

A qualitative assessment of the risks of transmission of microorganisms to humans resulting from the

consumption of raw milk and raw cream in Norway

Norwegian Scientific Committee for Food Safety Panel for Biological Hazards

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Summary

Pasteurisation of all consumer milk became mandatory in Norway in 1953, and this has been an important component of the protective measures that have reduced the incidence of milk and food borne diseases. In 2004, a complete recast of the hygiene legislation addressing both food hygiene and veterinary aspects was adopted by the European Union, the so-called "Hygiene package". According to this legislation, each member state may, on its own initiative, prohibit or restrict the marketing of some foods like raw milk or raw cream, intended for direct human consumption within its territory. In response to this, the Norwegian Food Safety Authority (Mattilsynet) commissioned the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety (Vitenskapskomitéen for mattrygghet), to prepare a risk assessment regarding the consumption of raw milk and raw cream. In response, an ad hoc Working Group of experts was appointed with the mandate to draft a risk assessment which should include the following components: identification and characterization of microbiological hazardous agents present in Norwegian raw milk; characterization of the public health consequences of these agents; assessment of the probability of transmission of these agents to humans by distribution of raw milk and cream. Additionally, the risk assessment should identify potential hazards to human health from the importation of raw milk, identify hazards associated with equipment used for production and storage of raw milk, and assess the risks associated with the potential transfer of antimicrobial resistance genes.

Observations concerning infections related to consumption of raw milk and raw cream in Norway, other European countries and North America, show that a number of pathogenic microorganisms, including emerging pathogens, can occur in raw milk and raw cream. These pathogenic microorganisms and their toxins may represent a real threat to human health. The panel concluded that the risks associated with *E. coli* O157:H7 and other EHEC, *C. jejuni* and *L. monocytogenes* in raw milk and cream are high. Furthermore the importation of raw milk to Norway may result in the (re)introduction of microorganisms, which have been eradicated, or never previously have been present, in Norway. This can have serious consequences for both human and animal health.

Background

According to EU legislation, "raw milk" is defined as milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than $40 \,^{\circ}$ C or undergone any treatment that has an equivalent effect (853/2004). In everyday speech, "raw milk" is usually understood to mean milk that has not been pasteurised. This report will use the latter meaning of the term.

In the Norwegian dairy industry, pasteurisation is defined as heat treatment of milk at 71.7°C for 15 seconds (Abrahamsen et al. 2003), also called HTST (High Temperature Short Time) pasteurisation. Certain dairy products are subjected to UHT (Ultra High Temperature) pasteurisation where the milk is heated to a much higher temperature for a few seconds, for example 138°C for 2 seconds, resulting in a sterile product. In a broader sense, the term pasteurisation may be given a wider definition within the food industry like "...the process of heating food for the purpose of killing pathogenic organisms". Hence pasteurisation must be recognised as being distinct from sterilisation, which involves killing all microorganisms present. On-farm pasteurisation usually utilises 63°C for 30 min. since this is more suitable for batch processing. Assessing the risk from consumption of unpasteurised milk is important. since unpasteurised milk may be consumed directly by dairy producers, farm employees and their families, neighbours, and raw milk advocates. Further, unpasteurised milk is consumed indirectly by a large segment of the population via several types of cheeses manufactured from unpasteurised milk (Oliver et al. 2005). In many countries, a poorly developed dairy industry means that milk is frequently consumed unpasteurised.

Advocates for the consumption of non-pasteurised milk and milk products purport that raw milk is preferable to pasteurised milk for several reasons, including: -raw milk contains antimicrobial substances that inhibit the growth of pathogenic bacteria,

-pasteurisation reduces the vitamin content of milk,

-pasteurisation reduces the amount of available calcium in milk,

-raw milk strengthens human health,

-pasteurisation denatures whey proteins.

However, these statements are not based on scientific assessments and any attributes are considered to be insignificant (Abrahamsen *et al.* 2003).

There are approximately 18 700 dairy farmers (2005) in Norway and 1.5×10^9 litres cows' milk and 2×10^7 litres goats' milk are produced annually. The volume of milk produced by sheep, horses and reindeer in Norway is unknown.

Legislation

Pasteurisation of all consumer milk became mandatory in Norway in 1953. The regulations stipulate that the milk is heated to 70-72°C for 15 seconds (The Norwegian Ministry of Health 1953), and this time/temperature combination is also used internationally. Current Norwegian legislation states that all milk intended for the

market must be heat-treated¹. Exception can be made for unpasteurised milk that is sold from a farm to a consumer for personal consumption. This exception is only valid if the sale is occasional, and does not resemble ordinary commercial trade² (Forskrift av 30.06.1995 om produksjon og omsetning mv. av rå melk, varmebehandlet melk og melkebaserte produkter, (melkeforskiften)). In 2004, a complete recast of the hygiene legislation dealing with both food hygiene

and veterinary aspects was adopted by the European Union (EU), the so-called "Hygiene package". This new legislation involved a fundamental revision of the EU's food safety hygiene rules, under which food operators along the food chain bear the primary responsibility for food safety. The new regulations were_intended to merge, harmonise and simplify the previous hygiene requirements in EU

(<u>http://europa.eu.int/comm/food/food/biosafety/hygienelegislation/index_en.htm</u>). The regulations in the Hygiene Package that are relevant to the current assessment are:

- Regulation (EC) 852/2004 on the hygiene of foodstuffs (H1)
- <u>Regulation (EC) 853/2004</u> laying down specific hygiene rules for food of animal origin (H2).

H1 and H2 give provision for market sale of raw milk for human consumption. However, a Member State may, on its own initiative and subject to the general provisions of the Treaty, maintain or establish national rules that prohibit or restrict the marketing of raw milk or raw cream intended for direct human consumption within its territory; (H2).

Terms of reference

The Norwegian Food Safety Authority (Mattilsynet) commissioned the Norwegian Scientific Committee for Food Safety (Vitenskapskomitéen for mattrygghet) to prepare a risk assessment regarding the consumption of raw milk and raw cream, and addressing the following specific questions²:

1- Which microbiological hazards may occur in Norwegian raw milk?

2- What is the probability that these microorganisms can be transmitted to humans by the distribution of raw milk and cream?

3- What are the risks associated with the transmission of these microorganisms to humans by consumption of raw milk and cream?

4-Which hazards to human health may occur from importation of raw milk?5- Which hazards to human health are associated with transmission of infections from equipment being used for production and storage of raw milk?

¹ 'heat treatment': any treatment involving heating that causes, immediately after it has been applied, a negative reaction to the phosphatase test

²All melk som omsettes skal være varmebehandlet. Som unntak fra dette krav kan det fra gård eller seter omsettes upasteurisert melk direkte til forbruker for bruk i egen husholdning. Dette unntaket gjelder bare dersom omsetningen skjer tilfeldig og ikke har preg av butikksalg.

³1-Hvilke ulike mikrobiologiske farer finnes i norsk rå melk?

²⁻Hvilken fare er det for at disse mikroorganismene kan overføres til menneske ved distribusjon av rå melk og fløte? 3-Hvilke risiko er knyttet til overføring av disse mikroorganismer til mennesker ved konsum av rå melk og fløte?

⁴⁻ Hvilke farer vil det medføre for human helse ved eventuelt import av rå melk?

⁵⁻ Hvilke farer for humanhelse er knyttet til overføring av infeksjoner fra utstyr som blir brukt til produksjon og oppbevaring av rå melk?

⁶⁻ Hvilke farer er knyttet til overføring av antibiotikaresistensgener til bakterier hos mennesker?

6- What are the probable risks associated with the transfer of antibiotic resistance genes to bacteria normally occurring in humans?

Processing of unpasteurised milk and cream

Milking

In Norway, milking is usually performed by a milking machine, directly connected to a cooled storage tank. The hygienic status of the stall, cow, udder and the milking machine are all aspects that must be considered when assessing possible risks from this system. On mountain dairy farms milking may be by hand, in which the animal is milked into an open bucket, and the milk is then filtered into 30L milk churns or a cooling tank. It is important to be aware that hand-milking provides an increased risk of contamination of the milk with human pathogens, such as *Streptococcus pyogenes*, from the human reservoir and subsequent transmission.

Tank/Bucket/Milk churn

The milking machine and cooling tank should be washed and disinfected according to written procedures provided by the manufacturer and approved by the food authority. The time for the required refrigeration temperature to be reached is dependent upon whether pre-cooling is installed, the capacity of the tank, the amount of cooled milk already in the tank and the amount of un-cooled milk added. Stainless steel buckets are usually used when manual milking is employed. The milk is sieved through a milk-filtering device into aluminium milk churns of approximately 30 litres. The churns are cooled in water, or in some instances, are set to air cool in a cold place e.g. a cellar.

Filling device and milk bottles/Bucket

In occasional sales from dairy farming, traditional aluminium or plastic milk buckets are used, and are provided and cleaned by the customer. In more systematic sales of unpasteurised milk, buckets or tanks equipped with a tap, and single use bottles may be used.

Separator

Cream is produced in different ways. In occasional sales from mountain dairy farming, where there is no fat cheese, sour cream or butter making, the milk is left to stand without stirring and skimmed off manually into small glass jars or small pails. Where the milk is used for making fat cheese or butter, a separator is employed. Milk separators consist of a stack of concentric cones that revolve at a pre-defined speed and separate the cream from the skimmed milk by centrifugal force. A separator must be fully dismantled before washing, disinfection and drying.

