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Attachment 2
A Risk Profile of Dairy Products in Australia

Appendices 1-6

DRAFT ASSESSMENT REPORT

PROPOSAL P296

PRIMARY PRODUCTION AND PROCESSING STANDARD FOR DAIRY

VII APPENDICES

1. IMPACT OF PROCESSING ON DAIRY PRODUCT SAFETY

1.1 Milk and cream
1.2 Cheese
1.3 Dried milk powders
1.4 Infant formulae
1.5 Concentrated milk products
1.6 Butter and butter products
1.7 Ice cream
1.8 Cultured and fermented milk products
1.9 Dairy desserts
1.10 Dairy based dips
1.11 Casein, why products and other functional milk derivatives
1.12 Colostrum

2. EPIDEMIOLOGICAL INFORMATION ON OUTBREAKS OF FOODBORNE ILLNESS ASSOCIATED WITH DAIRY PRODUCTS

3. OCCURRENCE OF MICROBIOLOGICAL HAZARDS ASSOCIATED WITH DAIRY PRODUCTS

4. CONSUMPTION FIGURES OF DAIRY PRODUCTS FOR AUSTRALIAN CONSUMERS

5. HAZARD IDENTIFICATION/HAZARD CHARACTERISATION OF PATHOGENS

5.1 *Aeromonas* Spp.
5.2 *Bacillus* cereus
5.3 *Brucella* Spp.
5.4 *Campylobacter jejuni/coli*
5.5 *Clostridium* Spp.
5.6 *Coxiella burnetii*
5.7 *Corynebacterium ulcerans*
5.8 *Cryptosporidium*
5.9 *Enterobacter sakazakii*
5.10 Pathogenic *Escherichia coli*
5.11 *Listeria monocytogenes*
5.12 *Mycobacterium bovis*
5.13 *Mycobacterium avium subsp. paratuberculosis*
5.14 *Salmonella* Spp.
5.15 *Shigella* Spp.
5.16 *Staphylococcus aureus*
5.17 Streptococcus Spp.
5.18 *Yersinia enterocolitica*

6. PREVIOUS RISK ASSESSMENTS ON MICROBIOLOGICAL PATHOGENS IN DAIRY PRODUCTS

7. CHEMICAL RISK ASSESSMENT FRAMEWORK

8. REGULATORY FRAMEWORK FOR AGRICULTURAL AND VETERINARY CHEMICALS

9. MAXIMUM RESIDUE LIMITS

10. CHEMICAL RESIDUES MEASURED IN BOVINE DAIRY PRODUCTS

11. REGISTERED ANTIMICROBIAL AGENTS

12. REVIEW OF ANTIBIOTICS AND ANTIMICROBIAL RESISTANCE IN AUSTRALIA

13. FEEDS AND FEED COMMODITIES FOR CATTLE

14. THERAPEUTIC PRODUCTS USED IN GOAT PRODUCTION AND REGISTRATION STATUS FOR USE IN GOATS

APPENDIX 1

Impact of processing on dairy product safety

1.1 Milk and cream

1.1.1 Description

Milk is defined in Standard 2.5.1 of the Food Standards Code as "the mammary secretion of milking animals, obtained from one or more milkings for consumption as liquid milk or for further processing but excludes colostrums".

In this Dairy Risk Profile, milk refers to the fluid form of milk derived from cow, sheep, goats, buffaloes, camels, horses and other mammalian animals, and available for human consumption through retail sale in Australia.

Milk may be sold in many forms, including whole milk, skim milk, low-fat milk, flavoured milk and other modified milks. Some of these products require the removal of the fat portion as cream. Under Standard 2.5.2, cream is defined as "a milk product comparatively rich in fat, in the form of an emulsion of fat-in-skim milk, which can be obtained by separation from milk". Cream is produced from whole milk by skimming or other separation means.

Milk is subjected to a range of processing operations before being sold. Typical processes include standardisation or formulation of milk, which may include: separation steps such as filtration, centrifugation, and sometimes clarification; homogenisation; and various forms of heat treatment such as thermisation, pasteurisation, sterilisation and UHT (ultra-high temperature) processing. The key processing operations are shown in Figure 1.

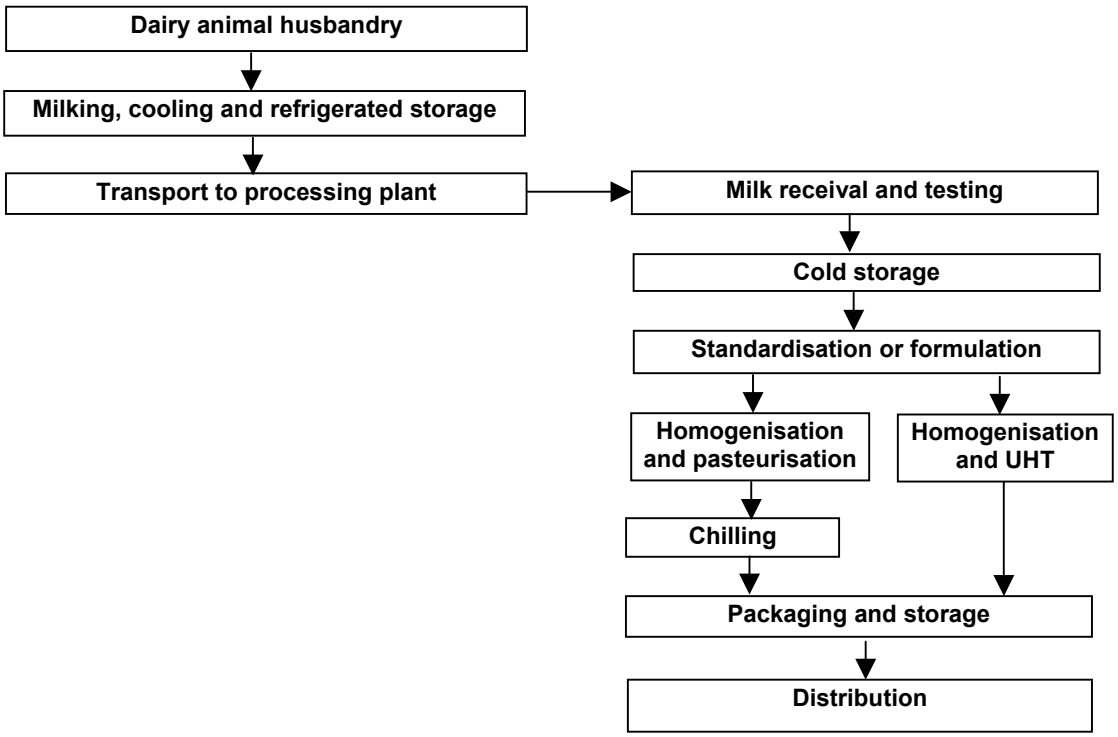

Figure 1: Indicative processing of fluid milk and cream products

Figure 1 simplifies the major steps in the processing of liquid milk products. The raw milk for low fat, skim milk and modified milk products is subjected to centrifugal separation to remove some of the fat phase. Flavoured milks have the addition of flavours and colouring agents, which are sometimes added post-pasteurisation, while ultra-high temperature processing (UHT) results in milk products which are shelf-stable and do not require refrigeration or chilling.

The cream that is separated from whole milk may be used in the production of liquid cream products, butter, and anhydrous milk fat. The cream may be subjected to additional processes including vacuum pasteurisation, which involves heat treatment and deodorisation of the fat.

1.1.2 The Microbial Flora of Milk

The microbial status of raw milk is influenced by various factors associated with milk production on farm. These factors impact on both the numbers of microorganisms present in raw milk and the type of bacterial flora. Generally, few bacteria are present in milk drawn from the udder of a healthy animal, but bacteria may enter milk if it is drawn from an infected animal or if it is contaminated by unhygienic milking practices and poor milk handling.

Bramley and McKinnon (1990) identified the main groups of microorganisms comprising the microflora of raw milk as follows:

Table 1: Major flora of raw milk

Group	Incidence (%)
Micrococci *e.g. Micrococcus*, *Staphylococcus*	30-99%
Streptococci *e.g. Enterococcus*	0-50%
Asporogenous Gram +ve rods *e.g. Corynebacterium, Microbacterium, etc*	<10
Gram –ve rods *e.g. Klebsiella, Escherichia, Enterobacter, Pseudomonas, etc*	<10
Sporeformers *e.g. Bacillus* spores or vegetative cells	<10
Miscellaneous *e.g. Streptomycetes,* yeasts, moulds, etc	<10

Various pathogenic microorganisms may also be associated with raw milk. These include organisms shed by an infected animal (pathogens will predominate in milk from mastitic cows) or organisms that enter the milk from contaminated equipment and poor milking hygiene. Surveys of raw cow's milk, mainly conducted overseas, have detected *Aeromonas* spp., *B. cereus*, *Brucella* spp., *Campylobacter* spp., *Coxiella burnetii*, pathogenic *E. coli*, *L. monocytogenes*, *Mycobacterium* spp., *Salmonella* spp., *S. aureus*, *Streptococcus* spp. and *Y. enterocolitica*. Raw goat's and sheep milk have been shown to contain *Aeromonas* spp., *Brucella* spp., *Campylobacter* spp., pathogenic *E. coli, L. monocytogenes*, *Mycobacterium* spp., *S. aureus* and *Y. enterocolitica.* In Australian surveys, potential pathogens detected in raw cow's milk have included *Aeromonas* spp. and *S. aureus*. Detections in raw goat's milk have included *E. coli*, *L. monocytogenes*, and *Y. enterocolitica* (Appendix 3).

Overseas surveys of cream have detected *Aeromonas* spp. and *Shigella* spp., while Australian surveys have indicated the presence of *E. coli* and *Salmonella* spp. (Appendix 3).

1.1.3 Effect of milk processing on the growth and survival of microbial pathogens

To minimise microbiological activity, raw milk harvested from animals at dairy farms is cooled rapidly to temperatures of 7°C or lower within 2 hours of milking (White, 2003). In Australia, milk is cooled to 4°C.

After a period of storage on farm, raw milk is transported by insulated bulk milk tankers to milk processing facilities. Traditionally, milk transportation was conducted in the early part of the day to minimise the impact of heat from sunlight, but nowadays milk collection may occur at any time during the day or night and insulated tankers minimise the impact of ambient temperatures on milk.

When the raw milk reaches the processing plant, it is sampled and its status assessed by measuring parameters such as temperature, presence of antibiotics, somatic cell counts, standard plate count, bactoscan, fat content, etc. Information on the microbial content of milk is helpful in judging its sanitary quality and the conditions of production, and is used in payment schemes that reward producers of high quality raw milk. The raw milk is normally transferred to large insulated storage silos and maintained at temperatures of less than 4°C before decisions are made about how it will be processed.

Milk for drinking is usually homogenised, a process where the milk fat globules are physically reduced in size and then remain in suspension throughout the milk for long periods of time. In unhomogenised milk, fat globules may coalesce to form a compact cream layer. Homogenisation has little effect on the microbiology of fluid milk, but may predispose the fat to oxidation reactions that affect its quality, hence homogenisation occurs simultaneously with pasteurisation. Practically all drinking milk in Australia is homogenised, preventing the milk from separating and giving it a more uniform colour.

Pasteurisation involves heat treatment of milk with the aim of ensuring a microbiological safe product, as well as to extend the shelf-life during refrigerated storage. Milk pasteurisation may be carried out either as a batch holding heat treatment or a high-temperature-short-time (HTST) heat treatment. The batch process involves low-temperature-holding for 30 minutes or longer at temperatures of approximately 63°C. This has been largely replaced by HTST treatment at temperatures of ≥72°C for at least 15 seconds. The Food Standards Code states: Milk must be pasteurised by:

(a) heating to a temperature of no less than 72°C and retaining at such temperature for no less than 15 seconds and immediately shock cooling to a temperature of 4.5°C; or
(b) heating using any other time and temperature combination of equal or greater lethal effect on bacteria;Where dairy products contain elevated levels of fat or solids, the specified temperature is increased to compensate for the protective effect of these fat and solids on microorganisms.

These specifications are sufficient to reduce populations of vegetative bacterial pathogens to a level considered safe for public health. The pasteurisation process used by processors of milk often employs temperatures and times in excess of 72°C for 15 seconds. This is to provide a higher margin of safety and to extend the shelf-life of liquid milk.

From survey data on industry pasteurisation practices in Australia HTST treatment of milk for liquid milk products was mostly in the range of 74-78°C for 15-30 seconds[1].

Pasteurisation processes for cream products utilise higher temperatures because of the protective effects of fat on microorganisms. From survey data on industry pasteurisation practices in Australia the pasteurisation temperatures and times for table cream varied in the range from 75-80°C for 20-30 seconds[2]. Internationally recognised heat treatments for pasteurisation of cream is 65°C for 15 seconds for cream with 10-20% fat, and 80°C for 15 seconds for cream with >20% fat[18]. Thickened cream has thickeners such as alginates and/or carragenans added before pasteurisation.

The same survey data indicates that batch pasteurisation is still widely used in Australia for the pasteurisation of raw cows' milk used in the manufacture of dairy products such as cheese, cream, ice cream and yoghurt, particularly by the smaller processors, many of whom are processing milk in on-farm situations. However, batch pasteurisation accounts for only a small percentage of all milk pasteurised in Australia. Temperatures and times of heat treatment for batch pasteurisation range from 62-90°C and from 15 seconds to 30 minutes[18].

Further detail on pasteurisation and conditions used in Australia are described in Section 10.

Pathogens such as *Salmonella*, *Campylobacter*, *Staphylococcus*, pathogenic *E. coli* (particularly enterohaemorrhagic *E. coli*), *Y. enterocolitica* and *Listeria monocytogenes* which may be present in raw milk are inactivated by pasteurisation. However pasteurisation will not destroy heat stable enterotoxins such as those produced by *Staphylococcus aureus*, if the organism has grown and produced enterotoxin in raw milk prior to pasteurisation. Inadequate chilling of raw milk is one of the key factors for the build up of *Staphylococcus* enterotoxins (ICMSF, 1998).

Pasteurisation also has the advantage of destroying many of the spoilage microorganisms present in raw milk, especially psychrotrophic bacteria which may proliferate during low temperature storage of liquid milk products. Pasteurisation, however, cannot be relied upon to destroy some of the more heat resistant bacteria (thermodurics) or bacterial spores produced by bacteria of the Genera *Bacillus* and *Clostridium*. After pasteurisation, milk and milk products still contain low numbers of thermoduric microorganisms such as *Micrococcus* and *Enterococcus* species and some lactic acid bacteria. For this reason, pasteurised milk and milk products have a limited shelf-life even when stored at refrigeration temperatures (ICMSF, 1998).

To minimise growth of the surviving microbes, and to minimise post-process recontamination the steps of cooling pasteurised milk, filling and packaging, and refrigerated storage of pasteurised milk and cream must be well managed. Pasteurised milk is particularly vulnerable to post-pasteurisation contamination, and asepsis and good hygiene is essential for preventing contamination by pathogenic microorganisms and for defending its shelf-life.

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1 Pasteurisation times and temperatures are from the report to FSANZ **Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products (**Juffs, H and Deeth, H, 2005)

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The shelf life of milk is influenced by the number of psychrotrophic bacteria that survive pasteurisation or subsequently contaminate the pasteurised product and grow at low temperatures in the liquid during storage. Although these contaminants are initially present in low numbers they can, under certain conditions, grow quickly and produce enzymes that break down protein and fat and generate off flavours and odours. The typical shelf life for pasteurised milk is from 7-14 days, although there are seasonal and regional variations.

UHT processing of milk involves heating milk at a temperature higher than 130°C with a holding period of 1-10 seconds with subsequent aseptic packaging. Usually the temperature and time combination is 138-145°C for 3-5 seconds (Deeth et al., 2003). Sterilisation treatment of milk is similar to that of UHT but at a higher temperature and is usually applied to condensed milk. The term '*sterilisation*', as used here, refers to commercial sterility of the milk or milk product. Milk and milk products of commercial sterility are not absolutely sterile in microbiological terms. However, those microorganisms and spores that may survive the sterilisation treatment are incapable of development under normal conditions of storage (Hersom et al., 1980). Temperature and time combinations for the sterilisation of milk and milk products range from 105-120°C for 10-40 minutes (Hinriches et al., 2003).

Both UHT treatment and sterilisation destroy bacterial endospores (Deeth et al., 2003). Milk and milk products after UHT treatment or sterilisation can be stored without refrigeration for extended periods of time.

Available epidemiological data indicates that illness resulting from the consumption of pasteurised milk and cream is rare, although outbreaks involving *Campylobacter* spp., *Salmonella* spp., *E. coli* O157:H7, *L. monocytogenes* and *Yersinia* spp. have been linked to consumption of pasteurised milk (See Appendix 2). These outbreaks have usually been traced to inadequate pasteurisation and/or post-pasteurisation contamination and/or temperature abuse (ICMSF, 1998) and not to any failure of the pasteurisation process.

Surveys conducted overseas on pasteurised milk have indicated the presence of *Aeromonas* spp. and *B. cereus* in Turkey. Australian surveys have indicated the presence of *Aeromonas* spp. which was introduced during subsequent handling of the milk and *Yersinia* spp. (Appendix 3).

1.2 Cheese

1.2.1 Description

The term cheese covers over 1,000 varieties of fermented dairy products with significant variations in their flavour, texture and appearance. The process of converting liquid milk into cheese involves a series of steps that are modified to produce a cheese of the desired characteristics.

Cheeses manufactured from the milk of all species, including bovine, ovine and caprine animals undergo similar processing steps. The production of all cheese varieties generally follows a similar process comprising the following general stages:

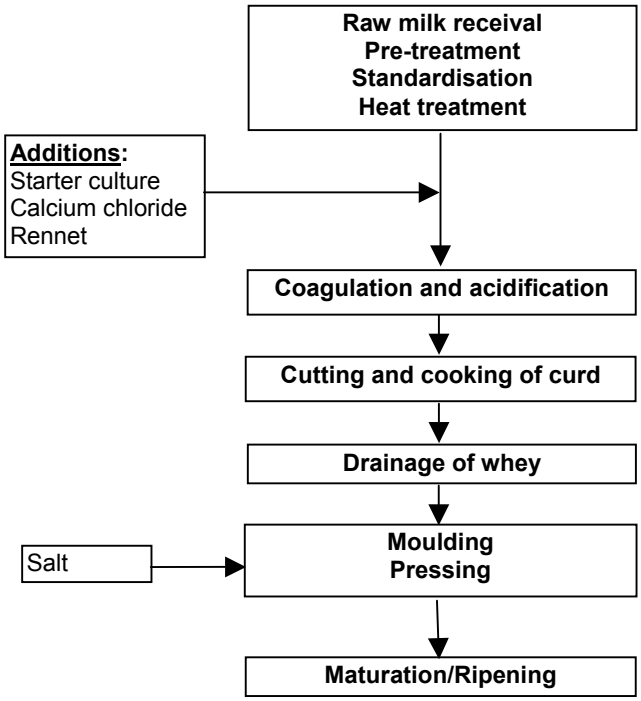

Figure 2: Overview of major steps in the manufacture of Cheddar-style cheese

Starter culture and rennet are added to milk resulting in the production of a cheese curd through a process of coagulation and acidification. The curds are usually cut and with mild (38-43ºC) heating there is separation of the whey, which is drained from the curds. The curds are salted before they are pressed into moulds and then stored under controlled conditions to ripen the cheese.

The actual processes vary enormously between cheese types. Some cheeses are manufactured without the use of rennet, while others are acidified by the addition of acid. Different starter cultures impart different physical and organoleptic properties on the cheese. Calcium chloride solution is added when milk is calcium deficient to assist cheesemakers. Calcium assists the milk coagulation process for rennet set cheeses. In some fresh cheese, the curds are not heated resulting in less expression of whey and a moister final product. Cheese curd may be washed with water, dry salted or brine salted, pressed or unpressed. The moisture content is influenced by the pH, salt concentration, amount of pressing, maturation conditions, etc. The addition of mould spores is required for surface ripened cheeses such as brie and camembert, while spores are incorporated in the curd for interior and surface ripened

blue cheeses. During maturation a range of conditions are used, with particular attention to the temperature of the ripening rooms.

Historically, cheese was made from raw milk, and this practice still prevails amongst traditional cheese makers in countries such as France, England, and Italy. However heat treatment of raw milk reduces the populations of microorganisms, both pathogenic and spoilage, before cheese making commences and provides the cheese maker with greater control over the microbial flora in the milk. Typically this involves pasteurisation, but heating milk to 64-70°C for 15-20 seconds *i.e.* thermisation is gentler than pasteurisation and does less damage to some of the intrinsic enzymes in the raw milk. These enzymes are considered relevant in the development of specific cheese flavours similar to those in cheeses made from raw milk (ICMSF, 1998).

Production of raw milk cheese is generally not permitted in Australia. The Food Standards Code (Clause 2 of Standard 1.6.2) prescribes that cheese made with thermised milk must undergo a maturation period of 90 days, at a temperature of no less than 2°C, from the date of manufacture. Alternatively, cheese can be made with pasteurised milk, *i.e.* milk subject to heat treatment at 72°C for 15 seconds or any equivalent of this temperature and time combination.

Several different schemes are used to classify cheeses. Cheeses may be grouped according to manufacturing or processing procedures, consistency or rheology (softness or hardness), country of origin, general appearance (size, shape, colour, surface ripening), source of milk, and chemical analysis (See Table 7).

Table 2: Classification of cheeses by type and moisture content

Cheese Type	Moisture	Description/Style
Very hard	<36%	Ripened by bacteria *e.g.* Parmesan, Asiago, Romano
Hard	<42%	Ripened by bacteria, without eyes *e.g.* Cheddar
		Ripened by bacteria, with eyes *e.g.* Emmental, Gruyère
Semi-soft	43-55%	Ripened principally by bacteria *e.g.* Gouda, Edam, Provolone
		Ripened by bacteria and surface microorganisms *e.g.* Limburger
		Ripened principally by interior blue mould *e.g.* Roquefort, Stilton, Danablu
Soft	>55%	Ripened by surface mould *e.g.* Brie, Camembert
		Unripened (also referred as fresh cheese) *e.g.* Cottage cheese, Quark, Cream cheese
		Salt cured or pickled *e.g.* Feta

1.2.2 Microbial pathogens of major concern

Cheese has been the vehicle in a number of outbreaks of foodborne illness, involving pathogenic microorganisms such as *S. aureus*, *Bacillus* spp., *Salmonella*, *L. monocytogenes, E. coli*, *Shigella*, *Cl. botulinum* and *Brucella* spp. (Cogan, 2003; ICMSF, 1998). A full list of the outbreaks resulting from cheese consumption is provided at Appendix 2.

Evidence from outbreak investigations suggests that illness resulting from consumption of cheese is often the result of faulty controls in cheese production; use of contaminated starter

cultures or contaminated ingredients; post-pasteurisation contamination; or mishandling during transportation and/or distribution.

In microbiological surveys conducted overseas and Australia, a number of potential pathogens have been detected in cheeses made from pasteurised milk, namely *L. monocytogenes* and *S. aureus*. Additional pathogens have been detected in raw milk cheese (*B, cereus*, *Brucella* spp., pathogenic *E. coli* and *Y. enterocolitica*). Detections of *Bacillus* spp. and *L. monocytogenes* have occurred in pasteurised milk cheeses in surveys conducted in Australia (Appendix 3).

1.2.3 Effect of cheese processing on the growth and survival of microbial pathogens

Several factors act as hurdles influencing the growth and or survival of pathogenic microorganisms in cheese. These include the amount of heat applied at various stages during the manufacture of cheese; the extent of acidification by the starter culture, salt levels, reduced water availability resulting from salting and ripening/maturation, production of bacteriocins by starter cultures, and the effect of selected food additives used in cheese making.

Survival of pathogenic microorganisms is also dependent on the status of the organisms including their initial population, their physiological condition and their characteristics such as tolerance of low pH, salt, reduced water activity, heat, and resistance to bacteriocins produced by lactic acid bacteria, etc.

While each of these factors has an effect, it is their combined effect that has the greatest impact on the growth or survival of microbial pathogens in cheese.

1.2.3.1 Effect of temperature and time

Heat treatments in cheese making include pasteurisation, thermisation, and cooking of the curd. Of all the factors, heat treatment applied to raw milk (*e.g.* pasteurisation) is the most important factor in survival and growth of potential microbial pathogens in raw milk used for cheese making (Cogan, 2003). Pasteurisation of raw milk is sufficient to reduce populations of vegetative bacterial pathogens to a level that is considered safe for public health (Cogan, 2003). Spores of *Cl. botulinum* may be present in milk and survive pasteurisation, but interactions between water activity, salt, pH and production of antimicrobial agents by starter cultures in the cheese, usually prevent germination or growth.

Thermisation is a milder heating process than pasteurisation and, on its own is unlikely to lead to a safe milk product. As such, extended ripening or maturation is required to reduce the level of microbial pathogens to an extent that is considered safe for public health. The extent of ripening or maturation varies according to the type and characteristics of the cheese, particularly its final pH, water activity, salt content and type and concentration of additives.

The temperature employed to facilitate coagulation in cheese making is referred to as the curd setting temperature. Curd setting temperature usually matches the optimum growth temperature of the starter culture, which varies between 32-37°C (Broome et al., 2003). Setting temperature has little role in reducing the level of microbial pathogens in cheese and may contribute to an increase in the population of microbial pathogens in the case of a failure of the starter culture, *e.g.* due to poor viability of the starter or the presence of a phage or antibiotics in the milk, etc. These factors may all contribute to slow growth of the starter, but may not slow the growth of microbial pathogens present in the milk.

Curd cooking refers to a heat treatment in cheese making that is aimed at stopping the growth of the starter culture and facilitate the contraction of the curd and expulsion of whey (syneresis). Curd cooking temperature varies according to the type of cheese and the way acidification is carried out. For soft and semi-soft cheese, the temperature ranges from 30-38ºC (Banks, 2003; van den Berg et al., 2003); for hard cheese, curd cooking ranges from 38-55ºC (Bachmann et al., 2003); and for acid/heat coagulated cheeses such as Cottage cheese, Cream cheese, Quark, Queso Blanco, Ricotta, Mascarpone and Paneer cheese, curd cooking can be as high as 90°C (Fox, 2003; Lucey, 2003). Where the cooking temperature is below 40°C, there is a good chance that microbial pathogens will grow, until the acidity of the curd becomes sufficiently high.

The combination of the curd cooking temperature and time applied in making hard cheeses and acid/heat coagulated cheeses may be sufficient to inactive most, if not all, of the vegetative cells of microbial pathogens present in the cheese.

1.2.3.2 Effect of pH

Selection of a starter culture that is a fast acid producer is vitally important in cheese making. If the starter culture (usually added at 10^6-10^7 cfu/mL) immediately dominates the population of microorganisms in the milk, the chance of an increase in the population of pathogenic microorganisms in the milk is substantially reduced. This is due to the dominance of starter culture in utilising the available nutrients in milk and the inhibitory effect of declining pH increasing organic acids.

In case of a starter culture failure due to infection by bacterial phage or inhibitory substances in milk, such as residues of antibiotics, pathogenic and spoilage microorganisms may dominate the microbial population (Cogan, 2003). This can lead to undesirable consequences, such as the build up of *Staphylococcus* enterotoxins that are likely to remain in the cheese.

Reaching the appropriate end point pH in acidification plays a critical role in reducing the level of microbial pathogens in cheese making. As illustrated by Cogan (2003), the level of *Salmonella* is reduced to zero during ripening of a Cheddar cheese if the pH of the cheese is 5.23 or lower, but remains constant throughout the 160 days of ripening when the cheese pH is 5.7.

During cheese maturation/ripening, cheese pH changes very slowly, and only a minor increase may occur during a long ripening time. The pH of surface-ripened cheeses, such as Camembert, Brie, Blue, and Tilsit, generally increases as a result of production of ammonium ions on the surface of the cheese. In other cases, the increase of cheese pH is a result of oxidation of lactate to water and carbon dioxide, which forms bicarbonate ions (Cogan, 2003).

1.2.3.3 Effect of salt on microflora

The level of salt (%w/w) in cheese ranges from approximately 0.7-6%. Salt, along with pH, redox potential and water activity contribute to the minimisation of spoilage and prevention of growth of pathogens in cheese.

Salt affects the water activity of cheese as well as microbial growth, water activity and ripening rate.

Salt is often reported as salt-in-moisture (S/M) level in the curd, and the level influences the growth of the starter culture, other organism, cheese flavour and properties.

It is important to recognise that salt is not distributed evenly throughout the cured mass. Dry salt applied to the surface of milled cured required time to diffuse throughout the curd mass, hence microorganisms in the curd will continue to grow. A salt gradient exists from the surface to the centre of cheeses where salt has been applied to the surface.

With 4% salt, many pathogenic microorganisms will not grow, with the exception of *S. aureus* and *L. monocytogenes*. *S. aureus* can grow in the presence of 6.5% of sodium chloride and *L. monocytogenes* can grow in the presence of 10% sodium chloride (Cogan, 2003). However, near these maximum levels, growth would be slow and require optimal conditions for other parameters.

1.2.3.4 Effect of water activity/moisture on microflora

The water activity is lowered during cheese ripening as the cheese loses moisture (whey) and as added salt binds free moisture and makes it unavailable for bacterial growth. The ability of bacteria to grow or survive is largely dependent on available moisture (in combination with other factors such as pH and temperature). Cheeses with relatively high a_w may readily support the growth of pathogenic bacteria compared to a low moisture cheese *i.e.* a_w less that 0.92 will inhibit the growth of bacterial pathogens, with the exception of *S. aureus*.

1.2.3.5 Effect of maturation/ripening on microflora

In hard cheeses, the combined effects of pH, salt, moisture and storage temperature come into play during ripening, inhibiting pathogen growth and promoting die off. Other than slow changes in pH and salt concentration, ripening/maturation leads to reduction in moisture content of the cheese (Sutherland, 2003). During the ripening/maturation process, pathogens in the cheese generally die off because of the low pH and low moisture content (a_w<0.96) combined with the relatively long ripening period at a low temperature. The physical-chemical characteristics of the cheese will also influence the decline of pathogens during this time. Long storage or ripening of cheese under controlled temperatures is likely to reduce any microbiological populations present.

Significant growth of pathogenic microorganisms may occur with the ripening of soft cheeses because of their relatively high moisture (a_w varies from 0.97-0.99), high pH and the often high ripening temperature (Cogan, 2003). Hard cheeses, by virtue of their low moisture content and long maturation periods, are unlikely to support the survival and proliferation of microbial pathogens.

1.2.3.6 Post-process contamination:

Recontamination of cheese by pathogens can occur at all stages post-pasteurisation. Cheese products that are on-processed such as cut, cubed, shredded and grated cheeses are more susceptible to post-process contamination and significant numbers of *L. monocytogenes* have been found on these types of products. The introduction of *L. monocytogenes* into these products follows inadequately cleaned and sanitised shredding/grating equipment.

In particular the dominance of *L. monocytogenes* on soft cheeses can be attributed to the psychrotrophic nature of this pathogen, its tolerance of reduced water activity, tolerance to salt, and its ability to grow well at ripening temperatures (10-12ºC), once the pH has

increased significantly above 5. In addition *L. monocytogenes* will continue to grow during retail storage (5ºC).

L. monocytogenes is ubiquitous in nature and can easily become established in processing plants. Its psychrotrophic nature allows it to colonise and grow where conditions tend to be wet and cool with areas of pooled water or liquid, including condensation on walls, ceilings and equipment surfaces; drains and floor puddles, condensate collected in refrigeration units and compressed air lines.

Specifically, brining tanks used in cheese making could be expected to be vectors for recontamination, depending upon the salt concentration, as *L. monocytogenes* is tolerant to salt.

1.3 Dried milk powders

1.3.1 Description

Whole milk, skim milk, whey, buttermilk, cheese and cream may be dried into powders by the application of heat. The fluid is initially concentrated by evaporation, then spray dried to form a powder. A description of the process of manufacturing dried milk powders is diagrammatically illustrated in Figure 3.

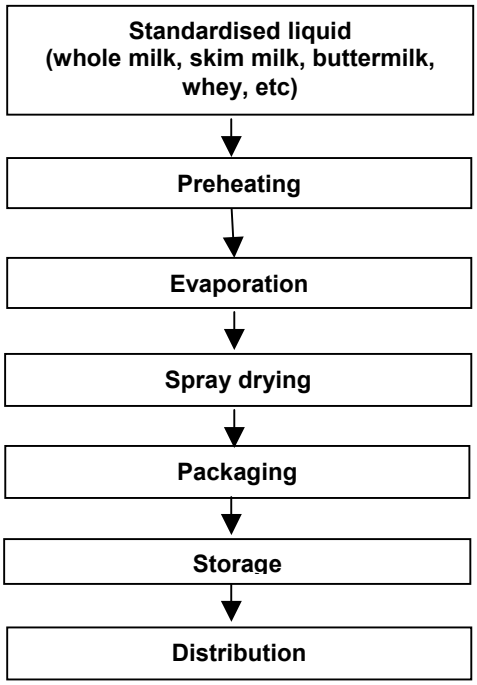

Figure 3: Manufacturing process for dried milk powders

1.3.2 Microbial pathogens of major concern

Microbial pathogens of major concern in dried milk powders include *Salmonella, L, monocytogenes, B. cereus, Cl. perfringens*, *S. aureus* and more recently *Enterobacter sakazakii.* While these organisms will not grow in powders, they may remain viable for long periods of time and resume growth when the powder is reconstituted and stored at favourable temperatures.

Surveys conducted overseas have shown the presence of *B. cereus* and *E. sakazakii* in dried milk powders, while Australian surveys have detected *Salmonella* spp. and *S. aureus* (Appendix 3).

The microflora of dried milk powders depend on many factors including the number and type of bacteria present in the raw milk or milk by-product, preheating temperatures, operating conditions of the evaporator and dryer, and plant hygiene. High numbers of microorganisms in the raw milk may result in high numbers in the milk powder. For example, raw milk counts in excess of 10^5 cfu/ml have resulted in counts in the powder of more than 10^4 cfu/g (Varnam and Hall, 1994). The decline in numbers as a result of exposure to heat, is offset by the removal of water and the concentration of bacteria in the powder. Post-processing contamination is a major factor impacting on contamination of milk powders, as the raw

material is often subjected to lethal temperatures, which eliminate vegetative cells of pathogens.

Dried milk powders have been implicated in a number of foodborne disease outbreaks (Appendix 2) involving *Salmonella* and *Cl. perfringens* (ICMSF, 1998; Anonymous, 1982). In an outbreak due to consumption of milk powder contaminated with *S. aureus* (ICMSF, 1998), it was considered likely that illness was a result of preformed *S. aureus* enterotoxin surviving the heating process. Illness has also been attributed to *S. aureus* contamination and abuse of reconstituted non-fat dried milk (El Dairouty, 1989). More recently a large outbreak of illness from *S. aureus* in Japan caused more than 13,000 cases and was due to preformed staphylococcal enterotoxin in the milk powder. This was traced back to poor hygienic and manufacturing practices during processing of liquid milk in particular the storage conditions (Asae et al., 2003).

Outbreaks demonstrate that failures in preventive systems, such as presence of water allowing multiplication or the presence of zones difficult to maintain and to clean (isolation from a drying tower), were the origin of contamination (ICMSF, 1998). In other cases illness has been due to contamination and abuse or reconstituted products. *S. aureus*, if present, may grow and produce enterotoxin when dry milk powder is rehydrated, if it is subject to time/temperature abuse (Umoh et al., 1985; El Dairouty, 1989). *Cl. perfringens* and *B. cereus* are able to produce spores that can survive pasteurisation and survive the manufacture of powdered milk production. They represent a problem when powdered milks are reconstituted and stored for prolonged periods at incorrect temperatures. Most *B. cereus* strains isolated from dairy products are able to grow and produce toxins below 10°C (Institute of Environmental Science & Research Limited, 1995).

Although there have been no outbreaks of listeriosis linked to dry dairy products, the persistence of *Listeria* spp. in the dairy plant environment and the association of listeriosis with other dairy products indicate the potential for *Listeria* contamination of dry dairy products (ICMSF, 1998). There is evidence that *L. monocytogenes* can survive a typical spray-drying process in the manufacture of dried milk powders (Doyle et al., 1985). Although dried milk powders will not support microbial growth due to their low water activity, *L. monocytogenes* is one of the few foodborne pathogens that can grow at refrigeration temperatures and, if present in the dried milk powders, it could possibly multiply when made up and stored in the refrigerator for a long period.

1.3.3 Effect of dried milk powder processing on the growth and survival of microbial pathogens

Before milk is dried, it is submitted to preliminary treatments such as separation, standardization, and concentration. Milk is usually concentrated as a preliminary step in the production of milk powders by evaporation. Milk is usually held at 0-4°C prior to evaporation and consequently requires pre-heating that can vary from <70 - 135°C for 15-30 seconds (TetraPak, 2003) before it can be concentrated/evaporated. Water is progressively removed from the milk to effect the concentration of milk solids. Modern evaporators are predominantly falling film evaporators in which the liquid to be evaporated passes as a film down the inner surface of a vertical steel tube through which heat is transferred from steam applied to the outer surface of the tube. To minimise damage to heat-sensitive components in milk, evaporation takes place under vacuum with approximate temperatures beginning at 70°C and reaching approximately 45°C at the last stages of evaporation (Gekas et al., 2003; personal communication, 2005).

The concentrate from the evaporation process is then dried. There are two types of drying treatments, spray drying or roller drying. Roller drying is sometimes referred to as drum-drying. Spray drying is now overwhelmingly used in the manufacture of dried milks because concentrated milk does not contact the steam-heated rotating rollers that adversely affect heat sensitive components of milk, especially proteins and lactose (Caric et al., 2003).

The drying temperature varies between 130ºC and 150°C for roller drying and 180-240°C for spray drying (Caric et al., 2003). In the latter situation, the residence time of concentrated milk in the spray-drying chamber is less than 30 seconds. The extent of microbial destruction during drying depends on the types of microorganisms present and on the drying temperature of the exit air in spray-drying or on the drum temperature and retention time for roller-drying.

Skim milk is subjected to different heating processes prior to drying, depending on the intended use of the product. High heat skim milk powder is usually pre-heated using direct steam injection to temperatures of approximately 115-120°C for 3 minutes. For use in the baking industry, a high temperature preheat (5 minutes at 95ºC) is usual. However, low heat treatments are applied to skim milk powder (15 seconds at 72ºC) which is to be used in cheese making and standardisation of liquid milk.

Vegetative cells of bacteria including those of the family Enterobacteriaceae have been shown to survive drying process in manufacturing milk powder (Daemen et al., 1982). Therefore, milk for drying must be given a heat treatment equal to or greater than pasteurisation prior to drying and then contamination must be avoided after the drying process.

Subsequent processing steps such as cooling, intermediate storage, instantising, blending and packaging may also influence the microbiological quality by increasing the risk of contamination from the production line or the environment. The presence of food pathogens in a properly pasteurised and dried milk powder is indicative of post-process contamination.

During storage of dried milk powder, surviving organisms slowly die, but spore-formers, being the most resistant, retain viability for long periods of time (ICMSF, 1998).

Since the water activity of dried milk powders is too low to permit microbial growth, the occasional microbiological problems with instant milk powder are largely associated with handling during reconstitution. Microorganisms present in milk powder, or those introduced at reconstitution, may proliferate in case of temperature abuse or mishandling of the reconstituted milk (ICMSF, 1998).

Outbreaks due to *Salmonella* usually share a common factor, the accumulation of contaminated dust and powder deposits in the factory environment, which are eventually, transferred to the product by mechanical fault. The most common hazard reported is the accumulation of powder deposits in the drier insulation, which having become contaminated by environmental salmonellae, gains access to the product via stress cracks in the inner skin of the dryer. The second most important hazard is due to contaminated air and may occur during the secondary drier stages, transport of powder to silos, or during filling and packing operations (Early, 1998).

The effect of processing on pathogens in dried milk powders is described in Table 4.

Table 4: Effect of processing for dried milk powders on selected pathogens

Pathogen	Effect of processing
Salmonella	*Salmonella* should not be present in dried milk powders as it is destroyed by heat treatment. *Salmonella* may enter powders from foci of contamination within the plant where organisms are able to persist and multiply over extended periods. Contamination can originate from parts of the powder handling system and cracks in the dryer walls and powder storage silos.
Staphylococcus aureus	*S. aureus* is destroyed in the heat process. Organisms present are a result of post-processing contamination or due to preformed toxins surviving the heat-processing step (ICMSF, 1998). Growth and enterotoxin production by *S. aureus* occurs either in raw milk before heat treatment or in the concentrated milk before drying. Staphylococcal toxins in dried milk powder indicate unhygienic ingredients or unacceptable processing conditions.
Listeria monocytogenes	*L. monocytogenes* has been reported to survive a typical spray-drying process (ICMSF, 1998), however, it will not survive heat treatment prior to spray drying. Gradual die-off has been reported for dried milk product after the drying process (ICMSF, 1998), but there is a risk of post-heat treatment contamination as *Listeria* is ubiquitous.
Bacillus cereus	Vegetative cells of *B. cereus* are destroyed in the heat process used to produce dried milk powder, but spores can survive the processing. Generally low numbers of *B. cereus* and/or its spores do not cause problems unless growth is permitted to occur (Doyle, 1989). Spore germination is greatly reduced by unfavourable conditions such as low temperatures, however psychrotrophic strains can grow at 4-5ºC (AIFST, 1997). Growth and toxin production can be prevented by storing reconstituted products at temperatures below 4ºC (AIFST, 1997).
Clostridium perfringens	Although *Cl. perfringens* spores can survive heat-treatment processing, the temperature range for the growth of *Cl. perfringens* is 15-50ºC. At cold temperatures of 0-10ºC, vegetative cells die rapidly (AIFST, 1997).
Enterobacter sakazakii *(infant formulae)*	*E. sakazakii* will not survive pasteurisation and temperatures of 70ºC or above should provide virtually instantaneous inactivation (Codex). Recontamination of powdered infant formulae during handling and filling processes is a risk. Other sources of recontamination are the ingredients added to the formulation. *E. sakazakii* cannot grow in a dry substrate, but it can survive a long period of time and is potential hazard when the powder is reconstituted.

1.4 Infant formulae

1.4.1 Description

Powdered infant formula belongs to a special sub-set of powdered milks. These products are formulated to be as similar to human milk as is possible then concentrated and spray dried. In some cases, specific heat-labile ingredients are added after drying. Typically, infant formulae contain milk, or soy proteins, or protein hydrolysates together with those forms of fat, carbohydrate, vitamins, and minerals that are bioavailable to the infant.

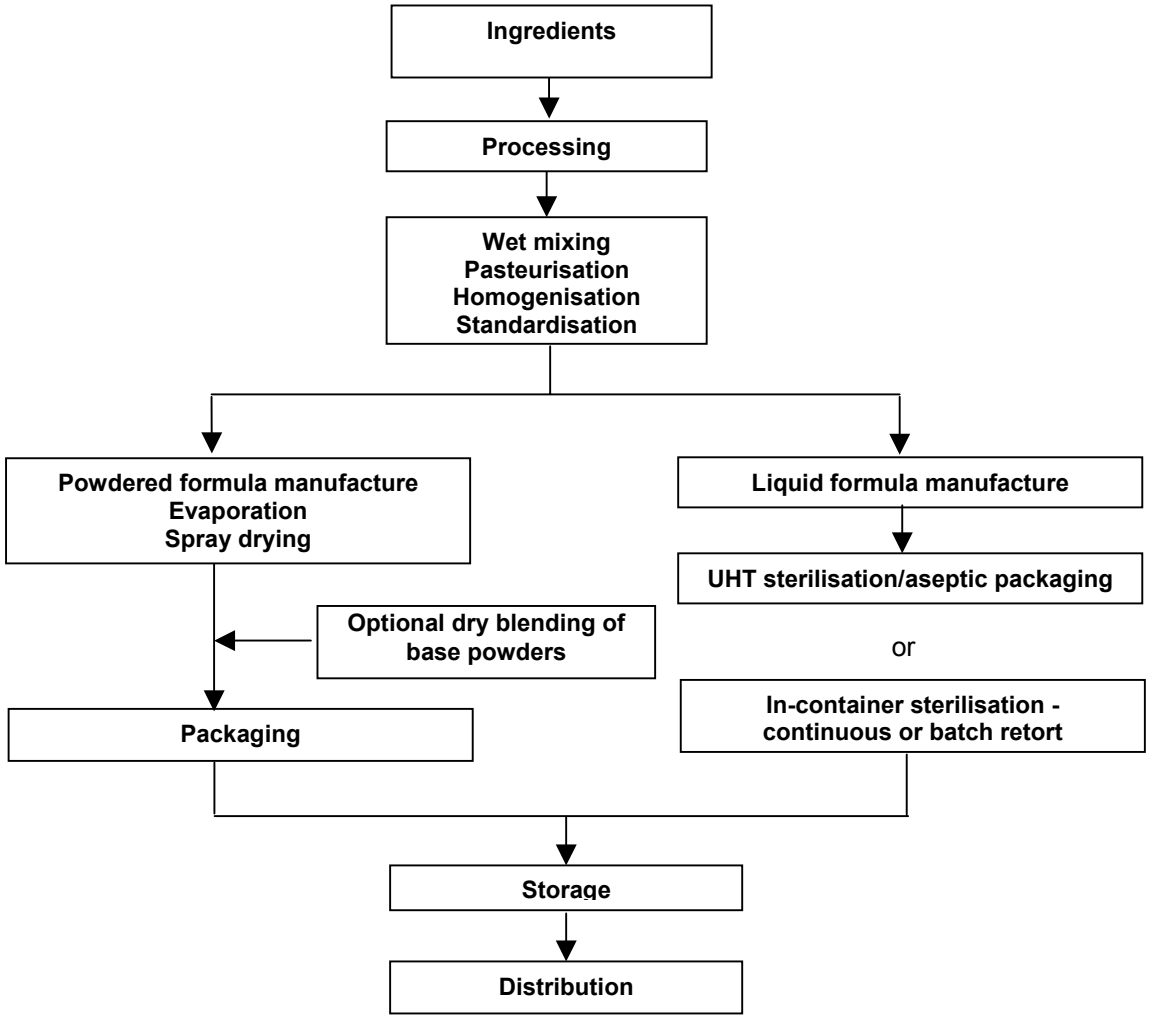

Figure 4: Manufacturing process for infant formulae

1.4.2 Microbial pathogens of major concern

Microbial pathogens of concern with powdered infant formulae are the same as those for dried milk powders, including *Salmonella, L, monocytogenes, B. cereus, Cl. perfringens*, *S. aureus* and more recently *Enterobacter sakazakii.* However, control over the microbiological status of these products is essential because of the vulnerable status of infants.

Surveys of infant formula overseas have indicated the presence of *B. cereus* and *E. sakazakii* (Appendix 3).

Several outbreaks have been associated with infant formulae (Appendix 2). While *Salmonella* is rarely found in surveys of powdered infant formula, low-level contamination of powdered infant formula with *Salmonella* has been epidemiologically and microbiologically associated with infections in infants (Picket and Agate, 1967, Rowe et al., 1987, CDC, 1993, Usera et al., 1996, Threlfall et al., 1998, Olsen et al 2001, Bornemann et al, 2002). .

Illness has also been attributed to *S. aureus* contamination and to abuse of reconstituted infant powdered milk (Umoh et al, 1985).

More recently a growing number of reports has linked *E. sakazakii* infection in infants to powdered infant formula (Biering et al., 1989,=; Simmons et al., 1989; Van aAcker et al., 2001; CDDC, 2002) In several investigations outbreaks of *E. sakazakii* infection has occurred among neonates in neonatal intensive care units. Mortality rates from *E. sakazakii* infection have been reported to be as high as 50% or more, but this figure has declined to <20% in recent years (Codex).

In Australia, an outbreak involving *Salmonella Bredeney* was traced to contamination of powdered milk-based infant formulae (Forsyth JR et al, 2003).

Liquid, ready-to-feed infant formulae are commercially sterile, generally do not support the growth of microorganisms and are shelf stable.

1.4.3 Effect of infant formulae processing on the growth and survival of microbial pathogens

The manufacture of infant formulae involves the blending of water-soluble proteins, carbohydrates, vitamins and minerals with vegetable oils to achieve a homogenous solution, followed by sufficient heat treatment or dehydration to provide microbiological safety. The final steps in the technology used for infant formula manufacture has seen little change in the past 20 years, as powder manufacture, typically via spray-drying, or heat sterilization (ultra-high heat or retort sterilisation) of liquids are the processes of choice (O'Callagahan and Wallingford, 2002). Development of rewet agglomeration (instantisation) processes in the art of spray-drying has contributed to improved reconstitution ability of infant formula powders. Dry blending of prepared base powders provides flexibility for the manufacture of market-specific formulations, which may include, among others, heat-sensitive components, such as starches, flavours, probiotics or bioactive proteins.

In the dry blending process, the ingredients are received from suppliers in a dehydrated powdered form and are mixed together to achieve a uniform blend of the macro and micronutrients necessary for a complete infant formula product. This process does not involve the use of water in the manufacturing process, and therefore the processing line can be kept dry for long periods of time. In a dry environment, pathogens are denied the water needed to support growth, thereby reducing the possibility of these organisms becoming established in the plant environment in sufficient numbers to cause further product contamination. However, the microbiological quality of a dry-blended product is largely determined by the microbiological quality of the constituent dry ingredients as there is no heat treatment to destroy bacteria in the final product. Thus, if one or more ingredients in a dry-blended product are contaminated with pathogens these bacteria are likely to be present in the finished product.

The wet-mixing-spray drying process involves blending of ingredients, homogenisation, pasteurisation and spray drying to produce a powdered product. The ingredients either in liquid or powdered form are typically mixed with water to form a liquid mix which is dried to a powder in large spray driers. Prior to drying the liquid mix is pasteurised (71.6°C for 15 seconds or 74.4°C for 25 seconds for products containing starches or thickeners or at higher temperatures such as 105-125ºC for at least 5 seconds) (Codex). The severity of the pasteurisation process varies among manufacturers, but is always sufficient to destroy *Salmonella* and *E. sakazakii* and vegetative cells of pathogens such as *S. aureus* and *B. cereus*.

The liquid is homogenised then concentrated by passing through an evaporator or pumped directly to the spray dryer. Prior to spray drying, the product is pre-heated (approximately 82ºC) and passed through a high-pressure pump to the spray dryer nozzles. The inlet air temperatures of spray towers are normally between 150ºC and 220ºC (Becker *et al.*, 1994). Due to the cooking of the rapidly evaporating water, the inner temperature of the sprayed particles only reach 40-50ºC (Kessler , 1988) or up to 70ºC (Eschamann, 1970).

Enterobacteriaceae are ubiquitous in the processing environment. *E. sakazakii* is relatively resistant to osmotic and dry stress compared with other members of the Enterobacteriaceae family (Breeuwer *et al.,* 2003). The survival of *E. sakazakii* at elevated temperatures (45°C) and its capacity for growth up to 47°C, illustrate that in warm and dry environments, such as in the vicinity of drying equipment this bacterium has a competitive advantage compared with other members of the Enterobacteriaceae. Condensation in the drying and filling areas can lead to an increase in the normally very low numbers of *E. sakazakii* n that environment.

After spray drying the product may be agglomerated to increase the particle size and improve its solubility. Accidental microbiological contamination can occur during the agglomeration process. The finished powder is sifted and packaged. Airborne *E. sakazakii* can re-contaminate the powder during the handling and filling processes.

Bactofugation is used by some producers and removes bacteria, especially spores (up to 95%), from milk by high speed centrifugation.

The spray drying process requires processing equipment to be regularly wet cleaned, therefore providing a moist environment for bacterial growth in the plant environment. If not controlled, these bacteria can be a source of product contamination. *B. cereus* spores and *E. sakazakii* bacteria are able to adhere to several types of surfaces and hence it is difficult to remove them from equipment during cleaning. These spores also possess appendages and/or pili that are, at least in part, involved with adhesion (Doyle *et al*., 1997).

