

# 1 On-Farm Hygienic Milk Production

M.M.M. Vissers and F. Driehuis

## 1.1 Introduction

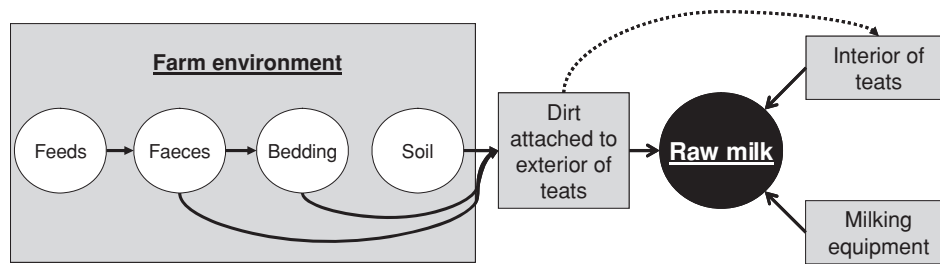
Food producers are responsible for the safety of their products, and to guarantee food safety of dairy products, the dairy industry has implemented hazard analysis of critical control points (HACCP) systems. This enables quality assurance of final products via a chain management approach (European Commission, 2004b). The quality and safety of raw milk is essential for the quality and safety of milk and dairy products. The quality and safety of milk is related to the contamination of milk with microorganisms, chemical residues and other contaminants. This chapter focuses on microbial contamination.

Human microbial pathogens that can be found in raw milk include *Listeria monocytogenes*, *Salmonella* spp. and *Campylobacter jejuni* (Jayarao & Henning, 2001). In addition to their significance for public health, a very good microbial quality of raw milk is also important to prevent production losses and to achieve an optimal shelf life of dairy products. For example, spore formers of butyric acid bacteria in raw milk are responsible for defects in semi-hard cheeses (Klijn *et al.*, 1995), and the contamination of raw milk with spores of *Bacillus cereus* limits the shelf life of pasteurised dairy products (Te Giffel *et al.*, 1997). To ensure a good microbial quality of bulk tank milk, quality assurance systems for dairy farms are being developed and bacteriological schemes are being implemented in payment systems of farm raw bulk milk (IDF, 2006). In addition, hygienic milk production by dairy farmers is important with respect to animal welfare and the image of the dairy sector. Pathogenic microorganisms can infect cows (e.g. gastrointestinal tract, udder tissue), and result in reduced milk yields and even the death of animals. Thus, in summary, control of the microbial ecology at the dairy farm resulting in on-farm hygienic milk production is important for all elements of the dairy production chain.

In this chapter, on-farm hygienic milk production is defined as the control of the microbial contamination of bulk milk tank. Microbial control includes minimisation of microbial sources in the farm environment, minimisation of microbial transmission, prevention of microbial growth and infection of animals and maximisation of microbial inactivation and removal. Microorganisms are present in all parts of the farm environment. Many aspects of farm management (e.g. feed management, facility hygiene and milking operations) are involved in the control of the microbial contamination of bulk tank milk. However, the total bacterial count will also be affected by factors that are independent of farm management, such as seasonal variations.

This chapter discusses the origin of microorganisms in bulk tank milk (Section 1.2), various aspects of microbial control at the dairy farm (Section 1.3), some future developments (Section 1.4) and draws some general conclusions (Section 1.5).

## 2 Chapter 1



**Fig. 1.1** Possible routes for the contamination of raw milk with microorganisms. Adapted from Akam *et al.* (1989).

## 1.2 Sources of microbial contamination of bulk tank milk

### 1.2.1 Background

Milk is sterile when secreted into the alveoli of the udder. Microbial contamination occurs mainly during and after milking (Figure 1.1). Microorganisms in bulk tank milk originate from the interior of teats, the farm environment and surfaces of the milking equipment (Bramley & McKinnon, 1990; Chambers, 2002). Microorganisms are mainly transferred from the farm environment to milk via dirt (e.g. faeces, bedding and soil) attached to the exterior of teats; in addition, microorganisms attached to the exterior of the teats can enter the teat canal and cause mastitis (Makovec & Ruegg, 2003). Finally, contamination can originate from insufficiently cleaned milking equipment when, during milking, microorganisms adhered to surfaces of the milking equipment are released into the milk (Bramley & McKinnon, 1990; Chambers, 2002). Aerial contamination is insignificant under normal production conditions (Akam *et al.*, 1989). The concentration of microorganisms in bulk tank milk can further increase due to their growth.

The microbial population in bulk tank milk consists of a variety of bacterial species. Most species have a specific origin. For example, the presence of *Staphylococcus aureus* in bulk tank milk will, generally, be traced back to cows suffering from mastitis, and silage is the most likely origin of spores of butyric acid bacteria in bulk tank milk (Stadhouders & Jørgensen, 1990; Haven *et al.*, 1996). Table 1.1 lists the origin and dominant contamination route(s) for various microorganisms found in bulk tank milk.

In the case of high microbial concentrations in bulk tank milk, determination of the composition of the microbial flora can reveal the cause of the elevated concentration. Holm *et al.* (2004) examined 73 samples of bulk tank milk with more than  $4.5 \log_{10}$  colony-forming units (cfu)  $\text{mL}^{-1}$ . In 48 samples, one microbial species dominated, for example *Lactococcus* spp. or *S. aureus*. In these samples, high microbial concentrations were in 64% of the cases, which were traced back to poor hygiene (dirty teats and insufficiently cleaned milking equipment). Psychrotrophic microorganisms, which could have grown during the storage of cooled bulk tank milk, overshadowed other microorganisms in 28% of the samples. Mastitis bacteria were found in 48% of all samples, and formed the dominant flora in 8% of the samples tested.

**Table 1.1** Main source of microorganisms occurring in milk and associated spoilage and safety issues in dairy products.