Pathogenic microorganisms in raw milk and raw cream

The modern food industry, including the dairy industry, has established efficient production processes and a wide product distribution network. This also provides a route for the rapid and efficient dispersal of microorganisms to a large population of consumers if any products are contaminated. Even relatively small numbers of bacteria within a food vehicle may be distributed widely in this way. Typically, within many bacterial species, some clones are more virulent than others, and able to establish themselves in the normal flora and persist. Whilst only a minor proportion of consumers may be exposed to a microbiological hazard, and often only a few of these may develop an overt clinical infection, others may be colonized without becoming ill. However, such symptomless colonization may later lead to clinical infection, for instance in connection with surgery or reduced general health. Today, more individuals with immunity dysfunction due to disease and/or treatment live fairly normal lives in ordinary society, and are more vulnerable to infections that may have only relatively small consequences for healthy people.

Much of our knowledge on how bacteria are disseminated with food is due to distinct outbreaks of some specific, clinically recognisable disease such as salmonellosis or listeriosis. Much less is known about the spread and colonization by microorganisms that do not cause such relatively easily recognised syndromes with distinct microbiological diagnoses. Such phenomena are naturally much more difficult to detect, and our insights into them are consequently meagre. Distributing contaminating bacteria with food for per oral consumption without heat treatment may also be unfavourable due to other risks. Distribution of foodborne bacteria may contribute to the spread of virulence factors by the horizontal exchange of genetic factors with bacteria in the human normal flora. Whether microbes containing antimicrobial resistance genes originate from animal or human microflora, it would seem prudent to attempt to curb any routes for dissemination. Although the occurrence of antimicrobial resistance in bacterial isolates is low or moderate in Norway, especially those of veterinary importance, there is no doubt that the incidence of resistance is increasing.

Milk is an excellent growth medium for many microorganisms, including some growth and spoilage microorganisms, because of its high water content, near neutral pH, and variety of nutrients (Doyle *et al.* 2001). Historically, illnesses from contaminated milk and milk products have probably occurred ever since cows were milked. The milkborne transmission of illnesses such as tuberculosis, brucellosis, and scarlet fever has been recognised since the beginning of the 20th century.

Microorganisms present in raw milk may include normal flora and pathogenic microorganisms and they may originate from: 1) the udder itself; 2) the skin of animal (including faecal material); 3) the environment; 4) contamination by human flora during handling; 5) storage; and 6) other pre-processing and post-processing activities.

A range of human diseases, caused by microorganisms, may be transmitted by consumption of raw milk. Those that the *ad hoc* group considers to be the most relevant to this risk assessment, either because of their relative frequency or because they cause particularly problematic illnesses, are listed in Table 1.

Furthermore, new, emerging pathogens may occur in raw milk.

TABLE 1. Pathogenic microorganisms that have the potential to be transmitted by the consumption of raw milk and cream.

EHEC syndrome (HUS) Meningitis, blood infections, intrauterine infections, intrauterine in Norway since 1963. Mycobacterium bovis Tuberculosis Fever, chills, weight los, abdominal pain, diarrhoea or constipation Eradicate from cattle in Norway since 1963. Brucella spp. Brucellosis Septicemia, undulant fever, systemic disease Bradita from animals in Norway since the 1950s, while Bruellensis has never been isolated from animals in Norway since the 1950s, while Bruellensis has never been isolated from animals in Norway since the 1950s, while Bruellensis has never been isolated from animals in Norway since the 1950s, while Bruellensis has never been isolated from cases Streptococccus spp. Streptococcal infections S. pyogenes: scarlet fever, pharyngitis, impetiog. cellulitis, bacteraemia S. zooepidemicus: meningitis, pneumonia, endocarditis Has never been isolated from cattle in Norway Coxiella burnetti Q fever Diarrhoea, addominal symptoms; may be fatal in theimmunocompromised as no effective chemotherapy available Not isolated in Norway <	Organism	Disease	Symptoms and Complications	Comments
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Norovirus Diarrhoea Human reservoir	TRF virus	Tick borne encenhalitis		Not isolated in Norway
			Diarrhoea	-
	Hepatitis A and E	Hepatitis		Human reservoir

Characterization of important pathogenic microorganisms in raw milk and cream

Data on outbreaks associated with consumption of raw milk and cream in Europe and United States are presented in Appendix I and II.

Staphylococcus spp.

Staphylococci are mainly found on the skin and mucous membranes of mammals. The genus *Staphylococcus* has been divided into two groups: coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS). While CPS like *S. aureus*, *S. intermedius* and some strains of *S. hyicus* are considered pathogenic, the CNS are part of the normal flora, especially on the skin, and are regarded either as saprophytes or as of low pathogenicity (Kloos and Bannerman 1994). Among the major group of bacteria causing mastitis, *S. aureus* is of particular significance (Aarestrup *et al.* 1995;Bergonier *et al.* 2003).

Staphylococcal enterotoxins (SE) are heat-stable proteins produced by some strains of *S. aureus* and also some other coagulase-positive staphylococci, e.g. *S. intermedius* and *S. hyicus. S. aureus* can grow over the wide temperature range of 7-48°C. Therefore, raw milk that is not cooled rapidly supports the growth of *S. aureus* and may result in toxin production. The minimum growth temperature for SE production has been shown to be 14 °C (Schmitt *et al.* 1990). SE is thermo-stable and if toxins are produced in the raw milk, active toxin will remain after thermal processing such as pasteurisation. In fresh milk, at temperatures of around 15°C, the generation time of *S. aureus* is close to 4 hrs.

Symptoms and disease in humans

Staphylococcal intoxication is a common bacterial foodborne disease in many countries (Balaban and Rasooly 2000). The symptoms have a rapid onset (1-6 h) after exposure and include nausea, vomiting, abdominal pain and diarrhoea. Since recovery is rapid and intoxication due to SE is self-limiting, outbreaks are often not reported.

Shedding in milk

S. aureus may be shed into milk and is a frequent contaminant of raw milk. When present, this is usually due to the presence of mastitis within the herd. In Norway, *S. intermedius* has never been reported in association with mastitis in animals, and *S. hyicus* has only infrequently been isolated in cows with mastitis (Jarp 1991). Several studies have reported SE among *S. aureus* isolates in Norway (Jørgensen *et al.* 2005b;Loncarevic *et al.* 2005a;Loncarevic *et al.* 2005b;Mørk *et al.* 2003;Tollersrud *et al.* 2000). In a study from 1999 to 2000, *S. aureus* was isolated from 65 and 85% of Norwegian bovine and caprine bulk milk, respectively (Mørk *et al.* 2003); 38% of the *S. aureus* isolates recovered from bovine milk and 64% of the *S. aureus* isolates recovered from caprine milk were SE-producing. In a previous study of Norwegian raw milk products, 17% of the *S. aureus* isolates were SE producing (Kruse 2000). A more recent study which analysed bovine and caprine bulk milk and that isolates frequently produce SE (Jørgensen *et al.* 2005b).

Upon proper cooling, the risk of SE production in fresh milk and cream is negligible. However, the use of unpasteurised milk as an ingredient in products that may be stored for prolonged periods at ambient temperatures must be considered to represent a much greater risk for toxin production.

Effect of pasteurisation

S. aureus in milk is inactivated by pasteurisation (Bergdoll 1989).

Escherichia coli (including serotype O157:H7)

E. coli is a natural inhabitant of the lower small and large intestines of all mammals. *E. coli* is able to grow at temperatures between 7-45°C, although optimal growth is at 37°C. At 15°C the doubling time is approximately 2 h, while at 8°C it is 10 h. Strains of *E. coli* causing diarrhoea are divided into several groups based on virulence properties, mechanisms of pathogenicity, clinical symptoms and distinct serogroups.

Symptoms and disease in humans

Diarrhoeagenic *E. coli* is divided into pathotypes based on virulence factors and clinical symptoms. Shigatoxin-producing *E. coli* (STEC) is the only pathotype of significant zoonotic importance, as a range of animal species, particularly ruminants, are regarded as the host reservoir. STEC of serotype O157, O26, O111, O103 and O145 are the most common STEC serogroups associated with severe disease (including haemorrhagic colitis and haemolytic uraemic syndrome) in humans, and are often designated EHEC (enterohaemorrhagic *E. coli*). Faecally-contaminated foods, including milk and milk products, are the most common vehicles for transmission of STEC from animals to humans.

Enteropathogenic (EPEC) and enterotoxigenic *E. coli* (ETEC) are also causes of human diarrhoea. These pathogens have, however, a human intestinal reservoir and milk containing these pathotypes must have been contaminated by human carriers.

Shedding in milk

Whilst *E. coli* is a well-recognised cause of mastitis in dairy herds, *E. coli* O157:07 has not been reported as a cause of mastitis. In Norway, 2.8 % of subclinical mastitis and 13.5% of clinical mastitis in cattle is caused by coliform (dominated by *E. coli*) bacteria (Veterinærinstituttet 2004). The presence of *E. coli* in milk is considered an indicator of faecal contamination. Faecal contamination of raw milk during the milking process is a possible source of *E. coli* in the raw milk. Consumption of raw milk contaminated with *E. coli* O157:H7 has resulted in haemolytic uraemic syndrome in humans (Martin *et al.* 1986;Wells *et al.* 1991).

