, 2001) to 10^7 cells (Granum *et al.*, 1995).

Immune status

People most susceptible to yersiniosis and the subsequent complications are the very young, the debilitated, the very old and persons undergoing immunosuppressive therapy (FDA 2003). In 2000, notification rate of yersiniosis in Australia was 3.6 cases per 100,000 for the 0 - 4 years old sub-population (male) and 1.5 cases per 100,000 for 0-4 years old (female) sub-population, and the remaining populations was at 0 - 1 cases per 100,000 (Lin *et al.*, 2002).

Food matrix

Survival and growth of *Y. enterocolitica* in food is influenced by pH, water activity, salt content, temperature of storage, oxygen availability and carbon dioxide levels, competing microflora, and food additives in the food matrix. *Y. enterocolitica* has been found to multiply in cottage cheese that contained no sorbic acid. Conversely, *Y. enterocolitica* could not be isolated from ripening hard goats' milk cheeses (Tornadijo *et al.*, 1993) or Swiss-hard or semi-hard cheeses made with raw milk (Bachmann and Spahr, 1995). In the absence of competing microflora, *Y. enterocolitica* can multiply to high numbers in foods, such as pasteurised milk (Black *et al.*, 1978). However, the presence of starter culture on the other hand, had an inhibitory effect on the growth of *Y. enterocolitica* in Turkish Feta cheese (Bozkurt and Erkmen, 2001). It has been demonstrated that the growth of *Y. enterocolitica* in milk could be inhibited by the presence of a bacteriocin producing *Yersinia kristensenii* (Toora *et al.*, 1994) or propionicin producing *Propionibacterium thoenii* (Lyon *et al.*, 1993).

¹⁴ Refers to lipopolysaccharide-protein somatic antigens of the microorganism.

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17. *Yersinia pseudotuberculosis*

Yersinia pseudotuberculosis is a Gram-negative rod shaped bacterium that has the ability to cause human gastroenteritis. It is predominately a zoonotic disease of wild and domesticated mammals and birds, with humans being involved as incidental hosts. This organism has been implicated as a waterborne and foodborne pathogen and has been recognised as a significant pathogen in many mammalian and avian animals, including humans; specifically in New Zealand, Europe, Japan, northern North America and Scandinavia (Fukushima *et al.*, 2001; Barton and Ribins-Browne, 2003). Three species of the *Yersinia* genus are considered pathogenic to humans and these include *Yersinia pestis* (cause of the bubonic plague), *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (Revell and Miller, 2001).

Growth characteristics

Y. pseudotuberculosis has the ability to survive easily in water and the environment (Jalava *et al.*, 2004), and is often particularly found in environments with cool climates (Bakholdina *et al.*, 2004). *Y. pseudotuberculosis* is psychrotropic and can survive in a wide range of ecological niches, including both abiotic (water and soil) and biotic (infection of animals and humans) (Bakholdina *et al.*, 2004).

Pathology of illness

Symptoms of infection with *Y. pseudotuberculosis* include fever, abdominal pain, diarrhoea and vomiting. These symptoms are often self-limiting and have a very low case fatality rate. More serious complications, however, may include reactive arthritis and bacteraemia. The incubation period tends to be around 5 - 10 days. This organism is not recognised as part of the normal human micro-flora (FDA, 2003).

Mode of transmission

Y. pseudotuberculosis is transmitted to humans and animals through the ingestion of contaminated food-stuffs, such as meats and unpasteurised milk and drinking water (Prober *et al.*, 1979; Fukushima *et al.*, 1997; Jalava *et al.*, 2004).

Incidence of illness

There is a lack of data is available on the incidence of illness caused by *Y. pseudotuberculosis*. Infection, however, does seem to occur more frequently in the northern hemisphere and in cooler climate areas (Fukushima *et al.*, 2001).

Occurrence in foods

An outbreak in Finland was caused by contaminated iceberg lettuce (Jalava *et al.*, 2004). Other foods which have been implicated in previous outbreaks include drinking water and foods contaminated with infected water. It has also been postulated that this organism may be contained in the meat products of infected animals (Fukushima *et al.*, 1997). There is a lack of information on the occurrence of *Y. pseudotuberculosis* in other foods. There has also been no identified links made between *Y. pseudotuberculosis* and raw milk contamination in the literature extensively searched (AgriQ, 2002).

Virulence and infectivity

The major mechanism of virulence of *Y. pseudotuberculosis* is its invasiveness of cells (Martins *et al.*, 1998) and ability to survive in macrophages and avoid destruction by the body's immune response (Revell and Miller 2001).

Dose response

A large dose of around 10^9 organisms is generally required for infection to occur in humans (Martins *et al.*, 1998).

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Appendix 3: Occurrence of microbiological hazards associated with raw goat milk

1. Australian data

Table 1: *Salmonella* isolates from raw goat milk (NEPPS data 1983 - 2004)

Organism	Origin and times isolated
S. Anatum	NSW 1, Qld 1
S. Choleraesuis bv Kunzendorf Australia	WA 7
S. Saintpaul	NSW 3
S. subsp IIIb ser 61:l,v:z35	Qld 2

Table 2: Summary of data from State testing programmes (1993 - 2006)

State	<i>Campylobacter</i>	<i>Coag + Staph</i>	<i>Coliforms</i>	<i>E.coli</i>	<i>Listeria</i>	<i>Salmonella</i>
NSW 93 – 99*	-	5.9% (2/34)	17.2% (17/99)	2% (1/51)	-	50% (1/2)
NSW 02 – 05*	0% (0/263)	12.8% (34/266)	-	10.5% (28/266)	0% (0/266)	0% (0/266)
SA 95 – 01**	0% (0/38)	7.9% (3/38)	9.5% (26/274)	-	0% (0/38)	0% (0/38)
SA 00 – 05**	-	34.1% (30/88)	-	12.5% (3/24)	0% (0/77)	0% (0/77)
QLD 03 – 06 [#]	0% (0/19)	0% (0/24)	1.5% (1/65)	0% (0/39)	0% (0/21)	0% (0/21)
WA 03 – 06 ^{##}	5.3% (6/113)	21.6% (24/111)	31% (38/122)	4% (5/122)	0% (0/120)	0% (0/107)

