FOOD & BIOBASED RESEARCH
WAGENINGEN UR

Microbial hazards in the dairy chain

A literature study

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Report 1553

Colofon

Titel	Microbial hazards in the dairy chain
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Nummer	1553
ISBN-nummer	-
Publicatiedatum	1 oktober 2015
Vertrouwelijk	Ja, tot 1 dag na advies van BuRO aan de IG-NVWA
OPD-code	OPD-code
Goedgekeurd door	Ben Langelaan

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Summary

Background

The main task of the Dutch Food and Consumer Product Safety Authority (NVWA) is to protect human and animal health. For this purpose, the NVWA monitors food and consumer products for the presence of possible hazards for human and animal health. The Authority monitors food and feed producers in the Netherlands to check whether hygiene and safety standards are met and if production is according to legal requirements. In addition, the NVWA conducts import and export inspection of food products within Europe. As it is by far not possible to inspect all food and feed products in the Netherlands, the NVWA needs to prioritize its activities. Risk based monitoring can help to identify the most important food and feed safety hazards. Risk in this case is defined as the combination of the probability of a hazard occurring in the production chain and the severity of the effects of this hazard on human health. The NVWA aims to set up a risk based monitoring program for various food chains including the dairy chain, which is the focus of the current research project.

The aim of the project is to make an inventory, based on available scientific literature, of possible microbial hazards in the dairy chain, and to search for available literature data on the possible human health effects of the most relevant microbial hazards. This information will be used by the NVWA as input to their risk prioritization of the dairy chain. Focus of this desk research was on milk (products) from dairy cows, but information of goat and sheep as milk producing animals was included when available. Products included in the research are milk, cheese, butter and milk powder.

Results

Within the Netherlands, 12 billion kilos of milk per year is currently produced by approximately 19,000 dairy cow farmers. This number is expected to increase with two billion kilos of milk per year in the future with the ending of the milk quota. Dutch milk production is processed into cheese (56%), milk powder (13%), consumption milk and cream (9%) condensed milk (7%), butter (2%), and other products (14%). Goat milk production is with 220 million kilo/year relatively small compared to cow milk production, whereas sheep milk production is only a minor activity with a yearly production of 1.5 million kilo.

A systematic search was performed in the available scientific literature to derive the most important **microbiological hazards** in the dairy chain. In addition, expert reports and zoonoses reports yearly published by the European Food Safety Authority (EFSA) and the RIVM were included in this study.

Looking at **critical points** in the dairy chain where pathogens can be introduced, contamination routes reported in literature can be classified in three main categories: contamination via the primary chain of milk (products) that will not undergo further processing steps to reduce

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pathogens, contamination via the primary chain and survival of pathogens during further steps in the milk processing (either due to lack of pasteurization, or inadequate process or storage conditions of milk), and contamination from the production environment.

According to EFSA annual reports on **foodborne outbreaks**, dairy products can be the vehicle for foodborne outbreaks with cheese as most reported dairy product category. For example, in 2013, 839 strong-evidence outbreaks were reported in the EU, for which cheese was the vehicle in 1.3% of the outbreaks and other dairy product accounted for 1.3% of the total number of foodborne outbreaks. Reported outbreaks for cheese could be attributed to the presence of *Listeria monocytogenes, Salmonella*, *Staphylococcus aureus* enterotoxins, or shiga-toxin producing *Escherichia coli* (STEC). *Campylobacter* ssp. was in particular involved in outbreaks caused by milk which, although not always specified, most likely involved consumption of raw milk. This is also in line with findings of a European expert panel that referred to *Campylobacter* ssp. as the leading cause of outbreaks related to consumption of raw milk. In most European countries including the Netherlands, consumption of raw milk is very low, yet increasing.

For the Dutch situation, the Dutch National Institute for Public Health and the Environment (RIVM) publishes annual reports on the estimated **disease burden** and **attribution** to specific food categories including dairy products. Based on these data, dairy products contribute to ~8% of the total number of disease incidents involving food as vehicle (~55,000) according to estimates for the Netherlands in the period 2010-2013 for 14 food related pathogens. The majority of the 55,000 dairy related disease incidents in 2013 are attributed to *S. aureus* toxins (68%) followed by *C. perfringens* toxins (11%) and *Campylobacter* ssp. (7%) based on expert estimates. Expressed in DALYs, dairy as products group ranks, with 410 lost healthy years of life, fifth in comparison to other food groups (2013 data). *Campylobacter* contributes with 127 DALYs to 31% of the total number DALYs attributed by experts to dairy products followed by *S. aureus* toxin (98 DALYs) and *T. gondii* (89 DALYs).

Although most frequently reported in the Rapid Alert System for Food and Fees (RASFF) system in cheese, *L. monocytogenes* accounts for only 14 of total of 55,000 disease incident attributed to dairy by experts. However, expressed in mortality *L. monocytogenes* (20% for the dairy food group in 2013) ranks second after *Campylobacter* (34%) which reflects that although incidence of this pathogen in the dairy food category is relatively low, the impact on disease burden is high.

Human pathogens can contaminate milk in the primary chain via milk producing animals. Pathogens that may be introduced in dairy products via the primary chain and factors of influence are extensively reported in literature. A significant hazard recognized is mastitis which in particular forms a risk for transmission of *S. aureus* from the infected udder to the milk. Potential **interventions** (both corrective and preventive) reported in literature are numerous and include taking care of animal health, feed and water, housing, and milking equipment.

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Industrial processing of milk is typically based on pasteurizing milk with a heating regime designed to inactivate all vegetative pathogens in the milk. Local production at the dairy farm does not necessary include milk pasteurization when (raw milk) cheese or fresh dairy products are produced. The lack of pasteurization in such production processes significantly increases the risk on prevalence of pathogens in final products. Raw milk cheese is frequently involved in foodborne outbreaks in particular caused by *L. monocytogenes*, STEC and enterotoxins produced by *S. aureus*. In milk processing, process control is considered an important factor in the control of pathogens.

With regard to scientific reports about contamination routes via the dairy processing environment, the limited number of studies available in literature in particular focus on *L. monocytogenes* as most frequently encountered pathogen in process environments. Potential contamination routes are discussed but typically refer to the importance of an effective HACCP plan including implementation of GMP and GHP.
For milk powder including the intermediates produced for powdered infant formula (PIF) *Cronobacter* ssp. and *Salmonella* ssp. are of most concern with regard to infection, especially for vulnerable groups such as young infants. Contamination of these powdered products is considered to be caused by recontamination of the products after drying.

Future trends in the dairy chain were discussed based in published reports, interviews with experts from Wageningen University, dairy industry and dairy farmers. The results of our inventory of potential microbial hazards in the dairy chain, can be used by the NVWA as input to their risk prioritization of the dairy chain.

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1 Introduction

1.1 Background of the project

The main task of The Netherlands Food and Consumer Product Safety Authority (NVWA) is to protect human and animal health. For this purpose, NVWA monitors food and consumer products for the presence of possible hazards for human and animal health. As it is not possible to inspect all food and feed products in the Netherlands, the NVWA needs to prioritize its activities. Risk based monitoring will help to identify the most important food and feed safety hazards. Risk in this case is defined as the combination of the probability of a hazard occurring in the production chain and the effects of this hazard on human health. The NVWA will perform risk based monitoring in various food chains. One of these food chains is the dairy chain, which is the focus of this research.

The aim of the current study is to make an inventory based on available scientific literature of possible microbial hazards in the dairy chain, from farm-to-fork and to search for available literature data on the possible human health effects of the most relevant microbial hazards. This information will be used by the NVWA as input to the risk prioritization of the dairy chain. Focus of this study is on dairy cows, but goat and sheep are also taken into account. Products included in the research are milk, cheese, butter and milk powder. The NVWA delivered schematic representations of the productions chains which form the basis of this research.

The results of the following tasks are described in the project:

1. Literature study on the microbiological hazards that may occur in the dairy chain. Data will be collected from scientific literature, the RASFF database, expert reports (EFSA, FAO/WHO, RIVM etc.).
2. Analysis of critical points in the dairy chain. Furthermore, factors are identified that may influence the presence of these hazards.
3. Literature research on the human health effects of the microbial hazards that are most relevant according to the analysis in step 1 and information about the attribution to the total disease burden.
4. Intervention measures are indicated that can prevent or reduce the most relevant microbial hazards as identified in step 1.
5. Evaluating trends in developments within the dairy chain up to 2025 that may influence food safety hazards in the future.

1.2 Background of the dairy chain

Within the Netherlands, there are approximately 19,000 dairy cow farmers who have a total of 1.5 million cows, which produce 12 billion kilo of milk per year. This milk is processed within

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51 factories (see Appendix 1) into cheese (56%), milk powder (13%), consumption milk and cream (9%), condensed milk (7%), butter (2%) and other products (14%) (Figure 1) [1].

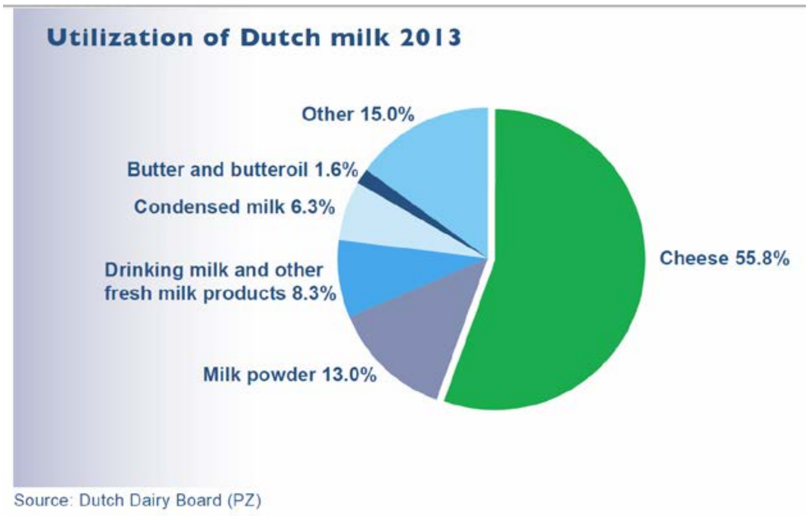

Figure 1. Utilization of Dutch milk in 2013 (Dutch Dairy Board 2013).

The 12 billion kg milk (2013 production) is mainly processed into cheese (approximately 10 kg milk is needed for 1 kg of cheese) and other dairy products (Figure 2, Figure 3). Less than 10% of the milk that is produced in the Netherlands is converted into drinking milk or other fresh milk products. Dry whey powder produced in the Netherlands reaches 200,000 tonnes per year (average production data over the period 2010-2013 extracted from FAOSTAT).
In 2012, 691,000 tonnes whey and whey powder were imported [2].

Dutch dairy production

	million kg		
	2012	2013	2013/2012
Milk delivered to factories	11 675	12 213	4.6%
Milk available for processing	11 849	12 346	4.2%
Drinking milk and other fresh milk products 1) 2)	1 044	1 028	-1.5%
Cheese (including quark)	766	794	3.6%
Butter and butteroil	195	199	2.1%
Non-skimmed milk powder	121	128	5.9%
Skimmed milk powder	66	65	-0.7%
Condensed milk 3)	371	360	-2.9%

1) excluding added ingredients 2) including cream 3) including coffee milk (evaporated milk)

source: Dutch Dairy Board (PZ)

Figure 2. Dutch Dairy production [3].

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Dairy goat production is much smaller with a production of 220 million kilo of goat milk per year, produced by 365 goat milk farmers (www.ngzo.nl). The volume of sheep milk is even lower with a yearly production of 1.5 million kilo, produced by around 6,000 sheep [4].
The composition of cow, goat and sheep milk is different, although the fat content of goat and cow milk is comparable with levels between 30-50 g/kg and between 35-40 g/kg, respectively. Sheep milk has a much higher fat content ranging from 60-82 g/kg [5].

Zuivelproductie per land 2014
Dairy production by country

Melkaanvoer en productie EU-28 — 2014
Milk deliveries and production EU-28 — index: 2013=100
(x 1.000.000 kg)

EU-28	jan	feb	mrt	apr	mei	jun	jul	aug	sep	okt	nov	dec	cumulatief	index
Melkaanvoer	11.986,0	11.176,2	12.777,3	12.936,2	13.499,4	12.769,1	12.758,4	12.372,2	11.790,1	11.842,4	11.238,1	11.729,8	**146.875,2** ↑	104,8
Consumptiemelk	2.735,6	2.548,1	2.745,8	2.695,8	2.698,7	2.503,0	2.565,4	2.545,1	2.549,4	2.629,6	2.561,6	2.702,9	**31.481,0** ↑	101,0
Room	216,5	205,1	224,2	244,2	237,3	236,1	227,0	215,6	221,8	238,8	220,1	243,8	**2.730,5** ↑	106,7
Kaas	750,2	665,7	745,5	761,9	761,5	720,3	744,3	713,5	706,6	726,3	682,1	691,5	**8.669,4** ↑	102,0
Boter	172,1	158,9	179,3	181,4	184,7	165,6	172,6	155,4	159,3	158,8	146,0	171,5	**2.005,6** ↑	102,3
Niet-mager melkpoeder	63,2	56,0	60,8	61,3	61,1	52,9	52,0	47,3	50,8	51,4	48,6	56,3	**661,7** ↑	111,3
Mager melkpoeder	105,9	99,3	116,8	125,3	139,2	129,7	125,9	114,5	100,9	:	:	:	**1.057,5** ↑	127,5
Gecondenseerde melk	91,4	87,1	91,1	95,1	87,7	78,1	86,3	82,2	80,9	86,5	86,7	87,7	**1.040,8** ↓	95,0
Weipoeder	:	:	:	:	:	:	:	:	:	:	:	:	:	:

Nederland*	jan	feb	mrt	apr	mei	jun	jul	aug	sep	okt	nov	dec	cumulatief	index
Melkaanvoer	1.073,5	979,8	1.083,8	1.081,3	1.094,0	1.045,2	1.051,2	1.036,1	997,9	1.015,6	976,8	1.033,0	**12.468,2** ↑	102,1
Consumptiemelk	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Room	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Kaas	68,6	61,0	66,6	65,2	66,7	61,9	66,7	63,4	61,0	63,6	59,9	63,8	**768,4** ↓	96,8
Boter	13,9	13,9	14,4	13,2	12,4	10,9	11,2	11,6	10,4	10,3	10,8	13,2	**146,2** ↑	106,9
Niet-mager melkpoeder	12,4	10,8	12,9	13,8	13,6	12,2	10,9	9,9	10,4	10,2	10,6	12,9	**140,6** ↑	109,8
Mager melkpoeder	5,6	5,5	6,4	5,7	6,3	5,2	5,3	5,4	4,4	4,6	4,7	6,5	**65,6** ↑	100,5
Gecondenseerde melk	31,4	31,4	28,5	28,9	28,0	29,8	33,6	30,3	30,5	33,1	32,8	33,4	**371,7** ↑	100,7
Weipoeder	:	:	:	:	:	:	:	:	:	:	:	:	:	:

Figure 3: Dairy production of the Netherlands in 2014. Data adopted from ZuivelNL [6].

The general production chain of dairy products is depicted in Figure 4.

