PASTEURISATION OF DAIRY PRODUCTS: TIMES, TEMPERATURES AND EVIDENCE FOR CONTROL OF PATHOGENS

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SUMMARY

A number of pathogenic micro-organisms can occur in raw milk from contamination by faeces or by being shed directly into milk as a result of mastitis in the cow. Before pasteurisation became mainstream the consumption of raw milk was therefore associated with a wide range of microbial diseases. The aim of pasteurisation is to control pathogens and spoilage organisms, without affecting the nutritional and organoleptic characteristics of the milk. This document collates information from New Zealand and overseas on:

- the prevalence of pathogens in raw milk;
- the efficacy of pasteurisation in controlling these pathogens.

The purpose of the document is to provide a benchmark against which the efficacy of alternative milk treatment systems can be assessed.

The thermal inactivation that pasteurisation inflicts on microbial pathogens is not always known with any high degree of certainty. The published scientific data may be old or incomplete, and/or the experimental methods may not be truly representative of what occurs in commercial pasteurisation systems. This is particularly noticeable for *Mycobacterium avium* subsp. *paratuberculosis* where a number of authors claim that the organism can survive pasteurisation and there is some evidence for the detection of the organism in pasteurised milk, while work in New Zealand using a pilot plant system with turbulent flow tends to indicate that the organism should not survive significantly.

Milk that has been properly pasteurised and handled correctly is not the cause of significant disease from any of the "traditional" foodborne bacteria, although the identification of emerging pathogens may challenge this view. Based on reported outbreaks, the consumption of raw milk products results in approximately an equal public health impact as their pasteurised counterparts while the amount consumed is only approximately 1% of pasteurised dairy products.

Alternative milk treatment processes include thermisation and aging. Thermisation produces a lesser inactivation of microbial pathogens when compared to pasteurisation, and aging may or may not result in a further inactivation. Equivalence with pasteurisation needs to be judged on a case by case basis.

1 INTRODUCTION

The use of relatively low temperature heat treatments (50-60°C) to destroy spoilage organisms in wine and beer is generally credited to Louis Pasteur in the 1860s and 1870s. There are competing claims for the first application of the technique to milk, but the general process of pasteurisation now bears his name (Holsinger *et al*., 1997). Initially the process was used to increase the shelf life of fluid milk, but its importance in controlling pathogenic bacteria, especially *Mycobacterium tuberculosis*, was recognised over the following 30-40 years.

Pasteurisation of milk is a microbiocidal heat treatment intended to:

- Reduce the number of any harmful microorganisms, to a level at which they do not constitute a significant health hazard;
- Reduce the level of undesirable enzymes and spoilage bacteria, and thus increase the keeping quality;
- Achieve the preceding two goals without destroying the original characteristics of the product.

The recognition of the public health importance of pasteurisation prompted the development of regulations that set times and temperatures to control pathogens. Initially pasteurisation conditions were devised to inactivate *Mycobacterium tuberculosis* (North and Park 1927) and were set as 61^{o}C for 30 minutes, but conditions have been changed subsequently to destroy the organism *Coxiella burnetii* which causes Q fever. *Coxiella burnetii* is the most heat-resistant non-sporulating pathogen likely to be present in milk. Pasteurisation is designed to achieve at least a 5-log reduction of *C. burnetii* in whole milk.

In recent times, however, there has been controversy among scientists about the ability of pasteurisation to inactivate the organism *Mycobacterium avium* subsp. *paratuberculosis*. This organism has become prominent because of its putative link with Crohn's disease in humans, a link which is also the subject of much debate and, currently, lack of consensus.

A bactericidal heat treatment applied to a food will result in the numbers of any one organism present being reduced. The amount of this reduction will depend on a number of factors, including:

- the properties of the organism;
- variation in the heat susceptibility of different strains of the organism;
- the physiological state of the organism prior to treatment; and,
- the chemical composition of the food.

In quantitative risk assessment terms the degree of inactivation of a particular organism that pasteurisation achieves will be represented by a probability distribution function. The numbers of a pathogen which may survive pasteurisation are dependent on the degree of kill and the initial load of the organism in the raw milk; hence survivors will also be represented by a probability distribution function.

A further consideration is that pathogen reduction is often described in terms of log reductions in cell numbers. It is important to recognise that 0 $\log_{10}$ pathogens /ml is

not the same as complete absence of the pathogen; it actually represents 1 pathogen /ml. Where negative $\log_{10}$ values are obtained, the numbers of organisms need to be considered in terms of pathogens per volume. For example a heat treatment that produces milk with 10^{-2} pathogens per litre means that one in 100 one litre packages of that food will contain a pathogen. Final exposure to the consumer will then depend on other variables such as the ability of the organism to grow in the food prior to consumption.

Pasteurisation is a widely accepted process for assuring the safety of milk and milk products. A number of alternative hygiene procedures have been proposed by the food industry and others, which may be able to achieve the same level of consumer protection. To assess these procedures, there needs to be a benchmark for pasteurisation, in terms of evidence for its control of pathogenic bacteria.

This report is intended to provide such a benchmark, by summarising evidence from the scientific literature that concerns:

- Times and temperatures for pasteurisation and the basis for their selection; and,
- Evidence for the control of pathogens likely to be encountered in dairy products.

The report is organised into the following sections:

Section 2: Relevant microbiological hazards in raw milk, with indicative information on their prevalence, and a brief overview of outbreaks associated with dairy products;
Section 3: Scientific data describing the control of these hazards in milk using pasteurisation conditions;
Section 4: An overview of current regulations and standards concerning pasteurisation, largely focused on New Zealand;
Section 5: A preliminary comparison of pasteurisation with alternative procedures, thermisation and aging;
Section 6: Risk assessments of such procedures, principally conducted by Food Standards Australia New Zealand (FSANZ).

Although pasteurisation has an important role in controlling spoilage organisms, these will not be covered in this report.

2 PASTEURISATION AND ITS EFFECT ON FOODBORNE PATHOGENS

2.1 Hazards to be Considered

Pathogens may be present in raw milk due to (ICMSF, 1998):

- Abnormal udder conditions caused by infection or disease, principally mastitis (an inflammatory disease of the mammary tissue);
- Contamination during milking, caused by faecal material, residues on milk-handling equipment, personnel and environmental sources.

The hazards which this review will consider are those micro-organisms that have been isolated from, or detected in, raw milk and which have caused human disease as a result of consumption of milk or dairy products. These are described in a number of publications (e.g. ICMSF, 1998; D'Aoust, 1989), and are listed in Table 1. This table also includes example reports from the literature of the occurrence and human disease caused by the pathogen.

One published list of potential pathogens in milk includes *Shigella dysenteriae* (Pearce, 2001), but shigellae are not natural inhabitants of the environment (ICMSF, 1996) and are more likely to arise from post-pasteurisation contamination by an infected person. Thus this organism has not been included in this report.

There are many types of pathogenic *Escherichia coli*, but the serotype most often associated with human illness is *E. coli* O157:H7. Since the physiological characteristics of this serotype are similar to other *E. coli*, the effects of pasteurisation on them can be considered equivalent.

Table 1 Significant Hazards in Raw Milk

Microbiological Hazard	Examples from the literature of:	
	Isolation from Raw Milk	**Human Disease resulting from Consumption of Milk or Dairy Products**
Bacillus spp.	Detected at all stages of processing (Crielly *et al.* 1994).	Dairy products: suspected food vehicles in 3 outbreaks in the Netherlands between 1991 and 1994 (Simone *et al.* 1997).
Brucella spp.	*B. melitensis* isolated from the milk of infected sheep (Banai *et al.* 1990).	Improperly pasteurised milk: 1 case (Mathur 1968).
Campylobacter	Isolated from 50 of 78 cows producing grade A milk in the USA (Doyle and Roman 1982).	Unpasteurised milk: 52 cases (Kalman *et al.* 2000)
Clostridium botulinum	At low levels (Collins-Thompson and Wood 1993).	Cream cheese: 8 cases (Aureli *et al.* 2000)
Coxiella burnetii[*]	Present in 33.1% of raw Japanese cow's milk (Muramatsu *et al.* 1997).	Unpasteurised goat's milk dairy products: 29 cases (Fishbein and Raoult 1992).
Cryptosporidium	Detected in unpasteurised milk and milk fat (Harper *et al.* 2002).	Unpasteurised cow's milk: 8 cases (Harper *et al.* 2002).
Escherichia coli O157:H7	In 1.46% of farm milk samples in Tennessee (Murinda *et al.* 2002).	Pasteurised milk: 114 cases (Goh *et al.* 2002).
Listeria monocytogenes	In farm milk (Meyer-Broseta *et al.* 2002).	Soft cheese: 57 cases, 18 died (Bula *et al.* 1995)
Mycobacterium spp.	In 35 of 51 samples (Hosty and McDurmont 1975).	Raw milk: Estimated to have caused 2,500 deaths from *M. bovis* and 4,000 new cases each year in the 1930s in the UK (O'Reilly and Daborn 1995).
Pathogenic streptococci	Detected at $>10^2$/ml in 2% of unpasteurised goats' and ewes' milk samples (Little and De Louvais 1999)	Whipped cream: 3 cases (Moore 1955)
Salmonella spp.	Detected in 6 of 268 bulk milk tank samples from 30 Tennessee farms (Murinda *et al.* 2002).	Cheese: 62 cases including 40 raw milk consumers, 6 secondary cases and 16 dairy workers (CDC 2003).
Staphylococcus aureus	100% of samples tested contained this organism (Adesiyun *et al.* 1995).	Responsible for 85.5% of dairy product outbreaks in France (Buyser *et al.* 2001)
Yersinia enterocolitica	In 2.7% of raw milk samples in Manitoba, Canada (Davidson *et al.* 1989).	Pasteurised milk: 10 cases. (Ackers *et al.* 2000).

[*]In a trial of 34 volunteers drinking milk naturally infected with the organism, none developed symptoms of Q fever (Krumbiegel and Wisniewski 1970).

2.2 Occurrence of Hazards in Raw and Pasteurised Milk

The following section considers each of the pathogens in Section 2.1 in more detail. Reports of the prevalence in milk are also given, where possible from New Zealand studies.

2.2.1 *Bacillus* spp.

This genus of Gram-positive bacteria is widespread in the environment and can be spread from soil and grass to the udders of dairy cows and into raw milk. Sources of milk contamination has been traced to storage of hay in milking sheds (Billing and Cuthbert, 1958), biofilm growth and sporulation in cans of milk allowed to stand after being emptied (Donovan, 1959), as well as mastitic cows (Turnbull *et al*., 1979).

Psychrotrophic strains of *B. cereus* are known to exist (Lechner *et al*. 1998) and these can grow at temperatures between 4 and 7ºC, well below the 10ºC lower minimum temperature at which most mesophilic strains of *B. cereus* grow .

Bacillus spp. form spores which are able to withstand pasteurisation. Isolation of *B. cereus* from samples of raw milk (9%) and pasteurised milk (35%) have been reported by Ahmed *et al*. (1983). Counts of up to 10^4 cfu/ml have also been recovered from fresh raw milk (12.4%) and bottled pasteurised milk (86.7%) by Ionescu *et al*. (1966). The high prevalence of *B. cereus* in milk reported by these older publications may be due to survival of spores during pasteurisation and subsequent re-growth. Improved temperature control of stored milk before sale may be reflected in the lower reported prevalences. *B. cereus* was detected in 2% of Chinese pasteurised milk samples at average counts of 280/ml (Wong *et al.* 1988).

Spores of this genus may also survive commercial milk sterilisation, with most survivors identified as *B. subtilis* (Franklin *et al.* 1956).

B. cereus and *B. subtilis* are both capable of causing food poisoning, and *B. cereus* can also cause "sweet curdling" and "bitty cream" which are spoilage problems. Untreated raw milk samples in Scotland have been shown to contain *Bacillus* spp. at numbers ranging from <10 cfu/ml to $>10^5$ cfu /ml. *B. licheniformis* and *B. cereus* were the most commonly isolated species (Crielly *et al.* 1994).

A British study showed 0-700 *Bacillus* spp. spores/100 ml in raw milk and a strong seasonal pattern with counts higher in the winter (Franklin *et al.* 1956). Mean counts from 10 dairies ranged from 3.9 to 143.7 mesophilic spores /100 ml. Of the organisms identified, most were *B. licheniformis*, and 7% of the isolates were identified as *B. subtilis*. Another study by Billing and Cuthbert (1958) in England showed a marked seasonal variation in the *B. cereus* index in raw milk supplies; maximum numbers were recorded from July to September (summer) and minimum numbers in April and May (late autumn). Counts of *B. cereus* in 38 samples of pasteurised milk in the Netherlands showed that all contained spores with a mean of around 100/100 ml (Notermans *et al.* 1997), with counts highest in the summer. Dutch data specific for *B. cereus* indicate prevalences of 7-35% for raw milk with a count of 10^1-10^2/ml (Notermans and Batt 1998).