* NSW Food Authority, ** Dairy Authority of South Australia, [#] Safefood Queensland, ^{##} Department of Health Western Australia

Table 3: Summary of outcomes of testing data from other programs (Pointon *et al.*, 2004)

State	<i>Campylobacter</i>	<i>Coag + Staph</i>	<i>Coliforms</i>	<i>E.coli</i>	<i>Listeria</i>	<i>Salmonella</i>	<i>Y. enterocolitica</i>
NSW 1972 (survey)	-	5.6% (4/72)	34.7% (25/72)	-	-	-	-
NSW 2001 (survey)	-	23.3% (14/60)	-	21.7% (13/60)	0% (0/60)	0% (0/60)	1.7% (1/60)
NSW 2002 (study)	0% (0/59)	-	-	20.4% (12/59)	6.8% (4/59)* 0% (0/59) [#]	0% (0/59)	0% (0/59)
SA 1995 – 2003 (testing)	0% (0/79)	12% (10/81)	7% (26/392)	-	0% (0/79)	0% (0/79)	0% (0/54)

* *L. innocua*, [#]*L. monocytogenes*

Table 4: Surveys from scientific literature in Australia

Organism	Sampling period	% Positive	Reference
<i>B. cereus</i>	Aug - Dec 1978	6.9% (20/291)	(Jensen and Hughes, 1980)
<i>E. coli</i>	Aug - Dec 1978	60.5% (176/291)	(Jensen and Hughes, 1980)
<i>Salmonella</i> spp.	Aug - Dec 1978	0.34% (Level=3.44E-05)	(Jensen and Hughes, 1980)
<i>S. aureus</i>	Aug - Dec 1978	5.5% (16/291)	(Jensen and Hughes, 1980)
<i>S. aureus</i>	-	<1% (<8/896)	(Ryan and Greenwood, 1990)
<i>L. monocytogenes</i>	-	1.4% (9/69)	(Arnold and Coble, 1995)
<i>Y. enterocolitica</i>	Aug - Dec 1978	12.8% (35/274)	(Hughes and Jensen, 1981)

Table 5: Food recalls notified to FSANZ (1990 –2009)

Product	Reason for recall
Frozen raw goat milk, soft cheese and fetta	Unacceptable levels of microorganisms
Fetta made from goat milk	<i>E. coli</i>
Goat milk yoghurt	<i>E. coli</i>
Unpasteurised frozen goat milk	<i>Salmonella Zanzibar</i>

2. International data

Table 6: Prevalence of *Brucella* sp. in raw goat milk

Organism	Country	Samples	% Positive	Reference
<i>B. abortus</i>	Mexico	24	6.4	(Acedo <i>et al.</i> , 1997)
<i>B. melitensis</i>	Mexico	24	8.4	(Acedo <i>et al.</i> , 1997)

Table 7: Prevalence of *Campylobacter* spp. in raw goat milk

Organism	Country	Samples	% Positive	Reference
<i>Campylobacter</i> spp.	UK	NA	0.04	(Burden, 1989)
<i>Campylobacter</i> spp.	Switzerland	344	0	(Muehlherr <i>et al.</i> , 2003)
<i>Campylobacter</i> spp.	UK	100	0	(Little and De Louvois, 1999)

Table 8: Prevalence of *Coxiella* in raw goat milk

Organism	Country	Samples	% Positive	Reference
<i>C.a burnettii</i>	US	29	7	(Ruppanner <i>et al.</i> , 1978)

Table 9: Prevalence of *E. coli* in raw goat milk

Organism	Country	Samples	% Positive	Reference
STEC	Switzerland	344	16.3	(Muehlherr <i>et al.</i> , 2003)
EHEC	UK	94	0.7	(Anon, 1999)
<i>E. coli</i>	UK	2462	10	(Roberts, 1985)
<i>E. coli</i>	US	2911	1.6	(White and Hinckley, 1999)
<i>E. coli</i>	Austria	204	1.5	(Pernthaler <i>et al.</i> , 1993)
<i>E. coli</i> O157:H7	Italy	60	1.7	(Foschino <i>et al.</i> , 2002)
<i>E. coli</i> O157:H7	UK	100	0	(Little and De Louvois, 1999)

Table 10: Prevalence of *Listeria* spp. in raw goat milk

Organism	Country	Samples	% Positive	Reference
<i>L. monocytogenes</i>	UK	100	0	(Little and De Louvois, 1999)
<i>L. monocytogenes</i>	India	64	1.56	(Barbuddhe <i>et al.</i> , 2000)
<i>L. monocytogenes</i>	UK	94	2.09	(Anon, 1999)
<i>L. monocytogenes</i>	Spain	1445	2.56	(Gaya <i>et al.</i> , 1996)
<i>L. monocytogenes</i>	US	450	3.8	(Abou-Eleinin <i>et al.</i> , 2000)
<i>L. monocytogenes</i>	Portugal	39	0	(Guerra <i>et al.</i> , 2001)
<i>L. innocua</i>	Spain	1445	1.73	(Gaya <i>et al.</i> , 1996)
<i>L. innocua</i>	US	450	5.8	(Abou-Eleinin <i>et al.</i> , 2000)
<i>Listeria</i> spp.	Portugal	39	5	(Guerra <i>et al.</i> , 2001)

Table 11: Prevalence of *Mycobacterium avium* subsp. *paratuberculosis* in raw goat milk

Organism	Country	Samples	% Positive	Reference
MAP (<i>Mycobacterium</i>)	Norway	340	7.1 (PCR)	(Djonne <i>et al.</i> , 2003)
			0 (culture)	
MAP	Switzerland	344	23	(Muehlherr <i>et al.</i> , 2003)
MAP (Goat identified was infected with Johne's disease)	India	20	1	(Singh and Vihan, 2004)
MAP	UK	90	<1 (PCR)	(Grant <i>et al.</i> , 2001)