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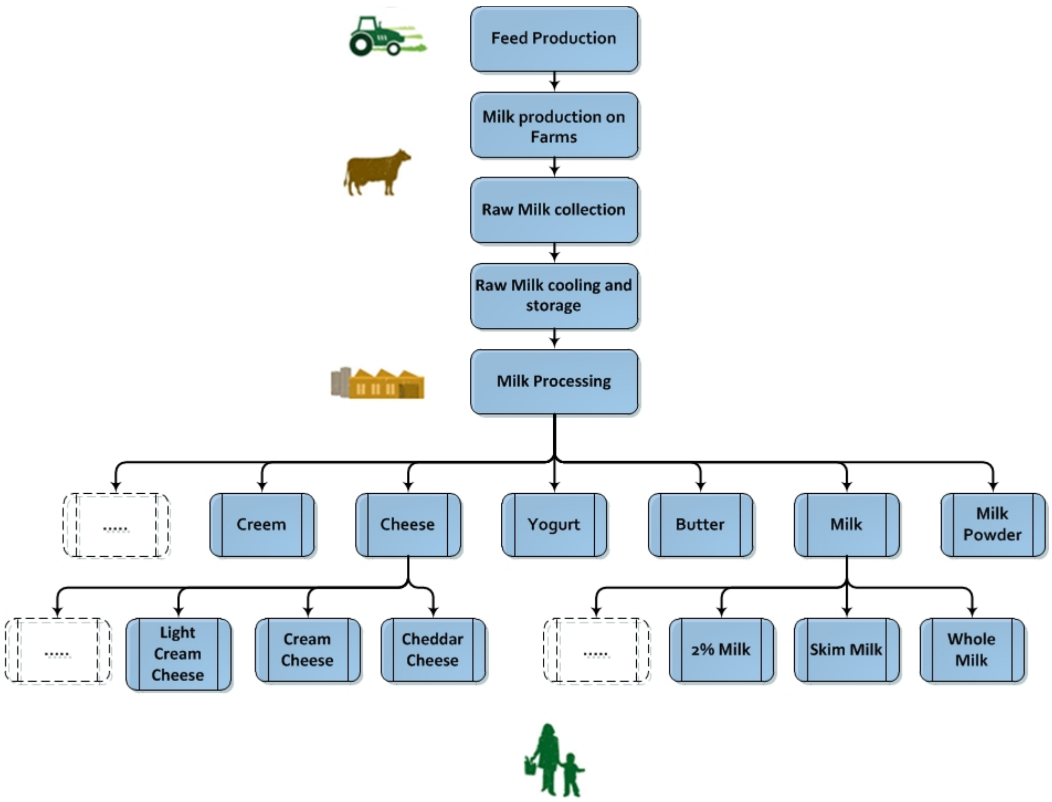

Figure 4. Various stages in the dairy production chain from farm-to-fork.

Most dairy cow products are exported to neighbouring countries with Germany as most important export country (see Figure 5). Cheese accounts for almost half of all exported dairy products. The Netherlands also imports around 2.7 billion euro of dairy products, primarily from neighbouring European countries. Total milk imports in 2012 were 849 million kg [2].
Germany is by far the largest supplier, accounting for 45% of the total import value.
Cheese is the main imported product (around 225 million kg in 2013) followed by skimmed milk powder and butter and butter oil (around 100 million kg in 2013) and non-skimmed milk powder (around 65 million kg in 2013). As export is larger than import, dairy products contribute with around 8% to the overall Dutch trade balance [7].

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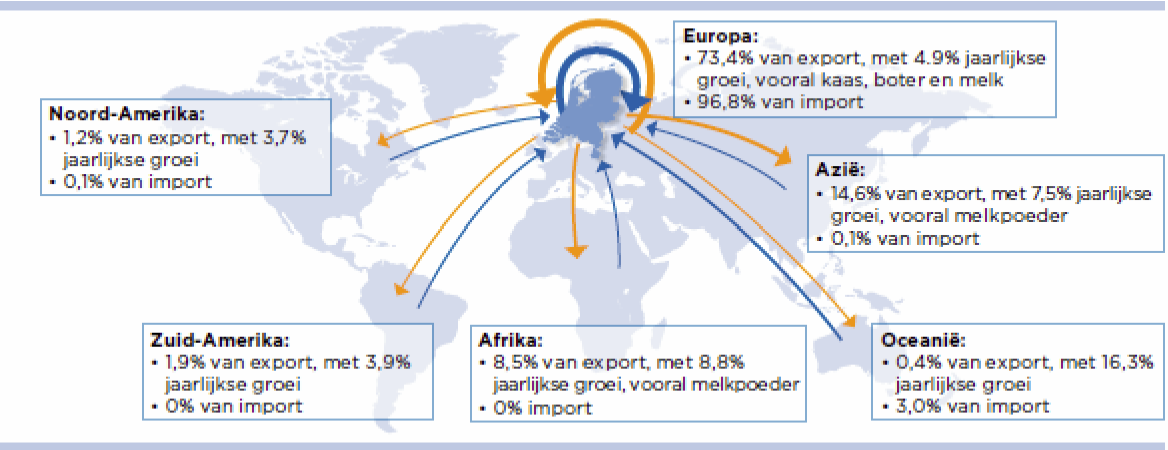

Figure 5. Import and export of dairy products to and from the Netherlands as percentage of the total value [7].

Import numbers related to dairy product types are given in Figure 6 (Eurostat, CBS).

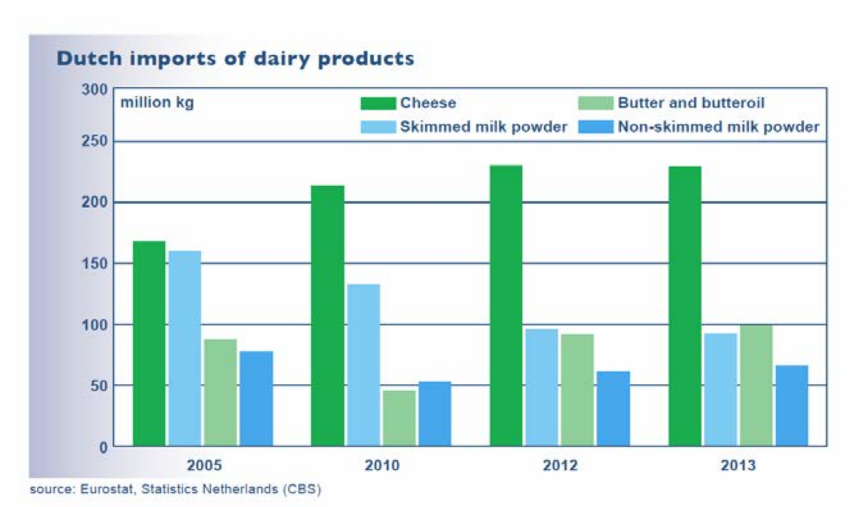

Figure 6. Dutch import specified by dairy product type expressed in million kg product(historical overview).

1.3 Materials and methods

In order to derive the most important microbial safety indicators for the dairy chain, a systematic literature search was performed using the Scopus database. Indicators were searched using the following search strings:

(TITLE-ABS-KEY ("micro* safety" OR pathogen* OR "bacteri* hazard" OR "micro* contamination" OR "disease burden" OR "foodborne disease" OR "food-borne disease" OR zoonoses OR "public health disease") AND TITLE (milk* OR cheese* OR "milk powder*" OR butter* OR dairy) AND TITLE-ABS-KEY (review* OR survey* OR overview*) AND NOT TITLE-ABS-KEY ("breast milk" OR allerg*)) AND PUBYEAR > 2005

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The relevance of the retrieved references was first determined based on the title of the reference. Further relevance assessment was based on the abstracts of selected references. Based on this selection, full text papers were downloaded.

In addition, the internet was used for additional relevant information such as expert reports as published by RIVM, EFSA, FAO or WHO.

The Rapid Alert System for Food and Feed (RASFF) portal was used to extract data with regard to notifications of pathogenic microorganisms in milk and milk products in the period 2009-2014 within the EU.

Experts from RIKILT, Wageningen University, CVI, NZO and industry were consulted to obtain expert input on future trends in the dairy chain that may affect microbiological hazards. In total 7 experts (3 scientists, 3 from the dairy sector, and 1 involved in coordination of the dairy chain (NZO)) were interviewed.
Additional experts from the Food Microbiology and Hygiene (FMH) and Food Quality and Design (FQD) chairs of the Agrotechnology & Food Sciences cluster of Wageningen University (were consulted for input and to review the final report).

1.4 Exclusion criteria

The aim of this desk research is to make an inventory of microbial hazards in the dairy chain and the possible human health effects of the most relevant microbial hazards based on available scientific literature. This analysis is based on dairy products starting from cow's milk (main stream), goat's milk and sheep's milk.
Not included in this study are:

- Dairy products from other milk sources than cow, goat or sheep;
- Dairy products other than consumption milk, cheese, butter and milk powder;
- The dairy chain is analysed until final product excluding retail and consumer phase;
- Mycotoxins are not included, this will be covered by the chemical hazard analysis that is executed by RIKILT.

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2 Microbiological hazards in the dairy chain

Using the search criteria as described in the materials and methods section to derive the most important microbiological hazards for the dairy chain, initially 253 publications were retrieved. After a first screening of title and abstract for relevance, 180 publications were selected that describe the microbiological hazards in the dairy chain. Seven publications contained the word 'goat' in the title and only two publications contained the word 'sheep' in the title. This indicated that limited information is available in the scientific literature about dairy products made from milk of these producing animals.

2.1 Microbial hazards in the primary cow milk chain

Pathogens may contaminate milk and dairy products via different routes in the chain from primary milk production by different producing animals at the farm level to retail. Contamination may already be introduced at the primary chain for cow's milk production. Pathogens that may be introduced at the primary chain and factors that affect their presence are described below.

2.1.1 Animal factors impacting on milk safety

Pathogenic bacteria can already contaminate raw milk via the farm environment. Several routes of contamination at the primary cow milk chain are in place. Zoonoses are diseases that are naturally transmittable from animals to humans (where it can be that the organism is also causing a disease in the animal, but that is not a prerequisite). Table 1 gives an overview of the human pathogens that can be present in milk (including raw milk) and milk products. Microorganisms including pathogens can already contaminate the milk before it leaves the teat, due to their presence on the teat skin and thereby also (partly) in the teat canal. In addition, the mammary gland can be colonized by *Staphylococcus* ssp., *Streptococcus, Bacillus* ssp., *Micrococcus*, *Corynebacterium,* and coliforms, without causing any disease symptoms with the cows [8, 9]. *Corynebacterium* ssp. are the causative agent of diphtheria. *Corynebacterium diphtheriae* is only transmittable via human contact [10]. *Corynebacterium ulcerans* is a zoonotic pathogen, with cattle being the most important reservoir, it can be transmitted to milk [10]. Data on prevalence of *C. ulcerans* in Dutch dairy cattle is lacking [10]. *Corynebacterium pseudotuberculosis* is also a zoonotic pathogen, with goat and sheep as reservoir. Direct contact with infected animals or their environment is considered the most important transmission route for *C. pseudotuberculosis*. In the US, one single incident is known associated with the consumption of raw milk. However, since 1953, a large-scale vaccination against diphtheria is in place in the Netherlands, and as a result the diphtheria disease incidence in the Netherlands is very low [10]. Santman-Berends *et al.* studied the incidence of heifer subclinical mastitis in the first 100 days in lactation of heifers and the associated risk factors in the Netherlands in 2008. Risk factors included transferring heifers close-to-calving to separate locations compared to housing with lactating cows [mean increase 4.5% with 0.2-8.7% as 95% confidence interval (CI)]; not removing supernumerary teats (teats in excess of the normal four teats; 7.0% CI 2.8-11.3%) in calves; and herds in which all lactating cows remained indoor

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compared to day and night grazing outside (5.9% CI 1.3-10.6%) [11]. When cows suffer from more severe mastitis (with visual symptoms), causative agents are typically *Staphylococcus aureus, Escherichia coli* and *Brucella* species, and these pathogens may end up in the milk [12, 13]. Brucellosis in humans is mainly caused via contact with contaminated animals, drinking of raw milk or consumption of other unpasteurized dairy products [14]. According to the World Organisation for Animal Health (OIE) and other official authorities, The Netherlands is officially brucellosis-free since 1999 [14]. The prevalence of intramammary infection in the Netherlands caused by coagulase-negative staphylococci was performed in 2003, based on 49 randomly selected herds with at least 40 lactating cows it was estimated that coagulase-negative staphylococci were the most frequently isolated group of pathogens accounting for 10.8% prevalence at quarter level and 34.4% at cow level [15]. Most significant risk factors for prevalence of intramammary infection by coagulase-negative staphylococci included housing dry cows in one group instead of multiple groups, and pasturing cows during outdoor season [16]. Additional risk factors were reported in a study involving 300 Dutch dairy farms showing that seasonal effects and grazing should be considered risk factors for clinical mastitis of both heifers and multiparous cows [17, 18].

2.1.2 Housing

In an EFSA risk assessment study on overall effects of dairy cow welfare and disease, the farming system was identified as major factor determining health problems and other aspects of welfare of dairy cattle. Genetic selection for high milk yield is an important factor affecting welfare and in particular health of dairy cows and has been shown to be positively correlated with the incidence of mastitis. However, housing, management and handling practices have been identified as factors with the most effect on dairy cattle welfare including udder problems [19]. In relation to housing the following hazards were described as being most important:

- the lack of facilities for cows with systemic mastitis, capable of causing poor welfare due to the increased discomfort, pain and disease duration [19].
- inadequate stall/cubicle design, especially in cubicles and tie-stalls.
- bedding hygiene as important factor for udder health. Infectious udder disorders may occur more in straw-yards where insufficient attention is given to hygiene of the bedding. If stocking density in straw yards is too high, this may lead to teat trampling [19].

2.1.3 Feed, water and faeces

Also the feed of the cows, including feed derived from crops, and the drinking water of cows can be a source of contamination. Poor drinking water quality is associated with a higher incidence of intramammary infection caused by coagulase-negative staphylococci [16]. It should be noted that a causal relation is not proven in this study [16]. Drinking water contaminated with *Salmonella* ssp.

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and *Yersinia* ssp. may also infect cows [13]. Water for both crops and cows should be of potable quality or retrieved from a deep well.

Pathogen-containing manure that is used as crop fertilizer can be a vehicle for pathogens [20]. The faeces of young Dutch dairy calves (1 to 21 days old) was reported to contain several enteropathogens, namely *E. coli* [2.6% prevalence with a CI of 1.3-4.6%], coronavirus (3.1%; 1.6-5.2%), *Cryptosporidium parvum* (27.8%; 23.6-32.4%), rotavirus (17.7%; 14.2-21.7%) and *Clostridium perfringens* (54.0%; 49.1-58.8%) [21]. Data show a relatively high prevalence of *C. parvum*, rotavirus, and *C. perfringens*. *C. parvum* is transmittable via milk of lactating cows, rotavirus and *C. perfringens* are considered not transmittable via this route [22]. Various animal species (cattle, pigs, sheep, mice, rodents, cats, mice) as well as humans can be a reservoir for *Cryptosporidium* spp. [13]. The oocysts of this organism can also be present in water and the environment [13]. A study on *Cryptosporidium* epidemiology in the Netherlands is currently ongoing. Preliminary results show that 30% of the incidents, mainly caused by *C. parvum*, can be attributed to exposure outside the Netherlands. Important risk factors for disease in the Netherlands are exposure to livestock [adapted odds ratio (aOR) 5.7; 95% CI: 2.3-14] and swimming in open water (aOR 5.7; 95% CI: 2.2-14) [14].

Cattle feed can be a source of human pathogens. Feed can be contaminated with pathogens similar to those present in faeces and other pathogens ubiquitously present in the environment (*B. cereus, Salmonella* ssp., *Listeria monocytogenes, Campylobacter* ssp.*, Yersinia* ssp.*,* and Shiga toxin-producing *E. coli* (STEC)) when the meadow is fertilized with contaminated manure [13, 23, 24]. Upon feed digestion, surviving (spore-forming) pathogens can end up in the barn bedding and attach to the udder and teats [25]. When attached dirt (consisting mainly of faecal material) is not fully removed from the teats, pathogens like *B. cereus, L. monocytogenes, Campylobacter* spp. can end up in the milk [13, 23, 26]. Also silage, fermented grass or corn crops, is reported as potential source of contamination when the fermentation process failed. Several studies identified silage as the main source of clostridial spores including butyric acid bacteria in cheese milk [26-28]. Most butyric acid bacteria are spoilage microorganisms like *Clostridium butyricum, Clostridium tyrobutyricum,* and *Clostridium beijerinckii*, however, the ability of some strains of *C. butyricum* to produce botulinum toxin makes the detection of this group of microbes in milk important [28]. Control of butyric acid bacterial spore counts in silage (see section 4.1.3) is therefore considered important in prevention of surface contamination of teats and eventually to limit spore counts in milk storage tanks [28-30].

The dairy farm environment is an important reservoir of foodborne pathogens such as *Salmonella*, *L. monocytogenes,* STEC*, Campylobacter jejuni*, *B. cereus*, *Yersinia enterocolitica* [23, 24, 31, 32]. *Clostridium botulinum* is present in the cattle environment and can be transferred via the feed to the gastro-intestinal tract of producing animals ([33] and references therein). An increasing number of outbreaks of botulism in cattle has been described in the last decades and the increased use of

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plastic wrapped or packed silage as cattle feed has been mentioned as possible root cause [33]. A few studies have shown that *C. botulinum* spores can be introduced in the milk via the cattle environment but studies on the contamination level and prevalence in raw milk are limited which hampers the assessment of botulism risk associated with dairy products [33]. It is not known whether cattle suffering from botulism secrete the neurotoxin in their milk.

