

1. LIQUID MILK PRODUCTS

1.1 Definitions

Milk is a complex biological fluid secreted in the mammary glands of mammals. Its function is to meet the nutritional needs of neonates of the species from which the milk is derived. This section of the handbook refers mainly to bovine milk, but the milk of other species, such as sheep and goats, is used for human consumption.

Typically, bovine milk is composed of approximately 87% water, 3.7 - 3.9% fat, 3.2 - 3.5% protein, 4.8 - 4.9% carbohydrate (principally lactose), and 0.7% ash. However, the exact composition of bovine milk varies with individual animals, with breed, and with the season, diet, and phase of lactation. Milk produced in the first few days post parturition is known as colostrum. Colostrum has a very high protein content, and is rich in immunoglobulin to help protect the newborn against infections. Colostrum is not generally allowed to enter the food supply in most countries.

Fresh milk products here refers to liquid milk, which accounts for about half of the total dairy market in the UK. Liquid milk is largely heat treated in developed countries, but a small quantity of raw (unpasteurised) milk is still sold in the UK. Skimmed and semi-skimmed milk, which are defined by their fat content (0.5%, and 1.5 - 1.8%, respectively), are increasingly important products in the liquid milk market.

1.2 Initial Microflora

1.2.1 Contamination from the udder

Although milk produced from the mammary glands of healthy animals is initially sterile, microorganisms are able to enter the udder through the teat duct opening. Gram-positive cocci, streptococci, staphylococci and micrococci; lactic acid bacteria (LAB), *Pseudomonas* spp. and yeast are most frequently found in milk drawn aseptically from the udder; corynebacteria are also common.

Where the mammary tissue becomes infected and inflamed; a condition known as mastitis, large numbers of microorganisms and somatic cells are usually shed into the milk. Mastitis is a very common disease in dairy cows, and may be present in a subclinical form, which can only be diagnosed by examining the milk for raised somatic cell counts. Many bacterial species are able to cause mastitis

infection, but the most common are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis* and *Escherichia coli*. These bacteria enter the udder by the teat duct, and *Staph. aureus* is able to colonise the duct itself. Although the organisms involved in mastitis are not usually able to grow in refrigerated milk, they are likely to survive, and their presence may be a cause of concern for health.

Diseased cows may also shed other human pathogens in their milk, including *Mycobacterium bovis*, *Brucella abortus*, *Coxiella burnetii*, *Listeria monocytogenes* and salmonellae. Recently, concerns have also been raised over the presence of *Mycobacterium avium* var. *paratuberculosis* (MAP) (the causative organism of Johne's disease in cattle) in milk from infected animals.

The outer surface of the udder is also a major source of microbial contamination in milk. The surface is likely to be contaminated with a variety of materials, including soil, bedding, faeces and residues of silage and other feeds. Many different microorganisms can be introduced by this means, notably salmonellae, *Campylobacter* spp., *L. monocytogenes*, psychrotrophic spore-formers, clostridia, and Enterobacteriaceae. Good animal husbandry and effective cleaning and disinfection of udders prior to milking are important in minimising contamination.

1.2.2 Other sources of contamination

Milking equipment and bulk storage tanks have been shown to make a significant contribution to the psychrotrophic microflora of raw milk if not adequately sanitised (1). Exposure to inadequately cleaned equipment and contaminated air are also sources of contamination (2). Milk residues on surfaces, and in joints and rubber seals can support the growth of psychrotrophic Gram-negative organisms such as *Pseudomonas*, *Flavobacterium*, *Enterobacter*, *Cronobacter*, *Klebsiella*, *Acinetobacter*, *Aeromonas*, *Achromobacter* and *Alcaligenes*, and Gram-positive organisms such as *Corynebacterium*, *Microbacterium*, *Micrococcus* and spore-forming *Bacillus* and *Clostridium* (3). These organisms are readily removed by effective cleaning and disinfection, but they may build up as biofilms in poorly cleaned equipment. Milk-stone, a mineral deposit, may also accumulate on inadequately cleaned surfaces, especially in hard water areas. Gram-positive cocci, some lactobacilli, and *Bacillus* spores can colonise this material and are then protected from cleaning and disinfection. Some of these organisms may survive pasteurisation and eventually cause spoilage (4).

Other, less significant, sources of contamination include farm water supplies, farm workers and airborne microorganisms.