Table 12: Prevalence of *Salmonella* spp. in raw goat milk

Organism	Country	Samples	% Positive	Reference
<i>Salmonella</i> spp.	Spain	1445	0	(Gaya <i>et al.</i> , 1996)
<i>Salmonella</i> spp.	Europe	50	0	(Abo-Elnaga <i>et al.</i> , 1985)
<i>Salmonella</i> spp.	UK	2463	0	(Roberts, 1985)
<i>Salmonella</i> spp.	Switzerland	344	0	(Muehlherr <i>et al.</i> , 2003)
<i>Salmonella</i> spp.	Italy	60	0	(Foschino <i>et al.</i> , 2002)
<i>Salmonella</i> spp.	UK	100	0	(Little and De Louvois, 1999)
<i>Salmonella</i> spp.	Bulgaria	60	0	(Vashin <i>et al.</i> , 1999)

Table 13: Prevalence of *Staphylococcus* spp. in raw goat milk

Organism	Country	Samples	% Positive	Reference
<i>S. aureus</i>	Europe	50	0	(Abo-Elnaga <i>et al.</i> , 1985)
<i>S. aureus</i>	US	2911	11	(White and Hinckley, 1999)
<i>S. aureus</i>	France	238	2	(De Buyser <i>et al.</i> , 1987)
<i>S. aureus</i>	Norway	213	96.2	(Jorgensen <i>et al.</i> , 2005)
<i>S. aureus</i>	Switzerland	344	31.7	(Muehlherr <i>et al.</i> , 2003)
<i>S. aureus</i>	Italy	60	43	(Foschino <i>et al.</i> , 2002)
<i>S. aureus</i>	Austria	359	17.6	(Deinhofer and Pernthaler, 1995)
<i>S. aureus</i>	UK	2,493	4	(Roberts, 1985)
<i>S. aureus</i>	UK	100	15	(Little and De Louvois, 1999)
<i>S. aureus</i>	Greece	1350	10	(Kalogridou-Vassiliadou, 1991)
<i>S. aureus</i>	Iraq	297	3	(Al-Graibawi <i>et al.</i> , 1986)
Coag -ve staph	Austria	204	55	(Pernthaler <i>et al.</i> , 1993)
Coag +ve staph	Austria	204	37.3	(Pernthaler <i>et al.</i> , 1993)
<i>Staphylococcus</i> spp.	Austria	204	1.5	(Pernthaler <i>et al.</i> , 1993)

Table 14: Prevalence of *Streptococcus* spp. in raw goat milk

Organism	Country	Samples	% Positive	Reference
<i>Streptococcus</i> spp.	US	2911	4.1	(White and Hinckley, 1999)
<i>Streptococcus</i> spp.	Austria	204	6.2	(Pernthaler <i>et al.</i> , 1993)
<i>Streptococcus</i> spp.	Austria	2243	1.6	(Deinhofer and Pernthaler, 1995)

Table 15: Prevalence of *Toxoplasma* spp. in raw goat milk

Organism	Country	Samples	% Positive	Reference
<i>T. gondii</i>	US	232	9	(Smith, 1993a)
<i>T. gondii</i>	US	-	7	(Walsh <i>et al.</i> , 1999)

Table 16: Prevalence of *Yesinia* spp. in raw goat milk

Organism	Country	Samples	% Positive	Reference
<i>Y. enterocolitica</i>	UK	2,493	0.08	(Roberts, 1985)
<i>Y. enterocolitica</i>	UK	-	0.08	(Burden, 1989)

Appendix 4: Foodborne illness associated with consumption of raw goat milk

1. Australian data

Table 1: Outbreaks of illness associated with raw goat milk

Year	Country	Cases	Causative Agent	Comment	Reference
1990	Australia	9	<i>Salmonella choleraesuis</i> var. Kunzendorf	Consumption of unpasteurised goats milk	(Iveson <i>et al.</i> , 1990)
1984	Australia	2	<i>Cryptosporidium parvum</i>	Consumption of unpasteurised goats milk five days prior to onset of illness - mother and 1yr old child	(Smith, 1993a) (WHO, 1984)

2. International data

Table 2: Outbreaks of illness associated with raw goat milk

Year	Country	Cases (deaths)	Causative Agent	Comment	Reference
2001	Austria	2	<i>E. coli</i> O157	Isolated from dairy cow and goat, raw milk	(Allerberger <i>et al.</i> , 2001)
2001	Canada	5	<i>E. coli</i> O157:H7	Source of implicated goat's milk was a co-operative farm	(McIntyre, 2001)
2001	Sweden	1	<i>E. coli</i> O157	Consumption of unpasteurised goats milk	(Steen <i>et al.</i> , 2001) SA risk assessment
1998	Scotland	1	<i>E. coli</i> O157	Unpasteurised goat milk from farm gate	(Handysides and Cowden, 1998)
1995	Czech Republic	5	<i>E. coli</i> O157	Unpasteurised goat milk from farm	(Bielaszewska <i>et al.</i> , 1997)
1992	France	40	<i>Coxiella burnettii</i>	Persons who worked on farm and consumed unpasteurised milk products	(Fishbein and Raoult, 1992)
1991	USA	3	<i>Campylobacter jejuni</i>	Consumed on farm	(CDC, 2002)
1990	UK	1	<i>Toxoplasma gondii</i>	Consumption of unpasteurised goats milk	(Skinner <i>et al.</i> , 1990)
1989	UK	3	<i>Campylobacter jejuni</i>	Consumption of unpasteurised goats milk	(Burden, 1989)
1988	Israel	3	<i>Staphylococcus aureus</i>	Milk from a goat with overt mastitis	(Gross <i>et al.</i> , 1988)
1985	Scotland	2	<i>Staphylococcus aureus</i>	Goats milk from farm - unpasteurised	(Sharp, 1989)
1985	UK	1	<i>Campylobacter jejuni</i>	Consumption of unpasteurised goats milk	(Hutchinson <i>et al.</i> , 1985a)
1985	UK	3	<i>Campylobacter jejuni</i>	Consumption of unpasteurised goats milk	(Hutchinson <i>et al.</i> , 1985b)
1984	Brazil	6	<i>Brucella melitensis</i>	Consumption of unpasteurised goats milk and / or cheese	(Chiari and Neves, 1984)
1984	USA	2	<i>Toxoplasma gondii</i>	Consumption of raw goats milk	(Smith, 1993a)
1984	USA	6	<i>Toxoplasma gondii</i>	Consumption of raw goats milk	(Smith, 1993a)
1983	USA	6	<i>Campylobacter jejuni</i>	Associated with dairy that produced raw goat milk	(Harris <i>et al.</i> , 1987)
1978	USA	10	<i>Toxoplasma gondii</i>	Consumption of unpasteurised goats milk	(Sacks <i>et al.</i> , 1982)
1975	USA	1	<i>Toxoplasma gondii</i>	Consumption of unpasteurised goats milk	(Riemann <i>et al.</i> , 1975)