2.1.4 Milking equipment and storage

Milking equipment can be an additional source of microbial contamination of the cow's milk. Most microorganisms have the capacity to adhere to surfaces and reside in surface-associated, multicellular communities called biofilms. The microbes embedded in biofilms often display increased resistance to antimicrobial agents because the self-produced matrix of extracellular polymeric material acts as a protective barrier against the effects of detergent and disinfectant solutions [34, 35]. Biofilms can be formed in the equipment, transport line, and storage tank when cleaning and disinfection programs are inadequate and not properly applied for a prolonged period. Spoilage microorganisms typically encountered in a dairy environment include micrococci, enterococci, *Pseudomonas* ssp., aerobic spore-formers, and certain lactobacilli. Although these microorganism are not a direct risk for food safety, they may form biofilms and once established, these may attract, shelter and/or protect pathogenic bacteria [35]. When fresh milk enters the storage tank, it should be cooled (< 6 °C) within maximal three hours after milking to inhibit outgrowth of micro-organisms. Milking with an automatic milking system was reported as risk factor for the incidence of subclinical mastitis of lactating heifers with on average 6.9% (95% CI: 2.2 to 11.5%) higher heifer subclinical mastitis incidence [11]. Poor hygiene of the milking equipment overall appeared to be a risk factor for mastitis of both heifers and multiparous cows, as well as barn type and post-milking teat disinfection [17, 18].

2.1.5 Veterinary drugs and antibiotic resistance

Antimicrobial agents are often used for treatment of clinical mastitis in dairy cattle [36]. The selective pressure introduced by the use of antimicrobials can lead to the emergence of antimicrobial resistant microorganisms [37]. The emergence of for example methicillin resistant *S. aureus* (MRSA) and occurrence in dairy cows, in particular in cases of clinical mastitis has been reported [38]. Several studies indicate that milk can be a reservoir of bacteria carrying resistance genes. A study performed in the Czech Republic indicated that for *E. coli* in raw cow's milk, 31.8% was resistant to β-lactam (31.8%) and 13% to tetracycline (13.0%) and 5.5% of the isolates was multi-resistant [39]. Antibiotic resistance has also been reported for *S. aureus* isolated from raw goat milk. A report on prevalence of antimicrobial-resistant *S. aureus* in goat milk in Poland showed that 86% of the raw goat milk samples is contains coagulase-positive *S. aureus* [40]. Of these isolates, 15.5% showed resistance to penicillin, 12.1% to sulphamethoxazole, and 6.3% to tetracycline and cefoxitin. Nineteen isolates (9.2%) were resistant to other antimicrobials (erythromycinum, streptomycin, gentamicin, chloramphenicol, florfenicol, and trimethoprim).

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MRSA was not identified in the tested samples. It should be remarked that above mentioned studies may not be directly applicable for other countries.

Further data on the occurrence of antimicrobial resistance is available in a recent EFSA report but focus is mainly on prevalence on meat [37]. In this EFSA report, data from one MS (Poland) on prevalence of MRSA in raw cow's milk is given, of the 12 samples analysed in a single study, one sample (8.3%) was positive for MRSA. No MRSA was found in raw goat's milk bit was based on a single study in Spain that involved only five samples. No information on prevalence in Dutch milk was reported, however, the prevalence of antibiotic resistant *E. coli* in dairy cows was reported for The Netherlands. Reported prevalence (2012) of antibiotic resistance for *E. coli* was between 0.4 and 1.5% for ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, nalidixic acid, streptomycin, sulfonamides, tetracyclines or gentamicin.

2.1.6 Other zoonotic diseases/infections

Besides the above mentioned factors, cattle itself can be a reservoir of *Coxiella burnetii* (causative agent of Q-fever), *Mycobacterium* spp., *Campylobacter* ssp., coliforms (including *E. coli* and *Salmonella enterica*), or the mouth-and-foot disease virus [41-46]. *C. burnetii* and the mouth-and-foot virus were considered not to be transmittable via milk and are therefore not considered a hazard upon consumption of (raw) milk (Table 1; [22]). Nevertheless, it should be remarked that there is no consensus among experts with respect to the question whether *C. burnetii* can be transmitted via milk or not. For example, recent model predictions in the UK suggests that the risk for *C. burnetii* infection via consumption of unpasteurized milk and milk products (including cheese) are not negligible but in comparison to transmission via inhalation of aerosols from parturient products and livestock contact the oral route represent a very low risk [47]. Exact numbers are not available due to lack of human oral dose–response data. Another pathogen that can infect cattle is the tick-borne encephalitis virus (TBEV). It is transmitted via ticks that can introduce the virus into cattle. Subsequently, the virus can be detected in the milk of infected animals [22]. However, until now TBEV is not detected in the Netherlands [48, 49] and therefore probably not endemic in the Netherlands. *Toxoplasma gondii* is an intra-cellular protozoan and the causative agent of toxoplasmosis [50]. *T. gondii* is widely prevalent in humans, warm-blooded animals and birds throughout the world [50]. The cat is the only definitive host in which sexual reproduction of the parasite occurs in the intestine, resulting in the shedding of oocysts into the environment [50]. Following sporulation of the oocysts, ingestion by humans and other animals results in release of sporozoites in the intestine [50]. *T. gondii* can be excreted in the milk of infected cows, goats and sheep and consequently drinking of raw milk from these animals can be considered a risk of toxoplasmosis [22]. Although *T. gondii* cannot multiply in milk, it has been shown to survive for 7 days at 4°C in cow's milk [50]. *T. gondii* prevalence in Dutch cows was estimated at 1.9% (95% CI: 0.7–3.5%) for the calves less than 8 months old, at 15.6% (95% CI: 10.3–21.0%) for calves between 8 and 12 months and at 54.5% (95% CI: 46.6–65.1%) for cattle over 12 months old [51]. Ruminants become infected by the ingestion of feed or water contaminated with *T. gondii*.

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No information on the prevalence of *T. gondii* in Dutch (raw) cow's milk was available in the consulted literature.

2.1.7 Key findings microbial hazards in the primary cow chain

Overall, good dairy farming practice improves the milk quality and includes taking care of 1) animal health, 2) milking equipment, 3) animal feeding and water, 4) animal welfare, and 5) farm environment [52, 53]. A recent report published by EFSA [22] lists the main microbiological hazards related to consumption of raw drinking milk identified as relevant in the EU by a panel of European experts (Table 1). The experts identified *Campylobacter* spp., *Mycobacterium bovis*, *Salmonella* spp., Shigatoxin-producing *E. coli* (STEC) and TBEV as main microbiological hazards in raw drinking milk from cows (see also section 2.2.2). From this list, *Campylobacter* spp., *Mycobacterium bovis*, *Salmonella* spp. and STEC are of main importance for raw milk produced in the Netherlands.

Table 1. Main microbiological hazards identified as relevant in the EU upon consumption of raw cow's milk. Adapted from [22].

Bacillus cereus
Brucella abortus[1]
Campylobacter* spp. (thermophilic)
Corynebacterium[2] ssp.
Listeria monocytogenes
Mycobacterium bovis
Salmonella spp.
Staphylococcus aureus
Streptococcus equi subsp. *zooepidemicus*
Shiga toxin-producing *E. coli* (STEC)
Yersinia enterocolitica
Yersinia pseudotuberculosis
Cryptosporidium parvum
Toxoplasma gondii
Tick-borne encephalitis virus (TBEV)[3]

The main microbiological hazards as identified by experts are indicated in bold font type. * *Campylobacter* was identified as the leading cause of outbreaks. [1] Not for raw milk produced in The Netherlands since The Netherlands is officially brucellosis-free [14]. [2]Since 1953, a large-scale vaccination program against diphtheria, of which *Corynebacterium* ssp. is the causative agent, is in place in the Netherlands, consequently, diphtheria disease incidence in The Netherlands is very low. [3]TBEV is not endemic in the Netherlands.

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2.2 Microbial hazards in the processing chain for cow's milk

2.2.1 Microbiological food safety criteria and process hygiene criteria relevant for processing of cow's milk

All foods including dairy products should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable risk for human health. Food safety is mainly ensured by a preventive approach (GHP, GMP, HACCP) but several microbiological food safety criteria and criteria for process hygiene for milk and dairy products have been set in European legislation. Table 2 gives an overview of all relevant criteria for food safety and process hygiene for dairy products as set by European legislation.

2.2.2 Heat treatment of milk

Virtually all industrial processes for milk and dairy products involve heat treatment as part of the HACCP (Hazard analysis and Critical Control Points) plan. HACCP has been developed to meet the safety standards indicated in EC No. 852/2005 and 853/2004 (replacing the Dairy Hygiene Directive 92/46/EEC) that describe regulations for the hygienic production and sale of raw milk, heat-treated milk and milk based products. These heat treatments mainly aim for inactivation of pathogens and prolong the shelf-life of milk. In general, thermal treatments and temperature control (cooling, freezing) are considered critical for production of a microbiological safe end-product [53]. Depending on the treatment time and heat load applied, thermal processes can be divided into:

- high temperature–short time (HTST) pasteurization (at least 15 sec at ≥ 72°C) is the international standard pasteurization regime as used by the Dutch dairy industry [54]. Bacterial spores are not eliminated by this heating regime and consequently the products depend on cold storage and have a limited shelf life.

- ultra-high temperature (UHT) sterilisation (135 - 150°C for 2-20 seconds) [55] and references therein. This process aims for inactivation of heat-resistant bacterial spores. UHT is a continuous process carried out by direct heating (steam injection and steam infusion), or in plate or tubular heat exchangers. In combination with aseptic packaging, the unopened product is shelf-stable at ambient temperatures.

Pasteurization processes were initially designed for inactivation of *Mycobacterium tuberculosis* which is a relatively heat-resistant, non-spore forming human pathogen present in milk. Pasteurization standards today aim for at least 6 log reduction of *C. burnetti*, which is the most heat-resistant milk-borne zoonotic pathogen known. Milk that has been pasteurized correctly is, therefore, unlikely to cause disease by non-spore forming pathogens [56].

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Table 2. Microbiological food safety and process hygiene criteria relevant for cheese, milk, butter and whey powders

Indicator	Food category	Limit	Type of criterion
S. aureus enterotoxins[1]	Cheese, milk powder and whey powder	Not detected in 25 g (n=5)	Safety
Salmonella ssp.[1]	Cheeses, butter and cream made from raw milk or milk that has undergone a lower heat treatment than pasteurization	Absent in 25 g (n=5)	Safety
Salmonella ssp.[1]	Milk powder and whey powder	Absent in 25 g (n=5)	Safety
Salmonella ssp.[1]	Dried infant formula and dietary foods (infants < 6 months)	Absent in 25 g (n=30)	Safety
Salmonella ssp.[1]	Dried follow-on formula	Absent in 25 g (n=30)	Safety
Cronobacter ssp.[1]	Dried infant formula and dietary foods (infants < 6 months)	Absent in 10 g (n=30)	Safety
Listeria monocytogenes[1]	Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes	Absent in 25 g (n=10)	Safety
Listeria monocytogenes[1]	Ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes	Maximum 100cfu/g (n=5) (Products placed on the market during their shelf-life)[3] or Absent in 25 g (n=5) (Before the food has left the immediate control of the food business operator, who has produced it)[4]	Safety
Listeria monocytogenes[1]	Ready-to-eat foods unable to support the growth	Maximum 100cfu/g	Safety

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	of *L. monocytogenes*, other than those intended for infants and for special medical purposes	(n=5) (Products placed on the market during their shelf-life)	
Coagulase-positive *Staphylococci* [1]	Cheeses made from raw milk	Maximum 2 out of 5 samples between 10^4 and 10^5 CFU/g (others below 10^4)	Process hygiene If values > 10^5 CFU/g are detected, the batch has to be tested for staphylococcal enterotoxins.
Coagulase-positive *Staphylococci* [1]	Cheese made from mildly heated milk and ripened cheese from pasteurized milk or whey	Maximum 2 out of 5 samples between 100 and 1000 CFU/g (others below 100)	
Coagulase-positive *Staphylococci* [1]	Unripened soft cheeses (fresh cheeses) made from milk or whey that has undergone pasteurization or a stronger heat treatment	Maximum 2 out of 5 samples between 10 and 100 CFU/g (others below 10)	
Coagulase-positive *Staphylococci* [1]	Milk powder and whey powder (without further processing)	Maximum 2 out 5 samples between 10 and 100 CFU/g	
Entero-bacteriaceae [1]	Pasteurized milk and other pasteurized liquid dairy products (without further processing)	Maximum 10 CFU/ml (n=5)	Process hygiene
Entero-bacteriaceae [1]	Milk powder and whey powder (without further processing)	Maximum 10 CFU/g (n=5)	Process hygiene
Entero-bacteriaceae [1]	Dried infant formula and dried dietary foods for special medical purposes intended for infants below six months of age	Absent in 10 g (n=10)	Process hygiene
Entero-bacteriaceae [1]	Dried follow-on formula	Absent in 10 g (n=5)	Process hygiene
E. coli [1]	Butter and cream made from raw milk or milk that has undergone a lower heat treatment than pasteurization	Maximum 2 out of 5 samples between 10 and 100 CFU/g	Process hygiene
E. coli [1]	Cheeses made from milk or whey that has undergone heat treatment	Maximum 2 out of 5 samples between 100 and 1000 CFU/g	Process hygiene
B. cereus [1]	Dried infant formula and dietary foods (infants < 6 months)	Maximum 1 out of 5 sample between 50 and 500 CFU/g	Process hygiene

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Total plate count (30 °C)[2]	Raw cow's milk	≤ 100,000 CFU/ml	Process hygiene
Total plate count (30 °C)[2]	Raw milk from other species	≤ 1,500,000 CFU/ml	Process hygiene
Total plate count (30 °C)[2]	Manufacture of products made with raw milk by a process that does not involve any heat treatment	≤ 500,000 CFU/ml	Process hygiene
Total plate count (30 °C)[2]	Raw cow's milk used to prepare dairy products	≤ 300,000 CFU/ml	Process hygiene
Total plate count (30 °C)[2]	Heat treated cow's milk used to prepare dairy products	≤ 100,000 CFU/ml	Process hygiene
Somatic cell count[2]	Raw cow milk	≤ 400,000 cells /ml	Process hygiene

[1] Consolidated version of Regulation (EC) No 2073/2005 – 01.06.2014

[2] Consolidated version of Regulation 853/2004 – 17.11.2014

[3] This criterion shall apply if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 cfu/g throughout the shelf-life. The operator may fix intermediate limits during the process that must be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of shelf-life.

[4] This criterion shall apply to products before they have left the immediate control of the producing food business operator, when he is not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf-life.

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Inadequate pasteurization or recontamination events in later processing steps can lead to the presence of *Salmonella* spp., *L. monocytogenes, C. jejuni, Yersinia enterocolitica*, pathogenic *E. coli, B. cereus*, *Mycobacterium* spp., *S. aureus* enterotoxins or *C. botulinum* in milk or milk products [34]. To ensure a safe product, strict cleaning and disinfection programmes are used to control cross-contamination and biofilm formation [35, 53]. Pasteurized milk should be refrigerated as soon as possible and maintained cold (< 6 °C) to prevent growth of potential pathogens that survived pasteurization or resulting from post-pasteurization contamination. The impact of temperature in the cold chain was further modelled by Koutsoumanis *et al.* [57] for a post-pasteurization contamination with *L. monocytogenes* from the process environment. Collected data on time-temperature conditions of pasteurized milk during transportation to retail, retail storage, and domestic storage and handling in Greece were used for *L. monocytogenes* growth modelling [57]. The model developed simulates a situation where pasteurized milk is recontaminated with 1 cell per litre. The predicted percentage of milk cartons, initially contaminated with 1 *Listeria* per package, in which the pathogen exceeds the safety criterion of 100 bacteria/ml (Regulation (EU) No 2073/2005) at the time of consumption (shelf life of 5 days) was 0.14% [57]. It should be remarked that time and temperature regimes used for this study were based on a survey in Greece and may not be applicable to the Dutch situation. For example, the temperature in the truck during transportation to retail varies between 3.6 and 10.9 °C degrees and transportation time was up to 10 hours. The temperatures observed in domestic refrigerators were comparable to those observed in other surveys in Europe (UK, France, Ireland and Greece) which showed a weighted mean of 6.4 °C with 64% of the refrigerators operating at temperature above 5 °C [57]. The door was the warmest position in the refrigerator and most frequently used to store milk cartons. The model predicts that a decrease of 2 °C of the mean temperature in combination with storage in the centre may extend the shelf life with days without affecting the current exposure of consumers to *L. monocytogenes* [57]. It should be remarked here that the model predictions were performed for a situation without competitive spoilage micro-organisms. Competitive micro-organisms cause premature , physical and sensorial spoilage of the milk and is therefore less likely to be consumed.