1.2.3 Natural antimicrobial factors

Raw milk contains a number of compounds that have some antimicrobial activity. Their purpose is to protect the udder from infection and also to protect neonates,

but they may also have a role in the preservation of raw milk during storage and transport.

Lactoperoxidase is an enzyme found in milk. It has no inherent antimicrobial activity, but, in the presence of hydrogen peroxide (usually of microbial origin), it oxidises thiocyanate to produce inhibitors such as hypothiocyanite. This is referred to as the lactoperoxidase system, and it has bactericidal activity against many Gram-negative spoilage organisms, and some bacteriostatic action against many pathogens. For this reason it has been investigated as a possible means of extending the life of stored milk (5)

Lactoferrin is also found in milk and is a glycoprotein that binds iron so that it is not available to bacteria. The chelation of iron in the milk inhibits the growth of many bacteria. In addition to producing an iron-deficient environment, lactoferrin is thought to cause the release of anionic polysaccharide from the outer membrane of Gram-negative bacteria, thereby destabilising the membrane.

Lysozyme acts on components of the bacterial cell wall, causing cell lysis. Gram-positive organisms are much more susceptible to lysozyme than Gram-negatives, although bacterial spores are generally resistant.

Immunoglobulins of maternal origin are often present in milk, and colostrum is a particularly rich source. These proteins may inactivate pathogens in milk, but their significance in preservation is uncertain.

1.3 Processing and its Effects on the Microflora

1.3.1 Raw milk transport and storage

In developed countries, raw milk on the farm is usually cooled quickly and stored in refrigerated bulk tanks at <7 °C prior to collection. Collection by insulated tanker is often on alternate days, or sometimes less frequently, and therefore some of the milk in the tank could be 48 hours old at the time of collection. Temperature control is thus critical to minimise microbial growth, and tanker drivers are usually permitted to refuse milk stored at too high a temperature, or which has an abnormal appearance or odour. Bacterial numbers in the milk may increase during transport, either as a result of contamination from inadequately cleaned tankers or from the growth of psychrotrophic organisms, particularly *Pseudomonas* spp.. Milk temperature and duration of the transport stage are therefore important factors.

On arrival at the processing site, the milk is transferred to bulk storage tanks, or silos, prior to processing. The milk may be stored in the silos for 2 - 3 days, and further growth of psychrotrophic bacteria is likely during this period. The degree of growth is dependent on the initial microbial load, and the storage time and temperature. Pseudomonads are the predominant organisms present in stored raw milk, with *Pseudomonas fluorescens*, *Pseudomonas fragi*, and *Pseudomonas lundensis* being commonly isolated (6), but Enterobacteriaceae, *Flavobacterium*, *Alcaligenes*, and Gram-positive species can also be found. The growth of psychrotrophic bacteria may also be accompanied by the production of heat-

stable, extracellular proteolytic and lipolytic enzymes. These enzymes are often capable of surviving pasteurisation and, in some cases, ultra high temperature (UHT) processing, and they may subsequently cause spoilage in the processed milk.

A number of techniques have been used to limit the growth of psychrotrophs during raw milk storage.

1.3.1.1 Thermisation

The most commonly used technique is to apply a mild heat treatment (thermisation), by heating to around 57 - 68 °C for 15 - 20 seconds and then cooling rapidly to <6 °C. This reduces the psychrotrophic population significantly and can extend the storage life of the raw milk by several days. However, thermisation cannot eliminate vegetative pathogens, and is therefore not a reliable control for the hazard. For example, *L. monocytogenes* can survive the process and could then grow during chilled storage (7).

1.3.1.2 Deep cooling

As the storage temperature is a key factor for the rate of growth of psychrotrophic spoilage organisms, storing milk at as low a temperature as possible can also extend the storage life significantly. Reducing the storage temperature from 6 °C to 2 °C has been shown to give a 2-day gain in storage life for milk of good microbiological quality (8).

1.3.1.3 Carbon dioxide addition

There has been some interest in extending the storage life of raw milk by the addition of carbon dioxide at a concentration of 20-30 mM. Three mechanisms are thought to be involved in carbon dioxide inhibition of microorganisms: the first is by the displacement of oxygen; the second is a lowering of the pH of the milk due to the dissolution of carbon dioxide and formation of carbonic acid, particularly for Gram-negative psychrotrophic aerobes; and the third is a direct effect on the metabolisms such as inhibiting the production of enzymes by these organisms. It has also been suggested that the technique could be used to extend the shelf life of pasteurised milk, but concerns have been raised that the use of carbon dioxide addition could allow growth and toxin production by psychrotrophic *Clostridium botulinum*. However, recent work indicates that the risk of botulism is not increased by the use of this treatment (9).