2.2.2 *Brucella*

Three species of *Brucella* can cause disease in man; *Bruc. abortus*, *Bruc. melitensis*, and *Bruc. suis*. New Zealand is free of these organisms (www.maf.govt.nz/biosecurity/pests-diseases/animals/brucella-melitensis/; www.maf.govt.nz/biosecurity/pests-diseases/animals/brucella-suis). A discussion of the history and evidence of New Zealand's *Bruc. abortus*-free status has recently been published (Anonymous 2003).

Many other countries now also claim *Brucella*-free status. In countries where *Brucella* still infects the animal population, the organism can still be detected in milk and dairy products. For example, in Turkey 5 of 35 samples of ewe's milk cheeses were positive for *Bruc. melitensis* (Kasimoglu 2002) with numbers ranging from $3.6x10^1$ to $9.3x10^3$ MPN/g. Of 35 raw milk samples and 35 cow's milk cheese samples, none contained the organism. More recently, up to 40 cases of brucellosis were reported in an outbreak in Thailand where consumption of unpasteurised goat's milk obtained from infected goat herds was the cause
(See: www.bangkokpost.com/news/10Sep2003_news12html)..

2.2.3 *Cryptosporidium*

The prevalence of this organism in raw milk has not been determined (Laberge *et al.* 1996).

2.2.4 *Campylobacter* spp.

Campylobacter in raw milk originates as a contaminant from unhygienic practices during milking. While it can be found in contaminated water and could get into milking equipment if the water source is not potable, the most likely source in raw milk is faecal contamination. The milking shed environment is the most likely area where cross contamination of milking equipment and raw milk with faecal material from dairy cows could occur. Thermophilic *Campylobacter* have been detected in the guts of dairy cattle in New Zealand and the UK (Meanger and Marshall, 1988; Stanley *et al*, 1998). In New Zealand a high carriage rate was found during summer (24%) and autumn (31%) (Meanger and Marshall, 1988) but unfortunately these authors did not include spring in the study. The UK study by Stanley et al (1998) showed two seasonal peaks in the carriage rate in dairy cattle occurring in spring and autumn.

The prevalence of *Campylobacter* in raw milk in New Zealand was 1 of 111 (0.9%) samples positive in a 1998 survey (Hudson *et al.* 1999), although a previous survey of 71 samples failed to detect this organism (Stone 1987).

In an Australian study, the prevalence of *Campylobacter* in pasture fed animals was higher in dairy cattle (6%) compared to beef cattle (2%), and this may be due to the high stocking rate (Anonymous 2003b).

This organism has been isolated from 1 of 108 (0.9%) bulk farm milk samples in the USA (Doyle and Roman 1982) and five of 610 (0.8%) raw milk samples in a British study (Food Standards Agency 2003). In samples from Manitoba, Canada, *Campylobacter* was detected in 1.6% of 192 farm and 0% of 64 dairy raw milk

samples (Davidson *et al.* 1989). Also in Canada, *Campylobacter* was detected in 0.47% (95% CI 0.22-0.95%) of 1,720 farm bulk tank milk samples. A slightly higher prevalence was found in milk collected from individual cows, where 2 from 40 (5.0%) were positive (Hutchinson *et al.* 1985). A British study detected the organism in 1.7% of 1097 raw milk samples (de Louvais and Rampling 1998), and a similar prevalence (1.5%) was reported from the USA (Lovett *et al.* 1983). Much higher prevalences of 9.2% and 12.3% have been reported in US surveys (Jayarao and Henning 2001;Rohrbach *et al.* 1992).

No isolates were obtained from 400 raw milk samples tested in the Netherlands (Oosterom *et al.* 1982), from 496 samples of Swiss raw milk (Bachman and Spahr 1994) or from 50 samples of raw milk in the USA (Wyatt and Timm 1982).

No quantitative data for *Campylobacter* in raw milk were located.

2.2.5 *Clostridium botulinum*

This species is capable of producing endospores which may germinate and produce neurotoxins in foods. Spores of the organism are rare in milk. Counts of less than 1 spore per litre have been indicated (Collins-Thompson and Wood 1993). None of 35 raw milk and 15 pasteurised milk samples were found to contain spores in an Italian study (Franciosa *et al.* 1999). However mozzarella cheese, soft cheese and processed cheese samples were found to contain spores, and these foods may be considered as concentrated milk samples. In particular, of 1017 mascarpone cheese samples analysed, 331 were positive, containing mostly type A and to a much lesser extent type B spores. The levels present were all <10 spores/g.

2.2.6 *Coxiella burnetii*

It is worth noting that a New Zealand survey of 2,181 bovine and 12,556 canine blood samples found them all to be seronegative for antibodies against *C. burnetii*. This information and previous reports were taken to indicate that New Zealand is free of the organism (Hilbink *et al.* 1993).

The distribution of this organism is thought to be worldwide and it is isolated most frequently from domestic animals, particularly cattle, sheep and goats. *Coxiella burnetii* grows well in the placenta of these animals, reaching levels of 10^9 organisms/g. It grows preferentially in the vacuoles of the host cell. Although not a spore-former, it has a high degree of resistance to heat comparable to that of sporogenic bacteria, partly due to their ability to assume endospore-like forms (Weiss and Moulder (1984).

Of 109 samples of milk arriving at a dairy in the USA eight (7.3%) contained this organism (Enright *et al.* 1957). A further 376 retail samples of milk and cream were examined and 14 raw and 1 pasteurised milk sample found to be positive. The maximum number of organisms found was 1,000 infective guinea pig units (the minimum number of cells required to infect a guinea pig when delivered in 2 ml intraperitoneally). Eighteen of 137 individual cows contained *C. burnetii* in their milk. Three of these samples contained 1,000 guinea pig units, 5 contained 10, and 5 contained 1 per 2ml. A dose of 10,000 units was considered to be the highest obtained in cow's milk.

The use of a PCR-ELISA technique detected the organism in 21 of 62 samples of raw cow's milk in Japan (Muramatsu *et al.* 1997).

2.2.7 *Escherichia coli* O157:H7

In a study of milk from 30 farms in Tennessee, 1.46% of milk samples contained the organism (Murinda *et al.* 2002). Of 1270 raw farm bulk tank milk samples tested in Canada, 47 (0.87 %, 95% confidence intervals 0.51-1.47%) were found to contain shiga toxigenic *E. coli* (STEC) by PCR (Steele *et al.* 1997). Since this assay detected any STEC, i.e. it was not targeted specifically at *E. coli* O157:H7, then an unknown proportion of non-pathogenic STEC may have been detected. A prevalence of 0.3% was found in a study of 1097 samples in a British survey (de Louvais and Rampling 1998), and a similar prevalence (0.2%) determined in another British survey (Food Standards Agency 2003).

In contrast no *E. coli* O157:H7 was isolated from 500 samples of raw cow's milk tested in south-east Scotland (Coia *et al.* 2001). Similar absences of the organism were found in 1,011 of raw milk samples in the Netherlands (Heuvelink *et al.* 1998), 42 raw milk samples from Wisconsin, USA (Ansay and Kaspar 1997), 23 samples of raw milk from two farms linked to sporadic cases of disease (Wells *et al.* 1991), 126 samples of goats' and ewes' milk samples from England and Wales (Little and De Louvais 1999) and 131 samples from South Dakota and Minnesota, although STEC were detected in 3.8% of the samples (Jayarao and Henning 2001).

A prevalence of 1.7% *E. coli* O157:H7 was detected in goats milk samples in Italy, and in this sample the *E. coli* concentration was 1.5 cells per ml although it is not clear if this level pertains to the O157 isolate exclusively (Foschino *et al.* 2002).

2.2.8 *Listeria monocytogenes*

A New Zealand study did not detect *L. monocytogenes* in any of 71 samples tested (Stone 1987). However other *Listeria* species were isolated.

Listeria, but not *L. monocytogenes*, was isolated from 1 of 120 (0.8%) samples of raw milk sampled from farm bulk tanks in Japan (Takai *et al.* 1990). A similar prevalence (0.6%) was determined for Swiss raw milk (Bachman and Spahr 1994).

A larger study in France (1459 bulk tank milk samples tested) determined a mean prevalence of 2.4% and a median of 0%. An enhanced testing programme was able to detect *L. monocytogenes* at double this prevalence. A seasonal pattern could be observed, with positive isolations tending to occur in the winter. Where enumeration was performed, eleven samples did not yield colonies when 2 ml of milk were enumerated. For the other three samples, counts of 210, 10 and 1 cfu/2ml were recorded (Meyer-Broseta *et al.* 2002).

In contrast an overall prevalence of 3.8% for *L. monocytogenes* in Scottish bulk tanks samples has been observed for samples taken in the summer (Fenlon and Wilson 1989), while the corresponding figure for the autumn and winter samples was 1.0%. It was concluded that where *L. monocytogenes* were present then the levels were low, at <1 cell/ml. A prevalence of 1.3% has been determined for raw milk in Ontario (Farber *et al.* 1988a).

A higher prevalence has been shown in the USA where 15 of 124 (12%) and 15 of 121 (12%) raw milk samples were positive (Fleming *et al.* 1985;Hayes *et al.* 1986), while in the Netherlands only 6 of 137 (4.4%) of raw milk samples were positive (Beckers *et al.* 1987) but the numbers present were all $<10^2$/ml. In samples from Manitoba, Canada, *L. monocytogenes* was detected in 1.0% of 192 farm and 3.1% of 64 dairy raw milk samples (Davidson *et al.* 1989). A similar prevalence (4.0%) was found in samples from Nebraska (Liewen and Plautz 1988), 4.2% in three areas of the USA although the prevalence ranged from 0 to 12.0% (Lovett *et al.* 1987), 4.6% in South Dakota and Minnesota (Jayarao and Henning 2001), 4.1% in Tennessee (Rohrbach *et al.* 1992) and Canada where the prevalence was 2.73% (Steele *et al.* 1997).

A high prevalence has also been reported for Spanish pasteurised milk, where 21.4% of samples from a single processing plant were positive (Fernandez Garayzabal *et al.* 1986).

No *L. monocytogenes* isolates were obtained from 60 samples of raw goats milk from Italy (Foschino *et al.* 2002).

In a comparison of raw and pasteurised milk in the UK, 101 of 610 raw milk samples contained *L. monocytogenes* while none of 1413 pasteurised milk samples contained the organism (Food Standards Agency 2003). No milk sample contained more than 2 $\log_{10}$ of the organism.

In cows with mastitis *L. monocytogenes* may be shed at 10,000-20,000 cells per ml of milk, with the appearance of the milk being normal and there being no inflammation of the affected quarter (Bunning *et al.* 1986).

Enumeration of *L. monocytogenes* Type Scott A has been carried out where cows had been inoculated with the organism by various means, followed by direct inoculation into the udder three weeks prior to the collection of milk (Doyle *et al.* 1987). Of twelve milk samples tested only 4 yielded *L. monocytogenes* on direct plating, with counts ranging from 3.0×10^2 /ml to 1.9×10^4 /ml. The remaining 8 samples had counts of $< 10^4$ /ml. Sonicated samples yielded counts 2-5 times higher. The number of *L. monocytogenes* present in polymorphonuclear leukocytes ranged from 0-26, equating to $<10^2$ to 4.8×10^4/ml.

In pooled milk from one cow which had been identified as shedding *L. monocytogenes*, counts in five replicate samples varied from $> 1.1 \times 10^3$ to 1.5×10^4 MPN/ml (Farber *et al.* 1988b).

2.2.9 *Mycobacterium avium* subsp. *paratuberculosis* (MAP)

MAP is shed in the milk of cows with advanced Johne's disease, with nine of 26 such cows in one study yielding the organism (Taylor *et al.* 1981). The prevalence of MAP in the milk of asymptomatic but shedding cows has been shown to be proportional to the pathogen load in the faeces. In "heavy" shedders 7/37 (19%) of milk samples were positive, which this reduced to 1/9 (11%) and 1/31(3%) in "intermediate" and "light" shedders respectively (Sweeney *et al.* 1992).

In cows which were symptomatic, MAP could be isolated from the milk of five of 11 cows tested, but the counts were all <100 CFU/ ml (Giese and Ahrens 2000). One other sample was positive by PCR, but not by conventional culture.

In 126 cows which were asymptomatic, only 3 (2.4%) yielded MAP in their milk, although 28.6% had the organism in their faeces (Streeter *et al.* 1995). In contrast to the data above there was no correlation between shedding in milk and degree of shedding in the faeces.