Table 3: Outbreaks of illness associated with raw goat milk cheese

Year	Country	Cases (deaths)	Product	Causative Agent	Comment	Reference
2006	Finland	7	Soft unpasteurised goats milk cheese	<i>Streptococcus equi</i> subspecies <i>zooepidemicus</i>	Small farm production	(Kuusi <i>et al.</i> , 2006)
2006	France	3	Raw goats milk cheese	<i>E. coli</i> 0157	Unpasteurised raw goat cheese produced by a local provider	(Espie <i>et al.</i> , 2006)
2005	France	18	Raw goats milk cheese	<i>Salmonella</i> Stourbridge	Cheese was made from the unpasteurised milk of a single herd of 260 goats	(Vaillant and Espie, 2005)
2004	Italy	4	Unpasteurised goats milk cheese	<i>Brucella melitensis</i>	Symptoms included fever and lumbar pain	(Taliani <i>et al.</i> , 2004)
2002	Spain	11	Raw goats cheese	<i>Brucella melitensis</i> serovar 3	Unpasteurised raw goat cheese produced in a farmhouse	(Mendez <i>et al.</i> , 2003)
1999	Canada	?	Cheese from goats milk	<i>Coxiella burnetii</i>	Associated with contact with goat placenta, smoking tobacco	(Hatchette <i>et al.</i> , 2001)
1995	Malta	135 (1)	Soft cheese made with raw goats milk	<i>Brucella melitensis</i>	Consumption of raw milk cheese	(Anon, 1995)
1994	France	4	Raw goats milk cheese	<i>E. coli</i> 0103	Goats milk suspected	(Ammon, 1997)
1993	France	273 (1)	Unpasteurised goats milk cheese	<i>S. enterica</i> Paratyphi B phage type 1 var 3	Brand A unpasteurised goat milk cheese, Out break was possibly related to contaminated goats milk cheese	(Desenclos <i>et al.</i> , 1996)
1992	France	4 (1)	Unpasteurised fromage frais goats/cows cheese	<i>E. coli</i>	Acute haemolytic uraemic syndrome (HUS)	(Deschenes <i>et al.</i> , 1996)
1990	France	277	Contaminated goats milk cheese	<i>S. enterica</i> Paratyphi B	Out break was possibly related to contaminated goats milk cheese	(Desenclos <i>et al.</i> , 1996)
1988	England	1	Goats milk soft cheese	<i>Listeria</i> spp.	Immuno-compromised case	(Azadian <i>et al.</i> , 1989)
1983	USA	31	Raw goats cheese	<i>Brucella melitensis</i>	Mexican raw goats milk cheese	(Thapar and Young, 1986)
1973	USA	3	Mexican fresh raw cheese	<i>Brucella melitensis</i>	Mexican raw goats milk cheese	(Eckman, 1975)
1973	Mexico	6	Fresh raw goats cheese	<i>Brucella melitensis</i>	Mexican raw goats milk cheese	(Young and Suvannoparrat, 1975)

Appendix 5: Qualitative framework for categorising hazards

Colour code

	High
	Moderate
	Low
	Very Low
	Negligible

Hazard characterisation (Severity of Hazard)

		Consequences of exposure			
		Mild	Moderate	Serious	Severe
"Infective dose"	<10				
	10 -100				
	100 - 1,000				
	>1,000				

Exposure assessment

Raw product contamination	Effect of processing					
	Eliminates	99% reduction	50% reduction	No effect	10 fold increase	1000 fold increase
Rare (1:1,000)						
Infrequent (1%)						
Sometimes (10%)						
Common (50%)						
Always (100%)						

Risk Characterisation

Exposure	Hazard Characterisation (Severity of Hazard)				
	Negligible	Very Low	Low	Moderate	High
Negligible					
Very Low					
Low					
Moderate					
High					

Figure 1: Example of risk categorisation of EHEC in raw goat milk for the general population

Colour code

	High
	Moderate
	Low
	Very Low
	Negligible

**Hazard characterisation
(severity of hazard)**

Consequences of exposure to EHEC from raw goat milk is considered to be serious

"Infective dose"	Consequences of exposure			
	Mild	Moderate	Serious	Severe
<10	Low	Moderate	High	High
10 - 100	Very Low	Low	Moderate	High
100 - 1,000	Negligible	Very Low	Low	Moderate
>1,000	Negligible	Negligible	Very Low	Low

Infective dose is <10?