Microbial species	Associated problem	Contamination source (main pathway <sup>a</sup> )	Possible growth in bulk milk tank
<i>Bacillus cereus</i> (spores)	Spoilage of pasteurised dairy products	Environment <sup>a</sup> (feeds → faeces + soil), milking equipment	Yes
<i>Bacillus sporothermodurans</i> (spores)	Spoilage of UHT-treated dairy products	Environment (feeds → faeces)	No
Butyric acid bacteria (spores)	Spoilage of Gouda and Emmenthal cheeses	Environment (feeds → faeces)	No
<i>Campylobacter jejuni</i>	Food safety (products made of raw milk)	Environment (faeces)	No
<i>Escherichia coli</i>	Spoilage and food safety (products made of raw milk)	Environment (faeces and bedding)	Yes
<i>Listeria monocytogenes</i>	Food safety (products made of raw milk and soft or surface ripened cheeses)	Environment <sup>a</sup> (e.g. feeds, faeces)	Yes
<i>Mycobacterium paratuberculosis</i>	Food safety (products made of raw milk) <sup>b</sup>	Environment <sup>a</sup> (faeces)	No
<i>Pseudomonas</i> spp.	Spoilage	Environment <sup>a</sup> (bedding, soil), milking equipment	Yes
<i>Salmonella</i> spp.	Food safety (products made of raw milk)	Environment <sup>a</sup> (faeces)	Yes
<i>Streptococcus thermophilus</i>	Spoilage	Environment <sup>a</sup> (faeces, bedding, soil), milking equipment	Yes
<i>Staphylococcus aureus</i>	Food safety (products made of raw milk)	Interior of teats	Yes

<sup>a</sup> For species having the environment as the major source of contamination and are the main microbial carries indicated between brackets.

<sup>b</sup> Relevance for human health is unclear.

Data compiled from Fenlon (1988), Haven *et al.* (1996), Slaghuis *et al.* (1997), Stadhouders & Jørgensen (1990), Te Giffel *et al.* (1995) and Vaerewijck *et al.* (2001).

### 1.2.2 Mastitis

Mastitis organisms enter the teat canal and infect the interior tissues of the teats. After inflammation, the levels of mastitis organisms within the teat increase significantly. Consequently, during milking, high concentrations of the infectious organisms can be transmitted to milk. The concentration of mastitic-associated microorganisms in bulk tank milk depends on the type of microorganism, infection status within a herd (clinical/sub-clinical), stage of infection and fraction of the herd infected (Bramley & McKinnon, 1990; Chambers, 2002).

A large variety of microorganisms causes mastitis. Table 1.2 presents the frequency of different mastitis organisms as the dominant flora in milk samples of infected cows. In general, contagious and environmental pathogens are distinguished, although a strict classification

## 4 Chapter 1

**Table 1.2** Frequency (%) of different mastitis organisms as the dominant flora in milk samples submitted for microbial analysis in the United States and the Netherlands.

Microorganisms	US 1994–2001 (Makovec & Ruegg, 2003)	The Netherlands 2000 (Sampimon <i>et al.</i> , 2004)
Contagious mastitis organisms		
<i>Staphylococcus aureus</i>	9.7	32.2
<i>Streptococcus agalactiae</i>	13.2	5.3
<i>Corynebacterium bovis</i>	2.7	0.0
Environmental mastitis organisms		
<i>Streptococcus uberis</i>	12.2 <sup>a</sup>	18.9
<i>Streptococcus dysgalactiae</i>		7.6
<i>Escherichia coli</i>	4.0	12.9
<i>Klebsiella</i> spp.	1.2	0.3

<sup>a</sup> Streptococci not including *Streptococcus agalactiae*.

is not possible for all species. Contagious pathogens are mainly transmitted from cow to cow, with or without an intermediate vector such as teat cup liners. The most important contagious pathogens are *S. aureus*, *Streptococcus agalactiae* and *Corynebacterium bovis* (Makovec & Ruegg, 2003).

Environmental pathogens are a natural part of the farm environment. They are, for example, present in faeces, bedding and mud. After the teats are soiled with (contaminated) faeces and bedding, these pathogens enter the teat canal and cause an infection (Smith, 1983). *Streptococcus uberis*, *Streptococcus dysgalactiae* and gram-negative bacteria, such as *Escherichia coli* and *Klebsiella* spp., are the most important environmental pathogens (Makovec & Ruegg, 2003; Ruegg, 2003b; Sampimon *et al.*, 2004). Unlike contagious pathogens, environmental pathogens cannot be eliminated entirely from the farm environment (Smith & Hogan, 1993). In amongst others in the Netherlands and the USA, the relative incidence of mastitis caused by environmental pathogens has increased in the recent decades, presumably due to the successful implementation of measures that reduce spreading of contagious pathogens (Makovec & Ruegg, 2003; Sampimon *et al.*, 2004).

Mastitis can be classified as clinical or sub-clinical. In the case of the former type, cows show recognisable and apparent symptoms, and their milk generally has a deviant colour. Since cows with clinical mastitis are relatively easy to recognise, they are generally removed from the milking herd and, thus, only accidentally contribute to the concentration of mastitis organisms in bulk tank milk. Cows suffering from sub-clinical mastitis show no apparent symptoms of mastitis and, in general, laboratory testing is necessary for diagnosis. The lack of apparent symptoms makes it difficult to recognise cows suffering from sub-clinical mastitis and, as a consequence, sub-clinical mastitis forms a greater threat for the microbial quality of bulk tank milk than clinical mastitis.

Depending on the stage of infection, a single cow can excrete up to  $7 \log_{10}$  mastitis pathogens  $\text{mL}^{-1}$ . In a herd of 100 milking cows, only 1 cow can thus be responsible for a total bulk tank count of  $5 \log_{10}$  cfu  $\text{mL}^{-1}$  (Bramley & McKinnon, 1990; Chambers, 2002). In theory, all mastitis organisms can increase the microbial contamination of bulk tank milk, and Zadoks *et al.* (2004) found that streptococci species to be responsible for 69% of the bacterial count variability at 48 dairy farms sampled, where *S. aureus* and gram-negative

bacteria were responsible only for 3% of the variation. Hayes *et al.* (2001) characterised sudden elevations of the total microbial count in bulk tank milk (i.e. spike values); *S. uberis* was responsible for 55% and *E. coli* for 20% of the spike values. However, both *S. uberis* and *E. coli* are environmental pathogens and, therefore, do not necessarily originate from the interior of infected teats.