A Norwegian survey carried out over a two-year period (1999-2000) analysed 346 bovine and 233 caprine bulk milk samples. In total, 109 (32%) cattle bulk milk samples and 55 (25%) goat milk samples contained *E. coli*, but *E. coli* O157:H7 was not found in any of the samples (Mørk *et al.* 2003). In a previous study in Norway, 576 raw milk samples were examined for the presence of EHEC O157:H7, but none were found to contain this bacterium. In a study at the National Veterinary Institute in 1998 and 1999, of 406 *E. coli* isolates recovered from bovines with clinical mastitis, none were EHEC O157:H7 (Mørk, 2003).

Effect of pasteurisation

All strains of *E. coli*, including *E. coli* O157:H7, are inactivated by pasteurisation.

Listeria monocytogenes

Listeria monocytogenes is the only *Listeria* species that has been regularly implicated as a human and animal pathogen. Both the natural reservoir and mode of transmission to man have been difficult to determine. *L. monocytogenes* survives intracellularly in leukocytes. *L. monocytogenes* is able to grow within the temperature range of 3-45°C. The generation time of *L. monocytogenes* at pH 6.7 is 3 hrs at 15°C and 5 hrs at 8°C.

L. monocytogenes has been the causative agent of major foodborne epidemics in which dairy products including raw milk, pasteurised milk and cheese have been identified as the contaminated foods (Oliver *et al.* 2005).

Symptoms and disease in humans

L. monocytogenes rarely causes severe illness (listeriosis) in healthy people, but susceptible individuals, including pregnant women and their foetuses and the immunocompromised, can develop serious infections (Schlech, III *et al.* 1983). The incubation time varies between a few days and several weeks.

Shedding in milk

L. monocytogenes is carried in the intestines of milk-producing animals and can cause disease in these animals (cattle, sheep, and goats). *L. monocytogenes* has been reported as a cause of mastitis and can be shed in milk and faeces. It has rarely been isolated in relation to mastitis in Norway. *L. monocytogenes* is infrequently detected in raw milk and the bacterium is able to grow in chilled milk. In a study of Norwegian bulk milk from 2003 (Mørk, 2003), *Listeria* spp. were not identified in any of the 346 bulk milk samples from cattle. However, *L. monocytogenes* was isolated from 2.2% of 233 bulk milk samples from goats. Since *L. monocytogenes* may be found on processing equipment, it is considered as a potential hazard for all cheese-producing processes, not just for products from raw milk, as a post-pasteurisation contaminant.

Effect of pasteurisation

Strains of *L. monocytogenes* differ in their heat resistance, but are generally somewhat more heat tolerant than many other pathogens. However, *L. monocytogenes* is considered to be inactivated by pasteurisation.

Salmonella spp.

The genus *Salmonella* comprises two species (*Salmonella enterica* and *Salmonella bongori*), subdivided into more than 2500 serovariants. *Salmonella* spp. can grow in food at temperatures 7-45 °C. The generation time of *Salmonella* spp. is usually around 3 hrs at 15°C and 10 hrs at 8°C.

Symptoms and disease in humans

In most cases, the symptoms of *Salmonella* infection, except the typhoid serovariants, are mild to moderate, causing a self-limiting gastroenteritis. However, the consequences for the immunocompromised, small children and elderly people can be severe, giving rise to systemic disease and are occasionally fatal. The bacteria are also known for their ability to cause post-infectious complications.

Shedding in milk

Salmonella spp. are infrequently isolated as a cause of mastitis in dairy cows (Fontaine *et al.* 1980), but has never been reported in association with mastitis in Norway. *Salmonella* spp. may also occur in raw milk, due to faecal contamination. The situation in Norway regarding *Salmonella spp.* in food producing animals is good, as they are virtually absent from domestically produced food. According to the nation-wide official control programmes for *Salmonella* spp., which were launched in 1995 and cover both live animals (cattle, swine and poultry) and meat (cattle, swine, sheep and poultry), the prevalence of *Salmonella* spp. in Norway is probably below 0.1% (The Norwegian Zoonosis Centre 2003). One exception is *"Salmonella diarizonae"*, *Salmonella* IIIb 61:k:1,5,(7), which is frequently isolated from healthy sheep in Norway. The pathogenicity of this bacterium for humans is, however, considered very low. In a national survey (The Norwegian Zoonosis Centre 2003) of milk and raw milk products, *Salmonella* spp. were not detected.

Effect of pasteurisation

Salmonella spp. are inactivated by pasteurisation (D'aost.J.Y. 1989).

Campylobacter spp.

Campylobacter spp. have a worldwide distribution and many species are commensals of the mucosa of the oral cavity and intestinal tract. *C. jejuni* and *C. coli* are the most common species and *C. jejuni* is most frequently associated with diarrhoea in humans. The most common species pathogenic to animals are *C. fetus* subsp. *fetus*, *C. fetus* subsp. *veneralis*, *C. jejuni*, and possibly *C. mucosalis* and *C. hyointestinalis*.

Symptoms and disease in humans

Symptoms due to *Campylobacter* include fever, abdominal pain and nausea, headache, and muscle pain. *Campylobacter* spp. are known for their ability to cause post-infectious complications such as arthritis.

Shedding in milk

C. jejuni is the most prominent *Campylobacter* spp. found in the gastrointestinal tract of cattle, sheep and birds, and may be transmitted to raw milk through faecal contamination. In a recently published study in Norway, 68.1% of calves carried *C. jejuni*, but the occurrence of the bacterium varied considerably between herds, and between young and adult cattle within herds (Valheim *et al.* 2005). *C. coli* is most frequently isolated from pigs. The role of *C. jejuni* in ovine mastitis is unclear, but it may infrequently cause sub-clinical mastitis in cattle. Although bacterial concentrations in raw milk are generally low, they are sufficient to cause disease, as the infective dose for campylobacteriosis is low (see Table 3).

Campylobacter spp. do not grow in raw milk, since their growth requires reduced oxygen tension and a temperature between 32-45°C. According to the International Commission on Microbiological Specifications for Foods (ICMSF), raw milk or inadequately pasteurised milk is one of the most frequently identified vehicles of foodborne human infection with *C. jejuni* (ICMSF 1996a). In a study performed by Mørk *et al.* (2003), *Campylobacter* spp. were not detected in bulk milk samples from cattle. There are no data on the occurrence of *Campylobacter* spp. in milk from sheep and goats in Norway.

Effect of pasteurisation

Campylobacter spp. are inactivated by pasteurisation.

Streptococcus spp.

Streptococci are, after staphylococci, the most frequent cause of mastitis in ruminants. *S. uberis*, and *S. dysgalactiae* are the *Streptococcus* species most frequently identified, but a few cases of *S. agalactiae* are also reported in Norway each year as the cause of mastitis. Also other species of streptococci, such as *S. pyogenes and S. zooepidemicus*, have been implicated in mammary gland infections.

S. agalactiae

S. agalactiae may cause serious diseases in humans including bacteraemia and meningitis, especially among the newborn and elderly, and very frequently causes asymptomatic gynaecological infections in women. *S. agalactiae* has been estimated to have caused 16,880 human infections, including 1650 deaths, in the United States alone in 1998 (Schrag *et al.* 2000). Transmission between cattle and humans is possible, and inoculation studies have shown that *S. agalactiae* isolated from humans can cause clinical mastitis in cattle (Jensen 1982;Van den Heever and Giesecke 1980). It is unknown whether *S. agalactiae* isolated from cows with mastitis are able to cause disease in humans. We are not aware of any reports that associate human infection with *S. agalactiae* with consumption of raw milk.

S. pyogenes

S. pyogenes may be transmitted to cattle from human carriers and then spread to other humans through raw milk from these cattle. Ingestion of contaminated foods (milk products) may result in explosive outbreaks (McArthur and Walker 2006). The risk of contamination of milk is particularly high during hand-milking.

S. zooepidemicus

Human infection with *S. zooepidemicus* can usually be traced to an animal source. Outbreaks of glomerulonephritis and septicaemia associated with ingestion of unpasteurised milk from cattle with mild and intermittent mastitis have been described (Las *et al.* 2002).

Effect of pasteurisation

The effect of pasteurisation on streptococci appears not to be directly documented, but it is likely that the pasteurisation temperature would destroy the bacteria.

Mycobacterium spp.

Species of the genus *Mycobacterium* that may occur in milk include *Mycobacterium avium* subsp. *paratuberculosis* (known as *M. paratuberculosis*) and *Mycobacterium bovis*. Mycobacteria are difficult to cultivate in the laboratory, and colonies are rarely visible to the naked eye in less than 4 weeks after cultivation on highly specific media. The temperature range for growth is 25-45°C with an optimum around 39°C.

Mycobacterium avium subsp. paratuberculosis

M. avium subsp. *paratuberculosis*, known as "Johne's bacillus", was first isolated and described by Johne and Frottingham in 1895 during the investigation of a

massive infiltration of the intestinal tract of cattle that had resulted in chronic diarrhoea (Johne 1895).

Symptoms and disease in humans

The role of *M. avium* subsp. *paratuberculosis* as a human pathogen is not proven. Crohn's disease is a chronic inflammatory disease of the digestive tract that primarily affects the ileum. The disease presents with clinical symptoms and a histopathology similar to that seen in cattle with Johne's disease (Chiodini 1989;Thompson 1994). Whether Crohn's disease may be caused by mycobacterial infection remains a subject of controversy. This complex subject has been comprehensively reviewed (Chiondini 1989; Thompson 1994). Several species of *Mycobacterium*, including *M. paratuberculosis*, have been isolated from intestinal biopsies in a small number of patients with Crohn's disease (Chiodini 1989;Sanderson *et al.* 1992). However, since a number of studies have not been able to demonstrate *M. avium* subsp. *paratuberculosis* DNA in the tissue of patients with Crohn's disease (Frank and Cook 1996;Rosenberg *et al.* 1991;Wu *et al.* 1991), this issue has yet to be resolved. The currently available evidence is insufficient to confirm or disprove that *M. paratuberculosis* is a causative agent of at least some cases of Crohn's disease (Scientific Committee on Animal Health and Animal Welfare 2000).