Hazard characterisation HIGH

Exposure assessment

Raw material infrequent contaminated

No effect during processing

Contamination	Effect of processing					
	Eliminates	99% reduction	50% reduction	No effect	10 fold increase	1000 fold increase
Rare (1:1,000)	Negligible	Negligible	Very Low	Very Low	Low	Moderate
Infrequent (1%)	Negligible	Negligible	Very Low	Low	Moderate	High
Sometimes (10%)	Negligible	Very Low	Low	Moderate	High	High
Common (50%)	Negligible	Very Low	Low	High	High	High
Always (100%)	Very Low	Low	Moderate	High	High	High

Exposure assessment LOW

Risk Characterisation

Severity HIGH

Exposure	Hazard Characterisation (Severity of Hazard)				
	Negligible	Very Low	Low	Moderate	High
Negligible	Negligible	Very Low	Low	Moderate	High
Very Low	Negligible	Very Low	Low	Moderate	High
Low	Very Low	Low	Moderate	High	High
Moderate	Low	Moderate	High	High	High
High	Moderate	High	High	High	High

Exposure Low

Overall risk HIGH

Appendix 6: Qualitative framework inputs

Table 1: Hazard and exposure

Hazard	Hazard Characterisation Module					Exposure assessment Module		
	Infective dose	Consequences of exposure ¹⁵		Severity of Hazard		Raw product contamination ¹⁶	Effect of processing	Exposure
		General	Susceptible	General	Susceptible			
<i>Bacillus cereus</i>	>1,000	Mild	Mild	Negligible	Negligible	Sometimes	No effect	Moderate
<i>Brucella melitensis</i>	10-100	Serious	Serious	Moderate	Moderate	Infrequent	No effect	Low
<i>Burkholderia pseudomallei</i>	>1,000*	Moderate	Severe	Negligible	Low	Rare	No effect	Very Low
<i>Campylobacter jejuni</i>	100-1,000	Moderate	Serious	Very low	Low	Infrequent	No effect	Low
<i>Clostridium perfringens</i>	>1,000	Mild	Mild	Negligible	Negligible	Sometimes	No effect	Moderate
<i>Coxiella burnetii</i>	<10	Mild	Serious	Low	High	Infrequent	No effect	Low
<i>Cryptosporidium parvum</i>	100-1,000	Moderate	Serious	Very Low	Low	Infrequent	No effect	Low
<i>Enterohaemorrhagic E. coli</i>	<10	Serious	Serious	High	High	Infrequent	No effect	Low
<i>Leptospira interrogans</i>	>1,000*	Moderate	Moderate	Negligible	Negligible	Rare	No effect	Very Low
<i>Listeria monocytogenes</i>	>1,000 (10-100)	Moderate	Severe	Negligible	High	Infrequent	No effect	Low
<i>Mycobacterium avium subs. paratuberculosis</i>	>1,000*	Mild	Moderate	Negligible	Negligible	Sometimes	No effect	Moderate
<i>Salmonella spp.</i>	10-100	Moderate	Serious	Low	Moderate	Infrequent	No effect	Low
<i>Staphylococcus aureus</i>	>1,000	Mild	Mild	Negligible	Negligible	Common	No effect	Moderate
<i>Streptococcus spp.</i>	>1,000	Mild	Mild	Negligible	Negligible	Sometimes	No effect	Moderate
<i>Toxoplasma gondii</i>	10-100*	Mild	Serious	Very low	Moderate	Sometimes	No effect	Moderate
<i>Yersinia pseudotuberculosis</i>	>1,000	Mild	Moderate	Negligible	Negligible	Infrequent	No effect	Low
<i>Yersinia enterocolitica</i>	>1,000	Mild	Moderate	Negligible	Negligible	Infrequent	No effect	Low

* assumed as no data

¹⁵ Refer to Table 2 – Consequences of exposure determinations

¹⁶ Refer to Table 4 – Raw Product Contamination

Table 2: Consequences of exposure determinations

Organism	Severity of illness (ICMSF)		Consequences of exposure (Qualitative Framework)	
	General population	Susceptible	General population	Susceptible
<i>Bacillus cereus</i>	Moderate		Mild	Mild
<i>Brucella melitensis</i> *	Severe		Serious	Serious
<i>Burkholderia pseudomallei</i>	-	-	Moderate	Severe
<i>Campylobacter jejuni/coli</i>	-	Severe	Moderate	Serious
<i>Clostridium perfringens</i>	-	Severe	Mild	Mild
<i>Coxiella burnettii</i> **	-	-	Mild	Serious
<i>Cryptosporidium parvum</i>	Serious	Severe	Moderate	Serious
Enterohaemorrhagic <i>E. coli</i>	Severe		Serious	Serious
<i>Leptospira interrogans</i>	-	-	Moderate	Moderate
<i>Listeria monocytogenes</i>	Serious	Severe	Moderate	Severe
<i>Mycobacterium avium subs. Paratuberculosis</i> ***	-	-	Mild	Moderate
<i>Salmonella</i> spp	Serious		Moderate	Serious
<i>Staphylococcus aureus</i>	Moderate		Mild	Mild
<i>Streptococcus</i> spp.	-	-	Mild	Mild
<i>Toxoplasma gondii</i>	-	-	Mild	Serious
<i>Yersinia enterocolitica</i>	Serious		Mild	Moderate
<i>Yersinia pseudotuberculosis</i>	-	-	Mild	Moderate

*Organism is not in Australian goat herds, **Foodborne transmission not proven, ***Role in human illness is not confirmed.

The qualitative framework was developed by Food Science Australia. It employs elements of Risk Ranger (Ross and Sumner, 2002) as well as using ICMSF (ICMSF, 2002) classifications for judging the severity of foodborne illness caused by selected pathogenic organisms. The descriptors used in the framework are an amalgamation of information from these sources combined with expert elicitation and information from epidemiological investigations.