During storage of infant formula, surviving organisms slowly die, but spore-formers, being the most resistant, retain viability for long periods of time (ICMSF, 1998).

The effect of processing on pathogens for infant formulae is the same as that for dried milk powders as described in Table 4.

1.4.4 Effect of preparation of infant formulae and possibility of growth of microbial pathogens after reconstitution

Recontamination of infant formulae with *E. sakazakii*, may take place during preparation or reconstitution of infant formulae due to poorly cleaned baby bottles and poorly maintained equipment at home and hospitals (Codex doc).

Incorrect storage and temperature abuse of reconstituted infant formulae may lead to multiplication of pathogens such as *E. sakazakii*, *B. cereus*, and *S. aureus* if present.

1.5 Concentrated milk products

1.5.1 Description

Concentrated milk products have reduced water content and include evaporated milks and sweetened condensed milks. Sweetened condensed milk is characterised by its high sugar content, which varies from 61-64% calculated as sucrose/(sucrose + water) in the product (Nieuwenhuijse, 2003b). Unlike evaporated milk, which is preserved by heat treatment (UHT treatment or sterilisation), sweetened condensed milk is preserved by its sugar content. Figure 4 shows the principal processing steps involved in the manufacture of concentrated milks.

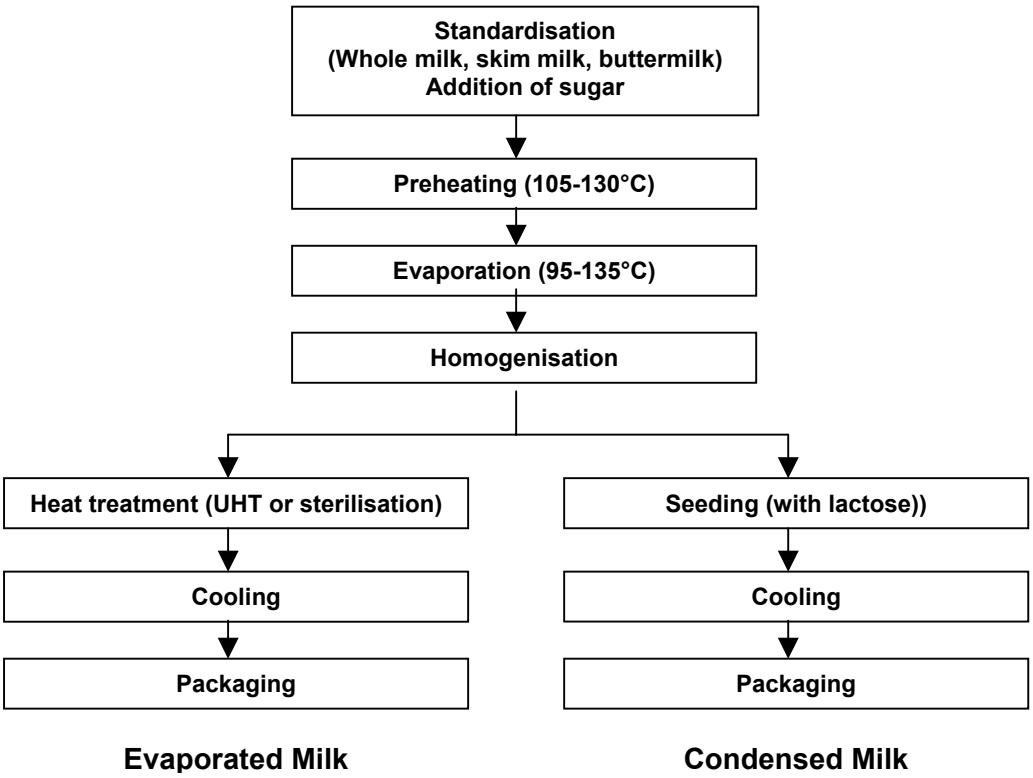

Figure 5: Indicative steps in the manufacture of evaporated milk and sweetened condensed milk

1.5.2 Microbial pathogens of major concern

No reported cases of foodborne disease outbreak have been attributed to the consumption of sweetened condensed milk or evaporated milk (ICMSF, 1998). These products generally do not support the growth of microorganisms and are shelf stable.

The main microbiological concern with evaporated milk is primarily non-pathogenic thermophilic spore-forming bacteria such as *Bacillus stearothermophilus*, which spoil the product (Nieuwenhuijse, 2003a). The main concern for sweetened condensed milks is *S. aureus*.

1.5.3 Effect of processing of concentrated milk on the growth and survival of microbial pathogens

Both evaporated milk and sweetened condensed milk have had a considerable portion of the water removed through an evaporation process. For evaporated milk, the milk is initially preheated to temperatures between 110-130°C for 1-3 minutes (Nieuwenhuijse, 2003a) then concentrated in a multistage falling film evaporator at 50-95°C. Milk is homogenised, heat treated and cooled before being packed into cans. Alternatively the product is homogenised and canned, then heat treated.

For sweetened condensed milk, milk is preheated to temperatures are between 105-120°C for 15-60 seconds. Preheating applied in producing sweetened condensed milk is aimed at inactivating all enzymes, osmophilic yeasts, micrococci and moulds, and to regulate viscosity. Sweetened condensed milk is concentrated also in a multistage falling film evaporator at 35-50°C. Sugar can either be dissolved in cold milk before preheating, or added as syrup after preheating or at the end of evaporation. Following evaporation, the concentrate milk is homogenised and then seeded with lactose to prevent the formation of large crystals. Sweetened condensed milk does not get subjected to a heat treatment process such as UHT or sterilisation that is applied to evaporated milk and is not commercially sterile (Nieuwenhuijse, 2003b).

The preheating treatment of evaporated milk and sweetened condensed milk through a continuous flow heating is considered to be equal to or better than the process of pasteurisation and destroys the vegetative forms of microbial pathogens, but not bacterial spores. Subsequent heat treatment, either UHT or sterilisation after homogenisation and stabilisation in the manufacture of evaporated milk destroys any remaining microorganisms, including spores, and leads to a product of commercial sterility. In the case of sweetened condensed milk, pathogenic or spoilage microorganisms are unlikely to proliferate because of its high sugar content and thus low water activity (Nieuwenhuijse, 2003a; Nieuwenhuijse, 2003b).

Although sweetened condensed milk is not a sterile product, the low water activity (between 0.83-.85) makes it unlikely to support the growth of pathogenic bacteria. Likewise, spores of *Clostridium* and *Bacillus* spp. present in sweetened condensed milk will also not be able to grow (Nieuwenhuijse, 2003b). The exception is *S. aureus*, which can grow at a water activity of around 0.85. However vegetative cells of *S. aureus* will not survive the pre-heat treatment given to sweetened condensed milk, and growth and toxin production of any spores is severely limited because of the anaerobic environment of sweetened condensed milk (ICMSF, 1998).

1.6 Butter and butter products

1.6.1 Description

Butter is produced from cream by churning or an equivalent process. Butter spreads are based on vegetable fats, a blend of vegetable and butter fat, or butterfat alone (light butter). The main steps in the manufacturing butter are illustrated in Figure 5.

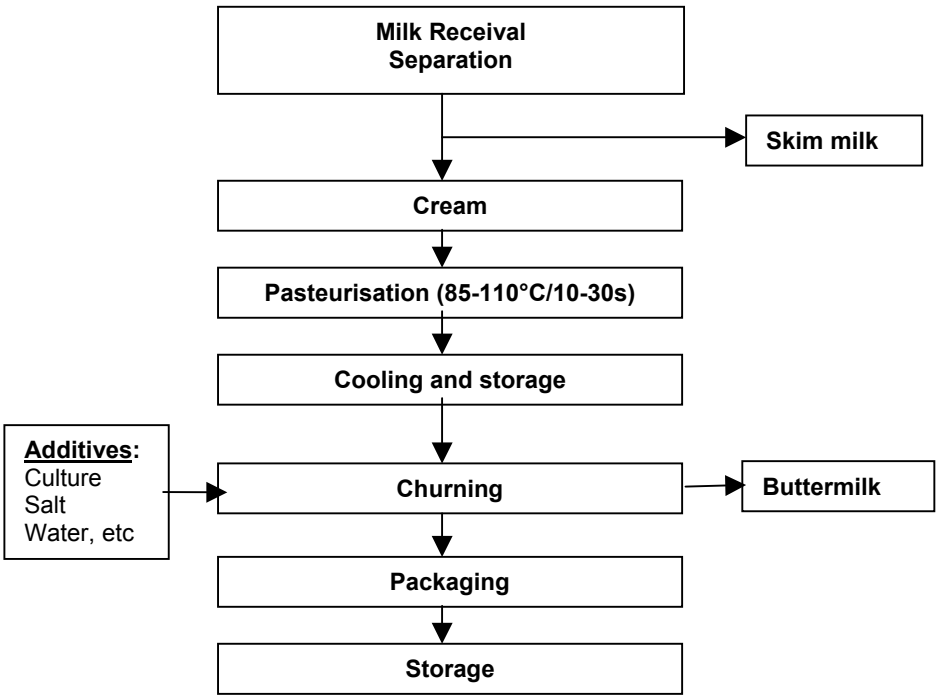

Figure 6: Indicative Manufacturing process for butter

1.6.2 Microbial pathogens of major concern

While butter represents a dairy product of low risk to public health there have been incidents of foodborne illness attributed to this product. Staphylococcal food poisoning has been traced to whipped butter in the United States, although temperature abuse was a contributory factor (ICMSF, 1998; Varnam and Sutherland, 1994).

There have also been two outbreaks of listeriosis linked to the consumption of butter. *L. monocytogenes* was isolated from several points in a production facility packaging small butter packages in an outbreak in Finland in 1998-1999 (Eurosurveillance, 1999). More recently a cluster of listeriosis cases occurred in England implicating butter (CDR Weekly, 2004). Mishandling may have been a contributing factor in this outbreak.

In addition in the US, there have been several recalls issued for *L. monocytogenes* contaminated butter (Ryser and Marth, 1999).

1.6.3 Effect of butter processing on the growth and survival of microbial pathogens

The process of butter manufacture begins with the separation of milk into cream and skim milk. The cream is typically pasteurised at 85°C for 15 seconds under vacuum (ICMSF, 1998). Pasteurisation destroys vegetative microorganisms, especially pathogens, but will not

eradicate bacterial spore-formers and some of the more heat-resistant vegetative spoilage flora (ICMSF, 1998). Preformed *S. aureus* enterotoxin resulting from poor sanitary conditions in the pre-pasteurisation stage may carry over to butter.

The pasteurised cream is then churned, a process where fat distributed in the aqueous phase of cream is converted into a substrate where water is dispersed through the butterfat. Additives such as flavour concentrates and seasonings such as garlic or herbs may be added during this stage.

During the butter making process, microorganisms are retained in the aqueous phase, the buttermilk, which is drained from the butter. The butter continues to be worked mechanically to create the right physical properties, with water being dispersed into minute droplets generally less than 10 μm in diameter in the fat matrix (ICMSF, 1998).

The microbiological stability of butter depends on its moisture content; the physical distribution of the aqueous phase and its nutrient content, and the presence, in the aqueous phase, of inhibitors (ICMSF, 1998; Varnam and Sutherland, 1994). Typically butter contains around 16 % moisture, and 2 % salt in salted butter. The extent of microbial growth is severely restricted by the very tiny physical size of the water droplets and extensive multiplication is not possible and will start to die off (Varnam and Sutherland, 1994; ICMSF, 1998). Salt is also an important inhibitor in some butters and at 2 % (will equal 12.5% in the aqueous phase) will be strongly inhibitory to most microorganisms (Frede, 2003; Varnam and Sutherland, 1994).

Unsalted or low- salt butter is much more likely to support the growth of spoilage microorganisms than salted butter as it presents a more favourable substrate for bacteria survival. Low fat spreads have a higher moisture content which requires that the droplet distribution be adequately spread throughout the product. Ideally, the moisture droplets should be in the range of 1μ - 10μm to reduce spoilage and ensure product safety. Any increase in the size of moisture droplets provides greater opportunity for microbial growth which places a greater reliance on any microbial inhibitors that may be present.

Butter does not appear to be a good growth medium for *L. monocytogenes*, as salt added during manufacture and distributed in the water phase is at or close to the limit for growth at refrigeration temperatures. However, growth has been demonstrated experimentally in butter during storage and it appears that *L. monocytogenes* favours the water rather than the lipid phase during butter making (Ryser and Marth, 1999). This is supported by the outbreaks and recalls that have been associated with *L. monocytogenes* and butter.

Anhydrous milk fat (AMF) is a purified form of butterfat made directly from cream or butter by centrifugal separation. The product has exceeding low moisture levels (0.1 – 0.3 %) and will not support the growth of bacteria. It is mixed with skim milk powder in the reconstitution of liquid milk. AMF may be further separated into various fat fractions, based on melting point, by fractional crystallisation. These products can be used in combination with skimmed milk powder to produce various milk products, and are used in the baking, confectionery, ice-cream and chocolate industries.

1.7 Ice-cream

1.7.1 Description

Ice-cream is a frozen aerated emulsion made from cream or milk products or both, and other food components. Manufacture of ice-cream involves the preparation of an ingredient mix comprising milk fat, milk solids, sweetener, water and other ingredients which are pasteurised and homogenised, aged, then whipped to incorporated air while being frozen. The final product is then packaging and hardened during frozen storage prior to distribution (Goff, 2003).

Other types of ice cream are available in many forms, flavours and packages. Different products prepared both from edible fats and milk or milk products; include gelati, soft serve, stick ice creams and confections, *etc.*

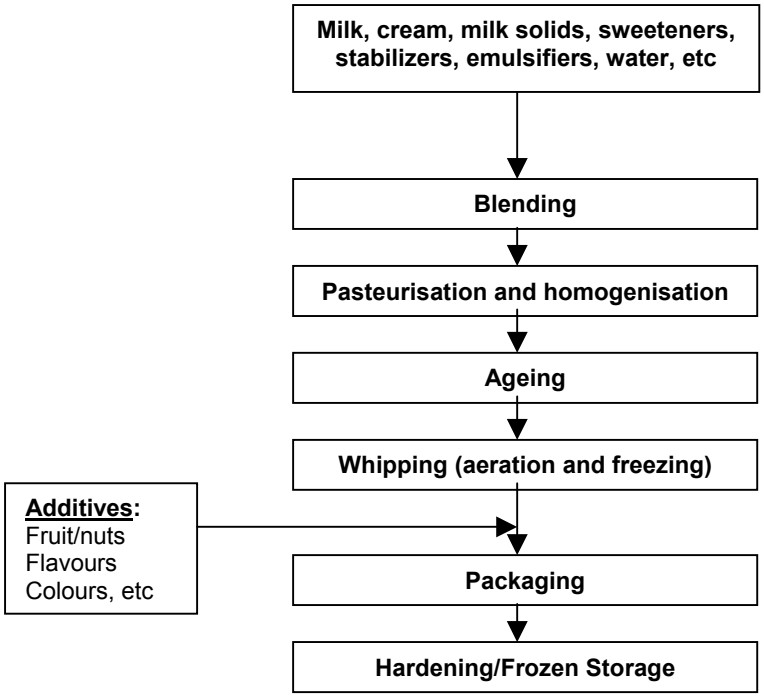

Figure 7: Manufacturing process for ice-cream

1.7.2 Microbial pathogens of major concern

While pathogenic bacteria will not grow in ice-cream, some pathogens, if present, may survive long periods of frozen storage. Therefore, any pathogens present in ice-cream as a result of post-process contamination may pose a potential hazard to consumers.

The major microbial pathogens of concern associated with ice-cream are *L. monocytogenes*, *Salmonella* spp. and *S. aureus*, (ICMSF, 1998). Reported outbreaks of foodborne illness attributed to ice-cream have typically involved home-made ice-cream where raw milk and/or raw egg were used and the heat treatment was inadequate (Taylor et al., 1984).

Outbreaks involving consumption of commercially manufactured ice-cream have been found to be the result of post-processing contamination. In the 1994 outbreak of *S. enteritidis* foodborne illness, a transport tanker previously used to transport unpasteurised raw egg was used to transport ice-cream mix that was not subsequently repasteurised (Hennessy et al., 1996).

Several recalls of ice-cream due to contamination of *L. monocytogenes* have occurred in the US since 1985. However no direct link to listeriosis has been documented. In Australia, chocolate-coated ice-creams were recalled in 1995 because of *L. monocytogenes* contamination.

An outbreak of *S. Oranienburg* associated with gelati was reported in South Australia in 1998. Contamination of the gelati most likely resulted from equipment contaminated with the *Salmonella* (Milazzo *et al.,* 1998). Gelati differs from ice-cream in that it has a very low (dairy) fat content (varying from 1.4 - 8%). Milk based gelati also has less air incorporated (approximately 35-40%) compared to ice cream which is approximately 50%.

Aeromonas spp. and *Br. abortus* have been detected in surveys of ice cream and ice cream products overseas, while *L. monocytogenes* has been detected in surveys of ice cream in Australia (Appendix 3).

1.7.3 Effect of ice-cream processing on the growth and survival of microbial pathogens

A wide range of ingredients are blended to prepare the liquid ice-cream mix. This mix is then subjected to a heat treatment process to reduce bacterial numbers, and specifically to destroy pathogenic organisms (Goff, 2003). Time and temperature of processing is typically greater than that for pasteurisation because of the high fat and high solids content of ice-cream mixes.

The heat treated mix is then homogenised to enhance the body and texture of the frozen product by reducing the size of fat globules in order to prevent the fat from churning during the freezing process. Flavours may be added before or after pasteurisation and homogenisation. However, colours, fruits, confectionery, chocolate and nuts are generally added after pasteurisation, and these may represent a source of contamination of the final products.

The pasteurised mix is cooled to 4°C or lower and aged to allow physical changes to occur. After aging, the ice-cream mix is frozen, unless it is to be used for soft-serve ice-cream. Hardened ice-cream is frozen in a two step process. The first step involves partial freezing of the mix to -5° to -8°C while the air is beaten into the mix. The partially frozen mix is packaged and immediately placed in a hardening room or freezing tunnel where it is frozen to -25° to -30°C (ICMSF, 1998).

The microbiological quality of ice-cream depends upon the interaction of factors such as the:

- microbiological status of the ingredients and additives;
- processing conditions (*e.g.* heat treatment) which the mix has been exposed to during the manufacture of ice-cream; and
- hygienic control and cleaning of the manufacturing equipment (Robinson, 1985) and the hygienic status of packaging materials.

Ingredients used in the manufacture of ice-cream must be of a high microbiological standard. Milk, cream, skim milk and skim milk concentrate should have been heat treated, must be kept under refrigeration, and used promptly to ensure satisfactory quality. The main organisms present after heat treatment will be spore-forming bacilli, although there may be some psychrotrophic organisms surviving if the initial population was high, together with some micrococci and other thermoduric bacteria. Normally, none of these groups constitute a health hazard (Robinson, 1985).

Butter and butter oil (anhydrous milk fat) are made from heat treated cream under carefully controlled conditions, and should be of good microbiological quality.

Granulated sugar, glucose syrup solids and dextrose should be almost free of contaminating organisms. Similarly, sugar syrups should also be virtually sterile. Emulsifying and stabilising agents could prove a hazard unless purchased from a reputable supplier and kept under good storage conditions (Robinson, 1985).

Other ingredients that are added to ice-cream or used as coatings may be added after heat-treatment of the mix and may introduce potential hazards. These ingredients include fruits (canned, fresh, or frozen and usually in concentrated sugar syrups), nuts, chocolate, pieces of toffee and biscuit, colours and flavours and may contribute significant contamination (ICMSF, 1998). Careful control of these ingredients is essential (Robinson 1985) although they are often difficult to decontaminate.

Heat treatments applied to ice-cream mixes are frequently more severe than pasteurisation requirements for liquid milk. As a result, vegetative cells are normally destroyed, with spores usually the only survivors. From survey data on industry pasteurisation practices in Australia HTST treatment of ice-cream mixes were in the range of 78-85°C for 13-45 seconds[3].

Where pathogens are present in ice-cream they may survive for many months. Ice-cream is stored frozen from the time of manufacture to the time of consumption. The low temperature of frozen ice-cream completely prevents microbial growth (ICMSF, 1998) but pathogens may survive.

Soft-serve ice-cream presents a special case as the mix must be transported to retail soft-serve outlets where it is stored until soft-frozen and dispensed to consumers. Contamination and temperature abuse of the mix may easily occur, plus procedures for the cleaning and sanitation of the freezer and associated equipment often are inadequate. Soft serve ice-cream is usually drawn from the freezer at about -6°C to -7°C (ICMSF, 1998).

The microbiological safety of ice-cream is ensured by eliminating vegetative pathogens by pasteurisation and the prevention of recontamination at all stages until the point of sale; control of the microbiological status of ingredients; and the prevention of microbial growth before freezing (Varnam and Sutherland, 1994). Pathogens are unable to grow in ice-cream/confection when stored at correct temperatures.

[3] Pasteurisation times and temperatures are from the ***Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products Report***

1.8 Cultured and fermented milk products

1.8.1 Description

Yoghurt and fermented milk products are prepared by fermentation of milk or milk products using specific microorganisms that reduce the pH and coagulate milk proteins. Yoghurt is characterised by fermentation with thermoduric *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* with or without other lactic acid producing bacteria. Fermented milk products include yoghurt, cultured buttermilk, cream (sour cream), and acidophilus milk (Surono et al., 2003).

Figure 8 shows the basic steps involved in manufacture of stirred-style yoghurt. Manufacturing processes for other fermented milk products vary from product to product, but the common steps are fermentation of pasteurised milk with or without addition of flavour substances.

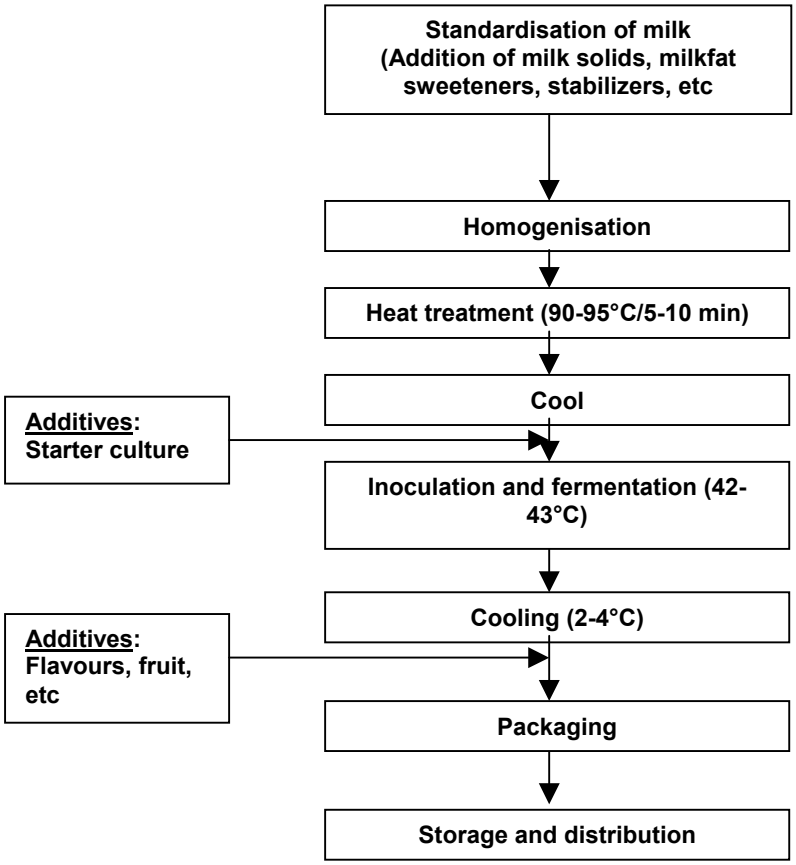

Figure 8: Indicative manufacturing process for stirred-style yoghurt

Products range from traditional yoghurts, to drinkable yoghurts, kefir, yoghurt cups and yoghurt tubes.

1.8.2 Microbial pathogens of major concern

Fermented products are rarely associated with foodborne disease as their pH is too low and the lactic acid concentration too high to permit growth of vegetative pathogens and death of non-growing cells is likely to be rapid (Varnam and Sutherland, 1994). However, consumption of yoghurt containing large numbers of yeasts can lead to digestive disturbances.

The limited outbreaks of foodborne illness that have been reported typically have involved *S. aureus*, *Cl. botulinum* and *E. coli* 0157:H7 (ICMSF, 1998; O'Mahoney et al., 1990; Morgean et al., 1993).

Slow growth by the starter culture provides an opportunity for growth of pathogens that contaminate the milk or ingredients, for example, staphylococcal toxin may accumulate in the ingredients where too much sugar inhibited the growth of starters but not the growth of *S. aureus*, resulting in illness (Mocquot et al., 1970). In another yoghurt outbreak, under processing of canned hazel-nut puree used to flavour the yoghurt caused growth and toxigenesis of *C. botulinum* spores in the puree. In addition the sugar in the ingredients was replaced by aspartame, leading to an increase in water activity to a level allowing growth of the pathogen (O'Mahony et al., 1990).

From a number of microbiological surveys of cultured and fermented milk products identified in the literature, only one reported the positive identification of a pathogen (*Y. enterocolitica* in fermented cow's milk) (Appendix 3).

1.8.3 Effect of cultured and fermented milk processing on the growth and survival of microbial pathogens

Fermented milks can be subdivided into three groups: lactic bacteria, lactic yeast and lactic mould fermentations, which are based on the metabolism of the respective groups of microorganisms. All products are the result of fermentation of lactose into mainly lactic acid. Yoghurt is the dominant fermented milk product on the retail market.

Sour cream (or cultured cream) is manufactured using *Streptococcus lactis* as a starter culture. The process for making sour cream is largely equivalent to that of other fermented products. Cream is standardised, homogenised, heat treated for 5 minutes at 90°C, cooled, inoculated and packaged. The final pH value of a freshly produced sour cream is about 4.5 (Hoffmann, 2002).

Fermented milks based on probiotic strains are processed in a similar manner to yoghurt. The most commonly used strains include *Bifodobacterium* spp., *Lb. acidophilus* and *Lb. casei*. A specific starter medium is used to grow the fastidious organisms.

The initial steps involved in the manufacture of fermented milks are the same as those applied to milk (*i.e.* homogenisation and heat treatment). Most of the manufacturing process for cultured and fermented milk products involves a fortification step where different solids are added to the milk. Skim milk powder or whey protein concentrates are most widely used, although alternative protein sources are used for supplementation *e.g.* ultrafiltration retentate.

Heat treatments of blended mix prior to the addition of starter culture vary from HTST pasteurisation to a full UHT process. After cooling to the desired optimal fermentation temperature, starter cultures are added.

Sweeteners, colouring and flavouring are usually added after pasteurisation, but may be added pre- or post-fermentation. Fruit and nuts added to yoghurt are usually supplied as heat-treated purees in large cans or bulk containers for direct connection to the yoghurt handling

line. Additions to stirred yoghurt[4] are made after fermentation, but in the case of set yoghurt[5] a layer of fruit in a viscous gel is placed in the container before addition of the inoculated ingredient mix.

The ingredient mix is fermented until the desired pH is reached, typically around a maximum pH of 4.5. Growth of the inoculated organisms and further acidification are minimised by cooling. The time taken to reach the required pH varies depending on whether a short or long set regime is used in manufacture. Most modern large-scale production uses the 'short-set' method, in which starter culture is added at 2%, permitting the fermentation to be completed within four hours at an incubation temperature of 42-43°C. However, a small amount of yoghurt is still made using a long-set process, in which starter culture is added at 0.5% and the fermentation continues for 14-15 hours at 30°C (Varnam et al., 1994).

Acidophilus milk is obtained after slow fermentation with *Lb. acidophilus*. Due to the poor competitiveness of the starter, a high heat treatment or even UHT is required to eliminate spore-forming microorganisms. The milk is cooled and inoculated with the starter and incubated for up to 24 hours.

Other fermented milks including traditional products such as Kefir and Koumiss, which are based on a combined fermentation of lactic acid bacteria and yeasts and are characterised by the presence of ethanol up to 2% and CO_2. A similar product is the Finnish yoghurt Viili that is made from the combination of mould and lactic acid bacteria. Concentrated or strained fermented milk products such as Ymer (Denmark), Skyr (Iceland) and Labneh (Lebanon) are popular in their respective regions. The whey in these products is drained off after fermentation.

Yeasts can become a problem in fermented milk products. The high moisture content of these products can allow excessive yeast growth causing gas production. Yeasts are not inhibited by the acidic conditions found in fermented dairy products (Stanly, 1998).

Pasteurisation is sufficient to kill vegetative cells including *Salmonella* and *Campylobacter* and the rapid development of the starter cultures is sufficient to inhibit outgrowth and development of spore-formers (ICMSF, 1998). The low pH, the presence of lactic acid and other organic acids as well as, in certain cases, inhibitory compounds such as bacteriocins generate an unfavourable environment for pathogenic organisms(ICMSF, 1998). However, if slow-fermenting strains are used, the outgrowth of spore-formers can occur and sterilisation or UHT treatment of the milk is necessary (ICMSF, 1998).

Fermented milks are stored at refrigeration temperatures after production, during distribution and during storage in the home. Low temperature storage minimises growth of surviving microbes and those present due to possible post-process contamination (Robinson, 2003).

Production of high-quality fermented products relies on application of GMP and reliable HACCP programs. Milk must be pasteurised and the fruits and flavours added after pasteurisation must be of high quality and free from vegetative pathogenic bacteria.

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[4] With **stirred yoghurt**, the background flavour of yoghurt is usually modified by the addition of fruit/flavours and sugar (Robinson, 2003), the fermentation is usually carried out in bulk, and the curd formed as a result of fermentation is stirred and cooled before being packaged into retail cartons.

[5] **Set yoghurt** is fermented in its retail cartons and the yoghurt formed as a result of fermentation has a firm, gel-like structure together with a clean, mildly acidic and slightly aromatic flavour (Robinson, 2003).

1.9 Dairy desserts

1.9.1 Description

In recent years there has been rapid proliferation in the range of dairy-based desserts available in the marketplace. These are typically branded, ready-to-eat products that are sold through retail outlets such as from supermarket cabinets with products ranging from medium to long shelf-life. These products often include probiotic bacteria, fibre, vitamins, minerals, and include flavours and colours that appeal to children and adults.

Dairy-based desserts include acidified and non-acidified products. Examples of these types of products include custards, crèmes and mousses, crème fraiche, puddings and sachet desserts. Difficulties with differentiation are increasingly common with a blurring of the lines which differentiate yoghurt (fermented) products, from crème desserts, and products containing probiotics.

Dairy-based desserts can be based on fresh milk (skim or full-fat), milk powder (skim or whole) or on milk protein concentrates. Flavours, colours and sweeteners may be added, along with a wide variety of hydrocolloid thickening agents to improve texture, of which, starches and carrageenan are most common. Other additives used include emulsifiers and binding agents.

A typical flow sheet for the manufacture of these products includes the following processing operations:

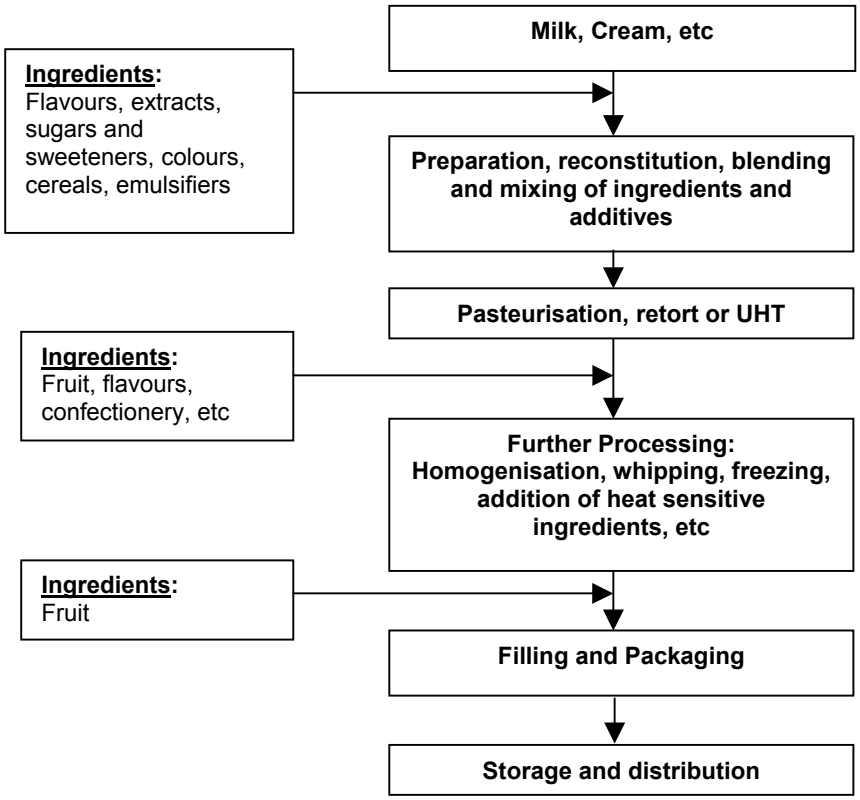

Figure 9: Major steps in the manufacture of dairy-based desserts

1.9.2 Microflora of major concern

The microbiological profile of these products is extremely varied, reflecting the nature of the ingredients incorporated into these products and variations in the preservation and processing operations employed in their manufacture.

Published microbiological data on these types of products is limited. Survey data typically indicates these products conform to national regulations (Pable-Busto, 2000) and are of acceptable microbiological quality (Rodriguez *et al*., 1994).

In determining the potential pathogens associated with these products, the typical microflora associated with milk and creams are combined with microflora originating from ingredients that vary from fruit to flavourings. Of particular concern is the survival of spores from *B. cereus* in the milk or presence their in ingredients such as thickeners.

1.9.3 Effect of processing on the growth and survival of microbial pathogens

The manufacturing process for dairy desserts involves heat and mechanical treatments.

The manufacture of ready to eat dairy desserts involves three basic steps, mixing of the ingredients into a homogenous fluid, heat treatment of the fluid and filling of the product into containers. Mousse-type products also require an aerations step before filling into containers.

Three basic types of heat treatment are used in the production of dairy desserts including pasteurisation, retort sterilisation, or UHT. Pasteurisation temperature/time combinations will vary depending upon the solids content, but must be sufficient to achieve product safety. Risks are also influenced by whether the product is hot or cold-filled. Shelf-life varies from about 3-28 days at 7ºC and may be shorter if cold filling is used.

Retort sterilisation is used for canned custards, while UHT treatments are used for long life creamy desserts. The time/temperature combination for UHT products is approximately 140ºC for 3 seconds, and the product usually undergoes preheating before the UHT treatment is applied. Product is generally packed aseptically with either hot (around 70ºC) or cold (<7ºC) filling.

Dry-mix sachet dairy desserts generally consist of milk powder, starch texturiser (generally carragenan), sugar, flavour and colour and are manufactured simply by dry blending.

The major risk in chilled dairy desserts is that they will become contaminated with pathogens which could grow during the products shelf-life. Components of this type of product such as cream and custards, are by formulation (pH and water activity) and method of manufacture (i.e. exposed to the factory environment) high risk. Custard and cream rely on proper heat treatment to eliminate pathogens that may be present in the raw materials used. Where these products are heat treated, non-sporeforming vegetative cells will be destroyed whereas, spores of *B. cereus*, may surive and become activated. The rapid cooling of products that have a heating step will help prevent growth of these spores. Another major public health concern can arise from post-pasteurisation contamination, particularly from heat labile ingredients and during filling and packaging. Points in the process where product can become re-contaminated is during assembly of the final product. Items such as roasted nuts added as decorative toppings to desserts can also be a route of contamination.

In addition, great care must be taken to avoid the addition of psychrotrophic bacteria such as *L. monocytogenes* which may grow during prolonged refrigerated storage. Another concern relates to spores of psychrotrophic *B. cereus* that may survive pasteurisation and grow and elaborate toxin during the extended storage of some types of dairy desserts (Beattie and Williams, 2002).

A risk to the consumer from these products if contaminated, is if they are temperature abused and consumed at or beyond end of normal shelf-life.

With the trend toward extended shelf-life dairy based desserts marketed in the chill chain, aseptic or ultra clean fillers are used, however in Australia UHT and hot-fill is more commonly used. These products comprise multilayed mousse type desserts that are heat processed at conditions between traditional pasteurisation and UHT conditions for ambient stable products. Where these products are UHT treated, vegetative cells and spores of pathogens are destroyed, and the product is typically shelf stable for extended periods of time.

1.10 Dairy-based dips

1.10.1 Description

As with dairy desserts there has been an increase in the number of dairy-based dips in the marketplace. These products are very diverse and typically ready-to-eat commodities and are sold from cabinets in retail outlets.

Dairy-based dips range from processed cheese-type products and starch-thickened bases flavoured with cheese solids to sour cream or yoghurt based and flavoured dips. A wide range of condiments can be added to the dairy dip base including herbs and spices, dehydrated vegetables and flavouring agents. These products range from medium to long shelf-life.

A typical flow sheet for the manufacture of these products includes the following processing operations:

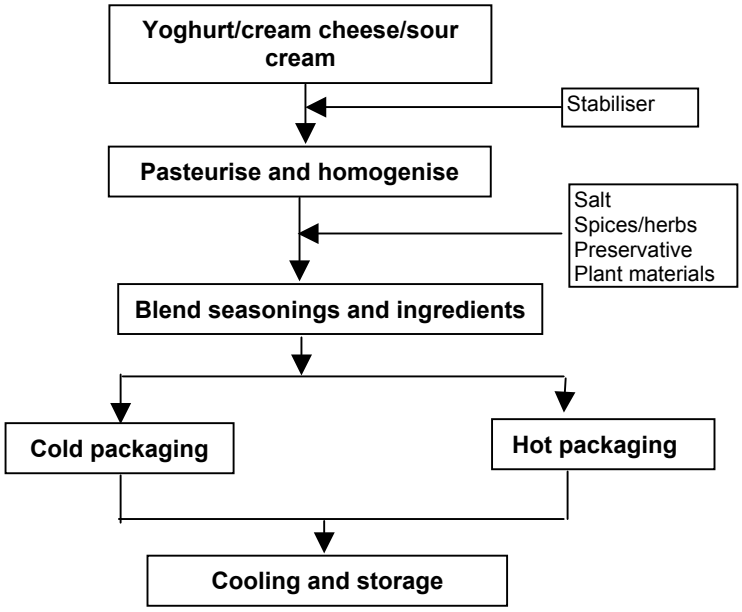

Figure 10: Major steps in the manufacture of dairy-based dips

1.10.2 Microflora of major concern

The microbiological profile of these types of products is extremely varied, reflecting the nature of the various components incorporated into these products and variations in the preservation and processing operations employed in their manufacture. Cold filling is frequently practiced, and careful management is essential to avoid contamination. This is especially important where heat labile ingredients are added to the product after a terminal heat process.

Published microbiological data on these types of products is limited. Survey data typically indicates these products conform to national regulations (Pable-Busto, 2000) and are of acceptable microbiological quality (Rodriguez *et al*., 1994).

In determining the potential pathogens associated with these products, the typical microflora associated with milk and creams are combined with microflora originating from ingredients that vary from vegetables and fruit to flavourings, herbs and spices. Where heat labile ingredients are added after a heat treatment steps, great care must be taken to avoid the addition of psychrotrophic bacteria such as *L. monocytogenes*.

1.10.3 Effect of processing on the growth and survival of microbial pathogens

Sour cream-based dips can either be produced using a traditional culturing of a cream base, or by direct acidification. Both processes drops the pH to a range of 4.5 – 4.75. Heat treatments and homogenisation are applied to the sour cream. At this pH range, growth of vegetative pathogens is unlikely. In addition the presence of lactic acid is inhibitory to vegetative pathogens. Other additives such as potassium sorbate are also often used as a preservative which imparts an antimicrobial effect. Stabilisers are also added to cultured cream to prolong shelf life.

Cultured products can be heated and packaged (hot-fill) at high temperatures to obtain and extended shelf-life (up to 120 days), or cold-filled resulting in a much shorter shelf-life (e.g. 45 days).

The pH in yoghurt based dips is ranges from 4.1 to 4.5, and thus is also inhibitory to vegetative pathogens.

Some cheeses-based dips have the right combination of solids, salt and pH to inhibit bacterial growth, creating a shelf-stable product. Other shelf-stable cheese dips typically undergo a heat process, such as retorting.

Where these products are pasteurised, non-sporeforming vegetative cells will be destroyed, and the major public health concern arises as a result of post-pasteurisation contamination, introduced in heat labile ingredients and during filling and packaging operations.

1.11 Casein, whey products and other functional milk derivatives

1.11.1 Description

An increasing awareness of the nutritional and health benefits of dairy products has driven the development of markets for a wide array of functional and nutritional ingredients derived from milk. Improvements in fractionation technologies have allowed the manufacture of these on a commercial basis from surplus milk and other dairy by-products.

The production of functional milk derivatives is summarised in Figure 11. The process typically uses pasteurised milk as a starting material. Separation of milk into cream and skim milk leads to processes for enrichment of components derived from the fat- and protein-enriched fractions, respectively.

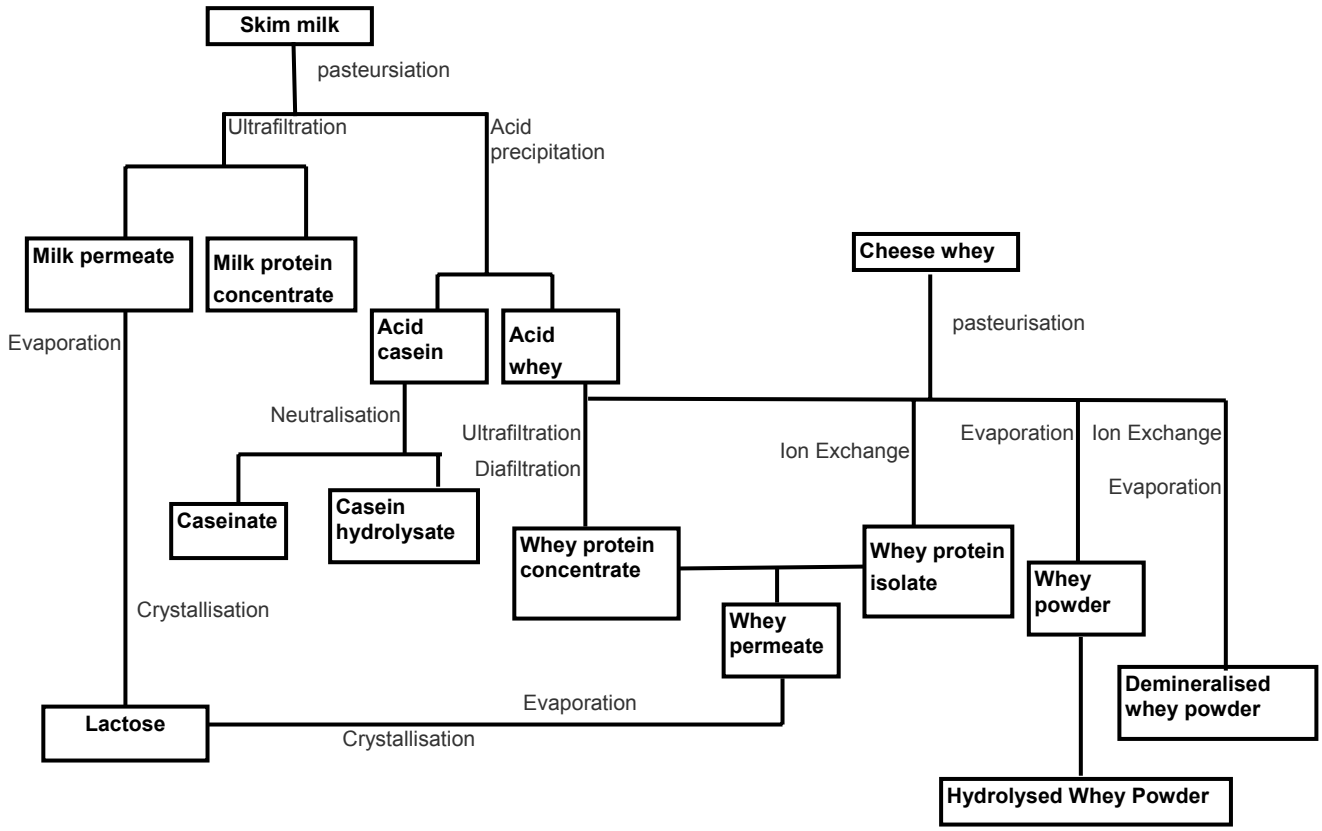

Figure 11: Indicative steps in the manufacture of casein, whey products and functional milk derivatives

For production of casein and caseinates, a low heat treatment is used (up to 1 minute at 85ºC).

Ultrafiltration of skim milk is used to produce milk protein concentrate (MPC), a casein and whey protein-enriched fraction used in dietetic and clinical nutritional supplements such as infant formula, adult medical foods, weight management products, liquid nutritional beverages, cheese products, cultured foods, powdered dietary supplements and sports nutrition products.

Aside from a wide variety of non-food and industrial uses (*e.g.* in adhesives, paints, textile fabrics, paper coatings, plastics, toothpastes, cosmetics, pharmaceuticals, nutritional and personal care products) casein and caseinates are used as functional ingredients in many food products. Edible acid casein is highly nutritious, low in fat and cholesterol, and flavourful, and is used in whiteners, infant formulas and processed cheeses. Ammonium caseinate is used in bakery products, while calcium caseinate is used as a nutrient supplement in creamed cottage cheese, powdered diet supplements, nutritional beverages, processed cheese, and frozen desserts. Potassium caseinate is used in frozen custard, ice-cream, ice milk, and fruit sherbets, and sodium caseinate is used as an emulsifier in coffee whiteners, cottage cheese, cream liqueurs, yoghurt, processed cheeses, and some meat products. It is also used to improve the whipping properties of dessert whips. Hydrolysed casein may be used as a binder in canned tuna.

Whey is usually recovered as a by-product from cheese making, with small amounts produced as a by-product of casein production. A variety of processes are used to recover components from whey. Lactose and lactose derivatives are recovered by crystallisation after evaporative concentration. Whey proteins can be recovered by ultrafiltration and ion exchange, and may be further processed by enzymatic hydrolysis.

Food grade whey powder is used in the manufacture of ice-cream, bakery products (cakes, biscuits), chocolate flavouring, infant formula, yoghurt, beverages and processed meat. It may also be used as an ingredient in animal feed and as a calf milk replacer. Whey protein fractions are increasingly used as functional or nutritional components in foods, *e.g.* in health foods for high-energy diets and in bakery and confectionary products (Horton 1995). Demineralised whey powder is used in infant formulae manufacture.

There are also various high value, small volume components derived from whey, including α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulins, lactoferrin and lactoperoxidase. These products require additional purification, but show promise as functional and nutritional food ingredients as well as having potential therapeutic uses (Horton, 1995).

A further by-product of whey fractionation are milk salts, recovered from whey protein ultrafiltrates and electrodialysates (Horton 1995; de Wit 2003). This mix of minerals may be found in bakery products, health drinks and as a table salt substitute.

AMF may be separated into various fat fractions, based on melting point, by fractional crystallisation. These derivatives can be used in combination with skimmed milk powder to produce various milk products, and are used in the baking, confectionery, ice-cream and chocolate industries.

1.11.2 Microflora of major concern

Pathogens of concern in the production of skim milk and skim milk powder include *Salmonella, L. monocytogenes, B. cereus, Cl. perfringens* and *E. sakazakii*. Casein and whey products produced from skim milk might contain spores of the bacilli and clostridia, and vegetative cells of other pathogens might survive extended periods in the dried products if present, although growth will not occur. A microbiological survey of dairy products conducted overseas detected *B. cereus* in whey powder (Appendix 3).

Products formed from severely temperature-abused milk might contain *Staphylococcus aureus* enterotoxin, which is exceptionally heat stable (ICMSF 1998), but this is unlikely to occur in a well-regulated processing environment.

Fat-enriched milk fractions *e.g.* AMF may protect pathogenic microorganisms such as *E. coli*, *Salmonella* or *Listeria* if present, however this is unlikely given the low moisture content of the product.

1.11.3 Effect of processing on the growth and survival of microbial pathogens

Milk fats, casein and whey protein components are derived from milk or cream that has been heat-treated to at least the level required by pasteurisation standards (de Wit 2003) and will be free from vegetative cells of pathogenic microorganisms, but not bacterial spores (ICMSF, 1998). Recontamination by pathogens occurs via post-processing contamination.

AMF is produced from pasteurised cream or from butter made from pasteurised cream. The final step in production, vacuum drying at around 90ºC (Munroe et al in Early 1998), is also lethal to surviving vegetative cells of pathogens. In addition, the low moisture content of the product ensures that outgrowth of pathogens will be limited and they will eventually die off in these products during storage (ICMSF 1998).

Membranes used in filtration and/or concentration steps during production of milk protein concentrate and whey proteins present hygiene problems due to the potential for concentrating pathogens and the vast surface area available for biofilm formation (Varnam and Sutherland 1994 - book). Modern plants tend to run membrane processing at lower temperatures to reduce bacterial growth and extend run times in ultrafiltration plants. Ultrafiltration during production of high protein whey powder is conducted at around 12-17ºC.

Some processes, such as lactose production, evaporation and reverse osmosis, use temperatures suitable for growth of mesophilic bacteria (Varnam and Sutherland, 1996).

1.12 Colostrum

1.12.1 Description

Bovine colostrum is the initial mammary secretion after the birth of a calf. It is produced for about 1-2 days (depleted usually within 4-5 days or 8-10 milkings), and provides the newborn animal with a concentrated source of factors that boost its immune status and support physical and physiological development (Marnila and Korhonen 2003).

Immediately post-partum, the colostrum obtained from cows is excluded from bulk milk collection and was normally fed to farm animals. Until recently, it has not been widely commercially exploited, although the high concentration of bioactive substances in colostrum have attracted increasing interest in the last few years because of their potential pharmaceutical and dietary uses (Marnila and Korhonen 2003). The sports food market is rapidly expanding, due to the perceived benefits of colostrum in providing an immune and performance boost to athletes (Sanders and Van Gammeren, 2001). The use of colostrum as passive immune protection for humans has been reviewed recently (Van Hooijdonk et al., 2000; Gill 2003).

Important biologically active substances contained in colostrums include immunoglobulins, leucocytes, lactoferrin, lysozyme, cytokines (interleukin (IL)-1β, IL-6. IL-10, tumour necrosis factor-α and granulocyte-, macrophage- and granulocyte/macrophage colony-stimulating factors) and other hormones / growth factors (*e.g.* insulin-like growth factors I and II). Some of the bioactive substances found in bovine colostrum provide specific (antibody) or non-specific (*e.g.* lactoferrin and lactoperoxidase) defences against infectious agents and foreign antigens.

1.12.2 Microflora of major concern

The microflora in powdered bovine colostrum will be similar to that in other milk powder products, and include *Salmonella, L. monocytogenes, B. cereus, Cl. perfringens* and *S. aureus*. Post-pasteurisation, colostrum may contain viable spores. Microorganisms present in the dried product will also arise from post-processing contamination, and vegetative cells of pathogens might survive extended periods in the dried product, although growth will not occur.

The final microbiological quality of colostrum powder will be influenced by the microbial load of the colostrum after milking, processing and the maintenance of good hygiene post-processing. *S. aureus* if present in the raw colostrum may grow and produce enterotoxin if the colostrum is subjected to temperature abuse prior to pasteurisation. The persistence of *Listeria* spp. in the dairy plant environment and the association of listeriosis with other dairy products (ICMSF, 1998) indicates the potential for contamination of dry dairy products such as colostrum powder.

1.12.3 Effect of processing on the growth and survival of microbial pathogens

Typically, cows produce more colostrum than is required by their calves (Marnila and Korhonen 2003), and the excess is collected by milking and stored either frozen or refrigerated. Processing usually involves a fat separation stage before pasteurisation, the liquid is then concentrated (often by membrane technology) and either spray dried or freeze-dried to produce a free-flowing, pale yellow powder. The manufacturing process is similar to that used for the production of skim milk powder.