Following storage, the milk then undergoes further processing.

1.3.2 Separation

If necessary, the milk is separated into skimmed milk, cream and sediment fractions, using centrifugal separators. The sediment may contain a comparatively high number of microorganisms and must be carefully discarded. The agitation involved may also break up clumps of bacteria, potentially producing an apparent increase in the number of colony-forming units. This process also allows the milk to be standardised to a specified fat content by adding back the correct quantity of cream.

1.3.3 Homogenisation

The fat globules in milk can coalesce and form a cream layer. Homogenisation reduces the size of the milk fat globules (to an average diameter of $<1 \mu\text{m}$) by using a pump to force milk through a valve under pressure. The fat globules are then small enough to remain in suspension. This process has little microbiological effect, although clumps of bacterial cells may be broken up. Homogenisers used for pasteurised milk may be linked to the pasteuriser, and run at raised temperature in order to minimise possible microbial contamination. UHT processed milks are homogenised in sterile conditions after heat treatment and before aseptic filling. Effective cleaning and sterilising of the homogeniser are then critical to product safety.

1.3.4 Pasteurisation

Some form of heat process is commonly applied to milk to ensure microbiological safety, and to extend shelf life. In the UK, the most commonly used process is pasteurisation. Time-temperature requirements for pasteurisation vary between countries, and are often specified in legislation. In the UK, both low-temperature, long time (LTLT, 63 - 65 °C for 30 minutes), and high-temperature, short time (HTST, 71.7 - 72 °C for at least 15 seconds) minimum processes are permitted. However, in practice, the HTST process is now generally used. Recent concern about the possible survival of MAP in pasteurised milk (discussed further in section 1.7.2.8: *MAP*) has seen many dairies increase the length of the HTST process to 25 seconds. Higher processes (such as ultra-pasteurisation at 138 °C for at least 2 seconds) (3) may also be applied to products with high fat and solids content. Plate heat exchangers are the most common method for milk pasteurisation, but it is essential that they are designed, constructed and operated in such a way as to minimise the possibility of recontamination of the pasteurised milk by raw milk. Most commercial pasteurisers are fitted with sensors that continuously monitor the pasteurisation temperature, and are linked to automatic divert valves. If the pasteurisation temperature falls below a specified value, the valve opens and diverts the under-processed milk away from the post pasteurisation section of the plant and the filling line, into a divert tank. The

correct operation of these monitoring systems is critical and should be regularly checked. It is also essential that there are no cross-connections between the raw and pasteurised sides of the process, and this should include separate clean-in-place (CIP) systems. It is also usual to maintain a higher pressure in the pasteurised milk to minimise the risk of cross contamination in the heat exchanger. Recontamination of this kind may have serious public health consequences (discussed further in section 1.7.1: *Pathogen growth and survival in raw milk*).

Accepted pasteurisation processes are designed to reduce the numbers of vegetative microbial pathogens to levels that are considered acceptable, although bacterial spores are not destroyed. Most of the potential psychrotrophic spoilage bacteria are also eliminated. However, certain heat-resistant mesophilic organisms, referred to as thermoduric, are able to survive pasteurisation. Thermoduric species commonly isolated from pasteurised milk include *Micrococcus* spp., *Enterococcus faecium* and *Enterococcus faecalis*, *Bacillus subtilis*, *Bacillus cereus*, and certain lactobacilli. Psychrotrophic strains of these organisms may be able to grow slowly in the pasteurised milk at 5 °C, and, if present initially in high numbers, could eventually cause spoilage. Effective cleaning of the cooling sections of pasteurisers is important to ensure that these organisms do not build up on surfaces.

1.3.5 UHT or sterilisation processes

Milk may also be subjected to more severe heat processes sufficient to achieve "commercial sterility". This may be done by batch heating in closed containers, or continuously with aseptic filling into sterile containers. Both conventional retort sterilisation and UHT processes must achieve a minimum F_0 of 3 minutes to ensure product safety. These processes destroy all vegetative cells in the milk, and the majority of spores, although certain very heat-resistant spores may survive. This results in a long shelf life without the need for refrigeration, but also causes organoleptic changes in the milk, such as browning.