### 1.2.3 Environment

As mentioned elsewhere, the most common microbial sources in the farm environment are feeds, faeces, bedding material and soil. Microorganisms from these sources are transferred to milk in a number of steps (see Figure 1.1). The consecutive steps from source to milk are referred to as the contamination pathway. A crucial step in the contamination pathway is the transmission of dirt, composed of, for example, faeces, bedding and/or soil, to milk. Microorganisms from transmitted dirt dilute in the milk and pass the filter of the milking system (Akam *et al.*, 1989). Dirt is mainly transmitted to milk when it is attached to the exterior of teats and rinses off during the milking operations (Stadhouders & Jørgensen, 1990; Murphy & Boor, 2000). Additional dirt and microorganisms can be transmitted from the farm environment to bulk tank milk when the teat cups (that fall on the ground or are kicked off the teats) get contaminated or even suck up dirt from the milking parlour floor (Stadhouders & Jørgensen, 1990). The mass of transmitted dirt per unit of volume can be calculated using a marker method (Stadhouders & Jørgensen, 1990). At eleven farms, Vissers *et al.* (2007c) found between 3 and 300 mg of dirt per litre of milk with an average of 59 mg L<sup>-1</sup>.

The strains and concentrations of microorganisms transmitted from the farm environment to milk via the exterior of teats depends on the composition of the attached dirt and microbial concentration in the dirt. When cows are at pasture, the teats are predominantly contaminated with soil, whereas teats of cows housed in the barn are mainly contaminated with faeces and bedding material (Christiansson *et al.*, 1999; Magnusson *et al.*, 2007). The contamination of teats with soil during the grazing period is considered to be the main cause of elevated concentrations of spores of *B. cereus* in bulk tank milk (Slaghuis *et al.*, 1997; Vissers *et al.*, 2007a,d).

Table 1.3 lists concentrations of important microbial groups observed in feeds, faeces, bedding and soil. Microorganisms in faeces include natural inhabitants, infectious microorganisms and microorganisms or their spores that originate directly from the feeds. Spore concentrations in faeces are between 2 and 10 times as high as the concentration in the ration of the cows (Hengeveld, 1983). This increase is explained by digestion of feed components while spores pass the gastrointestinal tract unaffected.

Different materials are used for bedding in barns, for example, straw, sawdust, wood shavings and shredded paper. Fresh bedding contains a large variety of microorganisms. Microbial concentrations in fresh bedding are usually much lower than concentrations in used bedding (Hogan *et al.*, 1990; Te Giffel *et al.*, 1995; Hogan & Smith, 1997; Slaghuis *et al.*, 1997). Especially, during the first day when the bedding is laid down, the concentrations in bedding material seem to increase significantly due to contamination with faeces and microbial growth (Hogan *et al.*, 1990, 1999; Hogan & Smith, 1997). However, high coliforms counts (7–9 log<sub>10</sub> cfu g<sup>-1</sup>) have also been measured in unused bedding material (Knappstein *et al.*, 2004b).

## 6 Chapter 1

**Table 1.3** Concentration ( $\log_{10} \text{g}^{-1}$ ) of aerobic microorganisms, spores of aerobic microorganisms and spores of gas-forming anaerobic microorganisms in feeds, faeces, bedding and soil.

Source		Aerobic microorganisms	Spores of <i>Bacillus</i> spp.	Spores of (gas-forming) clostridia <sup>a</sup>
Feed	Roughage	4.5 to more than 9.0	2.5–8.7	<1.7–6.2
	Concentrate	2.3–7.5	<1.0–6.7	<1.7–2.9
Faeces		5.6–8.0	3.3–6.8	<1.7–6.6
Bedding	Fresh	3.1–5.7	2.1–6.0	2.2–5.8 <sup>b</sup>
	Used	7.4–9.7	3.9–7.2	
Soil		6.0–7.9	4.8–6.6	3.4–4.4

<sup>a</sup> Enumerated using most probable number (MPN) method.

<sup>b</sup> No separate data for unused and used bedding material. After NIZO Food Research (unpublished data).

Feeds introduce a large variety of microorganisms to the farm environment, and subsequently to milk. The impact of feed as a hazard of microbial contaminants of raw milk is twofold: *firstly*, feed can be a source or transmission vehicle of pathogens causing infection in cattle, and *secondly*, feed is an important source of bacterial spores in raw milk.

Basically, the diet of high-yielding dairy cows consists of two categories of feedstuffs, roughages and concentrate. The former feed provides the animal with dietary fibre, which is essential for the normal functioning of the cow's rumen. The most important roughage crops are grass, maize and lucerne (Wilkinson & Toivonen, 2003). Ensiling and haymaking are the two most common methods to conserve the nutritional value after harvesting. A special situation exists for grass, for example during the growing season, it is usually fed fresh, and outside the growing season, it usually fed as silage or hay. To meet the high nutritional requirements of high-yielding dairy cows, roughage-based diets are supplemented with concentrate feeds, which are high in energy and/or protein. Some examples include cereal grains, bran of cereals and pulses and by-products of the processing of soybeans, rapeseed and other oilseeds. These feeds have low moisture content and may be fed as individual ingredients or blended into particular formulations by compound feed manufacturers. In addition, concentrate feeds with high moisture content are also utilised (e.g. sugar beet pulp, brewers' grains and other co-products of crop-processing industries). These products are usually supplied directly by the processor to the farmer and, subsequently, conserved as silage.