Because many of the active components in colostrum are extremely heat labile, processors face the challenge of minimising exposure to high temperatures, while ensuring sufficient heat to produce a safe product.

In Australia, bovine colostrum for human consumption is regulated by the Therapeutic Goods Administration (TGA), which produces a compositional guideline including microbiological specifications.

Bovine colostrum is treated by pasteurisation or an equivalent process before drying. Re-introduction of pathogens via post-processing contamination is also of concern, although the low moisture content of the product ensures that outgrowth will be limited and vegetative cells will eventually die off during storage (ICMSF 1998).

Both *Cl. perfringens* and *B. cereus* are able to produce spores that can survive pasteurisation and even ultra-high temperature processing (Institute of Environmental Science and Research Limited, 1995). If colostrum is inadequately stored when made up, the spores of *Cl. perfringens* and *B. cereus* can germinate and rapidly multiply, creating a potential health risk.

The toxin of *S. aureus* is heat stable and, if poor sanitary conditions allow the organisms to proliferate and produce toxin in the pre-pasteurisation stage, the toxin will carry over to the final product.

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APPENDIX 2

Epidemiological Information on Outbreaks of Foodborne Illness Associated with Dairy Products

2.1 OzFoodNet data: 1995-June 2004

Table 1: Outbreaks associated with milk and milk products in Australia (1995-December 2004)

State	Year	Setting food prepared	Ill	Hosp	Food Vehicle	Aetiology	Comments
	1977				Infant formula	*Salmonella* Bredeney	
SA	1997	Caterer	27		Cheese sauce	*Clostridium perfringens*	Infection likely due to poor food handling and preparation.
WA	1998	Camp	9		Unpasteurised milk	*Campylobacter*	Infection due to consumption of unpasteurised milk
SA	1998	Commercially manufactured food	111		Gelati	*Salmonella* Orianberg	Gelati made with pasteurised milk
SA	1999	Farm	12		Unpasteurised milk	*Salmonella* Typhimurium 44	Infection due to consumption of unpasteurised milk
SA	2000	Farm	12		Unpasteurised milk	*Campylobacter*	Infection due to consumption of unpasteurised milk
Vic	2000	Camp	21		Unpasteurised milk	*Campylobacter*	Infection due to consumption of unpasteurised milk
Vic	2001	Camp	12	0	Unpasteurised milk	Unknown	Relative risk was higher for those that had consumed unpasteurised milk
Qld	2001	Community	8	4	Unpasteurised milk	*Cryptosporidium*	Strong epidemiological evidence of association between consumption of unpasteurised milk and cryptosporidium.
Vic	2003	School	13	0	Unpasteurised milk	*Campylobacter*	The risk of illness was 3.7 times higher among people who had drunk any unpasteurised milk.
SA	2003	Camp	14	0	Unpasteurised milk	*Campylobacter*	Unpasteurised milk was supplied for drinks and cereal.

Table 2: Outbreaks associated with foods containing dairy products in Australia (January 2001-December 2004)

State	Year	Setting food prepared	Ill	Hosp	Food Vehicle	Aetiology	Comments
NSW	2000	Restaurant	41	2	Fried ice-cream	*Salmonella* Typhimurium 9	
SA	2001	Bakery	16	3	Custard Tarts	*Salmonella* Typhimurium 126	Unable to identify original source of infection
WA	2001	Restaurant	38	4	Ice cream and sponge	*Salmonella* Typhimurium 64	Food handler positive for STM 64.
Vic	2002	Take away	10	1	Cream filled cakes	*Salmonella* Typhimurium U290	Illness strongly associated with eating at bakery.
SA	2002	Bakery	22	7	Cream filled cakes	*Salmonella* Typhimurium 99	Piping bags in the bakery reused/used for sausage meat and cream.
NSW	2002	Take Away	29	4	Cream filled cakes	*Salmonella* Typhimurium 135a	14 of 22 primary cases had eaten cream-filled cake
SA	2003	Bakery	6	1	Cheesecake	*Salmonella* Typhimurium 4	
Qld	2004	Bakery	5	0	Custard Tarts	*Salmonella* Typhimurium 135a	Almond sauce suspected source of infection.
SA	2004	Home	5	1	Ice Cream	*Salmonella* Typhimurium 9	Raw egg used in ice cream
SA	2004	Bakery	13	0	Cream filled cakes	*Salmonella* Typhimurium 108	Epidemiological evidence suggested cream filled cakes as source of infection
NSW	2004	Institution	43	17	Custard	Unknown	
NSW	2004	Institution	43	10	Custard	*Salmonella* Typhimurium 135	

2.2 International data on dairy-related outbreaks of foodborne illness

There have been a number of reports of outbreaks of illness associated with consumption of dairy products. A literature search was undertaken to identify and outline outbreaks of foodborne illness attributed to dairy products internationally. The search looked at peer-reviewed literature, as well as other relevant literature such as government documents, reports, electronic citations and follow up of reference lists on documents.

The information from the search describes 163 outbreaks associated with dairy products. Outbreaks of illness associated with pasteurised dairy products are 22 with pasteurised milk (13.5%) (Table 1), 17 with cheese from pasteurised milk or pasteurisation not stated so assumed pasteurised (10.4%) (Table 3). Faults with the pasteurisation process or a post pasteurisation contamination has been identified or suspected as the source of infection in each case. These pasteurised or undefined products are a total of 39/163 outbreaks (23.9%).

Unpasteurized dairy products are the most common cause of dairy associated outbreaks of illness, 30 due to unpasteurised milk (18.4%) (Table 2), 18 unpasteurised cheese (11.0%) (Table 4), 13 unpasteurised non-bovine species (8.0%) (Table 10) this brings the total number of dairy outbreaks associated with unpasteurized products to 61/163 (37.4%).

Ice cream was responsible for 23 outbreaks (14.0%) (Table 7) in which 14 identified raw eggs as an ingredient, the eggs may be the source of infection. Butter was associated with 6 outbreaks (3.7%) (Table 5). Yoghurt and fermented products were associated with 2 outbreaks (1.2%) (Table 6). Dried milk products, which did not identify whether they were manufactured from pasteurised milk, were associated with 5 outbreaks (3.0%) (Table 8). Eight outbreaks of illness from foods where a dairy product was a component were identified (5.9%) (Table 11). Infant formula was associated with 19 outbreaks of illness (11.7%) (Table 9).

This epidemiological evidence supports the microbiological evidence that pasteurisation is an effective method of reducing the risk of human illness from dairy products.

Table 3: Outbreaks of illness associated with Pasteurised Liquid Milk

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2003	UK	114	Pasteurised milk	*E. coli* O157	On-farm pasteurisation.	(Goh *et al*., 2003)
2000	Japan	14000	Low fat milk	Staphylococcal enterotoxin (SE) (identified by PCR)	Caused by a bacteria contaminated valve at a milk packaging factory and ineffective temperature control	(Yamashita *et al*., 2003)
2000	USA	38	Pasteurised milk	*Salmonella* Typhimurium	Likely contaminated containers or milk contact surfaces after pasteurisation because of environmental conditions in plant	(Olsen and et al, 2004)
1998	UK	40	Pasteurised milk	*Salmonella*	Pasteurisation failure	(Brown, 1998)
1997	UK	50	Pasteurised Milk	Cryptosporidiosis	Faulty pasteuriser on farm	(Gellietlie *et al*., 1997)
1997	USA	54	Chocolate milk	*L. monocytogenes*	*L. monocytogenes* isolated from implicated chocolate milk, and from tank drain at dairy	(Dalton *et al*., 1997)
1996	UK	9	Raw/Pasteurised milk	*E. coli* O157 (VTEC 0157)	Unpasteurised milk from the farm was consumed also pasteurised milk with a faulty pasteuriser	(Clark *et al*., 1997)
1995	USA	10	Pasteurised milk	*Y. enterocolitica* O: 8	Post pasteurisation contamination of milk bottles	(Ackers *et al*., 2000)
1995	UK	110	Milk	*C. jejuni*	Inadequately pasteurised milk from a local dairy	(Fahey *et al*., 1995)
1994	USA	11	Pasteurised	*E. coli* serotype O104:H21	Inspection of dairy plant producing the implicated brand of milk revealed faecal coliform contamination of post pasteurisation equipment	(Moore *et al*., 1994)
1994	USA	4	Milk	*Bacillus cereus*	Hot chocolate consumed at work	(CDC 2002)
1994	USA	18	Milk	*E. coli* 0157:H7	Consumed in a number of private homes	(CDC 2002)
1992	USA	23	Milk	*C. jejuni*	Consumed on a farm	(CDC 2002)
1991	USA	37	Milk	*Salmonella* Typhimurium	Chocolate milk consumed on a farm	(CDC 2002)
1990	UK	32	Pasteurised milk	*C. jejuni*	Due to the consumption or handling of milk from bottles that had been attacked by birds.	(Southern *et al*., 1990)
1990	USA	19	Milk	Unknown	Consumed at a bowling alley	(CDC 2002)
1990	USA	7	Milk	Unknown	Consumed in a restaurant	(CDC 2002)
1987	USA	16000	Pasteurised milk	*Salmonella* Typhimurium	Pasteurisation equipment had been modified to facilitate the running off of raw milk	(Ryan *et al*., 1987)
1985	USA	1500	2% Pasteurised milk	*Salmonella* Typhimurium	2% pasteurised milk ("Blue Brook" brand) from one processing plant	(MMWR, 1985)
1985	USA	49 (14)	Pasteurised Milk	L. *monocytogenes*	At the plant where the milk was processed, inspections revealed no evidence of improper pasteurisation	(Fleming *et al*., 1985)
1984	USA	16	Pasteurised milk	*Salmonella* Typhimurium	Inadequately pasteurised milk	(MMWR, 1984b)
1983 - 1984	UK	32 O/B 714 cases (8)	27 Raw milk 2 pasteurised milk 1cheese 1cream 1 ice cream	22 Salmonellosis 7 *Campylobacter* 1 *S. aureus*, 1 *Y. enterocolitica* 1 *Streptococcus zooepidemicus*	There were eight deaths, all associated with the *S. zooepidemicus* outbreak	(Barrett, 1986)

Table 4: Outbreaks of illness associated with Unpasteurised Liquid Milk

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2003	USA	62	Raw milk	*Salmonella* Typhimurium	Unpasteurised milk at dairy/petting zoo	(Mazurek *et al*., 2004)
2003	USA	13	Raw Milk	*C. jejuni*	Unpasteurised milk	(Peterson, 2003)
2001	USA	75	Unpasteurised milk	*C. jejuni*	Unpasteurised milk obtained at a local dairy farm	(Harrington *et al*., 2001)
2001	Austria	2	Raw cows/ goats milk	*E. coli* O157	isolated from dairy cow and goat, raw milk	(Allerberger *et al*., 2001)
2000	Austria	38	Unpasteurised milk	*C. jejuni*	Unpasteurised milk distributed by a local dairy	(Lehner *et al*., 2000)
2000	Germany	31	Raw milk	*C. jejuni*	Consuming raw milk farm visit	(Thurm *et al*., 2000)
1998	Hungary	52	Raw Milk	*C. jejuni* and *C. coli*	Unpasteurised milk	(Kalman *et al*., 2000)
1996	UK	33	Unpasteurised Milk	*C. jejuni* resistotype 02	Educational farm visit, exposure to raw milk	(Evans *et al*., 1996)
1995	USA	3	Raw milk	*S*. Typhimurium, variate Copenhagen	Consumed in private home	(CDC 2002)
1993	USA	4	Raw milk	*E. coli* 0157:H7	Consumed in a nursing home	(CDC 2002)
1993	USA	6	Raw Milk	*E. coli* O157:H7	Commercially distributed Unpasteurised milk	(Keene *et al*., 1997)
1992	USA	50	Raw milk	*C. jejuni*	Consumed at church	(CDC 2002)
1992	USA	11	Raw milk	*Campylobacter* spp.	Consumed in private home	(CDC 2002)
1992	Australia	3	Raw milk	*Streptococcus zooepidermicus*	Unpasteurised milk from a house cow	(Francis *et al*., 1993)
1990	USA	13	Raw milk	*Campylobacter* spp.	Consumed at school	(CDC 2002)
1990	USA	5	Raw milk	Unknown	Consumed in private home	(CDC 2002)
1990	USA	42	Raw milk	*C. jejuni*	Consumed at a dairy	(CDC 2002)
1986	Austria	28 (5)	Raw milk	*L. monocytogenes*	Consumption of raw milk and biologically grown vegetables as possible source of infection	(Allerberger and Guggenbichler, 1989)
1985	USA	25	Raw Milk	*C. jejuni*	Unpasteurised milk	(Korlath *et al*., 1985)
1984	USA	23	Raw milk	not identified	Associated with drinking raw milk from local dairy	(MMWR, 1984a)
1984	Canada	9	Raw milk	*C. jejuni*	A raw milk dairy	(MMWR,1984)
1983	USA x 2 OB	31 26	Raw milk	*C. jejuni*	A raw milk dairy	(MMWR, 1983)
1983	USA	122	Raw Milk	not identifed	Associated with consumption of raw milk from a single dairy	(Osterholm *et al*., 1986)
1983	USA	? (1)	Raw Milk	*S*. Typhimurium	Unpasteurised milk	(Tacket *et al*., 1985)
1983	UK	130	Raw Milk	*S*. Typhimurium	Unpasteurised milk	(Shanson *et al*., 1983)
1982	USA	38	Raw Milk	*C. jejuni* and thermo-tolerant strain (*C. fetus* subsp *fetus*)	Unpasteurised milk	(Klein *et al*., 1986)
1981-83	USA	46 70 123(32)	Raw milk	*S*. Dublin		(Potter *et al*., 1983)
1981	USA	250	Raw Milk	*C. jejuni*	Unpasteurised milk	(Kornblatt *et al*., 1985)
1979	UK	700	Unpasteurised milk	*S*. Dublin	Milk which had not been subjected to heat treatment	(Small and Sharp, 1979)
1973-1992	USA	40 out-breaks	Raw Milk	Various	In states with legal raw milk	(Headrick *et al*., 1998)

Table 5: Outbreaks of illness associated with Cheese from Pasteurised Milk or unknown Pasteurised/Unpasteurised

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2001	France	45	Brie cheese	Salmonellosis serotype infantis	Milk and factory workers contaminated with Salmonella serotype infantis	(Simon *et al*., 2002)
1999	Canada	700	Cheese	*S*. Enteritidis	Lunch pack products	(CCDR, 1999)
1996	USA	8 (1)	Cheese Sauce	*Clostridium botulinum*	A commercial, canned cheese caused a botulism outbreak	(Townes *et al*., 1996)
1996	Italy	8	Mascopone cheese	*Clostridium botulinum* type A	Beak in cold-chain at retail likely caused germination of C. botulinum spores contaminating the products	(Aureli *et al*., 2000)
1996	UK	84	Cheddar cheese	*S*. Goldcoast		(Health Protection Agency 1997)
1995	Switzerland	57 (16)	Soft cheese	Listeriosis	Consumption of a soft cheese	(Bula *et al*., 1995)
1995	USA	9	Cheese	*Clostridium perfringens*	Consumed in restaurant	(CDC 2002)
1994	USA	5	Goats cheese	Salmonella enteridis	Consumed in a private home	(CDC 2002)
1993	USA	12	Cheese slices	Unknown	Consumed at a picnic	(CDC 2002)
1991	USA	25	Shredded cheese	Unknown	Consumed in a restaurant	(CDC 2002)
1990	USA	15	Cheese	Hepatitis A	Consumed in a private home	(CDC 2002)
1990	USA	23	Cheese sauce	*S*. Braenderup	Consumed in restaurant	(CDC 2002)
1990	USA	12	Processed Cheese	*S*. Enteritidis	Consumed in hospital	(CDC 2002)
1989	USA	167	Contaminated cheese	*S*. Javiana and S. Oranienburg	Mozzarella cheese manufactured at a single cheese plant	(Hedberg *et al*., 1992)
1983	USA	45	French Brie cheese	*E. coli* O27:H20	Three clusters of gastrointestinal illness, after office parties	(MacDonald *et al*., 1985)
1982	Canada	?	Cheddar Cheese	S. Typhimurium		(D'Aoust, 1985)
1976	USA	28,000 to 36,000	Cheddar cheese	*S*. Heidelberg	Consumption of cheddar cheese from a single shipment of a single manufacturer	(Fontaine *et al*., 1980)

Table 6: Outbreaks of illness associated with Raw Milk Cheese

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2005	France	18	Raw goats milk cheese	*S*. Stourbridge	Cheese was made from the unpasteurised milk of a single herd of 260 goats	(Vaillant V, 2005)
2003	Sweden	15	Cheese	*Listeria monocytogenes*	On farm manufactured fresh cheese	(Carrique-Mas *et al*., 2003)
2002	Canada	17	Raw milk cheese	*Listeria monocytogenes*	Environmental contamination	(CCDR, 2003)
2002	Canada	13	Unpasteurized gouda cheese	*E. coli* O157:H7	Implicated cheese was found to be contaminated with E. coli O157:H7 104 days after production, despite having met regulated microbiological and aging requirements	(Honish *et al*., 2005)
2001	France	190	Cantel cheese	*S*. Enteritidis	Cheese made from raw milk	(Haeghebaert *et al*., 2003)
2001	France	25	Cantel cheese	*S*. Enteritidis	Cheese made from raw milk	(Haeghebaert *et al.,* 2003)
2000	USA	13 (5 still births)	Mexican style cheese	*Listeria monocytogenes*	Mexican-style cheese made from contaminated raw milk traced to 1 local dairy	(MacDonald *et al*., 2005)
1998	USA	55	Fresh cheese curds	*E. coli* O157:H7	Produced during manufacture of Cheddar cheese from unpasteurised milk and had been incorrectly labelled as pasteurised	(Durch *et al*., 2000)
1997	USA	54	Mexican-style soft cheese made with unpasteurised milk	*S*. Typhimurium DT104	Raw milk samples from nearby dairies yielded Salmonella Typhimurium DT104	(Villar *et al*., 1999)
1997	France	113	Raw milk soft cheese	*S*. Typhimurium	From a single processing plant	(de Valk *et al*., 2000)
1997	USA	31	Unpasteurised Mexican-style soft cheese	*S*. Typhimurium DT104	Fresh Mexican-style cheese from street vendors and from cheese samples and raw milk	(Cody *et al*., 1999)
1996	Spain	81	Raw cheese	*Brucella mellitensis*	Home-made cottage cheese	(Castell *et al*., 1996)
1995	France	20	Raw milk cheese	*Listeria monocytogenes*		(Goulet *et al*., 1995)
1995	France	25	Raw milk cheese	*S*. Dublin		(Vaillant *et al*., 1996)
1994	Canada	82	Unpasteurised soft cheese	*S*. Berta	Cheese was contaminated by chicken carcasses during production	(Ellis *et al*., 1998)
1994	Scotland	22	Raw milk Cheese	*E. coli* 0157		(Ammon, 1997)
1988	UK	155	Stilton Cheese	Suggestive of a staphylococcal illness	Stilton cheese, produced from unpasteurised cow's milk	(Maguire *et al*., 1991)
1973-1992	USA	58 deaths	Cheese	*Salmonella*, *Listeria*, and *E. coli* O157:H7	Manufacturing errors caused most illnesses, manufacturing cheese with raw or improperly pasteurized milk and post pasteurization contamination	(Altekruse *et al*., 1998)

Table 7: Outbreaks of illness associated with Butter and Butter products

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2003	UK	17	Butter	*Listeria monocytogenes*	Listeria isolated from a drain at the dairy & from butter but not from other dairy products from the dairy	(ACMS and Advisory Committee on the Microbiological Safety of Food, 2003)
1999	Finland	25(6)	Butter	*Listeria monocytogenes*		(Lunden *et al*., 2004)
1995	USA	29	Butter	Unknown	Consumed in Pre release centre	(CDC 2002)
1991	USA	15	Whipped butter blend	*Staphylococcus aureus*	Consumed in a Hotel	(CDC 2002)
1991	USA	8	Butter	Unknown	Consumed in restaurant	(CDC 2002)
1990	USA	40	Butter	Unknown	Consumed on educational summit	(CDC 2002)

Table 8: Outbreaks of illness associated with Yogurt and Fermented Milk

Year	Country	Cases	Product	Causative Agent	Cause	Reference
1991	UK	16	Yoghurt	*E. coli* O 157	Consumption of a locally produced live yoghurt	(Morgan *et al*., 1993)
1989	UK	27(1)	Hazelnut flavoured yoghurt	*Clostridium botulinum* type B toxin	Can of hazelnut conserve, opened and unopened cartons of hazelnut yoghurt	(O'Mahony *et al*., 1990)

Table 9: Outbreaks of illness associated with Ice cream

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2000	Italy	113	Home made ice cream and Italian pastry	**S*.* Eenteritidis	Not confirmed but contamination appeared to be due to raw eggs in ice cream	(Lopalco *et al*., 2000)
1998	Australia	111	Gelati	*S*. Oranienberg	Gelati made from pasteurised milk	(2004)
1998	USA	3	Ice cream	**S*. Enteritidis		(Vought and Tatini, 1998)
1995	USA	27	Home made ice cream	**S*. Enteritidis	Consumed at a church	(CDC 2002)
1994	USA	2150	Ice cream	**S*. Enteritidis	Illness associated with consumption of a specific brand of ice cream	(Dept. Human Services, 1994)
1994	USA	6	Home made ice cream	**S*. Enteritidis	Consumed at a private home	(CDC 2002)
1994	USA	186	Ice cream	**S*. Enteritidis	Consumed at a home	(CDC 2002)
1994	USA	743	Ice cream	**S*. Enteritidis	Commercially produced ice cream	(CDC 2002)
1994	USA	5	Home made ice cream	**S*. Enteritidis	Consumed at a home	(CDC 2002)
1993	USA	8	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed at a home	(CDC 2002)
1993	USA	12	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed in a hospital	(CDC 2002)
1992	USA	10	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed at a home	(CDC 2002)
1992	USA	15	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed at a social function	(CDC 2002)
1992	USA	9	Home made ice cream with raw eggs	**S*. Heidelberg	Consumed at a home	(CDC 2002)
1992	USA	31	Home made ice cream with raw eggs	**S*. Typhimurim	Consumed at a home	(CDC 2002)
1991	USA	22	Home made ice cream with cooked eggs	**S*. Enteritidis	Consumed at school	(CDC 2002)
1991	USA	11	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed at a Picnic	(CDC 2002)
1991	USA	25	Home made ice cream with raw eggs	**S*. Typhimurium	Consumed at a church	(CDC 2002)
1990	USA	30	Home made ice cream with raw eggs	**Salmonella* spp .	Consumed at a church	(CDC 2002)
1990	USA	2	Home made ice cream with raw eggs	**S*. Typhimurium	Consumed at a home	(CDC 2002)
1990	USA	9	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed at a Picnic	(CDC 2002)
1990	USA	96	Home made ice cream with raw eggs	**S*. Heidelberg	Consumed at a home	(CDC 2002)
1982	USA	8 (1)	Ice cream	**S*. Typhimurium	S. Typhimurium isolated from all cases,leftover ice cream, and family's hens eggs used to prepare ice cream.	(Taylor *et al*., 1984)

NB. Salmonella infection has a strong association with eggs, ice cream is usually made with raw eggs therefore the outbreaks marked * are probably a result of the egg component of the ice cream rather than the dairy component.

Table 10: Outbreaks of illness associated with Dried Milk Products

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2005	France	49	Milk powder	*S*. Worthington	Consumption of contaminated powdered milk	(Institut de Veille Sanitaire, 2005)
1981	UK	77	Dried milk	*Clostridium perfringens*	Consumption of dried milk	(Anon, 1981)
1979	USA	?	Milk powder	*Salmonella*		(ICMSF, 1998)
1977	UK	?	Milk powder	*Bacillus cereus*	Suspected milk powder	(Pinegar and Buxton, 1977)
1964	USA	?	Milk powder	*Salmonella*		(ICMSF, 1998)

Table 11: Outbreaks of illness associated with Infant Formula

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2005	UK	1	Infant formula	*Clostridium botulinum*	C. botulinum type B isolated from opened infant formula milk pwd	(Brett *et al*., 2005)
2004	Korea	31	Infant formula	*S*. London	Highly likely source of the infection was infant formula	(Park *et al*., 2004)
2003	India	16	Infant formula	Enterotoxigenic *E. coli*	Contaminated by infected food handler	(Taneja *et al*., 2003)
2002	USA	?	Infant formula	*Enterococci sakazakii*		(Weir, 2002)
2002	Switzerland	11	Infant formula	*Serratia marcescens*	Cultures of milk from used milk bottles yielded *S. marcescens*	(Fleisch *et al*., 2002)
2002	USA	11	Infant formula	*S*. Saintpaul	Formula mixed by the hospital appears to have been the source of this Salmonella outbreak	(Bornemann *et al*., 2002)
2001	Belgium	12	Infant formula	*E. sakazakii*	E. sakazakii isolated from implicated prepared formula milk and from several unopened cans	(van Acker *et al*., 2001)
2001	USA	1(1)	Powdered infant milk formula	*E. sakazakii*	Infection associated with presence of organism in commercial powdered formula	(MMRW, 2002)
1999	Israel	?	Infant formula	*E. sakazakii*	Recovered from prepared formula and kitchen blender.	(Block *et al*., 2002)
1998	UK	17	Infant formula	*S*. Anatum	Formula-dried milk responsible for outbreak	(Threlfall *et al*., 1998)
1995	Spain	3	Infant formula	*S*. Virchow	Dried-milk formula was confirmed as the source of the infection	(Ruiz *et al*., 1995)
1994	Spain	48	Infant powdered milk	*S*. Virchow		(Usera *et al*., 1998)
1990	USA (2 outbreaks)	?	Infant formula	*E. sakazakii*	*E. sakazakii* from intrinsically contaminated dried infant formula was source of neonatal infection	(Clark *et al*., 1990)
1990	USA (2 outbreaks)	?	Infant formula	*E. sakazakii*	*E. sakazakii* from intrinsically contaminated dried infant formula source of neonatal infection	(Clark *et al.,* 1990)
1989	USA	4	Infant Formula	*E. sakazakii*	Infant formula contaminated during the manufacturing process	(Simmons *et al*., 1989)
1989	Iceland	3	Infant formula	*E. sakazakii*	Organism grown from several lots of the powdered-milk formula used in the hospital	(Biering *et al*., 1989)
1985	UK	?	Infant formula	*S*. Ealing	The source of infection was traced to the factory spray-drier	(Rowe *et al*., 1987)
1984	Chille	35	Infant formula	*Bacillus cereus*		(Cohen *et al*., 1984)
1977	Australia	17	Infant formula	*S*. Bredeney	Contamination of powdered infant formulae during manufacture	(Forsyth *et al*., 2003)

Table 12: Outbreaks of illness associated with Other Species

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2003	Spain	11	Raw goats cheese	*Brucella melitensis* serovar 3	Unpasteurised raw goat cheese produced in a farmhouse	(Mendez *et al*., 2003)
2001	Canada	5	Unpasteurised goats milk	*E. coli* 0157:H7	Source of implicated goat's milk was a co-operative farm	(McIntyre *et al*., 2001)
1999	Canada	?	Cheese from goats milk	*Coxiella burnetii*	Asociated with contact with goat placenta, smoking tobacco	(Hatchette *et al*., 2001)
1995	Czech Republic	5	Raw goats milk	*E*. coli O157	Unpasteurised goat's milk from the same farm	(Bielaszewska *et al*., 1997)
1994	France	?	Raw goats milk cheese	*E. coli 0103*		(Ammon 1997)
1993	France	273 (1)	Unpasteurised goats milk cheese	*S*. Paratyphi B phage type 1 var 3	Brand A unpasteurised goats' milk cheese	(Desenclos *et al*., 1996)
1992	France	40	Raw goats milk	*Coxiella burnetii*	Persons who worked on farm and consumed unpasteurised milk products	(Fishbein and Raoult, 1992)
1991	USA	3	Raw goats milk	*C. jejuni*	Consumed on farm	(CDC 2002)
1988	Czech Republic	74	Non pasteurised sheep milk cheese	*C. jejuni/coli*	Cheese prepared from unpasteurised sheep's milk	(Kourilova and Kultan, 1990)
1988	England	1	Goats milk soft cheese	*Listeria*	Immunocompromised case	(Azadian *et al*., 1989)
1983	USA	6	Raw goats Milk	*C. jejuni*	Associated with dairy that produced raw goat's milk	(Harris *et al*., 1987)
1983	France	20	Ewe milk cheese	*S. aureus*	Made with raw sheep milk, shepherd asymptomatic carrier of S. aureus	(DeBuyser *et al*., 1985)
1975	Mexico	3	Raw cheese	*B. melitensis*	Mexican raw goats milk cheese	(Eckman, 1975)

Table 13: Outbreaks of illness associated with Mixed Food including a Dairy Product

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2005	Israel	43	Cream cake	*Salmonella enterica* (Group D Salmonella)	Ingestion of cream cake at birthday party	(Hefer *et al*., 2005)
2002	Spain		Ravioli With cheese sauce	*Clostridium perfringens*	Ravioli With cheese sauce, consumed at a restaurant	(Sanz *et al*., 2002)
2001	UK	138	Cream, mints, or profiteroles	Small round structured virus (SRSV)	At a charity function	(Steel *et al*., 2001)
1999	UK	80	Ham, coleslaw, bread rolls, cheese and pineapple	Small round structured virus (SRSV)	Contamination appears to be due to poor food handling	(Fone *et al*., 1999)
1996	Mexico	83	Chilli rellenos ingredients included shelled eggs and cheese	*S*. Enteritidis phage type 4	Salmonella was isolated from the leftover cheese but the isolate was not serotyped	(Shane *et al*., 2002)
1995	UK	?	Kebabs with yogurt sauce	*S*. Typhimurium DT170	Raw kebab mince positive for *S*. Typhimurium DT170, yogurt stored under mince stained with blood	(Evans *et al*., 1999) S.
1990	Thailand	400	Eclairs	*Staphylococcus aureus* producing toxins A and C and Bacillus cereus	Eclairs which were prepared during the night before the dinner and kept at room temperature for at least 12 hours	(Thaikruea *et al*., 1995)
1981	USA		Macaroni Cheese	*Bacillus cereus*	Epidemiologic investigation incriminated macaroni and cheese as a cause of the illness and samples of this food contained large numbers of Bacillus cereus	(Holmes *et al*., 1981)

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APPENDIX 3
Occurrence of microbiological hazards associated with dairy products

3.1 Occurrence of microbiological hazards in Dairy products in Australia

The occurrence of microbiological hazards in Australian finished dairy products is extremely low, due largely to all products originating from pasteurised milk. Of dairy samples sent to the Melbourne Diagnostic Unit, and collated by the National Enteric Pathogen Surveillance Scheme (NEPSS) over a 20-year period (1983-2004), *Salmonella* has been isolated from a total of 1,156 samples. Although the data presented in Table 1 does not provide the prevalence of *Salmonella* spp. in Australian dairy products (only positive samples) it does give information on the range of *Salmonella* serovars associated with these products.

Surveys undertaken by the South Australian Dairy Authority confirm the high compliance of dairy products in Australia (Table 2). Samples are analysed for a number of microbiological and chemical contaminants. However, results are reported as either pass or fail, without identifying which specific test the product failed (Table 3). Testing undertaken by Dairy Food Safety Victoria on dairy products are summarised in Tables 4-6.

Table 1: *Salmonella* isolates from raw milk and milk products, NEPPS data 1983-2004.

Product	Organism	State of origin and no. of times isolated
Liquid raw cows milk	*S.* Agona	Vic 1
	S. Anatum	Vic 1
	S. Bovismorbificans 24	WA 24
	S. Dublin	Vic 12
	S. Kiambu	WA 24
	S. Mbandaka	WA 1
	S. Ohio	NSW 1
	S. Typhimurium 13	Vic 1
	S. Typhimurium 44	SA 6, Vic 1
	S. Typhimurium 135	Vic 2
	S. Typhimurium RDNC	NSW 1, Qld 1
	S. Zanzibar	Vic 4
Raw goats milk	*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
Dried milk powders*	*S.* Adelaide	Vic 2, WA 1
	S. Agona	Vic 79
	S. Anatum	Vic 125, Qld 8
	S. Anatum var 15+	Vic 14
	S. Bredeney	Vic 24
	S. Chester	Vic 1
	S. Derby	Vic 78, NSW 1
	S. Dublin	Vic 5, NSW 1
	S. Emmastad	Vic 4
	S. Havana	Vic 254, Qld 27, NSW 9, Tas 2
	S. Havana H2S negative	Vic 8
	S. Johannesburg	Vic 16
	S. Kottbus	Vic 1
	S. Mbandaka	Qld 1, Vic 1

Table 1: (cont.)

Product	Organism	State of origin and no. of times isolated
Dried milk powders*	*S.* Muenchen	Qld 1
(*Cont*)	*S.* Newport	Vic 15
	S. Ohio	Vic 59, NSW 1
	S. Ohio var 14+	NSW 2
	S. Oranienburg	Vic 6, Qld 4
	S. Orion	Qld 18, Vic 2
	S. Orion var 15+	NSW 1
	S. Sachsenwald subsp IV	Qld 1
	S. Senftenberg	Vic 6
	S. Senftenberg z27 phase	Qld 2
	S. Singapore	Qld 1
	S. Tennessee	Qld 14
	S. Typhimurium 6	Vic 1
	S. Typhimurium 44	Vic 1
	S. Typhimurium 135	Tas 2
	S. Typhimurium 170	NSW 4
	S. Typhimurium RDNC	Vic 1
	S. Zanzibar	Qld 1
	S. subsp I ser 4,12:d:-	Qld 1
Infant formula	*S.* Agona	NSW 1
	S. Anatum	Vic 1
	S. Bredeney	Vic 1
	S. Potsdam	Qld 1
Ice-cream	*S.* Anatum var 15+	Vic 1
Concentrated milk	*S.* Schwarzengrund	Tas 1
Whey powder	*S.* Agona	Vic 14
	S. Anatum	Vic 62, Tas 1
	S. Anatum var 15+	Vic 13
	S. Chester	Vic 1
	S. Give var 15+	Qld 2
	S. Havana	Vic 21
	S. Mbandaka	Vic 1
	S. Newport	Vic 5
	S. Senftenberg	Vic 7
	S. Wandsworth	Qld 1
Casein	*S.* Adelaide	Vic 24, NSW 3
	S. Agona	Qld 5, Vic 1
	S. Anatum	Vic 3
	S. Anatum var 15+	Vic 19
	S. Bareilly	NSW 1
	S. Bovismorbificans 14	WA 3
	S. Braenderup	Vic 4
	S. Derby	Vic 2
	S. Gaminara	Qld 2
	S. Havana	Vic 4
	S. Infantis	Vic 4
	S. Ohio	Vic 33, Qld 1
	S. Senftenberg	Vic 2, Qld 1
	S. Virchow (not typed)	Qld 4
	S. Virchow PT19	Qld 1, NSW 1
	S. Virchow PT19 var	Qld 3
	S. Virchow PT34	Qld 1

* dried milk powders include buttermilk, skim milk, full cream milk, unspecified powdered milks

Table 2: Dairy Authority of South Australia survey results

Product	Year	No of tests	Number failed (%)
Pasteurised milk	1998	22	1 (4.5)
	1999	26	0
	2000	26	0
	2001	26	0
	2002	29	0
	2003	44	3 (6.8)
	2004	59	8 (13.6)
	TOTAL	**232**	**12 (5.2)**
Cheese	1998	101	13 (12.9)
	1999	123	11 (8.9)
	2000	122	12 (9.8)
	2001	111	2 (1.8)
	2002	119	5 (4.2)
	2003	141	10 (7.1)
	2004	140	0
	TOTAL	**857**	**53 (6.2)**
Dip/Dessert	1998	ND	ND
	1999	ND	ND
	2000	7	0
	2001	13	1 (7.7)
	2002	25	0
	2003	27	0
	2004	33	0
	TOTAL	**105**	**1 (1.0)**
Yoghurt	1998	32	5 (15.6)
	1999	22	1 (3.3)
	2000	34	1 (2.9)
	2001	31	0
	2002	30	1 (3.3)
	2003	25	0
	2004	26	0
	TOTAL	**200**	**8 (4.0)**

Table 3: Tests conducted by the Dairy Authority of South Australia

Product	Test	Standard
Pasteurised milk	Standard Plate Count Coliforms Antimicrobial substances	<50,000 cfu/ml <1 cfu/ml <0.003 ug/ml
Cheese	Coliforms and *E. coli* Coagulase positive *S. aureus* *Listeria monocytogenes*	<10 cfu/ml <100 cfu/ml Not detected in 25g
Dip/Dessert*	Standard Plate Count Coliform Coagulase positive *S. aureus* *Listeria monocytogenes*	<50,000 cfu/ml <10 cfu/ml <100 cfu/ml Not detected in 25g
Yoghurt	Coliforms Yeasts Moulds	<10 cfu/ml <100 cfu/ml <100 cfu/ml

*Dip/Dessert includes Gelati, ice cream, cheese-based dip, yoghurt-based dip, cream.

Table 4: Summary of outcomes from Dairy Food Safety Victoria product testing program for 2004/05

Product	No. samples tested	Coliforms	*E. coli*	*L. monocytogenes*	*CP Staph*	*Salmonella*
High Moisture Cheese	102	41 (40%)	13 (13%)	4 (4%)	5 (%%)	0
Low Moisture Cheese	48	12 (25%)	4 (8%)	5 (10%)	0	0
Ice Cream	39	37 (95%)	0	0	0	0
Dips	14	5 (36%)	0	1 (7%)	0	0
Desserts	2	1 (50%	0	0	0	0

Table 5: Summary of outcomes from Dairy Food Safety Victoria product testing program for 2003/04

Product	No. samples tested	Coliforms	*E. coli*	*L. monocytogenes*	*CP Staph*	*Salmonella*
High Moisture Cheese	150	47 (31%)	14 (9%)	2 (2%)	2 (2%)	0
Low Moisture Cheese	57	16 (28%)	8 (14%)	1 (2%)	0	0
Ice Cream	59	31 (53%)	1 (2%)	0	0	0
Dips	22	5 (23%)	0	1 (5%)	0	0
Yoghurt	28	4 (14%)	0	0	0	0
Desserts	10	4 (40%)	0	0	0	0

Table 6: Summary of outcomes from Dairy Food Safety Victoria product testing program for 2002/03

Product	No. samples tested	Coliforms	*E. coli*	*L. monocytogenes*	*CP Staph*	*Salmonella*
High Moisture Cheese	129	52 (40%)	10 (8%)	2 (2%)	0	0
Low Moisture Cheese	80	23 (29%)	4 (5%)	2 (3%)	0	0
Ice Cream	50	19 (38%)	0	0	0	0
Dips	29	8 (28%)	6 (21%)	1 (3%)	0	0
Desserts	6	1 (17%)	0	0	0	0
Powder	33	4 (12%)	0	1 (3%)	0	0
Milk	19	0	0	0	0	0

3.1.1 Imported Foods Inspection Scheme testing results

The Imported Food Program, operated by the Australian Quarantine and Inspection Service (AQIS), tests a large number of samples of dairy products entering Australia each year. In the period 2002 to 2004, failures were recorded for imported cheeses for *E. coli*, and *L. monocytogenes* (Table 7). No failures were recorded in tests for *Salmonella* spp.

Table 7: Significant imported food testing failures for dairy products 2002-2004.

Product	Test	Number sampled	Number failed*
Cheese	*E. coli*	53	2 (3.8%)
	L. monocytogenes	15	0
	Salmonella spp.	2	0
Soft Cheese	*E. coli*	330	21 (6.4%)
	L. monocytogenes	894	21 (2.3%)
	Salmonella spp.	288	0

* All imported dairy products must meet the microbiological limits specified in Section 1.6.1 of the Food Standards Code

3.1.2 Food recalls

FSANZ is responsible for the coordination and monitoring of food recalls in Australia. FSANZ collates and disseminates information on recalls in consultation with the senior food officers or their deputies in the States and Territories, and the product' s supplier such as the manufacturer or the importer.

There were 43 recalls for dairy products due to microbiological concerns for the period 1990 to 2005 (Table 8). There were a total of 716 food recalls notified to FSANZ during this period, thus recalls attributed to dairy products only represents 6% of the total number of recalls notified to FSANZ.

Table 8 Summary of Food recalls notified to FSANZ 1990 – August 2005

Product	Number of recalls
Milk	16
Cheese	7
Cream	6
Ice cream	4
Dips	3
Dairy desserts	2
Yoghurt	2
Milk powder	2
Custard	1

Table 9 Food recalls notified to FSANZ 1990 – August 2005

Product	Reason
Skim milk	*Lactobacillus* spp.
Various flavoured UHT milk	Coliforms
Ice cream party cakes	Suspected *Salmonella* contamination
Chocolate flavoured UHT milk	*B. cereus*
Chocolate coated ice cream sticks	*L. monocytogenes*
Milk	*L. monocytogenes*
Full cream milk powder (1kg pack)	*Salmonella* anatum
Spring onion dip	*L. monocytogenes*
Smoked trout dip	*L. monocytogenes*
Ice cream and ice cream sticks	*E. coli*
Choc coated ice cream bars	*L. monocytogenes*
Gourmet dips (various flavours)	*L. monocytogenes*
Frozen unpasteurised goat milk, soft cheese and fetta	Unacceptable levels of microorganisms
Thickened cream	*L. monocytogenes*
Cheese	*Staphylococcus*
Milk (plain and flavoured)	*L. monocytogenes*
Whole milk	*L.* monocytogenes

Table 9: (cont.)

Product	Reason
Cream	*E. coli*
Fetta made from goat milk	*E. coli*
Goat milk yoghurt	*E. coli*
Goat milk fetta cheese, yoghurt, pasteurised milk	*E. coli*
Ice cream (berry flavour)	*L. monocytogenes*
Flavoured milk	*L. monocytogenes*
Chocolate milk	*L. monocytogenes*
Yoghurt	*E. coli*
Cheese	*L. monocytogenes*
Cappuccino topping (skim milk powder (1kg and 500g packs)	*Salmonella*
Parmesan and skim milk fetta wedges	*L. monocytogenes*
Chocolate mousse	*L. monocytogenes*
Various mousse and cheese cake products	*L. monocytogenes*
Ice cream cake	*E. coli*
Ice cream cake	*E. coli*
Custard	*Bacillus*
Whipping cream	*E. coli*
Chocolate flavoured UHT milk	*Bacillus*
Petit fromage	*E. coli*
UHT Banana flavoured milk	Spoilage
Whole milk and fresh cream	Pasteurisation fault
Flavoured milk	Spoilage
UHT milk	Premature spoilage – faulty packaging
UHT milk	Premature spoilage
UHT milk	Premature spoilage – faulty packaging

3.1.3 Data submitted by Industry to Dairy Australia (2005)

The following data was complied by Dairy Australia, and represents the larger processors of dairy products in Australia.

Table10: Prevalence of *Bacillus* spp. in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Cheese	*B. cereus*	2004	31	0	Dairy Australia
Cheese	*Bacillus* spp.	2003/04	31	25.8	Dairy Australia
Cream and butter	*Bacillus* spp.	2003/04	65	0	Dairy Australia
Dried milk	*Bacillus* spp.	2003/04	250	2	Dairy Australia

Table 11: Prevalence of *Campylobacter* spp. in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Milk and milk products	*Campylobacter* spp.	2004	25	0	Dairy Australia

Table 12: Prevalence of *Escherichia coli* in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Cream and cream products	*E. coli*	2004	1,672	0.54	Dairy Australia
Custard	*E. coli*	2004	58	0	Dairy Australia
Ice cream products	*E. coli*	2004	8,015	0	Dairy Australia
Milk and milk products	*E. coli*	2004	22,440	0	Dairy Australia
Yoghurt, fermented milk	*E. coli*	2004	260	0	Dairy Australia

Table 13: Prevalence of *Listeria* spp. in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Milk, cream, cream products, yoghurts	*Listeria* spp.	2004	748	0	Dairy Australia
Cream and butter	*Listeria* spp.	2003/04	4	0	Dairy Australia
Cheese	*Listeria* spp.	2003/04	1,957	0.05	Dairy Australia
Cheese	*Listeria* spp.	2004	184	0	Dairy Australia
Cheese	*Listeria* spp.	2004	253	0	Dairy Australia
Cheese	*Listeria* spp.	2004	12	0	Dairy Australia
Cheese	*Listeria* spp.	2004	31	0	Dairy Australia
Dried milk	*Listeria* spp.	2003/04	126	0	Dairy Australia
Ice cream products	*L. monocytogenes*	2004	4	0	Dairy Australia
Milk	*Listeria* spp.	2003/04	95	0	Dairy Australia
Milk	*L. monocytogenes*	2004	1,560	0	Dairy Australia

Table 14: Prevalence of *Salmonella* in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Dried milk	*Salmonella* spp.	2003/04	100	1	Dairy Australia
Dried milk	*Salmonella* spp.	2003/04	1,658	0	Dairy Australia
Dried milk	*Salmonella* spp.	2004	1,797	0	Dairy Australia
Cream and cream products	*Salmonella* spp.	2004	200	2	Dairy Australia
Cream and butter	*Salmonella* spp.	2003/04	65	0	Dairy Australia
Cheese	*Salmonella* spp.	2003/04	691	0	Dairy Australia
Cheese	*Salmonella* spp.	2004	40	0	Dairy Australia
Cheese	*Salmonella* spp.	2004	40	0	Dairy Australia
Cheese	*Salmonella* spp.	2004	31	0	Dairy Australia
Cheese	*Salmonella* spp.	2004	35	0	Dairy Australia
Milk powder	*Salmonella* spp.	2004	285	0	Dairy Australia
Milk powder	*Salmonella* spp.	2004	12	0	Dairy Australia
Ice cream products	*Salmonella* spp.	2004	4	0	Dairy Australia
Ice cream products	*Salmonella* spp.	2004	100	0	Dairy Australia
Milk and milk products	*Salmonella* spp.	2004	424	0	Dairy Australia
Yoghurt products	*Salmonella* spp.	2004	7	0	Dairy Australia
Dairy desserts	*Salmonella* spp.	2004	4	0	Dairy Australia

Table 15: Prevalence of *Staphylococcus aureus* in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Dairy based dips	*S. aureus*	2003/04	50	0	Dairy Australia
Custard	*S. aureus*	2004	27	0	Dairy Australia
Cheese	*S. aureus*	2003/04	3,457	0	Dairy Australia
Cheese	*S. aureus*	2004	1,265	0	Dairy Australia
Cheese	*S. aureus*	2004	12	0	Dairy Australia
Cheese	*S. aureus*	2004	31	0	Dairy Australia
Cheese	*S. aureus*	2004	274	0	Dairy Australia
Cream and butter	*S. aureus*	2003/04	66	0	Dairy Australia
Dried milk	*S. aureus*	2003/04	696	0.29	Dairy Australia
Ice cream products	*S. aureus*	2004	4	0	Dairy Australia
Milk	*S. aureus*	2003/04	102	1.96	Dairy Australia
Milk	*S. aureus*	2004	0	0	Dairy Australia
Milk Powder	*S. aureus*	2004	285	0	Dairy Australia

Table 16: Prevalence of *Yersinia enterocolitica* in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Milk and milk products	*Y. enterocolitica*	2004	25	0	Dairy Australia

3.1.4 Surveys from the scientific literature

Table 17: Prevalence of *Aeromonas* spp. in dairy products in Australia

Sample type	**Organisms Isolated**	**Sampling period**	**Samples**	**% Positive**	**Reference**
Pasteurised cow's milk	*Aeromonas*	-	183	4	Kirov et al., 1993
Raw cow's milk	*Aeromonas*	-	72	60	Kirov et al., 1993
Raw cow's milk	*Aeromonas*	-	150	27	Ibrahim and Mac Rae, 1991

Table 18: Prevalence of *Listeria* spp. in dairy products in Australia

Sample type	**Organisms Isolated**	**Sampling period**	**Samples**	**% Positive**	**Reference**
Raw cow's milk	*L. monocytogenes*	-	600	0	Anon, 2003
Cheese - soft	*L. monocytogenes*	-	437	3.4	Arnold and Coble, 1995
Ice cream and ice cream products	*L. monocytogenes*	-	166	13.8	Arnold and Coble, 1995
Pasteurised cow's milk	*L. monocytogenes*	-	33	0	Arnold and Coble, 1995
Raw goat's milk	*L. monocytogenes*	-	69	1.4	Arnold and Coble, 1995
Raw cow's milk	*L. monocytogenes*	-	150	0	Ibrahim and MacRae, 1991
Cheese	*L. monocytogenes*	-	255	2	Venables, 1989
Ice cream	*L. monocytogenes*	-	277	6	Venables, 1989

Table 19: Prevalence of *Yersinia enterocolitica* in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Raw goat's milk	*Y. enterocolitica*	-	274	12.8	Hughes and Jensen, 1981
Pasteurised cow's milk	*Yersinia* spp.	-	551	2.0	Hughes, 1987

3.2 Occurrence of microbiological hazards in Dairy products overseas

The majority of published reports on the occurrence of microbiological hazards in dairy products are for raw milk. As these hazards are inactivated by pasteurisation, the prevalence of these organisms in finished product is extremely low.

The following tables provide a summary of the reported prevalence of microbiological hazards in dairy products overseas. It is difficult to directly compare results between individual studies due to differences in the number of type of samples analysed, the stage of production that samples were taken, and the methodology used to isolate and/or enumerate the organisms. In general, the reported prevalence of microbiological hazards in raw milk is highly variable and is dependant on local factors. With the exception of *Bacillus cereus* spores, pasteurisation effectively inactivates these hazards.

Table 20: Prevalence of *Aeromonas* spp.in dairy products

Sample type	**Organisms Isolated**	**Country**	**Samples**	**% Positive**	**Reference**
Raw cow's milk	*A. caviae, A. hydrophila, A. sobria*	Turkey	157	47.7	Yucel and Citak, 2003
Pasteurised cow's milk	*A. hydrophila*	Turkey	31	5	Yucel and Citak, 2003
Cheese - soft	*A. hydrophila*	Brazil	45	17.7	Araujo et al., 2002
Cheese – homemade minas frescal	*A. caviae, A. hydrophila, A. schubertii*	Brazil	160	51.2	Bulhoes and Junior, 2002
Raw cow's milk	*A. caviae, A. hydrophila, A. sobria*	Egypt	60	75	Ibrahim, 2001
Cheese - Domiati	*A. caviae, A. hydrophila, A. sobria*	Egypt	50	36	Effat at el., 2000
Cheese - Kareish	*A. caviae, A. hydrophila, A. sobria*	Egypt	50	58	Effat at el., 2000
Cheese - Ricotta	*A. caviae, A. hydrophila, A. sobria*	Italy	20	45	Villari et al., 2000
Cheese - Mascarpone	*A. caviae, A. hydrophila, A. sobria*	Italy	20	0	Villari et al., 2000
Cheese - Mozzarella	*A. caviae*	Italy	20	5	Villari et al., 2000

Table 20: (cont.)