Given the lack of agreement as to the ability of this organism to survive pasteurisation two surveys of pasteurised milk have been carried out in order to determine the prevalence in this food (Grant *et al.* 2001). The first used PCR, and detected target DNA, in 7% of packages of pasteurised milk on retail sale in the UK. However no conclusive evidence for the presence of viable cells could be obtained, although evidence for acid fast organisms in some samples was shown (Millar *et al.* 1996). A follow up study detected the organism by culture in 1.6% of raw milk and 1.8% of pasteurised milk samples (Food Standards Agency 2003) The higher isolation rate in pasteurised milk was attributed to the overgrowth of MAP by spoilage organisms in cultures of raw milk.

This information has been claimed to show that pasteurisation is inadequate for the destruction of MAP. However, the presence of an organism in a retail package of milk is not necessarily the result of inadequate pasteurisation. Other possible explanations include; inadequate operation of the pasteuriser, leaks in valves or heat exchange plates (allowing raw milk to mix with pasteurised milk), and post pasteurisation contamination (Lund *et al.* 2002). The phosphatase test, which is used to indicate that pasteurisation has been achieved, is only sensitive enough to detect contamination by raw milk at levels >0.05-0.1%.

2.2.10 *Mycobacterium bovis*

Prevalences in milk at the turn of the last century in America and Europe have been quoted at 6-15% (Park 1927), and specific data for England in 1923 indicated a prevalence of 9.88%. Given the subsequent attempts to control this organism contamination rates are likely to be much lower today, and in the UK none of 765 samples of raw and pasteurised milk contained the organism (Food Standards Agency 2003).

2.2.11 Pathogenic streptococci

Some of the streptococci are important causes of mastitis in cows, and so the presence of the organism in milk is not unexpected. Haemolytic streptococci were detected at levels exceeding 10^2/ml in 2% of goats' milk but not in any of 26 ewes' milk samples tested in England and Wales (Little and De Louvais 1999).

2.2.12 *Salmonella*

In a survey of 268 bulk tank milk samples from 30 Tennessee farms, *Salmonella* was detected in six (2.24%) samples (Murinda *et al.* 2002). A lower prevalence was found in Canadian farm bulk tank samples, where 0.17% (95% confidence intervals 0.05-0.55%) of 1720 samples were positive (Steele *et al.* 1997), and similar values (0.5% and 0.3%) determined in a British study of 1097 and 610 raw milk samples

respectively (de Louvais and Rampling 1998;Food Standards Agency 2003). A higher prevalence (6.1%) was found in milk samples from South Dakota and Minnesota (Jayarao and Henning 2001), and an even greater proportion (8.9%) in a study of 292 samples from Tennessee (Rohrbach *et al.* 1992).

No *Salmonella* isolates were obtained from 60 samples of raw goats milk from Italy (Foschino *et al.* 2002), or from 456 samples of Swiss raw milk (Bachman and Spahr 1994).

2.2.13 *Staphylococcus aureus*

In a survey of 287 milk samples from Trinidad all contained *Staph. aureus* at levels ranging from 1.4×10^4 to 1.2×10^5 /ml (Adesiyun *et al.* 1995). However, this range is likely to be larger than might be expected from milk in developed countries since milk collection in Trinidad at the time of the report was at ambient temperature.

In the United Kingdom 1.1% of 1097 raw milk samples contained *Staph. aureus* at levels in excess of 500/ml (de Louvais and Rampling 1998), but in Switzerland 100% of samples contained the organism (Bachman and Spahr 1994).

Of 60 samples of Italian raw goats milk, 26 (43%) contained more than 10^2 *Staph. aureus* per ml, with a mean of 1.2×10^3 /ml (Foschino *et al.* 2002). Of the isolates 23% were able to produce enterotoxin, but *Staph. aureus* was detected at greater than 10^2/ml in 7% of 126 goats' and ewes' milk samples tested in the UK (Little and De Louvais 1999).

The United Kingdom has recently reported the results of a nationwide study into milk quality (Food Standards Agency 2003). Data for coagulase producing staphylococci are presented below in Table 2.

Table 2. Coagulase producing staphylococci in British milk

	Number (%) of milk samples				
Coagulase positive staphylococci Log_{10} count/ml	**Raw**	**Pasteurised whole**	**Pasteurised semi-skimmed**	**Pasteurised skimmed**	**Total pasteurised**
<1.00	497 (81.5)	555 (99.6)	438 (100.0)	418 (100.0)	1411 (99.9)
1.00-1.99	49 (8.0)	2 (0.4)	-	-	2 (0.1)
2.00-2.99	45 (7.2)	-	-	-	-
3.00-3.99	18 (3.0)	-	-	-	-
4.00-4.99	1 (0.2)	-	-	-	-
Total	610	557	438	418	1413

2.2.14 *Yersinia enterocolitica*

A survey of 71 raw New Zealand milk samples detected *Y. enterocolitica* in 3 (4.2%) of the samples (Stone 1987).

A low prevalence (0.6%) was determined in a survey of 352 raw milk samples in Switzerland (Bachman and Spahr 1994). In samples from Manitoba, Canada, *Y. enterocolitica* was detected in 1.6% of 192 farm and 6.3% of 64 dairy raw milk samples (Davidson *et al.* 1989). There was some evidence that the prevalence may have been higher in the colder months. A prevalence of 6.1% was found in milk samples from South Dakota and Minnesota (Jayarao and Henning 2001). A higher prevalence was found in samples from Tennessee (Rohrbach *et al.* 1992).

The organism has also been isolated from pasteurised milk associated with yersiniosis cases on a hospital ward (Greenwood and Hooper 1990). In a survey of 73 pasteurised milk samples from 15 commercial dairies in Scotland *Y. enterocolitica* was isolated from 23% of the samples examined (Bruce *et al.* 1995), although the isolates were mostly non-pathogenic. The possible source of these organisms was not discussed.

An Australian study detected *Y. enterocolitica* in raw milk from one farm at "approx. 2000/ml" (Hughes 1979).

2.3 Outbreaks of illness associated with dairy products

An attempt to analyse foodborne disease involving dairy products as the vehicle has been carried out for France and other industrialised countries (Buyser *et al.* 2001). Four pathogens were considered; *Salmonella*, *Staph. aureus*, *L. monocytogenes* and pathogenic *E. coli*. Examination of data from 60 published outbreaks and four single cases indicated that 32.8% of the food vehicles were made from pasteurised milk, 37.5% from raw milk, 10.9% from "unpasteurised" (heat treated but at conditions less bactericidal than standard pasteurisation) milk and 18.8% from milk whose provenance was unspecified. Overall 2-6% of outbreaks could be attributed to dairy products in the countries examined, and *Staph aureus* was the organism most often associated with outbreaks involving cheeses made from raw or unspecified milk. A problem with the data analysed was that for 51% of the food vehicles, the heat treatment applied to milk was unspecified. The data also need to be interpreted in relation to the volumes of foods produced using the different types of heat treatment; for example while at least 48.4% of the outbreaks were from foods made from milk that had received a lesser heat treatment than pasteurisation this is likely to be disproportionately high if most dairy products are produced from pasteurised milk. The data needed to assess this are not given in this paper.

Between 1983 and 1984 32 outbreaks of disease in England and Wales were attributed to the consumption of milk and dairy products (Barrett 1986). Of these vehicles 27 were attributed to raw milk, two to pasteurised milk and one each to cheese, cream and ice cream. Of interest is one outbreak among 12 people caused by *Strep. zooepidemicus* present in raw milk and found to be present in the milk produced by cows on the implicated farm. This outbreak was of significance as the twelve cases were hospitalised with meningitis or endocarditis, and eight died. The cases were in at risk groups (elderly and young).

An analysis of milkborne outbreaks of infectious intestinal disease in England and Wales for 1992-2000 (Gillespie *et al.* 2003) identified unpasteurised milk as the most common vehicle (52% of milkborne outbreaks), and pasteurised milk as the second most frequent (37%). Of the outbreaks attributed to pasteurised milk, inadequate heat treatment was the most common fault responsible, followed by cross contamination and inappropriate storage. The paper cited data indicating that in the North West of

England 4% of farm-bottled pasteurised milk failed the phosphatase test, indicating under-processing, and that 18% of on-farm dairies produced milk that both failed the phosphatase test and that was potentially microbiologically unsafe.

A specific study has been made of raw milk associated outbreaks from 1973 to 1992 in the USA (Headrick *et al.* 1998). A total of 46 outbreaks occurred in 21 states during this period. Most (57%) were caused by *Campylobacter*, with the others caused by *Salmonella* (26%), staphylococci (2%), *E. coli* O157:H7 (2%), and 13% were of unknown aetiology. In 1995 54% of the 52 states permitted the sale of raw milk, and it was estimated that in these states 1% of the milk sold was raw. Of the 46 raw milk associated outbreaks, 87% occurred in states where raw milk sales were legal at the time (inter-state sales of raw milk were permitted prior to 1987). It was concluded that "…the results of this study illustrate the dramatically higher rate at which raw-milk associated outbreaks are reported from states that allow the sale of this product…".

A review of cheese associated outbreaks was able to identify only six outbreaks in the USA from 1948-1988 (Johnson *et al.* 1990). However, when outbreaks occur they can be large. In one Canadian outbreak implicating cheddar cheese made from thermised milk more than 1500 people suffered from *Salmonella* Typhimurium PT10 infections (D'Aoust *et al.* 1985). In this outbreak the number of organisms consumed by some cases was very low, from 0.7 to 6.1 from MPN and self-reported consumption data (D'Aoust 1985).

3 REDUCTION IN MICROBIAL NUMBERS FROM PASTEURISATION

This section discusses information from the scientific literature on the inactivation of pathogens by pasteurisation, focussing on the pasteurisation conditions most commonly applied.

3.1 Measures of microbial reduction

Most assessments of heat inactivation of microorganisms are described as a log-linear relationship, i.e. a plot of the $\log_{10}$ of the number of survivors versus the exposure time at a given temperature is a straight line. This approach allows the calculation of two parameters, D time and z value.

3.1.1 D time

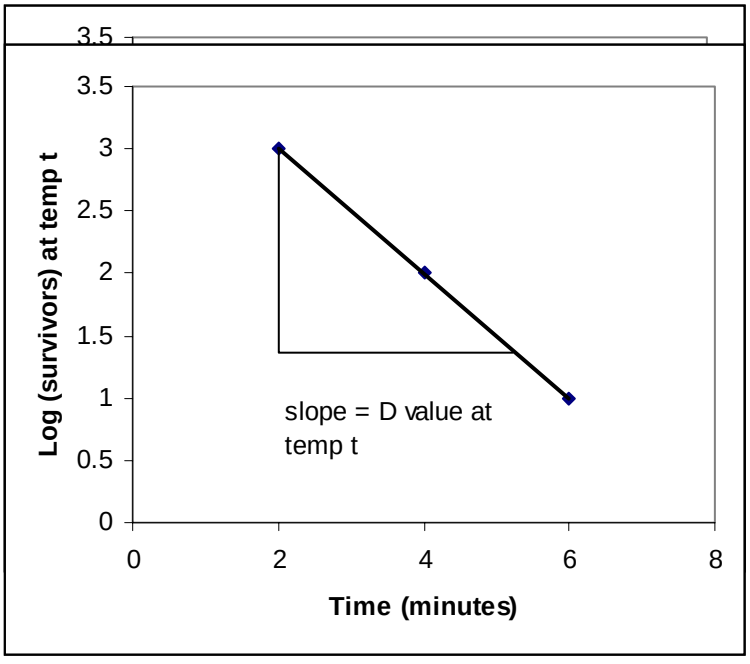
The D time is defined as the time at a given temperature required to reduce the population of organisms present by 90% or 1 $\log_{10}$ unit. Figure 1 illustrates the calculation.

Figure 1 Example of Calculation of D time at temperature t

D value at temperature t = 2/1 = 2 minutes

3.1.2 Z value: The *z* value is defined as the temperature change required to alter the D value by a factor of 10. Figure 2 illustrates the z value determination.

Figure 2 Illustration of *z* value calculation for pasteurisation



Z value for pasteurisation = 72-63/log (30x60)-log (15) = 9/2.079 = 4.33

More recently, papers have reported the presence of "shoulders" and "tails" (Ross *et al.* 1998) in thermal destruction curves that complicate the kinetics and reduce the value of easily understood concepts such as the D time. Shoulders represent an initial period of time where the organism is not being destroyed as quickly as in the subsequent section of the thermal death curve. Tails seem to represent a reduced rate of destruction (increased D value) in a hypothesised heat resistant sub-population. A method has been proposed to describe the effects of pasteurisation when considering this kind of inactivation kinetics. The method involves determining an organism's PE or "pasteurisation effect", equivalent to the log reduction that pasteurisation would deliver (Ross *et al.* 1998).