Animal pathogens associated with feed include *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella enterica*. Outbreaks of listeriosis in cattle herds have been associated with the feeding of poorly conserved silages contaminated with *L. monocytogenes* (Fenlon, 1988; Wiedmann *et al.*, 1996). Furthermore, there is evidence supporting a role of silage in the contamination of raw milk with *L. monocytogenes* (Sanaa *et al.*, 1993). In addition, recent studies suggest that cattle feed can be a vehicle for transmission of *E. coli* O157:H7 and *S. enterica* (Fenlon & Wilson, 2000; Davis *et al.*, 2003; Dodd *et al.*, 2003; Dargatz *et al.*, 2005). However, the significance of feed in the ecology of the bacterium in the farm environment and colonisation of cattle remains to be quantified.

Spore-forming bacteria isolated from feeds belong to the genera *Clostridium* and *Bacillus*. In contrast to vegetative cells, spores can survive the passage through the alimentary tract of the dairy cow, and are excreted with the faeces. *Clostridium* species, with particular dairy relevance, are *C. tyrobutyricum*, *C. butyricum*, *C. beijerinckii* and *C. sporogenes*. In cheeses such as Gouda and Emmenthal, the growth of these species, *C. tyrobutyricum* in particular, can cause off-flavours and excessive gas formation; a defect called late-blowing (Klijn *et al.*, 1995; Cocolin *et al.*, 2004; Le Bourhis *et al.*, 2005). Species of *Bacillus* organisms are associated with spoilage of heat-treated dairy products (Te Giffel *et al.*, 1997; Huemer *et al.*, 1998). Spores of *Clostridium* and *Bacillus* species are ubiquitous, and can be isolated from a wide variety of sources in the dairy farm environment, including soil, plants, bedding materials, concentrate feeds, roughages and cattle faeces (Te Giffel *et al.*, 1995; Vaerewijck *et al.*, 2001; Pahlow *et al.*, 2003). Silage is generally recognised as the most important source of *C. tyrobutyricum* spores in raw milk (Stadhouders & Jørgensen, 1990; Vissers *et al.*, 2006). Several studies indicate that silage is also a significant source of contaminating the milk with *Bacillus* spores (Slaghuis *et al.*, 1997; Te Giffel *et al.*, 2002; Vissers *et al.*, 2007b), which is due to growth of spore-forming bacteria in poorly conserved silages. This topic is further discussed in Section 3.3.

#### 1.2.4 Milking equipment

Contamination of milk via the milking equipment occurs when (a) microorganisms adhere to surfaces of the milking equipment and (b) milk residues that remain in the equipment after the cleaning cycle (Figure 1.2). Under these conditions, growth of adhered microorganisms may occur, especially in cracked and decayed rubber parts, that are sensitive to accumulation of microorganisms (Akam *et al.*, 1989). During the next milking, adhered microorganisms can be released into the milk.

The level and type of contamination of milk via the milking equipment depends largely on the cleaning procedure applied. The milking machine is cleaned after each milking or in the case of automatic milking systems at regular intervals, to remove residues and prevent contamination during milking. In general, microorganisms originating from the farm environment (e.g. soil, faeces, bedding and feeds) are found on equipment surfaces, but also *S. aureus* has been recovered from surface of milking equipment (Bramley & McKinnon, 1990; Zadoks *et al.*, 2002). Cleaning the milking equipment at low temperatures or cleaning without sanitisers gives rise to fast growing gram-negative rods like coliforms and *Pseudomonas* (Murphy & Boor, 2000). Increasing the times between two milking

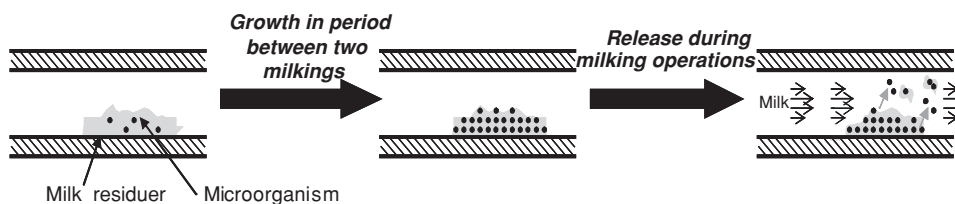


Fig. 1.2 Contamination of milk via the surfaces of the milking equipment.

intervals (i.e. more time available for growth) and higher temperatures during this period (i.e. increased growth rate) increase the number of microorganisms present in the equipment prior to milking and, thus, the level of contamination of milk.

### 1.2.5 Microbial growth during milk storage

It is common practice to collect milk of several milkings in a bulk tank before transportation of the milk to the dairy (e.g. milk of about four milkings in the UK and milk of six milkings in the Netherlands). To prevent microbial growth in the farm bulk tank, milk has to be cooled during storage. The European Union requires cooling of bulk tank milk to a temperature below 8°C when milk is collected daily, and to a temperature below 6°C when milk is not collected daily (European Commission, 2004b). However, cooling of milk does not prevent the growth of microorganisms completely. Some psychrotrophic organisms, such as *Pseudomonas* spp. and *L. monocytogenes*, still grow at temperatures below 6°C, although at reduced rates (Ratkowsky *et al.*, 1982; Te Giffel & Zwietering, 1999). Modelled studies showed that, in adequately functioning bulk tanks, the concentrations of psychrotrophic *L. monocytogenes* and *B. cereus* will not increase significantly (Albert *et al.*, 2005; Vissers *et al.*, 2007a).

## 1.3 Control of microbial contamination of bulk tank milk

### 1.3.1 Good farming practice

The HACCP approach has been implemented throughout the food and dairy industry, and it is a science-based quality management system developed to ensure the production of safe foods. Guidelines for the application of HACCP can be found in the Codex Alimentarius Code of Practice (FAO, 2003). Application of HACCP principles to dairy farms is discussed, but considered to be not yet generally feasible. The necessity for critical multidisciplinary review of management processes, difficulties in establishing limits via the identification of critical control points, the use of routine surveillance procedures and effective record keeping and documentation of standard processes restrict the widespread adoption of HACCP programme to dairy farms (Ruegg, 2003a). Furthermore, adequate monitoring is an essential principle in the HACCP methodology. Application of HACCP programmes for dairy farms is limited by the absence of adequate and low-cost monitoring tests (Gardner, 1997).