Table 3: Definitions used for consequence of exposure determinations

ICMSF	Risk Ranger	Qualitative Framework
SEVERE Life threatening, or substantial sequelae, or long duration	SEVERE Causes death to most victims	SEVERE – Life threatening, with substantial sequelae, or long duration, Causes death to many victims, with a case fatality rate of >10%
SERIOUS Incapacitating but not life threatening; sequelae infrequent; moderate duration	MODERATE Requires medical intervention in most cases	SERIOUS – Incapacitating and potentially life threatening, with or without substantial sequelae, or long duration. Requires medical intervention in >20% of cases
MODERATE Not usually life threatening; no sequelae; normally short duration; symptoms are self-limiting; can be severe discomfort	MILD Sometimes requires medical intervention	MODERATE - Incapacitating but not life threatening, sequelae infrequent and of moderate duration. Require medical attention in <20% of cases
	MINOR Patient rarely seeks medical intervention	MILD - Not usually life threatening; no sequelae; normally of short duration; symptoms are self-limiting, although may cause severe discomfort. Patient rarely seeks medical attention (<5% cases)

Table 4: Raw product contamination

Organism	Raw product contamination	Justification
<i>Bacillus cereus</i>	Sometimes (10%)	No data. Expert panel consultation.
<i>Brucella melitensis</i>	Infrequent (1%)	International data 6.4 – 8.4%, Disease not found in Australia. Expert panel consultation.
<i>Burkholderia pseudomallei</i>	Rare (1:1000)	No data. Expert panel consultation.
<i>Campylobacter jejuni</i>	Infrequent (1%)	Australian data 1.39%, International data 0 – 0.04%. Expert panel consultation.
<i>Clostridium perfringens</i>	Sometimes (10%)	No data. Expert panel consultation.
<i>Coxiella burnettii</i>	Infrequent (1%)	International data 7%. Expert panel consultation.
<i>Cryptosporidium parvum</i>	Infrequent (1%)	No data. Expert panel consultation.
<i>Enterohaemorrhagic E. coli</i>	Infrequent (1%) (Assumed from <i>E.coli</i> data)	Australian data 7.37% (<i>E. coli</i>), International data 0 – 16.3%. Expert panel consultation.
<i>Leptospira interrogans</i>	Rare (1:1000)	No data. Expert panel consultation.
<i>Listeria monocytogenes</i>	Infrequent (1%)	Australian data 0-6.8 %, International data 0 – 5.8%. Expert panel consultation.
<i>Mycobacterium avium subs. paratuberculosis</i>	Sometimes (10%)	International data 0 – 23%. Expert panel consultation.
<i>Salmonella</i> spp.	Infrequent (1%)	Australian data 0.2 %, International data 0 %. Expert panel consultation.
<i>Staphylococcus aureus</i>	Common (50%)	Australian data up to 23.3%, International data 0 – 96.2%. Expert panel consultation.
<i>Streptococcus</i> spp.	Sometimes (10%)	International data 1.6 – 6.2%. Expert panel consultation.
<i>Toxoplasma gondii</i>	Sometimes (10%)	International data 7 – 9%. Expert panel consultation.
<i>Yersinia pseudotuberculosis</i>	Infrequent (1%)	No data. Expert panel consultation.
<i>Yersinia enterocolitica</i>	Infrequent (1%)	International data 0.08%. Expert panel consultation.

Appendix 7: Outcomes of State risk assessments

1. Overview

South Australia

This study was undertaken following a risk profile of the primary industry sector commissioned by the Department of Primary Industries and Resources (Sumner, 2002). The study aimed to identify appropriate food safety risk management options, both policy and regulation for the dairy goat milk industry. The study adopted a qualitative risk ranking approach, based on ICMSF principles and considered hazard severity; occurrence of the hazard in foods; potential for growth; effects of production, processing and handling (including a consumer terminal step); and epidemiological data.

Queensland

The risk assessment utilised methodology developed to rank food safety hazards based on internationally accepted principles of risk assessment including Codex Alimentarius Commission CAC/GL-30 1999. The semi-quantitative approach assigned risk scores (maximum of 100) to hazards and determined total assessed risk scores for each of four population segments based on exposure and severity of consequence.

New South Wales

A risk assessment was conducted to analyse the risks associated with the production, sale and consumption of products made from goat milk. Conclusions are based on the assessment of risk relating to the 11 licensed operators working in the goat industry in NSW. The risk assessment was conducted as two separate parts; a qualitative analysis of risk for each hazard identified in the Hazard Analysis, and; a stochastic semi-quantitative model (Excel-@Risk) to scope the public health significance of *Salmonella*, *Staphylococcus aureus* and *Listeria monocytogenes*.

2. Uncertainty and assumptions

South Australia

The study reported several areas of uncertainty in the assessment:

- susceptibility of the exposed population (an assumption was made that goat milk is fed disproportionately to infants)
- frequency of consumption and distribution of consumption within the population
- probability of raw product contamination
- the effect of existing controls
- dose response
- frequency of use of a terminal kill step (e.g. boiling)
- underreporting of illness
- emergence of new microbiological hazards

Queensland

The Queensland study reported the following assumptions:

- it was estimated that consumption of raw goat milk in the ‘general population’ was between 1 and 20%
- it was estimated that consumption of raw goat milk in the ‘niche population’ was between 81 and 99%
- dose response data used was in the form of published point estimates of infective dose where available

New South Wales

Several areas of constraint were identified in the study including:

- knowledge of current industry hygiene and processing practices for dairy goat farmers outside of the industry quality assurance scheme
- significant lack of information in the literature on the incidence, prevalence and growth of organisms in goat’s milk
- information pertaining to the consumer population and consumption patterns

An estimate of 8% of the adult population in New South Wales was used to calculate consumption figures however it was noted that very little data was available. The study further stated that evidence suggested that products were consumed on a daily basis in some households and more frequently where goat’s milk was being used to feed infants.

Assumptions for growth predictions modelled using the USDA Pathogen Modelling Program included; growth conditions, competitive microflora, temperature ranges, initial microbial load, composition of goat’s milk and the pathogen level of concern (LOC). The LOC for pathogens was based on 1×10^6 cfu/g, except for pathogens with a reported low infective dose, such as *E. coli* and *Salmonella* spp.

Assumptions for the stochastic semi-quantitative model included:

- conditions for the milk harvesting, processing and storage/transport of product
- hazard ranking utilising decision analysis-like methodology
- consumption and exposure information

3. Conclusions

South Australia

The study reported that a broad range of microbiological hazards have been recorded internationally and domestically as causing illness due to consumption of raw goat milk. Many of these hazards are capable of causing severe illness and death.

A number of hazards were rated as **high** risk for susceptible populations (*C. parvum*, *EHEC*, *L. monocytogenes*, *Salmonella* and *T. gondii*). *C. jejuni/coli*, *Salmonella* and *EHEC* were rated as **medium** risk for the general population. *C. parvum*, *L. monocytogenes*, *S. aureus* and *T. gondii* were all rated as **low** risk for the general population.