Many of the papers cited below use a value of "thermal death time", i.e. the time taken for a given number of cells to become non-detectable by the method used at a given temperature. Given a variety of reported temperatures *z* values can be calculated. A curve of thermal destruction can then be compared to the curve of pasteurisation conditions and, given an initial number of organisms, statements made about the adequacy of pasteurisation.

3.2 General pasteurisation conditions

A fuller discussion of pasteurisation standards is given in Section 4. However, to provide context for the data reported in this section, it is necessary to discuss pasteurisation times and temperatures in general terms.

The most commonly used standards are the low temperature long time (LTLT) (63.5°C for 30 minutes) method (also known as the "holding method"), and the high temperature short time method (HTST) (71.7°C for 15 seconds). The most commonly used pasteurisation method for milk products in New Zealand is the HTST method. Extended shelf life and ultra heat treated products are pasteurised at 120-124°C and 134-135°C (or higher) respectively, for short periods. The "holding method" is occasionally used for batch pasteurisation of certain products. The efficacy of pasteurisation is always checked by phosphatase enzyme based assays (Chris Erikson, Mainland Products, personal communication).

3.3 Data on inactivation of pathogens by pasteurisation

3.3.1 *Bacillus* spp.

Pasteurisation will not inactivate *Bacillus* spores. D times for ***B. cereus*** spores at 95.0^{o}C ranged from 1.2 to 36.0 minutes, and the *z* value was 9.6^{o}C. At 100^{o}C the D time was 2.0 to 5.4 minutes (Wong *et al.* 1988). *Z* values of 8.2-8.5^{o}C have been reported elsewhere (Wescott *et al.* 1995). Most work has concentrated on heat inactivation of spores; data regarding vegetative cells are lacking.

3.3.2 *Brucella* spp.

A study of *Brucella abortus* focused on the thermal destruction of this organism under HTST and LTLT conditions (Kronenwett *et al.* 1954). It was found that the *z* value was not influenced by conditions prior to heat treatment, but that there were some differences between isolates. In this case the z value varied from 4.3 to 4.8^{o}C. Given a starting concentration of 2 x 10^{8} organisms/ml of the most heat resistant isolate, there

was a considerable margin of safety when compared with pasteurisation time/temperature combinations.

The level of detail is not present in the data provided, but some estimates on D times can be made. For the isolate 2308 the following were D times derived:

Temperature (^{o}C)	Approximate D Values (s)
61.5	< 18.7
63.3	< 6.5
66.5	< 1.8

For isolate 2016 the following D times were derived:

Temperature (^{o}C)	Approximate D Values (s)
64.3	< 8.6
65.5	< 4.8
66.6	< 2.5

N.B. These assume log linear inactivation kinetics.

Cultures containing 1.5 x 10^{8} *Bruc. abortus* per ml were destroyed by heating at 61.5^{o}C for 23 minutes and at 72^{o}C for 12-14 seconds (Foster *et al.* 1953). The calculated *z* value was 5.3^{o}C.

Experiments using guinea pig models as a detection system showed that naturally contaminated milk became unable to infect the animals after both LTLT (batch) and HTST pasteurisation (Heever *et al.* 1982).

3.3.3 *Cryptosporidium*

Where 10^{5} oocysts were heat treated at 71.7^{o}C for 15, 10 or 5 seconds in milk they were unable to infect infant mice (Harp *et al.* 1996). It was concluded that HTST pasteurisation is sufficient to destroy this organism.

3.3.4 *Campylobacter* spp.

Milk inoculated with 1.6 x 10^{6} *C. jejuni*/ml did not yield post pasteurisation survivors under HTST conditions, but the organism did withstand 10 seconds exposure at this temperature (Gill *et al.* 1981).

Data from the ICMSF give D times of 1.3-5.4 minutes in skim milk at 50^{o}C, and 0.74-1.0 minute at 55^{o}C in the same medium (ICMSF 1996). In physiological saline D times were 0.71-0.78, 0.24-0.28, 0.12-0.14 minutes at 56, 58 and 60^{o}C respectively (Sorqvist 1989).

3.3.5 *Clostridium botulinum*

As for *Bacillus* spores, pasteurisation is inadequate to inactivate spores of *Clostridium botulinum*. It appears that endospores of this organism have variable degrees of

natural heat resistance. At 100ºC, it would take 240 minutes to kill 72 x 10^9 spores compared to 40 minutes to kill 328 endospores. At 120ºC, it would need 5 minutes to kill 60 x 10^9 endospores (Jay 1986). Pasteurisation conditions of 125^{o}C for 5 seconds have been shown to be necessary for the destruction of spores of this organism (Collins-Thompson and Wood 1993) when found in low numbers.

3.3.6 *Coxiella burnetii*

It was reported in the late 1940s to early 1950s (Lennette *et al.* 1952) that this organism was capable of surviving pasteurisation conditions. Three samples showed survival, one processed at 62.2^{o}C for 30 minutes and two at 73.4^{o}C for 17 and 15.2 seconds respectively. Work was therefore undertaken under laboratory and commercial HTST conditions to examine this further (Enright *et al.* 1957). The work described produced thermal destruction times where 100,000 guinea pig units (10 times that considered the maximum possible in cow's milk) became non-infectious under the conditions used. The results showed that heating milk to 61.6^{o}C for 30 minutes would not inactivate the organism, while holding for the same time at 63^{o}C would. Under HTST conditions (72^{o}C for 15 seconds) it was concluded that these conditions are "..adequate to eliminate viable C. burnetii (sic) from whole raw milk". Because of the nature of the data provided it is not possible to calculate D times or *z* values for this organism.

3.3.7 *Escherichia coli* O157:H7

Very little information is available concerning the thermal inactivation of this organism in milk. Most of the data pertains to inactivation in meat and apple juice. In the only study identified, at 63^{o}C with an exposure time of 16.2 seconds and using a cocktail of isolates, D times of 4.3, 13.8 and 2.8 seconds were recorded (D'Aoust *et al.* 1988).

Z values of 4.1^{o}C in minced beef (Doyle and Schoeni 1984), 4.3 to 4.7^{o}C also in minced beef and 4.8^{o}C in apple juice (Splittstoesser *et al.* 1996) have been reported.

The similarity of this organism to *Salmonella* means that it is likely to have similar inactivation kinetics, i.e. it is not particularly heat resistant.

3.3.8 *Listeria monocytogenes*

The inactivation of *L. monocytogenes* by pasteurisation has been the subject of considerable scientific debate, especially in the mid 1980s. This followed an outbreak of listeriosis in Massachusetts in 1983 where pasteurised milk was identified as the vehicle (Fleming *et al.* 1985). Fourteen patients died, representing a case fatality rate of 29%. No fault in the pasteurisation could be identified, and the abstract of the paper comments "These results.…raise questions about the ability of pasteurisation to eradicate a large inoculum of *L. monocytogenes* from contaminated raw milk".

Even prior to this there had been controversy over the ability of *L. monocytogenes* to withstand pasteurisation. Survival of inocula in excess of 5 x 10^4 after batch (61.7^{o}C for 35 minutes) pasteurisation was demonstrated, and a D time at this temperature of 9.5-10.8 minutes calculated (Bearns and Girard 1958).

It was postulated that *L. monocytogenes* may be protected from pasteurisation after being ingested by phagocytes in raw milk (although the survival of intact phagocytes in the process has been questioned) (Fleming *et al.* 1985). Experiments using *L. monocytogenes* Scott A, isolated from the Massachusetts outbreak, however demonstrated that there was little difference in the thermal resistance of this isolate either freely suspended in milk or internalised by phagocytes (Bunning *et al.* 1986). D and *z* values are shown below:

Temperature (^{o}C)	D value (intracellular) (s)	D value (free) (s)
63.3	33.3	33.4
68.9	7.0	7.2
71.7	ND	1.3

z values: 6.0^{o}C for intracellular cells and 6.1^{o}C for freely suspended cells.

Data showing similar values have been reported in another paper (Bradshaw *et al.* 1985). These data are summarised below:

Temperature (^{o}C)	D value (s)	Range (s)
52.2	1683.7	1612.9-1754.4
57.8	289.6	269.5-309.6
63.3	19.9	13.4-28.4
66.1	7.3	6.2-10.1
68.9	3.0	2.1-4.2
71.7	0.9	0.8-1.1
74.4	0.7	0.5-0.9

The *z* value was 6.3^{o}C.

Variation in the D value at 62.7^{o}C has been shown for different isolates (Donnelly and Briggs 1986). The values ranged from 0.4 to 1.0 minute, and the *z* value for the most heat resistant isolate was 4.3^{o}C. Given a 30 minute holding time in the LTLT or batch process, this would give a 30 D reduction in numbers of *L. monocytogenes* during pasteurisation.

D times at 62^{o}C of 0.1-0.4 minute in sealed tube inactivation experiments have been determined (Donnelly *et al.* 1987). This paper also demonstrated that experiments where test tubes containing milk which are placed in waterbaths can give misleading results (see also information regarding *M. avium* subsp. *paratuberculosis* below). This phenomenon, whereby a constant number of surviving listeriae are present in the lid or on the walls of the tube above the waterline, was used to explain the results of Berans and Girard (1958). A similar finding was reported by Beckers *et al*., 1987.

In contrast to the information above, evidence was then presented which appeared to demonstrate that *L. monocytogenes* could survive the HTST process (Doyle *et al.* 1987). The organism, which was being shed in milk within polymorphonuclear leukocytes, was shown to survive pasteurisation when temperatures of 71.7-73.9^{o}C were used, but not at temperatures higher than this. Other work, also carried out using a pilot scale pasteuriser detected survivors when milk was pasteurised to 72^{o}C for 15

seconds with an inoculum of 6.5 $\log_{10}$ organisms/ml (Garayzabal *et al.* 1987). Cold enrichment was the best technique for detecting cells surviving the heat treatment.

Further work concerning the location of *L. monocytogenes* in milk failed to demonstrate increased heat resistance in bacterial cells located intracellularly (Bunning *et al.* 1988). The same paper reported a 33.4 D reduction in numbers during an LTLT process, but an unsafe 3.7 D reduction during HTST pasteurisation. A *z* value of 8^{o}C was derived. Work where *L. monocytogenes* was subjected to different pre-pasteurisation heat treatments indicated that the organism may survive HTST processing under some circumstances, but it was concluded that under normal conditions HTST would be effective (Farber *et al.* 1992).

Work which produced inactivation models for *L. innocua* showed that *L. monocytogenes* was more heat sensitive than the non-pathogenic species (Piyasena *et al.* 1998). This analysis of thermal inactivation took into account variability between experiments and built this into a stochastic model. The average reduction for a heat treatment of 72^{o}C for 16 seconds was 28.1 $\log_{10}$ units while the 95th percentile was 11.4 $\log_{10}$ units.

As a possible explanation of some of the discrepant findings, D values for *L. innocua* were found to be different when assessed using a pilot scale pasteuriser and a capillary tube method (Fairchild *et al.* 1994). D times at 65, 68 and 70^{o}C were 11.5, 3.5 and 1.6 s respectively when measured in the pasteuriser, and 16.5, 3.9 and 1.5 when measured in capillary tubes. Consequently the *z* values produced by each approach were different, being 5.9^{o}C for the pasteuriser and 4.8^{o}C when using capillary tubes. It was concluded that batch type lethality determinations are appropriate for batch processes (i.e. LTLT), while the laboratory scale pasteuriser is more appropriate for determining lethality in continuous flow systems.

The efficacy of HTST processing was demonstrated in four different experiments using up to 10^5 *L. monocytogenes*/ml (Lovett *et al.* 1990).

Should any cells survive pasteurisation they will most likely be heat injured. It has been shown that cells injured by pasteurisation cannot compete with surviving thermoduric organisms and do not grow in milk held under refrigerated storage (Crawford *et al.* 1989).

3.3.9 *Mycobacterium avium* subsp. *paratuberculosis (MAP)*

There has been a great degree of interest in this organism recently because of its possible link with Crohn's disease in humans (Bull *et al.* 2003;Chiodini 1989). In addition there have been reported isolations of the organism from pasteurised milk (Grant *et al.* 2001). The controversy surrounding the effect of pasteurisation on the organism has recently been reviewed (Lund *et al.* 2002) and it is apparent that much of the disagreement revolves around methodological problems which include the propensity of the organism to clump and so make the preparation of dilutions difficult, and the slow rate of growth of the organism in culture.