Shedding in milk

Low numbers of *M. paratuberculosis* have been isolated from milk and colostrum samples from clinically and subclinically infected cattle (Streeter *et al.* 1995;Taylor *et al.* 1981). In a study of carton and bottled milk from central and southern England, 22 of 312 (7%) of the samples tested positive for *M. paratuberculosis* by PCR amplification of insertion element IS*900* (Millar *et al.* 1996). However, this method does not distinguish dead from viable bacteria. In the same study, 9 of the retail milk samples yielded long-term liquid cultures that tested positive for *M. paratuberculosis* after 13 to 40 months of incubation indicating that some of the milk samples contained viable *M. paratuberculosis*.

While the occurrence of *M. paratuberculosis* in cattle in Norway has been sporadic since 1979, infection in Norwegian goats has been demonstrated on several occasions (Holstad *et al.* 2003). A more recent study (Djønne *et al.* 2003) also showed that *M. paratuberculosis* may occur in raw goats' milk in Norway.

Effect of pasteurisation

M. paratuberculosis may survive heat treatment at 63°C for 30 min and 71.7°C for 15 s (HTST pasteurisation), when present in high numbers $(10^4-10^7 \text{ CFU/ml})$ prior to heat treatment (Chiodini and Hermon-Taylor 1993;Grant *et al.* 1996). Both these studies used milk inoculated with high numbers of *M. paratuberculosis*. However, *M. paratuberculosis* could not be detected following pasteurisation if <10 CFU/ml were present in the raw milk. Commercial methods of pasteurisation used in the dairy industry have been shown to inactivate *M. paratuberculosis* (Stabel 1998). However, a study performed by (Millar *et al.* 1996) demonstrated the presence of *M. paratuberculosis* in pasteurised milk. Whether or not *M. paratuberculosis* survives pasteurisation remains equivocal, but is probably dependent on the number of bacteria present.

Mycobacterium bovis

Bovine tuberculosis is a contagious disease of cattle caused by the bacterium *M. bovis*, which may be excreted in respiratory discharge, faeces, milk, urine and semen (Grange and Yates 1994). According to the National Surveillance Programme (The Norwegian Zoonosis Centre 2003), *M. bovis* was eradicated from cattle in Norway in 1963. However, detection in cows occurred a few times subsequently, due to transmission from a human (Kåre Fossum, personal communication).

Disease in humans

M. bovis used to be a major source of tuberculosis in humans through consumption of unpasteurised milk from infected cows. Currently tuberculosis due to *M. bovis* infection is comparatively rare, but in countries with a high endemic level in cattle it still remains a cause for concern in persons at high risk, such as abattoir workers (Grange and Yates 1994).

Shedding in milk

M. bovis may be found in milk and meat from infected animals.

Effect of pasteurisation

M. bovis is inactivated by pasteurisation.

Brucella spp.

Brucella species are obligate parasites, and each species has a preferred natural host that serves as a reservoir of infection. *B. abortus* may be isolated from cattle and *B. melitensis* from sheep and goats. *B. ovis*, which can be isolated from sheep, is not considered to be zoonotic agent. *Brucella* spp. causes infectious abortion in animals. *B. abortus* has not been isolated from animals in Norway since the 1950s, while *B. melitensis* has never been detected in Norway (National Veterinary Institute 2004). However, imported human cases have occurred. Of the different Brucella species, *B. melitensis* is the most pathogenic to humans. *B. abortus* and *B. melitensis* are included in the mandatory National Surveillance Programme for zoonotic agents.

Symptoms and disease in humans

Humans can be infected by *Brucella* spp. (except *B. ovis* and non-pathogenic *B. neotemae*) and develop undulant fever. Brucellosis in humans may be fatal. The incubation time can be 1-2 months. The impact of disease in small ruminants is greatest in terms of the adverse effects it may have on human health following consumption of unpasteurised traditional products produced from sheep and goats' milk.

Shedding in milk

Transmission of *Brucella* species is most common through direct contact with infected animals/tissues, *B. suis* and *B. abortus* in particular gain access through breaks in skin. Ingestion of raw milk and raw milk products (especially cheese) from infected cows, sheep and goats can also result in transmission, especially of *B. melitensis*.

In the surveillance and control programme for occurrence of *Brucella* spp. in cattle in Norway, *Brucella* antibodies were detected in neither bulk milk nor blood samples.

Additionally *B. abortus* was not identified in samples from aborted foetuses older than 5 months (National Veterinary Institute 2002).

Effect of pasteurisation

Brucella spp. are inactivated by pasteurisation.

Coxiella burnetti

C. burnetti is an obligate intracellular pathogen within the phagolysosome of eukaryote phagocytes, and causes Q-fever in humans and animals. *C. burnetti* has an estimated long generation time of 12-20 hours (Zamboni *et al.* 2001;Zamboni *et al.* 2002). It forms spore-like structures, which may contribute to its persistence in soils and the environment (Marrie 2003).

Symptoms and disease in humans

In humans, infection is usually acquired from aerosols, but infection from the ingestion of contaminated dairy products is possible (Woldehiwet 2004). The virulence and pathogenic mechanisms are not clearly understood, but the bacterial lipopolysaccharides are important in the pathogenesis of Q-fever in man and animals (Baca and Paretsky 1974). The clinical picture is "flu-like", and may vary from a self-limiting, non-specific fever to a severe systemic disease which may include atypical pneumonia, endocarditis and neurological manifestations (Marrie *et al.* 1996).

Shedding in milk

C. burnetti is excreted in the milk of infected cattle, sheep and goats, although the shedding period is shorter in sheep than in cows (Woldehiwet 2004). Chronically infected cows may shed the organism in their milk and birth secretions for successive years (Biberstein *et al.* 1974) and may represent a major source of human infection. *C. burnetti* has never been isolated from cattle in Norway.

Effect of pasteurisation

C. burnetti is inactivated by pasteurisation (Baca and Paretsky 1983).

Yersinia enterocolitica

Y. enterocolitica has been divided into more than 70 serovars (Wauters *et al.* 1991), of which only a few have been conclusively associated with human or animal disease. The organism has been isolated from humans in many countries of the world, but occurs most frequently in cooler climates. *Y. enterocolitica* causes enteritis, the symptoms of which sometimes resemble appendicitis, and due to its association with serious post-infectious complications has been the subject of considerable attention.

Y. enterocolitica is able to multiply at temperatures approaching 0°C, allowing it to grow in properly refrigerated foods. In fresh milk or cream at pH 6.7, growth will proceed at a generation time of 2 hours at 15°C, and 4 hours at 8°C. A milkborne outbreak occurred in Kristianstad in Sweden in 1988 (Alsterlund *et al.* 1995) in which 75 persons were infected with *Y. enterocolitica* O:3, and was probably caused by recontamination of pasteurised milk due to lack of chlorination of the water supply. Pigs are considered the main reservoir of *Y. enterocolitica* O:3. In USA there have been several outbreaks *Y. enterocolitica* O:8 related to the consumption of milk (Ackers *et al.* 2000).

Shedding in milk

There is no information on the occurrence of Y. enterocolitica in raw milk in Norway.

Effect of pasteurisation

Y. enterocolitica is inactivated by pasteurisation and normal cooking at boiling, baking, and frying temperatures. Heat-treatment of milk at 60°C for 1-3 min effectively inactivates the bacterium(Lee *et al.* 2000).

Bacillus spp.

Aerobic and facultative anaerobic spore-forming bacteria of the genus *Bacillus* present a challenge to the dairy industry. *Bacillus* spp. are common in the agricultural environments and may contaminate milk from different sources during production, storage and processing. Raw milk may be contaminated due to inadequate udder hygiene, or from soil, feed, dust and faeces (Christiansson *et al.* 1999). In addition, inadequately cleaned milking equipment, pipelines, and farm bulk tanks may be important sources of contamination (Phillips and Griffiths 1986). Depending on the toxin that is produced, infection can be either associated with diarrhoea or vomiting. *B. licheniformis, B. pumilis,* and *B. subtilis* generally constitute the predominant mesophilic spore-forming species (Nazina *et al.* 2001), while *B. cereus* is often the most psychrotrophic species (Sutherland and Murdoch 1994). Vegetative cells of *B. cereus* have a generation time of 2 hours at 15°C and 5 hours or more at 8°C.

Effect of pasteurisation

Spores of *Bacillus* spp. can survive pasteurisation and pasteurised milk provides suitable conditions for germination and growth. Indeed, heat treatment commonly initiates spore germination. Thus, *Bacillus* spp. is more of a problem in pasteurised milk than in unpasteurised milk. *B. cereus* causes sweet curdling of milk and changes to milk odour and taste due to the production of proteinases, lipases, and phospholipase.

Pathogenic viruses

Viruses like norovirus, hepatitis virus (hepatitis A and hepatitis E) and tick borne encephalitis virus (TBEV) may contaminate raw milk either by human-derived direct contamination of milk, or via water used in the dairy farm (Heinz and Kunz 2004). Incidents for potential mass exposure to rabies through drinking unpasteurised milk from Massachussets, USA have been reported (see Appendix I; www.cdc.gov/mmwr/preview/mmwrhtml/00056759.htm). A recent announcement from Oklahoma, USA (31 December 2005) reported treating people identified as at risk of contracting rabies after drinking raw milk from a rabid cow (www.promedmail.org). However, the transmission of rabies virus to humans via unpasteurised milk has never been demonstrated.