Conventional sterilisation processes involve heating the milk in thick-walled glass bottles, closed with a crimped metal cap, at about 120 °C for approximately 30 minutes. However, modern large-scale production methods often use an initial UHT treatment prior to filling the container, followed by retorting for a reduced time (10 - 12 minutes), and then a rapid cooling process. This is said to give a product with improved organoleptic properties.

UHT processes may be direct or indirect. Direct systems inject high-pressure steam directly into the milk to obtain the desired temperature, and then employ flash cooling under vacuum to remove the resulting excess water. Indirect systems utilise heat exchangers and holding tubes. Direct systems are said to give better organoleptic properties, as the heating and cooling processes are very rapid, but they are more complex and expensive to install. UHT processed milk involves preserving milk by holding at a temperature of 140 - 150 °C for 1 - 2 seconds (minimum treatment is 130 °C for 1 sec) (3, 10). Heat treatment is usually followed by aseptic filling into sterile cartons or other containers. The

maintenance of sterility in filling is vital to prevent recontamination of the treated milk. As with pasteurised milk, it is also vital to ensure that raw milk cannot recontaminate the UHT-treated milk.

Certain very heat-resistant spores of mesophilic bacilli, classified as *Bacillus sporothermodurans* (11) are able to survive UHT processes and may subsequently grow in the final product. However, this organism has been shown not to be pathogenic (12) and does not seem to cause any detectable changes to the product. Thermotolerant *Bacillus stearothermophilus* are able to survive UHT processes and cause flat-sour spoilage (3).

1.4 Other Methods of Treating Milk

Because of the relatively short shelf life of conventional pasteurised milk, and the undesirable organoleptic changes in milk subjected to more severe heat processes, there has been much interest in alternative methods, both to improve product quality and to extend shelf life. Some of these processes are now being applied on a commercial scale in North America and Europe.

Microfiltration, usually using ceramic membrane filters, can be used in combination with a minimum HTST pasteurisation process to remove significant numbers of bacteria from milk, and give a substantial extension to shelf life over conventional pasteurised milk (13). The fat is separated from the milk before filtration and is heat treated separately before being added back to the milk after processing. Milk produced by this method is on sale in several countries, and is said to have a shelf life of at least 20 days.

Bactofugation is a centrifugation process that is also able to remove bacteria (including endospores) from milk. It has been used in the cheese industry for some years to minimise contamination with the spores of lactate-fermenting clostridia that cause 'late blowing'. The centrifugate produced by the process contains most of the microbial cells present initially in the milk, and this can be sterilised separately and then recombined with the treated milk, which is conventionally pasteurised, to restore its composition. A shelf life of 30 days or more is claimed for milk treated in this way.

Microwaving refers to dielectric heating due to polarisation effects at a selected frequency band (300 MHz to 300 GHz) in a nonconductor. It has been in commercial practice for milk pasteurisation for quite a long time as it provides the desired degree of safety with minimum quality degradation. Plate counts of raw milk undergoing continuous-flow microwave pasteurisation, at 2450 MHz, were negative while the temperature reached was 82.2 °C (14).

Other methods that have been applied to milk processing include irradiation, high-pressure processing, ultra sound treatment, ultra high-pressure homogenisation (UHPH), and pulsed-electric field (PEF).

1.5 Filling and Packaging

Although cleaning and hygiene of all processing equipment downstream of the heat treatment are essential to prevent recontamination of the product, for pasteurised milk it is the filling operation that is most likely to introduce microorganisms (15, 16). Psychrotrophic spoilage organisms may well be present on fillers, and these can then contaminate the milk and cause a significant reduction in shelf life. Microorganisms may also be present in the packaging, especially in poorly cleaned re-usable bottles, and this may also compromise the shelf life of the milk. For UHT-processed milk, aseptic filling into sterile containers is necessary for the maintenance of commercial sterility. Aseptic filling is not generally used for pasteurised milk, although it would be expected to have a significant influence on shelf life.

1.6 Spoilage

1.6.1 Pasteurised milk

Pasteurised milk provides a very suitable environment for microbial growth and is therefore highly susceptible to microbiological spoilage. Spoilage may result from either the growth of psychrotrophic thermophilic organisms that survive pasteurisation, or post-pasteurisation contamination by psychrotrophs. The latter is considered to be by far the most common cause of spoilage (17).