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Cheese - Fiordilatte	*A. caviae, A. hydrophila, A. sobria*	Italy	20	0	Villari et al., 2000
Cheese - Treccia	*A. caviae, A. hydrophila, A. sobria*	Italy	20	0	Villari et al., 2000
Ice cream	*A. caviae, A. hydrophila, A. sobria*	Italy	20	0	Villari et al., 2000
Raw cow's milk	*Aeromonas*	Greece	138	40.6	Melas at al., 1999
Raw ewe's milk	*Aeromonas*	Greece	57	35.1	Melas at al., 1999
Pasteurised cow's milk	*Aeromonas*	Greece	80	0	Melas at al., 1999
Cheese - Anthotyros	*Aeromonas*	Greece	39	10.3	Melas at al., 1999
Cheese - Manouri	*Aeromonas*	Greece	36	8.3	Melas at al., 1999
Cheese - Feta	*Aeromonas*	Greece	23	0	Melas at al., 1999
Raw cow's milk	*A. hydrophila*	Turkey	200	0.5	Uraz and Citak, 1998
Pasteurised cow's milk	*A. caviae, A. hydrophila, A. sobria*	Turkey	100	19	Sarimehmetoglu et al., 1998
Cheese - Villalón	*Aeromonas*	Spain	8	25	Santos et al., 1996
Raw cow's milk	*A. caviae, A. hydrophila, A. sobria*	Turkey	80	28.7	Akan et al., 1996
Raw cow's milk	*A. hydrophila*	Germany	200	14	Schweizer et al., 1995
Ice cream	*A. hydrophila*	India	42	7.1	Shankar et al., 1994
Raw cow's milk	*Aeromonas*	Spain	5	20	Pin et al., 1994
Cheese - unripened	*Aeromonas*	Spain	5	20	Pin et al., 1994
Cheese - soft	*Aeromonas*	England	43	2	Walker and Brooks, 1993
Milk and other dairy products	*Aeromonas*	England	97	2	Walker and Brooks, 1993
Pasteurised cow's milk	*Aeromonas*	Brazil	35	28.5	Freitas et al., 1993
Cheese - white	*Aeromonas*	Brazil	25	32	Freitas et al., 1993
Raw cow's milk	*Aeromonas*	Sweden	4	0	Krovacek et al., 1992
Cream - whipped	*Aeromonas*	Denmark	32	28	Knøchel and Jeppesen, 1990
Pasteurised cow's milk	*A. hydrophila*	China	248	0.4	Chen et al., 1988
Raw cow's milk	*A. hydrophila*	China	53	20	Chen et al., 1988
Milk and milk products	*A. hydrophila*	Canada	41	2.4%	Banerjee and Black, 1986

Table 21: Prevalence of *Bacillus cereus* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Infant formula - dried	*B. cereus*	Italy	30	16.7	Pizzin et al., 2003
Infant formula - liquid	*B. cereus*	Italy	20	5	Pizzin et al., 2003
Pasteurised cow's milk	*B. cereus*	Turkey	120	46.6	Ozdemir, 2003
Bulk cow's milk	*B. cereus*	Czech Republic	111	0	Schlegelova, 2002
Cheese - ricotta	*B. cereus*	Italy	50	0	Brindani et al., 2001
Milk powder	*B. cereus*	Germany	1,365	10.7	Hammer at al., 2001
Cheese - ricotta	*B. cereus*	Italy	50	0	Brindani et al., 2001
Raw cow's milk	*B. cereus*	Germany	149	8.1	Hahn et al., 1999
Raw cow's milk	*B. cereus*	Sweden	144	69.4	Christiansson et al., 1999
Cheese – Pichtogalo Chanion	*B. cereus*	Greece	62	14.5	Papageorgiou et al., 1998
Cheese – ricotta, raw cow's milk	*B. cereus*	Italy	32	6.25	Cosseddu et al., 1997
Infant formula - dried	*B. cereus*	UK	100	17	Rowan et al., 1997
Infant formula - prepared	*B. cereus*	UK	24	8.3	Rowan et al., 1997
Pasteurised cow's milk and cream	*B. cereus*	Denmark	458	56	Larsen and Jorgensen, 1997
Raw cow's milk	*B. cereus*	Denmark	115	25	Larsen and Jorgensen, 1997
Raw cow's milk	*B. cereus*	Canada	298	0.7	Odumeru et al., 1997
Dried milk powder	*B. cereus*	UK	45	53	Crielly et al., 1994
Infant formula - imported	*B. cereus*	Germany	92	52	Becker et al., 1994

Table 22: Incidence of *Bacillus cereus* in infant formula (Extracted from Becker *et al*., 1994)

Product	Positive/total samples examined	MPN/g		
		0.3-10	>10-100	>100
Infant formula (milk protein)	48/92 (52%)	37	10	1
Infant formula (soy protein)	11/16 (69%)	11	-	-
Follow-on formula	45/86 (52%)	35	7	3

Table 23: Incidence of *Bacillus cereus* in milk based infant food (extracted from Becker *et al*., 1994)

Country	Product	Samples	% positive	*B. cereus* /g	Authors
Romania	Milk products[a]	?	(25.8)	?	Ionescu and Ionescu, 1971
FRG	Infant Food	60	8 (13.3)	100-400	Könning, 1972
Korea	Dried milk[a]	3 Brands	?	1.5-100	Kwun et al., 1979
India	Infant food	10	9 (90.0)	200-2,000	Singh et al., 1980
Poland	Infant food	25	15 (60.0)	10-1000	Stec and Burzynska, 1980
USSR	Infant food	?	?	5-650	Kirilenko et al., 1983
FRG	Infant food	90	39 (43.3)	<1500	Döll, 1983
Egypt	Infant food	10	10 (100)	10-3,000	Helmy et al., 1984
FRG	Infant food Whey powder[a] Skim milk powder[a] Lactose[a]	140 10 6 50	54 (38.6) 6 (60.0) 4 (66.7) 0	3-460 3-100 3-100 0	Becker et al., 1984
Egypt	Infant food	30	24(80.0)	<500	Moustafa et al., 1984

[a] intended for infant feeding

Table 24: Prevalence of *Brucella* spp. in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Ice cream	*Br. abortus*	Turkey	217	6.25	Kuplulu and Sarimehmetoglu, 2004
Cheese – cow's milk	*Brucella* spp.	Turkey	35	0	Kasimoglu, 2002
Cheese – ewe's milk	*Brucella* spp.	Turkey	35	14.2	Kasimoglu, 2002
Raw cow's milk	*Br. melitensis*	Turkey	35	0	Kasimoglu, 2002
Cheese – ewe's and goat's milk	*Brucella* spp.	Italy	46	46	Tantillo et al., 2001
Cheese – mozzarella	*Brucella* spp.	Italy	150	0	Serpe et al., 2000
Cheese - ricotta	*Brucella* spp.	Italy	100	0	Serpe et al., 2000
Cheese – white, fresh	*Br. abortus and Br. melitensis*	Mexico	335	7.5	Acedo et al., 1997
Raw cow's milk	*Br. abortus and Br. melitensis*	Mexico	265	2.3	Acedo et al., 1997
Raw goat's milk	*Br. abortus and Br. melitensis*	Mexico	24	4.2	Acedo et al., 1997
Raw camel's milk	*Br. abortus*	Sudan	153	10.5	Obied et al., 1996
Raw cow's milk	*Br. abortus*	New Zealand	115	31	Blair, 1948

Table 25: Prevalence of *Campylobacter* spp. in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Raw cow's milk	*Campylobacter*	UK	610	0.8	Food Standards Agency, 2003
Pasteurised cow's milk	*Campylobacter*	UK	1413	0	Food Standards Agency, 2003
Raw cow's milk	*Campylobacter*	Ireland	62	1.6	Whyte et al., 2004
Raw goat's milk	*Campylobacter*	Switzerland	344	0	Muehlherr et al., 2003
Raw ewe's milk	*Campylobacter*	Switzerland	63	0	Muehlherr et al., 2003
Raw cow's milk	*Campylobacter*	US	131	9.2	Jayarao and Henning, 2001
Raw cow's milk	*Campylobacter*	Turkey	211	8.1	Uraz and Yucel, 1999
Raw goat's milk	*Campylobacter*	UK	100	0	Little and De Louvois, 1999
Raw sheep's milk	*Campylobacter*	UK	26	0	Little and De Louvois, 1999
Raw cow's milk	*Campylobacter*	Canada	1,720	0.47	Steele et al., 1997
Bulk cow's milk	*Campylobacter*	Trinidad	177	0	Adesiyun et al., 1996
Raw cow's milk and other dairy products	*Campylobacter*	Switzerland	93	6.5 (PCR) 0(culture)	Wegmuller et al., 1993
Raw cow's milk	*Campylobacter*	US	292	12.3	Rohrbach et al., 1992
Raw cow's milk	*Campylobacter*	UK	-	6	Humphrey and Hart, 1988
Raw cow's milk	*C. jejuni*	Netherlands	904	4.5	Beumer et al., 1988
Raw goat's milk	*Campylobacter*	-	2,493	0.04	Roberts, 1985
Raw cow's milk	*C. jejuni*	US	195	1.5	Lovett et al., 1983
Raw cow's milk	*Campylobacter*	US	108	0.9	Doyle and Roman, 1982

Table 26: Prevalence of *Coxiella burnetii* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Bulk tank milk	*C. burnetii*	UK	373	21.2 (ELISA)	Paiba et al., 1999
Raw cow's milk	*C. burnetii*	Japan	62	33.9 (PCR-ELISA)	Muramatsu et al., 1997
Raw cow's milk	*C. burnetii*	Nigeria	169	24	Adesiyun et al., 1985
Raw cow's milk	*C. burnetii*	US	109	7.3	Enright et al., 1957

Table 27: Prevalence of *Enterobacter sakazakii* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Cheese product	*E. sakazakii*	UK	82	2.4	Iversen and Forsythe, 2004
Milk powder	*E. sakazakii*	UK	72	4.2	Iversen and Forsythe, 2004
Infant formula - dried	*E. sakazakii*	UK	62	62	Iversen and Forsythe, 2004
Infant formula	*E. sakazakii*	UK	58	13.8	Leuschner et al., 2004
Infant formula	*E. sakazakii*	Netherlands	40	2.5	Heuvelink et al., 2001
Milk powder	*E. sakazakii*	Netherlands	170	4.1	Heuvelink et al., 2001
Infant formula - cans	*E. sakazakii*	Canada	120	6.7	Nazarowec-White and Farber, 1997

Table 28: Prevalence of pathogenic *Escherichia coli* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Pasteurised bovine products	*E. coli* O157	Italy	657	0	Conedera et al., 2004
Raw bovine products	*E. coli* O157	Italy	811	0	Conedera et al., 2004
Pasteurised ovine products	*E. coli* O157	Italy	477	0	Conedera et al., 2004
Raw ovine products	*E. coli* O157	Italy	502	0	Conedera et al., 2004
Cheese – buffalo milk mozarella	*E. coli* O157	Italy	501	0	Conedera et al., 2004
Raw cow's milk	*E. coli* O157	Northern Ireland	420	2.14	McKee et al., 2003
Raw cow's milk	*E. coli*	US	77,172	3.1-6.7	Makovec and Ruegg, 2003
Raw goat's milk	STEC	Switzerland	344	16.3	Muehlherr et al., 2003
Raw ewe's milk	STEC	Switzerland	63	12.7	Muehlherr et al., 2003
Raw cow's milk (bulk tank)	*E. coli* O157:H7	US	268	0.75	Murinda et al., 2002
Raw goat's milk	*E. coli* O157:H7	Italy	60	1.7	Foschino et al., 2002
Raw cow's milk (bulk tank)	*E. coli* O157:H7	US	131	0	Jayarao and Henning, 2001
Raw cow's milk (bulk tank)	STEC	US	131	3.8	Jayarao and Henning, 2001
Raw cow's milk	*E. coli* O157	Scotland	500	0	Coia et al., 2001
Cheese- raw milk	*E. coli* O157	Scotland	739	0	Coia et al., 2001
Cheese	STEC	France	603	1	Pradel et al., 2000
Raw cow's milk	*E. coli* O157:H7	Italy	100	0	Massa et al., 1999
Raw ewe's milk	*E. coli* O157:H7	UK	26	0	Little and De Louvois, 1999
Raw goat's milk	*E. coli* O157:H7	UK	100	0	Little and De Louvois, 1999
Raw cow's milk	*E. coli* O157:H7	Netherlands	1,011	0	Heuvelink et al., 1998
Raw cow's milk	VTEC	Canada	1,720	0.87	Steele et al., 1997
Raw cow's milk	*E. coli* O157:H7	UK	329	0	Mechie et al., 1997
Raw cow's milk	*E. coli*	France	69	~80	Desmasures et al., 1997
Cheese – raw milk, soft	Toxigenic *E. coli*	Spain	221	1.4	Quinto and Cepeda, 1997
Cheese– pasteurised, soft	Toxigenic *E. coli*	Spain	75	0	Quinto and Cepeda, 1997
Raw cow's milk	*E. coli* O157:H7	US	115	10	Padhye and Doyle, 1991
Raw cow's milk	*E. coli* O157:H7	US	23	4.3	Wells et al., 1991

Table 29: Prevalence of *Listeria monocytogenes* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Raw cow's milk	*L. monocytogenes*	UK	610	17	Food Standards Agency, 2003
Pasteurised cow's milk	*L. monocytogenes*	UK	1413	0	Food Standards Agency, 2003
Cheese,pasteurised ewe's milk, soft	*L. monocytogenes*	Portugal	63	46	Pintado et al., 2005
Raw cow's milk	*L. monocytogenes*	US	861	6.5	van Kessel et al., 2004
Raw cow's milk	*L. monocytogenes*	Brazil	6	16.7	Silva et al., 2003
Raw cow's milk	*L. monocytogenes*	US	474	4.9–7.0	Muraoka et al., 2003
Bulk raw cow's milk	*L. monocytogenes*	Sweden	294	1	Waak et al., 2002
Raw cow's milk	*L. monocytogenes*	US	131	4.6	Jayaroo and Henning, 2001
Raw cow's milk (farm milk filters)	*L. monocytogenes*	US	404	12.6	Hassan et al., 2000
Raw cow's milk	*L. monocytogenes*	Spain	774	3.62	Gaya et al., 1998
Raw cow's milk	*L. monocytogenes*	Canada	1,720	2.7	Steele et al., 1997
Raw cow's milk	*L. monocytogenes*	France	69	5.8	Desmasures et al., 1997
Bulk cow's milk	*L. monocytogenes*	Trinidad	177	1.1	Adesiyun et al., 1996
Raw cow's milk	*L. monocytogenes*	Scotland	160	15.6	Fenlon et al., 1995
Cheese - soft	*L. monocytogenes*	UK	251	0.4	MacGowan et al., 1994
Raw cow's milk	*L. monocytogenes*	US	292	4.1	Rorhbach et al., 1992
Raw cow's milk (bulk tank)	*L. monocytogenes*	Finland	134	2.9	Husu, 1990
Raw cow's milk	*L. monocytogenes*	Scotland	180	1.0 – 3.8	Fenlon and Wilson, 1989
Raw cow's milk	*L. monocytogenes*	Canada	445	1.3	Farber et al., 1988

See also "Quantitative Assessment of Relative Risk to Public Health and Safety from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods" (2003)
http://www.foodsafety.gov/~dms/lmr2-toc.html

Table 30: Prevalence of *Mycobacterium* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Raw cow's milk	MAP	Ireland	389	13 (PCR) 0.3 (culture)	O'Reilly et al., 2004
Pasteurised cow's milk	MAP	Ireland	357	9.8 (PCR) 0 (culture)	O'Reilly et al., 2004
Raw goat's milk	MAP	Norway	340	7.1 (PCR) 0 (culture)	Djonne et al., 2003
Raw goat's milk	MAP	Switzerland	344	23	Muehlherr et al., 2003
Raw sheep's milk	MAP	Switzerland	63	24	Muehlherr et al., 2003
Pasteurised cow's milk	MAP	Canada	710	15 (PCR) 0 (culture)	Gao et al., 2002
Raw cow's milk	MAP	UK	244	7.8 (PCR) 1.6 (culture)	Grant et al., 2002
Pasteurised cow's milk	MAP	UK	567	12 (PCR) 1.8 (culture)	Grant et al., 2002
Pasteurised cow's milk	MAP	Ireland	77	0	O'Doherty et al., 2002

Table 30: (cont.)

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Pasteurised goat's milk	MAP	Ireland	9	0	O'Doherty et al., 2002
Raw sheep's and goat's milk	MAP	UK	104	1 (PCR) 0 (culture)	Grant et al., 2001
Raw cow's milk	*M.* spp.	Tanzania	805	3.9	Kazwala et al., 1998
Pasteurised cow's milk	MAP	UK	312	7 (PCR) 3.5 (culture)	Millar et al., 1996
Raw cow's milk	MAP	Pakistan	72	8.3	Sabir et al., 1993
Cow's milk	*Mycobacterium* spp.	Russia	127	25.9	Gertman et al., 1990
Pasteurised cow's milk	*Mycobacterium* spp.	Germany	290	2.1	Beerwerth, 1970
Raw cow's milk	*Mycobacterium* spp.	Germany	1,764	7.9	Beerwerth, 1970

Table 31: Prevalence of *Salmonella* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Raw cow's milk	*Salmonella*	UK	610	0.3	Food Standards Agency, 200?
Pasteurised cow's milk	*Salmonella*	UK	1,413	0	Food Standards Agency, 200?
Raw cow's milk	*Salmonella*	US	861	2.6	Van Kessel et al., 2004
Raw goat's milk	*Salmonella*	Switzerland	344	0	Muehlherr *et al*., 2003
Raw ewe's milk	*Salmonella*	Switzerland	63	0	Muehlherr *et al.,* 2003
Raw goat's milk	*Salmonella*	Italy	60	0	Foschino et al., 2002
Raw milk (bulk tank)	*Salmonella*	US	268	2.2	Murinda et al., 2002
Raw milk (bulk tank)	*Salmonella*	US	131	6.1	Jayarao and Henning, 2001
Raw cow's milk	*Salmonella*	US	131	6.1	Jayarao and Henning, 2001
Raw cow's milk (farm milk filters)	*Salmonella*	US	404	1.5	Hassan et al., 2000
Raw goat's milk	*Salmonella*	UK	100	0	Little and De Louvois, 1999
Raw ewe's milk	*Salmonella*	UK	26	0	Little and De Louvois, 1999
Raw cow's milk	*Salmonella*	France	69	2.9	Desmasures et al., 1997
Raw cow's milk	*Salmonella*	Canada	1,720	0.17	Steele et al., 1997
Bulk cow's milk	*Salmonella*	Trinidad	177	1.7	Adesiyun et al., 1996
Raw cow's milk	*Salmonella*	England and Wales	1,673	0.36	O'Donnell, 1995
Raw milk	*Salmonella*	Switzerland	456	0	Bachmann and Spahr, 1995
Raw cow's milk	*Salmonella*	US	292	8.9	Rohrbach et al., 1992
Cheese – Turkish white	*Salmonella*	Turkey	38	0	Turantas et al., 1989
Raw cow's milk	*Salmonella*	US	678	4.7	McManus et al., 1987

Table 32: Prevalence of *Shigella* spp. in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Cheese - white pickled	*Shigella* spp.	Turkey	50	8	Sik et al., 2004
Cheese – raw milk (Palermitano)	*Shigella* spp.	Italy	12	0	Turtura and Grasselli, 2001
Frozen yoghurt	*Shigella* spp.	Spain	170	0	Lopez et al., 1997
Cheese – ripened, pasteurised milk	*Shigella* spp.	Spain	37	0	Massa-Calpe, 1996
Cheese – fresh, pasteurised milk	*Shigella* spp.	Spain	23	0	Massa-Calpe, 1996
Raw cow's milk	*Shigella* spp.	India	65	0	Singh et al., 1996
Flavoured ice cream	*Shigella* spp.	Canary Islands	150	0	Rodriguez-Alvarez et al., 1995
Cheese - farmouse	*Shigella* spp.	Ireland	25	0	Coveney et al., 1994
Cheese – non-farmhouse	*Shigella* spp.	Ireland	2	0	Coveney et al., 1994
Cheese - imported	*Shigella* spp.	Ireland	4	0	Coveney et al., 1994
Commercial curd	*Shigella* spp.	Spain	21	0	Jordano et al., 1987
Pasteurised cream	*Shigella* spp.	Spain	20	0	Jordano et al., 1987
Sheep's milk cream product (kishfa)	*Shigella* spp.	Iraq	90	2.2	Al-Rajab et al., 1986
Spoiled UHT milk	*Shigella* spp.	China	37	2.7	Lee, 1984
Market milk	*Shigella* spp.	Poland	135	0	Maciejska-Roczan and Burzynska, 1981
Raw buffalo's milk	*Shigella* spp.	India	240	0	Kumar et al., 1978

Table 33: Prevalence of *Staphylococcus aureus* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Bulk cow's milk	*S. aureus*	Norway	220	75	Jorgensen et al., 2005
Goat's milk	*S. aureus*	Norway	213	96.2	Jorgensen et al., 2005
Bulk cow's milk	*S. aureus*	US	118	60	Sato et al., 2004
Bulk cow's milk	*S. aureus*	Denmark	40	55	Sato et al., 2004
Raw cow's milk	*S. aureus*	Malaysia	930	>60	Fook-Yee-Chye et al., 2004
Raw cow's milk	*S. aureus*	US	77,172	9.7-17.7	Makovec and Ruegg, 2003
Raw ewe's milk	*S. aureus*	Switzerland	63	33.3	Muehlherr et al., 2003
Raw goat's milk	*S. aureus*	Switzerland	344	31.7	Muehlherr et al., 2003
Bulk cow's milk	*S. aureus*	Czech Republic	111	34.2	Schlegelova, 2002
Raw goat's milk	*S. aureus*	Italy	60	43	Foschino et al., 2002
Cheese – cottage	*S. aureus*	Slovakia	35	0	Belickova et al., 2001
Cheese - Ondava	*S. aureus*	Slovakia	29	0	Belickova et al., 2001
Cheese - ricotta	*S. aureus*	Italy	50	2	Brindani et al., 2001
Whole acidophilus milk	*S. aureus*	Slovakia	18	0	Belickova et al., 2001
Raw cow's milk	*S. aureus*	Canada	21	90.4	Tondo et al., 2000
Raw ewe's milk	*S. aureus*	UK	126	7	Little and De Louvois, 1999
Composite milk	*S. aureus*	Trinidad	287	97.6	Adesiyun et al., 1998
Raw cow's milk	*S. aureus*	Italy	794	34.3	Moretti et al., 1998
Cheese – ricotta, raw cow's milk	*S. aureus*	Italy	32	0	Cosseddu et al., 1997
Raw cow's milk	*S. aureus*	France	69	62	Desmasures et al., 1997
Cheese – pasteurised milk, ripened	*S. aureus*	Spain	37	2.7	Massa-Calpe, 1996
Cheese – Minas Frescal	*S. aureus*	Brazil	18	22.2	Gomes and Galla, 1995
Raw cow's milk	*S. aureus*	Brazil	19	57.9	Gomes and Gallo, 1995
Raw cow's milk	*S. aureus*	Denmark	4,645	10.2	Aarestrup et al., 1995
Raw cow's milk	*S. aureus*	Trinidad	287	100	Adesiyun et al., 1995
Raw goat's milk	*S. aureus*	UK	2,493	4	Roberts, 1985

Table 34: Prevalence of *Streptococcus* spp. in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Raw cow's milk	*S. agalactie*	US	77,172	3.0-8.1	Makovec and Ruegg, 2003
Raw milk	*Streptococcus* spp.	Venezuela	200	9.5	Faria-Reyes et al., 2002

Table 35: Prevalence of *Yersinia enterocolitica* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Pasteurised cow's milk	Y. enterocolitica	Iran	40	0	Soltan-Dallal et al., 2004
Raw cow's milk	Y. enterocolitica	Iran	310	1.6	Soltan-Dallal et al., 2004
Raw cow's milk	Y. enterocolitica	US	131	6.1	Jayaroo and Henning, 2001
Raw cow's milk	Y. enterocolitica	Turkey	211	3.8	Uraz and Yucel, 1999
Raw cow's milk	Y. enterocolitica	France	69.	36	Desmasures et al., 1997
Bulk cow's milk	*Y. enterocolitica*	Trinidad	177	1.1	Adesiyun et al., 1996
Fermented cow's milk	Y. enterocolitica	Morocco	63	6.3	Hamama et al., 1992
Raw cow's milk	Y. enterocolitica	Morocco	30	30	Hamama et al., 1992
Cheese – raw milk	Y. enterocolitica	Morocco	94	4	Hamama et al., 1992
Pasteurised cow's milk	Y. enterocolitica	Russia	120	35.8	Kuznetsof and Bagriantsev, 1992
Raw cow's milk	Y. enterocolitica	US	292	15.1	Rohrbach et al., 1992
Raw cow's milk	*Yersinia* spp.	Ireland	589	39	Rea et al., 1992
Pasteurised buffalo's milk	Y. enterocolitica	India	60	0	Toora et al., 1989
Raw buffalo's milk	Y. enterocolitica	India	207	24.1	Toora et al., 1989
Raw bulk cow's milk	Yersinia *spp.*	Northern Ireland	150	22.7	Walker and Gilmour, 1986
Farm bottled raw cow's milk	Yersinia *spp.*	Northern Ireland	20	25	Walker and Gilmour, 1986
Creamery pasteurised cow's milk	Yersinia *spp.*	Northern Ireland	100	6	Walker and Gilmour, 1986
Farm pasteurised cow's milk	Yersinia *spp.*	Northern Ireland	50	8	Walker and Gilmour, 1986
Raw cow's milk	Y. enterocolitica	Bulgaria	286	11.9	Pavlov, 1985
Raw goat's milk	Y. enterocolitica	UK	2,493	0.08	Roberts, 1985
Raw cow's milk and raw cow's milk products	Y. enterocolitica	Japan	374	3.2	Fukushima et al., 1984
Raw cow's milk	Y. enterocolitica	Nigeria	319	4.4	Umoh et al., 1984
Raw cow's milk	Y. enterocolitica	France	75	81.4	Vidon and Delmas, 1981

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APPENDIX 4

Consumption figures of dairy products for Australian consumers

4.1 Milk And Cream Consumption Data For Australia

Table 1: Australian average daily consumption of milk and milk products by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming milk (% of no. surveyed)		Average amount of milk consumed per day (g)	% of total food consumed
Male	2 - 3	170	150	(88)	457	20.3
Male	4 - 7	416	338	(81)	412	15.5
Male	8 - 11	385	321	(83)	419	13.5
Male	12 - 15	349	277	(79)	495	12.4
Male	16 - 18	215	163	(76)	536	10.6
Male	19 - 24	485	382	(79)	417	7.7
Male	25 - 44	2140	1795	(84)	314	6.2
Male	45 - 64	1554	1312	(84)	273	5.7
Male	65+	902	794	(88)	249	6.5
Female	2 - 3	213	193	(91)	420	20.9
Female	4 - 7	383	317	(83)	316	13.4
Female	8 - 11	354	273	(77)	352	11.9
Female	12 - 15	304	212	(70)	349	9.4
Female	16 - 18	218	145	(66)	298	7.1
Female	19 - 24	575	460	(80)	269	6.8
Female	25 - 44	2385	2048	(86)	234	6.0
Female	45 - 64	1752	1513	(86)	227	5.9
Female	65+	1058	907	(86)	215	6.3

Table 2: Australian average daily consumption of goat milk by gender and age (National Nutrition Survey, 1995).

Gender	Age	No. consumers surveyed	No. consuming goat milk (% of no. surveyed)		Average amount of goat milk consumed per day (g)	% of total food consumed
Male	2 - 3	170	1	(0.59)	371	0.110
Male	4 - 7	416	-		-	-
Male	8 - 11	385	-		-	-
Male	12 - 15	349	-		-	-
Male	16 - 18	215	-		-	-
Male	19 - 24	485	-		-	-
Male	25 - 44	2140	-		-	-
Male	45 - 64	1554	1	(0.06)	526	0.008
Male	65+	902	-		-	-
Female	2 - 3	213	-		-	-
Female	4 - 7	383	-		-	-
Female	8 - 11	354	-		-	-
Female	12 - 15	304	1	(0.33)	258	0.033
Female	16 - 18	218	-		-	-
Female	19- 24	575	-		-	-
Female	25 - 44	2385	3	(0.13)	337	0.013
Female	45 - 64	1752	3	(0.17)	120	0.006
Female	65+	1058	2	(0.19)	128	0.008

Table 3: Australian average daily consumption of cream and cream products by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming cream (% of no. surveyed)		Average amount of cream eaten per day (g)	% of total food consumed
Male	2 - 3	170	4	(2.3)	15.77	0.02
Male	4 - 7	416	8	(1.9)	11.39	0.01
Male	8 - 11	385	13	(3.4)	24.33	0.03
Male	12 - 15	349	18	(5.2)	32.08	0.05
Male	16 - 18	215	8	(3.7)	19.95	0.02
Male	19 - 24	485	38	(7.8)	46.07	0.08
Male	25 - 44	2140	155	(7.2)	47.87	0.08
Male	45 - 64	1554	137	(8.8)	39.13	0.09
Male	65+	902	88	(9.8)	37.35	0.11
Female	2 - 3	213	9	(4.2)	20.09	0.05
Female	4 - 7	383	16	(4.2)	16.39	0.04
Female	8 - 11	354	22	(6.2)	35.35	0.10
Female	12 - 15	304	14	(4.6)	20.92	0.04
Female	16 - 18	218	20	(9.2)	32.36	0.11
Female	19 - 24	575	40	(7.0)	35.01	0.08
Female	25 - 44	2385	207	(8.7)	33.32	0.09
Female	45 - 64	1752	159	(9.1)	28.46	0.08
Female	65+	1058	107	(10.1)	27.92	0.10

4.2 Cheese Consumption Data For Australia

Table 4: Australian average daily consumption of cheese (for all cheeses) by gender and age (National Nutrition Survey, 1995).

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)		Average amount of cheese consumed per day (g)	% total food consumed
Male	2-3	170	67	(39.41)	26.36	0.5240
Male	4-7	416	173	(41.59)	28.06	0.5406
Male	8-11	385	126	(32.73)	36.06	0.4549
Male	12-15	349	125	(35.82)	45.11	0.5092
Male	16-18	215	97	(45.12)	45.68	0.5351
Male	19-24	485	190	(39.17)	49.62	0.4551
Male	25-44	2140	957	(44.72)	43.51	0.4554
Male	45-64	1554	618	(39.77)	36.79	0.3646
Male	65+	902	339	(37.58)	26.87	0.3005
Female	2-3	213	86	(40.38)	25.87	0.5736
Female	4-7	383	141	(36.82)	27.18	0.5123
Female	8-11	354	152	(42.94)	29.44	0.5535
Female	12-15	304	115	(37.83)	30.51	0.4450
Female	16-18	218	95	(43.58)	34.94	0.5414
Female	19-24	575	236	(41.04)	36.69	0.4770
Female	25-44	2385	1027	(43.06)	32.28	0.4141
Female	45-64	1752	761	(43.44)	31.83	0.4144
Female	65+	1058	411	(38.85)	24.82	0.3283

Table 5: Australian average daily consumption of very hard cheese by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)		Average amount of cheese consumed per day (g)	% total food consumed
Male	2-3	170	2	(1.17)	4.25	0.0025
Male	4-7	416	8	(1.92)	4.55	0.0040
Male	8-11	385	7	(1.82)	2.51	0.0017
Male	12-15	349	2	(0.58)	5.95	0.0011
Male	16-18	215	6	(2.79)	11.50	0.0083
Male	19-24	485	11	(2.27)	32.26	0.0171
Male	25-44	2140	57	(2.66)	10.67	0.0066
Male	45-64	1554	32	(2.06)	13.93	0.0071
Male	65+	902	13	(1.44)	9.72	0.0042
Female	2-3	213	1	(0.47)	1.70	0.0004
Female	4-7	383	7	(1.82)	8.30	0.0078
Female	8-11	354	9	(2.54)	5.01	0.0056
Female	12-15	304	9	(2.96)	1.78	0.0019
Female	16-18	218	8	(3.67)	6.80	0.0089
Female	19-24	575	11	(1.91)	7.38	0.0045
Female	25-44	2385	79	(3.31)	7.96	0.0078
Female	45-64	1752	41	(2.34)	9.86	0.0069
Female	65+	1058	14	(1.32)	8.42	0.0038

Table 6: Australian average daily consumption of soft cheese by gender and age (National Nutrition Survey, 1995).

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)		Average amount of cheese consumed per day (g)	% total food consumed
Male	2-3	170	3	(1.76)	17.43	0.0155
Male	4-7	416	9	(2.16)	23.01	0.0231
Male	8-11	385	13	(3.38)	30.49	0.0397
Male	12-15	349	8	(2.29)	48.84	0.3528
Male	16-18	215	3	(1.39)	68.33	0.0248
Male	19-24	485	23	(4.74)	28.48	0.0316
Male	25-44	2140	106	(4.95)	43.20	0.0501
Male	45-64	1554	75	(4.83)	39.05	0.0470
Male	65+	902	32	(3.55)	38.12	0.0402
Female	2-3	213	6	(2.82)	10.24	0.0158
Female	4-7	383	8	(2.09)	13.84	0.0148
Female	8-11	354	14	(3.95)	32.49	0.0562
Female	12-15	304	14	(4.60)	29.97	0.5323
Female	16-18	218	13	(5.96)	27.44	0.0582
Female	19-24	575	36	(6.26)	42.07	0.0834
Female	25-44	2385	158	(6.62)	33.61	0.6634
Female	45-64	1752	144	(8.22)	36.15	0.0891
Female	65+	1058	63	(5.95)	31.65	0.0642

Table 7: Australian average daily consumption of semi-soft cheese by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)	Average amount of cheese consumed per day (g)	% total food consumed
Male	2-3	170	-		
Male	4-7	416	2 (0.48)	16.20	0.0036
Male	8-11	385	1 (0.26)	8.40	0.008
Male	12-15	349	2 (0.57)	31.66	0.0057
Male	16-18	215	2 (0.93)	55.55	0.0134
Male	19-24	485	4 (0.83)	11.24	0.0022
Male	25-44	2140	41 (1.92)	41.81	0.0187
Male	45-64	1554	34 (2.19)	23.06	0.0126
Male	65+	902	21 (2.33)	22.23	0.0154
Female	2-3	213	-		
Female	4-7	383	2 (0.52)	28.80	0.0077
Female	8-11	354	2 (0.56)	16.35	0.0040
Female	12-15	304	1 (0.33)	14.40	0.0018
Female	16-18	218	3 (1.38)	52.30	0.0256
Female	19-24	575	6 (1.04)	28.70	0.0095
Female	25-44	2385	43 (1.80)	25.15	0.0135
Female	45-64	1752	38 (2.17)	27.72	0.0180
Female	65+	1058	24 (2.27)	17.48	0.0135

Table 8: Australian average daily consumption of processed cheese by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)	Average amount of cheese consumed per day (g)	% total food consumed
Male	2-3	170	34 (20)	27.43	0.2767
Male	4-7	416	81 (19.47)	27.12	0.2446
Male	8-11	385	39 (10.13)	30.53	0.1192
Male	12-15	349	29 (8.31)	39.68	0.1039
Male	16-18	215	20 (9.30)	54.70	0.1321
Male	19-24	485	27 (5.57)	39.80	0.0519
Male	25-44	2140	187 (8.74)	35.79	0.0732
Male	45-64	1554	107 (6.88)	28.72	0.0493
Male	65+	902	56 (6.21)	25.70	0.0475
Female	2-3	213	40 (18.78)	27.57	0.2843
Female	4-7	383	62 (16.19)	25.02	0.2081
Female	8-11	354	41 (11.58)	23.59	0.1196
Female	12-15	304	38 (12.50)	22.37	0.1078
Female	16-18	218	24 (11.10)	27.40	0.1073
Female	19-24	575	47 (8.17)	26.56	0.0688
Female	25-44	2385	199 (8.34)	26.87	0.0669
Female	45-64	1752	174 (9.93)	24.80	0.0738
Female	65+	1058	104 (9.83)	22.09	0.0740

4.3 Dried Milk Consumption Data For Australia

Table 9: Australian average daily consumption of dried milk powder by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming dried milk powder (% of no. surveyed)		Average amount of dried milk powder consumed per day (g)	% of total food consumed
Male	2 - 3	170	-		-	-
Male	4 - 7	416	2	(0.48)	13.74	0.0031
Male	8 - 11	385	2	(0.52)	7.15	0.0014
Male	12 - 15	349	3	(0.86)	33.33	0.0090
Male	16 - 18	215	-		-	-
Male	19 - 24	485	3	(0.62)	9.47	0.0014
Male	25 - 44	2140	11	(0.51)	17.92	0.0022
Male	45 - 64	1554	26	(1.67)	16.34	0.0068
Male	65+	902	23	(2.55)	14.41	0.0109
Female	2 - 3	213	-		-	-
Female	4 - 7	383	2	(0.52)	40.74	0.0109
Female	8 - 11	354	2	(0.56)	10.95	0.0027
Female	12 - 15	304	2	(0.66)	7.35	0.0019
Female	16 - 18	218	-		-	-
Female	19- 24	575	1	(0.17)	2.00	0.0001
Female	25 - 44	2385	22	(0.92)	17.07	0.0047
Female	45 - 64	1752	36	(2.05)	20.09	0.0124
Female	65+	1058	40	(3.78)	21.98	0.0283

4.4 Condensed And Evaporated Milk Consumption Data For Australia

Table 10: Australian average daily consumption of condensed & evaporated milk by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming condensed milk (% of no. surveyed)		Average amount of condensed milk consumed per day (g)	% of total food consumed
Male	2 - 3	170	-		-	-
Male	4 - 7	416	-		-	-
Male	8 - 11	385	-		-	-
Male	12 - 15	349	2	(0.57)	107.25	0.0193
Male	16 - 18	215	-		-	-
Male	19 - 24	485	3	(0.62)	120.82	0.0175
Male	25 - 44	2140	16	(0.75)	24.54	0.0043
Male	45 - 64	1554	29	(1.87)	37.67	0.0175
Male	65+	902	16	(1.77)	38.93	0.0205
Female	2 - 3	213	-		-	-
Female	4 - 7	383	2	(0.52)	10.56	0.00282
Female	8 - 11	354	-		-	-
Female	12 - 15	304	1	(0.33)	102.38	0.0129
Female	16 - 18	218	1	(0.46)	3.25	0.0005
Female	19- 24	575	2	(0.34)	104.00	0.0115
Female	25 - 44	2385	11	(0.46)	36.40	0.0182
Female	45 - 64	1752	17	(0.97)	62.68	0.0124
Female	65+	1058	22	(2.08)	41.92	0.0297

4.5 Butter Consumption Data For Australia

Table 11: Australian average daily consumption of butter by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming butter (% of no. surveyed)		Average amount of butter consumed per day (g)	% of total food consumed
Male	2 - 3	170	16	(9)	7	0.03
Male	4 - 7	416	37	(9)	11	0.04
Male	8 - 11	385	41	(11)	11	0.04
Male	12 - 15	349	29	(8)	13	0.03
Male	16 - 18	215	17	(8)	20	0.04
Male	19 - 24	485	62	(13)	17	0.05
Male	25 - 44	2140	319	(15)	18	0.06
Male	45 - 64	1554	229	(15)	19	0.07
Male	65+	902	160	(18)	19	0.1
Female	2 - 3	213	23	(11)	7	0.04
Female	4 - 7	383	50	(13)	8	0.05
Female	8 - 11	354	44	(12)	10	0.06
Female	12 - 15	304	34	(11)	9	0.04
Female	16 - 18	218	24	(11)	10	0.04
Female	19- 24	575	70	(12)	11	0.04
Female	25 - 44	2385	385	(16)	11	0.05
Female	45 - 64	1752	271	(16)	13	0.06
Female	65+	1058	171	(16)	15	0.08

4.6 Ice Cream Consumption Data For Australia

Table 12: Australian average daily consumption of ice cream by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming ice cream (% of no. surveyed)		Average amount of ice cream consumed per day (g)	% of total food consumed
Male	2 - 3	170	24	(14.1)	80.6	0.57
Male	4 - 7	416	106	(25.5)	94.8	1.12
Male	8 - 11	385	93	(24.2)	139.6	1.30
Male	12 - 15	349	81	(23.2)	178.2	1.30
Male	16 - 18	215	45	(20.9)	216.7	1.78
Male	19 - 24	485	68	(14.0)	150.9	0.50
Male	25 - 44	2140	283	(13.2)	131.6	0.41
Male	45 - 64	1554	248	(16.0)	118.6	0.47
Male	65+	902	157	(17.4)	83.9	0.43
Female	2 - 3	213	39	(18.3)	59.2	0.59
Female	4 - 7	383	81	(21.1)	89.0	0.96
Female	8 - 11	354	81	(22.9)	115.8	1.16
Female	12 - 15	304	83	(27.3)	122.2	1.29
Female	16 - 18	218	26	(11.9)	121.1	0.51
Female	19- 24	575	75	(13.0)	98.4	0.41
Female	25 - 44	2385	263	(11.0)	88.4	0.29
Female	45 - 64	1752	209	(11.9)	72.4	0.26
Female	65+	1058	133	(12.6)	64.2	0.27

4.7 Yoghurt Consumption Data For Australia

Table 13: Australian average daily consumption of yoghurt and cultured milk products by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming yoghurt (% of no. surveyed)		Average amount of yoghurt consumed per day (g)	% of total food consumed
Male	2 - 3	170	22	(12.9)	154	1.00
Male	4 - 7	416	41	(9.6)	132	0.60
Male	8 - 11	385	24	(6.2)	192	0.46
Male	12 - 15	349	19	(5.4)	257	0.44
Male	16 - 18	215	15	(7.0)	243	0.44
Male	19 - 24	485	26	(5.4)	170	0.21
Male	25 - 44	2140	132	(6.2)	182	0.26
Male	45 - 64	1554	109	(7.0)	170	0.30
Male	65+	902	72	(8.0)	146	0.35
Female	2 - 3	213	23	(10.8)	150	0.89
Female	4 - 7	383	41	(10.7)	154	0.84
Female	8 - 11	354	22	(6.2)	179	0.49
Female	12 - 15	304	26	(8.6)	237	0.78
Female	16 - 18	218	17	(7.8)	200	0.55
Female	19- 24	575	43	(7.5)	168	0.40
Female	25 - 44	2385	250	(10.5)	162	0.51
Female	45 - 64	1752	238	(13.6)	155	0.63
Female	65+	1058	136	(12.9)	137	0.60

4.8 Dairy Based Dips Consumption Data For Australia

Table 14: Australian mean daily consumption of dairy dips* by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming dairy dips (% of number surveyed)		Mean amount of dairy dips consumed per day (g)
Male	2 - 3	170	1	(0.6)	20
Male	4 - 7	416	5	(1.2)	38
Male	8 - 11	385	6	(1.6)	64
Male	12 - 15	349	4	(1.1)	91
Male	16 - 18	215	3	(1.4)	52
Male	19 - 24	485	7	(1.4)	67
Male	25 - 44	2140	44	(2.1)	54
Male	45 - 64	1554	16	(1.0)	36
Male	65+	902	5	(0.6)	33
Female	2 - 3	213	4	(1.9)	13
Female	4 - 7	383	2	(0.5)	25
Female	8 - 11	354	4	(1.1)	35
Female	12 - 15	304	1	(0.3)	43
Female	16 - 18	218	6	(2.8)	46
Female	19 - 24	575	12	(2.1)	58
Female	25 - 44	2385	54	(2.3)	49
Female	45 - 64	1752	27	(1.5)	26
Female	65+	1058	5	(0.5)	24

* Includes yoghurt, cream cheese, and sour cream based dips, including eggplant and guacamole.

4.9 Dairy Based Dessert Consumption Data For Australia

Table 15: Australian mean daily consumption of dairy desserts* by gender and age (National Nutrition Survey, 1995**)**

Gender	Age	No. consumers surveyed	No. consuming dairy desserts (% of number surveyed)		Mean amount of dairy desserts consumed per day (g)
Male	2 - 3	170	15	(8.8)	105
Male	4 - 7	416	36	(8.7)	153
Male	8 - 11	385	24	(6.2)	180
Male	12 - 15	349	17	(4.9)	195
Male	16 - 18	215	14	(6.5)	162
Male	19 - 24	485	15	(3.1)	182
Male	25 - 44	2140	69	(3.2)	152
Male	45 - 64	1554	74	(4.8)	161
Male	65+	902	59	(6.5)	159
Female	2 - 3	213	15	(7.0)	103
Female	4 - 7	383	27	(7.0)	157
Female	8 - 11	354	23	(6.5)	168
Female	12 - 15	304	11	(3.6)	113
Female	16 - 18	218	6	(2.8)	148
Female	19 - 24	575	17	(3.0)	148
Female	25 - 44	2385	81	(3.4)	122
Female	45 - 64	1752	78	(4.5)	126
Female	65+	1058	71	(6.7)	133

* Includes custard, junket, dairy dessert, blancmange, flummery, mousse, cheesecake and creamed rice.

4.10 Whey Consumption Data For Australia

Table 16: Australian mean daily consumption of whey powder by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming whey powder (% of number surveyed)		Mean amount of whey powder consumed per day (g)
Male	2 - 3	170	0		-
Male	4 - 7	416	1	(0.2)	24
Male	8 - 11	385	0		-
Male	12 - 15	349	1	(0.3)	68
Male	16 - 18	215	0		-
Male	19 - 24	485	1	(0.2)	6
Male	25 - 44	2140	0		-
Male	45 - 64	1554	1	(0.1)	3
Male	65+	902	0		-
Female	2 - 3	213	0		-
Female	4 - 7	383	0		-
Female	8 - 11	354	0		-
Female	12 - 15	304	0		-
Female	16 - 18	218	0		-
Female	19 - 24	575	0		-
Female	25 - 44	2385	0		-
Female	45 - 64	1752	2	(0.1)	25
Female	65+	1058	0		-

Table 17: Australian mean daily consumption of whey based frozen dessert by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming whey-based frozen dessert (% of number surveyed)		Mean amount of whey-based frozen dessert consumed per day (g)
Male	2 - 3	170	0		-
Male	4 - 7	416	0		-
Male	8 - 11	385	0		-
Male	12 - 15	349	0		-
Male	16 - 18	215	0		-
Male	19 - 24	485	0		-
Male	25 - 44	2140	0		-
Male	45 - 64	1554	0		-
Male	65+	902	0		-
Female	2 - 3	213	0		-
Female	4 - 7	383	0		-
Female	8 - 11	354	0		-
Female	12 - 15	304	0		-
Female	16 - 18	218	0		-
Female	19 - 24	575	1	(0.2)	133
Female	25 - 44	2385	0		-
Female	45 - 64	1752	2	(0.1)	154
Female	65+	1058	0		-

APPENDIX 5

Hazard identification / hazard characterisation of pathogens

5.1 *Aeromonas* spp.

Aeromonas spp. are ubiquitous and occur worldwide, but are most frequently isolated from treated and untreated water and animals associated with water such as fish and shellfish. They may be pathogenic to amphibians, reptiles and fish. Although not yet definitively proven, there is epidemiological and clinical evidence that implicates aeromonads as causes of foodborne illness in humans.

Aeromonas hydrophila[6] is a gram-negative, facultatively anaerobic, non-spore forming rod-shaped bacterium that is present in all freshwater environments and in estuarine environments. It is also found in a wide range of foods, including seafood products and shellfish, raw foods of animal origin (for example, poultry, ground meat, raw milk), and raw vegetables and salads (Kirov, 2003).

5.1.1 Growth characteristics

Aeromonads are psychrotrophic and grow rapidly at refrigeration temperatures. Temperature range for growth is 2–45°C, with an optimum range between 28°C and 35°C (ICMSF, 1996). The organism is heat-sensitive, being easily destroyed by pasteurisation and cooking (Kirov, 2003).

Growth is optimal in the presence of 1–2 per cent NaCl (a_w = 0.991–0.986) and has been found to be inhibited completely at a NaCl concentration of 6.0 per cent (a_w = 0.96) or pH 5.5 (ICMSF, 1996). Optimal pH for growth is in the range 6.5-7.5 although high pH, up to 8.8, can be tolerated (Kirov, 2003).

5.1.2 Pathology of illness

Aeromonas spp. cause a broad spectrum of infections in humans, usually in immunocompromised patients. While identified as waterborne pathogens, *Aeromonas* spp. have not been definitively implicated as a significant cause of foodborne illness. *A. hydrophila* may cause gastroenteritis in healthy individuals or septicaemia in individuals with impaired immune systems or various malignancies. Two distinct types of gastroenteritis have been associated with *A. hydrophila*: a cholera-like illness with a watery (rice water) diarrhoea; and a dysenteric illness characterised by loose stools containing blood and mucus.

Symptoms associated with *Aeromonas*-related gastroenteritis include diarrhoea, abdominal pain, nausea, chills and headache, dysentery-like illness and colitis. Symptoms usually occur within 24–48 hours of exposure and generally last from one to 7 days (Kirov, 2003). On rare occasions, the dysentery-like syndrome is severe and may last for several weeks (Anon 2003).

[6] While many authors use the name of *A. hydrophila* as a general term to include *A. sobria* and *A. caviae* as well as the main species of *A. hydrophila* (ICMSF, 1996), in this document, *A. hydrophila* refers to this species only, unless otherwise indicated.

5.1.3 Mode of transmission

Water is considered the main source of human aeromonad infections, with foodborne illness still not firmly established for this genus (Kirov, 2003).

5.1.4 Incidence of illness

To date, there have been only a few incidents implicating *A. hydrophila* in foodborne illness. Most cases of illness attributed to *A. hydrophila* have been sporadic, rather than associated with large outbreaks. A summary of known incidents is given in Table 1.

Table 1: Foodborne illness associated with *Aeromonas* species.

Location	No. of people involved	Suspect food	Reference
Russia	'mass' poisoning	Fish (pre-frozen)	(Kalina, 1997)
Hungary	'several cases	Soups, starchy broths	(Janossy and Tarjan, 1980)
Nigeria	1	Edible land snails (pre-frozen)	(Agbonlahor *et al*., 1982)
USA	472	Oysters	(Abeyta *et al*., 1986)
USA	7	Oysters	(Abeyta *et al*., 1986)
USA	29	unknown (school lunch)	(Kobayashi and Ohnaka, 1989)
Japan	4	Seafood (sashimi)	(Kobayashi and Ohnaka 1989)
Scotland	>20	Cooked prawns	(Todd *et al*., 1989)
England	3	Oysters	(Todd *et al.,* 1989)
England	14	Cooked prawns	(Todd *et al.,* 1989)
England	2	Cooked prawns	(Todd *et al.,* 1989)
Switzerland	1	Shrimp cocktail	(Altwegg *et al*., 1991)
USA	2	Egg salad	(Bottone, 1993)
Sweden	24	shrimps, smoked sausage, liver pate, ham	(Krovacek *et al*., 1995)
Norway	3	Raw fermented fish	(Granum *et al*., 1998)
France	10	Dried fish sauce	(Hansman *et al*., 2000)

Source: (Kirov, 2003).

Suspect foods have been principally prawns and oysters, or other foods consumed with little or no cooking. In only one case, which was linked to ready to eat shrimp cocktail, has the isolate from the suspect food and from the diarrhoeal faeces been shown to be the same by ribotyping (Kirov, 2003). Most recently, reported *Aeromonas*-associated outbreaks have occurred in Sweden, Norway and France (Krovacek *et al*., 1995; Granum *et al.,* 1998; Hansman *et al.,* 2000). They are, however, still insufficiently documented to definitively established *Aeromonas* spp. as the causative agents.

5.1.5 Occurrence in foods

Aeromonas spp. are ubiquitous throughout the environment (particularly fresh and marine waters) and have been isolated from a variety of foods, including vegetables, raw and pasteurised milk and dairy products, meat and seafood (Chen *et al*., 1988; Knochel and Jeppesen, 1990; Ibrahim and Macrae, 1991; Krovacek *et al*., 1992; Shin *et al*., 1993; Craven and Macauley, 1993; Walker and Brooks, 1993; Kirov *et al*., 1993b; Freitas *et al*., 1993b; Shankar *et al*., 1994; Pin *et al*., 1994; Schweizer *et al*., 1995; Santos *et al*., 1996; Penchev *et al*., 1996; Akan *et al*., 1996; Zahran and AlSaleh, 1997; Lindberg, 1997; Eneroth *et al*., 1998; Uraz and Citak, 1998; Borrell *et al*., 1998; Sarimehmetoglu *et al*., 1998; Mauro *et al*., 1999; Melas *et al*., 1999; Villari *et al*., 2000; Neyts *et al*., 2000; Effat *et al*., 2000; Ibrahim, 2001;

Bulhoes and Junior, 2002; Araujo *et al*., 2002; Grassi *et al*., 2002; Birkenhauer and Oliver, 2002; Aly and Abo-Al-Yazeed, 2003; Yucel and Citak, 2003; Gran *et al*., 2003; Alisarli, 2003).