As an alternative to HACCP, the formulation of guides to good farming practices has been proposed (European Commission, 2004a). These guides should encourage the use of appropriate hygiene practices at farm level; however, the International Dairy Federation (IDF) and the Food and Agriculture Organisation (FAO) of the United Nations have developed such a guide (Morgan, 2004). The central objective is that the milk should be produced from healthy animals under generally accepted conditions. Good dairy farming practices require that people working and supervising at the farm are skilled in animal husbandry, hygienic milking of animals and administration of veterinary drugs. The guide contains guidelines with respect to different aspects of farm management.



In the next sections of this chapter, different aspects of farm management and their relations to hygienic milk production are discussed (Sections 1.3.2–1.3.7), whilst in Section 1.3.8, the use of mathematical models to identify effective control measures is briefly discussed.

### 1.3.2 *Animal health management*

Animal health management is extremely important for hygienic milk production. Mastitis infections lead to contamination of milk via the interior of teats, and gastrointestinal infections will increase the contamination via the exterior of teats. Furthermore, regulations of the European Union require that raw milk comes from animals that do not show any symptoms of infectious diseases that are communicable to humans via milk, and are in a good state of health and do not have udder wounds likely to affect milk; separation of milk of animals treated with authorised treatment products is also required (European Commission, 2004b).

Basically animal health management is aimed at achieving and sustaining a disease-free herd (Hillerton, 2004). This can be achieved when infected animals are cured or removed (e.g. culling) from the herd, and new infections are prevented. A closed herd, i.e. no import of animals from other farms, is an important measure to sustain a disease-free herd. Treatment and separation of infected animals from the rest of the herd prevents transmission of pathogens from cow to cow (Hillerton, 2004). In addition, a high feed quality, facility hygiene and hygienic milking operations are important to prevent infection of healthy cows with pathogens present in the farm environment.

As an example, mastitis control is an important issue for the dairy sector. In many countries, mastitis control programmes have been developed and implemented (Ekman *et al.*, 2005; Olde Riekerink *et al.*, 2005; Van der Zwaag, 2005). These programmes are usually based on five basic principles:

- Post-milking teat disinfection (see Section 3.5)
- Dry cow antibiotic therapy
- Appropriate treatment of clinical cases
- Culling of chronically infected cows
- Regular milking machine maintenance (Akam *et al.*, 1989).

In Norway, a successful udder health programme was implemented in 1982, and the main focus in this programme was on milking operations and correction of milking machines; however, less emphasis was put on dry cow therapy and teat dipping. In combination with changed farming attitudes and breeding programmes, this has led to a 50% reduction of treatments of clinical mastitis, a reduction of somatic cell counts (an indicator of sub-clinical mastitis) from 250 000 to 114 000 mL<sup>-1</sup>, and a significant reduction in mastitis costs between 1994 and 2004 (Ósteras & Sólveród, 2005).

### 1.3.3 *Control of feed*

Control of microbiological contaminants in feed is a critical factor, in particular for the contamination of raw milk with microbial spores. Because of the fundamental differences

in microbiological hazard properties and control measures between concentrate feeds and roughages, these feed categories will be reviewed separately.

Factors of importance for the microbiological quality of concentrate feeds are the initial contamination level, of (cross-)contamination during processing, and contamination during storage (ICMSF, 2005). Commonly applied processing methods used in feed manufacturing, such as solvent extraction, extrusion and pelleting, reduce the concentration of vegetative bacteria, but generally do not inactivate spores. The low moisture content of concentrate raw materials and compound feeds prevents microbial growth. However, unintentional rehydration during storage can create conditions permitting microbial growth. In many countries, feed manufacturers have developed quality assurance systems, either individually or on a national level, aiming to control chemical and microbiological safety hazards in feed (Den Hartog, 2003).

The microbiological quality of roughages depends strongly on the effectiveness of the conservation, and the conservation principle of hay is low in moisture content. High moisture conditions in hay cause deterioration, especially by moulds and bacilli. Rapid drying of the crop in the field to at least 85 g dry matter 100 g<sup>-1</sup> is important to achieve a high-quality product. The main principles of conservation by ensilage are a rapid achievement of a low pH by lactic acid fermentation involving lactic acid bacteria and the maintenance of anaerobic conditions. The pH after fermentation is determined mainly by the concentration of water-soluble sugars, buffer capacity and dry matter content of the crop and the activity of the lactic acid bacteria.

Undesirable microorganisms in silage are involved in anaerobic spoilage (primarily *Clostridium* species, especially *C. tyrobutyricum*) and aerobic/facultative anaerobic spoilage (e.g. acid-tolerant yeasts, moulds, *Bacillus* and/or *Listeria* species). The final concentration of spores of anaerobic microorganisms in silage is determined by the ensiling conditions, permitting or inhibiting growth of clostridia. Growth of clostridia can be prevented when a sufficiently low pH is achieved by fermentation. The pH needed for an anaerobically stable silage decreases with decreasing dry matter content (which is inversely related to water activity), and ranges from pH 4.1 to pH 5.0 for silage with dry matter content of 150–500 g kg<sup>-1</sup> (Pahlow *et al.*, 2003). Silage additives are available that aim to control the fermentation process (Driehuis & Oude-Elferink, 2000). Wilting is commonly used to increase the dry matter content of grass and lucerne prior to ensiling. Another important factor, which affects the survival and growth of clostridia, is the nitrate concentration of the crop. Crops low in nitrate are more susceptible to spoilage by clostridia than crops high in nitrate (Kaiser *et al.*, 1999). The initial concentration of clostridia spores in the crop at ensiling is of minor importance (Hengeveld, 1983; Rammer, 1996).