Cryptosporidium spp.

Cryptosporidium spp. are protozoan parasites belonging to the Phylum *Apicomplexa.*. *Cryptosporidium* spp. infections are common in humans and calves, and also occur in various other domestic and wild animal species. Currently 4 species of *Cryptosporidium* spp. are considered to be naturally infective to cattle: *C. parvum*, *C. andersoni*, *C. felis* and *C. bovis*. *C. parvum* and *C.bovis* (in the intestine)

and *C. andersoni* (in the abomasum), are the species most commonly detected. *C.parvum* is also infective for a wide range of other mammals, including humans, and is particularly common in ruminants. C. bovis was only described as new species in the summer of 2005, and therefore its potential to infect animals and humans, is not yet completely determined. C. andersoni appears to have a restricted host range (cattle, Bactrian camels and sheep) which does not include humans. C. felis infections have also been found in cattle, but this species is principally a parasite of cats, with both cattle and humans as minor hosts. Cattle have also been experimentally infected with C. canis (dogs are the major host, although human infections occur rarely) and *C. hominis* (humans and other primates are the major hosts), but natural infections with these two species have not been found in cattle. C. parvum is an enteric pathogen with a worldwide distribution and causes cryptosporidiosis, characterized by abdominal pain, weight loss, diarrhoea, loss of appetite and anorexia. In healthy individuals, the infection is usually self-limiting and resolves within a few weeks (Fayer 2004), but in the immunocompromised host the infection does not readily resolve. As there is no effective chemotherapy, the infection may be fatal for some patients. The parasite is transmitted by the faecal-oral route and infection may be acquired in a number of ways, including unpasteurised milk. Water used for washing of the equipment (e.g. milking machine, cooling tanks, etc) may also be a source of contamination of raw milk. Although various cases of cryptosporidiosis among humans have indicated raw milk and other raw foods as possible sources of infection, conclusive demonstrations of foodborne cryptosporidiosis are infrequent (Laberge et al. 1996).

Cryptosporidium spp. oocysts_are infective at the time of excretion from the host. Large numbers of oocysts are excreted by an infected host (e. g. 30 billion oocysts from a single infected calf over a 2-week period has been reported) and the oocysts are environmentally robust and can survive for long periods outside the host, particularly in moist environments. The infective dose for *Cryptosporidium* spp. is considered to be low.

A number of North American and European studies have shown these parasites to be highly prevalent in dairy calves with infection rates as high as 100 % in some herds. A study in Norway demonstrated that over 50% of the dairy farms studied had calves infected with *Cryptosporidium* spp., (Hamnes *et al.* 2006) and a small human outbreak of cryptosporidiosis associated with infected calves has also been documented from Norway (Robertson *et al.* 2006).

Shedding in milk

Cryptosporidium spp. do not multiply in milk, but the oocyst transmission stage can survive in milk. There is no information on the occurrence of *Cryptosporidium* spp. in raw milk in Norway.

Effect of pasteurisation

Cryptosporidium oocysts are inactivated by pasteurisation (Harp et al. 1996).

Mycotoxins

Mycotoxins are toxins produced by moulds, which may grow on cereals and other materials used as animal feeds (Bennett and Klich 2003). In dairy cattle some of these toxins may be metabolised, and their metabolites may be absorbed and transferred into milk. A temperature between 12-47°C and a humidity level of >70% are prerequisites for fungal growth and mycotoxin production. Mycotoxins which may

pose a risk to human health include aflatoxins and ocratoxin A, both of which are potential contaminants of animal feeds (Diekman and Green 1992). Ruminants are relatively resistant to aflatoxins, but aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* can cause liver damage and cancer in humans. The EU has prepared legislation for the legal limit of mycotoxins per kg feed or food (http://193.132.215/eman2/fsheet6 1.asp)

Many mycotoxins are relatively heat-tolerant and are often only partly broken down by pasteurisation and even sterilisation. Data are lacking regarding the occurrence of mycotoxins in raw milk in Norway.

Dose-response assessments

Dose-response (i.e. infectious dose) assessments are used to convert the final exposure to a pathogen population, into a health response risk in the consumer population. Different doses of the same microorganism can produce variable responses within the same individual at different times, or between two individuals at the same time. The differences in response among various susceptible populations (young, old, immunocompromised, and pregnant women) are important variables. Ultimately, dose-response assessment is very difficult because of the shortage of data on pathogen-specific responses and because those responses depend on the immune status of the host (consumer) (Lammerding and Paoli 1997) and the food matrix. A number of dose-response experiments have been carried out in humans for different microorganisms that may occur in milk (Table 2).

It should be emphasized that raw milk was not the food involved in any of these dose-response studies. The high fat content of raw milk may result in different dose-responses than those presented in this table. Most dose-response experiments have insufficient numbers of dose groups and insufficient numbers of subjects per dose group to enable accurate definition of complete dose-response curves (Oscar 2004).

Micro-organism	Comments	Reference
<i>S. aureus</i> (toxins)	Toxin dose below 10 μ g in contaminated food will produce symptoms of staphylococcal intoxication. This toxin level was reached when <i>S. aureus</i> populations exceeded 100,000 cells/gram food.	(Food and Drug administration (U.S.) 2003)
E. coli	Infective doses of most pathogenic <i>E. coli</i> are not clearly defined. EHEC may cause illness with fewer than 50 cells. A Monte Carlo simulation predicted a probability of haemolytic uraemic syndrome of 3.7×10^6 and a probability of mortality of 1.9×10^{-7} per meal for the very young.	(Mead and Griffin 1998) (Cassin <i>et al.</i> 1998)
L. monocytogenes	The morbidity (serious/potential listeriosis) and mortality is determined by a number of factors including the number of cells consumed, host specific factors and the pathogenicity of the strains. A review of dose-response modes for <i>L</i> . <i>monocytogenes</i> shows that the number of cells required to give a significant probability of infection is probably relatively high i.e. > 10,000 cells.	(Food and Agriculture Organization of the United Nations and World Health Organization 2004)

TABLE 2. Dose-response data for different microorganisms, which may occur in milk.

	Some serovars can cause illness in animals, but infections with other serovars are asymptomatic. Different serovars vary in their pathogenicity. Therefore it is difficult to determine the infective dose.	
Salmonella spp.	In human volunteers, 10 ⁷ salmonella were required to have a significant likelihood of causing disease.	(ICMSF 1996b)
	Cheese implicated in salmonellosis outbreaks has been found to contain low numbers of bacteria (0.36-9.3 cells / 100g).	(D'Aoust <i>et al.</i> 1985)
<i>Campylobacter</i> spp.	The heat-labile toxin from <i>Campylobacter</i> may cause diarrhoea. In one experiment a dose of 500 cells ingested with milk caused illness in one volunteer. In a study involving 111 young adults from Baltimore, a dose ranging from 800 to 20×10^6 cells caused diarrhoeal illness.	(Robinson 1981) (Black <i>et al.</i> 1988)
<i>Brucella</i> spp.	No data available. The number of cells required to cause infection is thought to be very low.	
Coxiella burnetti	No data available. Humans are very susceptible, and very few microorganisms (as few as 10) may be required to cause infection.	
Y. enterocolitica	Very little is known about the dose-response relationship. Szeita <i>et al.</i> , reported the consumption of 3.5×10^9 organisms by one of the authors. This resulted in symptomatic infection with enterocolitis and fever, and lasted 4 weeks. Since it was only one dose, one strain, used in one subject, and the dose administrated was very high, these data add little information for elucidation of the dose-response relationship.	(Szita <i>et al.</i> 2005)
<i>M. avium</i> subsp. paratuberculosis	No data available	
M. bovis	Results from animal experiments in 1934 and earlier, indicate that for <i>M. bovis</i> , thousands or millions more organisms are required for infection via the oral route as compared to infection via the inhalation route.	(O'Reilly and Daborn 1995)
<i>Cryptosporidium</i> spp.	Human infection trials using 29 healthy volunteers without previous evidence of <i>C.parvum</i> infection, has indicated a median infectious dose of 132 oocysts. At the lowest dose provided (30 oocysts), 1 of 5 subjects became infected, whereas at doses higher than 1000 oocysts, all of 7 subjects became infected. Enteric symptoms occurred in all those that became infected.	(DuPont <i>et al.</i> 1995)
	Human infection trials using different isolates of <i>C.parvum</i> have demonstrated that different isolates	(Okhuysen <i>et al.</i> 1999;Teunis <i>et al.</i>
	have different ID 50	2002)

No data available The main concern is for the potential carcinogenic effect of long term exposure	

Outbreak data

Since the sale of unpasteurised milk is prohibited in many countries, documented outbreaks associated with the consumption of unpasteurised milk usually comprise of small local groups. A list of raw milk-related outbreaks in North America during 1973-2002 is provided in Appendix I. A summary of data (1996-2005), available on Eurosurveillance (www.eurosurveillance.org), which mainly presents the outbreak data from the European countries, is provided in Appendix II. Most of the data from Europe are from countries where the sale of unpasteurised milk is legal. The data presented in these tables only include outbreaks originating from consumption of unpasteurised milk and not from milk products made from raw milk.