1.6.1.1 Thermophilic spoilage

The thermophilic microflora of milk consists largely of Gram-positive spore-formers, mainly *Bacillus* spp., *Clostridium* and organisms with heat-resistant vegetative cells, such as *Micrococcus*, *Lactobacillus*, *Enterococcus*, *Streptococcus*, *Corynebacterium* and *Alcaligenes*. Of these, the spore-formers are most important in spoilage, since the other species are not generally psychrotrophic and are unable to grow in refrigerated milk. Several *Bacillus* spp. contain psychrotrophic strains, notably *B. cereus* and *Bacillus circulans*, which may grow at temperatures as low as 2 °C. These organisms may become dominant in milk containing very low numbers of Gram-negative psychrotrophs, but even so, they rarely cause spoilage at <5 °C. However, at slightly higher temperatures (7 - 8 °C), *B. cereus* in particular may grow quite rapidly, producing a type of spoilage known as 'bitty cream' or 'sweet curdling', caused by the action of lecithinase on the phospholipids in fat globules. This produces small particles that stick to surfaces. Bitter taints may also be produced as a result of spoilage by *Bacillus* spp. These organisms are thought to originate from the raw milk, and the level of contamination has been shown to vary with the season, the highest numbers of spores being present between April and September (18).

1.6.1.2 *Post-process contamination*

The majority of post-process contaminants are Gram-negative bacteria, which may have some resistance to sanitisers and be able to colonise milk contact surfaces downstream of the pasteuriser. Initially, Enterobacteriaceae, such as *Enterobacter*, *Cronobacter*, and *Citrobacter*, predominate, but Gram-negative psychrotrophs, principally pseudomonads, but also *Alcaligenes*, *Klebsiella*, *Acinetobacter* and *Flavobacterium*, are more important in terms of eventual spoilage. Although these organisms may only contaminate the product in low numbers, they have a competitive advantage over Enterobacteriaceae at low temperatures and may grow rapidly to high levels (19). Spoilage by Gram-negative psychrotrophs usually takes the form of off-flavours, often described as unclean, fruity, rancid or putrid, formed as a result of proteolytic and lipolytic activity. Ropiness and partial coagulation may also occur occasionally. The time for spoilage to occur depends on the numbers and composition of the initial microflora, and the storage temperature (20). Milk produced with good hygienic practices in a modern facility should have a shelf life of more than 10 days at refrigerated storage temperatures.

Under conditions of mild temperature abuse, Enterobacteriaceae may predominate and cause acid clotting or the development of 'faecal' taints. At still higher temperatures, souring by LAB is possible.

Yeast and mould are also indicators of post-process contamination. Their presence and growth contribute to fruity and yeasty flavours in milk (2, 3).

1.6.2 *UHT or sterilised milk*

Spoilage of UHT-processed products is usually caused by post-process contamination. Spoilage caused by survival of heat-resistant *Bacillus* spores is rare, unless very large numbers of spores are present initially, although reports of sterility failure caused by *B. sporothermodurans*, as previously mentioned, are becoming more common.

Post-process contamination usually occurs as a result of a failure in the integrity of the aseptic filling system, or, more likely, as a result of packaging defects, such as pinholes or faulty seals. The product may then become contaminated with a variety of environmental organisms and the type of spoilage will be dependent on the nature of the contaminant. A spoilage rate of 1/10,000 units is a realistic target for producers using modern, well operated equipment.

A particular problem associated with UHT-processed milk is spoilage by heat-resistant, extracellular, proteolytic and lipolytic microbial enzymes. These will have been produced by psychrotrophic organisms growing in the raw milk prior to processing, particularly pseudomonads, *Acinetobacter*, and *Achromobacter*, which are then able to survive the thermal process, even though all viable cells have been destroyed. In the course of the long shelf life that these products are given, proteolytic enzymes can cause bitter flavours and gelation, whilst lipases cause the development of rancid flavours (21).