1.1.6 Virulence and infectivity

Illness caused by aeromonads is thought to be mediated partly by production of several virulence factors including elastases, lipases, lipopolysaccharides, adhesins, flagellae and cytolytic enterotoxins (Wadstrom and Ljungh, 1991; Kirov and Brodribb, 1993; Freitas *et al*., 1993a; Kirov *et al*., 1993a; Kirov *et al.,* 1993b; Krovacek *et al.,* 1995; Handfield *et al*., 1996; Granum *et al.,* 1998; Chopra and Houston, 1999; Kingombe *et al*., 1999; Grassi *et al.,* 2002; Sen and Rodgers, 2004). Some virulence factors are optimally expressed at lower temperatures (Kirov, 2003).

5.1.7 Dose Response

The infectious dose of *A. hydrophila* is unknown. It is possible that illness can result from a low dose, as scuba divers who have ingested small amounts of water have become ill, and *A. hydrophila* has been isolated from their stools (Anon 2003). However, it is likely that illness requires $>10^6$ cfu, based on one human trial (Morgan *et al*., 1985) and limited data from suspected foodborne incidents (Kirov, 2003).

5.1.8 Host Factors

All people are believed to be susceptible to *Aeromonas*-related gastroenteritis, although it is most frequently observed in very young children. People with impaired immune systems or underlying malignancy are considered more susceptible to infection, which often results in more severe clinical symptoms compared with the general population (Anon 2003).

5.1.9 Food Matrix

Aeromonads are unlikely to grow at refrigerated storage temperatures in foods with pH below 6.0 and water phase salt higher than about 3.0-3.5% (*w/w*) (Kirov, 2003). Organic acids, nitrite and polyphosphates reduce the growth rate of aeromonads in foods (Palumbo and Buchanan, 1988; Kirov, 2003).

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5.2 *Bacillus cereus*

The genus *Bacillus* encompasses a great diversity of species and strains. *B. cereus* is a Gram-positive, facultatively aerobic spore-forming bacteria whose cells are 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.

5.2.1 Growth characteristics

Strains of *B. cereus* vary widely in their growth and survival characteristics. Psychrotrophic strains are able to grow at 4 – 5ºC but not at 30 – 35ºC, whereas mesophilic strains grow between 15ºC and 50 or 55ºC. The optimum growth temperature ranges from 30 – 40ºC. The minimum pH at which growth will occur is 5.0, maximum 8.8 and optimum 6.0-7.0 (ICMSF, 1996). 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 30-35ºC (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 $D_{85ºC}$ = 33.8-106 min in phosphate buffer; $D_{95ºC}$ = 1.5-36.2 min in distilled water and 1.8-19.1 min in milk (ICMSF, 1996). 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 (EDTA) and polyphosphates (Jenson and Moir, 2003).

Spores are more resistant to radiation than vegetative cells (Farkas, 1994).

5.2.2 Pathology of illness

There are two types of *B. cereus*-mediated intoxication. 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 56ºC (but not 45ºC for 30 min). 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 32ºC, one week at 4ºC and several weeks at –20ºC (Andersson, 1995).

The emetic toxin is preformed during 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 126ºC (ESR, 2001). It as also very resistant to pH and proteolysis, but is not antigenic.

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.

Illness caused by the ingestion of emetic toxin generally has a shorter incubation period. Acute nausea and vomiting often occur 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 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, 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 illness caused by this organism (Hui et al., 2001).

5.2.3 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).

5.2.4 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). 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 & 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 & 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, 1981; Pinegar & 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.

5.2.5 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 (Jenson and Moir, 2003). Rice is a well recognised source, with most samples

containing low levels of the organism (Jenson and Moir, 2003). Spices are also frequently 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 its widespread distribution. 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 $<10^3$/g and mostly $<10^2$/g (ICMFS, 1996).

The presence of *B. cereus* in processed foods results from contamination of raw materials and the subsequent spore resistance to heat treatment processes during manufacture.

5.2.6 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 (HBL), consists of three protein components (L2, L1, and B), and causes the destruction of red blood cells. The second enterotoxin, non-haemolytic enterotoxin (NHE), also consists of 3 protein moieties (B, L1 and L2) and all components are needed for maximum cytotoxicity. Both HBL and NHE 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) recently 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 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 is also unclear, but data suggest the peptide is enzymatically produced (Hui et al., 2001).

5.2.7 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 food containing less than 10^5 cfu/g (Kramer and Gilbert, 1989).

Becker et al. (1994) indicated that concentrations of *B. cereus* of 10^3 to 10^5/g can result in illness in infants or aged and infirm individuals, although such illness was rare

Granum and Lund (1997) reported that concentrations ranging from 200 to 10^9/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}organsims. 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, Granum and Lund (1997) suggest an average serving of food containing more than 10^3 *B. cereus*/g cannot be considered completely safe for consumption.

Rowan et al., (1997) and Notermans and Batt (1998) also suggest that the infectious dose for *B. cereus* may vary from about 1×10^5 to 1×10^8 viable cells or spores. 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.

5.2.8 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.

5.2.9 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, the $D_{121°C} = 30$ min) and in foods with lower water activity (Jenson and Moir, 2003).

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5.3 *Brucella* spp.

There are six species in the genus *Brucella*, these being *B. abortus* with eight biovars; *B. melitensis* with three biovars; *B. suis* with five biovars and *B. ovis*, *B. neotomae* and *B. canis* (Corbel 1984).

Brucellae are non-motile, short, Gram-negative coccoid to rod-shaped cells. Brucellae grow aerobically, but many strains of *B. abortus* and *B. ovis* require an atmosphere of increased carbon dioxide tension (5-10%) for primary isolation. They are catalase-negative and usually also oxidase negative (ICMSF 1996). *Brucella* species are pathogenic for humans and a wide range of animals. Brucellae are located intracellularly in infected animals (Tantillo *et al.* 2001).

Brucella abortus causes bovine brucellosis, a highly contagious disease. The dominant feature is late-term abortion and infertility in cattle. The disease is also a serious zoonosis, causing undulant fever in humans.

Until recently, bovine brucellosis was present throughout the world. However, a number of countries have now succeeded in eradicating this disease. These include Australia, Canada, Israel, Japan, Austria, Switzerland, Denmark, Finland, Norway, Sweden and New Zealand.

Australia has been free of bovine brucellosis since 1989. It was prevalent throughout Australia by the 1920s, particularly in dairy herds where it was a source of major economic loss and public health concern. Various regional control schemes were in operation from the 1930s, and a nationally coordinated brucellosis eradication program commenced in 1970 as a component of the brucellosis and tuberculosis eradication campaign (BTEC). Freedom from bovine brucellosis freedom was achieved progressively - Tasmania in 1975; Western Australia in 1985; the Australian Capital Territory, New South Wales, Victoria and South Australia in 1988; and Queensland and the Northern Territory in July 1989. Australia officially declared its freedom from bovine brucellosis to the Office International des Epizooties (OIE) in August 1989. There have been no recurrences of the disease since 1989 (Animal Health Australia, 2005a).

Brucella melitensis is a major cause of brucellosis in sheep and goats. The disease affects mainly adult female animals, causing abortion and udder infection. It is also a serious zoonosis and is more pathogenic to man than *Brucella abortus*.

B. melitensis infection has never been reported in sheep or goats in Australia. However, overseas travellers occasionally arrive in Australia suffering from *B. melitensis* infection, and they may travel widely in Australia. Since the organism is excreted in the urine of infected humans, infection of sheep and goats from this source is possible, although highly unlikely (Animal Health Australia, 2005b).

5.3.1 Growth and survival characteristics

Temperature

Whilst the optimum temperature for growth on artificial media is 37ºC, Brucellae can grow at temperatures between 20 and 42ºC (ICMSF 1996). There is some discrepancy in what time and temperatures are adequate to kill the bacteria. Encev (1965) found that heat treatment at 60ºC for 30 minutes or 70ºC for more than one minute was sufficient to kill *B. suis* (4×10^8

CFU/mL in glycerol-dextrose broth). However, a time/temperature combination of 75 minutes at 85ºC was necessary to kill all 40 tested strains of *B. abortus* 1, 2 and 3 in studies conducted by (Swarm *et al.* 1981).

Survival of *Brucella* in milk and milk products declines with increasing storage temperature. Brucellae at a concentration of 8×10^9 CFU/mL survived for 800 days at –40ºC, but were eliminated within 2 days at 25ºC (Kuzdas and Morse 1954). An increase in storage temperature from 2-4ºC to 18-22ºC reduced survival time by approximately 50% for *Brucella* in Egyptian white cheese of the Domiati and Tallaga variety (Salem *et al.* 1977). In addition, high fat content of products may have a protective effect. *B. abortus* survived in higher fat cheese types for 3-4 times longer than lower fat content cheeses held at the same temperature of between 2 and 4ºC (Salem *et al*. 1977).

ICMSF reports that most published data is presented as lethal or non-lethal time/temperature combinations, with the result that D- and z-values are not quoted. One reference (Stumbo 1973) reports Brucellae in milk as having a D-value at 65.5ºC of 0.10-0.20 minutes and a z-value between 4.4 and 5.6ºC. Kronenwett *et al.* (1954) experimentally determined the z-values for eight strains of *B. abortus* in milk with the range being 4.3 to 4.8 ºC.

Water activity
A NaCl content of >3% for *B. suis* or 4% for *B. melitensis* will prevent growth of *Brucella* spp on liver agar (Lerche *et al.* 1960).

The survival rate of *Brucella* appears to decrease with increased sodium chloride in milk products (ICMSF 1996 Table 2). A survival time of 6 months was reported for salted butter (2.3% NaCl), whereas in unsalted butter Brucellae remained viable for 13 months (ICMSF 1996). However, Brucellae may resist high salt concentrations at lower temperatures. A survival time of 45 days was reported for *Brucella* in a sheep cheese brine containing 27% salt and stored at a temperature of between 11 and 14ºC (ICMSF 1996).

Water content
There is a positive correlation between the survival of *B. abortus* in different cheeses and the water content of the cheeses (Kästli and Hausch 1957). Brucellae 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 Camenbert; water content 50%).

pH
The optimum pH for growth in artificial media for all *Brucella* spp. ranges between 6.6 and 7.4 at 37ºC (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 and 6.8, whilst Lerche and Entel (1959) reported a lower growth limit of between pH 4.1 and 4.5.

Kästli and Binz (1948) recorded a lower limit of pH of 5.3 for growth of *Brucella* in sterilised milk held at a temperature of 37ºC. As pH falls, the survival time of Brucellae in milk and milk products decreases (Lerche 1931; Kästli and Binz 1948). When stored at 38ºC and a pH of 5.0 survival of *B. abortus* is less than 24hr (Kästli and Binz 1948).

5.3.2 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 (Kasimoğlu 2002).

The signs and symptoms of foodborne illness associated with *B. abortus*, *B. melitensis* and *B. suis* include fever, chills, sweating, weakness, headache, muscle and joint pain, diarrhoea and bloody stools during the acute phase (CDC website). The incubation period ranges from seven to 21 days, with the duration of illness being in the order of weeks. Raw milk, goat cheese made from unpasteurised milk and contaminated meats are the foods most commonly associated with foodborne transmission. The laboratory testing utilised is blood culture and positive serology. Treatment is usually with a combination of tetracyclines, streptomycin and sulphonamides/trimethoprim (ICMSF 1996).

5.3.3 Mode of transmission

Zoonotic transmission from infected animals to humans may be either via direct or indirect transmission (Kasimoğlu 2002). Direct transmission occurs via close contact with infected animals and involves the respiratory, conjunctival and cutaneous routes. Airborne transmission of brucellea 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.

5.3.4 Incidence of illness

There have been several outbreaks of brucellosis in humans in various parts of the world. An outbreak in 1991 in Italy of brucellosis in humans involving a total of 60 cases was traced to consumption of fresh ewe's milk cheese, particularly ricotta. The majority of the cases (46) were from the town of Termoli. Patients of all ages were involved in the outbreak. A veterinary survey conducted at the same time indicated a high incidence of brucellosis in local sheep flocks (Montanaro *et al.* 1992).

Unpasteurised raw goat milk cheese contaminated with *B. melitensis* was implicated in an outbreak in Spain during 2002 which resulted in 11 cases of brucellosis (Méndez et al., 2003). Eckman (1975) also reported an outbreak of brucellosis in the US associated with consumption of unpasteurised raw goat milk cheese obtained from Mexico which contained *B. melitensis*.

A case of *Brucella* meningitis in Mexico in 1987 was due to consumption of milk and cheese made from unpasteurised goats milk. *B. melitensis* was identified in the blood and cerebrospinal fluid cultures from the patient (Challoner, Riley and Larsen 1990).

In Germany during 1995 there were 34 reported cases of brucellosis. consumption of raw milk cheese was implicated as the possible source of the infection in 14 of the cases (Rasch *et al.* 1997).

5.3.5 Occurrence in foods

Brucella is most commonly transmitted via raw milk or raw milk products, such as cheeses (Kasimoglu 2002). Ewes milk has been found to be a more significant source of *Brucella* spp than cows milk.

In a survey of 217 ice cream samples collected from small-scale producers in Turkey, *B. abortus* was isolated in 5 (6.25%) of the vanilla flavoured ice cream samples (Kuplulu and Sarimehmetoglu 2004). The levels of contamination ranged from 1.1×10^2 to 2.3×10^3 MPN/g.

The incidence of *Brucella* spp. was investigated in 289 samples of raw milk (265 from cows' milk and 24 of goats' milk), and in 335 samples of soft, white fresh cheese made in the Cajeme Sonora municipality of Mexico. Seven samples of milk and 25 of cheese samples were positive for *Brucella* spp. The species in the milk and cheese samples were identified as *B. abortus* and *B. melitensis* (Acedo, Diaz and Leon 1997).

A survey of 46 cheese samples produced from goats' and ewes' milk to detect *Brucella* spp. resulted in 46% of the samples being positive, in particular those cheeses made from ewe's milk (Tantillo *et al.* 2001).

Prevalence of animals infected with Brucella
53% of representative herd milk was found to react positively to the *Brucella* Milk Ring Test (BMRT) and 17% of herd milk provided to the Johannesburg, South Africa was found to contain viable *B. abortus* (Van Den Heever, Katz and Te Brugge 1982).

In a survey of 763 Sudanese camels from 400 herds, 16 of the 153 samples tested contained *Brucella abortus* agglutinating antibodies (Obied, Bagadi and Mukhtar 1996).

A survey of 6472 cows found that 397 were positive for *B. abortus.* In one herd alone, 30% of the cows with positive blood serum excreted *Brucella* in their milk (Ebadi, Ardalan and Zoughi 1981).

5.3.6 Virulence and infectivity

Brucella can infect and multiply in both phagocytic and nonphagocytic cells (Sarinas and hitkara, 2003). The exact mechanism of *Brucella* pathogenesis is not fully understood, with no specific cell components specifically promoting cell adhesion and invasion being characterised (Corbel, 1997).

5.3.7 Dose response

There are no quantitative data on the infective dose (ICMSF1996). 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).

5.3.8 Host factors

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

5.3.9 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 Brucellae during the production of hard cheeses, however, Brucellae may survive conditions during the production of other types of cheeses such as soft cheeses.

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5.4 *Campylobacter jejuni/coli*

Campylobacter cells are mostly slender, spirally curved rods, with a single polar flagellum at one or both ends of the cell, and typically motile with a characteristic rapid darting corkscrew-like mobility (Smibert, 1984; Vandamme, 2000). They are Gram-negative and non-spore forming bacteria. Their cells are 0.2-0.8 μm wide and 0.5 to 5 μm long. Campylobacters are classified under *Campylobacteraceae*, a bacterial family comprised of genera *Campylobacter*, *Arcobacter* and *Sulfurospirillum* (Vandamme, 2000).

Among the 16 species and six subspecies of *Campylobacter*, two are most commonly isolated from stool samples of human gastroenteritis (campynet[7]; (Vandamme, 2000). They are *Campylobacter jejuni* subspecies *jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*). *C. jejuni* accounts for approximately 95% of *Campylobacter* caused human gastroenteritis, and *C. coli* is responsible for approximately 3-4% of the human illness. Other species causing human gastroenteritis include *C. lari*, *C. upsaliensis*, *C. fetus* subsp. *fetus*, and subsp. *venerealis*, *C. hyointestinalis* subsp. *hyointestinalis*, *C. concisus*, *C. jejuni* subsp. *doylei*. All these species share a common feature, which is their ability to grow at, or tolerate 42^{o}C. As such, these pathogenic *Campylobacter* species are collectively referred to as thermophilic campylobacters. For the purpose of this risk evaluation, *C. jejuni* and *C. coli* are collectively referred as campylobacters in the following text.

Campylobacters are normal intestinal flora of young cattle, sheep, goats, dogs, rabbits, monkeys, cats, chickens, turkeys, ducks, seagulls, pigeons, blackbirds, starlings and sparrows (Smibert, 1984), pigs (Nielsen et al., 1997), and in blood and faecal material from humans with *Campylobacter* enteritis. They are also found in the reproductive organs and oral cavity of man and animals[8]. Healthy puppies and kittens, rodents, beetles and houseflies may also carry campylobacters (Hartnett et al., 2002). *C. jejuni* is predominantly associated with poultry and *C. coli* is found predominantly in pigs.

5.4.1 Growth characteristics

Most campylobacters possess oxidase, are able to utilise all the organic acids in the tricarboxylic acid cycle, and are capable of deamination of glutamate and aspartate. Campylobacters however do not catabolise carbohydrates, have no lipase or lecithinase activity and do not require serum or blood for growth. They do not hydrolyse gelatin, casein, starch or tyrosine, but can reduce nitrate to nitrite.

Campylobacters require microaerophilic conditions for growth and different degrees of oxygen tolerance (3 to 5%) exist among different species of *Campylobacter* (Forsythe, 2000). For optimal growth, campylobacters require microaerophilic condition with 5% oxygen and 2-10% carbon dioxide (Park, 2002; CFSAN[9]). Most *Campylobacter* strains do not grow in the presence of air, other than a few that occasionally 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).

[7] http://campynet.vetinst.dk/ accessed 05 May 2004.
[8] http://campynet.vetinst.dk/ accessed 05 May 2004.
[9] Centre for Food Safety and Applied Nutrition, US Food & Drug Administration. http://www.cfsan.fda.gov/~mow/chap4.html Accessed 02 April 2004.

Campylobacters grow optimally at 42-43^{o}C. *C. jejuni* can grow in the temperature range of 30-45^{o}C, in the pH range of 4.9-9.5 and at water activity above 0.990. At 32^{o}C, *C. jejuni* may double its biomass in approximately 6 hours (Forsythe, 2000). Campylobacters do not multiply at temperatures below 30^{o}C (Park, 2002), which means that the number of campylobacters in foods will not increase at normal room temperatures (20 – 25^{o}C). Although unable to grow below 30^{o}C, campylobacters remain metabolic active, are able to generate ATP, and are motile at temperatures as low as 4^{o}C (Park, 2002).

Although thermotolerant, campylobacters are sensitive to heat and readily inactivated by pasteurisation treatment or domestic cooking processes. Cooking at 55-60^{o}C for several minutes readily destroys campylobacters. The D value for *C. jejuni* at 50^{o}C is 0.88-1.63 minutes (Forsythe, 2000).

Low temperature treatment leads to inactivation of campylobacters. Zhao et al. (Zhao et al., 2003) reported that a short-term (72 hours) exposure to –20^{o}C and –30^{o}C can lead to 1.3 $\log_{10}$ and 1.8 $\log_{10}$ reduction of campylobacters on the surface of poultry meat respectively. A long-term (52 weeks) exposure to –20^{o}C and –80^{o}C can result in 4 $\log_{10}$ and 0.5 $\log_{10}$ reductions respectively. The study noted however that either short-term or long-term freezer storage could leave sufficient levels of campylobacter organisms on the surface of poultry meat to cause illness in humans if the initial contamination was high.

Other than temperature, a range of other environmental factors including desiccation, oxidation, and osmotic stress influences the survival of campylobacters.

- Campylobacters are highly sensitive to desiccation and do not survive well on dry surfaces (Fernandez, 1985).
- The microaerophilic nature of campylobacters means that these organisms are inherently sensitive to oxygen and its reduction substances (Park, 2002).
- Campylobacters are much less tolerant to osmotic stress than a number of other foodborne pathogenic bacteria. For example, campylobacters are not capable of multiplication in an environment where sodium chloride concentration is 2% or higher (Doyle et al., 1982).

Due to their sensitive nature to environmental conditions and inability of growth at temperatures below 30^{o}C and under air, the ability of campylobacters to multiply outside of an animal host is severely restricted. Campylobacters are not normally capable of multiplication in food during either processing or storage, although they have the ability to survive outside optimal growth conditions. (Park, 2002).

5.4.2 Pathology of illness

Both *C. jejuni* and *C. coli* cause fever and enteritis in human. *Campylobacter* enteritis is acute inflammatory diarrhoea with clinical signs similar to those of other acute bacterial infections of the intestinal tract, such as salmonellosis or shigellosis. Detecting campylobacters in the faeces is the only way to confirm the diagnosis.

Principal symptoms caused by campylobacters are diarrhoea, abdominal pain, fever, myalgia, headache, vomiting and blood in faeces with approximate mean frequencies of 84%, 79%, 50%, 42%, 41%, 15% and 15% respectively (Lastovica et al., 2000). Nausea is also a common symptom of *Campylobacter* infection.

The onset of symptoms is often abrupt with cramping abdominal pains quickly followed by diarrhoea. Mean incubation period of *Campylobacter* enteritis is approximately 3.2 days with a range of 18 hours to 8 days. A particular feature of *Campylobacter* 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 et al., 2000).

An Australian multi-centre case control study identified the following symptoms of *Campylobacter* infection (Table 1). The study suggests that approximately 13.3% of *Campylobacter* enteritis patients are hospitalised, and remained in hospital for 3 nights per person (median). This figure is similar to the estimation of FoodNet (13.2%)[10]. The study also indicates that 84% of people developed *Campylobacter* enteritis misses 5 days per person (median) from work/school/recreational/holiday activities(Hall, 2003)[11]).

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

Table 1: Clinical symptoms of *Campylobacter* infection[12]

Symptoms	**% (case number 881)**
Diarrhoea	100
Stomach cramps	88
Fever	72
Nausea	70
Muscle/body aches	66
Headache	63
Vomiting	35
Blood in stool	34

Other than *C. jejuni* and *C. coli*, *C. fetus* subsp. *fetus* has been found in cases of human diarrhoea, septicaemia, abortion and meningitis. *C. hyointestinalis* subsp. *hyointestinalis*, *C. lari*, *C. concisus*, *C. jejuni* subsp. *doylei* have been found in association with human enteritis. *C. fetus* subsp. *venerealis*, *C. hyointestinalis* subsp. *hyointestinalis*, *C. lari, C. consisus and C. jejuni* subsp. *doylei* have been found in association with human septicaemia (Lastovica et al., 2000). *C. upsalienesis* has been isolated from cases of human diarrhoea, septicaemia, spontaneous abortion and haemolytic-uremic syndrome. A number of *Campylobacter* species such as *C. concisus*, *C. curvus*, *C. rectus*, *C. showae* and *C. sputorum* occur in human oral cavity causing periodontal diseases[13].

5.4.3 Mode of transmission

In ascertaining transmission of *Campylobacter* cases in the USA, Friedmann et al., examined data from 111 outbreaks of *Campylobacter* enteritis due to food or water occurred in the

[10] http://www.cdc.gov/foodnet/annual/2002/2002executive_summary.pdf accessed 7 July 2004
[11] Personal communication (Russell Stafford, July 2004)
[12] Personal communication (Russell Stafford, July 2004)
[13] http://campynet.vetinst.dk/ accessed 05 May 2004.

period of 1978 to 1996. Other than unknown foods, milk and water are the most common vehicles associated with transmission of *Campylobacter* (Table 2). Raw (unpasteurised) milk is largely responsible for dairy-related transmission. Of four milk-borne outbreaks of *Campylobacter* enteritis in the period of 1990 to 1992, three were caused by raw cows' milk and raw goats' milk[14].

Surveys in other developed economies, including the United Kingdom, Sweden, Germany, New Zealand, Denmark, US and Norway, indicate milk is the most frequent cause of foodborne *Campylobacter* infection (Friedman et al., 2000). Outbreak data of foodborne campylobacteriosis recorded in Australia (Table 3) between 1992 and 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 accounts for approximately 80% of milk-borne Campylobacter outbreaks.

Table 2: Transmission vehicles for *Campylobacter* enteritis (US, 1978-1996)[15]

Vehicle	Proportion of total outbreaks
Unknown food	38%
Milk	27%
Water (community and others)	11%
Multiple food	9%
Fruits	4%
Poultry meat (chicken and turkey)	3%
Other meat	2%
Beef	1%
Eggs	1%
Other foods	4%
Total	100%

Table 3: Reported outbreaks of *Campylobacter* enteritis in Australia

Year	Number falling ill	Vehicle	Location	Reference
2001	10	A number of foods*	Restaurant	(Raupach et al., 2003)
2001	3	Chicken kebabs	Takeaway food premise	NRVP[16]
2000	12	Milk (raw)	Farm – retail dairy	(Sumner, 2002)
2000	3	Chicken kebabs	Takeaway food premise	(Anonymous, 2000)
2000	~25	Milk (raw)	Farm – school camp	(Anonymous, 2001)
1999	16	Unknown	Caterer – function	NRVP
1998	9	Milk or water	Food caterer	NRVP#
1997	171	Chicken, beef salad	Food caterer – function	NRVP
1996	40	Unknown	Residential college	(Liddle, 1997)
1995	78	Cucumber salad	Catering facility – camp	(Kirk et al., 1997)
1993	21	Milk (raw)	Church caterer – camp	(Watson et al., 1993)
1992	4	Milk (raw)	Prison	(Bates et al., 1992)

* Most likely foods were a chicken dish, spring rolls and fried rice.
# *Clostridium perfringens* was part of the cause of foodborne outbreak.
^ Other than *Campylobacter*, *Salmonella* Virchow PT 34 and *S.* Typhimurium PT 64 were involved.

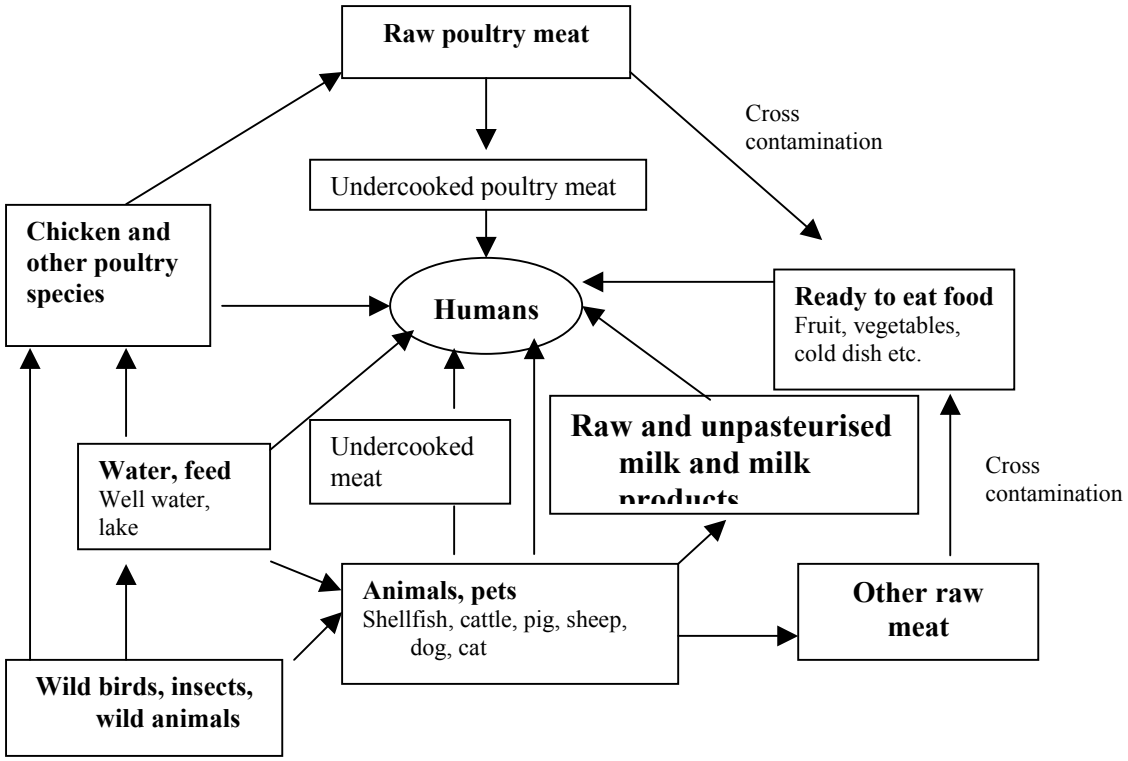
[14] http://www2.cdc.gov/ncidod/foodborne/OutbreaksReport.asp accessed 9 July 2004.
[15] Modified from Friedmann et al., 2000.
[16] NRVR for National risk validation report ((Food Science Australia & Minter Ellison Consulting, 2002)

Published information suggests that major routes of *Campylobacter* transmission to humans (Eberhart-Phillips et al., 1997; Friedman et al., 2000; Vellinga et al., 2002; World Health Organisation, 2000) 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.; and
- contact with infected domestic animals, such as a pet dog, cattle and bird.

These possible routes of transmission of *Campylobacter* spp. are summarised in Figure 1. In any case of the mentioned routes of transmission, *Campylobacter* infection is a result of oral ingestion of *Campylobacter* through food or water or animals, and faecal contamination is the common source of campylobacter transmission.

Figure 1. Possible route of transmission of *Campylobacter* spp. to humans[17]



5.4.4 Incidence of illness

Among different pathogenic bacteria associated with foodborne illness, campylobacters cause the highest number of human gastroenteritis cases in developed economies, such as Australia (Fig. 2), United Kingdom (Park, 2002) and the US (Mead et al., 1999). In the US,

[17] Adopted from Friedmann et al., (2000) and "Campylobacter" data sheet of Ministry of Health, New Zealand (May 2001).

approximately 80% of all the cases of human campylobacteriosis are foodborne (Mead et al., 1999). Campylobacteriosis accounts for approximately 2 to 2.4 million cases of foodborne illness annually in the US (Friedman et al., 2000; Mead et al., 1999). *Campylobacter* caused foodborne illness accounts for approximately 47% of all the foodborne illnesses caused by bacteria associated with food, some 29% of hospitalisation and about 8% of death due to foodborne illness caused by bacteria.

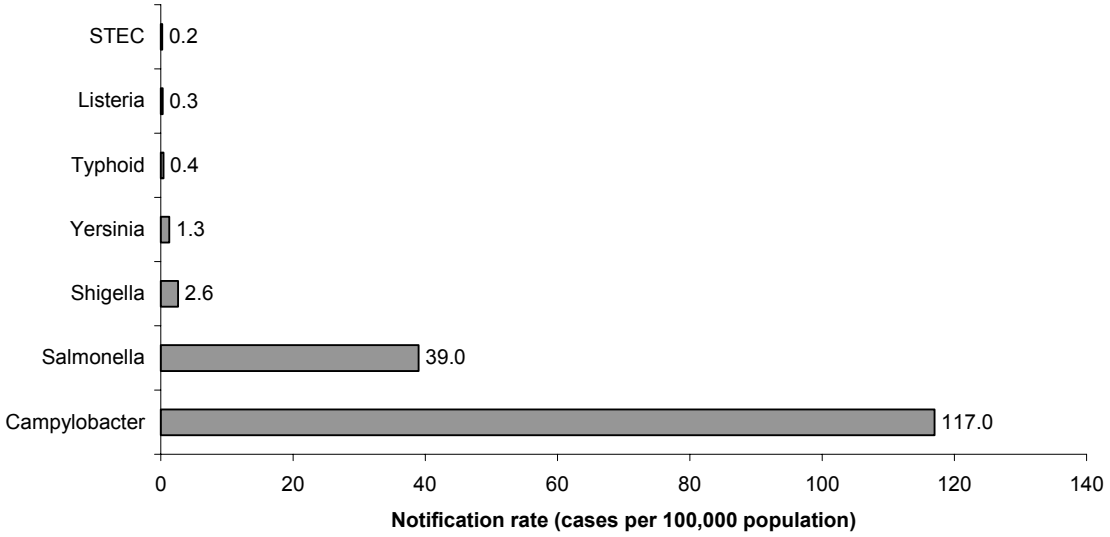


Figure 2. Notification rate for infections in Australia, 2004 (OzFoodNet, 2005)

From 2000 onwards, the notification rate of campylobacteriosis has been 100 – 120 cases per 100,000 population (Fig. 3).

Applying a multiplier of 15 (Food Science Australia & Minter Ellison Consulting, 2002) to account for underreporting of foodborne illness, the total number of campylobacteriosis cases would be at approximately 333,600 per annum in Australia. Assuming 80% of the total number of campylobacteriosis cases is transmitted by food (Food Science Australia & Minter Ellison Consulting, 2002; Mead et al., 1999), the number of foodborne campylobacteriosis cases in Australia is approximately 266,880 per year[18]. This figure is comparable to 247,351 cases of foodborne campylobacteriosis cases estimated by the Australian national risk validation project.

Although *Campylobacter* leads bacterial pathogens in causing the highest number of foodborne illness in developed economies, it is a low profile pathogen because most patients infected by campylobacters recover without treatment and large outbreaks of campylobacteriosis case are uncommon[19].

The trend of nationwide notified cases of *Campylobacter* enteritis (Fig. 4) suggests that the extent of campylobacteriosis as a communicable disease in Australia is yet to show clear signs of abating.

[18] This estimate is influenced strongly by the use of underreporting multiplier of 15. The underreporting multiplier may vary from 7.6 to 100 as suggested by the Preliminary Report of "Hazard identification, hazard characterization and exposure assessment of *Campylobacter* spp. in broiler chickens" prepared by the Joint FAO/WHO activities on risk assessment of microbiological hazards in foods (2001)

[19] http://www.fda.gov/fdac/features/1999/599_bug.html, accessed 07 July 2004

Figure 3: Notification rate for Campylobacteriosis in Australia (cases per 100,000)[20]

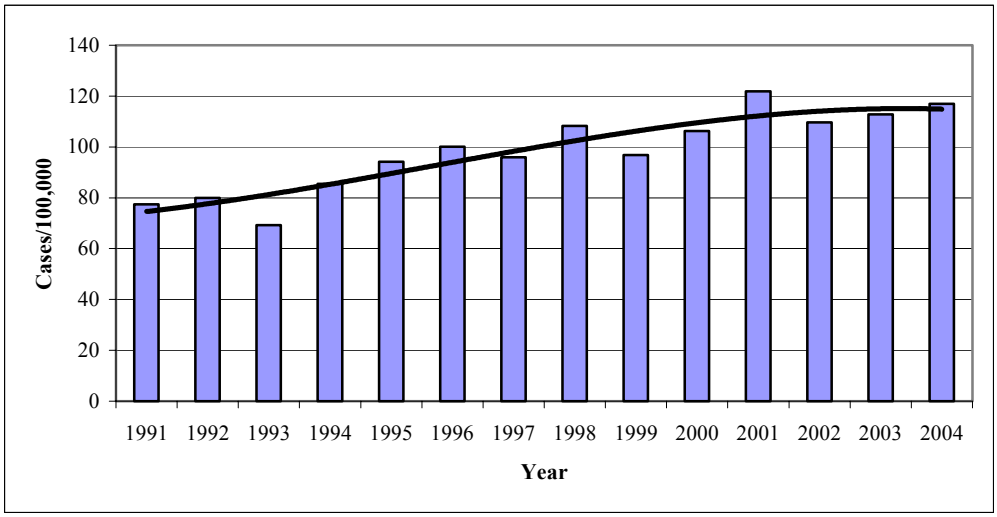
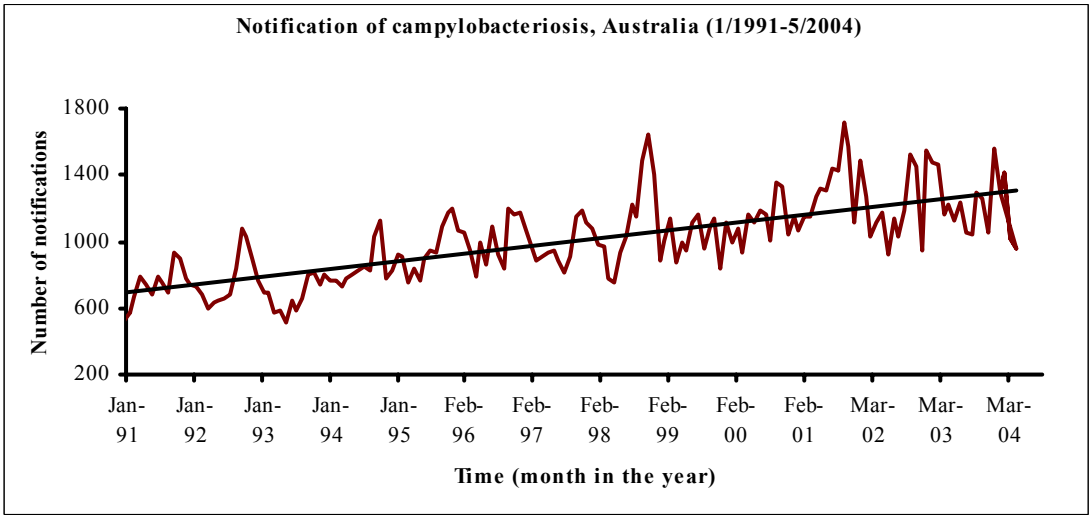


Figure 4 Annual cases of notified Campylobacteriosis in Australia[21]



There have been no reported cases of campylobacteriosis associated with the consumption of pastuerised milk or from dairy products made with pasteurised milk in Australia. A number of outbreaks of campylobacteriosis have been reported in the UK that were associated with consumption of raw milk or improperly pasteurised milk (Table x).

Table x. Outbreaks of campylobacteriosis resulting from consumption of unpasteurised and improperly pasteurised milk in the United Kingdom in 1987-1989 (Sockett, 1991) and 1992-1996(Djuretic et al., 1997).

	Number of outbreaks	Total number of cases

[20] National Notified Diseases Surveillance System. http://www1.health.gov.au/cda/Source/CDA-index.cfm Accessed 05 July 2004. Data presented does not include the State of New South Wales where campylobacteriosis is not reported separately. Population for the year 2003 is based on the estimation of Australian Bureau of Statistics.

[21] National Notified Diseases Surveillance System. http://www1.health.gov.au/cda/Source/CDA-index.cfm Accessed 05 July 2004. Data presented does not include the State of New South Wales where campylobacteriosis is not reported separately.

Unpasteurised milk	11	484
Improperly pasteurised milk	4	650

5.4.5 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 (Institute of Food Technologists, 2002).

5.4.6 Virulence and infectivity of campylobacters

The pathogenic mechanisms of *Campylobacter* causing human illness have not been fully elucidated. Two possible mechanisms are reflected in the literature. One is the genetic heterogeneity, *i.e.* different strains of *Campylobacter* may possess different ability to cause diseases (Park, 2002). The other is the involvement of microbial toxins in causing diseases. Published information indicates *Campylobacter* infection may involve production of microbial toxins. An enterotoxin[22] (Wassenaar, 1997), abbreviated, as CJT for *C. jejuni* toxin, is immunologically similar to the *Vibrio cholerae* toxin and the *E. coli* heat-liable toxin. At least six cytotoxins[23] have been observed in campylobacters. They are a 70-kDa cytotoxin, a Vero/HeLa[24] 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, 2000). Active CDT toxin has been found in roughly 40% of the over 700 *Campylobacter* strains tested (Johnson & Lior, 1988). However, the role of enterotoxin and the cytotoxins in *Campylobacter* pathogenesis has not been fully identified.

5.4.7 Dose Response

Campylobacter infection has been induced with a minimum dose of 800 cells in an experimental human feeding trial (Black et al., 1988). Taking into consideration the limited data in the human feeding trial and an infection rate of 50% resulting from the minimum dose, it has been proposed that the lowest infective dose would be somewhere close to 100 cells (Tribble[25]). This prediction is comparable with epidemiological data of campylobacteriosis where the number of milk-borne and waterborne outbreaks of *Campylobacter* enteritis is high.

Based on the human trail data (Black et al., 1988), dose-response relationships discussed or established in various risk assessments of *Campylobacter* in poultry meat (Teunis et al., 1996, Hartnett et al., 2002, Rosenquist et al., 2003) came to a conclusion that (1) a single pathogen cell has the ability to initiate an infection and (2) the probability of causing infection increases as the level of the pathogen increases. Such dose-response relationship differs to

[22] Enterotoxins are defined as secreted proteins with a capacity to bind to a cellular receptor, enter the cell and elevate intracellular cyclic AMP levels.

[23] Cytotoxins are defined as proteins that kill target cells. Cytotoxinis can act intracellularly or form pores in the cells.

[24] Vero cells refer to African green monkey kidney cells and HeLa cells are human tumour epithelial cells used in cell toxicological studies.

[25] Tribble D (1998) Suitability of experimental infections in volunteers to measure pathogenesis of foodborne pathogens. http://www.foodriskclearinghouse.umd.edu/Aug1988/Talks/tribbletalk.htm Accessed 11 February 2004

some degree from the traditional dose-response relationship where an infection/illness is not established until a minimum dose is ingested.

Dose-response relationships have been developed based on results from human feeding studies, whereby human volunteers are fed known numbers of Campylobacter cells and then monitored for their response (Black et al., 1988). These models make the assumption that (1) a single pathogen 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* infection correlates proportionally to the dose ingested and gradually reaches saturation. For example, when the dose ingested increased from 3.9 log to 5.9 log (a 100 fold increase in cell numbers), *Campylobacter* infection[26] increased correspondingly by a rate of 13%[27]. 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 Group 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 that the probability of illness is 20% to 50% after the establishment of an infection by campylobacters.

5.4.8 Immune status

The incidence of *Campylobacter* 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* infections resulted in bacteraemia in these immuno-compromised patients, a rate much higher than those occurring in the general population.

Literature data suggest that people with existing diseases have a higher susceptibility to campylobacteriosis than the general population. Pigrau et al., (1997) demonstrated in a study involving 58 patients with bacteraemia resulting from *Campylobacter* infections, 54 of the patients had existing diseases including human immunodeficiency virus infection, immunosuppressive therapy, liver cirrhosis and neoplasia.

Available data suggests that young children under the age of four and young adults in the age range of 20 to 30 years old are most susceptible to *Campylobacter* infection. Population groups that are very young (0-4 years), and that have an existing immuno-suppressed condition due to another serious disease, are likely to suffer more severe consequences as a result of *Campylobacter* infection.

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[26] Infection is determined by positive detection of campylobacters in stool sample(Black et al., 1988).
I[27] illness is determined by signs of diarrhea or fever(Black et al., 1988)

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5.5 *Clostridium* spp.

Most of the clostridia are saprophytes, and only a few species are pathogenic for humans. Those that are pathogens also have primarily a saprophytic existence in nature and, in a sense, are opportunistic pathogens. For the purposes of this assessment, *Clostridium botulinum* and *Clostridium perfringens* are considered to be the principal species likely to be transmitted to humans by the foodborne route. Less common pathogenic species, such as *C. difficile*, will not be explicitly considered. *Clostridium tyrobutyricum*, a late-blowing agent in high pH and semi-hard cheeses (Klijn *et al*., 1995; Ingham *et al*., 1998; Rilla *et al*., 2003), is a food spoilage organism and is considered non-pathogenic to humans.

C. botulinum is an anaerobic, gram positive, spore-forming rod shaped bacterium that produces a potent neurotoxin. Seven types of *C. botulinum*, (types A-G) are recognised, grouped according to the antigenic specificities of their toxins (Szabo and Gibson, 1997). *C. botulinum* has also been classified phenotypically into Groups I-IV, with foodborne botulism mainly associated with Group I (proteolytic) or Group II (non-proteolytic) strains (Szabo and Gibson, 1997). The organism is ubiquitous and is found in almost all foods, whether of plant or animal origin. Spores of *C. botulinum*, although usually in low numbers, are widely distributed in soil, the sediments of lakes and coastal waters and in the intestinal tracts of fish and animals. It can cause illness in cattle (Cobb *et al*., 2002).

C. perfringens is an anaerobic (microaerophilic) Gram-positive bacillus with a square-ended rod morphology and oval central or sub-terminal spores. It is widely distributed in soil and vegetation and is part of the normal intestinal flora of humans and animals (Labbe, 1989).

C. perfringens is grouped into five types (A - E) according to the particular soluble antigens (exotoxins) produced (Labbe, 1989). Only types A, C and D are human pathogens, 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; CPE) associated with the acute abdominal pain, nausea and diarrhoea of *C. perfringens* food poisoning.

5.5.1 Growth characteristics

<u>*C. botulinum*</u>
C. botulinum requires anaerobic conditions for growth. Both the spores and the toxins of *C. botulinum* are tolerant of freezing, while toxin is destroyed rapidly at temperatures of 75–80°C (ICMSF, 1996). All strains of *C. botulinum* produce toxin to about pH 5.2 under optimal conditions (ICMSF, 1996).

Group I (proteolytic) *C. botulinum* strains have a temperature range for growth of about 10-50°C, with an optimal range of 35-40°C. Toxin production is very slow below about 12°C (ICMSF, 1996). Group I spores are the most heat-resistant of all *C. botulinum* spores and this property led to the development of the botulinum cook or '12D process' for low-acid canned foods.

Strains of Group I will not grow below pH 4.6 or if the water phase NaCl concentration exceeds 10 per cent (a_w = 0.935). Toxin production is very slow below an a_w of 0.95 (ICMSF, 1996).

Group II strains such as *C. botulinum* type E are capable of growth and toxin production at refrigeration temperatures (≥ 3.3°C) (ICMSF, 1996) but generally need weeks of growth to produce sufficient toxin to cause foodborne illness (Lyon and Reddmann, 2000). Optimum temperature for growth is 28-30°C.

Strains of Group II will not grow below pH 5.0 or if the NaCl concentration exceeds 5 per cent (a_w = 0.97) in the water phase (ICMSF, 1996).

<u>*C. perfringens*</u>
C. perfringens also requires anaerobic conditions for growth. Cells of *C. perfringens* will grow between 12°C and 50°C, with an optimum temperature of 43-45°C (Solberg and Elkind, 1970; Labbe, 1989). The organism is capable of rapid growth. Generation times as short as 7.1 min at 41°C were reported in a study of a number of strains having an average generation time of 13 min 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 to 7.0, with growth inhibited below pH 5.5 and cell death occurring slowly below pH 5.0 (Bates and Bodnaruk, 2003). Growth is also inhibited below an a_w of 0.93.

In general, conditions for sporulation are more limited than for growth. The optimal temperature range is 35 - 40°C, and good sporulation can be obtained between pH 6.0 and 8.0 (Labbe and Duncan, 1974). The a_w must be above 0.98 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.

5.5.2 Pathology of illness

<u>*C. botulinum*</u>
Illness caused by *C. botulinum* can be of three types: foodborne, so-called "infant", and wound botulism (Anon 2003). Foodborne botulism is caused by ingestion of preformed toxin. The mortality rate depends on the type of *C. botulinum* toxin ingested. Infant botulism affects infants under the age of 12 months and results from the ingestion of spores, which release cells that subsequently [D1]colonise the alimentary tract and produce toxin. Wound botulism occurs after infection of a wound with *C. botulinum* cells, or germination of spores within a wound, followed by toxin production.

C. botulinum neurotoxin causes muscle paralysis, beginning in the upper body and progressing downward, paralysing the chest muscles, eventually leading to asphyxiation and death. Mortality rates are still quite high, in the range 5-15% in most countries but up to 40% in some (Hauschild, 1989).

Onset of symptoms in foodborne botulism is usually 18–36 hours after ingestion of the food containing the toxin, although cases have varied from 4 hours to 8 days. Early signs of intoxication consist of marked lassitude, weakness and vertigo, usually followed by double vision and progressive difficulty in speaking and swallowing. Difficulty in breathing, weakness of other muscles, abdominal distension and constipation may also be common symptoms (Anon 2003). All people are believed to be susceptible to the foodborne intoxication.

<u>*C. perfringens*</u>
Only the symptoms of *C. perfringens* food poisoning and enteritis necroticans are described in this section. Symptoms of *C. perfringens* food poisoning include diarrhoea and abdominal cramps (sometimes severe), typically without fever. There is normally no vomiting, fever, 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.

Symptoms of enteritis necroticans 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. It can be fatal.

5.5.3 Mode of transmission

<u>*C. botulinum*</u>
Foodborne botulism, the ingestion of preformed toxin in foods, typically results from the presence of vegetative cells or spores of *C botulinum* in raw foods due to their presence in the growing environment or through contamination. Subsequent germination and/or growth and toxin production occur prior to ingestion of the food.

Infant botulism usually results from ingestion of spores, generally associated with the consumption of honey.

<u>*C. perfringens*</u>
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.

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 in cooked meat

and poultry dishes. Outgrowth of spores commonly occurs after the heat shock encountered during cooking, and is favoured in anaerobic microenvironments within the food. 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.

Type A strains also cause gas gangrene, a wound necrosis associated with poor hygiene which was widespread in troops in both world wars (Labbe, 1989).

5.5.4 Incidence of illness

C. botulinum

Botulism caused by consumption of commercial foods has been rare, with most cases involving improperly canned food (usually home-canned) and semi-preserved foods (Hauschild, 1992). Data for the years 1994-2003, inclusive, from the Foodborne Outbreak Response and Surveillance Unit of the United States Centers for Disease Control and Prevention indicate that this trend has continued (CDC 2003), with most outbreaks occurring in the private home setting and many involving home-canned and home-preserved foods.

However, outbreaks associated with commercial foods occur occasionally, *e.g.* a 1993 outbreak of type A botulism associated with a commercial cheese sauce (Townes *et al*., 1996) and a 2001 outbreak associated with inadequately refrigerated "chili" (Kalluri *et al*., 2003).

In Canada 61 outbreaks occurred in the period 1971–84, most (113/122) cases involving native peoples eating raw, parboiled or 'fermented' meats from marine mammals. Fermented salmon eggs or fish were responsible for 23 per cent of these outbreaks (Hauschild and Gauvreau, 1985). A similar pattern of illness occurs in Alaska.

In Italy, a 1996 outbreak of botulism affecting 8 people (1 death) was associated with consumption of a commercial cream cheese (mascarpone), either alone or as the (uncooked) ingredient of a dessert, tiramisu (Aureli *et al*., 2000).

In New Zealand, there have been two cases of illness (one death) due to botulism type A involving home-bottled fermented mussels and watercress, a traditional Maori food (Hauschild, 1992).

There have been no reported cases of foodborne botulism in Australia since national notification commenced in 1991 (Blumer *et al*., 2003). From 1942–83 there were five reported outbreaks of botulism in Australia (Hauschild, 1992), of which one (two cases) was linked to consumption of Australian canned tuna (Murrell, 1979).

C. perfringens

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.

A summary of the epidemiology of foodborne disease outbreaks in Australia from 1995 to 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 to 171. Meats were the food vehicles in 60% (18 of 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 2001-2002 OzFoodNet, Australia's enhanced foodborne disease surveillance network, catalogued a further 10 outbreaks of *C. perfringens* food poisoning involving 102 cases. Dairy products were not implicated in any of these outbreaks.

The US Centres for Disease Control and Prevention (CDC) listings of foodborne disease outbreaks for 1990 to 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 to 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 1 outbreak due to *C. perfringens* related to a vegetable product was identified in the period 1969 to 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 are few 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.