The reported stated the low general exposure must be weighed against the exposure of lactose intolerant, susceptible infants.

Queensland study

Findings were reported for four population segments within each of two groups. The two groups consisted of the general population group where consumption of raw goat milk was likely to be consumed by less than 20% of the population, and the “niche” market where raw goat milk is the milk of choice. Each of these groups was then categorised into four segments: general population, babies/infants, immunocompromised/very old and hypersensitive/intolerant.

For the normal population where goat milk is generally not consumed there was an overall **low** risk, with *E. coli* O157:H7 and other pathogenic *E. coli* a **medium** risk to babies and infants, and *L. monocytogenes* a **medium** risk to babies/infants and the immunocompromised.

For the niche market where raw goat milk is consumed as the milk of choice, there was an overall increase in risk compared to the normal market population. *S. aureus* enterotoxin and *Burkholderia pseudomallei* were considered a **medium** risk to all populations segments, while *E. coli* O157:H7 was a **medium** risk to the general population and immunocompromised and a **high** risk to babies/infants. *L. monocytogenes*, while considered a **low** risk to the general population, was a **high** risk to both babies/infants and the immunocompromised.

NSW study

The study concluded that insufficient data was available to make any statement, qualitative or quantitative on the risks and public health significance associated with the hazards identified in the hazard analysis.

Particular mention was made of the paucity of information relating to the incidence of specific hazards in goat’s milk, incidence of food poisoning outbreaks, consumption data, consumer handling of product prior to consumption and the lack of information pertaining to unlicensed operators.

Modelling of the public health significance for *Salmonella*, *S. aureus* and *L. monocytogenes* indicates that a single contamination event resulting from contamination and subsequent abuse of the product could lead to severe public health consequences. A major contamination of product (all product from one farm) over 2 to 3 days resulted in some deaths expected for *Salmonella* and *L. monocytogenes*, while contamination with *S. aureus* could result in 30 to 40 people requiring intensive medical treatment. Item contamination (a single package of cheese or litre of milk) would be less serious with *Salmonella* and *S. aureus* causing 2 to 3 people to experience mild illness, although one high risk event with *L. monocytogenes* and a pregnant woman could not be dismissed.

Appendix 8: Regulation of dairy products in Australia

Australia currently has State-based regulations for the dairy sector that cover on-farm activities, milk collection and dairy product manufacture. For most jurisdictions this includes the requirement for HACCP-based food safety programs for on-farm and dairy processing activities. The Authorities responsible for maintaining and implementing requirements are:

- NSW Food Authority
- Safe Food Queensland
- Dairy Authority of South Australia
- Tasmanian Dairy Industry Authority
- Dairy Food Safety Victoria
- Health Department of Western Australia

The Risk Profile of Dairy Products in Australia outlines the requirements for the regulation of dairy products in Australia.

Table 1 provides a summary of each State's legislative requirements for the dairy industry, and covers the production of goat milk. No requirements are listed for the Northern Territory and the ACT as there are no dairy farms in these localities. Requirements for the processing and packaging of milk products in these jurisdictions are covered under the relevant Food Acts.

Table 1: Regulations for the dairy industry

State	Legislation	Requirements for Food Safety Programs
NSW	<ul style="list-style-type: none"> • Food Act 2003 (& Food Standards Code) • Food Production (Dairy Food Safety Scheme) Regulation 1999 	NSW Dairy Manual
QLD	<ul style="list-style-type: none"> • Food Production (Safety) Act 2000 • Food Production (Safety) Regulations 2002 • Food Act 1981 (QLD Health Dept) 	Food Production (Safety) Regulations 2002
SA	<ul style="list-style-type: none"> • Food Act 2001 and Regulations under the Food Act 2001 • Primary Produce (Food Safety Schemes) Act 2004 • Primary Produce (Food Safety Schemes) (Dairy Industry) Regulations 2005 	Code of Practice for Dairy Food Safety, 2005
TAS	<ul style="list-style-type: none"> • Dairy Industry Act 1994 • Food Act, 2003 	Tasmanian Code of Practice for Dairy Food Safety
VIC	<ul style="list-style-type: none"> • Dairy Act 2000 • Food Act, 1984 	Code of Practice for Dairy Food Safety, 2002
WA	<ul style="list-style-type: none"> • Health Act 1911 • Health (Food Hygiene) Regulations 1993 	Code of Practice for Dairy Food Safety (Under development)

1. Regulations for unpasteurised goat milk

The permission for the sale of unpasteurised goat milk is enacted under the relevant Food Acts of South Australia, Western Australia, New South Wales and Queensland.

In addition to the requirements currently prescribed for the production of dairy products, further requirements for the production of unpasteurised goat milk exist within each State. The requirements are briefly summarised in the following table (Table 2).

Table 2: Additional regulations

State (Responsible Authority)	Legislation	Additional Food Safety Program Requirements
NSW (NSW Food Safety Authority)	<ul style="list-style-type: none"> Regulations under the Food Production (Safety) Act 1998 Food Production (Dairy Food Safety Scheme) Regulation 1999 	<ul style="list-style-type: none"> Code of Practice for Dairy Buildings (Goat/Sheep Farms) Code of Practice for the Goat Milk Industry Goat Dairy Farm HACCP Manual NSW Dairy Manual – Unpasteurised Goat Milk Producer
QLD (SafeFood Queensland)	<ul style="list-style-type: none"> Food Production (Safety) Act 2000 	<ul style="list-style-type: none"> Part 3 of the Food Production (Safety) Regulation 2002
SA (Dairy Authority of SA)	<ul style="list-style-type: none"> Code of Practice for Dairy Food Safety 	<ul style="list-style-type: none"> Guidelines for Raw or Unpasteurised Goat Milk (under Section 3.4.2 of the Code of Practice for Dairy Food Safety)
WA (Health Department of WA)	<ul style="list-style-type: none"> Health Act 1911 Part VIIA Division 4 	<ul style="list-style-type: none"> Code of Practice for the Goat Dairy Industry Part 1 Building and Facilities 1990 revised 1995 Code of Practice for the Goat Dairy Industry Part 2 Hygiene 1990 revised 1995

Producers of unpasteurised goat milk are required to be licensed or accredited by the relevant Authority in New South Wales, Queensland and South Australia. Accredited goat milk producers in South Australia operate under food safety program system called “QDairy”. The main focus of QDairy is to minimise microbiological hazards in milk and this is achieved by controlling Good Manufacturing Practices (GMP) and Good Agricultural Practices (GAP). According to the risk assessment conducted for the Dairy Authority of South Australia, QDairy should not be considered a HACCP based program as it lacks a hazard analysis step and does not identify critical control points for the reduction or elimination of microbiological hazards (Pointon *et al.*, 2004).