1.7 Pathogens: Growth and Survival

1.7.1 Raw milk

Before the adoption of routine pasteurisation, milk was an important vehicle for the transmission of a wide range of diseases, including typhoid, brucellosis and diphtheria. Pasteurisation and improvements in veterinary medicine have seen a very large reduction in the incidence of such traditionally milkborne diseases. However, raw milk may still contain a very wide range of pathogens, including *Salmonella* spp. (particularly *Salmonella typhimurium* and *Salmonella dublin*, a virulent serotype in humans), *E. coli* O157, *L. monocytogenes* and *Campylobacter* spp. derived from the milk animals, the environment or from farm workers and milking equipment (22). Pathogens may be present even in hygienically produced milk of generally good microbiological quality. In short, raw milk is a potentially hazardous product, the microbiological safety of which cannot be assured without the use of pasteurisation or an equivalent process. Recent milk-associated outbreaks of infectious intestinal disease in the UK have been shown to be caused mainly by unpasteurised or inadequately pasteurised milk products (23).

1.7.2 Pasteurised milk products

1.7.2.1 *Salmonella*

Salmonellae are not able to survive the typical minimum pasteurisation processes generally prescribed in legislation. Therefore, their presence indicates that the process has not been carried out effectively, or that post-process contamination has occurred. For example, an outbreak of salmonellosis in Kentucky in 1984 was associated with pasteurised milk, but an investigation of the dairy concerned showed that pasteurisation temperatures were inadequate, and could have been as low as 54.5 °C for 30 minutes (24). An outbreak caused by *Salmonella braenderup* in the UK in 1986 was also associated with pasteurised milk, and on this occasion the pasteuriser was found to be poorly designed and incorrectly operated, probably resulting in the application of an inadequate heat treatment (25).

In 1985, one of the largest outbreaks of salmonellosis in US history occurred in Illinois. Almost 200,000 people were affected, and were associated with pasteurised low-fat 2% milk contaminated with *S. typhimurium* (26). Investigations at the dairy plant involved revealed no evidence of inadequate pasteurisation, and the outbreak strain was not found to be abnormally heat resistant (27). Although the cause of the outbreak has never been completely explained, the investigation did discover a possible cross-connection between raw and pasteurised milk, which may have been the source of contamination (28).

In 1998, an outbreak of salmonellosis in Lancashire, caused by a multiresistant strain of *S. typhimurium* DT104, affected 86 people. This outbreak was also linked to defective pasteurisation of milk at a dairy on a local farm (29).

Consumption of raw milk or raw milk products have been responsible for 62 and 29 cases of diarrheal illness caused by *S. typhimurium* in 2003 and 2007, respectively, in the states of Ohio and Pennsylvania (30, 31)

Since salmonellae are occasional contaminants of raw milk, they may sometimes enter the processing environment. It is very important that contamination of the post-pasteurisation plant is not allowed to occur and effective precautions and monitoring procedures, based on HACCP principles, are necessary to prevent this.

1.7.2.2 *Campylobacter* spp.

Campylobacter spp. are not capable of surviving milk pasteurisation treatments, and cannot grow in raw or pasteurised milk, although they are able to survive for long periods in milk at refrigeration temperatures. Nonetheless, outbreaks of campylobacteriosis associated with pasteurised milk have occurred. For example, a large outbreak in the UK in 1979 caused by *Campylobacter jejuni* was estimated to have affected at least 2,500 schoolchildren, and was associated with free milk provided in schools. Although conclusive evidence was absent, it seems likely that raw milk may have bypassed the pasteurisation process (32).

A more recent outbreak in 2001 involved 75 people and was linked to the consumption of unpasteurised milk procured through a cow leasing program (33).

Birds are known to be an important reservoir of *Campylobacter* infection, and the tendency of some birds to peck through the foil tops of doorstep-delivered milk bottles is becoming recognised as an important source of infection in parts of the UK. Some individual cases have been attributed to this cause, and, in one instance in 1990, the organism was isolated from the beaks of jackdaws and magpies as well as the contaminated milk (34). More recently, an outbreak thought to be associated with bird-pecked milk was reported (35).

1.7.2.3 *Listeria monocytogenes*

There has been some discussion regarding the potential for *L. monocytogenes* in milk to survive pasteurisation. An outbreak of listeriosis in Massachusetts during 1983 resulted in 49 cases, 14 of whom subsequently died. Epidemiological evidence strongly suggested an association with consumption of pasteurised whole and low-fat (2%) milk, although this could not be confirmed microbiologically. The investigation failed to reveal any evidence of inadequate pasteurisation (a process of 77.2 °C for 18 seconds was applied), and the organism could not be found in environmental samples in the dairy, suggesting that post-process contamination was unlikely. However, samples of raw milk taken from farms supplying the plant were found to be positive for *L. monocytogenes* serotype 4b, and the investigators concluded that survival of some organisms through pasteurisation was the most likely cause of the outbreak (36). Three deaths and a