5.5.5 Occurrence in foods

C. botulinum

There is a large amount of data relating to the presence of *C. botulinum* in fish, and some data relating to the level and prevalence of spores in meat, meat products, fruit and vegetables (Dodds, 1992). The incidence and level of contamination of prepared fish in Europe and Asia appears to be much lower than that in North America, but fish from Scandinavia and the Caspian Sea appear to be exceptions (Dodds, 1992).

There is a small possibility that *C. botulinum* spores may be present in raw milk from infected animals (Cobb *et al.,* 2002; Bohnel *et al*., 2005). Silage is also a significant source of contamination of raw milk with *C. botulinum* spores (Giffel *et al*., 2002).

A survey of the presence of *C. botulinum* spores in 236 samples of infant foods (honey, dry cereal, non-fat dry milk, evaporated milk, canned formula, and canned baby food) in New York City found that none of the products was contaminated (Guilfoyle and Yager, 1983).

A survey prompted by the 1996 outbreak of botulism due to mascarpone in Italy found a high prevalence (32.5%; 331/1017) of *C. botulinum* spores in mascarpone, and 7 (0.8%) of the 878 samples produced at the plant involved in the outbreak also contained toxin type A. In addition, 2.7% of 260 other dairy products tested contained spores (Franciosa *et al*., 1999).

C. perfringens
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 (FSIS) of the United States 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 2004).

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 cfu per gram *C. perfringens*, and the remaining 42 samples had less than 5 log cfu per gram (Carlin *et al.,* 2000).

5.5.6 Virulence and infectivity

C. botulinum
While most of the virulence factors in *C. botulinum* are chromosomally encoded (Shukla and Sharma, 2005), toxin production is complex and strain-dependent, with phages and plasmids implicated in control mechanisms (Hauschild, 1989; ICMSF, 1996).

C. perfringens
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 (υ-toxin) and a collagenase (κ-toxin). The β-toxin is implicated as the necrotic factor in enteritis necroticans ('pigbel').

C. perfringens types A and C also produce an enterotoxin (*Clostridium perfringens* enterotoxin; CPE) associated with the acute abdominal pain, nausea and diarrhoea of *C. perfringens* food poisoning.

5.5.7 Dose Response

C. botulinum
A very small amount (a few nanograms) of botulinum toxin can cause illness (Anon 2003). As little as 0.1–1.0 µg of type A toxin has been found to cause death in humans (ICMSF, 1996).

C. perfringens
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 $5x10^9$ 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.

5.5.8 Host Factors

C. botulinum
All people are believed to be susceptible to botulinal foodborne intoxication. Infant botulism is typically seen in children under 6 months of age, although adults have also been known to suffer intestinal colonisation prior to intoxication (McCroskey *et al*., 1991; Szabo and Gibson, 1997).

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

5.5.9 Food Matrix

C. botulinum
Botulism is primarily a concern when processes are used to extend the shelf life of a food, such as canning and vacuum- or modified atmosphere-packing. If *C. botulinum* spores survive treatment processes prior to packaging, they have the ability to proliferate and produce toxin, especially if the food is subjected to temperature abuse. The anaerobic environment produced by the canning process may further encourage the outgrowth of spores.

C. perfringens
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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5.6 *Corynebacterium ulcerans*

Corynebacteria are non-motile, rod-shaped, Gram-positive bacteria related to the genus Actinomycetes. They do not form spores or branch, but may form irregular shaped, club-shaped or V-shaped arrangements in normal growth (TOTB 2002). They may be aerobic or facultatively anaerobic and are catalase-positive (Frassetto 2004).

The genus *Corynebacterium* comprises a diverse group of bacteria, including both animal and plant pathogens. Some *Cornyebacterium* are part of the normal flora of humans (TOTB 2002).

The genus is composed of the species *Corynebacterium diphtheriae,* the causal agent of the disease diphtheria and the best known and most widely studied of the corynebacteria (TOTB 2002), and the nondiphtheria corynebacteria, collectively referred to as diphtheroids. Nondiphtheria corynebacteria have recently been recognised as pathogenic, particularly in immunocompromised hosts (Frassetto 2004).

The nondiphtheria coryenbacterium include *C. ulcerans*, *C. pseudotuberculosis*, *C. pyogenes*, *C. haemolyticum*, *C. aquaticum*, *C. pseudodiphtheriticum* (also known as *C. hofmannii*), *C. urealyticum* (Group D2), Group E and *C. jeikeium* (Group JK) (Frassetto 2004).

C. ulcerans is found more commonly in cattle than in other animals and can carry the same bacteriophage that codes for toxin elaborated by toxigenic strains of *C. diphtheriae* (Anon. 1997). In dairy cattle, *C. ulcerans* is a cause of mastitis. Infected cows may produce milk containing *C. ulcerans* for many months or even years (Hedlund and Pohjanvirta 1989; Hommez et al., 1999).

5.6.1 Growth characteristics

Corynebacterium spp. are classified as thermoduric, psychrotrophic bacteria (Coghill 1982; Shin *et al.* 1993). Very little published information is available on the specific growth characteristics of *C. ulcerans*.

5.6.2 Pathology of illness

C. ulcerans, a cause of bovine mastitis (Hedlund and Pojmanvirta 1989; Watts 1988), was not a recognised species until 1995 (Riegel 1996). In humans, *C. ulcerans* causes a zoonotic infection similar to diphtheria. Usually the symptoms are milder than the illness caused by *C. diphtheria,* however some strains of *C. ulcerans* produce potent diphtheria toxin and may cause severe symptoms (Hatanaka *et al.* 2003; Kisely *et al.* 1994). In addition to the usual respiratory and pharyngitis symptoms, *C. ulcerans* may also causes skin infections (Frassetto 2004).

5.6.3 Mode of transmission

C. ulcerans infections have occurred in humans after drinking unpasteurised milk or after coming into contact with infected dairy animals or their waste (Barrett 1989; Bostock *et al.* 1984; Frassetto 2004). Cats with nasal discharge have also been found to have diphtheria-toxin-producing *C. ulcerans* and may transmit the bacteria to humans via scratches (Taylor *et al.* 2002; Hatanaka *et al.* 2003). Person-to-person transmission has not been reported (Anon. 1997), and in some cases of infection, the route may not be clear (Pers 1987).

5.6.4 Incidence of illness

Infections with the nondiphtheria corynebacteria have been reported throughout the world (Frassetto 2004). Two cases of poisoning due to *C. ulcerans* were reported in England and Wales in 1981. Both cases were attributed to consumption of raw milk (Anon 1982). A further two sporadic cases of *C. ulcerans* infections were reported in the UK in 1983 (Barrett 1986). Neither patient had signs of toxigenic diphtheria. One patient had been immunised as a child. Raw milk from a pet goat had been consumed prior to the infection in one case. In the other case, illness was associated with consumption of cows' milk that was thought to be improperly pasteurised due to a malfunction at the processing believed. Illness due to *C. ulcerans* has also been associated with consumption of unpasteurised milk in the US (Bostock *et al.* 1984).

Between 1993 and 1999, *C. ulcerans* caused five of the 10 cases of phyaryngeal diphtheria in the UK (CDR Weekly 2000). In January 2000, three apparently unrelated cases of infection with toxigenic *C. ulcerans* were identified in the Northern and Yorkshire NHS Region. All three cases lived in rural areas, denied exposure to raw milk and had not travelled overseas. The three cases spanned a wide age range. All cases had similar symptoms, presenting with sore throats. One case, an elderly woman, was admitted to hospital with pneumonia and a pharyngeal membrane. This case was fatal. The other two cases, a girl and a woman who worked at a riding school, did not require hospitalisation (Anon. 2000).

For a number of reported cases of illness associated with *C. ulcerans*, the vehicle or source could not be identified. For example, a case of respiratory diphtheria caused by a toxin-producing strain of *C. ulcerans* occurred in Terre Haute, Indiana in 1996. The patient did not report consumption of unpasteurised milk products or exposure to farm animals. The health authorities indicated that acquisition of the organism occurred locally in the state. In a case in Japan, a previously healthy 52-year-old woman was reported suffering illness due to *C. ulcerans* infection in 1991 (Hatanaka *et al.* 2003). Again, the patient did not report prior consumption of unpasteurised milk. The source of the infection was thought to be a scratch from a stray cat, which had rhinorrhea and sneezing. The cat died before the patient became ill. The cat was thought to have picked up the bacterium at one of the more than 10 dairy farms in the vicinity of the patient's home.

5.6.5 Occurrence in foods

Very few surveys have been conducted in recent times for the presence of *Corynebacterium spp.* in food. *Corynebacterium* spp. has been isolated from 0.5% of 200 raw milk samples taken from a bulk tanker in Korea (Shin *et al.* 1993). In a separate study, *Corynebacterium* spp. was the predominant species isolated from raw farm bulk tank milk collected from dairy farms in the Kyungii area of Korea during the period of July to December 1996, accounting for 28% of standard plate counts (Choi *et al.* 1998).

Corynebacterium spp. have been isolated also from raw camel milk produced in Riyadh, Saudi Arabia (Zahran and Al-Saleh 1997).

5.6.6 Virulence and infectivity

Virulence of *C. ulcerans* is primarily associated with the production of diphtheria-like toxins (Wong and Groman, 1984).

5.6.7 Dose response

There is a lack of information on the dose-response relationship for *C. ulcerans*.

5.6.8 Host factors

Immunocompromised hosts are more susceptible to infection with the nondiphtheria cornyebacteria, as are the very young and the elderly (Frassetto 2004).

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5.7 *Coxiella burnetii*

Coxiella burnetii is a gram-negative-like (non staining) species of rickettsia. The organisms are variously described as coccobacillus (Vanderlinde 2004b) or rod-like (Weiss and Moulder 1984) and are of the size 0.2-0.4μm by 0.4-1.0μm. Coxiella is distributed globally and causes the zoonotic illness Q fever (Vanderlinde 2004b). The usual animal reservoirs of *C. burnetii* are cattle, sheep and goats. Coxiella 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). Wild mammals and birds are also likely to be infected, however, isolations from these animals have been rare (Weiss and Moulder 1984). In Australia, several isolations have been made from the bandicoot, *Isodon torosus* (Derrick 1953). Other domesticated animals may also become infected, for example, sheep dogs (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.

5.7.1 Growth characteristics

C. burnetii is an obligate intracellular microorganism - it will not grow in foods or outside host cells. However, it is 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).

Coxiella has a high resistance to drying, elevated temperatures and chemical agents including many common disinfectants (Vanderlinde 2004b; Weiss and Moulder 1984). Complete inactivation may not be attained at 63ºC for 30 minutes, or at 85-90ºC for a few seconds (Weiss and Moulder 1984). Studies conducted by Enright et al. (1957) 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 62.7ºC for 30 minutes, but holding milk at 61.6ºC for the same period of time was insufficient to inactivate the organism. They strongly recommended pasteurisation at 72ºC for 15 seconds to be sure of complete elimination of viable *C. burnetii* from whole raw milk. It is also able to retain viability at 4ºC for one or more years in dried fomites such as tick faeces or wool.

5.7.2 Pathology of illness

Of those people infected with *C. burnetii*, only about half develop clinical signs of illness. 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, chest pain (Vanderlinde 2004b). If a fever is present, it may last 1 to 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% (Vanderlinde 2004b).

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 at 65% (Vanderlinde 2004b).

5.7.3 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 (Vanderlinde 2004b; Maurin and Raoult 1999).

5.7.4 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 2004b).

Infected herd animals do not usually exhibit clinical disease. Abortion in goats and sheep may occur in some instances of infection though. Organisms are excreted in milk, urine and faeces. High numbers of the organism are present in amniotic fluids and placenta during birthing.

The incidence rate of Q fever in France is estimated at 50 cases per 100,000 inhabitants per year (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-Dupont *et al.*1992). The majority (61%) of these cases presented with hepatitis, which is linked with oral exposure rather than aerosol exposure (Vanderlinde 2004a).

In 1985, five cases of hepatitis were reported from workers at a meat packing plant in California. Further investigation of the workforce found that 31of 42 persons tested were positive during serological testing for Q fever rickettsiae, with eight of these having recently experienced clinical symptoms of Q fever (MMWR 1986). 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 and 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 and 4.99 cases per 100,000 inhabitants for the period 1991-4, whilst the hospital morbidity data for 2001-02 indicates a case rate of 1.3 cases per 100,000 (Australian Institute of Health and Welfare).

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).

5.7.5 Occurrence in foods

C. burnetii 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. burnetii* in seroprevalence studies (Rousset *et al.* 2001), with *C. burnetii* 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 2004a).

Tests on milk samples from Uttar Pradesh, India found 18 of 260 cows' milk samples and 2 of 84 buffaloes' milk samples were positive for antibodies to *C. burnetii*. The organism was isolated from 1 of 4 pooled buffaloes' milk samples and 1 of 8 pooled cows' milk samples from the LRC Dairy in Nagla (Sethi *et al.* 1978).

Milk from 20 herds of dairy cows from Zaria, Nigeria were screened for *C. burnetii*, with 16 (80%) of the herds containing cows shedding the organism in their milk. Of 169 individual cows tested, 41 (24%) were shedders of *C. burnetii* (Adesiyun *et al.* 1985).

Of 1052 dairy cows from 22 premises across 17 Californian counties, 82% tested positive to serum agglutinating antibodies to rickettsia. 51% of 1634 cows had specific agglutinating antibodies in their whey and 23% of 840 cows were actively shedding *C. burnetii* (Biberstein *et al.* 1974).

5.7.6 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. 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 2004b).

5.7.7 Dose response

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

5.7.8 Host factors

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

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5.8 *Cryptosporidium*

Cryptosporidium is an intestinal protozoan parasite that induces 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 was discovered in 1907 but the first recognised case of human *Cryptosporidium* infection was in 1976 (Berkelman, 1994). During the early 1980's the population most at risk of infection were immunodeficient individuals, such as those suffering from HIV/AIDS. Cryptosporidosis, the disease caused by infection from *Cryptosporidium*, is a severe diarrhoeal disease. In immunocompetent individuals the disease is self-limiting, usually lasting less than ten days. It is often accompanied with abdominal pain, nausea, vomiting, general malaise and low -rade fever (Berkelman, 1994). For immunocompromised individuals, however, the disease can be prolonged and life threatening (Duffy and Moriarty, 2003).

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 *C. parvum*, however this species also infects and causes disease in a range of mammals, particularly cattle and sheep (Dawson, 2003).

5.8.1 Growth and survival characteristics

Cryptosporidium will not grow outside an animal host. *Cryptosporidium* oocysts appear to be sensitive to heat, loosing infectivity rapidly at >60°C (Rose, 1997). Standard high-temperature-short-time (HTST; 72°C/15 sec) 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 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% and 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 (Robertson et al., 1992).

A number of studies have demonstrated survival of *C. parvum* oocysts in different media (such as yoghurt) down to pH 4.0 (Deng and Cliver, 1999; Dawson et al., 2004).

5.8.2 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 grated fever and headache (Smith, 1993). The disease is usually self-limiting, with symptoms normally lasting for two to four days (FDA 2003). Severity and duration of symptoms is considered greater for immunocompromised individuals. In these susceptible populations, infection may extend to other organs including the lungs and the bile duct and is considered life threatening (Dawson, 2003).

5.8.3 Mode of transmission

Cryptosporidium is transmitted via the faecal-oral route. Person-to-person contact to 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.

5.8.4 Epidemiological data

Cryptopsoridiosis became a notifiable disease in Australia in 2001. A total of 3,255 (16.6 cases per 100,000 population) cases were notified to health authorities during 2002 (Yohannes et al., 2004). Children under the age of four have the highest cryptosprodiosis notification rate (129 cases per 100,000 population). This may reflect an increased susceptibility of children to *Cryptosporidium* 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* 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* 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 (Anon, 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 consumption of unpasteurised milk during school camp was cause of infection.

A cryptosporidiosis outbreak (n = 48) 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* oocysts.

5.8.5 Occurrence in foods

Food may be contaminated via a number of sources such as direct contact with faecal material during production (eg slaughtering or during milking), exposure to contaminated water or exposure via infected food handlers. Once contaminated, *C. parvum* oocysts can 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 to isolate oocysts from samples, methods of detection and viability assays.

5.8.6 Virulence and infectivity

Cryptosporidium is considered highly infective. Once ingested, oocysts excysts 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 reinfect 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.

5.8.7 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 (ID50) was determined mathematically to be 132 oocysts. At the lowest dose of 30 oocysts, a probability of infection of 20% was observed.

When data was fitted with an exponential model, the probability of infection is described by:

$$P_i = 1 - e^{-rD}$$

where,

P_i = Probability of infection
r = 0.004005
D = dose ingested

5.8.8 Host factors

Severity and duration of cryptosporidiosis is generally more severe in immunocompromised individuals, including children under five. For example, it is estimated that approximately 1% of the immunocompetent population may be hospitalised with very little risk of mortality, *Cryptosporidium* infections are associated with high rates of mortality in the immunocompromised population (Rose 1997).

5.8.9 Food Matrix

Survival data for *Cryptosporidium* 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* oocysts are not able to survive the ice-cream making processes, largely due to its sensitivity to low temperature (Deng and Cliver

1999). Ooycsts inoculated into milk have been found to survive the yoghurt-making process (Deng and Cliver 1999).

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5.9 Enterobacter sakazakii

Enterobacter sakazakii is a gram-negative bacterium belonging to the Enterobacteriaceae family. This family contains a number of species of bacteria that are commonly found in the human and animal gut, and in the environment (Lehner and Stephan 2004). *E. sakazakii* are included within the coliform group of bacteria

E. sakazakii is considered an opportunistic pathogen and has been associated with sporadic cases or small outbreaks of meningitis, sepsis, cerebritis and necrotizing enterocolitis, especially in neonates and infants (FAO/WHO, 2004).

5.9.1 Growth characteristics

E. sakazakii generally grows between 6 – 45°C, although there have been reports of some strains being able to grow at as low as 3.4°C and as high as 47°C (Lehner and Stephan, 2004; Nazarowec-White and Farber, 1997). *E. sakazakii* is considered more heat tolerant than many other Enterobacteriacea, however, it is rapidly inactivated at temperatures obtained during HTST pasteurisation. Reported thermal inactivation rates for *E. sakazakii* vary between studies. Nazarowec-White and Farber (1997) calculated a $D_{72°C}$ of 1.3 seconds when heated in infant formula, whereas Iversen et al. (2004) calculated a $D_{71°C}$ of 0.7 seconds in infant formula.

Breeuwer et al. (2003) demonstrated that *E. sakazakii* cells are particularly tolerant to desiccation, which may provide a competitive advantage in dry environments such as those found in dried milk powder. It has also been demonstrated that *E. sakazakii* has the ability of forming biofilms on a range of surfaces which may act as a reservoir of infection (Iversen et al., 2004).

5.9.2 Pathology of illness

E. sakazakii has been implicated in cases of meningitis and enteritis. Urmenyi and Franklin (1961) reported the first two known cases of neonatal meningitis caused by *E. sakazakii* infection in 1961. Although the frequency of cases of *E. sakazakii* is low, it is the severity of the illness that is of concern, with neonates and infants being particularly affected by this organism. Neonatal meningitis can result in ventriculitis, brain abscess or cyst formation and development of hydrocephalus. The reported fatality rate for neonatal infections has been reported to be as high as 50%, with over half the reported patients dying within one week of diagnosis. All neonatal patients that recover from the central nervous system infection suffer mental and physical delays (Lehner and Stephan 2004).

Another clinical manifestation of infection with *E. sakazakii* is the development of neonatal necrotising enterocolitis (NEC) following consumption of re-constituted contaminated powdered infant formula. This disease is characterised by intestinal necrosis and pneumatosis intestinalis. It is the most common gastrointestinal emergency in newborns (Lehner and Stephan 2004).

E. sakazakii has been isolated from clinical sites such as cerebrospinal fluid, blood, sputum, lower and upper respiratory tracts, digestive tract, superficial wounds and urine of infected individuals (Lehner and Stephan 2004).

5.9.3 Incidence of illness

E. sakazakii is not a notifiable disease in Australia, and some cases of infection may go undetected due to the difficulties of identifying the organism in the laboratory. The frequency of disease in infants appears to be low, with 2 cases over a 14-year period being recorded by the Victorian Hospital Pathogen Surveillance Scheme.

In July 2004, a premature baby in New Zealand developed meningitis due to *E. sakazakii* infection and subsequently died. The source of the organism was attributed to mishandling of contaminated powdered infant formula.

An outbreak of necrotising enterocolitis occurred in an intensive care unit in a hospital in Belgium in 1998, with 12 neonates contracting the disease between June and July that year (Van Acker *et al.*, 2001). A significant correlation was found between the development of NEC, the consumption of reconstituted powdered infant formula from a specific manufacturer, and the isolation of *E. sakazakii* in neonates.

In a review of *E. sakazakii*-induced illness in infants in the United States between 1961 and 2003 there were 48 reported cases (Lehner and Stephan, 2004). During 2001, a number of *E. sakazakii* infections were associated with consumption of reconstituted powdered infant formula in Tennessee, USA (Himelright et al., 2002). Of 49 infants screened for *E. sakazakii*, 10 were positive. Of these ten infants, one had a confirmed infection of cerebrospinal fluid (and died 9 days post infection), two had suspect tracheal infections and there were seven cases of infection identified by *E. sakazakii*-positive stool and/or urine samples.

5.9.4 Occurrence in foods

Although *E. sakazakii* has been isolated from a wide range of food commodities, most research has been undertaken on the presence of the organism in dried infant formula (Lehner and Stephan, 2004). Muytjens et al. (1988) analysed 141 powdered infant formulas and isolated *E. sakazakii* from 20 (14%) of them (limit of detection 1 cfu/100g). Iversen and Forsythe (2004) isolated *E. sakazakii* in two of 82 powdered infant formulae sampled. Nazarowec-White and Farber (1997) reported the prevalence of *E. sakazakii* in powdered infant formulae made from five different manufacturers to be 0 – 12%.

E. sakazakii has also been isolated from cheese, meat, vegetables, grains, herbs and spices and ultrahigh-temperature milk (Lehner and Stephan, 2004).

5.9.5 Virulence and infectivity

Although not fully understood, virulence of *E. sakazakii* has been associated with the ability to produce enterotoxin (Pagotto et al., 2003). Another key mechanism required for extraintestinal infection of *E. sakazakii* is thought to be the ability to penetrate the epithelial layer of the intestinal mucosa.

5.9.6 Dose response

There is no epidemiological or experimental data to develop an accurate dose-response relationship for *E. sakazakii* infections in humans. It is assumed that the ingestion of one *E. sakazakii* cell has the ability, albeit small, to cause illness in infants at risk. Using this assumption, R-values (the probability of one ingested organism causing illness) have been estimated between 8.9×10^{-6} to 2.5×10^{-6} (EFSA, 2004).

Reported levels of *E. sakazakii* present in samples of powdered infant formula associated with outbreaks are often low. Counts between 1 - 20 coliforms/g have been observed (EFSA).

5.9.7 Host factors

Neonates and infants have been particularly affected by this organism. The outcome related to adult disease seems to be significantly milder than that for children. There have only been a few reports of infections in adults, with most adult patients with *E. sakazakii* infection also having serious underlying diseases such as malignancies (Lehner and Stephan 2004).

5.9.8 Food matrix

Stationary-phase *E. sakazakii* is remarkably resistant to osmotic stress and desiccation and can therefore survive in dry environments such as those observed in dried milk powder (Breeuwer et al., 2003)

E. Sakazakii can grow in the reconstituted products if stored at temperatures above 5ºC for a sufficient time and multiply very rapidly at room temperatures.

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5.10 Pathogenic *Escherichia coli*

Escherichia coli (*E. 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, facultatively 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).

This assessment is primarily concerned with human pathogenic *E. coli*. Pathogenic *E. coli* are characterised into specific groups based on virulence properties, mechanisms of pathogenicity and clinical syndromes (Doyle et al., 1997). These groups include enteropathogenic *E. coli* (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* (SLTEC), and verocytotoxin-producing *E. coli* (VTEC).

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).

5.10.1 Growth and Survival

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 (a_w). 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, a_w and composition of the food (Bell and Kyriakides, 1998). Due largely to its importance as a cause of foodborne illness in the United States, most studies on the growth and/or survival of pathogenic *E. coli* have been undertaken with *E. coli* O157:H7 (an EHEC organism). Studies on the thermal sensitivity of *E. coli* O157:H7 have revealed that it is no more heat sensitive than *Salmonella* (Doyle and Schoeni, 1984). Therefore, heating a product to kill typical strains of *Salmonella* will also kill *E. coli* O157:H7.

Numbers of pathogenic *E. coli* O157:H7 have been shown to remain stable in ground beef stored at –20°C for over 9 months (Doyle and Schoeni 1984). In contrast, a 10-fold reduction of non-pathogenic *E. coli* has been observed in ground beef stored at –25.5°C for 38 weeks (ICMSF, 1996).

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; Large *et al*., 2005). 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 (a_w) 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 a_w required for growth increases (Desmarchelier and Fegan, 2003).

5.10.2 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 food and water-borne 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 from three to 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 per cent of haemorrhagic colitis victims may develop haemolytic ureamic syndrome (HUS) (European Commission, 2000). This involves the rupture of red blood cells (haemolysis), subsequent anaemia, low platelet count and kidney failure. The case-fatality rate of HUS has been reported to be 3–7 per cent (Codex Alimentarius Commission, 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 4.1 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

5.10.3 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 faecally contaminated water) and direct contact with infected animals.

5.10.4 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). More recently, contaminated parsley was implicated in two ETEC outbreaks in Minnesota, USA during 1998 (Naimi et al., 2003). The source of the contamination was believed to be inadequately chlorinated wash water used on-farm.

A large ETEC outbreak, affecting over 800 people, occurred in Japan during 1996. The outbreak occurred at four elementary schools and was associated with consumption of tuna paste (Mitsuda et al., 1998). Analysis of patient stool samples and samples of the implicated tuna paste confirmed *E. coli* O25:NM as the cause of illness.

EPEC stains have caused infantile diarrhoea in hospitals and nurseries in the United Kingdom and the United States (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). Outbreaks associated with consumption of contaminated cold pork and meat pies have been reported in Britain (Doyle and Padhye, 1989).

Since its identification as a human pathogen in 1982, and implication in a number of outbreaks in the United States, *E. coli* O157:H7 has become identified as the most predominant cause of EHEC related disease (WHO/FAO, 2002). It is estimated that 85% of EHEC infections in the United States are foodborne (Mead et al., 1999).

In the United States, consumption of undercooked hamburger meat has been an important cause of EHEC outbreaks (Nataro and Kaper 1998). 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 United States, 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 0157: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 WHO 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 (Ashbolt *et al*., 2002; OzFoodNet, 2003; OzFoodNet, 2004, OzFoodNet, 2005). *E. coli* O157 has been the most commonly reported serotype.

Significant variations in notifications exists 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 haemolytic uraemic syndrome (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).

5.10.5 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 have 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 have been isolated from Brazilian fish and shrimp which were harvested from waters contaminated with raw sewage (Teophilo et al., 2002). ETEC have also been detected in sauces at Mexican-style restaurants, and in chilli sauce sold by street vendors in Mexico (Adachi et al., 2002; Estrada-Garcia et al., 2002). In general, these sauces had been prepared and handled under poor hygienic conditions.

The major reservoir of EHEC organisms appears to be the intestinal tract of ruminants, in particular cattle and sheep (Desmarchelier and Fegan, 2003). *E. coli* O157:H7 and other EHEC species have been isolated from both healthy and diarrhoeic animals, and individual animals can carry more than one serotype (Anon, 1998). Foods derived from these animals may become contaminated via exposure to faecal material during processing.

Prevalence of STEC in raw milk has been determined in a limited number of studies (Table 4.2). 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).

Table 4.2 EHEC isolation rates from a variety of dairy products

Sample	Organisms Isolated	Country	No. Sampled	% Positive	Reference
Raw goat's milk	STEC	Switzerland	344	16	(Muehlherr *et al.*, 2003)
Raw ewe's milk	STEC	Switzerland	63	13	(Muehlherr *et al.,* 2003)
Raw cow's milk (bulk tank)	O157:H7	USA	268	0.75	(Murinda *et al.*, 2002)
Raw cow's milk	O157:H7	UK	329	0	(Mechie *et al.*, 1997)

5.10.6 Virulence and infectivity

Clinical, pathological and epidemiological characteristics of disease caused by pathogenic *E. coli* vary between pathotypes and is 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 & Levine 1983). EPEC cause characteristic attaching and effacing lesions in the intestine, similar to those produced by EHEC, but do not produce Shiga toxins. Attachment to the intestinal wall is mediated by a plasmid-encoded outer membrane protein called the EPEC Adherence Factor in type I EPEC. However, pathogenicity is not strictly correlated to the presence of the EPEC Adherence Factor, indicating that other virulence factors are involved (ICMSF, 1996).

ETEC that survive passage through the stomach adhere to mucosal cells of the proximal small intestine and produce a heat-labile toxin (LT) and/or a heat-stable toxin (ST). 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 LT or ST. 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 EHEC group of *E. coli* comprises a subset of Shiga toxin-producing *E. coli* (STEC). The Shiga toxins (Stx1 and Stx2) 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.

5.10.7 Dose response

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

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

EIEC: Volunteer studies have shown that 10^8 EIEC cells are necessary to cause illness in adults, with the infectious dose reduced to 10^6 when ingested with sodium bicarbonate (DuPont *et al.,* 1971). However, the United States Food and Drug Administration (FDA) 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*. (1996), 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*., 1999). 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).

5.10.8 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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5.11 *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 (USFDA CFSAN, 2004a). *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).

5.11.1 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., 1990). The limits and optima for key factors are summarised in Table 4.7.

Table 4.7 Growth conditions for *L. monocytogenes* (ANZFA unpublished)

	Minimum	Optimum	Maximum
Temperature (°C)	-1.5	37	45
pH	4.39	7.0	9.4
Water activity (a_w)	0.90	-	-

Under conditions outside the growth range, the bacteria may survive and growth may recommence once suitable conditions are encountered.

Temperatures of >50ºC are lethal to *L. monocytogenes*. When in a suitable medium, *L. monocytogenes* can grow between ~0-45°C. Although *L. monocytogenes* does not grow below –1.5ºC, it can readily survive at much lower temps. Nonetheless, freezing and frozen storage will cause a limited reduction in the viable population of *L. monocytogenes*. Optimal conditions for growth are between 30 and 37ºC (Ryser, 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 most bacterial species, *L. monocytogenes* grows optimally at a water activity (a_w) of approximately 0.97. However, when compared with most foodborne pathogens, the bacterium has the unique ability to multiply at a_w 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, 1999).

L. monocytogenes is reasonably tolerant to salt and can grow in NaCl concentrations up to 10% (European Commission, 2003). 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).

5.11.2 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 (USFDA Centre for Food Safety and Applied Nutrition, 2004a).

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 et al., 2003). The onset of illness is usually greater than 12 hours (Anon, 2004).

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 (USFDA CFSAN, 2004a). 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 to 3 week incubation time, but onset time may extend to 3 months (FDA et al., 2003).

It is estimated that approximately 2-6 percent of the healthy human population harbour *L. monocytogenes* in their intestinal tract, which suggests that people are frequently exposed to *L. monocytogenes* (Rocourt and Bille, 1997; Farber and Peterkin, 1991a). 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 health individuals;(Anon, 2004; Hitchins, 1996; Marth, 1988).

5.11.3 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.

5.11.4 Incidence of illness

Most cases of listeriosis are sporadic. The number of reported cases of invasive listeriosis in Australia between 2001 and 2004 varied between 61 – 72 cases (Ashbolt et al., 2002; Anon., 2003a, Anon., 2004a; Anon., 2005a), which equates to approximately 3 – 4 cases per million persons per year. In Australia, the exact mortality rate is not known, although the data available would suggest a rate of approximately 23%.

The reported incidence of invasive listeriosis in New Zealand over the period of 2002 - 2004 was between five – six cases per million (range of 19 – 26 cases per annum) of the general population per year (Anon., 2003b; Anon., 2004b; Anon., 2005b). The case fatality rate in New Zealand is approximately 17% (Anon, 2004).

Table 4.8 Outbreaks of listeriosis (US: 1970-2002; Outside US: 1970-2000) with known food vehicle(s) (FDA et al., 2003).

Year	Food Vehicle	Country	Cases	Deaths (% total)	Sero-type	Reference
Not Specified	Frozen vegetables	US	7	Unknown	4b	Simpson.D.M., 1996
1983-1987	Vacherin Mont d'Or cheese	Switzer-land	122	31 (25.4)	4b	Bille, 1990a; Bula et al., 1995
1987-1989	Pâté and meat spreads	England	355	94 (26.5)	4b	McLauchlin et al., 1991
1986-1987	Ice cream, salami, brie cheese	US	36	16 (44.4)	4b,1/2b, 1/2a	Schwartz et al., 1989
1986-1987	Raw eggs	US	2	Unknown	4b	Schwartz et al., 1989
1998-1999	Hot dogs, deli meats	US	101	21 (20.8)	4b	Mead, 2004
2000-2001	Homemade Mexican-style cheese (raw milk)	US	12	5 (41.7)	unknown	CDC, 2001
1978-1979	Vegetables (raw)	Australia	12	0 (0)	Unknown	Le Souëf and Walters, 1981
1989-1990	Semi-soft Cheese (blue)	Denmark	23	0 (0)	4b	Jensen et al., 1994
1994-1995	Smoked Seafood (finfish and molluscs)	Sweden	9	2 (22.2)	4b	Ericsson et al., 1997
1998-1999	Butter	Finland	25	6 (0)	3a	Lyytikainen et al., 2000
1999-2000	Pigs tongue in aspic	France	26	7 (0)	Unknown	Dorozynski, 2000
1979	Raw vegetables or cheese	US	20	3 (15.0)	4b	Ho et al., 1986
1980	Raw seafood (finfish and mollusks)	New Zealand	22	6 (27.3)	1b	Lennon et al., 1984
1981	Miscellaneous Dairy Products	England	11	5 (45.5)	1/2a	Ryser, 1999
1981	Vegetables (raw)	Canada	41	17 (41.5)	4b	Schlech, III et al., 1983
1983	Pasteurized fluid milk	US	32	14 (43.8)	4b	Fleming et al., 1985
1985	Mexican-style cheese (raw milk)	US	142	48 (33.8)	4b	Linnan et al., 1988
1986	Unpasteurised milk, organic vegetables	Austria	28	5 (17.9)	Unknown	Allerberger and Guggenbichler, 1989
1987	Butter	US	11	Unknown	Unknown	Ryser, 1999
1990	Pâté and meat spreads	Australia	11	6 (54.5)	1/2a	Ryser, 1999
1991	Smoked mussels	Australia	4	0 (0)	1/2a	Mitchell, 2001; Misrachi et al., 1991
1992	Smoked mussels	New Zealand	4	0 (0)	1/2	Brett et al., 1998
1992	Pork tongue in jelly	France	280	63 (22.5)	4b	Jacquet et al., 1995
1993	Rillettes	France	38	11 (28.9)	4b	Goulet et al., 1998
1995	Soft Ripened Cheese, >50% moisture (brie, feta, camembert, mozzarella)	France	33	4 (20.0)	4b	Jacquet et al., 1995; Goulet et al., 1995
1996	Cooked chicken	Australia	5	1 (20.0)		Sutherland, 2003Hall et al., 1996
1997	Pon l'Eveque cheese	France	14	0 (0)	4b	Ryser, 1999
1999	Pâté	US	11	unknown	1/2a	Carter, 2000
2000	Deli turkey meat	US	29	7 (24.1)	unknown	CDC, 2000
2002	Deli turkey meat, sliceable	US	63	7 (11.1)	unknown	CDC, 2002

The estimated incidence of invasive listeriosis in European countries has been reported to between 0.3-7.5 cases per million of the general population per year (European Commission, 2003). In France, the estimated incidence is sixteen cases per million (general population) per year (ICMSF, 1996; Bille, 1990b). The annual incidence of listeriosis in the United States has been estimated to range from 3.4 per million (Centers for Disease Control and Prevention, 2000) to 4.4 per million (Tappero et al., 1995). Of all foodborne pathogens, *L. monocytogenes* results in the highest hospitalisation rate in the United States, 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/FSIS, 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).

An outbreak of listeriosis associated with consumption of pre-cooked, diced chicken occurred in South Australia during 1996 (Hall et al., 1996). There were five confirmed cases of listeriosis, including one death. The majority of cases were patients of health care facilities. Between September 1997 and January 1999, nine cases of listeriosis (resulting in six deaths) were reported in the Hunter region of NSW (Anon., 2000). All individuals were either immunocomprimised or elderly. Fruit salad was reported as the likely source of infection.

5.11.5 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* spp. in raw milk is estimated to be around 3-4% (Doores and Amelang, 1988; Hayes et al., 1986; Lovett et al., 1987). 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). 7% of imported cheeses, camembert and blue vein were positive for *L. monocytogenes* (Sutherland et al., 2003). 25% of European soft and surface-ripened cheeses have been found to be positive for *L. monocytogenes* (Terplan, 1988).

The incidence of *L. monocytogenes* in slaughter animals is generally low (0-9%) (Farber and Peterkin, 1999). Overseas studies have shown the prevalence of *L. monocytogenes* contamination in raw meat to be in the range 5-20% (Farber and Peterkin, 1999). In Australia, levels of 24% in beef, 16% in lamb and 10% in pork have been found (Ibrahim and MacRae, 1991). Other meat products from which *L. monocytogenes* has been isolated include minced meat products, sausages, salami, ham, mettwurst, pate, frankfurters and vacuumed packed meat (Farber and Peterkin, 1991b).

Prevalence in poultry meat products ranges from 12-60% (Ojeniyi et al., 2004), and has been isolated from fresh, frozen, cook-chilled and precooked ready to eat chicken products (Cox et al., 1999).

L. monocytogenes has been detected in fresh, frozen and processed seafood. Prevalence in fresh processed seafood ranges between 4-12% in published surveys (Sutherland et al., 2003).

Types of vegetable produce where the organism has been detected include radishes, cucumbers, cabbage, potatoes, lettuce, frozen broccoli and cauliflower and endive (Brackett, 1999; Heisick et al., 1989). Levels of 44% have been detected on fresh cut salad vegetables in the Netherlands, and 9% in prepared salads in Ireland (Harvey and Gilmour, 1993). Recent European surveys show the presence of *L. monocytogenes* to be less than 10% (Brackett, 1999).

5.11.6 Virulence and infectivity of L. monocytogenes

When ingested, *L. monocytogenes* penetrates the intestinal tissue and is taken up by macrophages and non-phagocytic cells in the host. *L. monocytogenes* is disseminated throughout the host via blood or lymphatic circulation to various tissues. Its presence intra-cellularly in phagocytic cells permits access to the brain and probably transplacental migration to the foetus in pregnant women. The pathogenesis of *L. monocytogenes* relies on its ability to survive and multiply in phagocytic host cells.

Not all strains appear to be equally virulent. The 4b and occasionally 1/2a and 1/2b serovars account for most cases of human listeriosis (ICMSF, 1996).

The virulence of *L. monocytogenes* is increased when the bacterium is grown at low rather than high temperatures. The possibility exists that cold storage may enhance virulence of some *L. monocytogenes* strains isolated from refrigerated foods (Ryser and Marth, 1999).

5.11.7 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. FDA et al. (2003) and WHO/FAO (2004) developed separate dose-response models for both healthy and susceptible populations by combining data from surrogate animal models with epidemiological data. For the healthy population (classified as "intermediate-age") the median mortality rate from ingestion of 10^9 organisms was estimated to be 1.0×10^{-6} (FDA et al., 2003). For neonatal and elderly groups the mean mortality rate at the same dose was estimated to be 1.4×10^{-3} and 3.3×10^{-6} respectively.

The infectious dose is unknown but it is believed to vary with 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 et al., 2003).

It is thought the consumption of food with exceptionally high levels of *L. monocytogenes* ($>10^7$/g) is required to cause the mild gastrointestinal form of illness in healthy persons (Sutherland et al., 2003).

5.11.8 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, HIV/AIDS patients and alcoholics). Less frequently reported, diabetic, cirrhotic, asthmatic and ulcerative colitis patients are also at more risk (USFDA CFSAN, 2004a).

Another physiological parameter thought to be relevant to susceptibility is a reduced level of gastric acidity (WHO/FAO, 2004).

5.11.9 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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5.12 *Mycobacterium bovis*

The genus Mycobacterium comprises approximately 95 species, of which over 30 have been associated with disease in humans (Katoch, 2004). Mycobacterium species are also pathogens of food producing animals such as cattle, sheep, other ruminants and fish, and some of those species have zoonotic potential in humans (Sutherland, 2003).

Mycobacteria 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).

The mycobacteria are widely distributed in the environment, being found in soil and water. They readily form biofilms in drinking water distribution systems (Falkinham, III, 2002; Sutherland, 2003). Mycobacteria 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).

M. bovis is related to *M. tuberculosis*, the agent of human pulmonary tuberculosis. *M. bovis* causes systemic infections in cattle and other animals, where it initially infects the gastrointestinal tract before spreading to other parts of the body, including the lungs. *M. bovis* can be shed directly from infected mammary glands into milk, and subsequently transmitted to humans via consumption of contaminated milk (Lake et al., 2002). Multidrug resistance is common. The current requirements for holding (batch) and high temperature short time (HTST) pasteurisation were developed, in part, to manage the risk to human health from the transmission of *M. bovis* through the milk supply.

M. bovis was introduced into Australia at the time of European settlement. A program to eradicate bovine tuberculosis began in 1970 and Australia was declared free of the disease in December 1997 (Animal Health Australia, 2005).

5.12.1 Growth characteristics

Mycobacteria are slow growing obligate aerobes which are difficult to culture as they do not grow on ordinary microbiological media (Anon 1998). Colonies are rarely visible to the naked eye in under 4 weeks of incubation on Dorset egg medium (Anon 1998).

This slow and fastidious growth habit, allied with the short shelf life of foods they are associated with, means that mycobacteria are unlikely to grow to any significant extent in food during production, processing, distribution and storage (Lake et al., 2002).

M. bovis is inactivated by sunlight (Lake et al., 2002). It has also been found to be relatively resistant to a wide range of disinfectants used in medical / hospital settings (Rutala et al., 1991; Gregory et al., 1999; Lake et al., 2002).

5.12.2 Pathology of illness

Symptoms associated with *M. bovis* gastrointestinal infection include fever, chills, weight loss, abdominal pain, diarrhoea or constipation. Symptoms of further infections depend on

the organs infected. Symptoms may last for months or years, and death may result (Lake et al., 2002).

Due to the slow growing nature of the organism, the onset time to elaboration of symptoms may be years after initial infection, and even in immunocompromised individuals the onset time may be several months (Lake et al., 2002).

5.12.3 Mode of transmission

M. bovis is considered to be transmitted to humans primarily through aerosols from infected animals and consumption of unpasteurised milk and dairy products (O'Reilly and Daborn, 1995; Cousins and Dawson, 1999; Lake et al., 2002; Anon 2005). Water is not considered to be a source of human infection with *M. bovis* (Lake et al., 2002), although transmission of other waterborne mycobacteria can occur through drinking or via inhalation as a result of aerosolisation.

There is disagreement as to whether consumption of meat from infected (tuberculous) cattle can lead to human infection.

5.12.4 Incidence of illness

M. bovis was responsible for 2.4% of human TB patients in Santa Fe province, Argentina, in the period 1984-1989 (Zumarraga et al., 1999). Eleven of 19 *M. bovis* strains isolated from humans were from rural or slaughterhouse workers.

M. bovis subsp. *caprae* was responsible for one third of 166 human isolates of *M. bovis* from TB cases in Germany between 1999-2001, and *M. bovis* was present in approximately 1% of human TB cases (Kubica et al., 2003).

The prevalence of human tuberculosis (TB) due to *M. bovis* was determined in urban areas in Madagascar in 1994-1995. A prevalence of *M. bovis* of 1.25% was observed among sputum smear-positive patients and 1.3% among extra-pulmonary TB patients (Rasolofo-Razanamparany et al., 1999).

M. bovis was isolated in 0.5% (38/7075) of cases of bacteriologically confirmed tuberculosis notified to the National Reference Centre (CNR) in France in 1995. Incidence rates increased with age, and were approximately equally split between pulmonary and extra-pulmonary sites. Occupational exposure was identified in 13 cases and ingestion of non pasteurised milk in three (Robert et al., 1999).

M. bovis was responsible for approximately 1% of cases of TB in the Australian population during 1970-1994 (at least 236 cases). The majority of cases (74%) involved pulmonary disease. Most cases were apparently due to reactivation of infection acquired through occupational exposure and had histories of employment in meat and/or livestock industries (Cousins and Dawson 1999).

About 1% of clinically diagnosed cases of TB in the UK are attributed to *M. bovis* (Gibson et al., 2004).

33.9% of 180 culture-positive paediatric cases (<15 years old) of TB in San Diego during 1980 to 1997 were attributed to *M. bovis* (Dankner and Davis, 2000). Between 1994 and 2000, 6.7% (129/1931) of all cases of culture-positive TB in San Diego County were identified as *M. bovis*, and 90% of these occurred in the Hispanic population (LoBue et al., 2003).

Lumb et al., (2002) and Lumb et al. (2003) reported that *M. bovis* accounted for only 2 of 765 new diagnoses of disease caused by tuberculosis-causing mycobacteria in 2000. In 2001, only 1 of 771 cases were due to *M. bovis* (Lumb et al., 2002; Lumb et al., 2003).

M. bovis subsp. caprae accounted for less than 1% (4/640) of human isolates of Mycobacterium species from patients in western Austria in the period 1994-2001 (Prodinger et al., 2002).

A study of 35 culture-confirmed cases of tuberculosis caused by *M. bovis* in New York, USA during 2001 – 2004, raw milk cheese from Mexico was implicated as the likely source of infection (Anon., 2005).

5.12.5 Occurrence in foods

Very few surveys have been conducted in recent times for the presence of *M. bovis* in pasteurised milk, presumably due to the expectation that current pasteurisation practices are sufficient to eliminate the pathogen from milk. A survey of milk samples in Brazil identified culturable *M. bovis* in 1 of 78 raw milk samples and no isolates from pasteurised and UHT milk (Leite et al., 2003). It is generally accepted that current pasteurisation practices are sufficient to inactivate *M. bovis* at the levels commonly found in raw milk.

5.12.6 Virulence and infectivity

The virulence factors of the mycobacteria remain largely unknown (Collins et al., 1995; Collins, 1996). Mycobacteria are intracellular pathogens, able to grow and multiply inside macrophage cells, thus effectively avoiding attack by the host's immune system. The unique structure of the mycobacterial cell wall, particularly a cell wall glycolipid containing mycolic acid, is thought to contribute to protecting the invading organism from the host's defence mechanisms. Pathology may also be due to the direct action of toxic chemical components of the mycobacterial cell wall.

The other main virulence factor identified is a catalase-peroxidase (KatG gene: (Collins 1996), which appears to help the cells to resist destruction by macrophages.

Most mycobacteria do not secrete exotoxins.

5.12.7 Dose Response

Results from animal experiments indicate that *M. bovis* infection via the oral route requires thousands or millions of organisms compared to less than ten via the inhalation route (O'Reilly and Daborn 1995; Lake et al., 2002).

5.12.8 Host Factors

The group at greatest risk of infection with *M. bovis* is those with a compromised immune system, whose resistance to infection is lowered

Biet et al. (2005) report that those at greatest risk of infection with *M. bovis* (and mycobacterial infections generally) include the very young and old, those with compromised immune systems (*e.g.* transplant patients, patients on corticosteroid treatments, HIV/AIDS patients and alcoholics), and those exposed due to occupation or lifestyle. Reactivation can occur under stress or in old age, when latent mycobacterial infections may become subject to less stringent control by host systems.

5.12.9 Food Matrix

M. bovis is a very slow growing, microaerophillic organism and given the usual short shelf-life of products that may be associated with transmission, eg raw milk and raw meat, it would appear that growth in foods would be insignificant.

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5.13 *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 species are also pathogens of food producing animals such as cattle, sheep, other ruminants and fish, and some of those species have zoonotic potential in humans (Sutherland, 2003).

Mycobacteria 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).

The mycobacteria are widely distributed in the environment, being found in soil and water. They readily form biofilms in drinking water distribution systems (Falkinham, III, 2002; Sutherland, 2003). Mycobacteria 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).

5.13.1 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 25ºC to 45ºC with an optimum of around 39ºC (Anon 1998). Batch (63ºC for 30 min) and HTST (72ºC for 15 sec) pasteurisation are sufficient to inactivate high levels of pathogenic mycobacteria in milk, although they will survive thermisation (treatment at 62ºC for 15 sec 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 one year or so (Anon 1998).

5.13.2 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 (reviewed by: 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).

5.13.3 Mode of transmission

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 calf ingesting colostrum, milk or faeces from an infect animal. MAP is also transmitted horizontally through the faecal-oral route (Streeter et al., 1995; Sweeney, 1996, 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; Stabel and Lambertz 2004; Pearce et al., 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).

Goats' 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).

5.13.4 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 per 100,000 (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).

5.13.5 Occurrence in foods

Infected cattle may shed MAP in their faeces at levels up to 10^8 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 of MAP in milk in England and Wales conducted by (Millar et al., 1996) raised significant concern regarding the possible survival of MAP during pasteurisation. Seven percent (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. Fifty percent of PCR positive samples and 16% of PCR negative samples yielded MAP-positive cultures. However, other workers questioned the conclusion drawn that MAP could survive pasteurisation (*e.g.* see 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 cows' milk samples, 244 bulk raw and 567 commercially pasteurised (228 whole, 179 semiskim, 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 cows' milk in the United Kingdom.

A similar 13-month study (to November 2001) of bulk raw (n = 389) and commercially pasteurised (n = 357) 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 cows' milk but, since no viable MAP was isolated from pasteurised milk samples, current pasteurisation procedures were considered to be effective.

5.13.6 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 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).

5.13.7 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.

5.13.8 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 (McGovern et al., 2001).

5.13.9 Food Matrix

Limited studies have investigated the survival of MAP in food, with most research being undertaken on dairy products. For cheddar cheese, Donaghy et al. (2003) observed an increased concentration of MAP in 1-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 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 descreased 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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5.14 *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 has 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: *S. enterica* (comprising six subspecies) and *S. bongori* (Brenner *et al*., 2000); Table 2.1). 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 *Salmonella 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.

Table 2.1: Species of the genus *Salmonella* (Brenner *et al.,* 2000).

***Salmonella* species/subspecies**	**No. of serotypes**	**Usual habitat**
S. enterica subsp. *enterica*	1,454	Warm-blooded animals
S. enterica subsp. *salamae*	489	Cold-blooded animals and environment [a]
S. enterica subsp. *arizonae*	94	Cold-blooded animals and environment
S. enterica subsp. *diarizonae*	324	Cold-blooded animals and environment
S. enterica subsp. *houtenae*	70	Cold-blooded animals and environment
S. enterica subsp. *indica*	12	Cold-blooded animals and environment
S. bongori	20	Cold-blooded animals and environment
Total	2,463	

[a] Isolates of all species and subspecies have occurred in humans.

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).

5.14.1 Growth and survival

Salmonellae have relatively simple nutritional requirements and can survive for long periods of time in foods and other substrates (Jay *et al*., 2003). 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 (a_w). Being facultatively 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).

Temperature
The growth of most salmonellae is substantially reduced at <15°C and prevented at <7°C (ICMSF, 1996). Growth generally does not occur at >46.2°C. The optimum temperature for growth is 35 – 43°C.

Freezing can be detrimental to *Salmonella* 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 the freezing point as a result of the freezing damage. However, at lower temperatures (-17 to -20°C) there is a significantly less rapid decline in the number of viable organisms. *Salmonella* have the ability to survive long periods of time at storage temperatures of < -20°C (Jay *et al*., 2003).

Heat resistance of *Salmonella* in foods is dependant on the composition, nature of solutes and 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).

pH
The minimum pH at which *Salmonella* 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 (Table 2.2). Volatile fatty acids are more bactericidal than acids such as lactic and citric acid.