Methodological problems also exist with the ways in which heat treatments are delivered to inoculated milk. For example there are many cases where not all of the milk may have reached the target temperature, and where pilot scale pasteurisers are used the milk flowing through the holding tube shows laminar flow rather that the

turbulent flow found in commercial machines (this has an impact on the variability of time that any particle may take to pass through the holding tube).

As a number of different methods have been used, the reported decimal reductions that LTLT and HTST treatments achieve varies (Lund *et al.* 2002). For LTLT the values ranged from <2 $\log_{10}$ units to >10 $\log_{10}$ units, while for HTST the range was <2 $\log_{10}$ units to 5.6-6 $\log_{10}$ units.

A publication has appeared since the review was published from New Zealand workers (Pearce *et al.* 2001). They used a validated pilot plant pasteuriser operating with turbulent flow in the holding tube. The data presented in this paper gave mean D times of 15.0 +/- 2.8 s at 63°C and 5.9 +/- 0.7 s at 66°C, with a mean *z* value of 8.6°C. Extrapolation to 72°C indicated a D time of <2.03 s, equivalent to a >7D kill under HTST conditions.

3.3.10 *Mycobacterium bovis*

Death kinetics of three isolates of *M. bovis* have been examined (Kells and Lear 1960). *Z* values of 4.8, 4.9 and 5.2°C were calculated. D times calculated from the data presented are as follows;

Isolate 12621	Temperature (°C)	D time (s)
	64	6.6
	65	3.4
	66	2.3
	67	1.4
	68	0.9
	69	0.6
Isolate 11756	64	4.8
	65	3.3
	66	2.4
	67	1.3
	68	0.8
	69	0.4
Isolates 854	64	4.8
	65	2.3
	66	1.8
	67	1.3
	68	0.9
	69	0.6

It was concluded that pasteurisation conditions gave a considerable margin of safety when the organism is present at the "maximum probable concentration" in raw milk (taken here to be 10^4/ml).

3.3.11 Pathogenic *Streptococcus*

Streptococci are not unusually heat resistant. The following are D times for *Strep. pyogenes* in milk (International Commission on Microbiological Specifications for Foods 1996):

Temperature (°C)	D time at stated temperature (mins)
60	0.44
62	0.33
65	0.15
70	0.02
72	0.01
75	0.01
78	0.007
79	0.005
65	0.1-0.2
66	0.1-0.2

3.3.12 *Salmonella* spp.

In an analysis of multiple isolates of *Salmonella*, D times of between 3.5 and 5.9 seconds were recorded at 60°C, and from 2.2 to 3.7 seconds at 63°C. *S.* Senftenberg was more thermally resistant, with D times of 3 to 3.2 seconds at 66°C (D'Aoust *et al.* 1987).

A review on the thermal resistance of salmonellae has been published recently (Doyle and Mazzotta 2000). Table 4 in that paper records D times for inactivation of salmonellae in raw milk, and the data are summarised below:

Temperature (°C)	D time at stated temperature (mins)
51.8	22.6 (mean of two values)
57.2	1.7
60.0	0.084 +/- 0.026 (mean and standard deviation of six values)
61.5	0.063 +/- 0.025 (mean and standard deviation of five values
62.8	0.11
63.0	0.05 +/- 0.015 (mean and standard deviation of five values)
64.5	0.051
67.5	0.046
68.3	0.015
71.7	0.004

Z values from the same paper are 5.3°C for two isolates

A D time of 147.0 s at 55.5°C was reported as the only datapoint in one paper (Moore and Madden 2000).

Salmonellae isolated from dried milk (7 serotypes) had D times of 3.6-5.6 s, 1.1-1.8 s and 0.28-0.52 s at 62.8, 65.6 and 68.3°C respectively. For *S.* Senftenberg D values of 34.0, 10.0, 1.2 and 0.55 s were recorded at 65.5, 68.3, 71.7 and 73.9°C respectively (Read *et al.* 1968).

Factors found to influence the thermal resistance of *Salmonella* in milk include total solids (higher total solids gave increased *z* and D values), pressure (reduced pressure

decreases heat resistance), and inoculum growth temperature (higher temperature increases heat resistance). The effects of other factors, such as water activity are beyond the scope of this report.

3.3.13 *Staphylococcus aureus*

D times for inactivation of this organism in milk are as below (International Commission on Microbiological Specifications for Foods 1996):

Temperature (^{o}C)	D time (mins)
50	10.0
55	3.0
60	0.9
65	0.2
70	0.1
75	0.02

A *z* value of 9.5^{o}C was reported.

3.3.14 *Yersinia enterocolitica*

There appears to be little information available on the thermal inactivation of *Y. enterocolitica*. D times obtained in physiological saline have been reported as 1.4-1.8, 0.40-0.51 and 0.15-0.19 minutes at 58, 60 and 62^{o}C. The *z* values reported ranged from 4.00 to 4.52^{o}C (Sorqvist 1989).

Considerable variability has been demonstrated in heat resistance of different isolates of the organism (Hanna *et al.* 1977). When five isolates, inoculated at 10^{6}-10^{7} cfu/ml were heat treated at 60^{o}C in skim milk, all became undetectable within 3 minutes.

The efficacy of LTLT pasteurisation (62.8°C for 30 minutes) has been shown in brain heart infusion broth, skim milk and whole milk with three different isolates of the organism added at levels of 10^{5} to 10^{6} per ml (Kushal and Anand 1999). None of the isolates survived pasteurisation treatment. However, these authors also reported recovery of injured *Y. enterocolitica* cells after post-pasteurisation storage at 10^{o}C for 8-10 days (but not before this time). While pasteurised milk is unlikely to experience these conditions the observation indicates that some cells survived pasteurisation, albeit in a sub-lethally damaged form.

D times at 62.8^{o}C varied from 0.7 to 17.8 s when 21 different *Y. enterocolitica* isolates were tested (Francis *et al.* 1980). Parallel inactivation curves were obtained for three *Y. enterocolitica* isolates, but for one isolate the D times were higher than for the other two with values at 62.8^{o}C of between 0.24 and 0.96 minute reported (Lovett *et al.* 1982). The *z* values reported were higher than those cited above, being in a range of 5.11 to 5.87^{o}C. Similar values for *z* were obtained in skim milk where data read from the graphs presented indicated *z* values of 5.3^{o}C when cells were incubated at 37^{o}C prior to testing and 6^{o}C when pre-incubation was at 4^{o}C (Pagan *et al.* 1999).

4 REGULATIONS AND STANDARDS CONCERNING PASTEURISATION

4.1 New Zealand

In New Zealand, legislation relating to the sale of food on the domestic market is embodied in the Food Act 1981. The Act allows the sale of small quantities of raw milk at farm premises. Otherwise the Food Act 1981 requires all milk and milk products manufactured for sale, used as ingredients in the manufacture of any food for sale, or sold by retail to be processed according to one of the recognised methods in the New Zealand (Milk and Milk Products Processing) Food Standards 2002.

The Dairy Industry Act 1952 governs the manufacture, sale and export of dairy produce. However the Dairy Industry Regulations 1990, made under the Act establish the food safety outcomes, which must be met for dairy products to be considered safe for domestic consumption and export. Before milk is pasteurised, the microbiological quality of raw milk collected from dairy farms by mobile tankers in New Zealand is safe-guarded and maintained by a series of dairy standards (D101 to 106). Together these make up the requirements for a registered farm to have a registered product safety programme (PSP), PSP reporting requirements, milk cooling systems and procedures, farm animal health and farm dairy water quality. All these aspects influence the microbial quality of raw milk as presented at the processing plant. Pasteurisation would be more effective if the microbial quality of raw milk is good from the outset. Raw milk collected at the farm must not have an aerobic plate count at 30ºC of more than 10^5 cfu/ml.

There are two basic variants of the pasteurisation process, the long time low temperature (LTLT), or holding, and high temperature short time (HTST) methods. These processes were not designed to deliver equivalent bactericidal effects (Bunning *et al.* 1986), but interpolation between them gives equivalence if the organism in question has a *z* value of 4.3^{o}C. Values different to this for individual organisms mean that the lethality delivered by the two approaches will not be equivalent, and possibly the only organism considered here with a *z* value around 4.3^{o}C is *Y. enterocolitica*. An organism with a *z* value >4.3^{o}C is killed more effectively under LTLT conditions than under HTST treatment as HTST conditions are less lethal than LTLT for such an organism. Most of the hazards discussed here have *z* values exceeding 4.3^{o}C.

Standards in this section and the one below include much ancillary information beyond the time and temperature requirements for pasteurisation.

Since September 1993, most milk treatment stations have been following the MRD Standard 3: Standard for Pasteurisation Heat Treatments. In New Zealand, this Standard is based on manuals MQD 1B and MDQ 12, the Food Regulations, Codex Alimentarius Commission code of hygienic practice for dried milk and the FDA Grade "A" milk ordinance (described in section 4.3).

The heat treatment portion of the standard is as below.

"Pasteurised milk is defined as that:

- Has been heat treated by one of the following minimum temperature and time combinations:
    - The batch holding method (63°C for 30 minutes), or
    - The HTST method (72°C for 15 seconds), or
    - The HHST method (89°C and above for 1 second and less), or
    - An equivalent heat treatment allowed in section 5 below".

Section 5 states (*inter alia*):
"Alternative heat treatments must have a bactericidal effect equivalent to the treatments in section 4.

Equivalent heat treatments for pasteurisation of milk up to 10% fat are one of the combinations below or an interpolation between them:

Minimum Holding Time (s)	Minimum Temperature (°C)
0.01	100
0.05	96
0.1	94
0.5	90
1.0	89
8	73.4
9	73.1
10	72.8
11	72.7
12	72.5
13	72.3
14	72.1
15	72.0
16	71.9
17	71.8
18	71.7
19	71.6
20	71.5
22	71.3
24	71.1
26	70.9
28	70.8
30	70.7
35	70.4
40	70.1
45	69.9
50	69.7
55	69.5
60	69.3"

Additional conditions apply to dairy products containing >10% fat or sweeteners, and condensed or concentrated dairy products.

On the 8 December 2002, the Food Regulations 1984 that regulated processing of milk and milk products were revoked and all the dairy regulations were updated and consolidated into the New Zealand (Milk and Milk Products Processing) Food Standards 2002.

In this new standard, processing method or methods are set against each milk and milk product:

Dairy Product	**Permitted methods of processing**
Milk (of any type)	Pasteurisation
Cream (of any type)	Pasteurisation
Fermented milk products, including yoghurt	Pasteurisation
Cheese	Pasteurisation
Cheese with moisture content <39% moisture and a pH level <5.6	Pasteurisation Cheese Treatment
Emmental, Gruyere or Sbrinz Cheese	Pasteurisation Cheese Treatment The method set out in the *Ordinance on Quality Assurance in the Dairy Industry* of the Swiss Federal Council of 18 October 1995
Butter	Pasteurisation
Ice cream	Ice cream treatment
Dried evaporated and condensed milk	Pasteurisation

(from New Zealand (Milk and Milk Products Processing) Food Standards 2002).

The interpretation of pasteurisation, ice cream treatment and cheese treatment must be taken in the following context:

Pasteurisation means treatment according to one of the following methods –

- The holding method – milk or milk product is rapidly heated to a temperature of not less than 63ºC and not more than 66ºC, retained at that temperature for not less than 30 minutes, and then –

  (A) Immediately and rapidly reduced to 5 ºC or less in the case of milk and milk products other than cream, or to 7ºC or less in the case of cream
  (B) Maintained at or below that temperature until the milk or milk product is removed from the premises for delivery;

- The high-temperature short time method – milk and milk product is rapidly heated to a temperature of not less than 72ºC, retained at that temperature for not less than 15 seconds, and then chilled according to (A) and (B) above;

- Any other heat treatment method that is as effective in terms of bacterial reduction as methods described above.

Ice cream treatment means heat treatment of an ice cream mix to be used in ice cream by retaining the ice cream mix –

- At a temperature of not less than 69ºC for not less than 20 minutes; or
- At a temperature of not less than 74ºC for not less than 10 minutes; or
- At a temperature of not less than 79.5ºC for not less than 15 seconds; or
- At a temperature of not less than 85.5ºC for not less than 10 seconds; or
- At another temperature for a time which achieves an equivalent result to the treatments above;
- and followed by freezing the ice cream mix.

Cheese treatment means –

- The rapid heating of milk or milk product to be used in the manufacture of cheese to a temperature of not less than 64.5ºC, retaining it at that temperature for not less than 16 seconds; and
- Storing the cheese prior to sale at a temperature of not less than 7ºC for not less than 90 days from the date of commencement of manufacture.