miscarriage in Boston, USA between 2007-8 have been linked to presence of *Listeria* in pasteurised milk. So far, investigations have found nothing wrong with its pasteurisation process (37). Furthermore, in a survey of pasteurised milk conducted in Spain, *L. monocytogenes* was recovered from six out of 28 samples (21.4%) heated at 78 °C for 15 seconds (38). The explanation offered for both these findings was that the organisms might have been protected during heat treatment within leucocytes in the milk. However, this effect has not been conclusively demonstrated, and *L. monocytogenes* has not yet been shown to have survived pasteurisation in milk subjected to minimum HTST pasteurisation requirements of 71.7 °C for 15 seconds. For these reasons, it is currently accepted that existing pasteurisation processes are adequate to inactivate the organism in milk.

L. monocytogenes is likely to be present in wet dairy processing environments, and post-process contamination is therefore a particular hazard. The organism has been shown to be capable of significantly more rapid growth in pasteurised milk than in raw milk at 7 °C, and is also capable of growth at 4 °C in pasteurised milk (39). Therefore, effective HACCP-based controls to prevent post-process contamination are critical, particularly the cleaning and sanitising of all milk-contact surfaces. Adequate temperature control is also important.

1.7.2.4 Verotoxigenic *Escherichia coli*

Dairy cattle are an important reservoir for *E. coli* O157:H7 and this organism may therefore be present in raw milk, usually through faecal contamination. For this reason, raw milk is a high-risk food for this serious intestinal pathogen, and there have been a number of small outbreaks of infection associated with its consumption. However, *E. coli* O157:H7 is not a heat-resistant organism and there is no evidence that it is able to survive pasteurisation. Despite this, there have been outbreaks associated with pasteurised milk. In 1994, an outbreak in Scotland affected over 100 people and was associated with consumption of pasteurised milk from a local dairy. The outbreak strain was eventually recovered from cows on one of the farms supplying the dairy, from a bulk milk tanker, and from a pipe transferring milk from the pasteuriser to the bottling machine (40). Whether this outbreak was the result of faulty pasteurisation or post-process contamination was unclear, but, in either case, the raw milk is likely to have been the original source of the organism. In 1999, a serious outbreak occurred in Cumbria in the north-west of England, which was also associated with pasteurised milk from a local dairy. There were at least 60 confirmed cases involved, and the cause was thought to be a fault in the operation of the pasteuriser (41, 42).

The first general outbreak of verocytotoxin-producing *E. coli* (VTEC) in Denmark occurred in 2004 and involved 25 patients; 18 children and seven adults. It was thought to be due to the consumption of a particular kind of organic milk from a small dairy. Environmental and microbiological investigations at the suspected dairy did not confirm the presence of the outbreak strain, but the outbreak stopped once the dairy was closed and thoroughly cleaned (43).

E. coli O157 is not reported to be able to grow in raw or pasteurised milk stored at 5 °C, but may grow slowly at higher temperatures (44). However, since the infective dose of this pathogen is thought to be very low (probably fewer than 100 cells), effective pasteurisation and the prevention of post-process contamination are critical to ensure product safety.

1.7.2.5 *Yersinia enterocolitica*

Although there has been a question about the ability of *Y. enterocolitica* to survive milk pasteurisation, the majority of the evidence indicates that it is inactivated. Three different strains of *Y. enterocolitica* were reported to have D-values of 0.24 - 0.96 minutes at 62.8 °C (45). Therefore, the presence of the organism in pasteurised milk is likely to be the result of post-process contamination. There have been several *Y. enterocolitica* outbreaks associated with pasteurised milk. In 1976, an outbreak affecting 36 children was associated with the consumption of contaminated chocolate milk. It was thought that the organism was introduced to the product during mixing of chocolate syrup with pasteurised milk, without any subsequent heat process (46). Another outbreak in 1982 was the largest foodborne yersiniosis outbreak ever recorded in the USA, and was also associated with pasteurised milk. It is thought that several thousand people may have developed illness, although the organism was not isolated from milk or environmental samples at the dairy. It was found that surplus milk was used to feed pigs and that the crates used to transport this milk were stored on the ground at the farm and could have become contaminated with pig faeces. Since pigs are a well known reservoir for *Y. enterocolitica*, it was thought that inadequate washing of the crates allowed the organism to survive in mud on them, and subsequently contaminate the external surfaces of milk cartons (47).