Aerobic spoilage of silage is associated with penetration of air into the silage during storage or feeding. Lactate-oxidising yeasts are generally responsible for the initiation of aerobic spoilage, and the growth of these microorganisms causes an increase in pH, which subsequently permits the growth of other organisms. This secondary spoilage flora consists of moulds, bacilli, *Enterobacteriaceae*, *Listeria* and even clostridia (Woolford, 1990; Pahlow *et al.*, 2003; Vissers *et al.*, 2007b). Under practical farming conditions, exposure of silages to air is inevitable after opening a silo for feeding. The extent of penetration of air into the silage mass mainly depends on the porosity and density of the material, pressure gradients in the silo and the rate of silage removal (Honig, 1991). However, aerobic spoilage often

starts during the storage period, for instance because in practice the silo construction and sealing materials, usually plastics, do not prevent air infiltration completely, and there may be damage to the sealing materials.

To a great extent, the success of silage conservation is dependent on management decisions. Silage management factors that are under control by the farmer are (a) time of harvest, (b) dry matter content at ensiling, (c) harvesting and ensiling machinery, (d) use of silage additives, (e) silo construction, (f) compaction and sealing and (g) rate of silage removal and face management. Not all farmers are aware of the effects that these factors can have on the quality and safety of silage and, ultimately, on milk quality.

Communicating relevant information on these topics to farmers is of great importance for hygienic milk production. In this context, the recent implementation in Europe of the Feed Hygiene Regulation is of importance too (European Commission, 2005). This regulation establishes requirements for feed hygiene on the basis of HACCP principles and applies to feed manufacturers and other operators in the feed production chain, including individual farmers.

#### 1.3.4 Facility hygiene

Facility hygiene comprises amongst others the cleanliness of the barn, access alleys and milking parlour, and is an integral part of hygienic milk production and quality control programme. The cleanliness of cows (e.g. udder and teats) and, thus, microbial contamination of milk via the exterior of teats and the incidence of mastitis are affected by measures related to facility hygiene (Ruegg, 2003b). They include, for example, regular removal of dung from the barn, regular refreshment of bedding materials, clean entries to the milking parlour, one or more cubicles per cow and non-crossing walking paths (Haven *et al.*, 1996; Ouweltjes *et al.*, 2003; Ruegg, 2003b).

Surveys have been performed to establish relations between measures related to facility hygiene and microbial counts in bulk tank milk. A correlation between the dirtiness of the access alley to the milking parlour and the concentration of spores of *B. cereus* in bulk tank milk has been reported by Christiansson *et al.* (1999). Relations between cleanliness of housing areas and the rate of clinical mastitis and high somatic cell counts in bulk tank milk have also been established (Barkema *et al.*, 1998). Herlin & Christannson (1993) compared tied and loose housing systems; remarkably, lower concentrations of clostridial spores in milk were detected at farms with tied housing systems despite the fact that cubicles, parlour floors and teats were considered less clean than at farms with loose housing systems. A more intensive care and management of the barn and thorough cleaning of dirty teats (prior to milking) in tied housing systems were considered to be the cause of these opposing observations. In their survey, Hutchinson *et al.* (2005) did not find any significant relation between various microbial parameters (total viable counts, coliforms, *Bacillus* spp., *Bifidobacteria* spp. and *Pseudomonas* spp.) and hygienic practices, such as milking parlour cleaning regime and barn cleanliness.

The lack of clear and significant relation between measured facilities and microbial counts in bulk tank milk may be due to various reasons. First, microbial concentrations in faeces, bedding and soil vary more than the amount of dirt transferred to milk (see Section 1.2.3 and Table 1.3). This means that the microbial concentration in bulk tank milk depends more

on the concentration of microorganisms in the dirt rather than the mass of transferred dirt. With respect to the contamination of bulk tank milk with spores of butyric acid bacteria and *B. cereus*, it has been concluded on the basis of mathematical models that the concentration of these spores in silage and soil are more important than hygienic measures associated with the cleanliness of teats and milking operations (Vissers *et al.*, 2006, 2007a). Second, hygienic practices comprise a large number of measures, and negligence of one or more of these measures can diminish the positive effect of other measures on microbial counts in bulk tank milk. The study of Herlin & Christiansson (1993) is an example of the complexity of the effect of facility hygiene on the microbiological quality of milk. Third, seasonal variations and outbreaks of mastitis can affect bulk tank milk counts independent of facility hygiene (Slaghuis *et al.*, 1997; Zadoks *et al.*, 2001).

### 1.3.5 Milking operations

Hygienic milking operations start with a clean and stress-free milking environment, teat cleaning, pre-dipping, fore-stripping, careful attachment of the teats cups and post-milking teat disinfection. Teat cleaning is performed to reduce the microbial load on the teats prior to milking. Pre-dip agents are often used to disinfect the teats prior to milking and reduce the risk of environmental mastitis. Pre-dipping should be applied with care since residues may contaminate milk. Fore-stripping is expressing two or three streams of milk before attachment of the milk liners in order to visibly check the milk quality and to stimulate milk let down. Post-milking teat disinfection is important to increase the hygienic defence against infection of the teats after milking is completed. An overview of literature and methods that were proved to be useful for improving milking performance were reported by Reinemann *et al.* (2005).

Table 1.4 summarises experimentally determined efficiencies of four commonly used manual cleaning methods. In general, cleaning with a moist plus a dry towel has the highest efficiency, whilst cleaning with a dry towel the lowest, although for the microbial contamination of bulk tank milk differences are marginal. The difference between the least and most effective method on bulk tank milk microbial counts is  $<0.5 \log_{10} \text{cfu mL}^{-1}$ ; however, in the light of mastitis, this difference can be of importance.