With the introduction of this new Milk and Milk Products Processing Standards, issued under the Food Act 1981, the MRD Standard 3 will be withdrawn from 14 April 2005. Meanwhile a new NZFSA Dairy and Plants Standard, D121.1 Dairy Heat Treatments, has been introduced on 14 April 2003 following issuance of Circular 77 under the Dairy Industry Regulations 1990. Most milk-produce processing in dairy plants would have switched or will be switching to this new standard pending an equipment upgrade. From 14 April 2005, standard D121.1 will supercede MRD Standard 3.

Pasteurisation, under Standard D121.1, is defined as:

- Rapidly heating milk to a temperature of no less than 72ºC and retaining it at that temperature for no less than 15 seconds; or
- Rapidly heating milk to a temperature of no less than 63ºC and retaining it at that temperature for no less than 30 minutes.
- Other heat treatment and holding time combinations of dairy produce equivalent to pasteurisation may be used, based on the equivalence illustrated in the following table:

Heat treatments equivalent to pasteurisation for common types of dairy produce:

	A1	A2	A3	B1	B2	B3	C
	All dairy produce (excluding ice cream) with						
	Milks with <10% fat and no added sweeteners and particles			Dairy produce with 10% fat and/or added sweeteners and concentrated dairy produce with 15% total solids and particles			Ice cream mixes with particles
Particle diameter	<200 µm ϕ	200 to <500 µm ϕ	500 to <1000 µm ϕ	<200 µm ϕ	200 to <500 µm ϕ	500 to <1000 µm ϕ	<1000 µm ϕ
Minimum holding time (seconds)	Minimum temperature (ºC)						
1.0	81.6	-	-	84.4	-	-	-
2.0	79.0	81.6	-	81.8	84.4	-	-
3.0	77.6	79.0	-	80.4	81.8	-	-
4.0	76.5	77.6	81.6	79.3	80.4	84.4	-
5.0	75.7	76.5	79.0	78.5	79.3	81.8	-
6.0	75.1	75.7	77.6	77.9	78.5	80.4	-
7.0	74.6	75.1	76.5	77.4	77.9	79.3	-
8.0	74.1	74.6	75.7	76.9	77.4	78.5	-
9.0	73.7	74.1	75.1	76.5	76.9	77.9	-
10.0	73.3	73.7	74.6	76.1	76.5	77.4	85.5
11.0	73.0	73.3	74.1	75.8	76.1	76.9	-
12.0	72.7	73.0	73.7	75.5	75.8	76.5	-
13.0	72.4	72.7	73.3	75.2	75.5	76.1	-
14.0	72.1	72.4	73.0	74.9	75.2	75.8	-
15.0	72.0	72.1	72.7	74.8	74.9	75.5	79.5
30.0	70.7	70.8	70.9	73.5	73.6	73.7	-
60.0	69.4	69.4	69.5	72.2	72.2	72.3	-
Minimum holding time (minutes)	**Minimum temperature (ºC)**						
1	69.4	69.4	69.5	72.2	72.2	72.3	-
2	68.1	68.1	68.1	70.9	70.9	70.9	-
5	66.4	66.4	66.4	69.2	69.2	69.2	-
10	65.1	65.1	65.1	67.9	67.9	67.9	74.0
15	64.3	64.3	64.3	67.1	67.1	67.1	-
20	63.8	64.8	64.8	66.6	66.6	66.6	69.0
25	63.3	63.3	63.3	66.1	66.1	66.1	-
30	63.0	63.0	63.0	65.8	65.8	65.8	-

(extracted from D121.1 Dairy Heat Treatments)

Notes:

1. ϕsignifies particle diameter
2. Minimum holding time. The minimum holding time is set at 1 second to give an adequate safety margin. Shorter holding times will require validation to demonstrate the effectiveness of the time temperature combination in controlling the hazard(s).

Immediately heating or cooling to a temperature that maintains the produce in a wholesome condition either until further processing or for the duration of its shelf life is an integral part of this process at the end of heat treatment. The range of temperature equivalents gives processors some flexibility in deciding which temperature/holding time combination to use when developing new products.

Following processing, the end products must comply with NZFSA Dairy Standard 107.2, “Dairy Product Safety”.

4.1.1 Dairy product safety criteria used in New Zealand

In New Zealand, surrogate bacterial indicators that are non-pathogenic are no longer used as criteria of safety in a milk treatment process. A generic criterion based on levels of pathogenic microorganisms that should be achievable using the process, has been adopted in May 2003 as the Dairy Standard 107.2: "Dairy Product Safety". This newly introduced standard provides generic microbial product safety limits (PSLs) for dairy products that are pasteurised or treated with an equivalent recognised process by industry. These new microbial product safety limits were adopted from a report recommended to the Technical Consultative Committee on PSLs for the NZ Dairy Industry (3 March 2003). There were slight modifications before final adoption as the NZFSA Dairy Standard D107.2, "Dairy Product Safety". The limits have a two-class PSL approach, one for the general public and the other for more susceptible members of the population. The following table is extracted from NZFSA Dairy Standard D107.2:

Product Safety Limits for Pathogenic Bacteria

Pathogen	**General PSL (1,3)**	**Specific PSL (2,3)**	**Explanatory notes/comments**
Salmonella	ND/25g	ND/250g	• ND = Not detected in the volume tested. • Composite of samples collected throughout the production run as defined by the manufacturer's PSP
Listeria monocytogenes	ND/25g (4)	ND/25g	• ND = Not detected in the volume tested. • Composite of samples collected throughout the production run as defined by the manufacturer's PSP
Coagulase-Positive Staphylococci (*S. aureus*)	1000/g	100/g	• It is critical that sampling and testing are performed in a way that correctly estimates the maximum number of *S. aureus* reached in a product. This is important because the risk posed by released enterotoxin is "estimated" by the bacterial load.
Bacillus cereus	1000/g	100/g (5)	
Escherichia coli	100/g	10/g	

(1) General PSLs: for product to be consumed by the general public
(2) Specific PSLs: For products that are specifically designated for, and are likely to form, a substantial part of the dietary intake of more susceptible members of the population (i.e. infants and young children, the old, pregnant and immuno-compromised).
(3) Sampling rates: If testing is required, the rate of sampling for each organism/product combination should be decided as part of a HACCP analysis performed on the manufacturing process.
(4) *Listeria monocytogenes*: 100/g may be adopted if NZFSA and the dairy industry are convinced that this level has become accepted by reputable food safety authorities worldwide.
(5) *Bacillus cereus*: This limit only applies to product designated as infant formula.

In tandem with these limits, the NZFSA Dairy Standard D110, "Dairy HACCP Plans" must first be followed for the development of a PSP. The PSP decides the sampling rate and testing regime performed on the manufacturing process. The pasteurisation process under the PSP will have to demonstrate compliance with these PSLs.

4.2 Australia

In Australia, the processing requirements for milk and liquid milk products must comply with Standard 1.6.2 Processing Requirements of the Food Standards Code. In this standard, the processing parameters are as follows:

1 Milk and liquid milk products:

a) Heating to a temperature of no less than 72ºC and retaining at such a temperature for no less than 15 seconds and immediately shock cooling to a temperature of 4.5ºC; or

b) Heating using any other time and temperature combination of equal or greater lethal effect on bacteria;

-unless an applicable law of a State or Territory otherwise expressly provides.

2 Liquid milk products must be heated using combinations of time and temperature of equal or greater lethal effect on the bacteria in liquid milk that would be achieved by pasteurisation or otherwise produced and processed in according with any applicable law of a State or Territory.

In the Food Standards Code, no standard is set for pasteurised milk but a microbiological guideline criteria for pasteurised milk is used. The criteria for pasteurised milk is as follows:

Campylobacter per 25 ml	n=5,	c=0,	m=0	
Coliforms per ml	n=5,	c=1,	m=1,	M=10
Psychrotrophic organisms per ml	n=5,	c=1,	m=10,	$M=10^2$
Listeria monocytogenes per 25 ml	n=5,	c=0,	m=0	
Salmonella per 25 ml	n=5,	c=0,	m=0	
SPC per ml (30ºC)	n=5,	c=1,	$m=5X10^4$,	$M=10^5$

4.3 USA

The FDA Grade "A" pasteurised milk ordinance (FDA 2000) define pasteurisation as "..the process of heating every particle of milk or milk product, in properly designed and operated equipment, to one of the temperatures given in the following chart and held continuously at or above that temperature for at least the corresponding specified time:

Temperature	Time
*63°C (145°F)	30 minutes
*72°C (161°F)	15 seconds
89°C (191°F)	1.0 second
90°C (194°F)	0.5 second
94°C (201°F)	0.1 second
96°C (204°F)	0.05 second
100°C (212°F)	0.01 second

* If the fat content of the milk product is 10% or more, or if it contains added sweeteners, the specified temperature shall be increased by 3°C (5°F)."

The same document states that bacteriological limits for Grade "A" raw milk for pasteurisation should have a bacteriological count not to exceed 100,000 per ml prior to commingling, and 300,000 per ml after commingling. Pasteurised milk itself has a limit of 20,000 per ml, and a coliform limit of 10 per ml. These limits did not change in the 2002 revision (www.cfsan.fda.gov/~ear/pmo01-2.html), and represent time and temperatures for treatment which are the same as those used in New Zealand.

4.4 Europe

EC Directive 92/46/EEC of June 1992 indicates that (*inter alia*) pasteurised milk must have been obtained by means of a treatment involving a high temperature for a short time (at least 71.7°C for 15 seconds or any equivalent combination) or a pasteurisation process using different time and temperature combinations to obtain an equivalent effect.

Raw milk intended for the production of heat-treated drinking milk, fermented milk, junket, jellied or flavoured milk and cream must meet a plate count standard of 100,000 per ml (when tested at 30°C). This is a geometric mean over a period of two months with at least two samples a month tested.

Standards applied to pasteurised milk are;

Pathogenic micro-organisms: absent in 25g n=5, c=0, m=0, M=0

Coliforms (per ml) n=5, c=1, m=0, M=5

Plate count at 21°C (per ml) (after incubation at 6°C for 5 days)
n=5, c=1, m=$5x10^4$, M=$5x10^5$

5 COMPARISON OF PASTEURISATION WITH ALTERNATIVE PROCEDURES

5.1 Alternatives to Pasteurisation

5.1.1 Thermisation (synonyms: "subpasteurisation", "heat treatment" and "cheese treatment")

In New Zealand, the New Zealand (Milk and Milk Products Processing) Food Standards 2002 include a requirement for raw milk (or a milk product) that is not subject to pasteurisation but will be made into cheese. The requirement is that the raw milk be subjected to a heat treatment of 64.5°C for not less than 16 seconds followed by storage for not less than 90 days from the date of commencement of manufacture at not less than 7°C. This is also reflected in the newly introduced D121.1 Dairy Heat Treatments, Circular No 77 of the Dairy Industry Regulations 1990. However the FSANZ Standard 1.6.2, Clause 2 allows two options, one of which is the high temperature short time method of pasteurisation (72ºC for 15 seconds), and the other remaining similar to the revoked Food Regulation (113.2b) i.e. heat treatment at a temperature of no less than 62ºC for a period of no less than 15 seconds and the final product stored at a temperature of no less than 2ºC for a period of 90 days from the date of manufacture of the cheese or cheese product.. Unlike the situation in the USA, where particular requirements are associated with particular cheese types, the New Zealand requirements only apply to the manufacture of cheese with moisture content less than 39% moisture (by mass), pH less than 5.6 and where the pH does not increase on ripening. ESR has produced a report on thermisation for the Ministry of Health (Baldwin 2001) which summarises data in the literature in relation to the ability of this process to inactivate pathogens which may be present in the raw milk. The report concluded that, provided the milk used is of good quality, the process will result in the production of hard cheese that is safe for consumption. The report also recommends that the process is not safe for the production of soft and fresh cheeses.

The process of thermisation has a rather loose meaning. Typical treatments reach 60-65°C for 10-20 seconds, although the range of temperatures used spans 57 to 68°C. The efficacy of thermisation in the destruction of pathogens appears to be controversial; from "recent thorough research has affirmed that milk heat-treatment at 65.0-65.6°C (149-150°F) for 16-18s will destroy virtually all known pathogenic microorganisms which are major threats to the safety of cheese" (Johnson *et al.* 1990) to a warning in regard to "The propensity of infectious agents to survive heat treatment of milk for 16-17s at subpasteurisation temperatures" (D'Aoust 1989).