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

Table 2.2: Limits for growth of *Salmonella* when other conditions (*e.g.* temperature, pH, a_w) are near optimum (ICMSF, 1996).

Condition	Minimum	Optimum	Maximum
Temperature (°C)	5.2*	35-43	46.2
pH	3.8	7.0-7.5	9.5
a_w	0.94	0.99	>0.99

* Most serotypes fail to grow at <7°C

5.14.2 Pathology of illness
Outcomes of exposure to *Salmonella* 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, 1999). In a small number of cases, *Salmonella* 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 risk of death (mortality within a year of infection) 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 (FAO/WHO, 2002; Hohmann, 2001). Illness is usually self-limiting, with patients fully recovering within a 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* 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* 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* gastroenteritis (Hohmann, 2001). Antibiotics should be used, however, for those who are severely ill and for patients with risk factors for extraintestinal 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*, 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* isolates in the USA. 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).

5.14.3 Mode of transmission

Salmonella 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.

5.14.4 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 (Hall, 2003). 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 (Figure 1). This varies 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.

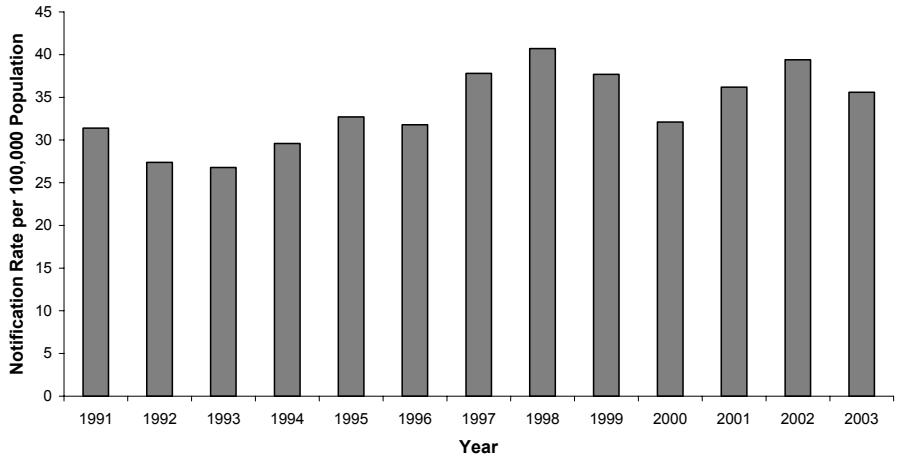

Figure 2.1 Salmonellosis notification rates in Australia by year (1991-2003; National Notifiable Diseases Surveillance System).

Of the total number of *Salmonella* serovars reported to Australian health authorities during 2002, *S*. Typhimurium 135 was the most commonly reported (Table 2.3). 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).

Table 2.3 Principal isolates in Australia, 2002 (Yohannes *et al.,* 2004)

	State or Territory									
Organism	ACT	NSW	NT	Qld	SA	Tas	Vic	WA	Aust	Total %
S. Typhimurium 135	11	238	8	117	14	18	178	91	675	8.8
S. Typhimurium 9	16	268	0	77	24	12	151	44	592	7.7
S. Typhimurium 170	5	161	0	135	1	1	152	3	458	5.9
S. Saintpaul	0	37	20	225	11	2	44	44	383	5
S. Virchow 8	0	21	0	268	0	0	11	2	302	3.9
S. Birkenhead	0	95	3	134	4	0	8	1	245	3.2
S. Typhimurium 126	1	62	2	28	39	4	61	8	205	2.7
S. Chester	1	29	16	82	11	2	5	32	178	2.3
S. Hvittingfoss	1	17	6	110	3	1	13	2	153	2

S. Muenchen	0	20	12	55	9	3	9	24	132	1.7
Other	60	1136	248	1354	405	117	588	470	4378	56.8
Total	**95**	**2084**	**315**	**2585**	**521**	**160**	**1220**	**721**	**7701**	**100**

It has been estimated that in the United States (Mead, 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 (Table 2.4). It is clear from Tables 2.4 and 2.5 that foods of animal origin (*e.g.* meat, eggs, dairy) are important sources of human salmonellosis.

Table 2.4 Major foodborne outbreaks of human salmonellosis (from D'Aoust, 1994)

Year	Country(ies)	Vehicle	Serovar	Number	
				Cases[a]	Deaths
1973	Canada, US	Chocolate	*S*. Eastbourne	217	0
1973	Trinidad	Milk powder	*S*. Derby	3,000[b]	NS
1974	United States	Potato salad	*S*. Newport	3,400[b]	0
1976	Spain	Egg salad	*S*. Typhimurium	702	6
1976	Australia	Raw milk	*S*. Typhimurium PT9	>500	NS
1977	Sweden	Mustard dressing	*S*. Enteritidis PT4	2,865	0
1981	Netherlands	Salad base	*S*. Indiana	600[b]	0
1981	Scotland	Raw milk	*S*. Typhimurium PT204	654	2
1984	Canada	Cheddar cheese	*S*. Typhimurium PT10	2,700	0
1984	France, England	Liver pâté	*S*. Goldcoast	756	0
1984	International	Aspic glaze	*S*. Enteritidis PT4	766	2
1985	United States	Pasteurised milk	*S*. Typhimurium	16,284	7
1987	China	Egg drink	*S*. Typhimurium	1,113	NS
1987	Norway	Chocolate	*S*. Typhimurium	361	0
1988	Japan	Cuttlefish	*S*. Champaign	330	0
1988	Japan	Cooked eggs	*Salmonella* spp.	10,476	NS
1991	US, Canada	Cantaloupe	*S*. Poona	>400	NS
1991	Germany	Fruit soup	*S*. Enteritidis	600	NS
1993	France	Mayonnaise	*S*. Enteritidis	751	0
1993	Germany	Paprika chips	*S*. Saintpaul, *S*. Javiana, *S*. Rubislaw	>670	0
1994	United States	Ice cream	*S*. Enteritidis	>645	0
1994	Finland, Sweden	Alfalfa sprouts	*S*. Bovismorbificans	492	0

[a] Confirmed cases unless stated otherwise.
[b] Estimated number of cases.
[c] Jay *et al*., 2003.
NS = not specified.

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 traceback, 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; Table 2.5). 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.

Table 2.5 Salmonellosis outbreaks in Australia, 1995-2000 (from Dalton *et al*., 2004).

Vehicle	Outbreaks		Cases	
	n	%	n	%
Meats	25	33	658	17
Chicken	10		335	
Beef	4		67	
Pork	2		37	
Processed meats – consumed cold	4		61	
Other meats*	5		158	
Eggs	8	11	701	17
Sandwiches	7	9	1,205	29
Desserts	6	8	254	6
Fruit	2	3	60	1
Seafood	2	3	14	<1
Dairy	1	1	26	<1
Fish	1	1	26	<1
Fruit juice	1	1	502	12
Salads	1	1	21	<1
Vegetables	1	1	54	1
Miscellaneous	18	24	573	14
Unknown	2	3	43	1
Total	**75**	**100**	**4,123**	**100**

* Includes meats in above categories that may be mixed together and meats not in above categories, or where type of meat was not known.

5.14.5 Occurrence of Salmonella *in food*

The primary reservoir of *Salmonella* 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* has 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* (Bryan and Doyle, 1995). *Salmonella* 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* in finished products (Bryan and Doyle 1995).

Table 2.6 summarises reported isolation rates of *Salmonella* from a variety of dairy products. It is difficult to directly compare results between different commodities due to variations in sample size, stage of production sampled and methodology used. Pasteurisation effectively inactivates *Salmonella* spp., however contamination of milk has occurred due to improper pasteurisation and/or post-processing contamination (Jay *et al*., 2003).

Table 2.6 Reported prevalence of salmonellae in dairy products.

Food	Country	Samples	% positive	Reference
Raw milk	Switzerland	456	0	(Bachmann and Spahr, 1995)
Raw goat's milk	Switzerland	344	0	(Muehlherr *et al*., 2003)
Raw ewe's milk	Switzerland	63	0	(Muehlherr *et al.,* 2003)
Raw milk (bulk tank)	USA	131	6.1	(Jayarao and Henning, 2001)
Raw goat's milk	UK	100	0	(Little and De Louvois, 1999)
Raw ewe's milk	UK	26	0	(Little and De Louvois 1999)
Raw milk (bulk tank)	US	268	2.2	(Murinda *et al*., 2002)
Raw bovine milk	France	69	2.9	(Desmasures *et al*., 1997)

5.14.6 Virulence and infectivity

Once ingested, *Salmonella* 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* possesses a number of structural and physiological virulence factors enabling it to cause acute and chronic disease in humans.

Virulence of *Salmonella* varies with the length and structure of the O side chains of lipopolysaccharide (LPS) molecules at the surface of the cell. Resistance of *Salmonella* 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* 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 (LT) 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*, infection is generally limited to a localised intestinal event.

5.14.7 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*, 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. Again, a beta-Poisson model was used to describe the dose-response relationship (Figure 2.2).

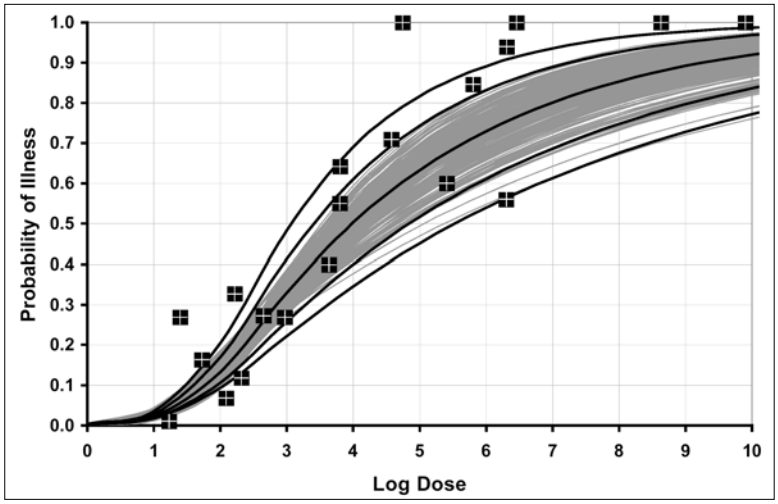

Figure 2.2 Uncertainty bounds for dose-response curves compared with expected value for the outbreak data (FAO/WHO, 2002).

Although not subject to some of the inherent flaws associated with using purely experimental data, 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.

Table 2.7 Beta-Poisson dose-response parameters that generate the approximate bounds shown in Figure 2.2 (FAO/WHO, 2002).

	Alpha	**Beta**
Expected Value	0.1324	51.45
Lower Bound	0.0763	38.49
2.5th Percentile	0.0940	43.75
97.5th Percentile	0.1817	56.39
Upper Bound	0.2274	57.96

5.14.8 Host factors

Individual susceptibility to *Salmonella* 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* include the very young, the elderly, pregnant women and the immunucompromised (organ transplant patients, cancer patients, AIDS patients) (Gerba *et al.,* 1996).

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5.15 *Shigella* spp.

Shigella is a genus of the Enterobacteriaceae family. The *Shigella* genus has four species, *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. *Shigella* spp. are Gram-negative, non-spore forming, rod shaped, non-motile, facultatively anaerobic organisms. All members of the species are able to cause bacillary dysentery. *Shigella* spp. share many biochemical and serological features with the genus of *Escherichia coli*.

5.15.1 Growth characteristics

Available information indicates *Shigella* species can survive and grow in a wide range of food including boiled rice, lentil soup, milk, cooked beef, cooked fish, raw cucumber, mashed potato, cheese, shredded lettuce, tofu, butter and margarine, lemon juice and wine (Lightfoot, 2003). Table 1 summarises the prevailing growth conditions of *Shigella* species. Under favourable conditions, the growth of *Shigella* in food is rapid. For example, *S. sonnei* can double its cells number in less than 1.5 hour when it is incubated with diced tofu at 32^{o}C (Lee et al., 1991).

Table 5 Growth conditions for *Shigella* species

	Minimum	**Optimum**	**Maximum**
Temperature (^{o}C)	6-7	Room temperature	45-47
pH	4.5	6-8	9.3
Water activity (a_w)	0.97	Not available	Not available

From (Lightfoot, 2003) and (International Commission on Microbiological Specification for Foods, 1996).

At temperatures around 65^{o}C, *Shigella* species are inactivated rapidly. At pH less than 4.0, shigellae die rapidly (International Commission on Microbiological Specification for Foods, 1996).

5.15.2 Pathology of illness

Shigellosis refers illness in humans caused by *Shigella*. It occurs principally as a disease to humans and rarely occurs in animals. Shigellosis is associated with symptoms of abdominal pain, cramps, diarrhoea, fever, vomiting, mucosal ulceration, rectal bleeding, and drastic dehydration. The onset time is between 12 to 50 hours. The fatality rate is high and for some of the virulent strains, the rate is close to 10-15%. Sequelae as a result of shigellosis include Reiter's disease, reactive arthritis and haemolytic ureamic syndrome (Lightfoot, 2003).

5.15.3 Mode of transmission

Shigellosis is prevalent in areas of inadequate sanitation and poor living conditions with overcrowding. Transmission of *Shigella* to humans is via the faecal-oral route. Faecal contaminated water and unhygienically prepared/handled food are the most common causes of transmission of *Shigella* organisms.

Contamination of food is generally the result of poor personal hygiene of food handlers. In Australia, contamination of food by an infected food handler resulted in a number of cases of *Shigella* infection in outbreaks of *S. flexneri* in Alice Springs in 1977 and in Wangaratta in 1978 (Lightfoot, 2003). Transmission of *Shigella* contamination can occur via contaminated flies. Inadequate treated contaminated water used of drinking or food preparation, inadequately disinfected swimming pool or recreational water contaminated by animal or human faeces are potential sources of transmission of shigellae.

Peron to person transmission is common, either via direct or indirect contact. There have been reports of *Shigella* infection in laboratory settings (Collins et al., 1999).

5.15.4 Incidence of illness

In 2003, 443 cases shigellosis of reported in Australia, representing 2.2 cases per 100,000 of the population. The majority of shigellosis infections probably were acquired by person-to-person transmission or overseas. Northern Territory recorded highest rate of notification, 67 cases per 100,000. Rates of shigellosis were considerably higher in the indigenous communities. The notified rate of shigellosis was 300 cases per 100,000 population in indigenous children aged 0–4 years of age in Western Australia (The OzFoodNet Working Group, 2004).

According to information of the Foodborne Outbreak Response and Surveillance Unit of the Centres for Disease Control and Prevention[28], during the period of 1990 and 1995, 39 outbreaks of foodborne illness caused by *Shigella* species were reported in the US. Average case number per outbreak was 53, ranging from the lowest number of 3 cases to the highest number of 400 cases in a single outbreak. Although the vehicle of transmission could not be identified for almost half of the outbreaks, food implicated as vehicles of transmission included vegetable or vegetable based food, turkey based noodles, raw oysters, wild rice salad, spaghetti salad, chicken meat salad, smoked salmon, cheese/lettuce/tomato mix and others. In reviewing *Shigella* outbreak investigations in the past 30 years, Lightfoot (Lightfoot, 2003) listed the following as main contributing factors to *Shigella* foodborne outbreaks, which are:

- poor hygiene in food preparation;
- food handlers who have developed shigellosis;
- use of uncooked food ingredient which may have been contaminated by *Shigella*;
- ready to eat food; and
- mass food service.

A typical example is the explosive outbreak of shigellosis caused by *S. sonnei* that affected an estimated 3175 people attending a five-day outdoor musical festival in August 1988 in the State of Michigan of the US. The vehicle of transmission was an uncooked tofu salad that was made with several hundred ponds of uncooked tofu and vegetables involving some 50 people in its preparation (Lee et al., 1991).

5.15.5 Occurrence in foods

Salads (lettuce, potato, tuna, shrimp, macaroni and chicken), raw vegetables, raw oysters, apple cider, milk and dairy products, and poultry have been identified as food sources that have been contaminated by *Shigella* organisms.

Fresh pasteurised milk cheese was implicated in an outbreak of shigellosis caused by *S. sonnei* in the winter of 1995-1996 in southwest Spain. More than 200 people from eight townships were affected during the outbreak. Investigation of the outbreak suggested that an infected food handler at the cheese factory might have been the source of contamination and the cheese processing method might have allowed cross contamination to occur post pasteurisation (Garcia-Fulgueiras et al., 2001).

[28] http://www2.cdc.gov/ncidod/foodborne/OutbreaksReport.asp Accessed 5 May 2005

5.15.6 Virulence and infectivity of Shigella *species*

Virulent *Shigella* organisms, once ingested and passed through the digestive system, attach to and penetrate the colonic epithelial cells of the intestinal mucosa. The organism then multiplies intracellularly, resulting in tissue destruction. Invasive strains of *S. dysenteriae* 1 produce high levels of cytotoxin referred as Shiga toxin. Shiga toxin has both enterotoxic and neurotoxic activity in addition to cytotoxiticity. Other serovars of *S. dysenteriae* and other *Shigella* spp. produce low levels of cytotoxic activities (Lightfoot, 2003).

5.15.7 Dose Response

Shigella spp. are considered relatively infectious, with an estimated infective dose as low as 10 cells (DuPont et al., 1989).

5.15.8 Immune status

Humans of all age and all health status are susceptible to *Shigella* infection. However, incidence of shigellosis is the highest among children of 1 to 4 years old (Clemens et al., 1999). The infants, the elderly and those suffering from immune deficiency, particularly people with AIDS and non-AIDS homosexual men are susceptible to the severest symptoms of shigellosis (CFSAN[29]).

5.15.9 Food Matrix

Shigella can survive in foods stored at –20^{o}C, at refrigerated temperature and at room temperature for long period of time. Their survival is greater at temperature of 25^{o}C or less. It has been shown that *Shigella* can survive in cheese for several weeks at room temperature (Nakamura, 1962) and in ground meat for at least 4 days at 6-8^{o}C (Smith, 1987). *S. flexneri* has been shown to be able to survive in prepared coleslaw, carrot and vegetable salad for at least 11 days and in crab salad for up to 20 days at 4^{o}C (Rafii et al., 1997).

Shigella species survive better in alkaline environment and there is a data to suggest that *S. sonnei* and *S. dysenteriae* can survive in acidic foods, such as grape juice, lemon juice and wine for 4 to 24 hours and in orange juice for six days and in carbonated beverages for a day (International Commission on Microbiological Specification for Foods, 1996).

S. flexneri and *S. sonnei* have been shown to be able to grow in less then 5.2% of sodium chloride and less than 700 mg/L of sodium nitrite (International Commission on Microbiological Specification for Foods, 1996).

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5.16 *Staphylococcus aureus*

The genus *Staphylococcus* is subdivided into 28 species and 8 subspecies. *S. 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 (USFDA Centre for Food Safety and Applied Nutrition, 2004b).

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 (Stewart, 2003). In addition, some strains of enterotoxin-producing staphylococci do not possess the coagulase enzyme.

5.16.1 Growth characteristics

The temperature range for growth of *S. aureus* is 7-48°C with optimum growth occurring at 35-40^{o}C. The temperature range for toxin production is 10-48°C with the optimum temperature being from 40-45^{o}C. *S. aureus* grows over a wide a_w range (0.83-0.99) with an optimum a_w 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 (a_w 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 D_{149}=100 min (a_w of 0.99) (ESR, 2001). Heat resistances for *S. aureus* vegetative cells have been reported at D_{60} = 0.43-8.0 min whereas a time/temperature equivalent for enterotoxin is 121^{o}C for 3-8 min (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 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).

5.16.2 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 (Stewart, 2003; Ash, 1997). Symptoms include nausea, vomiting, abdominal cramps of varying severity, and diarrhoea. Some individuals may not demonstrate all 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 of 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. *S. aureus* can also cause respiratory infections or may become established in the gut causing enteritis.

S. auerus 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.

5.16.3 Mode of transmission

Staphylococcal food poisoning is caused by the consumption of food containing enterotoxins produced by certain stains of *S. aureus*. Despite the wide-spread association of *S. aureus* with animals, humans are the main reservoir for *S. aureus* involved in human disease (Jablonski and Bohach, 1997). Hand contact with ready-to-eat foods is an important means by which *S. aureus* may enter food supply by food handlers.

Foods that present the greatest risk of causing illness are those in which the normal flora has been destroyed (eg cooked meats) or inhibited (eg cured meats containing high salt content) (Stewart, 2003).

5.16.4 Incidence of illness

Food poisoning caused by *S. aureus* is one of the most common type of foodborne diseases world-wide. 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 (especially ham, hot dogs, salami). Improper

storage/temperature abuse of food is 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. aureas* 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 (Anon 2003a; Ashbolt et al., 2002) . An outbreak was also reported in 2001 from consumption of BBQ chicken strongly suggesting an enterotoxin-producing bacterium as the causative agent, possibly *S. aureus* (Armstong *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, 2003b).

Mead *et al*. (1999) state 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 was 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 *Staphylococcus* 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 and 2000 (Le Loir *et al*., 2003). Of these outbreaks, milk products and especially cheeses were responsible for 32% of cases, meats 22%, sausages and pies, 15%, fish and seafood 11%, eggs and egg products 11% and poultry 9.5% (Haeghebaert *et al*., 2002).

In the United Kingdom for the years 1969-81, 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 1969-90 a study of 359 incidents of staphylococcal food poisoning was investigated (Table 4.4). Poultry and poultry products accounted for 22% of incidents, most attributed to cold cooked chicken and in nine incidents turkey was the food vehicle (Bertolatti *et al*., 1996; Wieneke *et al*., 1993).

Table 4.4: Foods implicated in staphylococcal food poisoning in the UK from 1969-1990 (Wieneke *et al*., 1993)

Type of Food	Number of incidents

Ham	65	53%
Meat pies	25	
Corned beef	20	
Tongue	16	
Jars of meat, chicken or fish paste	12	
Other meats and meat containing products	43	
Meat dishes	9	
Poultry (chicken, turkey, duck)	64	22%
Poultry dishes	15	
Fish and shellfish	24	7%
Milk and desserts containing milk or cream	23	8%
Cheese	5	
Boiled eggs and egg dishes	13	3.5%
Other foods	20	5.5%
Not known	5	1%
Total	**359**	

5.16.5 Occurrence in foods

Animals carry *S. aureus* on various parts of their bodies. Cows 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 10^5 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*, a, b 2001).

5.16.6 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 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, although it is thought that the vomiting response to ingestion of preformed toxin is the result of the stimulation of local neuroreceptors in the intestinal tract which transmit the stimuli to the vomiting centre of the brain via the vagus 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; Zschöck 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).

5.16.7 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).

5.16.8 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.

5.16.9 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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5.17 *Streptococcus* spp.

Streptococci are gram-positive, spherical or ovoid, non-motile bacteria. They grow aerobically or microaerophilically. Anaerobic species have no significance in food microbiology. The term 'streptococcus' was first used by Billroth (1874) to describe the chain-forming, coccoid bacteria that had been observed in wounds and discharges of animals (ICMSF 1996).

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 *S. pyogenes* and *S. agalactiae.* Enterococci include *E. faecalis* and *E. 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 (Bad Bug Book 1992). *S. zooepidemicus* (Group C) has also been implicated in several episodes of human illness, including death, in the UK following consumption of raw milk (Barrett 1986).

Streptococcus agalactiae is a major cause of bovine mastitis (ICMSF). It is a highly contagious obligate parasite of the mammary gland (Martinez et al., 2000).

5.17.1 Growth characteristics

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).

Limits for growth of *S. pyogenes* (ICMSF 1996)

	Minimum	Optimum	Maximum
Temperature (ºC)	10 - 15	37	>40, <45
pH	4.8 - 5.3	7	<9.3
NaCl (%)	-	-	>4, <6.5

Experiments conducted by Obiger (1976) found that *S. pyogenes* would not survive exposure to 66ºC for 20-40s resulting in a calculated D-value at 66ºC of 0.1-0.2min.

Heat resistance figures reported by Stumbo (1973) included a D-value at 65.6ºC of 0.2-2.0 and a z value of 4.4-6.7ºC. Based on these figures, ICMSF (1996) conclude that pasteurisation at 62ºC for 30 minutes and 70ºC for 30s would ensure only a 1.6-2.3 decimal reduction of *S. pyogenes*. However, using the D-value at 66ºC of 0.2 as per Obiger (1976), pasteurisation would result in a 20 decimal reduction of *S. pyogenes* in milk.

5.17.2 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, 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 (GAS) disease". Two of the least common but most severe forms of GAS disease are necrotising fasciitis and Streptococcal Toxic Shock Syndrome (STSS). 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 (Bad Bug Book 1992).

5.17.3 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. Group A streptococci may be carried in the throat on the skin of people with no symptoms of illness.

5.17.4 Incidence of illness

Outbreaks of septic sort 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.

An outbreak of food borne illness due to *S. zooepidemicus* (Group C) involving at least 11 cases occurred in the UK in 1984. Seven persons died 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).

Outbreaks of Group D streptococcal infections are not common and have usually been the result of unsanitary preparation, storing or handling of food (Bad Bug Book 1992).

Sixteen cases of invasive group C streptococcal infection were identified in northern Mexico between July 25 and 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 cows' 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 October 07, 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 12 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 mild were subsequently found to be excreting *S. zooepidemicus* in their milk (Barrett 1986).

5.17.5 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 (Bad Bug Book 1992).

Food sources for streptococcus Group D foodborne illness include sausage, evaporated milk, cheese, meat croquettes, meat pie, pudding, raw milk and pasteurised milk. Underprocessing and/or poor food preparation is the usual mechanisms for entrance into the food chain (Bad Bug Book 1992).

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 until 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 was 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 *S. 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).

5.17.6 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).

5.17.7 Dose response

The infectious dose for streptococcus Group A likely to be quite low, with less than 1,000 organisms required for infection (Bad Bug Book 1992). In contrast, it is estimated that foodborne Group D streptococcus has a high infectious dose of greater than 10^7 organisms.

5.17.8 Host factors

All individuals in a population are equally susceptible to streptococcal illness. (Bad Bug Book 1992). People with chronic illnesses such as cancer, diabetes and kidney dialysis and

those using medications such as steroid have a higher risk of getting invasive GAS disease (CDC webpage).

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5.18 *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. *Y. pestis* is the cause of the plague. *Y. pseudotuberculosis* and *Y. enterocolitica* are enteropathogenic strains. *Y. pestis* and *Y. pseudotuberculosis* do not frequently infect humans. *Y. pestis* mainly infects rats and other rodents which are the prime reservoir for the bacteria. Fleas are the prime vectors carrying the bacteria from one species to another. They bite rodents infected with *Y. pestis*, then they bite people and so transmit the disease to them. Transmission of the plague to people can also occur from eating infected animals such as squirrels. Once someone has the plague, they can transmit it to another person via aerosol droplets. *Y. pseudotuberculosis* is primarily a zoonotic disease of wild and domesticated birds and mammals, with humans as incidental hosts. *Y. enterocolitica* is more commonly found in human clinical specimens.

Y. enterocolitica are Gram-negative, small rods with dimension in the range of 0.5-0.81µm x 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 are 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 (CFSAN[30]).

5.18.1 Growth characteristics

Optimal growth temperature for *Y. enterocolitica* is at approximately 30°C, but the organism can grow at refrigerated temperatures and ability of growth at –5°C has been reported (Barton et al., 2003). *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.

Table 1 Growth conditions for *Y. enterocolitica*

	Minimum	**Optimum**	**Maximum**
Temperature (°C)	-5	22-28	44
pH	4.6	7- 8	10.0
Water activity (a_w)	0.945	Data not available	Data not available

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 min at 55°C, 0.5 min 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 min (Lovett et al., 1982). As such, cells of *Y. enterocolitica* in milk are readily inactivated by pasteurisation.

[30] Yersinia enterocolitica, http://www.cfsan.fda.gov/~mow/chap5.html Accessed 26 April 2005.

5.18.2 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 et al., 2003). Mesenteric lymphadenitis caused by *Y. enterocolitica* shows symptoms similar to appendicitis, 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 one to three days. Duration of excretion of the organisms in the stool of infected patients ranges from 14-97 days (Cover et al., 1989).

5.18.3 Mode of transmission

Cells of pathogenic *Y. enterocolitica* ingested and travelled through the gastrointestinal tract can bind to the epithelial cells of the ileum and penetrate the intestinal mucosa and colonise the Peyer's Patches. Cells multiplied may spread to the mesenteric lymph nodes via the lymphatics and in rare situations may spread to the bloodstream, liver and spleen (Barton et al., 2003).

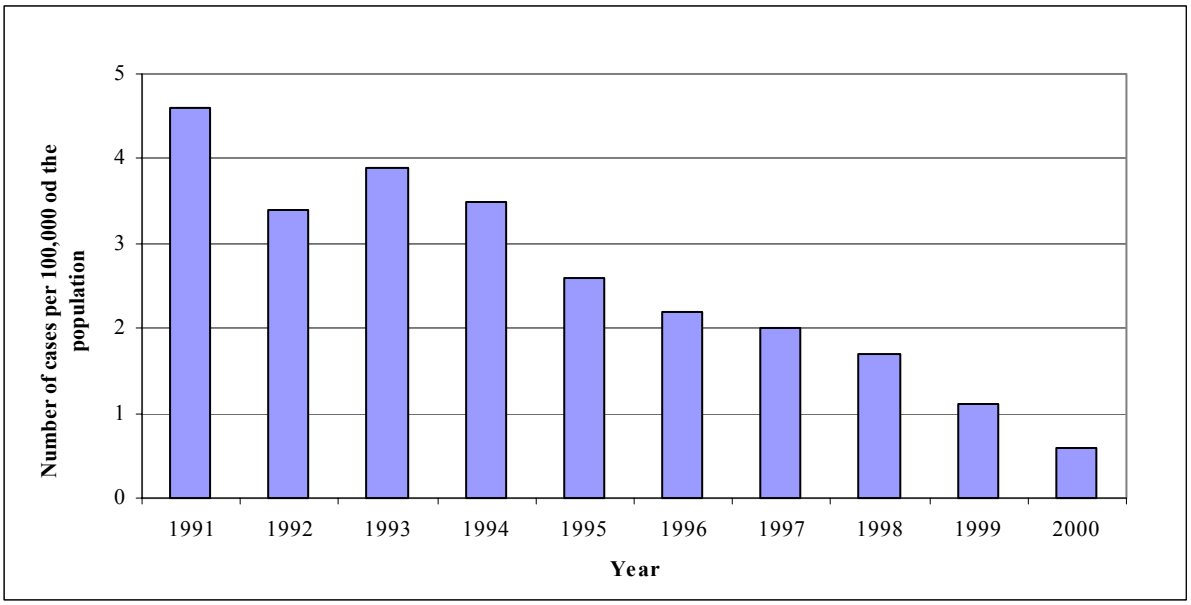
Pigs are the primary source of human infections of yersiniosis. *Y. enterocolitica* is carried in health pigs worldwide. Tonsils and oral cavities of pigs are generally heavily contaminated. Consumption and handling of raw pork meat are a primary source of human infection by *Y. enterocolitica* (Barton et al., 2003).

5.18.4 Incidence of illness

Since its peak in early 1990s, there has been a continuing decline in the number of yersiniosis in Australia, as reported by the National Notifiable Disease Surveillance Systems (Figure 1). 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 per 100,000 of the population.

Most cases of foodborne yersiniosis are sporadic but some outbreaks have been reported. In September and 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 Australia 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., 1991). Other than milk, tofu (Tacket et al., 1985), pig meat products and bean sprouts have been implicated as vehicles of outbreaks of yersiniosis.

Figure 1. Notified cases of yersiniosis in Australia



The above data does not include those of NSW and ACT where yersiniosis was reported as either a "foodborne disease" or "gastroenteritis in an institution". (To be qualified as some of the years do include ACT reports but none of the figure includes NSW reports.)

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 et al., 2003). The number of reported yersiniosis is high in New Zealand where the incidence of yersiniosis is 15.1 per 100,000 in 1998 and 13.9 cases in 1999 (ESR 2001[31]). In Finland, the reported varied from 11.7 to 17.5 per 100,000.

5.18.5 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. They have been found in many food sources like raw milk and cream, meat and meat products, oysters, vegetables, fish, and poultry (Barton et al., 2003). They have also been isolated from well water, streams, lakes, and soil.

5.18.6 Virulence and infectivity of Y. enterocolitica

There are 5 biotypes (described as biotype 1A, 1B, 2, 3, 4 and 5) and at least 60 O-antigen[32] 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 et al., 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 et al., 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

[31] ESR (2001) Fact sheet of *Yersinia enterocolitica*.
[32] Refers to lipopolysaccharide-protein somatic antigens of the microorganism.

North American biotype 1B strains carry a high pathogenicity island (HPI) on their chromosome, which enhances their virulence (Barton et al., 2003). In Australia, biotype O:3, O:6,30 have been reported in outbreak investigations (Butt et al., 1991).

5.18.7 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[33]) to 10^7 cells (Granum et al., 1995).

5.18.8 Immune status

Population most susceptible to yersiniosis and the subsequent complications are the very young, the debilitated, the very old and persons undergoing immunosuppressive therapy (CFSAN[34]). In 2000, notification rate of yersiniosis in Australia was 3.6 per 100,000 for the 0-4 years old sub-population (male) and 1.5 per 100,000 for 0-4 years old (female) sub-population, and the remaining populations was at 0 to 1 per 100,000 (Lin et al., 2002).

5.18.9 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. On the other hand, *Y. enterocolitica* could not be isolated from ripening hard goat's milk cheeses (Tornadijo et al., 1993) or Swiss-hard or semi-had cheeses made with raw milk (Bachmann et al., 1995). In the absence of competing microflora, *Y. enterocolitica* can multiply to high numbers in foods, such as pasteurised milk (Black et al., 1978). Presence of starter culture on the other hand, had an inhibitory effect on the growth of *Y. enterocolitica* in Turkish Feta cheese (Bozkurt et al., 2001). It has been demonstrated that the growth of *Y. enterocolitica* in milk could be inhibited by the presence of a bacteriocin producing *Y. kristensenii* (Toora et al., 1994) or propionicin producing *Propionibacterium thoenii* (Lyon et al., 1993).

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APPENDIX 6

Previous Risk Assessments on Microbiological Pathogens in Dairy Products

Organism	International Risk Assessment
Bacillus cereus	**Effect of dissolved carbon dioxide on thermal inactivation of microorganisms in milk**. Journal of Food Protection 65 (12) : 1924-1929, 2002 **Abstract:** Post pasteurization addition of CO_2 inhibits growth of certain microorganisms in dairy products, but few studies have investigated the effect of CO_2 on thermal inactivation of microorganisms during pasteurization. Concn. of CO_2, ranging from 44 to 58mM, added to raw whole milk significantly ($P < 0.05$) reduced the number of surviving standard plate count (SPC) organisms in milk heated over the range 67-93degreesC. A decrease in thermal survival rates (D-values) for Pseudomonas fluorescens RI-232 and Bacillus cereus ATCC 14579 spores in milk was positively correlated with CO2 concn. (1-36mM). D50degreesC-values for P. fluorescens significantly decreased ($P < 0.05$) in a linear fashion from 14.4 to 7.2 min. D89degreesC-values for B. cereus spores were significantly ($P < 0.05$) decreased from 5.56 min in control milk to 5.29 min in milk containing 33mM CO2. The Weibull function was used as a model to describe the thermal inactivation of P. fluorescens, B. cereus spores and SPC organisms in raw milk. Nonlinear parameters for the Weibull function were estimated, and survival data fitted to this model had higher R2 values than when fitted to the linear model, further providing support that the thermal inactivation of bacteria does not always follow first-order reaction rate kinetics. Results suggest that CO2 could be used as a processing aid to enhance microbial inactivation during pasteurization.
	A risk assessment study of Bacillus cereus present in pasteurized milk. Notermans S, Dufrenne J, Teunis P, Beumer R, te Giffel M, Peeters Weem P., Food Microbiology 14 (2) : 143-151, 1997 It is generally regarded that the presence of 105 toxigenic Bacillus cereus in pasteurized milk is hazardous to human health. A risk assessment study of the presence of B. cereus in pasteurized milk in the Netherlands was determined. Risk of exposure to B. cereus was determined using data collected on the storage conditions (temp. and time) of pasteurized milk in households and by storage tests at 6, 8, 10 and 12degreesC. Milk in households was held at <5-13degreesC and stored for 2-12 days. Storage tests revealed that spoilage occurred at 8, 10 and 12degreesC after 12, 8 and 6 days storage, respectively. Results suggested that >105 B. cereus/ml would be present in approx. 7% of the total portions of milk consumed. It is concluded that more information is required to evaluate human exposure to B. cereus in milk.
	Biological variability and exposure assessment. Delignette-Muller ML, Rosso L. Int J Food Microbiol. 2000 Jul 15;58(3):203-12. Predictive models are now commonly used for exposure assessment, with growth parameters defined for each microbial species. In this study, we tried to take into account microbial growth variability among strains of a single species. Bacillus cereus in pasteurized milk was chosen to illustrate the influence of the biological variability on the outcome of exposure assessment. Each parameter of the exposure assessment (growth parameters, shelf-life conditions) was characterized by a probability distribution describing variability and/or uncertainty. The impact of the intra-species variability on the result of the exposure assessment was then quantified and discussed. Two simple domestic shelf life conditions were tested. The results confirm that the biological variability has a great impact on the accuracy of the result and should not be systematically neglected.

	Bacillus sporothermodurans - a Bacillus forming highly heat-resistant spores. International Dairy Federation, Bulletin of the International Dairy Federation : No. 357, 3-27, 2000 **Abstract:** This monograph on research carried out on Bacillus sporothermodurans was originally presented at the International Dairy Federation Annual Sessions of 1998. After an introduction describing the problem of B. sporothermodurans contamination of UHT milk, a further 6 papers are included, covering: classification of milk isolates of B. sporothermodurans; isolation and methods of detection; pathogenicity/toxicology; heat resistance; UHT processes to inactivate heat-resistant sporeformers; and risk assessment/quality assurance.
Brucella	**A simulation model of brucellosis spread in British cattle under several testing regimes. England T, Kelly L, Jones RD, MacMillan A, Wooldridge M.** Prev Vet Med. 2004 Apr 30;63(1-2):63-73. Brucellosis is a widespread, economically devastating and highly infectious zoonosis. In cattle, infection predominantly is caused by Brucella abortus, and is usually detected in pregnant females through abortions. Great Britain (GB) has been declared free from brucellosis (officially brucellosis free (OBF)) since 1993 and as such is required by European Union (EU) regulations to test > or =20% of both beef and dairy cattle >24 months old routinely. Currently, however, GB serologically tests more cattle than required and the issue of reducing the level of testing has come under consideration. We developed a simulation model to determine the rate of spread of brucellosis under a variety of testing regimes. For dairy herds, we found that reducing the level of testing would have a major effect on the rate of spread of infection, should it be imported. For beef herds, reducing the level of testing would have much less effect. We also found that abortion notification is a very-important additional means of surveillance. As a result of our predictions, policy-makers decided not to reduce the level of testing and actively to promote abortion notification.
Campylobacter	**Savill M, Hudson A, Devane M, Garrett N, Gilpin B, Ball A. Elucidation of potential transmission routes of Campylobacter in New Zealand.** Water Sci Technol. 2003;47(3):33-8. Campylobacter is the most commonly reported notifiable disease in New Zealand. The cost of Campylobacter infections in the country during 1994 was estimated as dollar 61.7M although the true cost was probably higher. Investigation of the main environmental reservoirs and routes of transmission to humans is necessary to formulate the most appropriate intervention strategies. This project investigated the reservoirs of Campylobacter in a defined geographical area within New Zealand and compared strains isolated from humans and environmental sources within this area as a prelude to investigating the likely transmission routes to humans. Campylobacter jejuni was commonly found in faeces from dairy cows, beef cattle, sheep and ducks, chicken carcasses, sheep offal and surface waters and C. coli was commonly found in sheep faeces. Preliminary analysis of Penner types was suggestive of transmission to humans from dairy and beef cattle and possibly from sheep
	Environmental aspects of Campylobacter infections. Stelzer W, Jacob J, Schulze E. Zentralbl Mikrobiol. 1991;146(1):3-15. Epidemiological data indicate high incidence of campylobacteriosis. Improperly prepared poultry-products, unpasteurized milk as well as non-chlorinated drinking water were shown to be the main vehicles of Campylobacter transmission to man. There is a lack of knowledge concerning the role of various environments in transmission of Campylobacter. The review summarizes the present knowledge about occurrence and survival of Campylobacters in various environments (sewage, sludge, surface water, drinking water). In conclusion risk assessment for public health is discussed.
Coxiella burnetii	**Kloppert B, Wolter W, Zschock M, Kabisch D, Hamann HP, Frost JW. [Coxiella burnetii as zoonotic pathogen with special regard to food hygiene]** Dtsch Tierarztl Wochenschr. 2004 Aug;111(8):321-3 [Article in German] In Hesse, Germany, bulk milk of farms producing raw milk cheese is examined by PCR for Coxiella burnetii yearly. In 2003 the pathogen has been detected unusually frequent. By means of two examples the hygienic measures are shown, which were initiated by the veterinary administration. To detect Coxiella burnetii means always the preoccupation with unsolved questions. It is particularly uncertain, whether there is a risk of oral infection for the human being. From the point of view of food hygiene, surveys are needed urgently to work out a risk assessment. Based on this a uniform risk management and a reasonable risk communication can be fixed.
Enterobacter sakazakii	**Joint FAO/WHO Workshop on *Enterobacter Sakazakii* and Other Microorganisms in Powdered Infant Formula** http://www.who.int/foodsafety/publications/micro/feb2004/en/ **Source:** Food Safety Department, World Health Organization **Author:** Food and Agriculture Organization of the United Nations/World Health Organization **Summary:** This page links to the report and executive summary from this meeting held February 2-5, 2004 in Geneva. There is also a link to questions and answers regarding *Enterobacter sakazakii* in powdered infant formula. The report includes discussion of "Epidemiology and Public Health Aspects," "Hazard Identification," "Hazard Characterization," "Exposure Assessment," "Risk Characterization" for E. sakazakii and Salmonella enterica, "Risk Reduction Strategies for Formula-fed Infants," and more. Appendices include "List of Background Papers," "Data Received in Response to the FAO/WHO Call for Data," and "Risk Assessment," which discusses a risk

	assessment model for comparing the effectiveness of control measures for E. sakazakii and S. enterica in powdered infant formula **Resource type:** report, tables, charts, executive summary, fact sheet **Publication Date:** 2004
	<u>*Enterobacter sakazakii* in Powdered Infant Formula</u> http://www.who.int/foodsafety/fs_management/en/No_01_Esakazakii_Jan05_en.pdf **Source:** INFOSAN Information Note, No. 1/2005, 13 Jan. 2005/International Food Safety Authorities Network, World Health Organization **Author:** International Food Safety Authorities Network, World Health Organization **Summary:** Note to alert authorities to emerging issue of infections related to *E. sakazakii* in powdered infant formula, and to summarize current efforts toward determining the magnitude and resolution of the problem **Resource type:** notice **Publication Date:** January 13, 2005
	<u>Hazards Associated with *Enterobacter sakazakii* in the Consumption of Dairy Foods by the General Population</u> http://www.nzfsa.govt.nz/dairy/publications/information-papers/enterobacter-sak… **Source:** New Zealand Food Safety Authority **Summary:** Information for consumers about *Enterobacter sakazakii*. Contains links to other resources, including information regarding *E. sakazakii* in infant formula **Resource type:** fact sheet
	<u>Health Professionals Letter on *Enterobacter sakazakii* Infections Associated with Use of Powdered (Dry) Infant Formulas in Neonatal Intensive Care Units</u> http://www.cfsan.fda.gov/~dms/inf-ltr3.html **Source:** Office of Nutritional Products, Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition, Food and Drug Administration **Summary:** This letter describes the risk to newborns from *Enterobacter sukazakii* in milk-based powdered infant formulas. It is designed to communicate these risks to health care professionals, and provides steps to be taken to minimize the risk of infection from using this product **Resource type:** letter **Publication Date:** October 10, 2002
	<u>Isolation and Enumeration of *Enterobacter sakazakii* from Dehydrated Powdered Infant Formula</u> http://www.cfsan.fda.gov/~comm/mmesakaz.html **Source:** Center for Food Safety and Applied Nutrition, Food and Drug Administration **Summary:** This page provides methodology for testing for *Enterobacter sakazakii* in dehydrated powdered infant formula **Resource type:** web page **Publication Date:** August 2002
	<u>Joint FAO/WHO Workshop on *Enterobacter Sakazakii* and Other Microorganisms in Powdered Infant Formula</u> http://www.who.int/foodsafety/publications/micro/feb2004/en/ **Source:** Food Safety Department, World Health Organization **Author:** Food and Agriculture Organization of the United Nations/World Health Organization **Summary:** This page links to the report and executive summary from this meeting held February 2-5, 2004 in Geneva. There is also a link to questions and answers regarding *Enterobacter sakazakii* in powdered infant formula. The report includes discussion of "Epidemiology and Public Health Aspects," "Hazard Identification," "Hazard Characterization," "Exposure Assessment," "Risk Characterization" for E. sakazakii and Salmonella enterica, "Risk Reduction Strategies for Formula-fed Infants," and more. Appendices include "List of Background Papers," "Data Received in Response to the FAO/WHO Call for Data," and "Risk Assessment," which discusses a risk assessment model for comparing the effectiveness of control measures for E. sakazakii and S. enterica in powdered infant formula **Resource type:** report, tables, charts, executive summary, fact sheet **Publication Date:** 2004
	<u>Opinion of the Scientific Panel on Biological Hazards on the Request from the Commission Related to the Microbiological Risks in Baby Formulae and Follow-on Formulae</u> http://www.efsa.eu.int/science/biohaz/biohaz_opinions/691_en.html **Source:** EFSA Journal, Vol. 113, 2004, p. 1-35/Panel on Biological Hazards, European Food Safety Authority **Author:** Scientific Panel on Biological Hazards, European Food Safety Authority **Summary:** Opinion adopted on September 9, 2004 regarding microbiological risks in infant formulae and follow-on formulae, with emphasis on *E. sakazakii*. Includes discussion of hazard identification for *Salmonella*, *E. sakazakii*, and other microorganisms, hazard characterization, exposure assessment, and control measures **Resource type:** report **Publication Date:** November 17, 2004

	Risk Profile of *Enterobacter sakazakii* in Powdered Infant Formula ftp://ftp.fao.org/codex/ccfh35/fh03_13e.pdf **Source:** Food and Agriculture Organization of the United Nations **Author:** United States of America and Canada, Codex Committee on Food Hygiene, Codex Alimentarius Commission **Summary:** Risk profile for *Enterobacter sakazakii* in powdered infant formula, presented at the Thirty-fifth Session of the Codex Committee on Food Hygiene, held Jan. 27-Feb. 1, 2003, at Orlando, FL, USA **Resource type:** report **Publication Date:** January 2003
	Risk Profile of *Enterobacter sakazakii*, an Emergent Pathogen Associated with Infant Milk Formula http://dx.doi.org/10.1016/S0924-2244(03)00155-9 **Source:** Trends in Food Science and Technology, Vol. 14, Issue 11, Nov. 2003, p. 443-454/ScienceDirect **Author:** Iversen, C.; Forsythe, S. **Summary:** Risk profile for *Enterobacter sakazakii* in infant formula milk powder. Includes discussion of hazard identification, exposure assessment, hazard characterization, risk management, and detection methods. The abstract is freely available online, but access to the full text requires subscription or purchase **Resource type:** report **Publication Date:** August 01, 2003
Enterococci	**Antibiotic resistance and virulence traits of enterococci isolated from Baylough, an Irish artisanal cheese. Gelsomino R, Huys G, D'Haene K, Vancanneyt M, Cogan TM, Franz CM, Swings J.** J Food Prot. 2004 Sep;67(9):1948-52. Eight representative Enterococcus strains from a collection of over 600 previously isolated from an Irish artisanal cheese were subjected to phenotypic and genotypic analysis of antibiotic resistance and virulence properties. Genes encoding resistance to tetracycline (tet(M) and tet(L)) and/or erythromycin (erm(B)) were detected in five strains. In addition, all strains contained two or more of the virulence genes tested (agg, gel, cyl, esp, ace, efaAfs, and efaAfm). Further investigation into the transferability and environmental dissemination of these resistance and virulence traits will allow risk assessment and safety evaluation of artisanal cheeses.
	Phenotypic and genetic diversity of enterococci isolated from Italian cheeses. Andrighetto C, Knijff E, Lombardi A, Torriani S, Vancanneyt M, Kersters K, Swings J, Dellaglio F., Journal of Dairy Research 68 (2): 303-316, 2001 **Abstract:** This study aimed to identify the enterococci present in different Italian cheeses at the species and intra-species level and investigate some technologically relevant characteristics of these strains, in order to improve understanding of the significance and role of these microorganisms in dairy products. 124 enterococcal strains, isolated from traditional Italian cow, goat and buffalo cheeses, were characterized using phenotypic features and RAPD-PCR. RAPD-PCR profiles obtained with 4 primers and 5 different amplification conditions were compared by numerical analysis, and allowed inter- and intraspecific differentiation of the isolates. Whole-cell protein analysis by SDS-PAGE was used as a reference method for species identification. The strains were identified as Enterococcus faecalis (82 strains), E. faecium (27 strains), E. durans (9 strains), E. gallinarum (4 strains) and E. hirae (2 strains). Species recognition by means of RAPD-PCR was in agreement with SDS-PAGE results, except for 8 strains of E. faecium that clustered in separated groups. Phenotypic identification based on carbohydrate fermentation profiles, using the rapid ID 32 STREP galleries, gave different results from SDS-PAGE in 12.1% of cases. The majority of strains had weak acidifying and proteolytic activities in milk. 1 E. faecium strain showed the vanA (vancomycin resistance) genotype while 4 strains showed a beta-haemolytic reaction on human blood. Several strains showed antagonistic activity towards indicator strains of Listeria innocua, Clostridium tyrobutyricum and Propionibacterium freudenreichii subsp. shermanii. Results showed considerable genetic and phenotypic diversity among enterococcus strains isolated from Italian cheeses. It is concluded that information gained in this study could be used as a basis for selection of safe and useful strains for starters or protective cultures in cheese production.
Listeria monocytogenes	**Risk Assessment of *Listeria monocytogenes* in Ready-to-Eat Foods** http://www.fao.org/es/esn/food/risk_mra_riskassessment_listeria_en.stm **Source:** Food and Nutrition Division, Economic and Social Department, Food and Agriculture Organization of the United Nations **Author:** Food and Agriculture Organization of the United Nations/World Health Organization **Summary:** Interpretative summary and technical report of the FAO/WHO risk assessment of *Listeria monocytogenes* in RTE foods. The overall assessment included example risk assessments for the risk from *L. monocytogenes* in pasteurized milk, ice cream, fermented meats, and cold-smoked, vacuum-packed fish. The risk characterization includes discussion of the risk when different levels of *L. monocytogenes* are present in a food serving, the risks for susceptible population groups, and the effects of growth in foods on risk **Resource type:** report, summary