In automatic milking systems (for a description of automatic milking systems see Section 1.3.6), teats are cleaned automatically. Teat cleaning efficiencies of different brands and

**Table 1.4** Mean and range (%) of bacteria removal reported for different teat cleaning methods.

Teat cleaning method	Total viable count		Coliforms		Clostridia (i.e. spores)	
	Mean	Range	Mean	Range	Mean	Range
Dry towel	41	3–90	51	4–99	60	45–79
Moist towel	42 <sup>a</sup>	40–43	35 <sup>b</sup>	75	50–91	
Moist towel + dry towel	77	67–97	71	55–84	95	92–96
Pre-dip + dry towel	63	44–80	63	48–74	88 <sup>a</sup>	85–91

<sup>a</sup> Data based on two observations.

<sup>b</sup> Result of one observation.

After Galton *et al.* (1982, 1986), Pankey (1989), Rasmussen *et al.* (1991) and Magnusson *et al.* (2006).

types of automatic milking systems differ, but significant differences were also observed between farms using the same system (Jepsen *et al.*, 2004). Cleaning efficiencies range from 50 to 98% (Melin *et al.*, 2002; Knappstein *et al.*, 2004a), and are comparable with efficiencies of manual methods (Table 1.4). Teat cleaning intervals (2 or 3 times a day) do not affect microbial counts in bulk tank milk significantly (Benfalk & Gustafsson, 2004).

Finally, it should be realised that teat cleaning efficiencies determined under experimental conditions may not be translated directly to the practical situation on farms. The farmer's perception of hygiene may be more important; this is supported by the results of a survey in the UK demonstrating that teat cleaning efficiencies achieved in practice were lower than the theoretical efficiencies (Gibson *et al.*, 2005). Moreover, in 28% of the cases, teat cleaning resulted in an increase of the microbial concentration in milk. The use of the same towel for more than one cow, not using an effective disinfectant, insufficient general parlour hygiene, microbiologically dirty hands and contamination of cleaning materials and solutions were all considered to have the potential to increase microbial concentrations in milk.

### 1.3.6 Milking machine design and operations

In former days, hand milking was applied, but at present in developed countries, milking machines are more widely used. In these machines, milk is extracted from the udder and conveyed to the cooled bulk tank automatically using air–vacuum pulses to extract milk (Figure 1.3) (Akam *et al.*, 1989; Haven *et al.*, 1996). ISO standard 5707 (ISO, 2001) covers the construction and performance of milking machines, and important aspects of the design are the possibility to drain the different elements and the cleanability of the different parts. With respect to maintenance, it is important to regularly check and replace rubber parts; cracked and decayed rubber parts are very sensitive to accumulation of microorganisms.

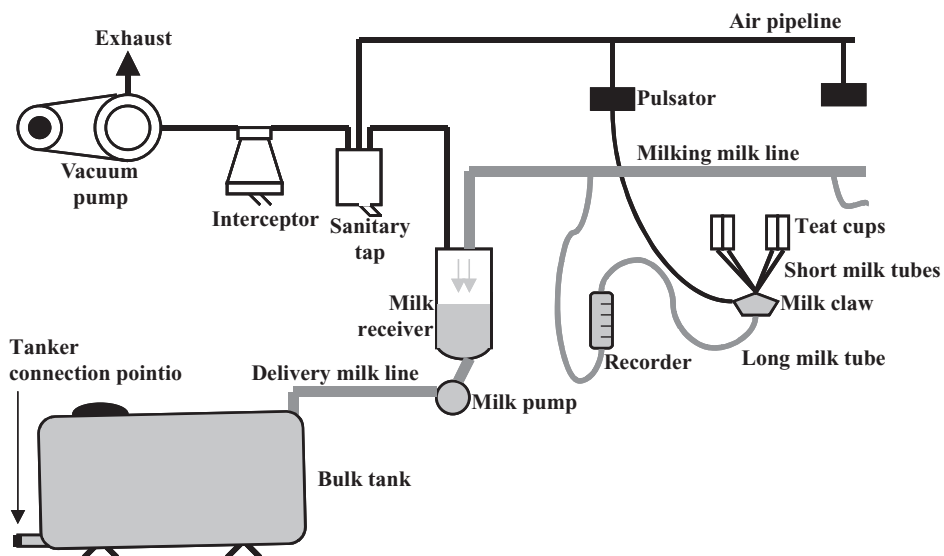


Fig. 1.3 Schematic representation of milking machines.

More and more, larger farms are implementing automatic milking systems. Cows can enter these systems freely and are milked without interference of the farmer. To assure a high hygienic level of the milk, automatic milking systems are equipped with animal recognition functions, online monitoring systems for pre-defined milk quality indicators and teat cleaning functions. Online monitoring systems are used to divert milk from pre-defined animals (e.g. cows treated with antibiotics), off-quality milk and foremilk from the main milk flow. More information can be obtained from the 'Code of Good Hygienic Practices for Milking with Automatic Milking Systems', which was published by the IDF (Jepsen *et al.*, 2004).

Contamination of milk via surfaces of milking equipment depends largely on the effectiveness of cleaning procedures. The choice of a cleaning regime depends on national regulations, the energy costs for heating water and chemicals and local habits. In most European countries, the standard cleaning regime starts with a pre-rinse with hot water (35–45°C), followed by 8 to 10 min cleaning with an alkaline detergent and disinfectant, and finally, a cold water rinse. Additionally, the equipment can be rinsed with an acid solution to remove milk stone, and in Denmark, an acid rinse is generally implemented directly after the cold water rinse, and an extra cold water rinse is performed before the start of each milking. In the USA, the most common routine consists of a pre-rinse, alkaline detergent, acid rinse and pre-milking disinfection. More information about cleaning and sanitation of milking machines have been reported by Reinemann *et al.* (2003).