Sales of raw milk are prohibited in Norway, but there are a number of farms where raw milk is consumed on a daily basis. It is possible that immunity against local milkborne pathogens may be high among the people who have consumed raw milk since childhood. There are limited data available regarding outbreaks related to consumption of raw milk in Norway. The three reported outbreaks since 1997 are presented in Table 3. It is probable, however, that several unreported outbreaks have occurred within the same period.

Infectious agent	Contaminated food	Outbreak details	Reference
Vest-Agder; 1997			
Campylobacter jejuni	Raw milk	Gastrointestinal infection in 31 (34%) cases (of 90 persons) who consumed raw milk from the Agricultural college's milk tank.	MSIS-report-The Norwegian Surveillance System for Communicable Disease (MSIS) (<u>www.msis.no</u>). MSIS 1998; 14
Trondheim; 1998			
Campylobacter jejuni	Raw milk	Gastrointestinal infection in 7 individuals from different families who bought and consumed raw milk from one particular farm.	MSIS-report-The Norwegian Surveillance System for Communicable Disease (MSIS) (<u>www.msis.no</u>). MSIS 1999; 10
Oslo; 2004			
<i>S. aureus</i> – staphylococcal enterotoxin group H	Mashed potato prepared with raw milk	8 persons, the majority children from a kindergarten, became ill with vomiting, stomach cramps and diarrhoea shortly after eating lunch together.	(Jørgensen <i>et al.</i> 2005a)

TABLE 3. Outbreaks of infection related to consumption of raw milk or raw milk products in Norway since 1997.

Exposure assessment

Exposure assessment requires both knowledge concerning the testing or sampling procedures and the prevalence and concentration of various pathogens, as well as information on the consumption of raw milk and cream. The infection routes, described in the next section (contamination of unpasteurised milk and cream) and processes as indicated in Figure 1 could be sources of contamination of raw milk.

Contamination of unpasteurised milk and cream

The contamination of raw milk by microorganisms may occur at various stages in the production process, as illustrated in Figure 1. These steps may be divided into:

Pre-milking

Feeding of cows varies according to season; in the summer months, the animals are at pasture, while in the cold season they are fed indoors. The hygienic quality of the feed (self-made or commercially available) is variable and organisms may contaminate the milk directly or indirectly via the animal and their environment. In HACCP systems the absence of hazards in feed would probably be considered a "prerequisite").

The udder is washed well before the milking in order to reduce contamination from the skin.

Subclinical infections (mastitis) may occur in apparently healthy animals and result in shedding of pathogens in milk. The occurrence of udder inflammation is monitored by regular quantitative analyses of somatic cells.

Somatic cell counts vary throughout the year, and the warm, grazing period is the most hazardous time of the year, as reflected by both the somatic cell count and the modulated seasonal trends Relative Risks (RR) for pathogenic microorganisms (Østerås *et al.*, 2005, personal communication)

Milking

Faecal contamination of milk may occur during milking, and the presence of faecesderived enteric pathogens does not necessarily reflect the general microbial quality of the milk. Depending on the microorganism in question, the numbers of pathogens required to cause infection in consumers may be small. The role of hand-milking as a potential source of milk contamination should be emphasized.

Post-milking

Post-milking equipment includes; tank/bucket/milk churn, milk bottles / bucket, filling device, and separator (Figure 1). Critical factors for reducing the risk of contamination of the milk with pathogens and preventing their subsequent growth during storage and transportation include: healthy, disease-free animals; good hygienic practices on farms; rapid reduction in milk temperature immediately after completion of milking. Inappropriate storage conditions and processing of milk may permit bacterial growth to an unacceptable level. Several types of pathogenic bacteria may grow in milk if the storage and processing temperatures are greater than 10 °C. The temperature of raw milk during storage is critical, and microbial load may increase significantly, if milk is not stored at the appropriate temperature (< 4°C).

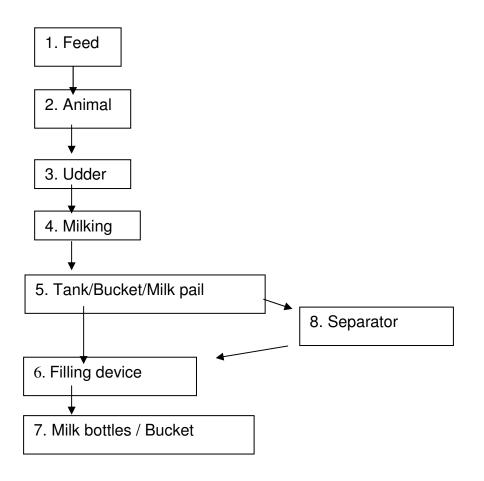


Figure 1. Milk production steps: Steps 1, 2, and 3 represent pre-milking, Step 4 represents milking, and steps 5, 6, 7, and represent post-milking procedures.

The amount of raw milk that is consumed in Norway is unknown, but is probably minimal in comparison to the consumption of pasteurised milk.

Answers to the questions

1- Which microbiological hazards may occur in Norwegian raw milk?

The bacteria *S. aureus* and *Streptococcus* spp. are the most prevalent microbiological hazards present in Norwegian raw milk, as these organisms are major causes of clinical and subclinical mastitis in cattle and sheep. Pathogenic bacteria like *E. coli* O157:H7 and other EHEC, *L. monocytogenes, Salmonella* spp., *C. jejuni/coli*, Y. *enterocolitica, M. avium* subsp.*paratuberculosis* and *B. cereus*, and the protozoan *Cryptosporidium parvum*, may occur in raw milk due to faecal or environmental contamination during the milking process. Of these, *Campylobacter* spp. and *Bacillus* spp. are probably the most prevalent, although there is limited data available to confirm this. EHEC and *L. monocytogenes* represent the highest risk due to the severity of the diseases they cause. Viruses like Norovirus, which occur frequently in the human population, may also occur in raw milk through indirect faecal contamination from humans during the milking process.

The bacteria *M. bovis, Brucella* spp. and *C. burnetti* are currently not endemic to Norway, but as these agents are important milkborne pathogens in other countries and able to cause severe disease in humans, they are included in this assessment.

S. aureus, E. coli O157:H7 and other EHEC, *Salmonella* spp., *C.jejuni/coli, Y. enterocolitica, B. cereus, Cryptosporidium parvum* and Norovirus all cause gastrointestinal disorders. Of these, *E. coli* O157:H7 and other EHEC are considered to cause the most severe disease, including the life-threatening haemorrhagic uraemic syndrome.

L. monocytogenes causes septicaemia, meningitis and abortions/infections in the newborn, intrauterine infections of foetuses, systemic disease of newborns, while *S. agalactiae* may cause meningitis, especially in the newborn. Other streptococci may cause diseases like glomerulonephritis, meningitis and septicaemia. It is currently uncertain whether *M. avium* subsp. *paratuberculosis* is associated with Crohn's disease. *Mycobacterium bovis* causes tuberculosis, the zoonotic *Brucella* spp. cause undulant fever, and *C. burnetti* causes Q fever, a flu-like disease with fever and pneumonia.

2- What is the probability that these microorganisms can be transmitted to humans by the distribution of raw milk and cream?

Outbreak data from NorthAmerica and Europe show that *E. coli* O157:H7 and *C. jejuni/coli* are the most common bacterial infections associated with the consumption of raw milk and cream.

C. jejuni has caused two of the three reported outbreaks associated with raw milk or cream in Norway, and as the organism is relatively frequent in dairy cattle, the probability of transmission of *C. jejuni* to humans by distribution of raw milk and cream is considered moderate to high.

The occurrence of *E. coli* O157:H7 and other EHEC is currently very low in the ruminant reservoir in Norway, and thus the risk of exposure through raw milk and cream is low.

S. aureus occurs frequently in raw milk, and the probability of human exposure to the enterotoxins is mainly associated with use of unpasteurised milk as an ingredient in products that may be stored for prolonged periods at ambient temperatures. Such a situation resulted in an outbreak in Norway in 2004. The probability for transmission of *L. monocytogenes* and *B. cereus* through raw milk and cream is relatively high, for *C. parvum* moderate, and for *Salmonella* spp., *Y. enterocolitica* and *Streptococcus* spp. low.

The probability for transmission of *M. avium* subsp. *paratuberculosis* to humans by distribution of raw milk and cream is difficult to assess, due to the limited data on occurrence of this organism in milk.

M. bovis, *Brucella* spp. and *C. burnetti* do not currently occur in Norwegian dairy animal reservoirs, so there is presently no risk of transmission of these pathogens to humans by distribution of raw milk and cream in Norway.

3- What are the risks associated with transmission of these microorganisms to humans by consumption of raw milk and cream?

Even though the probability of exposure to *E. coli* O157:H7 and other EHEC through raw milk and cream is low, as the infectious dose is low and the consequences for the individual infected may be very severe, or even fatal, the risk associated with *E. coli* O157:H7 and other EHEC in raw milk and cream is still be considered as high. Rapid changes in the epidemiological situation are possible, and may cause an increase in transmission risk.

For *C. jejuni*, the probability for exposure is moderate to high, and as the infectious dose is low and the disease caused is a severe form of diarrhoea, the risk should be considered high.

The risk of *S. aureus* intoxication in humans by distribution of raw milk and cream is considered moderate.

Although *L. monocytogenes* seldom causes disease in a healthy person, the consequences per case can be dramatic, and therefore the risk associated with consumption of raw milk and cream is considered high.

The risks associated with *B. cereus*, *Salmonella s*pp. and *C. parvum* by consumption of raw milk and cream are considered moderate in the immunocompetent population. In the immunosuppressed, the risks are higher. The usually self-limiting *B. cereus* infections/intoxications are associated with pasteurised milk and cream.