2.2.3 Consumption milk

In general, milk intended for human consumption must meet the requirements of the General Food Law (Regulation (EC) 178/2002) and must be free of pathogens. In many European countries including the Netherlands, raw cow's milk can be sold directly to the consumers at the farm, and in some EU countries via vending machines located on-farm or in retail settings [22]. Also in The Netherlands, raw cow's or goat's milk is sold via vending machines at some dairy farms. The levels and patterns of raw milk consumption in Europe are poorly documented, but only a small fraction of the milk that is produced is sold as raw drinking milk to date [22]. There is a clear link between drinking raw milk and human illness associated with pathogens that can be present in raw milk. The main microbial hazards associated with consumption of raw milk are mentioned in Table 1. A recent study [56] listed reported outbreaks that could be linked to

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consumption of raw cow's milk in Europe between 1970 and 2010. These outbreaks could be attributed to the following pathogens: *Salmonella* ssp. (5 outbreaks), *Campylobacter* ssp. (18) and human pathogenic *E. coli* (13). *L. monocytogenes* is frequently detected in raw milk but no outbreaks were reported in this period in Europe, but this may (in part) be linked to the low consumption of raw milk in Europe.

Pasteurization of milk according to the standard regime (at least 72 °C for minimal 15s) reduces the probability of vegetative pathogens to survive with at least a factor of 10^6 [56]. Pasteurization does not eliminate spores of bacteria including those of *C. botulinum* and *B. cereus*. Two cases of botulism caused by commercially retailed milk are known that date back to 1920 in Washington and one in 1931 in California [33] and at least one of them could be attributed to severe temperature abuse. To prevent outgrowth of surviving microorganisms or incidental recontaminations, pasteurization and temperature control (rapid cooling, chilled storage) are Critical Control Points (CCPs) for foodborne pathogens associated with milk [53]. Historical data support that pasteurization of milk has resulted in improved public health [56]. Filtration and bactofugation techniques can be used in combination with pasteurization for further reduction of microbial counts and spore-forming bacteria, i.e. *B. cereus* and *C. botulinum,* that survive pasteurization [53].

Hazards potentially present in shelf-stable milk (UHT treated) are *C. botulinum* and toxigenic bacilli [34] but cases are rare. The applied heating regime is sufficient to ensure a 12 $\log_{10}$ reduction of *C. botulinum* [34]. In case of process failure, survival of group I spores may occur and upon prolonged storage at room temperature, surviving spores may grow out in UHT-treated milk [33]. In general, the high redox potential of milk decreases the probability of germination and outgrowth of *C. botulinum* spores. However, sterilization decreases the redox potential and it has been demonstrated that milk autoclaved for 121 °C for 30 min could indeed support growth of Group I *C. botulinum* and growth was better compared to shorter (18 min) heat-treated milk. [33]. However, outbreaks involving *C. botulinum* were not reported for UHT treated milk in the literature review by Lindstrom et al. [33] and were not found in an additional search performed in the scientific literature for the current report.

2.2.4 Cheese

The majority of the milk produced in the Netherlands is processed into cheese (see section 1.2). Approximately 10 kg milk are required to produce 1 kg of cheese. Cheeses are ready-to-eat (RTE) food products that do not undergo further treatment to reduce pathogens before consumption and have to comply with European legislation concerning RTE food products (see Table 2). Most of the RASFF notifications of pathogens in dairy products (see Figure 12) involve cheeses contaminated with predominantly *L. monocytogenes, Salmonella* ssp., STEC, and *Bacillus* ssp. According to the annual EFSA reporting on foodborne-outbreaks, cheeses are the vehicle of foodborne outbreaks in ~1-1.5% of the total number of strong-evidence foodborne outbreaks

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and include the pathogens *L. monocytogenes*, *S. aureus*, *Salmonella* ssp. or STEC ([12], see section 3.1). An exception is 2012, cheese accounts for 5.4% of the foodborne outbreaks due to a high number of outbreaks reported by one member state (see also Table 6).

Contamination from the milk
Industrially produced cheese in the Netherlands is typically manufactured from pasteurized milk. However, at farm level, cheeses may be produced from raw milk and pathogens present in the raw milk (see Table 1) form a potential source of cheese contamination ([12] and references therein). Several studies performed in both European and non-European countries detected foodborne pathogens in raw milk stored at farm bulk tanks and dairy silo's, including *L. monocytogenes*, *Salmonella* ssp., *S. aureus*, *E. coli* O157 and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) as reviewed by [12]. *S. aureus* contamination of raw milk typically occurs when cows suffer from *Staphylococcal* mastitis (see section 2.1.1) although contamination routes for *S. aureus* via recontamination by food handlers ([12] and reference therein) have been described. S. *aureus* food poisoning is caused by ingestion of food containing enterotoxins formed by this species. Contamination of raw milk with the other listed pathogens is more likely via the farm environment.

Soft and semi-soft cheeses
Soft and semi-soft cheese contain a high moisture content and allow growth of different pathogens. In particular *L. monocytogenes* forms a risk in these type of cheeses as it can grow during refrigerator storage and most reports in literature focus on this pathogenic species. An European baseline study on the presence of *L. monocytogenes* in soft and semi-soft cheeses (excluding fresh cheese) was carried out in 2010 and 2011 for in total 3452 samples [58]. Most samples (65%) were made from pasteurized milk whereas 14% of the samples involved cheese made from raw milk. The observed EU prevalence of *L. monocytogenes*–contaminated soft or semi-soft cheeses at the end of shelf-life was 0.47%, which is remarkably low. This number is based on the pooled set of samples including cheese made from pasteurized milk and raw milk, no differentiation to the two types of cheese is provided. Two out of the 3452 samples analysed exceeded 100 CFU/g. A low prevalence of *L. monocytogenes* in semi-soft cheese (excluding fresh cheeses) at retail was also reported following a survey in Sweden, with 0.4% of the 525 samples analysed testing positive [59].

Recently, data for 2013 became available in the EU summary report on zoonoses and foodborne outbreaks [60] including data on the prevalence of *L. monocytogenes* in soft and semi-soft cheese and in hard cheeses in the EU. In this dataset, differentiation towards cheese made from raw or pasteurized milk is provided. Data on the proportion of *L. monocytogenes* positive cheese samples are provided in Figure 7. In general, *L. monocytogenes* was more often detected in soft or semi-soft cheeses made from raw or mildly heat treated samples compared to those made from pasteurized milk. The proportion of samples that exceeded levels of *L. monocytogenes* above 100 CFU/g was

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very low, in line with data over previous years [58]. For soft and semi-soft cheeses, lower levels were observed in cheese made from pasteurized milk (0.1 % of 8,895 samples positive and the proportion of samples exceeding 100 CFU/g was 0.1%) compared to those made from raw or mildly heat-treated milk (4.3% of 2,538 samples with 0.6% exceeding 100 CFU/g).

It is further reported that the proportion *L. monocytogenes* positive samples was higher in soft and semi-soft cheeses made from cow's milk compared to those made from milk from other animal species [60] but exact numbers are not mentioned in the report (but may possibly be extracted from data in the appendix). The proportion of samples with more than 100 CFU/g cheese was highest for cheese made from sheep's milk compared from cheese made with milk from other animal species.
The risk for human health arises from consumption of foods containing *L. monocytogenes* exceeding the level of 100 CFU/g ([61]. Samples of soft and semi-soft cheese exceeding this level were rare, however, high morbidity, hospitalisation and mortality in vulnerable populations may raise concern for public health, even in the case of a rare event.

Specified data for the Netherlands are reported for soft and semi-soft cheese made from raw or low heated milk from cows or goats. Of the 186 samples tested for cheese made from cow's milk, 0.54% tested positive for *L. monocytogenes* whereas for soft and semi-soft cheese made from goat's milk, none of the 27 samples tested positive [60]. This data suggest that for the Dutch situation, less than 1% of the soft and semi-soft cheeses made from raw milk are positive for *L. monocytogenes*. In addition to *L. monocytogenes*, a recent study by the Netherlands Food Safety Authority (NVWA) of 250 raw milk cheeses (soft and semi-soft) in 2013 did not demonstrate detectable levels of *Salmonella* [14]. This is in line with the relatively low contamination percentages as observed in the studies at the European level.

Soft and semi-soft cheeses allow also favourable environmental conditions for growth of *S. aureus*, in particular in the first phase of the process from inoculation to salting [62]. This increase can in part be explained by water loss during curd draining. The *S. aureus* population usually remains stable during the ripening but this is dependent on ripening temperature and pH of the products [62]. For example, growth is inhibited below pH 4 for aerobic conditions and pH 4.6 for anaerobic environments, minimal pHs for enterotoxin production are pH4 and pH 5.3 for aerobic and anaerobic conditions, respectively [63]. The temperature range allowing growth of *S. aureus* varies between 7 and 48 °C depending on the strain tested [63]. The temperature limit for enterotoxin production is around 14 °C and *S. aureus* can produce enterotoxins at salt concentrations up to 10%. In general, fermentation processes reaching high numbers of lactic acid bacteria result in a decline in *S. aureus* numbers and inhibit enterotoxin formation. According to available literature data, enterotoxins may be produced when fermentation is retarded due to starter culture failure or when *S. aureus* is already present in high numbers in the milk (> 10^3-10^5 cells/ml) [63]. Existing data suggest that internal mould ripened cheeses (e.g. Gorgonzola,

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Stilton, Blue Cheese) do not readily support growth of *S. aureus* probably due to the combined inhibitory effect of the *Penicillium* ssp. and starter bacteria [63].

Hard cheeses

For hard cheeses, *L. monocytogenes* was found in 0.6% of the 1,704 samples of raw or mildly heat treated samples analysed in 2013 whereas hard cheese made from pasteurized milk was positive in 0.4% of the 8,360 samples tested by EU member states [60]. Except for one sample of hard cheese made from pasteurized milk obtained from retail, levels of *L. monocytogenes* did not exceed 100 CFU/g in any of the hard cheese samples tested (made from either raw, low heat or pasteurized milk). Remarkably, the proportion of positive samples between the detection level and 100 CFU/g in pasteurized hard cheeses is at the same level as soft and semi-soft cheese made from raw or mildly heat treated milk. This was mainly influenced by data from one member state (Germany) [60] and suggest recontamination but further details with regard to type of cheese or processing steps of the cheese (e.g. slicing) are not mentioned.

Semi hard and hard fermented cheeses are characterized by a relatively quick draining step which permits growth of *S. aureus* [62]. The risk of growth is further influenced by the cooking process of curd which is dependent on the type of cheese produced. In general, semi hard and hard cheeses may allow growth of *S. aureus* and enterotoxin production if the initial population in the milk is high (above 10^3 CFU/ml). The manufacturing processes may allow *S. aureus* multiplication from 3 to 5 log CFU/g before the pH drops to inhibitory levels according to literature data [63].

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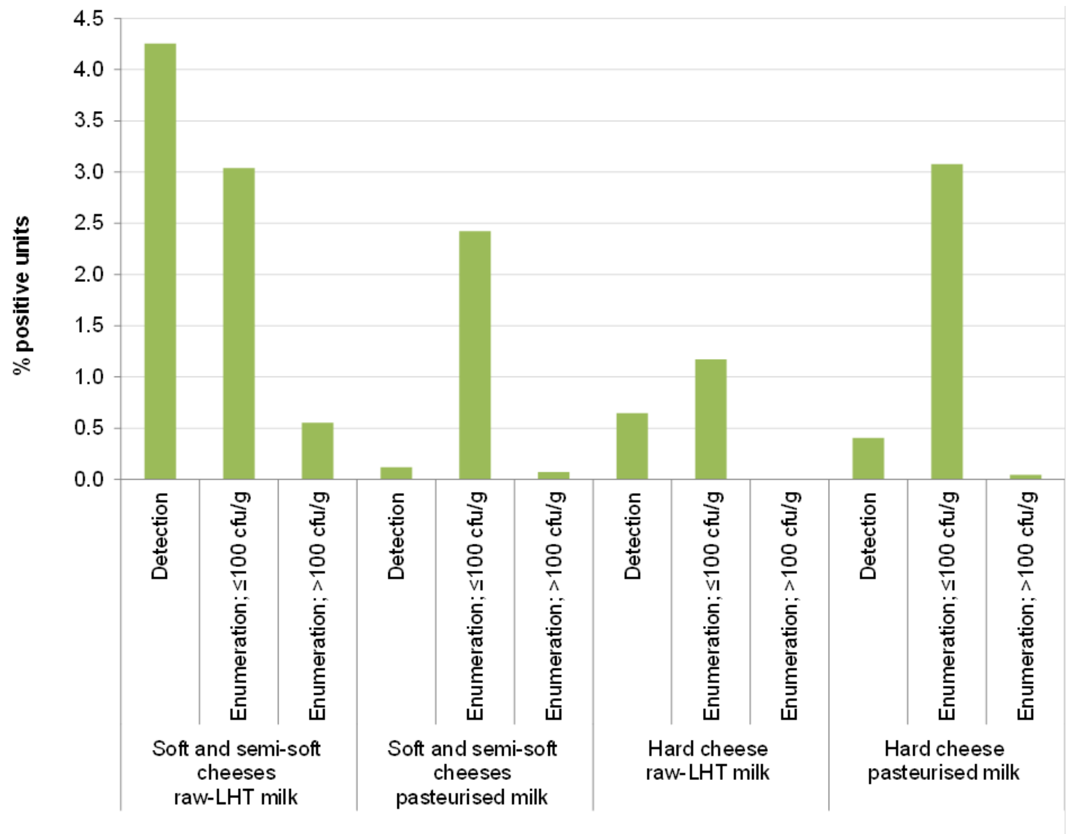

Figure 7: Proportion of *L. monocytogenes* positive units in hard cheeses and soft and semi-soft cheeses made from raw or low heat-treated milk and pasteurized milk, 2013 [60].

Two studies (in 2004 and 2005) for retail fresh, ripened and semi-hard cheese made from either raw, thermized or pasteurized milk were undertaken to assess the microbiological quality of these cheeses on the UK market [64]. Based on European microbiological criteria in place, 2% (37/1819 samples) of both raw, thermized milk cheeses and pasteurized milk cheeses (51/2618 samples) were of unsatisfactory quality. Cheese made from raw or thermized milk contained relatively high levels of *S. aureus* ($\geqslant 10^4$ CFU/g), *E. coli* ($\geqslant 10^5$ CFU/g), and/or *L. monocytogenes* ($\geqslant 10^2$ CFU/g), whereas pasteurized milk cheeses that were of unsatisfactory quality contained high levels of *S. aureus* ($\geqslant 10^3$ CFU/g) and/or *E. coli* ($\geqslant 10^3$ CFU/g) [64]. *Salmonella* was not detected in any of the 4437 samples analysed [64]. For raw or thermized milk cheeses, more samples of unripened cheese were of unsatisfactory quality (4.8%) compared to ripened (2.9%) or semi-hard cheese (1.2%). Moreover, cheese stored at a temperature above 8 °C was more frequently of poor quality (6.6% of all cheese samples) compared to those stored at 8 °C or lower (1.7%).
In the same study, also the origin of the producing country was considered. For raw and thermized milk cheeses of at least 30 samples, a higher proportion of unsatisfactory quality samples were found for the Republic of Ireland (6.7% of unsatisfactory quality) compared to France (2.4% of unsatisfactory quality) , UK (3.4%), and Switzerland (1.6%) [64]. Also retail

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cheeses originating from the Netherlands were included in this study but no samples of unsatisfactory quality were identified. It should be remarked here that the number of samples was relatively low and included only 7 raw or thermized cheese samples (0.4% of the total number of samples in this category) and 63 pasteurized cheese samples (2.4% of the total number of samples analysed).