Y. enterocolitica is capable of psychrotrophic growth, and could therefore multiply in pasteurised milk during storage. Measures should therefore be taken to prevent post-process contamination as with *L. monocytogenes*.

1.7.2.6 *Staphylococcus aureus*

Staph. aureus is only rarely involved in food poisoning associated with consumption of pasteurised milk, although enterotoxigenic strains can be found as contaminants in raw milk. This may be because *Staph. aureus* does not generally grow at temperatures below 7 °C, and enterotoxin production is inhibited at low temperatures. The organism is also known to be inhibited by the presence of competing species. Nevertheless, an outbreak in California affecting 500 school children was associated with chocolate-flavoured milk. The cause was thought to be growth of *Staph. aureus* in raw milk, and the subsequent persistence of the heat-stable enterotoxin through pasteurisation (48). In June and July 2000, a very large outbreak of staphylococcal food poisoning was reported in Japan, associated with consumption of pasteurised low fat milk. Over 14,500 people were said to

have been affected. The outbreak was unusual in that the thermal processes had destroyed staphylococci in milk but *Staphylococcus* enterotoxin A (SEA) had retained enough activity to cause intoxication. SEA exposed at least twice to pasteurisation at 130 °C for 4 or 2 s retained both immunological and biological activities, although it had been partially inactivated (49).

1.7.2.7 *Bacillus* spp.

As has already been mentioned, psychrotrophic *Bacillus* spp. present in raw milk may survive pasteurisation and then become dominant in the pasteurised milk, potentially causing spoilage. Concerns have been expressed that some psychrotrophic strains of *B. cereus* may be able to produce toxin in milk at refrigeration temperatures, but it seems likely that obvious spoilage would occur before sufficient toxin production had taken place to cause illness (50). Even so, *B. cereus* was isolated at levels of 4×10^5 /g from pasteurised milk associated with 280 food poisoning cases in the Netherlands in 1989 (51).

1.7.2.8 *Mycobacterium avium* subsp. *paratuberculosis*

MAP is the causative organism of Johne's disease in cattle, a chronic wasting disease, and may occasionally be present in raw milk. Evidence linking MAP to a chronic inflammatory bowel condition in humans, called Crohn's disease, is becoming increasingly compelling. Concerns have been raised that MAP might be able to survive pasteurisation if present at levels above 100 cells per ml, especially if clumps of cells are present, and that pasteurised milk may therefore be a vehicle for Crohn's disease (52). On the basis of new heat-resistance studies, many UK dairies have increased pasteurisation times to from 15 to 25 seconds (53). A survey of the level of contamination of pasteurised milk by MAP over a 17 month period, in 1999 - 2000, revealed a mean of 1.6% of raw and 1.8% of pasteurised samples were positive for MAP cultures indicating that commercially pasteurised milk may occasionally contain low levels of viable MAP. The potential public health impact of this situation is, however, still uncertain given that an association with Crohn's disease in humans remains unproven (54).

1.7.2.9 *Viruses*

A number of viruses have been shown to be present in raw milk, although many of these, such as Foot and Mouth Disease Virus (FMDV), are not pathogenic to humans. However, raw milk has been implicated in outbreaks of hepatitis and poliomyelitis. Some viruses, including poliovirus, are completely inactivated by pasteurisation, but this seems not to be the case with others, such as FMDV, if the virus is naturally present rather than inoculated. There is therefore the possibility that other viruses pathogenic to humans may survive at low levels, but, in bulk

milk processing systems, it is thought unlikely that sufficient viruses will be present to infect consumers (55).

1.7.2.10 Toxins

Mycotoxins may be present in milk as a result of the ingestion of mouldy and contaminated feed by cattle. Feed contaminated by aflatoxin B1 as a result of the growth of *Aspergillus flavus* or *Aspergillus parasiticus* has been shown to give rise to the presence of aflatoxin M1 in the milk of dairy cows consuming it. However, only a small percentage (0.4 - 2.2%) of the ingested toxin appeared in the milk (56). Aflatoxins are persistent compounds and are not greatly affected by milk processing, and could therefore be present in pasteurised, packaged milk. However, recent surveys suggest that contamination of the milk supply is very limited and well within acceptable levels (57).

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