	Publication Date: 2004
	Comparison of the U.S. and FAO/WHO *Listeria monocytogenes* Risk Assessments http://www.jifsan.umd.edu/csl2003.htm **Source:** Joint Institute for Food Safety and Applied Nutrition **Author:** Buchanan, Robert L. **Summary:** This presentation describes both the FDA/FSIS and the FAO/WHO risk assessments for *Listeria monocytogenes*, and discusses differences between the two approaches. It was presented at the 2003 CSL/JIFSAN Joint Symposium - Food Safety and Nutrition: Risk Analysis **Resource type:** presentation **Publication Date:** June 12, 2003
	Survey of Listeria Monocytogenes in Ready to Eat Foods http://www.foodrisk.org/listeria_survey.htm **Source:** Food Safety Risk Analysis Clearinghouse **Author:** National Food Processors Association **Summary:** The National Food Processors Association (NFPA) conducted this study with the purpose of addressing uncertainties in risk assessment regarding the occurrence of L. Monocytogenes in ready to eat foods. NFPA collected and tested about 31,700 samples in eight different categories of ready to eat foods. Samples were collected and tested over a period of 14 -23 months from retail markets in Maryland and California Food Net sites. The product categories on the access database include fresh soft "Hispanic" style cheeses, bagged salads, blue veined and soft mold ripened cheeses, smoked seafood and seafood salad. Lunch meats and Deli salads will be included in the database in the near future. The downloadable access database includes information on the product, such as location packaged, geographic area, date listed, date of purchase, date of assay, if vacuum packaged, etc. Test information also includes presumptive positives, number of tubes that are turbid at the 1, 10 and 100 dilution, most probable number, presumptive colonies, cfu/g, etc. **Resource type:** database/dataset, database/documentation **Publication Date:** March 2003
	Quantitative Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods http://www.foodsafety.gov/~dms/lmr2-toc.html **Source:** Center for Food Safety and Applied Nutrition, Food and Drug Administration/Food Safety and Inspection Service, U.S. Department of Agriculture/Centers for Disease Control and Prevention **Summary:** Assessment of the risks of serious illness and death from *Listeria monocytogenes* in 23 categories of ready-to-eat foods **Resource type:** risk assessment, tables, charts **Publication Date:** September 2003
	Program Information Manual: Retail Food Safety: Date Marking of Cheese http://www.cfsan.fda.gov/~ear/ret-chdt.html **Source:** Center for Food Safety and Applied Nutrition, Food and Drug Administration **Author:** Beaulieu, Raymond D. **Summary:** This document is an interpretation of the need to date mark all cheeses as described in the Food Code Section 3-501.17. It specifies which cheeses are and are not exempt to the date marking provisions in the Food Code, based on their potential for supporting the growth of *L. monocytogenes* and other foodborne pathogens **Resource type:** policy document **Publication Date:** December 15, 1999
	Risk Profile: *Listeria monocytogenes* in Ice Cream http://www.nzfsa.govt.nz/science-technology/risk-profiles/lmono-in-ice-cream.pd… **Source:** New Zealand Food Safety Authority **Author:** Lake, Rob; Hudson, Andrew; Cressey, Peter/Institute of Environmental Science and Research Limited **Summary:** Profile of the risks associated with *Listeria monocytogenes* in ice cream in New Zealand. Includes each step of a qualitative risk assessment as well as other information that will be useful for risk management. Also includes an appendix entitled "Categories for Risk Profiles" **Resource type:** report, tables, charts **Publication Date:** October 2003
	Sanaa M, Coroller L, Cerf O.Risk assessment of listeriosis linked to the consumption of two soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux. Risk Anal. 2004 Apr;24(2):389-99. This article reports a quantitative risk assessment of human listeriosis linked to the consumption of soft cheeses made from raw milk. Risk assessment was based on data purposefully acquired inclusively over the period 2000-2001 for two French cheeses, namely: Camembert of Normandy and Brie of Meaux. Estimated Listeria monocytogenes concentration in raw milk was on average 0.8 and 0.3 cells/L, respectively, in Normandy and Brie regions. A Monte Carlo simulation was used to account for the time-temperature history of the milk and cheeses from farm to table. It was assumed that cell progeny did not spread within the solid cheese matrix (as they would be free to do

	in liquid broth). Interaction between pH and temperature was accounted for in the growth model. The simulated proportion of servings with no L. monocytogenes cell was 88% for Brie and 82% for Camembert. The 99th percentile of L. monocytogenes cell numbers in servings of 27 g of cheese was 131 for Brie and 77 for Camembert at the time of consumption, corresponding respectively to three and five cells of L. monocytogenes per gram. The expected number of severe listeriosis cases would be < or =10(-3) and < or =2.5 x 10(-3) per year for 17 million servings of Brie of Meaux and 480 million servings of Camembert of Normandy, respectively.
	Bunning VK, Crawford RG, Tierney JT, Peeler JT. Thermotolerance of heat-shocked Listeria monocytogenes in milk exposed to high-temperature, short-time pasteurization. Appl Environ Microbiol. 1992 Jun;58(6):2096-8. The effect of prior heat shock (48 degrees C for 15 min) on the thermotolerance of Listeria monocytogenes at the minimal high-temperature, short-time (71.7 degrees C for 15 s) parameters required by the Pasteurized Milk Ordinance was examined. The mean D71.7 degrees C value for heat-shocked L. monocytogenes was 4.6 +/- 0.5 s (control D = 3.0 +/- 1.0 s); the ratio of D to control D was 1.5. The increased thermotolerance of heat-shocked Listeria cells was not significant and appeared unlikely to have practical implications, in terms of risk assessment, for the safety of pasteurized milk.
	Farber JM, Ross WH, Harwig J. Health risk assessment of Listeria monocytogenes in Canada. Int J Food Microbiol. 1996 Jun;30(1-2):145-56. In this review, the major steps used in the formulation of a health risk assessment for Listeria monocytogenes in foods are discussed. Data is given on the numbers of human listeriosis cases reported in Canada along with the current Canadian regulatory policy on L. monocytogenes. Four major steps in the health risk assessment of this organism in foods, namely, hazard identification, hazard characterization, exposure assessment and risk characterization, were examined. For hazard characterization, since it is known that no direct human dose response data is available for L.monocytogenes, a flexible dose response model called the Weibull-Gamma model was evaluated. For the exposure assessment, pate and soft cheese, both high-risk foods in terms of listeriosis infection, were used as prototypes in some of the models that were used. Using disappearance data for cheese and 100 g as a typical serving, the data suggested an average of 102 servings per capita, per year in Canada. As a rough approximation, for L. monocytogenes, reference ID10 and ID90 dose levels of response for both normal and high risk populations were given as 10(7) and 10(9) for normal individuals, and 10(5) and 10(7) for high-risk people. The corresponding dose response models were graphically displayed. These models exhibited a higher degree of susceptibility and less host/pathogen heterogeneity for the higher risk group. The range of doses between the ID10 and ID90 reference values corresponded roughly to levels associated with cases of listeriosis. In the risk characterization stage, dose response data was combined with some predictive growth modeling data of L. monocytogenes on pate, assuming an initial exposure of a single cell for food stored at 4 degrees and 8 degrees C. Storage of pate at 4 degrees C for more than 35 days resulted in a rapidly increasing risk for the high risk population, while storage at 8 degrees C produced a similar risk after about 13 days. In addition, an equation, used to calculate the average probability of acquiring human listeriosis in Canada from soft and semi-soft cheese consumption, was formulated. Computations derived from this equation indicated a substantial level consistency between reported data and assumptions of the risk assessment model. An important part of risk characterization or possibly risk management is characterizing the economic and social consequences of estimated risks. The total annual estimated cost of listeriosis illnesses and deaths in Canada was estimated to be between 11.1 and 12.6 million dollars.
	Estimation of uncertainty and variability in bacterial growth using Bayesian inference. Application to Listeria monocytogenes. Pouillot R, Albert I, Cornu M, Denis JB. Int J Food Microbiol. 2003 Mar 15;81(2):87-104. The usefulness of risk assessment is limited by its ability or inability to model and evaluate risk uncertainty and variability separately. A key factor of variability and uncertainty in microbial risk assessment could be growth variability between strains and growth model parameter uncertainty. In this paper, we propose a Bayesian procedure for growth parameter estimation which makes it possible to separate these two components by means of hyperparameters. This model incorporates in a single step the logistic equation with delay as a primary growth model and the cardinal temperature equation as a secondary growth model. The estimation of Listeria monocytogenes growth parameters in milk using literature data is proposed as a detailed application. While this model should be applied on genuine data, it is highlighted that the proposed approach may be convenient for estimating the variability and uncertainty of growth parameters separately, using a complete predictive microbiology model.
	Quantitative risk assessment of Listeria monocytogenes in ready-to-eat foods: the FAO/WHO approach. Rocourt J, BenEmbarek P, Toyofuku H, Schlundt J. FEMS Immunol Med Microbiol. 2003 Apr 1;35(3):263-7. Quantitative microbiological risk assessment is a very new and unique scientific approach able to link, for the first time, data from food (in the farm-to-fork continuum) and the various data on human disease to provide a clear estimation of the impact of contaminated food on human public health. The Food and Agriculture Organization of the United Nations (FAO) and the World Health

	Organization (WHO) have recently launched risk assessment studies of a number of pathogen-food commodity combinations (Salmonella in eggs and in broiler chickens, Listeria monocytogenes in ready-to-eat foods, Campylobacter in broiler chickens, Vibrio in seafood) to be used to lower the risk associated with these food-borne diseases and ensure fair practices in the international trade of food. The FAO/WHO Listeria risk assessment was undertaken in part to determine how previously developed risk assessments done at the national level could be adapted or expanded to address concerns related to L. monocytogenes in ready-to-eat foods at an international level. In addition, after initiation of the risk assessment, the risk assessors were asked by the Codex Committee on Food to consider three specific questions related to ready-to-eat foods in general, which are: (1). estimate the risk for consumers in different susceptible populations groups (elderly, infants, pregnant women and immunocompromised patients) relative to the general population; (2). estimate the risk for L. monocytogenes in foods that support growth and foods that do not support growth under specific storage and shelf-life conditions; (3). estimate the risk from L. monocytogenes in food when the number of organisms ranges from absence in 25 g to 1000 colonies forming units per gram or milliliter, or does not exceed specified levels at the point of consumption. To achieve these goals, new dose-response relationships and exposure assessments for ready-to-eat foods were developed. Preliminary data indicate that eliminating the higher dose levels at the time of consumption has a large impact on the number of predicted cases.
	Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk. Bemrah N, Sanaa M, Cassin MH, Griffiths MW, Cerf O. Prev Vet Med. 1998 Dec 1;37(1-4):129-45. Microbial hazards have been identified in soft cheese made from raw milk. Quantification of the resulting risk for public health was attempted within the frame of the Codex Alimentarius Commission, 1995 approach to quantitative risk assessment, using Monte Carlo simulation software. Quantitative data could only be found for Listeria monocytogenes. The complete process of cheese making was modeled, from milking to consumption. Using data published on the different sources of milk contamination (environment and mastitis) and bacterial growth, distributions were assumed for parameters of the model. Equations of Farber, J.M., Ross, W.H., Harwing, J. (1996) for general and at-risk populations were used to link the ingested dose of L. monocytogenes to the occurrence of listeriosis. The probability of milk contamination was estimated to be 67% with concentration ranging from 0 to 33 CFU ml-1. The percentage of cheese with a predicted concentration of L. monocytogenes greater than 100 CFU g-1 was low (1.4%). The probability of consuming a contaminated cheese serving was 65.3%. Individual annual cumulative risk of listeriosis, in a population each consuming 50 servings of 31 g, ranged from 1.97 x 10(-9) to 6.4 x 10(-8) in a low-risk sub-population and 1.04 10(-6) to 7.19 10(-5) in a high-risk sub-population. The average number of expected cases of listeriosis per year was 57 for a high-risk sub-population and one for a low-risk healthy sub-population. When the frequency of environmental milk contamination was reduced in the model and L. monocytogenes mastitis was eliminated, the expected incidence of listeriosis decreased substantially; the average number of expected cases was reduced by a factor of 5. Thus the usefulness of simulation to demonstrate the efficiency of various management options could be demonstrated, even if results should be interpreted with care (as many assumptions had to be made on data and their distributions.
	Studies on the risk assessment of Listeria monocytogenes. Notermans S, Dufrenne J, Teunis P, Chackraborty T. J Food Prot. 1998 Feb;61(2):244-8. Humans are frequently exposed to Listeria monocytogenes, and high numbers may be ingested during consumption of certain types of food. However, epidemiological investigations show that listeriosis is a rare disease. Risk assessment studies using an animal mouse model indicate that almost all L. monocytogenes serovars present in food have clear virulent properties. The intravenous dose causing infection in 50% (IV ID50) of mice not previously exposed to L. monocytogenes (nonprotected mice) was 1.8 log(10) units. For mice previously exposed to L.monocytogenes (immunologically protected mice was >9.0 log10 5.6 log(10) units. The ID(50) of orally exposed nonprotected mice amounted to 6.5 log10 units, and no significant effects of type of food (water/milk) and storage time at 5 degrees C (milk) were observed. The oral ID50 of immunologically protected mice was >9.0 log10 units. Furthermore, there was approximately 1-2 log10 difference between the ID50 and the lethal dose causing death in 50% (LD50). The results show that both the intestinal barrier and the specific immune defense mechanism are highly effective in preventing infection of mice orally exposed to L.monocytogenes. Delaying the immune defense had no effect on the protective activity of the intestinal barrier, indicating that these protective mechanisms independently. The risk assessment results obtained in the mouse model support the epidemiological finding that listeriosis is a rare disease in humans, despite frequent exposure to the organism.
	Predictive modelling of inactivation of Listeria spp. in bovine milk during high-temperature short-time pasteurization. Piyasena P, Liou S, McKellar RC. Int J Food Microbiol. 1998 Feb 17;39(3):167-73. A linear model was derived to describe the thermal inactivation of Listeria innocua in bovine whole milk in a high-temperature short-time pilot scale pasteurizer. Integrated lethal effect, or pasteurization effect (PE), was obtained by converting times at different temperatures in the various

	sections of the pasteurizer to the equivalent time at the reference temperature (72 degrees C). PE was then related by a simple linear function to the log10 of the % viable counts with a power transformation of the PE values to improve the linear fit. R2 values for the five L. innocua trials varied from 0.728 to 0.974. Validation of this model with Listeria monocytogenes confirmed that L. monocytogenes was more heat sensitive. Inter-trial variation was incorporated into the model using the @RISK simulation software. Output from simulations confirmed that pasteurization at the IDF standard conditions of 72 degrees C for 15 sec can ensure at least an 11-log reduction of L. monocytogenes. The results showed that L. innocua may be used as a model microorganism to assess the thermal inactivation of L. monocytogenes, since its heat resistance is at least equal to or greater than that of the pathogenic species.
	Health risk assessment of Listeria monocytogenes in Canada. **Farber JM, Ross WH, Harwig J.** Int J Food Microbiol. 1996 Jun;30(1-2):145-56. In this review, the major steps used in the formulation of a health risk assessment for Listeria monocytogenes in foods are discussed. Data is given on the numbers of human listeriosis cases reported in Canada along with the current Canadian regulatory policy on L. monocytogenes. Four major steps in the health risk assessment of this organism in foods, namely, hazard identification, hazard characterization, exposure assessment and risk characterization, were examined. For hazard characterization, since it is known that no direct human dose response data is available for L.monocytogenes, a flexible dose response model called the Weibull-Gamma model was evaluated. For the exposure assessment, pate and soft cheese, both high-risk foods in terms of listeriosis infection, were used as prototypes in some of the models that were used. Using disappearance data for cheese and 100 g as a typical serving, the data suggested an average of 102 servings per capita, per year in Canada. As a rough approximation, for L. monocytogenes, reference ID10 and ID90 dose levels of response for both normal and high risk populations were given as 10(7) and 10(9) for normal individuals, and 10(5) and 10(7) for high-risk people. The corresponding dose response models were graphically displayed. These models exhibited a higher degree of susceptibility and less host/pathogen heterogeneity for the higher risk group. The range of doses between the ID10 and ID90 reference values corresponded roughly to levels associated with cases of listeriosis. In the risk characterization stage, dose response data was combined with some predictive growth modeling data of L. monocytogenes on pate, assuming an initial exposure of a single cell for food stored at 4 degrees and 8 degrees C. Storage of pate at 4 degrees C for more than 35 days resulted in a rapidly increasing risk for the high risk population, while storage at 8 degrees C produced a similar risk after about 13 days. In addition, an equation, used to calculate the average probability of acquiring human listeriosis in Canada from soft and semi-soft cheese consumption, was formulated. Computations derived from this equation indicated a substantial level consistency between reported data and assumptions of the risk assessment model. An important part of risk characterization or possibly risk management is characterizing the economic and social consequences of estimated risks. The total annual estimated cost of listeriosis illnesses and deaths in Canada was estimated to be between 11.1 and 12.6 million dollars.
	Risk assessment of L. monocytogenes in Swiss Emmental cheese. Aebi R, Muehlemann M, Buehlmann G, Schaellibaum M., AgrarForschung 10 (8) : 306-311, 2003 **Language of Text:** German **Language of Summary:** English, French **Abstract:** Risk assessment of Listeria monocytogenes in Swiss Emmental cheese is discussed. Origin and spread of L. monocytogenes contamination through the whole production chain from raw milk to the final product reaching the consumer were assessed; a contamination profile of production and distribution was developed. The main factor governing reduction in L. monocytogenes count during cheesemaking was heat treatment temp. L. monocytogenes contamination of the rind may be reduced by rind removal or specific treatments. Presence of L. monocytogenes in the retail product is mainly due to recontamination during packaging, distribution, etc. It is concluded that consumers may be exposed to L. monocytogenes counts of 1-10 per cheese portion, and that consumption of traditionally made Emmental cheese presents an extremely low hazard.
Mycobacterium bovis	**Risk Profile: *Mycobacterium bovis* in Milk** http://www.nzfsa.govt.nz/science-technology/risk-profiles/mycobacterium-bovis-i… **Source:** New Zealand Food Safety Authority **Author:** Lake, Rob; Hudson, Andrew; Cressey, Peter/Institute of Environmental Science and Research Limited **Summary:** This risk profile includes elements of a qualitative risk assessment and other information that will be useful to risk managers. Includes an appendix entitled "Categories for Risk Profiles" **Resource type:** report, tables **Publication Date:** October 2002
Mycobacterium paratuberculosis	***Mycobacterium paratuberculosis* and Milk** http://www.ifst.org/hottop23.htm **Source:** Institute of Food Science and Technology

	Summary: This document provides an overview of *M. paratuberculosis*, including pathogenicity, potential sources for human infection, association with Crohn's disease, and heat resistance **Resource type:** report **Publication Date:** August 19, 1998
	***Mycobacterium paratuberculosis* -- Another Emerging Pathogen of the Human Gastrointestinal Tract?** http://www.wisc.edu/fri/briefs/paratb.htm **Source:** Food Research Institute, University of Wisconsin-Madison **Author:** Doyle, M. Ellin **Summary:** Review of information on the potential role of *Mycobacterium paratuberculosis* in causing Crohn's Disease. Includes discussion of possible vehicles of transmission **Resource type:** literature review **Publication Date:** April 1997
	Mycobacterium bovis versus Mycobacterium tuberculosis as a cause of acute cervical lymphadenitis without pulmonary disease. Fennelly GJ. Pediatr Infect Dis J. 2004 Jun;23(6):590-1. Bovine tuberculosis remains a common disease of cattle in countries such as Mexico. Children eating unpasteurized dairy products from Mexican cattle can develop Mycobacterium bovis cervical lymphadenitis. However, the bovine mycobacterium can be misdiagnosed as Mycobacterium tuberculosis based on standard laboratory testing. Accurate speciation is important for selection of the preferred antibiotic regimen for treatment of Mycobacterium bovis infection.
	Effects of prevalence and testing by enzyme-linked immunosorbent assay and fecal culture on the risk of introduction of Mycobacterium avium subsp. paratuberculosis-infected cows into dairy herds. Carpenter TE, Gardner IA, Collins MT, Whitlock RH. J Vet Diagn Invest. 2004 Jan;16(1):31-8. A stochastic simulation model was developed to assess the risk of introduction of Mycobacterium avium subsp. paratuberculosis infection into a dairy herd through purchase of female replacement cattle. The effects of infection prevalence in the source herd(s), number of females purchased, and testing by enzyme-linked immunosorbent assay (ELISA) alone or ELISA and fecal culture as risk mitigation strategies were evaluated. Decisions about negative test results were made on a lot and individual basis. A hypothetical dairy herd, free from M. a. paratuberculosis, which replaced 1 lot (10, 30, or 100) of cows per year, was considered. Probability distributions were specified for the sensitivities and specificities of ELISA and fecal culture, the proportion of infected herds and within-herd prevalence for randomly selected replacement source herds (high prevalence) and herds in level 2 (medium prevalence) and level 3 (low prevalence) of the Voluntary Johne's Disease Herd Status Program (VJDHSP). Simulation results predicted that 1-56% of the lots had at least 1 M. a. paratuberculosis-infected cow. Assuming that ELISA sensitivity was 25%, simulation results showed on a lot basis that between 0.4% and 18% and between 0.1% and 9% were predicted to have at least 1 infected cow not detected by ELISA and by a combination of ELISA and fecal culture, respectively. On an individual cow basis, between 0.1% and 8.3% of ELISA-negative cattle in ELISA-positive lots were estimated to be infected. In both the lot and individual analyses, the probability of nondetection increased with larger lot sizes and greater prevalence. Sensitivity analysis indicated that the effect of a lower ELISA sensitivity (10%) was a variable decrease in mean detection probabilities for all combinations of prevalence and lot size. The benefit of testing introduced cattle with ELISA alone or in combination with fecal culture was found to be minimal if cows were purchased from known, low-prevalence (level 3) herds. The value of testing by ELISA alone or in combination with fecal culture was greatest in high-prevalence herds for all lot sizes. Testing of random-source cattle, bought as herd replacements, can partially mitigate the risk of introduction of M. a. paratuberculosis but not as well as by using low-prevalence source herds (level-3 VJDHSP), with or without testing.
	Biosecurity on dairy operations: hazards and risks. Wells SJ. J Dairy Sci. 2000 Oct;83(10):2380-6. The objective here was to present a model for considering biosecurity related to infectious diseases on US dairy operations using a risk assessment framework. With the example of an important dairy cattle pathogen (Mycobacterium paratuberculosis), I followed risk assessment steps to characterize risks related to the use of certain management practices and possible risk reduction within an infectious disease biosecurity program. Biosecurity practices focus on the prevention of introduction of these pathogens to the dairy, and estimates of the risks associated with introduction of different sources of cattle are presented. In addition, biosecurity practices also limit the transmission of these pathogens within an infected dairy operation, especially those focused on sick cow management, calving area management, and manure management. Recent information from the National Animal Health Monitoring System (NAHMS) Dairy 96 Study indicates that many of these practices have not been adopted on US dairy operations, indicating both risk of disease and opportunity for animal health improvement.
	Significance of Mycobacterium paratuberculosis in milk. Hammer P, Knappstein K, Hahn G., Bulletin of the International Dairy Federation : No. 330, 12-16, 1998 The presence of Mycobacterium paratuberculosis in milk and possible links with Crohn's disease in

	humans are discussed. Aspects covered include: taxonomy and biological characteristics; Johne's disease, caused by M. paratuberculosis in all types of domestic and wild ruminants and in some species of laboratory animals; Crohn's disease (incidence, possible causative factors, transmission); heat resistance of M. paratuberculosis, with respect to survival under pasteurization conditions; risk assessment on M. paratuberculosis in pasteurized milk according to Codex Alimentarius procedures; and future research requirements.
Pseudomonas	**Researchers Work to Arrest Spoilage Organisms' Progress** http://www.cheesemarketnews.com/articlearch_old/2002/01mar02/01mar02_04.html **Source:** Cheese Market News Article Archive, March 1, 2002 **Author:** Sander, Kate **Summary:** This article describes the work of Dr. Kathryn Boor and Dr. Martin Wiedmann on the tracking and identification of spoilage organisms in dairy products, including research on fingerprinting methods for Pseudomonas spp. and Listeria monocytogenes **Resource type:** release **Publication Date:** March 01, 2002
Staphylococcus	**Quantitative microbial risk assessment exemplified by Staphylococcus aureus in unripened cheese made from raw milk. Lindqvist R, Sylven S, Vagsholm I.** Int J Food Microbiol. 2002 Sep 15;78(1-2):155-70. This paper discusses some of the developments and problems in the field of quantitative microbial risk assessment, especially exposure assessment and probabilistic risk assessment models. To illustrate some of the topics, an initial risk assessment was presented, in which predictive microbiology and survey data were combined with probabilistic modelling to simulate the level of Staphylococcus aureus in unripened cheese made from raw milk at the time of consumption. Due to limited data and absence of dose-response models, a complete risk assessment was not possible. Instead, the final level of bacteria was used as a proxy for the potential enterotoxin level, and thus the potential for causing illness. The assessment endpoint selected for evaluation was the probability that a cheese contained at least 6 log cfu S. aureus g(-1) at the time of consumption; the probability of an unsatisfactory cheese, P(uc). The initial level of S. aureus, followed by storage temperature had the largest influence on P(uc) at the two pH-values investigated. P(uc) decreased with decreasing pH and was up to a factor of 30 lower in low pH cheeses due to a slower growth rate. Of the model assumptions examined, *i.e.* the proportion of enterotoxigenic strains, the level of S. aureus in non-detect cheeses, the temperature limit for toxin production, and the magnitude and variability of the threshold for an unsatisfactory cheese, it was the latter that had the greatest impact on P(uc). The uncertainty introduced by this assumption was in most cases less than a factor of 36, the same order of magnitude as the maximum variability due to pH. Several data gaps were identified and suggestions were made to improve the initial risk assessment, which is valid only to the extent that the limited data reflected the true conditions and that the assumptions made were valid. Despite the limitations, a quantitative approach was useful to gain insights and to evaluate several factors that influence the potential risk and to make some inferences with relevance to risk management. For instance, the possible effect of using starter cultures in the cheese making process to improve the safety of these products.
	Staphylococcus aureus in raw milk and human health risk. Zecconi A, Hahn G., Bulletin of the International Dairy Federation : No. 345, 15-18, 1999 Staphylococcus aureus in raw milk and the associated human health risks are discussed. Aspects considered include: S. aureus identification; S. aureus polymorphism; S. aureus enterotoxins; and risk assessment for S. aureus in raw milk cheese. It is concluded that control measures should be implemented to reduce the prevalence of S. aureus in dairy cattle and thus the risk of toxins in raw milk and its products. [This paper was presented at a conference entitled Quality and safety of raw milk and its impact on milk and milk products, held in Athens, Greece, in Sept. 1999.]

Salmonella	**Salmonella and other Enterobacteriaceae in dairy-cow feed ingredients: antimicrobial resistance in western Oregon. Kidd RS, Rossignol AM, Gamroth MJ.** J Environ Health. 2002 Oct;65(3):7, 21. Several studies have suggested an association between the use of antimicrobial agents in animal feeds and an increased risk that humans will contract resistant strains of bacteria such as Salmonella species, Escherichia coli, and other enteric isolates. The authors of this study evaluated whether animal feeds might serve as sources of antimicrobial-resistant bacteria, especially bacteria that are pathogenic to humans. From July through August 1998, samples of feed ingredients were collected from a total of 50 feed piles located on 12 dairy farms in western Oregon. From a subset of 10 piles, repeated samples were collected over time until each pile was depleted. Analysis of the samples indicated that 42.0 percent of all 50 piles and 60.0 percent of the piles from which there was repeated sampling were presumptive positive for Salmonella. Sixty-two percent of 50 Enterobacteriaceae isolates showed ampicillin resistance, and 10.0 percent displayed tetracycline resistance. Other bacteria displayed varying degrees of resistance to ampicillin, streptomycin, tetracycline, or a combination of these antimicrobials. The extent of antimicrobial-resistant Enterobacteriaceae in feed ingredients observed in this study raises significant concerns about the potential for human health risks from food-producing animals such as dairy cows.
Yersinian	**Emerging food pathogens and bacterial toxins. Bielecki J.** Acta Microbiol Pol. 2003;52 Suppl:17-22. Many different foodborne diseases have been described. For example, Shigella bacteria, hepatitis A virus and Norwalk virus were shown as a unwashed hands microorganisms, but pathogen Campylobacter and Escherichia coli were named as raw and undercooked meat and poultry or raw milk and untreated water born bacteria. However, two of them: Listeria monocytogenes and Yersinia enterocolitica are known as growing at refrigerator temperatures. Essential virulence determinants of Listeria monocytogenes pathogenicity are well known as a bacterial toxins. Basic molecular mechanisms of pathogenicity depending from these toxins were presented. It was shown that other bacterial toxins may act as very danger food poisoning substances. This is why elimination of pathogenic microorganisms from foods is an obvious solution in some food processes, however this approach is not practical or even desirable in many processes. Thus, risk assessment and microbial monitoring will continue to play important roles in ensuring food safety. Some technological advances have the capability of delivering detection systems that can not only monitor pathogenic microorganisms, but also entire microbial populations in the food matrix.
General	MICROORGANISMS **IN** DAIRY-PRODUCTS **- FRIENDS AND FOES** **Author(s):** KEOGH BP **Publisher:** DAIRY INDUSTRY ASSN AUSTRALIA, PO BOX 20, HIGHETT VICTORIA 3190, AUSTRALIA **Subject Category:** AGRICULTURE, DAIRY & ANIMAL SCIENCE; FOOD SCIENCE & TECHNOLOGY **Source:** AUSTRALIAN JOURNAL OF DAIRY TECHNOLOGY 33 (2): 41-45 1978
	Microbial Pathogen Data Sheets http://www.nzfsa.govt.nz/science-technology/data-sheets/index.htm **Source:** New Zealand Food Safety Authority **Author:** Institute of Environmental Science and Research Limited **Summary:** Fact sheets on foodborne pathogens. Sections include "The Organism/Toxin," "Growth and Its Control," "The Illness," "Sources," "Outbreaks and Incidents," "Adequate Processing Guidelines," and others. Fact sheets may be available for *Bacillus cereus*, *Campylobacter*, *Clostridium botulinum*, *Clostridium perfringens*, *Cryptosporidium parvum*, enteric viruses, *E. coli* O157:H7, non-O157 STEC, *Giardia intestinalis*, hepatitis A virus, *Listeria monocytogenes*, *Mycobacterium bovis*, Norwalk-like viruses, *Salmonella* Typhi, non-typhoid *Salmonellae*, scombroid poisoning, *Shigella*, *Staphylococcus aureus*, *Toxoplasma gondii*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Yersinia enterocolitica* **Resource type:** fact sheets
	Microbiological risk analysis of milk and milk products in international trade. Kelly PM, Farm & Food 7 (3) : 23-28, 1997 **Abstract:** The Sanitary and Phytosanitary (SPS) Agreement arising from the latest initiatives of the World Trade Organisation (WTO) is a major challenge to food exporters and in particular to the Irish **dairy** industry. SPS compliance demands that food microbiological specifications are established on a scientific basis rather than on the current HACCP basis. Hazards analysis for milk and **dairy** products is explored in an attempt to explain the concept of risk analysis, procedures to be used, impact on the Irish **dairy** industry, and the work of the International **Dairy** Federation (IDF). Aspects considered include: definitions (hazard, risk, risk assessment); revised principles for establishment and application of microbiological criteria; WTO agreements on SPS and Technical Barriers to Trade (TBT); establishment of an expert group to study **microbiological risk assessment**; and examples illustrating the principles (Listeria monocytogenes (effective management strategies, criteria) and enterohaemorrhagic Escherichia coli (EHEC; effective management strategies, microbiological criteria)).

Other relevant information

Cheese	**Food Safety and Cheese: Institute of Food Science and Technology Position Statement** http://drinc.ucdavis.edu/dfoods5.htm **Source:** Dairy Research and Information Center, University of California, Davis **Author:** Professional Food Microbiology Group, Institute of Food Science and Technology **Summary:** This page gives a description of bacterial illnesses associated with cheese contamination and their effects on humans, possible pathways for cheeses to become hosts of these bacteria, and measures to ensure the production of safe cheese **Resource type:** statement **Publication Date:** November 15, 1996
	Food Safety and Cheese http://www.ifst.org/hottop15.htm **Source:** Institute of Food Science and Technology **Summary:** This report describes the hazards to human health associated with cheese. It includes discussion of foodborne outbreaks associated with cheese, the microbiological safety of cheese, control measures, and consumer awareness of health risks associated with cheese **Resource type:** report **Publication Date:** April 16, 1998
	Program Information Manual: Retail Food Safety: Date Marking of Cheese http://www.cfsan.fda.gov/~ear/ret-chdt.html **Source:** Center for Food Safety and Applied Nutrition, Food and Drug Administration **Author:** Beaulieu, Raymond D. **Summary:** This document is an interpretation of the need to date mark all cheeses as described in the Food Code Section 3-501.17. It specifies which cheeses are and are not exempt to the date marking provisions in the Food Code, based on their potential for supporting the growth of *L. monocytogenes* and other foodborne pathogens **Resource type:** policy document **Publication Date:** December 15, 1999
Data	***Escherichia coli* O157 on U.S. Dairy Operations** http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/dairy/Dairy02/Dairy02Ecoli.pdf **Source:** National Animal Health Monitoring System Program Unit, National Center for Animal Health Surveillance, Centers for Epidemiology and Animal Health, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture **Summary:** Brief report on the presence of *E. coli* O157 on U.S. dairy operations, from the Dairy 2002 study **Resource type:** report, charts **Publication Date:** December 2003
	***Salmonella* and *Listeria* in Bulk Tank Milk on U.S. Dairies** http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/dairy/Dairy02/Dairy02bulktank.pdf **Source:** National Animal Health Monitoring System Program Unit, National Center for Animal Health Surveillance, Centers for Epidemiology and Animal Health, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture **Summary:** Report from the National Animal Health Monitoring System Dairy 2002 study on the prevalence of *Salmonella* and *Listeria* in bulk tank milk on U.S. dairy operations **Resource type:** report, charts **Publication Date:** December 2003
	***Salmonella* and *Campylobacter* on U.S. Dairy Operations** http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/dairy/Dairy02/Dairy02SalCampy.pdf **Source:** National Animal Health Monitoring System Program Unit, National Center for Animal Health Surveillance, Centers for Epidemiology and Animal Health, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture **Summary:** Report on results from Dairy 2002 and previous studies on *Salmonella* and *Campylobacter* in cow feces from dairy operations **Resource type:** report, chart, table **Publication Date:** December 2003
	Home Delivery of Perishable Foods Project

	http://www.health.vic.gov.au/foodsafety/research/microbiological.htm **Source:** Food Safety Unit, Public Health Group, Rural and Regional Health and Aged Care Services Division, Department of Human Services, Victorian State Government **Author:** Microbiological Diagnostic Unit, Public Health Laboratory, University of Melbourne **Summary:** Report of a study on the potential for pathogen growth in home delivered foods. The study included determination of delivery times and temperature profiles for home delivered foods, challenge tests for the growth of *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* on **cheese**, sliced meat, savoury pastry, fruit, and **milk**, comparison of challenge test results with predictive models, and application of predictive models to the home delivery data. Appendices include "Temperature measurement trials - comparison of core vs surface temperature measurements," "Temperature profiles for Home deliveries," and "Challenge test results" **Resource type:** report, tables, charts **Publication Date:** November 2003
	Microbe growth in custard and cream products http://www.health.vic.gov.au/foodsafety/research/microbiological.htm **Source:** Food Safety, Victoria Government Health Information
Goat milk ***Enterobacter, Staphylococcus, Campylobacter,* EHEC, *Salmonella, Mycobacterium***	**Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. Muehlherr JE, Zweifel C, Corti S, Blanco JE, Stephan R.** J Dairy Sci. 2003 Dec;86(12):3849-56. A total of 407 samples of bulk-tank milk (344 of goat's milk and 63 of ewe's milk) collected from 403 different farms throughout Switzerland, was examined. The number of farms investigated in this study represents 8% of the country's dairy-goat and 15% of its dairy-sheep farms. Standard plate counts and Enterobacteriaceae counts were performed on each sample. Furthermore, the prevalence of Staphylococcus aureus, Campylobacter spp., Shiga toxin-producing Escherichia coli, Salmonella spp., and Mycobacterium avium ssp. paratuberculosis was studied. The median standard plate count for bulk-tank milk from small ruminants was 4.70 log cfu/ml (4.69 log cfu/ml for goat's milk and 4.78 log cfu/ml for ewe's milk), with a minimum of 2.00 log cfu/ml and a maximum of 8.64 log cfu/ml. Enterobacteriaceae were detected in 212 (61.6%) goat's milk and 45 (71.4%) ewe's milk samples, whereas S. aureus was detected in 109 (31.7%) samples of goat's milk and 21 (33.3%) samples of ewe's milk. Campylobacter spp. and Salmonella spp. were not isolated from any of the samples. However, 16.3% of the goat's milk and 12.7% of the ewe's milk samples were polymerase chain reaction (PCR)-positive for Shiga toxin-producing E. coli. Seventy-nine (23.0%) goat's tank-milk and 15 (23.8%) ewe's tank-milk samples were PCR-positive for insertion sequence 900, providing presumptive evidence for the presence of M. avium ssp. paratuberculosis. These results form the basis for determining the microbiological quality standards for goat's and ewe's milk. Moreover, the data presented form part of the risk assessment program for raw milk from small ruminants in Switzerland.
Milk powder	**Drying risk assessment strategies.** Markowski AS, Mujumdar AS, Drying Technology 22 (1-2) : 395-412, 2004 **Abstract:** Drying risks assessment strategies are discussed with particular reference to the methodology involved and the various tools used at risk assessment. The methodology suggested is to apply qualitative risks assessment with risks estimation used for qualitative ranking of recommendations. The methodology follows, to a large extent, the general procedure for risks assessment of machinery and the application of the modified 'what if' hazard method with the risks estimation by a multi-layer risk matrix technique. A case study of risks assessment for the typical 2-stage spray-fluid bed dryer to produce milk powder is presented. Analysis indicated how an acceptable risk level may be achieved by the introduction of risks reduction options.
Farm	**Pathogens and manure management systems: a review. Bicudo JR, Goyal SM.** Environ Technol. 2003 Jan;24(1):115-30. There has been an increasing concern about the effects of pathogens that are present in animal manure on humanand animal health. In recent years, outbreaks of food-borne diseases associated with the consumption of animal products havereceived much attention from the media in North America and Europe, leading to increased consumer concerns about the safety of their food supply. The health risks associated with animal operations depend on various factors. The most important ones appear to be related to the animal species being reared and the concentration of pathogenic microorganisms in animal manure.

	The ability of the pathogens to survive for long periods and through treatment to remain infective in the environment until ingested by human or animal host is an added concern. On the other hand, the role of livestock in most waterborne bacterial outbreaks has often been difficult to clarify since both humans and various wildlife species can shed the same microorganisms and thereby serve as sources of infection. This paper summarizes existing information on the main microbial pathogens present in livestock wastes, and discusses the impact of livestock wastes and agricultural drainage on microbiological quality of water, as well as available management and treatment technologies to minimize the prevalence of pathogens in animal wastes. Despite the fact that most disease outbreaks have been associated with food poisoning by cross-contamination during meat or milk processing and during finished product storage this review shows that a number of best management practices and technical solutions have been developed in the last few years that can be effective tools in minimizing the spread of pathogens from livestock operations in the environment.
FMD and BSE/TSE	**Statement of the EFSA Scientific Expert Working Group on BSE/TSE of the BIOHAZ Panel on the Health Risks of the Consumption of Milk and Milk Derived Products from Goats** http://www.efsa.eu.int/science/biohaz/biohaz_documents/catindex_en.html **Source:** Panel on Biological Hazards, European Food Safety Authority **Author:** EFSA Scientific Expert Working Group on BSE/TSE, Scientific Panel on Biological Hazards, European Food Safety Authority **Summary:** Statement regarding the likelihood of milk and milk products from goats to present a risk of TSE contamination **Resource type:** statement **Publication Date:** November 24, 2004
	Risks of spreading foot and mouth disease through milk and dairy products. **Donaldson AI.** Rev Sci Tech. 1997 Apr;16(1):117-24. A review of epidemics of foot and mouth disease (FMD) has highlighted the important role which raw (untreated) milk can play in the spread of the disease in a country which is normally free of FMD and whose cattle are not routinely vaccinated. The greatest hazard is likely to be in the early stages of an outbreak, before disease control measures have been implemented. The spread of FMD through milk can be prevented by the effective application of control measures combined with 'codes of practice' for the treatment of potentially infected milk. The author considers the probable mechanisms of transmission of FMD by milk and dairy products. These mechanisms are based on the quantities of virus excreted in milk, the survival of the virus under various management and manufacturing conditions and the minimum doses required to initiate infection in susceptible animals by different routes. The key points for consideration when making a risk assessment of the importation of milk and dairy products are also discussed.
	Safety of milk and milk derivatives in relation to BSE: the lactoferrin example. **Vetrugno V.** Biometals. 2004 Jun;17(3):353-6. Bovine Spongiform Encephalopathy (BSE) belongs to Transmissible Spongiform Encephalopathies (TSEs) or Prion diseases. BSE is a feed borne infection of cattle. Epidemiological and laboratory data suggest that the BSE infectious agent is responsible for the variant form of Creutzfeldt-Jakob Disease (vCJD) and that the oral route is the most plausible way of infection. Therefore there is concern that the BSE agent can be transmitted to humans by biological materials (*i.e.* meat products, blood, milk) from susceptible BSE animal species (mostly cows but possibly, sheep and goats). Lactoferrin (LF) can be produced by purification from large volumes of cow's milk or whey. Therefore, a potential BSE risk for milk and milk products needs to be evaluated by risk assessment. The Committee for proprietary Medicinal Products--CPMP of the European Commission and the WHO have categorized risk tissues from TSE susceptible ruminant species in different classes in relation to the BSE risk for medicinal products. Milk, colostrum, and tissues of the mammary gland have been classified in the category of no detectable infectivity. A secondary contamination of milk can be virtually excluded (*i.e.* milk is taken from living animals). In the light of current scientific knowledge and irrespective of the geographical origin, milk and milk derivatives (*e.g.* lactoferrin, lactose) are unlikely to present any risk of TSE contamination provided that milk is sourced from healthy animals in the same conditions as milk collected from human consumption. So the risk of milk and milk derivatives in relation to BSE is negligible.

	[BSE: milk and risk potential?] [Article in German] **Heeschen WH.** Dtsch Tierarztl Wochenschr. 2002 Aug;109(8):350-3 A potential BSE risk for milk and milk products has to be evaluated by means of risk analysis, especially risk assessment. The 3rd element of risk assessment--hazard exposition--is of decisive significance. In 1997, the Scientific Steering Committee of the European Commission has categorized risk materials in 4 classes. Colostrum, milk and tissues of the mammary gland have been classified in category 4, *i.e.* "infectivity not detected". A secondary contamination of the milk can be excluded (living animals). However, the term "not detected" refers also to the low sensitivity of the mouse test, which has to be taken into consideration. Therefore, in 2000 investigations started in Great Britain to test milk fractions, especially the fraction of somatic cells, for the possible occurrence of prions, using newly developed and highly sensitive methods. Results can not be expected before 2003 at the earliest. In case prions would be detected, their biological activity has to be demonstrated in order to develop an appropriate risk assessment for the consumer. Investigations in Great Britain in the early nineties of the last century with suckling cows under practical conditions have shown no indications of a BSE transfer via the milk to the calves. Therefore, the statement of national and international organizations is still valid, that milk can be regarded safe according to the present state of scientific knowledge.
General approach in RA	**Practical approaches to risk assessment. Brooke-Taylor S.** Biomed Environ Sci. 2001 Jun;14(1-2):14-20. The importance of using risk assessment in developing food regulations is growing with the globalization of our food supply. The World Trade Organization has entrenched the principles of science-based risk assessment in the Agreement on Sanitary and Phytosanitary Measures. The relevant international organization for food standards, the Codex Alimentarius Commission, recognises risk analysis, and its component parts risk assessment, risk management and risk communication, as the basis for scientific decision-making. Risk assessment comprises two activities: hazard evaluation; and exposure estimation. A hazard may be chemical, microbiological or nutritional in origin. The practical application of risk assessment in Australia is illustrated in this presentation by four examples involving: (1) food additives, (2) microbiological safety of imported raw milk cheeses, (3) genetically modified foods and (4) imported food inspection.
	ILSI Europe Risk Analysis in Microbiology Task Force.Recontamination as a source of pathogens in processed foods. Reij MW, Den Aantrekker ED; Int J Food Microbiol. 2004 Feb 15;91(1):1-11. Food products that have been submitted to an adequate heat-treatment during processing are free of vegetative pathogens and, depending on the treatments, of sporeformers and are generally regarded as safe. Processed products such as pate, ice cream, infant formulae and others have nevertheless been responsible for food-borne illnesses. Thorough epidemiological investigations of several of these outbreaks have demonstrated that the presence of vegetative pathogens such as Salmonella spp. or Listeria monocytogenes in the consumed products was frequently due to post-process recontamination. The majority of studies on pathogens in foods are devoted to investigations on their presence in raw materials or on their growth and behaviour in the finished products. Reference to recontamination is, however, only made in relatively few publications and very little is published on the sources and routes of these pathogens into products after the final lethal processing step. The investigation of an outbreak, including epidemiological studies and typing of strains, is very useful to trace the origin and source of the hazard. Published data demonstrate that the presence of pathogens in the vicinity of unprotected product in processing lines represents a significant risk of recontamination. Microbiological Risk Assessment studies can be conducted as part of governmental activities determining appropriate protection levels for populations. Although recontamination has been identified as a relevant cause of food incidences, it is often not considered in such studies. This paper advocates that an effort should be made to develop our knowledge and information on recontamination further and start using it systematically in the exposure assessment part of Microbiological Risk Assessment studies.
GMO	**Biosafety assessment of the application of genetically modified Lactococcus lactis spp. in the production of fermented milk products.** Klijn N, Weerkamp AH, de Vos WM. Systematic and Applied Microbiology 18 (4) : 486-492, 1996 **Abstract:** Safety assessment of the use of genetically modified Lactococcus lactis in

	dairy products (cheese, fermented milks, fermented dairy products) is discussed. Aspects considered include: the introduction of genetically modified microorganisms in the food industry; clearance of genetically modified microorganisms for use in foods; principles of biosafety assessment (definition of risk, risk assessment, biological containment); biosafety assessment of genetically modified L. lactis in fermented dairy products (retrospective studies, survival in specific ecosystems (fermentation in milk and in cheesemaking), gene transfer (transfer of pAMbeta1 between Lactococcus spp.)); and hazard identification and normalization.
HACCP & QA	**Application of hazard analysis and critical control point system in the dairy industry. Kassem M, Salem E, Ahwal AM, Saddik M, Gomaa NF.** East Mediterr Health J. 2002 Jan;8(1):114-28 This study aimed to assess the hygiene quality of some packaged milk (pasteurized or sterilized) and dairy products before and after application of a hazard analysis and critical control point (HACCP) system at a milk and dairy products company in Cairo, Egypt. The steps taken to put HACCP in place are described and the process was monitored to assess its impact. Assessment of the hygiene quality of the milk and dairy products before and after HACCP showed an improvement in quality and an overall improvement in the conditions at the company.
	Implementing a quality assurance program using a risk assessment tool on dairy operations. Sischo WM, Kiernan NE, Burns CM, Byler LI. J Dairy Sci. 1997 Apr;80(4):777-87. Concerns and perceptions about antibiotic residues in milk prompted the dairy industry to develop a voluntary program to support rational antibiotic use on dairy farms. One deficiency of this program is the inability of producers to identify easily the weaknesses in antibiotic management in order to develop control plans. To overcome this deficiency, an educational approach was designed. The program centered on an on-farm risk assessment tool used by the producer and an industry educator to determine the current risk for residue violation. The risk assessment tool was tested by 25 field personnel working with northeastern milk receivers and 250 producers in seven states. The participants in the study identified a lack of adequate treatment records as being the highest risk factor for antibiotic residues, followed by deficiencies in understanding how to use antibiotics and poor relationships between veterinarians and their clients. When field representatives utilized the risk assessment tool, for most producers, risk of antibiotic residue decreased by approximately 19%. In particular, more farms kept written records or more complete records. Finally, producers with reported histories of antibiotic residues were less likely to implement management changes to reduce the risk of antibiotic residue.
Import	**Risk assessment on the importation of milk and milk products (excluding cheese) from countries not free from foot and mouth disease. Heng NH, Wilson DW.** Rev Sci Tech. 1993 Dec;12(4):1135-46. The authors discuss the risk assessment conducted by the Australian Quarantine and Inspection Service (AQIS) on the importation of milk and milk products (excluding cheese) from countries not free from foot and mouth disease (FMD). This assessment was undertaken in response to requests from countries wishing to export dairy products for sale on the Australian market. AQIS conducted a public consultation on the proposal, in line with Australian Government policy on transparency and accountability in the quarantine decision-making process. The authors examine the procedures involved in the investigation of the likely presence of FMD virus in milk of vaccinated and non-vaccinated cows, and of the heat treatment parameters effective in the inactivation of the virus. The data provide a useful aid in the assessment of the risk factors associated with the importation of milk and milk products, and in the development of quarantine conditions for importation.
Microbiological criteria	**Scientific Criteria to Ensure Safe Food** http://www.nap.edu/catalog/10690.html **Source:** National Academies Press **Author:** Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food, National Research Council **Summary:** This report presents recommendations for improving the food safety system in the U.S. It includes recommendations related to specific government agencies and for specific food product types (meat and poultry products, seafood, produce, and dairy products). There is also discussion of public health surveillance and food safety tools. Appendices include "Current and Proposed Definitions of Key Food Safety Terms,"

	"Sanitation Performance Standards," "Food and Drug Administration and Environmental Protection Agency Guidance Levels in Seafoods," "Food Defect Action Levels in Produce," "International Microbiological Criteria," "International Microbiological Criteria for Dairy Products," "U.S. Department of Agriculture-Agricultural Marketing Service Standards for Milk and Dairy Products," and "Biographical Sketches of Committee and Subcommittee Members" **Resource type:** book, charts, tables **Publication Date:** 2003
Milk microbiological quality	**Grade "A" Pasteurized Milk Ordinance *2001 Revision*** http://www.cfsan.fda.gov/~ear/pmo01toc.html **Source:** Center for Food Safety and Applied Nutrition, Food and Drug Administration **Summary:** Recommended sanitary standards for Grade "A" raw milk for pasteurization and Grade "A" pasteurized milk and milk products for adoption by states, counties, and municipalities **Resource type:** standards **Publication Date:** May 15, 2002
Raw milk	**[Health risk due to the consumption of raw milk commercialized without due authorization]** [Article in Portuguese] **Badini KB, Nader Filho A, do Amaral LA, Germano PM.** Rev Saude Publica. 1996 Dec;30(6):549-52. Sixty raw milk samples commercialized without due authorization in the counties of Botucatu and S. Manuel, State of S. Paulo (Brazil), were submitted to mesophilic microorganism and coagulase-positive Staphylococcus and most probable number of total coliform and fecal coliform counts. Forty-one (68.3%) and 50 (83.3%) of the samples were found, respectively to contain mesophilic microorganisms and total coliforms above the maximum limits established by the Health Ministry for type C pasteurized milk. Thirty (50.0%) and 11 (18.3%) of the samples were found, respectively, to the contaminated by coagulase-positive Staphylococcus and fecal coliforms. Only 5 (8.3%) samples were found to comply with the required legal standards. The results showed the unsatisfactory hygienic and sanitary conditions of the raw milk and suggest the existence of great risk to the health of the consumers, especially when the product is taken without being boiled.
Resources	**Australian Journal of Dairy Technology** http://www.diaa.asn.au/Index.html **Source:** Dairy Industry Association of Australia, Inc. **Summary:** This journal is the official journal of the Dairy Industry Association of Australia, Inc. **Resource type:** publication **Dairy Industry Association of Australia** http://www.diaa.asn.au/ **Summary:** Professional organization for the Australian dairy industry **Resource type:** website **International Dairy Federation** http://www.fil-idf.org/ **Source:** International Dairy Federation **Summary:** Organization for the dairy sector that works to promote milk and milk products worldwide. IDF is also the source of draft standards for milk products for adoption as Codex standards **Resource type:** website **International Dairy Journal** http://www.elsevier.com/gej-ng/29/81/27/32/show/Products/FOOD/jnl_index.htt **Source:** Food Science and Technology Program, Elsevier Science **Summary:** This journal publishes information on dairy science and technology, including microbiology, enzymology, biotechnology and bioengineering, dairy engineering and new developments in processing, raw material quality, milk assembly, analytical, nutritional, environmental, and legal subjects and more **Resource type:** publication