Several studies are available reporting Shiga-toxin producing *E. coli* (STEC) in cheese and other dairy products [65]. Most studies focus on prevalence in cheese made from raw milk, a few on other dairy products including ice cream, cream, butter and yoghurt (see Appendix 2).
The prevalence of STEC in raw milk in Europe is typically between 0 and 2% over the period reviewed (2011-2012) [65]. One study in the Netherlands performed in 2005 in raw cow's milk (N=140) , bulk tank (N=13) and milk filter (N=4) samples could not detect STEC O157 [66]. Substantial diversity among occurrence of STEC in dairy products is reported varying from 0% - 27% in a selection of studies involving at least 100 samples. Samples in these studies were mainly taken from cheese made from raw milk. In one study performed in Italy, STEC O157 could not be detected in cheese made from pasteurized milk (N=60), dairy products from pasteurized cow's milk (N=811), and dairy products from pasteurized sheep's milk (N=477) [65].

Cheese processing plant
Most studies reported in literature refer to the presence of foodborne pathogens at the retail level but limited information is available on the presence of pathogens at the processing level.

Acidification, salting and/or brining ensure correct proliferation of microflora in the cheese [53]. Contamination with pathogens may occur at several stages of cheese production. Potential contamination sources reported in literature include starter cultures, drains, floor, packaging material, cheese vat, shelves, cheese cloth, curd cutting knife, brushes and coolers [67-73].
A challenge study with *L. monocytogenes* in Gouda cheese shows that this pathogen does not grow during the first 8 weeks of ripening [74]. Cheeses were artificially contaminated with three *L. monocytogenes* strains but only during curd formation there was a slight increase observed in cell count of 0.3 up to 0.8 log CFU per gram that could be attributed to concentration of the cells in the curd due to water loss [74]. During the first 8 weeks of ripening, the cell numbers did not increase by more than 0.5 log CFU per gram which was not considered significant growth.
For longer ripening times, viable counts declined with at least one log unit at week 28 of ripening.

In the cheese manufacturing process, brine solutions can be a reservoir of *L. monocytogenes* due to its survival capacity at high salinity and low temperature [75, 76]. The transfer of *L. monocytogenes* from brine to cheese was studied for Gouda cheese manufacturing [77]. Data show that, depending on the brining time, 0.1 up to 2.8 log decrease of *L. monocytogenes* occurs during brining and that concentration on the cheese surface was 100 fold lower compared to the concentration in the brine. Besides, a significant decrease in *L. monocytogenes* occurred in cheese after ripening

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times of 2 up to 12 weeks, therefore, it seems unlikely that a brine contamination will result in detectable levels (per 25 g) of *L. monocytogenes* in Gouda cheese during shelf-life [77].

Additional sources of pathogens can be biofilms formed in processing plants [78] or ingredients added to the cheese as for example herbs and spices. During the ripening process of hard and semi-hard cheeses, pathogens potentially present are reduced due to several unfavourable factors in the cheese such as reduction of the water activity (reduction in a_w), presence of salt, nitrate, low pH and temperature. Also the microbial ecosystem in cheese, especially the presence of lactic acid bacteria, can restrict growth of pathogens either by reduction of pH or by production of bacteriocins [62].

Herbs and spices
Some specialty cheese may contain spices and herbs (for example cumin, fenugreek, black pepper) which could introduce a microbiological hazard. In a joined FAO/WHO expert meeting the microbiological hazards in spices and dried aromatic herbs were reviewed (FAO/WHO report 2014). Pathogens typically associated with herbs and spices are *Salmonella* (77% of reported illnesses), and the spore forming micro-organisms *B. cereus* (20%) and *C. perfringens* (3%) should be considered the foodborne pathogens of particular concern for spices and dried aromatic herbs. Although other bacterial hazards have been reported in spices and dried aromatic herbs (for example *S. aureus*) no spice-associated outbreaks and or illnesses due to these bacteria are known. Viruses and parasites present a potential microbiological hazard but epidemiological data on these hazards or its occurrence is lacking.

2.2.5 Butter
Butter is a water-in-oil emulsion with at least 80% fat, in which microorganisms will be mainly concentrated within the aqueous phase [79, 80]. There are three main processes applied for butter production: traditional batch churning (not in use at an industrial scale anymore), continuous churning (also called the Fritz-process) and high-fat processes [79]. The latter is a niche process essentially based on hot cream processes that do not require cream to be aged but employed reseparation of the cream at temperatures varying from 52°C to 90°C [79].
In both the traditional and continuous churning processes, the pasteurization of the cream is in effect the critical control point, with all steps thereafter potentially leading to contamination of the final product. With the high-fat processes there is less potential for contamination as the heat treatment occurs later in the process just before packaging [79].
Psychrotrophic moulds may grow on the surface of the butter if the storage environment has a high humidity and permeable packaging is used [79]. The presence of coliforms is an indicator of poor hygiene and may be a potential risk of food poisoning [79]. Microbial hazards reported for butter include *Salmonella* spp., *E. coli* O157:H7, *L. monocytogenes*, and *S. aureus* [80]. However, there are relatively few reported cases of butter associated food-borne illness [80]. In The Netherlands, mainly cultured cream is made with a pH of 4.6 (expert input) which inhibits outgrowth of pathogens.

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2.2.6 Milk powder

Milk powder including the intermediates used for powdered infant formula (PIF) and dietary foods for medical purposes can be a source of pathogenic microorganisms or microbial toxins. Most information in literature can be found for powders intended for infant formula as this concerns an age group likely to experience higher disease rates. In general, identified microbiological risk apply for milk derived powders including those intended as food and feed ingredients.

Microorganisms or microbial toxins of concern with powdered infant formula, and the strength of the evidence of a causal associated between their presence in powdered infant formula and illness in infants were grouped in three categories in a FAO/WHO expert meeting in 2004 [81]:

<u>Category A organisms</u>

Microorganisms in this category are well established causes of illness in infants, have been found in infant formula, and it was convincingly (both epidemiologically and microbiologically) shown that the contaminated powder was the vehicle for infection in infants.

<u>Category B organisms</u>

Microorganisms in this category are well established causes of illness in infants and have been found in infant formula but it was not convincingly (either epidemiologically or microbiologically) shown that the contaminated powder was the vehicle for infection in infants.

<u>Category C organisms</u>

Despite causing illness in infants, they have not been identified in powdered infant formula or, although having been identified in infant formula they have not been implicated as causing illness in infants.

The table below (Table 3) shows the classification of microorganisms or microbial toxins of concern along the lines of these three categories.

Table 3: Categorization of the microorganisms or microbial toxins of concern in powdered infant formula based on the strength of evidence of a causal association between their presence in PIF and illness in infants.

Category	*Organisms included*
Category A organisms – clear evidence of causality	*Cronobacter* ssp., *Salmonella enterica*
Category B organisms – causality plausible, but not yet demonstrated	*Pantoea agglomerans* and *Escherichia vulneris* (both formerly known as *Enterobacter agglomerans), Hafnia alvei, Klebsiella pneumoniae, Citrobacter koseri, Citrobacter freundii, Klebsiella oxytoca, Enterobacter cloacae, E. coli,*

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	Serratia spp. and *Acinetobacter* spp.
Category C organisms – causality less plausible, or not yet demonstrated	*B. cereus, Clostridium difficile, Clostridium perfringens, C. botulinum, L. monocytogenes, S. aureus* and coagulase-negative *staphylococci*

Taken together, *Cronobacter* ssp.(formerly known as *Enterobacter sakazakii*) and *Salmonella* spp. [81-84] can be considered microbial hazards of most concern in PIF. Both bacteria do not grow in dry PIF, however they can survive for long periods in it [85] and may grow at a later stage, e.g. after rehydration of the powder.

Besides vegetative pathogens, spores formed by pathogenic microorganisms (*B. cereus, C. botulinum, C. perfringens*) can contaminate the dry powder products. In 2013, a New Zealand dairy company announced a whey protein concentrate produced for application in infant formula, beverages and animal feed was likely contaminated with *C. botulinum* leading to recalls of infant formula products but eventually turned out not *C. botulinum* but *C. sporogenes*. No report on consequences on public health are known but it raised a concern about the safety of these type of products with regard to *C. botulinum*. Although spores of *C. botulinum* have incidentally been found in powdered dairy products [86] they generally represent a low level of risk for illness [81, 82, 87]. Several studies have detected presence of *C. perfringens* in infant formula [88], however *C. perfringens* illness has never been associated with consumption of powdered infant formula [28, 81]. This has led WHO/FAO to classify this *Clostridium* species as "category C" organisms [81].
Evaluation of the International Commission on the Microbiological Specifications of Foods (ICMSF) of the usefulness of testing for *C. botulinum* presence in infant formula led to the recommendation that routine testing for this pathogen is not recommended and end-product testing should only serve a function in source identification during outbreaks.

European legislation (Regulation (EC) No 2073/2005) has set food safety criteria for dried infant formula and dried dietary foods for both *Salmonella* and *Cronobacter* ssp. For dried milk powder and whey powder, a food safety criterion for *Salmonella* has been set. In addition, a process hygiene criterion has been set in this regulation with regard to the presence of *Enterobacteriaceae,* and coagulase-positive *Staphylococci* in powders including milk, whey and those used in PIF and dietary foods. A *B. cereus* criterion has been set for dried infant formula and dietary food for special medical purpose intended for infants below six months of age in European legislation (Consolidated version of Regulation (EC) No. 2073/2005, see also Table 2 in this report).

The processing conditions (applied time and temperature combination) may vary over different manufacturers and product formulations but the heat load applied to produce the powder milk will provide reduction of *Cronobacter* ssp. and *Salmonella* in excess of 10 log units for example for a process temperature of 75 °C for 30 seconds [82]. Contamination of end-products is therefore

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considered to be caused by recontamination of the product after drying rather than survival of the microorganisms [82, 83].

Recontamination can be due to several contamination routes:
1. via addition of contaminated dry-mix ingredients after spray drying of the PIF [82, 83]
2. via the factory environment between spray drying of the PIF and packaging [83][56].
At formula production sites, microorganisms persist in the processing environment and contaminate the product after the drying step.

To prevent post-process contamination, strict hygiene measures are required including zoning and dry cleaning (see section 4.3 on interventions).

Contamination may also be introduced or handling conditions may allow outgrowth of low levels of contamination in the powder formula during reconstitution either at home or by caregivers in hospitals when hygienic principles are not properly followed. Guidelines for safe preparation, storage and handling of PIF have been developed [84] but will not be discussed further (not within scope of this desk study).

2.2.7 Summary microbial hazards in the processing chain for cow's milk

Pasteurization of milk eliminates vegetative human pathogens that can be present in the raw milk and incidents involved with drinking milk mainly involve non heat treated milk. Industrially processed cheese in the Netherlands starts from pasteurized milk and pathogens in final products are more likely to result from contamination from the process environment at several stages of the cheese production. Cheeses produced at the farm are more likely produced from raw milk or involve less well controlled heating processes and pathogens present in the raw milk pose a contamination risk. Soft and semi-soft cheese form a higher risk for presence of *L. monocytogenes* as composition and storage conditions may also allow growth of this pathogen. *L. monocytogenes* can be detected more frequently in cheese made from raw milk (4.3%) compared to pasteurized milk (0.1%) based on 2013 survey data within the EU. Reported data for the Netherlands suggest that less than 1% of the soft and semi-soft cheeses made from raw milk are positive for *L. monocytogenes*. Also *S. aureus* enterotoxins form a risk for this type of cheese, especially when the fermentation process is retarded or initial cell numbers in the milk are high. For hard cheese, *L. monocytogenes* levels are not likely to exceed the 100 CFU/g based on data in literature and surveys within the EU. Manufacturing conditions for hard cheese may also allow growth and enterotoxin formation by *S. aureus*. Most reported studies on STEC involve raw milk cheeses or dairy products. The prevalence of STEC varies substantially between the reported studies in literature and report values between 0 and 27%. For milk powder products, *Cronobacter* ssp.(formerly known as *Enterobacter sakazakii*) and *Salmonella* spp. can be considered microbial hazards of most concern, especially in PIF intended for infants younger than 6 months.

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2.3 Microbial hazards in the primary goat milk and sheep milk chains

As for dairy cows, clinical and subclinical udder infection (mastitis) may introduce pathogens in the milk of goats and sheep [89, 90]. Risk factors for subclinical mastitis in Dutch and American dairy goats are higher parity (the number of goats born by one goat), late lactation and low milk yield (Dutch data was collected in 2005-2007) [91]. The only significant and controllable risk factor was an udder base below the hocks according to the authors [91]. When milk is retrieved from healthy animals, contamination before processing occurs either during the milking process (e.g. machines and hands contaminated with *S. aureus* or milking machines) or during on-farm storage and transportation [92]. With respect to sheep, risk factors for increasing lactate fermenting *Clostridium* spp., i.e. butyric acid bacteria, spores in sheep milk and cheese are 1) farm-made total mixed food (compared to commercial total mixed food), 2) use of wet brewers' grains for feeding, and 3) the presence of dust in the milking parlour [93].

A recent article describes the microbial hazards associated with the consumption of raw milk from animals other than cows [94]. Samples from raw milk produced worldwide showed that goat milk and sheep milk can contain the following human pathogens: *Salmonella* spp. *Campylobacter* spp., human pathogenic *E. coli, L. monocytogenes*, *S. aureus*, *B. cereus*, *Streptococcus* spp., *C. burnetii*, *Helicobacter pylori*, *Mycobacterium* spp. or TBEV [65, 94]. However, only consumption of raw goat milk has a history of recorded human cases and outbreaks (Table 4) [65, 94]. *Brucella* has been reported as causative agent for two outbreaks in 2006 in the USA and in Spain, however, in the Netherlands brucellose was never detected in sheep or goats and, as mentioned above, the Netherlands is officially brucellose-free [14]. According to Verreas *et al.*, the major microbial risks associated with raw goat milk consumption are infection with human pathogenic *E. coli*, *Campylobacter* ssp. and TBEV [94]. As mentioned above, TBEV is until now not detected in the Netherlands [48, 49] and therefore probably not endemic in the Netherlands. A recent EFSA scientific opinion report lists microbiological hazards in milk of goats and sheep EU wide (Table 5). Table 5 also includes *T. gondii.* This parasite can infect both sheep and goats and end up in milk produced by these animals. The overall prevalence of *T. gondii* in Dutch sheep was estimated at 27.8% (25.6–29.9%), but was significantly higher in sheep over one year old, and in sheep from the central provinces (compared to the northern or southern provinces) [95].
The regional differences found may be due to a mixture of environmental contamination with *T. gondii* and farm management according to the authors [95]. Seventy-seven out of 380 lambs (sheep younger than one year) (20.4%), and 101 out of 188 sheep (older than one year) (53.7%) tested positive for *T. gondii* [95]. Information on the prevalence of *T. gondii* in Dutch goat's and/or sheep's milk was not found in the scientific literature.

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Table 4. Reported human cases and outbreaks due to raw goat milk consumption.