However, since the level of heat treatment afforded by thermisation is less than that of pasteurisation, the degree of assurance of destruction of pathogens is, logically, less. Data do exist showing that thermisation can allow the survival of pathogens "of significance to cheese". For example an inoculum of around 10^5 *L. monocytogenes*/ml survived treatment for an average 17.6 seconds at 60, 63, 64.5, 66.0 and 67.5°C, but not at 69 or 72°C (Farber *et al.* 1988b). Under the same conditions *Salmonella* (excluding *S.* Senftenberg, which has higher thermal resistance but appears not to be significant to the dairy industry) survived temperatures up to 64.5°C (D'Aoust *et al.* 1987). Also under the same conditions an inoculum of around 10^5 *E. coli* O157:H7 /ml became non detectable at 64.5°C, but not at 63.0°C, while *Campylobacter* and *Y.*

enterocolitica inoculated at the same level were both non-detectable after treatment at 63.0°C (D'Aoust *et al.* 1988).

The heat treatment constitutes only half of the process, since there is a period of aging that the cheese must undergo prior to sale. It is therefore possible that any surviving organisms would be inactivated during this period. This is covered under "aging" below.

In New Zealand, the Food Act 1981 does recognise an equivalence to pasteurisation of milk for cheese making; that is the New Zealand (Milk and Milk Products Processing) Food Standards 2002 also incorporates the method set out in the *Ordinance on Quality Assurance in the Dairy Industry* of the Swiss Federal Council of 18 October 1995 as a method for Emmental, Gruyere or Sbrinz cheese. FSANZ (Standard 2.5.4) has also recognised this method of cheese making from raw milk.

5.2 Aging

Cheeses of some types made from raw or thermised milk may also be held for a period prior to sale or consumption (Johnson *et al.* 1990). The "thermisation" regulation in New Zealand requires the aging of cheese from the date of manufacture for not less than 90 days at not less than 7ºC.

The period of this aging in North America is, usually, 60 days at >2°C (Johnson *et al.* 1990). The efficacy of this aging process is contentious, and there has been considerable debate in North America as to the safety of cheese produced by this process.

A survey was carried out in the USA of 127 raw milk Cheddar cheese samples that had been aged for 60 days. *S. aureus* was detected in two samples (1.6%) at levels exceeding 1000/g, while *Salmonella* and *Campylobacter* were not isolated. A non-pathogenic *Y. enterocolitica* isolate was recovered from one cheese (Brodsky 1984). The pH reached by the cheese ranged from 4.98 to 5.5, with a mean of 5.26. It was concluded that "60-d aged raw milk Cheddar cheese produced in the Province of Ontario does not pose a significant health risk". However a prevalence of 1.6% of cheeses with staphylococcal counts in excess of 1,000/g might be considered significant in its own right, but it is also known that staphylococci can produce toxin in foods and subsequently reduce in numbers, potentially becoming undetectable, leaving the toxin unchanged. On rare occasions staphylococcal enterotoxin in cheese has caused outbreaks in the absence of the viable organism. (Wieneke *et al.* 1993). Under these circumstances the aging process will not improve the safety of the cheese.

The degree of safety afforded by aging depends on the characteristics of the cheese concerned. Work with Swiss hard (350 g/kg water, 310 g/kg fat) and semi-hard cheeses (390 g/kg water, 285 g/kg fat) demonstrated that none of *Aeromonas hydrophila*, *C. jejuni*, *E. coli, L. monocytogenes, Pseudomonas aeruginosa*, *Salmonella*, *Staph aureus*, or *Y. enterocolitica* could be detected in inoculated hard cheese one week post manufacture (Bachman and Spahr 1994). However *L. monocytogenes* survived the 90 day semi-hard cheese aging process. Swiss hard cheese has a significant heating step during manufacture (53°C for 45 minutes), while that for semi-hard cheese is lesser (42°C for 15 minutes), but the final pH reached is around the same at 5.2.

Both *L. monocytogenes* and *E. coli* O157:H7 have been shown to survive in Feta and Camembert for 60 days at 2°C (Ramsaran *et al.* 1998).

D times for the inactivation of MAP in Swiss hard (Emmentaler) and semi-hard (Tisliter) cheeses made from raw milk were recorded as 27.8 days for the hard cheese and 45.5 days for the semi-hard cheese during ripening (Spahr and Schafroth 2001). Given ripening periods of 4 months for Emmentaler and 3 months for Tisliter then the numbers of MAP would reduce by 4.3 and 2.0 $\log_{10}$ respectively.

Salmonella has been shown to survive in naturally contaminated cheddar cheese made from thermised milk stored at 5°C for up to 8 months (D'Aoust *et al.* 1985) in one from seven batches of cheese. In this case the initial concentration was not high since all samples contained less than 10 salmonellae when initially tested (at 2-3 months of storage). This work showed that *Salmonella* could cause disease when present in cheese at very low levels. *Salmonella* can grow during cheddar cheese manufacture and is concentrated in the curd (Park *et al.* 1970). Therefore pathogens that might be present in milk prior to manufacture will increase in numbers prior to aging. Similarly when *Salmonella* was inoculated into milk used to make Cheddar cheese at a level of around 10^5/ml, 16 of 48 lots contained detectable organisms after 9 months storage at 4.5°C, and in 6 lots after storage at 10°C for the same period (El-Gazzar and Marth 1992).

Acid adaptation has been shown to result in increased survival in cheeses (Leyer and Johnson 1992). It was postulated that salmonellae living in processing environments may be acid adapted, and that critical control points require verification using organisms which have been acid adapted.

As a further example of the influence of the characteristics on the cheese of the survival of *L. monocytogenes* during aging, three reports concerning four different cheeses are considered here; a very hard cheese (Parmesan), a hard cheese (Cheddar), a semi-soft goat's cheese and a soft cheese (Camembert). Relevant data from the publications are summarised in Table 3 below.

Table 3 Survival of *L. monocytogenes* During the Ripening of Various Cheeses

Cheese name	% moisture	pH	Change in *L. monocytogenes* numbers during ripening	Reference
Parmesan	30.1-31.4	5.1	Approximate 3 $\log_{10}$ reduction over 6 weeks ripening (12.8°C).	(Yousef and Marth 1990)
Cheddar	35.9-38.5	5.1	Highly variable decline; from approx. 2 $\log_{10}$ over 45 days to approx. 1 $\log_{10}$ over >300 days ripening (6 and 13°C).	(Ryser and Marth 1987a)
Semi-soft goat's	NS	5.5-6.5	< 1 $\log_{10}$ decline in 18 weeks at 12°C in two samples where counts could be made.	(Tham 1988)
Camembert	44.1-60.0	7.5	Grew on surface and interior for 60 days of ripening (10 days at 15-16°C, then at 6°C).	(Ryser and Marth 1987b)

From this information it is evident that the efficacy of aging in the removal of pathogens in raw milk or surviving thermisation is dependent on the type of cheese. Those with less moisture and lower pH values (among other factors) produce the fastest inactivation rates. *L. monocytogenes* is able to grow in cheeses such as camembert because of the high moisture content and the high pH caused by mould growing on the surface of the cheese.

Overall, aging appears to be an unreliable means of ensuring the safety of cheese. Even in hard cheese such as cheddar, organisms such as *Salmonella* can survive for months. Since cheese is generally eaten without cooking even small numbers may cause disease in a proportion of consumers. Some cheeses may have characteristics which produce a high degree of inactivation of pathogens, but this needs to be determined on a case by case basis.

5.3 Comparison of Pathogen Inactivation from Pasteurisation and Alternatives

The data available for the effects of pasteurisation on any one organism is heterogeneous. This is due to factors such as strain variation, and because the methods used to derive point estimates of inactivation for any one set of conditions is difficult. A more rigorous comparison could be made using a stochastic approach which could take into account variability and uncertainty.

However from the data which have been obtained some idea of the effect of the various treatments can be summarised. . Table 4 below shows a summary of data as cited above in terms of the D kill expected under both LTLT and HTST conditions. Also included are the values of *z* where known. The table has been "filled in" where possible either by using provided *z* values or by linear regression of thermal inactivation data so that data information for both forms of pasteurisation is provided.

Table 4 Summary of Pasteurisation Inactivation Data for Selected Pathogens

Organism	**Decimal reduction for LTLT method (63^{o}C for 30 minutes) (*z* value used)**	**Decimal reduction for HTST method (72^{o}C for 15 seconds) (*z* value used)**	***Z* values reported (^{o}C)**
Brucella	>300[1]	>114 (4.55)[2]	4.3-4.8, 5.3
Campylobacter	395.6[3]	72.8[3]	5.1, 5.6, 6.1
E. coli O157:H7	600.0[1] 130.4[1] 428.6[1]		
L. monocytogenes	54[1] 90.5[1] 30-75[1] 33.4[4] 101.1 (5.91)[2]	11.5[1] 16.7[1] 20.8-8.3 (5.91)[2] 3.7[4] 28.1[4]	4.3, 4.8, 5.9, 6.0, 6.1, 6.3, 8.0 (mean 5.91)
MAP	<2->10[4] 120[1]	<2->7[4] 11.1 (8.6)[2]	8.6

M. bovis	>273[1]	>144 (5.0)[2]	4.8, 4.9, 5.2 (mean 5.0)
Salmonella	300[3] 272[1] 486-818[1]	93.8[3] 113.1 (5.3)[2] 202-340 (5.3)[2]	5.3
Staph. aureus	72.4[3]	5.5[3]	9.5
Y. enterocolitica	>200->158[1] >5[1] 101-2571[1] 31.3-125[1] 117.6[3]	>96.2->76.0[2] (5.11) >2.4[2] (5.11) 48.6-1236.4[2] (5.11) 15.0-60.1[2] (5.11) 16.1[3]	4.0-4.52, 5.11-5.78, 5.3, 6.0 (mean 5.11)

[1] Calculated from D times given for the appropriate temperature
[2] Calculated using *z* value indicated
[3] Calculated using linear regression of data points
[4] D values given in reference

The heterogeneity of the information available is evident from the figures in this table. For example the D reduction for *Y. enterocolitica* during pasteurisation may range between a 31 and 2571. Since all of the organisms in this table have *z* values exceeding 4.3°C then the efficacy in terms of D inactivation is noticeably less under HTST conditions than LTLT pasteurisation. In general LTLT gives a D reduction that can be measured in hundreds, while HTST is approximately an order of magnitude less.

Table 5 compares equivalent thermal inactivation of HTST pasteurisation and thermisation at 64.4°C for 16 seconds

Table 5 Summary of equivalent kill of thermisation (64.4oC for 16 seconds) compared with HTST pasteurisation

Z value (°C)	Decimal reduction at HTST (arbitrary value)	Decimal reduction under thermisation conditions
4	10	0.134
4.5	10	0.218
5.0	10	0.322
5.5	10	0.445
6.0	10	0.574
6.5	10	0.720
8.0	10	1.199

The data produced in this table are a mathematical extrapolation of decimal reductions under HTST conditions to thermisation conditions (64.4°C for 16 seconds). The difference in temperature between the two criteria is 7.6 seconds (72-64.4). With a *z* value of 4°C the reduction achieved at the lower temperature is 7.6/4 = 1.9 $\log_{10}$ less (i.e. 79.4 fold less inactivation per second). From the arbitrary value of a 10D kill in 15 seconds, the D time at 72°C is 10/15 =0.667 D/second. At 64.4°C the D time will be 0.667/79.4 = 0.0084D/second. This rate of kill is applied over 16 seconds and so the total inactivation is 16 x 0.0084=0.134 D total inactivation.

Depending on the *z* value, thermisation results in a reduction of only 1-10% of that which would be obtained by HTST pasteurisation.

6 RISK ASSESSMENTS AND RESEARCH ON PASTEURISATION AND ALTERNATIVE TREATMENTS

A specific safety assessment of dairy products made from raw milk has been produced (D'Aoust 1989). Much of the information above is given in this paper, but the final paragraph warrants reproducing:

"An assessment of the potential health hazard associated with the manufacture of dairy products from non-pasteurised milk should recognise the following facts:

The ability of bacterial pathogens to survive, grow, and produce toxins during refrigerated storage of raw milk.

The propensity of infectious agents to survive heat treatment of milk for 16-17s at subpasteurisation temperatures.

The ability of pathogens to grow during the manufacture and ripening of cheese.

The growth of *Listeria* spp. in refrigerated soft cheese with a generation time of 1.5d at 4^{o}C.

The survival of bacterial pathogens well beyond the 60-d mandatory refrigerated storage of cheese manufactured from unpasteurised milk.

The high fat content of milk products that tend to protect pathogens against human gastric acidity.

The low human infective dose for some bacterial pathogens.

Possible degeneration of diarrheal disease into more serious and costly chronic conditions.

The increasing antibiotic resistance in bacterial populations.