The diseases caused by pathogenic *Y. enterocolitica* and *Streptococcus spp.* range from self-limiting diarrhoea to severe systemic diseases. However, the milkborne route may contribute to increasing the endemic level of *S. agalactiae* and is therefore a matter of concern. The risks associated with consumption of raw milk and cream is considered low to moderate.

As an association between *M. avium* subsp. *paratuberculosis* and Crohn's disease is presently unresolved, it is not possible to assess the risk for humans associated with acquiring this infection from the consumption of raw milk and cream. *M. bovis, Brucella* spp. and *C. burnetti* do not currently occur in Norwegian dairy animal reservoirs, so there is presently no risk of transmission of these microorganisms to humans by consumption of raw milk and cream in Norway. The potential (re)introduction of these agents to the domestic reservoir may, however, alter this situation.

4-Which hazards to human health may occur from importation of raw milk?

To the best of our knowledge, raw milk and raw cream are currently not imported to Norway. Any risk related to the import of raw milk and raw cream has to be evaluated based on the endemic level of microbiological diseases in milk-producing animals in the country and region of export. For example, the prevalences of *E. coli* O157:H7 and other STEC/EHEC vary widely between countries and regions, and also with time, and the risks associated with import must be specifically evaluated with regard to such variable factors. The variation and uncertainty surrounding such factors means that reliable evaluation is problematic.

When evaluating the potential importation of raw milk, the consequences for an increased level, or (re)introduction, into the domestic reservoir of the pathogen in question must also be considered. Norway is currently considered free for some microorganisms like *B. abortus, B. melitensis* and *M. bovis*, which are categorised as "group A microorganisms" in the Norwegian legislation. The introduction of these microorganisms to Norway would have serious consequences for both human and animal health.

5- Which hazards to human health are associated with transmission of infections from equipment being used for production and storage of raw milk?

The hazards to human health associated with transmission of infections from equipment used for production and storage of raw milk are mainly those associated with the microbiological quality of water used for washing the utensils. Water can be faecally contaminated with all the microorganisms listed in the answer to Question 1 that have an animal or human intestinal reservoir. However, *C. parvum* and Norovirus are generally considered the most frequent waterborne pathogenic agents. The parasite *Giardia duodenalis* should also be considered, as there has recently been a large waterborne outbreak of giardiasis in Norway. The use of water of drinking water quality for food production is mandatory in Norway.

L. monocytogenes is able to establish itself in a production environment due to its ability to survive and replicate in biofilms.

6- What are the probable risks associated with the transfer of antimicrobial resistance genes to bacteria normally occurring in humans?

Raw milk and cream may be a source of bacteria that are resistant to antimicrobials, depending on the reservoir of antimicrobial resistant bacteria in the environments of the different farms and animals. The transfer of antimicrobial resistant strains of *Salmonella* spp. and *Campylobacter* spp. due to consumption of raw milk has been documented. Antimicrobial resistant bacteria may also transfer their resistance determinants to other bacteria. There are, however, no data that confirm the transmission of antimicrobial resistance determinants from bacteria in raw milk and raw milk products to bacteria in the human host *in vivo*. Nor are there any data that establishes raw milk as a suitable environment for transmission of genes. The presence of DNA in the mammalian intestinal tract and the ability of the intestinal microflora to pick up, stabilize and express free DNA (*blaZ*) has been studied in an *in vivo* mouse model (Sidhu *et al.* 2005), but no evidence of either persistence or transfer of the donor beta-lactamase gene into the competent recipient (*Enterococcus faecium*) was detected. It is highly unlikely that DNA is destroyed by pasteurisation.

Conclusions

- Many pathogenic microorganisms have the potential to occur in raw milk, and several of these have been involved in milkborne outbreaks of infection and are thus confirmed to represent a human health hazard.
- Outbreak data from North America and Europe show that *Campylobacter* spp. and *E. coli* O157:H7 are the most common infectious agents associated with outbreaks caused by raw milk and cream.
- With the current epidemiological situation in Norway, the risk for transmission of *E. coli* O157:H7 and other EHEC, *C. jejuni* and *L. monocytogenes* to humans by consumption of raw milk and cream should be considered high.
- Additionally, the risk that new emerging pathogenic microorganisms may be spread by consumption of raw milk cannot be excluded.
- The import of raw milk to Norway may result in the (re)introduction of microorganisms, which have been eradicated or never present in Norway. Such reintroduction could have serious consequences for both human and animal health.
- Pasteurisation of milk has been an important protective measure that has reduced the incidence of milkborne infectious diseases in Norway.

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Appendix I

Outbreaks of infection in NorthAmerica 1973-2002 associated with the

consumption of raw milk (Adapted after <u>www.foodsafetynetwork.ca/articles/384/rawmilkoutbreaksummary.pdf.</u>)

Date and location	Infectious agent	Contaminated food	Outbreak details	Reference
1973-1992; USA	Various	Raw milk	46 outbreaks associated with raw milk were reported during the study period; 40 outbreaks (87%) occurred in states where the intrastate sale of raw milk was legal. See table 1 below	Marcia L Headrick; ShahinKorangy; Nancy H Bean; Frederick J Angulo; et al. 1998. The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States, 1973 through 1992. Am J Publ Health; Washington
1981-83; California	Salmonella dublin	24% of patients reported using certified raw milk (CRM) (1981-2), 44% reported using CRM (1983)	1981: 46 cases 1982: 70 cases 1983: 123 cases were identified. 61% of patients were 40 years of age or over, and 17% were less than 20 years of age. Nearly 80% were hospitalized, and 26% died.	_http://www.cdc.gov
May 1983; Pennsylvania	Campylobacter jejuni	Raw milk	2 outbreaks are described: 1) 60 first-grade students and three teachers visited a dairy farm in south central Pennsylvania. 31 became ill, and 16 saw a physician; none were hospitalized. <i>C. jejuni</i> was found in the stool of one child. 2) 43 kindergarten children and 2 teachers visited a dairy farm in central Pennsylvania. Subsequently, 26 persons became ill, 4 children saw a physician, and one was hospitalized. <i>C. jejuni</i> was found in both of two stool specimens cultured.	ttp://www.cdc.gov/mm wr/preview/mmwrhtml/ 00000104.htm
March 1984; Kentucky	<i>Salmonella</i> typhimurium	Raw milk; pasteurisation failure at a	16 cases of gastroenteritis (predominantly	March 1984 http://www.cdc.gov/mm wr/preview/mmwrhtml/

		a a mi va mt		00000400 btm
		convent	diarrhoea) were associated with the convent between	00000400.htm
May 1984; California	Campylobacter jejuni	Certified raw milk (CRM), ice cream, and kefir	March 28 and May 2. 28 kindergarten children and 7 adults visited a CRM bottling plant in southern California, where they were given various products listed. 9 children and 3 adults became ill.	http://www.cdc.gov/mm wr/preview/mmwrhtml/ 00000412.htm
June 1984; Vancouver Island, BC, Canada	Campylobacter jejuni	Raw milk	 17 members of a kindergarten class on visited a raw milk dairy where13 drank raw milk. 9 persons became ill a median of 4 days after visiting the dairy. Stools from 10 persons were cultured and 3 yielded <i>C. jejuni.</i> 	(editor's note). Kindergarten field trip to a farm, June 25, 1984, Vancouver Island. Disease Surveillance, British Columbia 1984;5:201-3. http://www.cdc.gov/mm wr/preview/mmwrhtml/ 00000412.htm
July 1984 Minnesota	Not identified	Raw milk	20-50 patients had experienced onset of chronic diarrhoea since January 1984; investigation by the Minnesota Department of Health identified 23 persons who met the case definition of acute onset of diarrhoea, lasting at least 4 weeks. A case-control study of these patients and 46 gender- and age-matched neighbourhood controls revealed that illness was strongly associated with drinking raw milk from a local dairy; no other risk factors were identified. The dairy voluntarily stopped selling raw milk.	http://www.cdc.gov/mm wr/preview/mmwrhtml/ 00000406.htm
1985; Chicago	Salmonella spp.	Pasteurised milk contaminated with raw milk	16,000 confirmed cases of salmonellosis in 6 states; pasteurisation equipment at a Chicago dairy had been modified to facilitate the running off of raw milk, resulting in pasteurised milk being	http://www.cfsan.fda.go v/~mow/chap1.html