Pathogenic agent	*Country*	*Year*	*Comments*
E. coli O157:H7	Czech Republic	1995	4 cases of Haemolytic Uraemia Syndrome (HUS) (children), 1 case of mild diarrhoea and 4 asymptomatic cases
	Canada	2001	5 children, of which 3 with bloody diarrhoea and 2 with HUS and hospitalised
	United States	2008	2 cases of HUS (children aged 1 and 9 years) and 2 cases, of which 3 laboratory confirmed
E. coli O157:H-	Austria	2001	1 case of bloody diarrhoea (child of 9 years)
E. coli sp.	United States	2009	1 case of HUS (child)
E. coli O157:H7 and/or *C. jejuni*	United States	2010	30 cases, of which 2 cases of HUS and 47 asymptomatic cases, of which 11 cases laboratory confirmed: 6 *Campylobacter*, 2 *E. coli* O157, 3 *Campylobacter* and *E. coli* O157
C. jejuni	United States	1983	4 cases
		1991	3 cases
		2005	11 cases, of which 3 hospitalised
		2012	18 cases
Campylobacter sp.	United States	2007	60 cases (also by raw cow milk)
B. melitensis	Spain	2006	9 cases in 2 families of Moroccan immigrants
Brucella sp.	United States	2006	5 cases, of which 3 hospitalised
	Namibia		1 family and 1 case by consumption of raw goat milk, raw goat cheese and coffee with raw milk
C. burnetii	France	1992	9 cases (psychiatric facility)
Enterotoxins of haemolytic *S. aureus*	United States	1942	3 cases of vomiting and purging, of which 2 died (children aged 3 and 4 years); due to 1 goat with mastitis
Enterotoxin D of *S. aureus*	Switzerland	2008	3 cases (children)
Tick-borne encephalitis virus	Slovakia	1951	660 cases, of which 261 with encephalitis and 271 hospitalised
		1993	7 cases (family) of tick-borne encephalitis and hospitalised

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	not given	not given	2 cases of tick-borne encephalitis (elderly couple)
	Poland	1995	15 cases with neurological symptoms and hospitalized, 33 cases with flu-like symptoms and 15 asymptomatic cases
	Estonia	2005	15 cases with flu-like symptoms, 4 cases of vomiting and 8 cases with neurological symptoms and hospitalized
	Hungary	2007	154 people exposed, 31 cases of which 25 cases of tick-borne encephalitis
Toxoplasma gondii	Great Britain	1988	1 case with mononucleosis-like clinical picture (13 years of age) and 1 case with flu-like illness (15 years of age)
	United States	1973	1 case of toxoplasmosis (infant)
	United States	1978	1 case of retinochoroiditis and 9 asymptomatic cases

Table adapted from [94].

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Table 5. Main microbiological hazards identified as relevant in the EU for raw goat's and/or sheep's milk.

Brucella melitensis
Campylobacter* spp. (thermophilic)
Listeria monocytogenes
***Salmonella* spp.**
Staphylococcus aureus
Shiga toxin-producing *E. coli* (STEC)
Yersinia pseudotuberculosis
Toxoplasma gondii
Tick-borne encephalitis virus (TBEV)[1]

Table adapted from [22]. The main microbiological hazards as identified by experts are indicated in bold font type. * *Campylobacter* was identified as the leading cause of outbreaks. [1] TBEV is not endemic in the Netherlands.

2.4 Microbial hazards in processing chains for goat's and sheep's milk

Industrial processing of goat and sheep milk is essentially not different from that of cow milk and hazards associated with products based on milk of these producing animals is comparable to those listed for cow's milk processing. However, products originating from sheep's and to a lesser extend for goat's milk more likely result from small scale processing as for example at the dairy farm. Dairy products made from milk of those producing animals may be more likely to involve raw milk and could involve more traditional processes at non-optimal process control.

In 2014, a study was published on transmission of *Toxoplasma gondii* via cheese made from goat milk [96]. In this study, 8 goats were orally inoculated with oocysts of *T. gondii*. Milk samples were collected and assayed for presence of the parasite in a bioassay using mice and cats. Using the mouse bioassay, *T. gondii* could be detected in milk obtained from all eight goats. Fresh cheese was made from milk using a cold enzyme treatment of unpasteurized milk collected from *T. gondii* positive goats and fed to cats. One out of four cats, shed oocysts of *T. gondii* from day 7 up to day 11. This shows that goat milk and fresh cheese made of unpasteurized milk in a cold enzyme process can be a source of *T. gondii*.

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3 Overview of in literature reported foodborne outbreaks, disease incidents and disease burden and attribution to dairy products

In this chapter, an overview is presented of data described in literature for foodborne outbreaks reported in the EU in the period 2010-2013, reported disease incidents in the Netherlands, estimates of disease incidents and disease burden (Disability-Adjusted Life Years (DALYs) and attribution to dairy products as food category. Moreover, an overview is made of notifications of pathogens in milk and milk products that are imported and exported in the EU (RASFF).

The estimates presented in this chapter on incidences, disease burden and attribution to food categories based on estimations made by Dutch experts in the period 2007-2008 for type of food pathway [97](personal communication). No information is available about the arguments of the experts for estimated attributions.

3.1 Food-borne outbreaks reported in the EU

The European Food Safety Authority (EFSA) publishes annual reports on the number of reported food-borne outbreaks caused by zoonoses in Europe. In the EFSA report, food-borne outbreaks are defined as “incidents of two or more human cases of the same disease or infection in which the cases are linked or are probably linked to the same food vehicle. Situations in which the observed human cases exceed the expected number of cases and where the same food source is suspected are also indicative of a food-borne outbreak”.

In 2013, 839 strong-evidence food-borne outbreaks were reported within the EU. Of these 839 outbreaks, cheese was the vehicle in 1.3% of the outbreaks, other dairy products involved milk (1.3% of the total number of outbreaks) and dairy products other than cheese and milk (0.8%). *Salmonella* is a causative agent most frequently reported for foodborne outbreaks (22.5% of the total amount of 5,196 foodborne outbreaks with either strong or weak evidence in that year). Of the 314 strong-evidence *Salmonella* outbreaks in 2013 (Figure 8), eggs and egg products were most frequently reported as vehicle (45% of the total number of outbreaks). Reported outbreaks for *Salmonella* with strong-evidence vary between 283 and 347 cases per year in the period between 2010-2013 in the EU (Table 6). Of the reported strong-evidence *Salmonella* outbreaks, cheese was the vehicle in ≤ 1.1% of the cases in the period 2010-2013. An exception is 2012 where a relatively high number of outbreaks was reported for cheese, all resulting from France as member state (see also below). No additional information was provided on the type of cheese implicated and on the contributing factors.

Dairy products other than cheese accounted for 0-0.6% (milk, including raw milk) and 0.6-2.1% (dairy products other than cheese), relative to the total amount of strong-evidence *Salmonella* outbreaks in the period 2010-2013 in the EU. No outbreaks for the Netherlands were reported for 2013.

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Human listeriosis is a relatively rare but serious zoonotic disease, with high morbidity, hospitalisation and mortality rates in vulnerable populations. The number of *Listeria* outbreaks is relatively low, but the attribution to cheese is relatively high. In 2013, seven strong-evidence food-borne outbreaks caused by *L. monocytogenes* were reported, of which one outbreak was related to cheese. No information is given about details of this cheese outbreak. In 2012 and 2011 also one *L. monocytogenes* outbreak was reported for cheese as food category.
No *L. monocytogenes* outbreaks were reported for dairy categories other than cheese in the period 2010-2013.

In 2013, 32 strong-evidence *Campylobacter* ssp. outbreaks were reported in the EU, one outbreak was reported for the Netherlands. Of the *Campylobacter* outbreaks, between 9% (2013) up to 20% (2012) could be attributed to milk. It is not reported whether this involved raw or heat treated milk but it can be expected that raw milk was involved in the majority of the outbreaks.

In 2013, nine member states(MS) tested 860 raw milk samples from bovine animals intended for direct human consumption and 2.3 % were VTEC-positive and included serotypes O157, O103, O26, O145 and O111 [60]. VTEC was also tested in non-raw milk and non-raw dairy by eight MS products such as cheeses, and, respectively 5.0 % and 0.2 % were positive for VTEC and for VTEC O157.

Staphylococcus enterotoxins in cheese were involved in 6.4% (2013) up to 20% (2012) of the outbreaks for all food categories. It is not specified what type of cheese was involved and whether the cheese was made from raw or pasteurized milk.

Outbreaks due to *C. botulinum* contaminated dairy products are rare compared to incidents linked to vegetables, meat and fish products [28] [33]. Nevertheless, at least 20 human botulism outbreaks have been reported due to milk or milk products between 1912 and 2010 [33].
Apart from three milk incidents, on hazelnut yogurt and one commercial infant formula, the majority includes outbreaks related to soft cheeses or cheese products. Hard cheese generally are not a vehicle for botulism as the relatively low pH and a_w prevent growth of this species [33].
The majority of the dairy related outbreaks were caused by processing or handling failure such as insufficient heating and/or post process contamination [33]. Assuming that most of outbreaks involved proteolytic *C. botulinum*, an abused storage temperature of 10-12 °C or higher is most likely a leading cause for most of the outbreaks [33]. In the period 2010-2013, no incidences have been reported within Europe for outbreaks involving *C. botulinum* in cheese or other dairy products.

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Table 6. Reported food-borne outbreaks with strong evidence in the EU in 2010-2013. Sources: [60, 98-100].

Pathogen (total number of outbreaks reported for specific pathogen)	*Year*	*Cheese*	*Dairy products other than cheese*	*(raw) Milk*
Salmonella (314)	2013	0.3% (n=1)	1.3% (n=4)	0.6% (n=2)
(347)	2012	7.8% (n=27)	0.6% (n=2)	-
(283)	2011	1.1% (n=3)	2.1% (n=6)	-
(341)	2010	0.9% (n=3)	0.6% (n=2)	0.3% (n=1)
Campylobacter (32)	2013	-	-	9.4% (n=3)
(25)	2012	-	4% (n=1)	20% (n=5)
(37)	2011	2.7% (n=1)	5.4% (n=2)	13.5% (n=5)
(27)	2010	3.7% (n=1)	-	18.5% (n=5)
E. coli (12)	2013	17% (n=2)	-	-
(12)	2012	-	-	8.3% (raw)
(14)	2011	-	7.1% (n=1)	-
(2) one household outbreak	2010	50% (raw milk cheese) (n=1)	-	-
L. monocytogenes (7)	2013	14% (n=1)	-	-
(5)	2012	20% (n=1)	-	-
(3) one household outbreak	2011	33% (n=1)	-	-
(3)	2010	-	-	-
Brucella (0)	2013	-	-	-
(1) Household outbreak	2012	100% (n=1)	-	-
(0)	2011	-	-	-
(1)	2010	100% (n=1)	-	-
Bacillus toxins (54)	2013	-	-	1.9% (n=1)
(38)	2012	5.3% (n=2)	-	-
(47)	2011	-	-	2.1% (n=1)
(26)	2010	-	-	3.8% (n=1)
Staphylococcus enterotoxins (94)	2013	6.4% (n=6)	2.1% (n=2)	3.2% (n=3)
(35)	2012	20% (n=7)	-	2.9% (n=1)
(35)	2011	8.6% (n=3)	5.7%	-
(38)	2010	18.4% (n=7)	-	-
Calicivirus * (76)	2013	-	-	-
(97)	2012	2.1% (n=2)	-	-
(87)	2011	-	1.1% (n=1)	-
(84)	2010	-	-	-

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* Including norovirus. Waterborne outbreaks not included. Listed are those causative agents for which a direct link to dairy products is known. It cannot be excluded that in the mixed food category dairy ingredients are involved.

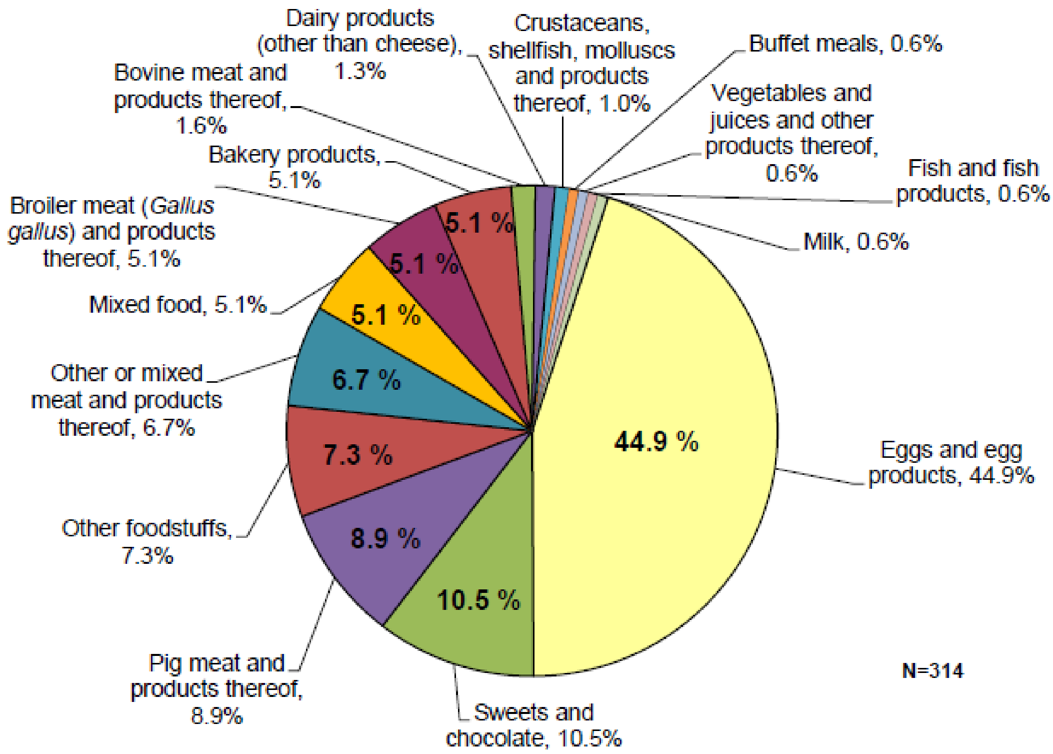

Figure 8. Distribution of food vehicles in strong-evidence outbreaks caused by *Salmonella* in the EU, 2013. Figure adopted from [60].

3.2 Reported disease incidences in the Netherlands

Annually, the Dutch National Institute for Public Health and Environment (RIVM) publishes an overview of the incidence of zoonotic diseases in the Netherlands. According to surveys in 2002-2003 and 2010-2012, *Campylobacter*-isolates of Dutch patients, were mainly transmitted via poultry meat (~60-70%) and cattle meat (~20-25%) [101]. These numbers encompass all transmission routes including contact with infected animals and are not limited to consumption of infected food. Consumption of chicken meat can explain 28% of the human *Campylobacter* infections in the Netherlands [14]. Raw milk can be contaminated with *Campylobacter* ssp. and was reported as the leading cause of outbreaks upon consumption in the EU between 2007 and 2012 [22]. In the Netherlands, two outbreaks of campylobacteriosis associated with the consumption of raw cow's milk have been reported [102]. For both outbreaks (2005 and 2007) epidemiological and bacteriological findings support raw cow's milk, consumed at a dairy farm, as the vehicle.

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In 2010, an outbreak was reported of *Campylobacter* ssp. in the Netherlands associated with raw goat's milk. [22].

In 2013, nine patients were reported with Q-fever. The incidence level (0,11 patients per 100.000 inhabitants) was for the first time comparable with that of years before the Q-fever epidemic (2007-2010) [14]. According to experts, this indicates that veterinary precautions established in 2011 in the goat and sheep sectors are effective and that there are no other transmission routes for Q-fever in the Netherlands [14].

Approximately 85% of the *Salmonella*-infections in the Netherlands are caused by the consumption of *Salmonella*-contaminated food, including undercooked eggs, raw meat products and incidentally raw, minimally-processed vegetables and/or fruit [14]. Incidentally, dairy products are reported as vehicle for *Salmonella*-outbreaks in the Netherlands (Figure 9).
Two outbreaks of *Salmonella* associated with the consumption of dairy products occurred in 2006 involving hard cheese made from raw milk [103] and in 2008 involving cream cheese [104] (Figure 9). Hard cheese made from unpasteurized milk is not a common source of *Salmonella* infections, although occasionally outbreaks caused by consumption of *Salmonella* contaminated cheddar cheese have been described [104].The hard cheese was aged for 9 months and quantitative analysis of two whole cheeses showed that the contamination could be as low as 4.3 *Salmonella* per kg [104].The reported outbreaks shows that although rarely involved in outbreaks, hard cheeses made from unpasteurized milk cannot be excluded as vehicle for *Salmonella* infections.

Shiga-toxin-producing *E. coli* (STEC) O157:H7-infections and STEC non-O157:H7-infections are mainly associated with raw and/or insufficiently heated meat products and therefore surveys focus on this food category [14]. Overall STEC O157 incidence in 2013 was 5.4 illnesses per 1 million inhabitants [14] which is an increase over previous year's most likely caused by the use of PCR based detection technology. It is not mentioned whether this included dairy products as potential source. Although raw milk consumption and consumption of cheeses, especially those made from unpasteurized, cow's and goat's milk have been associated with several STEC outbreaks within Europe [65](Table 6), no outbreaks involving milk and dairy products have been reported for the Netherlands.