The staggering cost of foodborne outbreaks to the manufacturer and to the public purse.

From these strong elements of risk, the critical question on whether or not we can afford any longer to manufacture dairy products from unpasteurised fluid milk needs to be resolved."

A three part review of unpasteurised milk cheese, however, came to a contradictory conclusion, with the executive summary stating "Recent thorough research has affirmed that milk heat-treatment at 65.0-65.6^{o}C (149-150^{o}F) for 16-18 s will destroy all pathogenic micro-organisms which are major threats to the safety of cheese" (Johnson *et al.* 1990;Johnson *et al.* 1990). However, the effectiveness of the 60 day holding period is not endorsed by this review. It was recommended that a minimum heat-treatment of 64.4^{o}C for 16 seconds, or equivalent, be adopted as a guideline.

Part three of this review (Johnson *et al.* 1990) produced the following prioritisation of foodborne hazards for cheese;

High risk:	*Salmonella*
	L. monocytogenes
	Enteropathogenic *Escherichia coli*
Medium risk:	*Streptococcus*
	Yersinia enterocolitica
	Brucella abortus
	Mycobacterium tuberculosis
	Pseudomonas aeruginosa
	Coxiella burnetii
	Vibrio spp.
	Aeromonas hydrophila
Low risk:	*Staphylococcus aureus*
	Clostridium botulinum
	Clostridium perfringens
	Corynebacterium diphtheriae
	Bacillus cereus
	Campylobacter jejuni
	Viruses (Hepatitis A, polio, retrovirus Coxsackie, Adenovirus, Herpes, Oncogenic, Foot and Mouth disease).

It is interesting to note the low risk associated with *Staph. aureus*, which is reported above to be the organism most often associated with disease produced by unpasteurised milk cheeses in France and other industrialised countries (see Section 2.30..

A quantitative risk assessment has been published which is focused entirely on soft cheeses made from raw milk (Bemrah *et al.* 1998). The probability of a resident of France consuming contaminated raw milk cheese was estimated at 65.3%, but the probabilities of consuming cheese containing greater than 10^2, 10^3 and 5 x 10^3 *L. monocytogenes* were 41%, 8.3% and 0.08% respectively based on a 31 g typical cheese serving size. An estimate of risk of listeriosis, based on the consumption of 50 portions of 31g per annum ranged from 1.97 x 10^{-9} to 6.4 x 10^{-8} in the low risk population subgroup, to between 1.04 x 10^{-6} and 7.19 x 10^{-5} in the high risk subpopulation. In a population of 50 million people this equates to 34 to 90 (mean 57) cases and 1 to 23 (mean 21) deaths per annum in the high risk subpopulation, and 0 to 4 cases (0 to 3 deaths) in the low risk subpopulation.

By eliminating the effects of mastitis from the model, exposure to *L. monocytogenes* was much decreased. (e.g. 99th percentile was around 100 *L. monocytogenes*/g when mastitis was modelled, and around 20/g when it was not included). The authors discuss at length the assumptions made and the fact that the results need to be treated with care because of these assumptions.

A risk assessment has been performed concerning the presence of *B. cereus* in pasteurised milk (Notermans *et al.* 1997). However this is really an exposure assessment as a conclusion reached is that the dose response information for this organism needs to be re-evaluated. The study combined data for the presence and numbers of *B. cereus* in pasteurised milk, studies on the growth of the pathogen in this food and information on time and temperature of storage from Dutch consumers

and consumption data to model the likely levels at the time of consumption. Results of the modelling showed that 7% of milk consumed would contain $>10^5$ *B. cereus* /ml, and 4% would contain $> 10^6$ /ml. These levels of *B. cereus* are those conventionally thought to be associated with disease, but given the large amount of milk consumed (10^9-10^{10} portions) and the lack of epidemiological information linking milk consumption with *B. cereus* intoxication it was concluded that the risk modelled was not congruent with the observed risk, and that the dose response model was likely to be the part of the model to be in error and it needed "to be considered".

FSANZ have approved the importation of some Swiss cheeses made from unpasteurised milk (www.foodstandards.gov.au/_srcfiles/A357%20FAR.pdf), following application A357 from the Swiss Federal Veterinary Office. The New Zealand (Milk and Milk Products Processing) Food Standards 2002 also recognise and permit this method of processing. The very-hard cheeses Emmentaler, Sbrinz and Gruyère were considered to pose no greater risk to public health than cheeses made from pasteurised milk. This determination was based on the fact that these cheeses are ripened for between 90 and 360 days. The semi-hard cheeses Tilsiter, Appenzeller and Vacherin Fribourgeois could all be manufactured from thermised milk and so met the standards. One semi-hard cheese, Tete de Moine, which is always made from raw milk, was not allowed to be imported.

This decision follows a FSANZ decision not to allow the production in Australia of specialty cheeses from raw milk (rejection of Application A270). This application was for the manufacture of hard dry and soft moist cheeses.

FSANZ have allowed the importation into Australia of extra hard grating cheeses (<36% moisture, including Parmigiano, Reggiano, Grana Padano and other Parmesan-style cheeses) made from raw milk (Food Standards Australia New Zealand 2003). The manufacture of these cheeses includes a step during which the curd is heated, and there is a long period of maturation which, it was concluded, amounted to a 5 $\log_{10}$ kill "of the pathogens of concern" which is equivalent to thermisation and aging.

A hazard assessment has been reported which examines the behaviour of *L. monocytogenes* in milk from the farm to immediately post pasteurisation. While the model uses stochastic distributions to represent variability, a Monte Carlo simulation was not carried out and results presented as the values for the 50th and 95th percentiles at each, and the probabilities of these values occurring (Peeler and Bunning 1994). At the median, there was a probability of 0.016 that grade A milk would contain *L. monocytogenes* at 1.7×10^{-11} cells per gallon, while at the 95th percentile the concentration was 1.0×10^{-4} cells per gallon, although the probability of this occurring was 1.6×10^{-8}.

An exposure assessment for MAP in pasteurised milk has been produced for the Netherlands (Nauta and van der Giessen 1998). The assessment gives a simple point estimate based on direct shedding of the organism into milk plus possible faecal contamination of milk. The model assumes three sub-populations of animal, healthy, subclinical and clinical, the last two of which are shedding MAP in their milk and faeces. A point estimate of 5.4 cfu/litre was derived for MAP in pasteurised milk from a farm with a high prevalence of infection. The paper also considers probability distributions but states that the data are not available to model variability with any accuracy. In assessing interventions to reduce the number of MAP in milk, preventing cows with clinical signs of MAP infection reduced exposure by 99%. Given the

assumed herd prevalence in the Netherlands (20%) and an equal division between sub-clinically infected and clinically infected animals, a point estimate exposure of 0.5 cfu/litre was derived. Some discussion surrounds the probabilistic approach, and how that would seem to suggest a higher median contamination rate, but a) the probability distributions were constructed in the absence of information on variability and b) there seems to be confusion between the median and the mean.

A quantitative risk assessment model has been produced that simulates the probability of an "unsatisfactory cheese" containing >6 $\log_{10}$ *S. aureus* per g (Lindqvist *et al.* 2002). The assessment was for unripened cheese made from raw milk. This number of cells had to be used as there is no satisfactory dose/response relationship for preformed toxins in food. Survey data were available for the organism in cheese at the time of sale, and growth was simulated during domestic storage. The data required for the model to be able to estimate risk were not available, with numerous datagaps being identified, and the model was used to estimate the changes of various parameters, e.g. pH and storage temperature, on the simulated potential risk. Interestingly a "negative" high pH cheese, modelled as containing the organism below the limit of detection at the point of sale could still, according to the model, produce an unsatisfactory cheese. The model demonstrated that a low initial pH was important in determining the probability of an unsatisfactory cheese.

Two draft risk assessments for *L. monocytogenes* in ready-to-eat foods have been produced, one by the USDA/FSIS in January 2001:

http://vm.cfsan.fda.gov/~dms/lmrisk1.html

and the other by FAO/WHO:

http://www.who.int/fsf/mbriskassess/Scientific_documents/mra001.pdf.

After the most recent round of revisions the FAO/WHO model has combined aspects of the FDA/FSIS one and almost merged the two. However, since the latest draft of the FAO/WHO assessment is not yet publicly available only the FDA/FSIS assessment will be discussed here.

It should be noted that this is very much a North American risk assessment and so used an exposure assessment which is particular to that part of the world (even though data from anywhere in the world were used to calculate prevalences in food). We might assume that the hazard characterisation (essentially dose response) would be the same in New Zealand as North America, but the derived risk characterisation will be different because of the different exposure assessments.

The relative risks predicted for the various ready-to-eat food categories in the FDA/FSIS risk assessment are given in Table 6. These risk rankings are quite consistent with results from case control studies.

Table 6: Predicted relative risks of listeriosis based on median values for the North American population on a per serving basis (1 represents the highest ranked risk and 20 the lowest)

Food Categories	Sub-Population		
	Intermediate Age	Elderly	Perinatal
	Relative Rank (1-20)		
SEAFOOD			
Smoked seafood	3	3	3
Raw seafood	14	14	14
Preserved fish	7	7	6
Cooked ready-to-eat crustaceans	6	5	5
FRUIT AND VEGETABLES			
Vegetables	17	17	17
Fruits	18	18	18
DAIRY PRODUCTS			
Soft mould ripened & blue vein cheese	9	9	9
Goat, sheep and feta cheese	16	16	16
Fresh soft cheese (e.g. queso fresco)	2	1	1
Heat-treated natural/process cheese	15	15	15
Aged cheese	19	19	19
Fluid milk, pasteurised	10	10	10
Fluid milk unpasteurised	11	11	11
Ice cream and frozen dairy products	20	20	20
Miscellaneous	12	13	13
MEATS			
Frankfurters*			
All frankfurters	8	8	7
Only reheated frankfurters	[15]	[15]	[15]
Only non-reheated frankfurters	[1]	[2]	[2]
Dry/semi dry fermented sausages	13	12	12
Deli meats	4	4	4
Pâté and meat spread	1	2	2
COMBINATION FOODS			
Deli salads	5	6	8

*Numbers in square brackets are for sub frankfurters either eaten raw or cooked.

Source: USDA/FSIS (http://vm.cfsan.fda.gov/~dms/lmrisk1.html)

The highest ranking risk assigned to a dairy product was to soft cheese, which ranked the second highest risk out of 20 foods. This was because of the high contamination frequency, high contamination levels at retail, moderate growth rate during storage and long storage time.

Ranking 9th was soft mould ripened and blue vein cheese. While consumption of these cheeses was low, the long storage time which they experience gives *L. monocytogenes* time to grow to high numbers.

It is interesting to note that pasteurised and unpasteurised cheese are ranked very closely (10th and 11th). For pasteurised milk the contamination rate was low, but this was offset by the amount consumed. For unpasteurised milk consumption was infrequent, but this was offset by a moderate risk of contamination and large serving sizes when consumed. It is estimated that that less than 1% of the milk sold in the USA is unpasteurised.

Three cheeses were ranked at 15 (heat treated natural/processed), 16 (goat, sheep and feta) and 19th (aged). These rankings were derived primarily from the ability of *L. monocytogenes* to grow or survive in these cheeses; the organism can grow in the heat treated/processed cheese, but is low if any in aged cheese. These rankings demonstrate well that risk posed by various dairy products are very product specific; it is simply not possible to generalise to products groups such as cheese.

7 CONCLUSIONS

Raw milk contains a variety of foodborne pathogens as is evident from the survey work that has been carried out. Much of the data on the effectiveness of pasteurisation is old, and methodology can be problematic. There is debate about the efficacy of pasteurisation in controlling MAP, and to some extent *L. monocytogenes.* However, the degree of control, as shown by the inactivation data in Table 4, appears to be sufficient to control most pathogens at the likely levels of contamination indicated by survey data.

The fact that New Zealand legislation (Food Act 1981) only allows limited quantities of raw milk to be sold at farm gates and that all dairy products must be produced from milk that is pasteurised or treated by a recognised equivalent method, will certainly add to the safety of New Zealand manufactured dairy products.

While outbreaks of disease occur approximately equally with dairy products made from pasteurised and unpasteurised milk, the very small quantity of dairy products available which are unpasteurised means that the risk of foodborne disease from such products is comparatively high. When disease occurs following consumption of a product made from pasteurised milk it is almost invariably the result of post-pasteurisation contamination or failure of the pasteurisation process.

Alternatives to pasteurisation using lesser heat treatments, which are seen as desirable because of improved organoleptic qualities imparted to cheese and other dairy products, do not by definition give the same level of protection against foodborne disease. Assessment of the risk posed by such alternative treatments will need to be made on a case-by-case basis.

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