	1	[
			contaminated with raw	
			milk under certain	
			conditions	
March 1985; Illinois	Salmonella typhimurium, resistant to ampicillin and tetracycline	2% pasteurised milk ("Blue Brook" brand)	1,500 culture- confirmed cases of salmonellosis in northern Illinois which investigations linked to one processing plant.	http://www.cdc.gov/mm wr/preview/mmwrhtml/ 00000520.htm
October 1985; California	Campylobacter jejuni	Raw milk	23 (46%) became ill after drinking raw milk on a field trip to a San Joaquin County dairy.	1. Korlath JA, Osterholm MT, Judy LA, Forfang JC, Robinson RA. A point- source outbreak of campylobacteriosis associated with consumption of raw milk. J Infect Dis 1985;152:592-6. 2.CDC. Campylobacteriosis associated with raw milk consumption - http://www.cdc.gov/mm wr/preview/mmwrhtml/ 00000734.htm
1990; Washington	<i>Campylobacter</i> spp	Raw milk	13 people ill after consuming raw milk at school	1990 CDC Outbreak Surveillance Unit Report
1992; Minnesota	Campylobacter jejuni	Raw milk	50 people ill after consuming raw milk at a church function	CDC Outbreak Surveillance Unit Report
1997;	Salmonella	Mexican-style	31 persons became ill	http://jama.amaassn.
California	serotype Typhimurium definitive type 104 (DT104), with resistance to 5 drugs	cheese cheese samples and raw milk.	after eating various fresh products from street vendors	org/issues/v281n19 /abs/joc81201.html
1996–1998; Massachusetts	Rabies virus	Unpasteurised milk	2 incidents have been reported since 1996 of potential mass exposures to rabies through drinking unpasteurised milk. 1) On November 12, 1998, the Virology Laboratory of the Massachusetts Department of Public Health (VLMDPH) diagnosed rabies in a 6-yearold Holstein dairy cow from a farm in Worcester County. Public health investigations identified 66 persons who drank	Noah DL, Smith GM, Gotthardt JC, Krebs JW, Green D, Childs JE. Mass human exposure to rabies in New Hampshire: exposures, treatment, and cost. Am J Public Health 1996;86:1149– 51. http://www.cdc.gov/nci dod/dvrd/rabies/professi onal/MMWRtext/mmwr 4811.htm

			unpasteurised milk	
			collected from this	
			dairy during October	
			23–November 8.	
			All 66 received rabies	
			post-exposure	
			prophylaxis (PEP).	
			2) In 1996, the VLMDPH diagnosed	
			rabies in a 14-year-old	
			Jersey dairy cow from	
			a different farm in	
			Worcester County. An	
			investigation identified	
			14 persons who drank	
			unpasteurised milk collected from this cow	
			during this period. All	
			14 persons received	
			rabies PEP	
September	Salmonella	Raw milk	40 people – 50%	Jackie Brown, PA
1998;			children – were	News. SALMONELLA
Lancashire			infected following	
			pasteurisation failure at Preston dairy	TO MILK Sept. 9/98 PA News.
			Ambrose Hall Farm.	TANEWS.
August	E. coli	Goats' milk	3 children from the	Wang G, Zhao T,
2001;	O157:H7		same family (ages 1, 2	Doyle MP. Survival and
Vancouver			and 7) became ill with	growth of Escherichia
Island, BC,			bloody diarrhoea within	<i>coli</i> O157:H7 in
Canada			2 to 4 days following consumption of goats'	unpasteurized and pasteurized milk. J
			milk from a co-	Food Protection 1997;
			operative farm south of	60: 610-13.
			Nanaimo, on	http://www.hcsc.
			Vancouver Island. 2	gc.ca/pphbdgspsp/
			children from another	publicat/ccdrrmtc/
			family also became infected. 2 of these 5	02vol28/dr2801eb. html
			infected children were	num
			hospitalized and	
			developed haemolytic-	
			uraemic syndrome.	
December	Campylobacter	Unpasteurised	A cow-leasing	2001. Morbidity and
2001; Wisconsin	jejuni	milk from an	programme was used to distribute	Mortality - Vol 51(25) :548-549.
44190011910		organic dairy	milk.	.546-549. www.cdc.gov/mmwr/pr
			75 people, ages 2-63	eview/mmwrhtml/mm5
			yrs, developed enteritis	125a2.htm
			and 23 cases of	
			Campylobacter	
			infection were	
			confirmed. No patients were	
			hospitalized.	
			Campylobacter was	
			also isolated in the	
L			milk samples.	
February	Campylobacter	Unpasteurised	5 individuals of an	2002. Communicable
2002;		milk	Edmonton-based	Disease Corner. 6(2):5.

Edmonton	group became ill with diarrhoea following consumption of unpasteurised milk served on a farm field trip. <i>Campylobacter</i> was also isolated in the milk samples from the
	farm.

Other useful references:

Robinson DA, Jones DM. Milk-borne Campylobacter infection. Br Med J 1981;282:1374-6: In 1981 and 1982, five of 10 and six of 11 foodborne *Campylobacter* outbreaks reported to CDC were traced to raw milk consumption. Outbreaks of campylobacteriosis have followed consumption of raw milk on school-sponsored trips in Michigan, Minnesota, and Vermont; a field trip in Maryland resulted in an outbreak of salmonellosis and campylobacteriosis. These, and similar occurrences in England, demonstrate the necessity of protecting school children from exposure to unpasteurised dairy products while on outings.

The epidemiology of raw milk-associated foodborne disease outbreaks reported in the United States,

1973 through 1992

American Journal of Public Health; Washington; Aug 1998; Marcia L Headrick;Shahin Korangy;Nancy H, Bean;Frederick J Angulo;et al;

Pathogen	No. of Outbreaks (%)	No. of Cases
Campylobacter	26 (57)	1100
Salmonella	12 (26)	331
Staphylococci	1 (2)	15
Escherichia coli O157:H7	1 (2)	6
Unknown	6 (13)	281
Total	46 (100)	1733

Appendix II

Outbreaks of infection in Europe 1992-2004 associated with the consumption of raw milk.

Date and location	Infectious agent	Contaminated food	Outbreak details	Reference
1992; France	Coxiella burnetti	Unpasteurised goats' milk	Patients and staff of a psychiatric institution in southern France went to a goat farm, where some drank unpasteurised goats' milk. Nine people developed Q fever	Am J Trop Med Hyg 1992;47: 35-40. http://www.ncbi.nlm.ni h.gov/entrez/query.fcgi? cmd=Retrieve&db=Pub Med&list_uids=163688 1&dopt=Abstract
1992-1996; England and Wales*	Campylobacter spp. (4 cases), S. Typhimurium DT104 (3 cases), S. Typhimurium DT12 and DT193 (2 cases), E. coli O157 (1 case).	Unpasteurised milk	218 persons became ill in10 outbreaks of infectious intestinal disease associated with unpasteurised milk	Eurosurveillance 1998. 2(9). Eurosurveillance 1998. 2(11).
1996; Finland	E. coli O157	Unpasteurised cows' milk	One child died	Eurosurveillance 1998. 2(9).
1996; Finland	S. Infantis	Unpasteurised cows' milk	<10 persons became ill	Eurosurveillance 1998. 2(9).
1997; Finland	S. Poona	Unpasteurised cows' milk	<10 persons became ill	Eurosurveillance 1998. 2(9).
1997; England	<i>E. coli</i> O157:H/	Raw goats' milk	Three children in south east England developed infection with <i>E. coli</i> O157 following consumption of raw goats' milk from an open farm in Hertfordshire	Eurosurveillance 1997. 1(13).
1997; England and Wales	S. Java	Fortified milk for toddlers	14 children under 3 years of age became ill	Eurosurveillance 1997. 1(14).
1998; England (south east)	Verocytotoxin producing <i>E.</i> <i>coli</i> O157	Unpasteurised cream from a small local farm	7 persons became ill (3 primary cases and 4 secondary cases)	Eurosurveillance 1998. 2(43). Eurosurveillance 1998. 2(44).
1999; England (North)	Verocytotoxin producing <i>E.</i> <i>coli</i> O157	Milk from a local farm in Cumbria	38 cases (18 adults and 20 children)	Eurosurveillance 1999. 3(11).
1999; Germany	Campylobacter spp.	Chocolate drink made with raw milk	5 teachers and 14 children from a kindergarten visited a local farm in North-Rhine- Westphalia. Gastrointestinal infection occurred in 11 cases (3 adults and 8 children)	Eurosurveillance 1999. 3(48).
2000 England; (North west)	Verocytotoxin producing <i>E.</i> <i>coli</i> O157	Unpasteurised milk	 2 outbreaks occurred. 1) 3 adults and one child became ill. 2): 4 children became ill, one consumed raw milk from family farm, one was in the same class at school and two others were known to have held hands with each other. 	Eurosurveillance 2000. 4(23).
2000; Austria	Campylobacter jejuni	Unpasteurised milk	38 children became ill at an Austrian youth centre.	2000. Epidemiology and Infection; 125 (1)13-16.
2001; Austria	Vero cytotoxin producing <i>E.</i> <i>coli</i> O157	Raw cows' milk and raw goats' milk	2 outbreaksin which 2 children became ill	Eurosurveillance 2001. 6(10).
2004; Denmark	Vero cytotoxin producing <i>E.</i> <i>coli</i> O157	Raw milk	11 persons became ill	Eurosurveillance 2004. 8(20).

* Scotland differs from England and Wales in that sale of unpasteurised milk is prohibited. The only incident with unpasteurised milk in recent years was the case of a child, who died in 1996 from Vero cytotoxin producing *E. coli* O157 infection linked to consumption of unpasteurised milk sold at a farm gate (Eurosurveillance 1998. 2(9)). Sale of unpasteurised milk was prohibited in Scotland in 1983. In the seven years previous to compulsory heat treatment, from 1975-1982, 42 outbreaks were attributed to the consumption of unpasteurised milk: 27 in the general community and 15 in the farm community. In the seven years after heat treatment became compulsory, 1984-1991, the incidence was halved to 21, and all but one of the cases occurred in the farming community. No outbreak reported from Scotland since 1992 has been attributed to unpasteurised milk (Eurosurveillance 1998. 2(11)).

Retail sale of unpacked and unpasteurised milk is prohibited in the following countries: Finland, Italy, Sweden, Denmark, Norway, Scotland (since 1983), Ireland (since 31.07.97) In the Netherlands, sale of unpasteurised milk is discouraged but not prohibited (Eurosurveillance 1998. 2(9)).

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