Data for foodborne outbreaks *S. aureus* reported for the Netherlands between 1993 and 1998 published in the WHO surveillance programme in Europe show that this pathogen was the causative agent of 0.9% of the total number of foodborne disease outbreaks in that period [63]. Milk products (not further specified) were involved in 5% of the staphylococcal foodborne outbreaks, data for cheese as vehicle were not available.

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No outbreaks for *L. monocytogenes* involving dairy products as vehicle were found in the literature for the Netherlands.

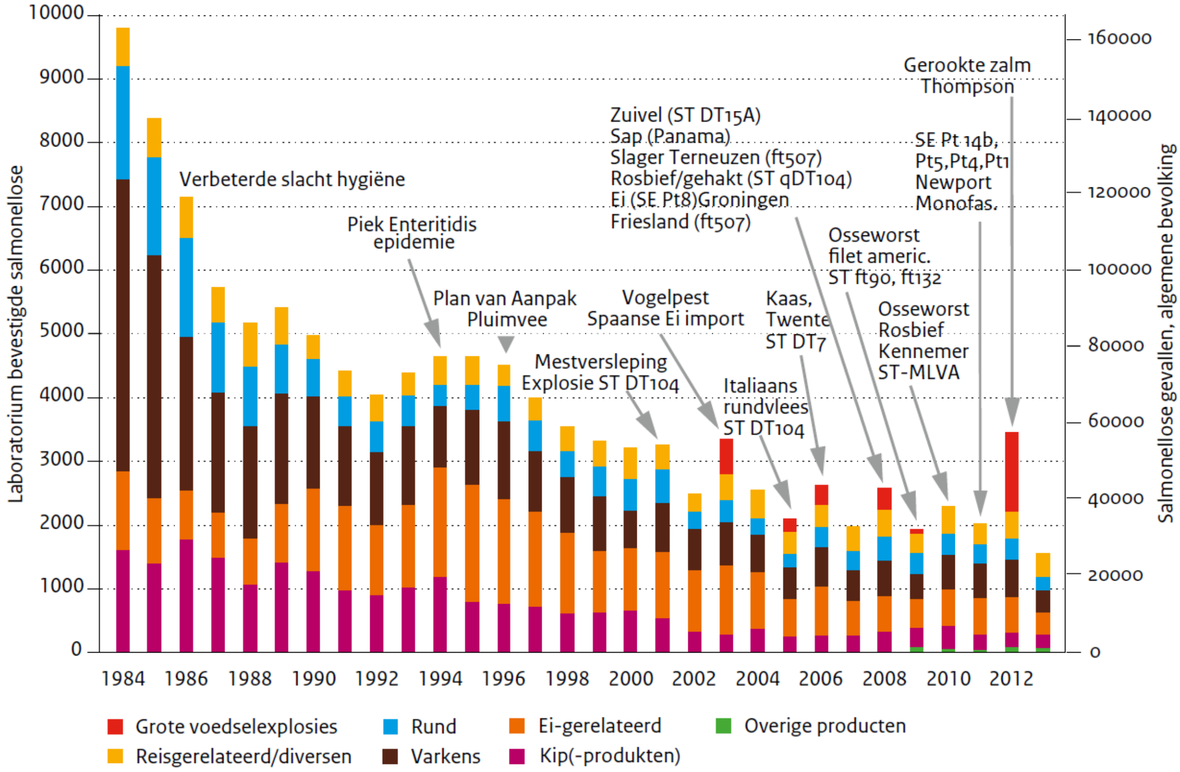

Figure 9. Attribution of laboratory-confirmed (left y-as) and estimated (right y-as) human Salmonellosis caused by travelling (or unknown), cattle, pigs, eggs, chicken and/or their products. Extensive explosions in 2003, 2005, 2006, 2008, 2009 and 2012 that are not representative for the *Salmonella*-status of the Dutch livestock are depicted in red. Figure from adopted from [14].

As mentioned in section 2.1.1, the Netherlands is officially brucellosis free and since 1997, no *Brucella*-contaminated cows are detected [14]. In the Netherlands, brucellosis in humans is rarely reported. Yearly, between 1 to 6 patients infected with *Brucella* ssp. were reported of which infections were mainly acquired in foreign countries [14]. There were no reported BSE (bovine spongiform encephalopathy) cases in the Netherlands in 2013 and 2012 [14].

3.3 Estimated disease burden of pathogens and attribution to dairy products

Table 7 presents an overview of the estimated total disease burden and the attribution to dairy products in the period 2010-2013 [105-108]. For the interpretation of this numbers it should be realised that attributions to food categories are based on expert opinions. For certain pathogens, relatively few experts have been involved and individual opinions have a relatively large impact on the outcome. For example, for the transmission of *T. gondii* , 3 experts contributed with attributions varying from 0-14% and on average a 5% attribution to dairy products (see Havelaar et al. [88]. This implies that 2 out of the 3 experts estimated a close to zero attribution to dairy

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products and one expert 14%. This may have large impact if the total disease burden of a specific pathogen is high (DALY is product of number of cases and severity and mortality). This results in that even a very small estimated fraction attribution of a specific product group still can result in a high estimate, since for that pathogen the overall disease burden is high.

In 2013, 54,600 disease incidences are estimated for dairy products as food category. This corresponds to 8.1% of the overall food-related disease incidences. Most disease incidents for dairy products were attributed to *S. aureus* toxins (68%), *C. perfringens* toxins (11%), and *Campylobacter* (7%) by the consulted experts (Figure 10). According to a French study on outbreaks involving milk and milk products in France between 1988-1997, *S. aureus* ranks first with 25% of the total food related outbreaks, followed by *Salmonella* (1.8%) and *C. perfringens* (1.1%) [109]. This shows that estimates for *S. aureus* and *C. perfringens* in the Netherlands are higher than reported cases in the French surveillance system. It should be remarked here that notifications in France are likely under-reported, hospitalisations are included but milder disease is possibly not included [109]. Overall, milk and milk products contribute to ~8% of the total number of disease incidences involving food as vehicle according to estimates for the Netherlands (Table 7), this is slightly higher than the 2-6% according to published reviews for other countries [109].

Table 7: Overall disease incidence in the Netherlands in the period 2009-2013 (based on 14 pathogens that can be transmitted via food) and attribution to dairy food category as estimated by the disease model of RIVM based on attribution data from an expert survey. Sources: [105-108].

Disease incidence	2010	2011	2012	2013
Total for the Netherlands	1,993,000	1,749,000	1,724,00	1,684,000
Food related	725,000	689,000	703,300	671,500
Dairy products	57,500	55,800	55,400	54,600
S. aureus toxin in dairy	38,100	37,400	37,500	37,300
C. perfringens toxin in dairy	6,200	6,300	6,300	6,250
Campylobacter in dairy	4,000	4,000	3,800	3,700
Norovirus in dairy	2,700	2,350	2,350	2,200
Salmonella in dairy	1,600	1,300	1,300	1,000
Rotavirus in dairy	1153	665	569	658
Gardia lamblia in dairy	745	643	572	487
C. parvum in dairy	304	311	306	306
STEC O157 in dairy	60	63	63	210
T. gondii in dairy	22	20	20	20
L. monocytogenes in dairy	13	15	13	14

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Below the estimated attribution of incidence by pathogen to dairy products in 2010 through 2013 is visualised (Figure 10). Over these years, the relative contribution of different pathogens remained constant.

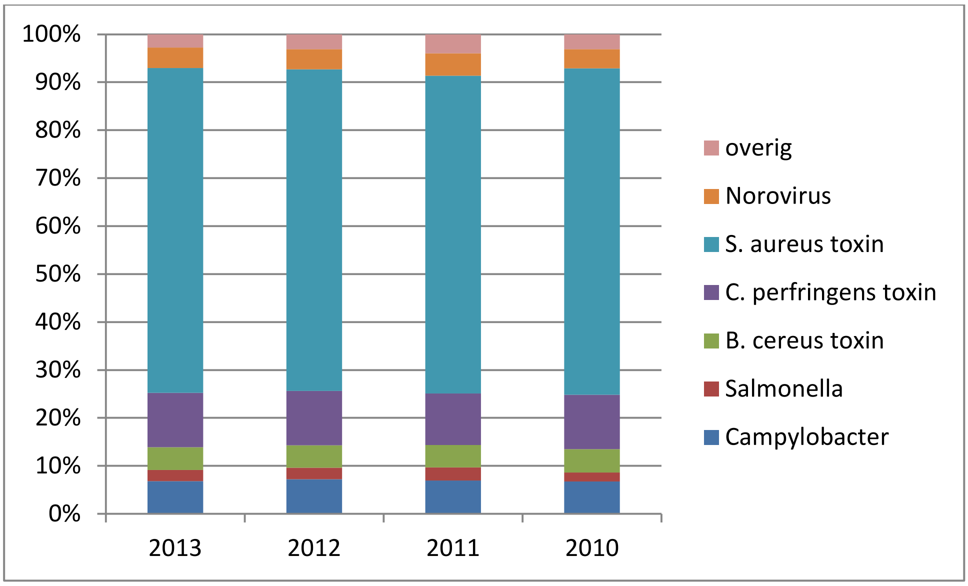

Figure 10: attribution of incidence by pathogen to dairy products in 2010 through 2013. Sources: [105-108].

3.4 Disability-adjusted life years (DALYs)

A different way to quantify the disease burden is by Disability-Adjusted Life Years (DALYs). DALYs are so called years of life corrected for disability, the number of healthy years of life lost because of people living in disease states and death. This number includes the time lived with disability and the time lost due to premature mortality [84].

Expressed in DALY's, *Campylobacter* (127 years in 2013) , followed by *S. aureus* toxin (98 years) and *T. gondii* (89 years) have the highest contribution to the disease burden attributed to the dairy food group (Table 8). Expressed in mortality (Figure 11), *Campylobacter* ranks first (34% for the dairy food group in 2013) but is followed by *L. monocytogenes* (20%) which reflects that although incidence of this pathogen in the dairy food category is relatively low, the impact on disease burden is high. *Salmonella* and *S. aureus* toxin follow *L. monocytogenes* in ranking with 16% and 15%, respectively, of the total number of deaths for the dairy food category.

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Table 8. Disease burden in the Netherlands in disability adjusted life years (DALYs, undiscounted) based on 14 pathogens that can be transmitted via food and attribution to food, dairy products and pathogens as estimated by the disease model of RIVM based on attribution data from an expert survey. Sources: [105-108].

DALYs	2010	2011	2012	2013
Total for the Netherlands[1]	14,900	13,940	13,961	13,196
Food related	6,420	6,231	6,543	5,852
Dairy products	473	449	427	411
Campylobacter in dairy	137	136	130	127
S. aureus toxin in dairy	100	98	98	98
T. gondii in dairy	101	92	90	89
Salmonella in dairy	54	47	46	40
L. monocytogenes in dairy	27	34	22	16
C. perfringens toxin in dairy	20	20	20	20
Norovirus in dairy	6	6	6	6
STEC O157	4	4	4	5
Rotavirus in dairy	6	4	3	4
Gardia lamblia in dairy	1	1	1	0
C. parvum in dairy	1	1	1	0

[1] Total includes following pathways: food, environment, human, animal and travel.

In comparison to other product groups, the dairy product category ranks fifth both based on disease incidents and on DALYs (Table 9). Data over previous year's show a similar pattern (Appendix 3).

Expressed in DALYs *S. aureus* toxin appears second after *Campylobacter* for disease incidence and disease burden (DALYs). In addition, EFSA reporting shows that *S. aureu*s toxins in cheese account for 6-20% of the outbreaks (Table 6). *S. aureus* toxins are not reported in the RASFF notifications but this may be explained by the fact that testing for this toxin is not performed on a routine basis. Data extracted from reported outbreaks in France in the period 1992-1997 also show *S. aureus* as the most frequent reported pathogen associated with cheese from raw or unspecified milk [109]. Of the total 53 reported outbreaks for cheese, *S. aureus* was involved in 24 raw milk cheese and 23 cheeses of unspecified milk.

T. gondii ranks third expressed in DALYs which is relatively high but here should be mentioned again that attributions to food categories are based on expert opinions. For the transmission of *T. gondii* only three experts were consulted which differed largely in their estimates (see above) . The consequences of infection are quite severe and consequently affects the disease burden in terms of DALYs. However, *T. gondii* is more frequently associated with consumption of undercooked meat or direct contact with cats. It can be transmitted to the milk but was not

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considered an important risk factor for consumption of raw drinking milk by an expert panel [22] and no outbreaks involving this parasite were reported in the EU in 2010-2013. Moreover, also no information on the prevalence of *T. gondii* in Dutch (raw) cow's milk was available in the consulted literature to support this (see also 2.1.6).
It is not possible to trace back arguments of consulted experts for attributions (personal communication) and it should be remarked that these values are based on estimates made in 2007-2008 [97].

For norovirus, the disease incidence for dairy was with 2,200 in 2013 relatively high whereas the disease burden ranks relatively low. The total disease incidence for norovirus is with 110,595 high. Five experts were consulted who estimated between 0-5% attribution for dairy with on average 2%. This implies that at least a few experts estimated a close to zero attribution to dairy products. The average of 2% then still accounts for over 2,200 incidents. No data on prevalence are available in the scientific literature and it appears more likely that norovirus incidents associated with dairy arise from contamination introduced during preparation of food for example at food services or kitchens.

For *C. perfringens* toxins, it remains unspecified which dairy products were considered high in estimation of disease burden in the Netherlands. It should be remarked that the number of experts consulted was only four with estimates between 0-21% and on average 4%. *C. perfringens* can be present at relatively high numbers in faeces of Dutch dairy cattle (see 2.13), however, *C. perfringens* is considered not transmittable via milk from producing animals. This pathogen was found in 2 out of 13 raw milk samples analysed in an Australian study [110]. In the UK, a large *C. perfringens* associated outbreak occurred in 1981 affecting 77 school children due to consumption of contaminated milk shakes [28]. More recently, the presence of *C. perfringens* was detected in infant formula (PIF) [88] but illness has never been attributed to consumption of PIF [28]. The lack of disease incidents for PIF also resulted in a "category C" classification by WHO/FAO for this species in PIF (see also section 2.2.6) [81].

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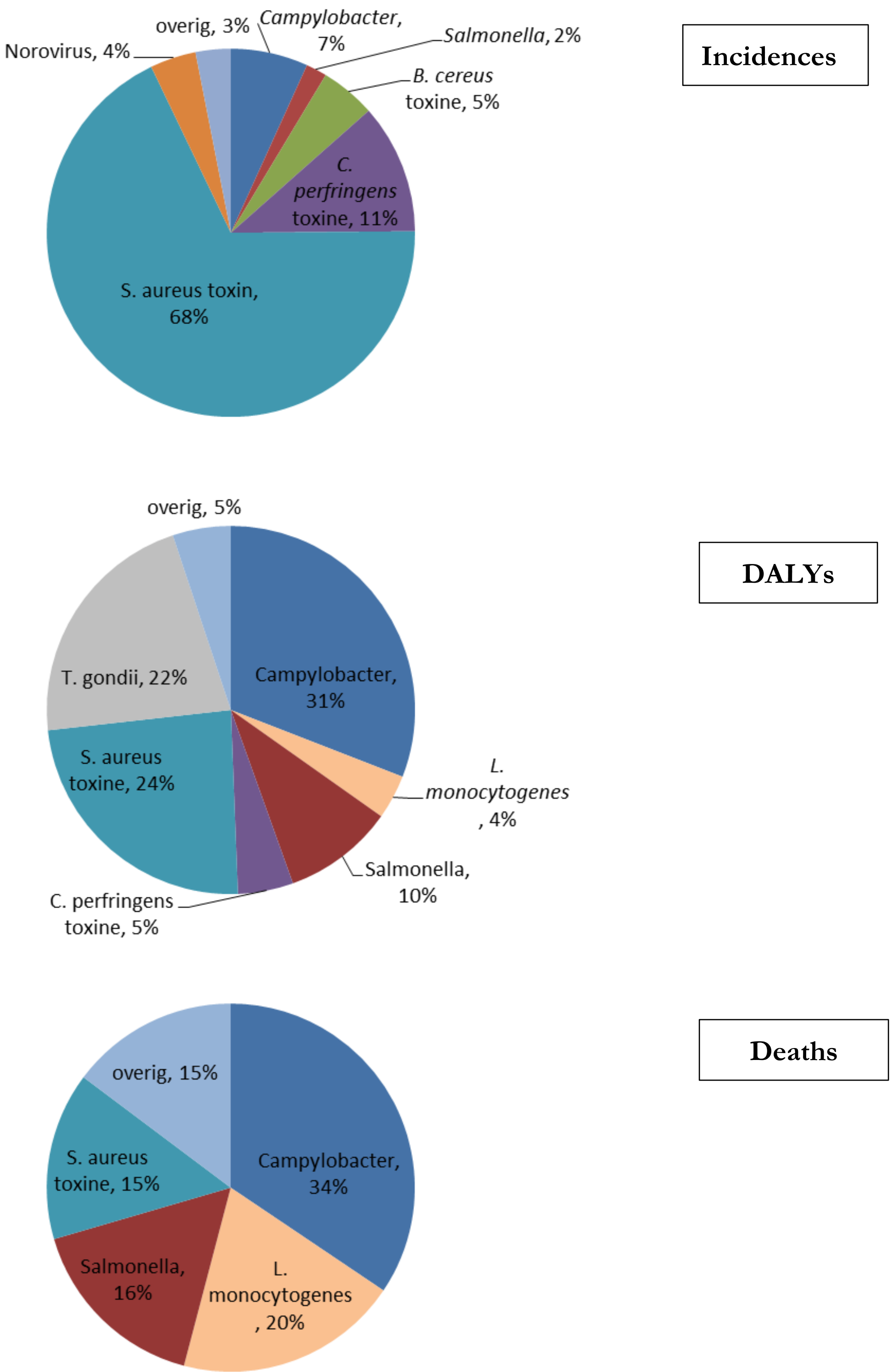

Figure 11: Attribution of the disease incidents (panel A, n= ~55,000), DALYs (panel B, n= 411) and deaths (panel C, n= ~6) for the dairy food group over disease causing pathogen.
Data represent estimated values based on expert survey attribution data [97].
Data presented in the figure are for 2013 [105].