### 1.3.7 Bulk tank design and operations

Milk has to be cooled during storage in bulk tank at the farm, and in the ISO standard 5708 (ISO, 1983), requirements for design and operation of refrigerated bulk tanks milk are described together with methods for testing the performance. The materials used and design of bulk tank should allow proper cleaning of the tank using an automatic (or semi-automatic) system and fast drainage by gravity. The cooling system of the tank must be able to cool a full tank from 35 to 4°C within 2.5–3.5 h, and milk of the second milking from 10 to 4°C within 0.8–1.75 h. Installation of a plate cooler in the milk line could further reduce cooling time; however, plate coolers increase the risk of contamination of the milk via the surfaces of the equipment and extra attention should be paid when cleaning it. Isolation of the tank should prevent heating of the milk with more than 0.25°C h<sup>-1</sup> when the cooling system does not work. Tanks should be equipped with a system to monitor the milk temperature, and important parameters of bulk tank cleaning procedures involve the disinfectants used, temperature at which cleaning is performed and the rinsing procedure to remove disinfectants (Reinemann *et al.*, 2003).

### 1.3.8 Identification of effective control measures

In the field of food safety, there has been a shift from qualitative approaches like good manufacturing practices (comparable with guidelines for good farming practices) and HACCP to more quantitative risk analyses using mathematical models (Cassin *et al.*, 1998;

Van Gerwen, 2000). Quantitative risk analyses are used to assess the risks throughout the food chain, and to identify effective measure to improve food safety.

The use of mathematical models at the farm level is also useful to identify effective measures to control animal welfare and the microbial contamination of bulk tank milk. Over the years, various models describing microbial process at the farm have been developed starting with models to simulate and identify measure to control animal diseases. The development of epidemiological models to simulate outbreaks of mastitis and to identify measures to control spreading of mastitis started more than 25 years ago, and are still in progress (Oltenucu & Natzke, 1975; Allore & Erb, 1999; Grinberg *et al.*, 2005). Groenendaal *et al.* (2002) developed a simulation model to improve control of Johne's disease, an infection of the gastrointestinal tract of cows. These models, amongst other, increase the insight into the dynamics of the spread of infectious pathogens within a herd, and reveal whether it is, for example, more effective to cull or treat infected animals. Also, microbial processes in silage have been modelled extensively (Leibensperger & Pitt, 1987; Ruxton & Gibson, 1993, 1995; Kelly *et al.*, 2000). An interesting example is a model that links aerobic deterioration of big-bale silage with growth of *L. monocytogenes* in silage (Ruxton & Gibson, 1995). Strategies to control the contamination of bulk tank milk with spores of butyric acid bacteria and *B. cereus* have been identified using models based on the contamination pathway of these microorganisms (Vissers *et al.*, 2006, 2007a). Controlling the silage quality is most important to prevent concentrations of butyric acid bacteria above 1 spore mL<sup>-1</sup>. To control the contamination of bulk tank milk with spores of *B. cereus*, it is most important to keep teats clean during the grazing period, and to assure a high feed quality during the housing period.

## 1.4 Future developments in handling of the milk on the farm

Since prehistoric times, humans have kept animals for the production of milk for human consumption. Economical, social and technological developments have forced dairy farmers and dairy producers to continuously change and improve their production processes. Nowadays globalisation puts milk prices under pressure; also, the quality and safety of raw milk receives more and more attention from consumers and governmental bodies. On the other hand, the increasing sizes of dairy farms and technological developments, such as the introduction of automatic milking systems, generate new opportunities for improving the production processes at the farm. In the future, three relevant technological trends for milk handling on the farm will be:

### 1.4.1 Concentration of milk

Milk consists for more than 90% of water. This means that transportation of milk from the dairy farm to the processing dairy, for a large part, is transportation of water. Concentration of milk at the dairy farm, using membrane filtration, is a way to decrease costs of transportation and energy use. Especially, with increasing herd sizes, this option becomes more and more

attractive to dairy farmers. In New Zealand, concentration of the milk at the farm is already applied, and in many countries application of this technology is under discussion.

#### **1.4.2** *Heat treatment of the milk*

In general, raw milk undergoes a preliminary heat treatment ( $\pm 10$  s,  $65^{\circ}\text{C}$ ) directly after arriving at the dairy processing plant and before storage in a silo. This treatment is known as thermisation, and it is applied to inactivate psychrotrophic microorganisms. These organisms produce heat-stable enzymes like lipases and proteinases at low temperatures, and are responsible of spoilage of dairy products. Thermisation or pasteurisation at the dairy farm decreases the time for psychrotrophic microorganisms to multiply and produce heat-stable enzyme and, thus, decreases risks of spoilage.

#### **1.4.3** *In/online monitoring of bulk tank milk quality*

Sensors and measuring systems to automatically analyse quality indicators are getting faster and more accurate. Especially, automatic milking systems offer possibilities for the implementation of online monitoring tools. Online application of these automatic measuring systems combined with modern information and communication technology solutions enables the farmer and the industry to gain more information about raw milk quality during milking operations and storage of the milk in the bulk tank. Based on these data, the farmer can react; for example, milk from cows under medical treatment can be separated, and batches of the bulk tank milk can be selected and sent to dedicated dairy plants. In addition, if the concentration of spores of butyric acid bacteria can be measured at the dairy farm and the count is high, the milk of this farm can be sent to a processing plant where no cheese is produced.

### **1.5 Conclusions**

On-farm hygienic milk production is important for farmers, the dairy industry and consumers. For farmers, hygienic milk production is not only important with respect to the quality of the bulk tank milk, but also for animal welfare. Microorganisms in bulk tank milk at the farm originate from the interior of teats, the farm environment and surfaces of the milking equipment. Different microorganisms have different origins and, hence, require different control measures. Therefore, hygienic milk production involves many aspects of farm management, varying from animal welfare and feed management to bulk tank design. Mathematical models are useful means to identify effective measures for control of microbial contamination. It should be kept in mind that complete control is not possible. The contamination of bulk tank milk is also affected by uncontrollable aspects, such as seasonal variations in microbial concentrations in, for example, the soil and periodic stress, such as calving. The awareness of farmers of the impact of hygiene in various aspects of farm management on milk quality and their attitude towards hygiene in everyday practices are key factors in hygienic milk production. Therefore, to make progress in this field, more attention to education of and communication with farms is needed.



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22 Chapter 1

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