New South Wales had previously employed a raw milk quality scheme which focused on end-point testing and inspections rather than systems designed to prevent contamination. During the reign of the Raw Milk Quality Scheme there was no obligation to implement HACCP programs in NSW. Under the current requirements raw goat’s milk producers must be licensed by the NSW Food Authority and abide by strict requirements for both facilities and manufacturing practices. Buildings or equipment must comply with the Code of Practice for Dairy Buildings (Goat/Sheep Farms) and premises used for bottling raw milk must comply with Standard 3.2.2 – Food Safety Practices and General Requirements and Standard 3.2.3 Food Premises and Equipment of the Food Standards Code. Operators must also develop an approved HACCP based food safety program.

The Code of Practice for the Goat Dairy Industry in Western Australia is currently not enforceable under any legislation in WA but is used by local government enforcement officers who may be required to approve a goat dairy if a development application is received by their local authority.

Appendix 9: Testing requirements for unpasteurised goat milk

Microbiological limits for unpasteurised milk in the *Australia New Zealand Food Standards Code* Standard 1.6.1, Microbiological limits in Foods, lists the maximum permissible levels of foodborne microorganisms that pose a risk to human health in nominated foods, or classes of foods. This Standard includes mandatory sampling plans used to sample lots or consignments of nominated foods or classes of foods, and the criteria for determining when a lot or consignment of food poses a risk to human health and therefore should not be offered for sale, or further used in the preparation of food for sale. The microbiological standards included in the Schedule to this Standard are applicable to the foods listed in the Schedule.

The below table describes the microbiological requirements set out in the Schedule for unpasteurised milk.

Table 1: Microbiological sampling plan for unpasteurised milk from the *Australia New Zealand Food Standards Code*

Criteria	n	c	m	M
<i>Campylobacter</i> spp./25 ml	5	0	0	
Coliforms/ml	5	1	10 ²	10 ³
<i>Escherichia coli</i> /ml	5	1	3	9
<i>Listeria monocytogenes</i> /25 ml	5	0	0	
<i>Salmonella</i> spp./25 ml	5	0	0	
SPC/ml	5	1	2.5x10 ⁴	2.5x10 ⁵

n means the minimum number of sample units which must be examined from a lot of food.

c means the maximum allowable number of defective sample units.

m means the acceptable microbiological level in a sample unit.

M means the level which when exceeded in one or more samples would cause the lot to be rejected.

Dairy Authority of South Australia

The Dairy Authority of South Australia implemented its Code of Practice for Raw or Unpasteurised Milk on 1 July 1995, which included a requirement for testing for total plate count and coliforms on a monthly basis. Testing for coagulase positive *Staphylococci*, *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni* and *Yersinia* spp. were required on a six monthly basis and subsequently increased to three monthly, however testing for *Yersinia* spp. was discontinued as of 1 July 1996.

Table 2 outlines the testing requirements and standards applicable to unpasteurised goat milk in South Australia.

Table 2: Tests conducted by the Dairy Authority of South Australia

Test Frequency	Test	Standard
Monthly	Standard Plate Count	<50,000 cfu/ml
	Coliforms	<100 cfu/ml
	Antibimicrobial substances	Nil
Three Monthly	<i>Salmonella</i> spp.	Nil
	<i>Campylobacter jejuni</i>	Nil
	Coagulase positive <i>S. aureus</i>	<100 cfu/ml
	<i>Listeria monocytogenes</i>	Nil

Department of Health, Western Australia

The Western Australian Code of Practice for the Goat Dairy Industry Part 2 – Hygiene refers to the Australian Food Standards Code for testing parameters for Standard Plate Count (SPC) and Coliforms. The limits referenced include a standard of less <150,000 cfu/ml for SPC and a coliform count of <100 cfu/ml. The Code of Practice states that a well run goat dairy should produce milk with a SPC below 10,000 cfu/ml and a coliform count of < 10 cfu/ml.

The New South Wales Food Authority

Microbiological standards for goat milk were adopted as a regulation under the Pure Food Act, 1908 (now the Food Act 1989) of New South Wales on 31 January 1989. The NSWFA carries out testing according to the limits contained within the Food Standards Code. The now defunct Goat Milk Quality Scheme recommended a farm gate total plate count of < 20,000 cfu/ml and a farm gate coliform count of < 10 cfu/ml. This standard was elected by industry to remain the same after the Goat Milk Quality Scheme ceased to exist.

Microbiological testing requirements are specified within the NSW Food Authority's Dairy Manual – Unpasteurised Goat Milk Producer documentation and are detailed in the following table.

Table 3: Microbiological analysis of goat milk in NSW

Test Frequency	Test	Standard
Fortnightly	Standard Plate Count	<25,000 cfu/ml
	Coliforms	<10 cfu/ml
Three Monthly	<i>E. coli</i>	<10 cfu/ml
	<i>Salmonella</i> spp.	Nil/25 ml
	<i>Campylobacter</i>	Nil/25 ml
	<i>Listeria monocytogenes</i>	Nil/25 ml

SafeFood Queensland

It appears that testing limits in Queensland are in accordance with the criteria listed in the Food Standards Code.

Appendix 10: References

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