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Table 9. Comparison of estimated disease incidents, DALYs (discounted) and deaths attributed to food groups. Presented data are based on numbers for 2013 [105].

Product group	Incidence	DALYs	death
Other foods	121.300	450	5
Beef and lamb	105.900	910	8
Poultry	59.800	1.060	16
Fish and shellfish	55.400	370	7
Dairy	**54.900**	**410**	**6**
Pork	44.700	1.250	9
Grains	41.100	180	3
Produce	40.000	360	6
Egg and egg products	21.200	225	5
Beverages	15.900	90	2
Total	560.200	5.305	67

3.5 Rapid Alert System for Food and Feed (RASFF)

Notifications of pathogenic microorganisms in milk and milk products that are imported or exported within EU member countries were retrieved from the Rapid Alert System for Food and Feed (RASFF) portal. In total, 629 notifications of pathogenic microorganisms in food products were retrieved from the RASFF portal for the period 2009-2014. The contribution of milk and milk products as product category to this number was 122 notifications (19%). Most notifications (101) involved cheeses, 5 consumption milk, 6 other milk products and for 10 notifications no product specification was provided (Figure 12 and Table A in Appendix 4). With regard to the milk producing animal, 9 notifications could be attributed specifically to products derived from goat's milk, 7 from sheep milk, 6 from cow's milk and 2 from buffalo's milk. The remaining notifications lacked a specificaton of the producing animal but most likely involved products derived from cow's milk.

Two notifications involved raw milk and 30 notifications were related to raw milk cheeses. From these products containing raw milk, 2 originated from goat's milk and 4 from sheep's milk. Looking at the specific pathogens, most notifications involved *L. monocytogenes* (72 notification), followed by *Salmonella* spp. (18), pathogenic *E. coli* (14), *Pseudomonas* spp.(6), *Bacillus* spp.(5), one *Brucella* in raw cow's milk from France, one *Cronobacter sakazakii* (together with *Salmonella*) in dried milk formula from Belgium and one suspicion of *Mycobacterium tuberculosis* in raw milk from Germany.

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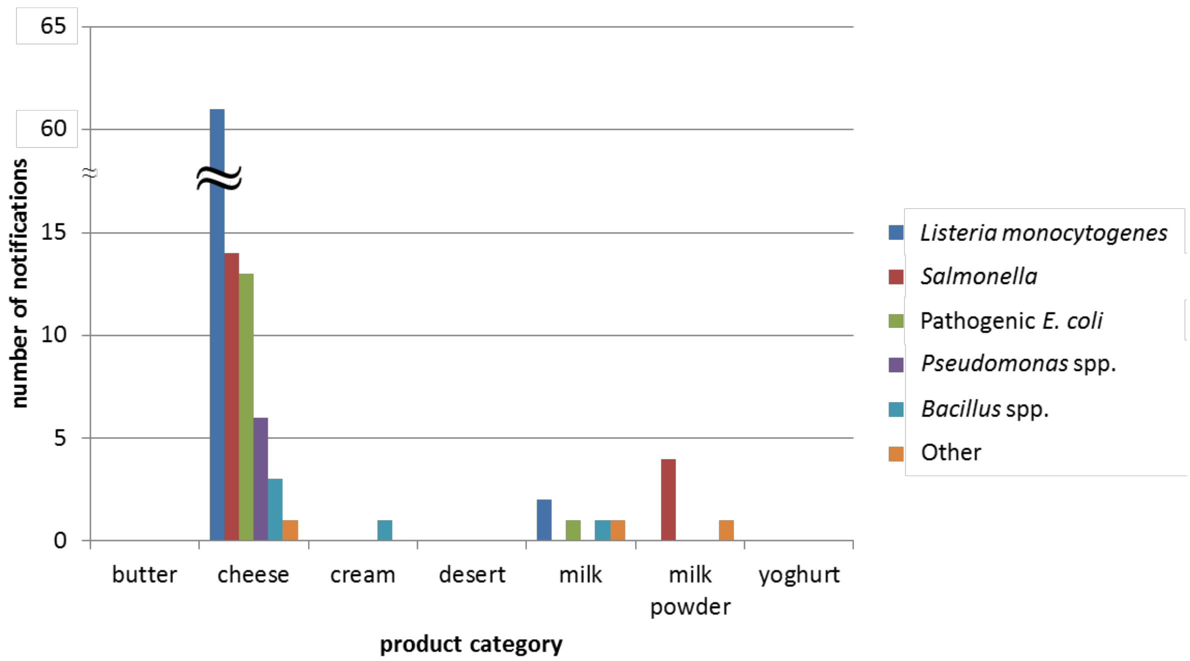

Figure 12. Number of RASFF notifications of pathogens in various dairy products retrieved over the period 2009 to 2014 within the EU.

3.6 Attribution disease burden to dairy product categories

Raw Milk

There is a lack of epidemiological data to assess the burden of disease linked to the consumption of raw milk. Published quantitative microbiological risk assessment (QMRA) models are published from Australia, New Zealand, USA and Italy, but cannot be translated to the European situation [22]. Between 2007 and 2012, 27 outbreaks were reported involving consumption of raw drinking milk in the EU. Four of these 27 outbreaks could be attributed to the consumption of raw goat's milk, the remaining 23 cases were due to consumption of raw cow's milk. *B. melitensis*, *Campylobacter* spp., *M. bovis*, *Salmonella* spp., Shigatoxin-producing *E. coli* (STEC) and tick-born encephalitis virus (TBEV) were identified as main microbiological hazards in raw drinking milk (see also section 3.1) [22]. This selection was based on the criterion that the incidence was at least 10 per 100,000 population and/or the severity of the illness was at least 0.1% of deaths in confirmed cases. *Campylobacter* spp., *Salmonella* spp., and STEC were considered more widely distributed in the EU with *Campylobacter* spp. resulting in most outbreaks [22]. No outbreaks involving *L. monocytogenes* in raw milk were reported in the period 2007-2012 although *L. monocytogenes* is detected in 2.2 -10.2 % of the raw cow's milk in Europe [56][1].

[1] This may in part be explained by the relatively long incubation time until onset of disease and the fact that only outbreaks are included and not individual cases with disease attribution

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Cheese
According to the EU annual monitoring report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the EU, in total 347 strong-evidence *Salmonella* outbreaks occurred in the EU in 2012 (Table 6) and cheese was the vehicle for 7.8% of these outbreaks. This is a remarkably high number given the fact that percentages are between 0.32% and 1.1% over the period 2010-2013 (Tables 6). The in 2012 reported outbreaks caused by cheese all originated from one member state (France). No specific information is available on the type of cheese implicated. Based on an analysis of a *Salmonella* outbreaks associated with the consumption of mozzarella cheese made of pasteurized milk in the USA, Hedberg et al. [111] reported that the dose causing infectious of the outbreak was relatively low (most probable numbers of *Salmonella* organisms in these samples were 0.36/100 g and 4.3/100 g) and indicates that the low levels likely to cause disease may be difficult to detect given the fact that the sample size for *Salmonella* ssp. detection analyses is 25 g. Also in The Netherlands, a *Salmonella* outbreak caused by hard chees made of unpasteurized milk was reported for 2006 that involved very low numbers of this pathogen [104].
Outbreaks reported for *L. monocytogenes* in the EU recorded by the member states over the years are typically one or no outbreaks. Nevertheless, cheese was the main implicated vehicle reported in 2009 and 2011 ([58]). The outbreak in 2009 involved cheese and the one in 2011 involved domestically produced cheese. However, reported outbreaks over earlier years (1983-2005) published by [12] indicated that *L. monocytogenes* is involved in different types of mainly soft cheese.

Butter
In 1999, there was an outbreak caused by *L. monocytogenes* reported in Finland [112]. The strain could be traced back to butter delivered initially to a tertiary care hospital and 25 cases were identified. Recall of the product ended the outbreak [112]. In the period between 2010-2013 (Table 6) no outbreaks associated with the consumption of butter were reported.

Milk powder
With respect to powdered infant formula (PIF), two outbreaks were reported involving *Cronobacter* spp. in 2004; one in New Zealand and one in France [82]. The outbreak in New Zealand was relatively small, one premature infant died, further investigation in the neonatal intensive care unit identified four more babies that had been colonized with the organism.
The outbreak in France in the same year involved nine cases with two deaths. All infants were premature and below 2000 gram birth weight with the exception of one infant with colitis that weighted 3250 gram and was born at week 37 [82].

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4 Potential interventions to reduce the microbial hazards in the dairy chain

This section describes interventions reported in the scientific literature to reduce the microbial hazards in the dairy chain. The focus will be on the cow's milk chain; When available, information about the goat and sheep dairy sectors is included.

4.1 Potential interventions in the primary cow milk dairy chain

As a basis, good dairy farming practice is required for optimal milk quality and this includes taking care of animal health, proper milking equipment, animal feeding and water, animal welfare, and farm environment. Additional interventions with impact on microbiological hazards reported in literature are described below.

4.1.1 Animal factors impacting on the microbiological safety of milk

Since subclinical and clinical mastitis is recognized by experts as a significant hazard for the presence of specifically *S. aureus* , control of mastitis should be considered a major intervention. Based on the scientific opinion of an EFSA expert panel, interventions at the level of farming systems and dairy cow welfare to reduce the prevalence of mastitis include: 1) treatment of clinical and subclinical disease, 2) dry cow therapy, 3) identification and elimination of carrier cows, 4) prevention of transmission of infection from cow to cow or through the environment, and 5) improvement of the immune system by minimising stress factors and by a controlled and nutritionally-balanced feed intake [113]. Dairy herd health management aspects identified as particularly relevant are:

- Establishing of, and adhering to, effective mastitis prevention programmes, including through maintenance of animal cleanliness (particularly the hide and tail);
- Avoiding lactating cows from coming into contact with other animal species that are potential carriers of pathogens such as *Salmonella* and *Campylobacter* (e.g. pigs and poultry);
- Separation of cows with mastitis, enteritis and metritis from healthy cows and discarding their milk during and after related treatments;
- Assuring that the feed contains enough roughage to reduce faecal contamination of animals/environment and the occurrence of environment-mediated mastitis; in contrast, deficient roughage rations in combination with high (>18%) crude protein content are known to reduce faeces consistency ("firmness") making faeces more spreadable [38].

In addition, pain management should be part of the treatment of severe lameness and clinical mastitis. The expert panel also emphasises that farmers should be well trained in recognizing signs of disease at early stages and veterinary advice should be obtained at an early stage of disease in dairy cattle [113]. Access to the outdoors has a number of implications for both cow's health/welfare and food safety aspects. At the moment of publication in 2009, it was not possible for another EFSA expert panel to make a universal judgement on the superiority/inferiority of

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either indoor or outdoor farming practices from the overall food safety perspective [19]. See for the discussion about animal welfare and microbial food safety also below (housing). The EFSA risk assessment study stressed out the importance of the presence of facilities for cows with systemic mastitis, cubicles in the stall that are big enough and bedding hygiene as significant factors for good cow's udder health [19].

4.1.2 Housing

In order to reduce microbial risks at the cow housing level, temperature and humidity in housing facilities should be controlled [19]. In addition, the floor of the housing facilities should be designed in such a way that it enables regular and thorough removal of faecal matter and that effective sanitation is possible. The provision and use of facilities for sick animals allowing segregation by adhering to biosecurity principles, is considered essential from a food safety perspective [19]. It should be noted that dairy farming practices, including housing practices, that are considered effective to reduce the risks of foodborne pathogens may compromise dairy cow's welfare. Since also an increased cow's welfare can reduce microbial hazards, it is not always clear what the overall effect is of an intervention. Examples of opposing measures favouring animal welfare but compromising safety are the use of bedding that can serve as a vector for microbial cross-contamination and the use of grooved/non-slippery floors that are difficult to clean/sanitise [19].

4.1.3 Feed, water and faeces

Water and feed should preferably be of potable quality. With respect to spore-forming butyric acid bacteria and *B. cereus*, Vissers developed a practical guide with advice to reduce the numbers of the spores in silage and milk storage tanks (Tables 10 and 11) [114]. With respect to manure, it should be free of pathogens when it is used as fertilizer [20] and it is important to eliminate or minimize faecal contamination during milking [115].

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Table 10. Reported measures to reduce the number of spore-forming butyric acid bacteria in the dairy storage tank at the farm. Table adapted from [114].

Target	Precaution
Fast and good fermentation of the corn and grass to silage with a pH below 4.5	Harvest with dry weather Use additives like molasses and bacterial cultures Cover the silage on the day of harvest
Prevention of oxygen in the silage	Make sure the silage is stored in a high density through sufficient compacting during ensilaging The covering plastic should be well connected with the silage Place a layer of soil on the covering plastic Prevent holes in the covering plastic Sufficient feeding speed (over 1.5 m/week)
Prevention of cross-contamination of spores from badly fermented silage parts to well fermented parts	Remove silage with (visible) fungi on it Remove the outer 20 cm of the silage that show signs of self-heating

Table 11. Potential measures to reduce the number of *B. cereus* spores in the dairy storage tank at the farm. Table adapted from [114].

Target	Control measure
Prevention of high numbers of *B. cereus* via feed in storage tank	Feed should contain less than 1,000 spores/g pH of the feed and bedding material should be preferably below pH 5 Change feed and bedding daily
Prevention of high numbers of *B. cereus* via soil in storage tank	Prevent soil containing teats Keep the cow's walking routes free from soil Keep the cows indoors when it rains
Prevention of high numbers of *B. cereus* in milking installation	Hygienic design of the milking machinery Correct maintenance of equipment Clean/sanitize equipment according the instructions
Prevention of growth of *B. cereus* during storage	Cool the milk within 2 hours after the first milking period Ensure that the milk temperature does not exceed 5 °C between two milking periods Prevent failure of the cooling equipment

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4.1.4 Barn, milking equipment and storage

Another EFSA Scientific Opinion [38] on dairy cow housing and husbandry systems describes that the main hygienic measures to minimise microbial contamination of raw milk during milking include:

- Effective cleaning and disinfection of corridors and driveways to the milking parlour, as well as the milk parlour itself (walls, floor);
- Applying appropriate milking techniques;
- Thoroughly cleaning of udders before milking (may be preceded by hair clipping), as well as effective cleaning-disinfecting teats followed by subsequent drying, both pre- and post-milking;
- Proper maintenance and cleaning-disinfection of milking equipment between milking cycles by a combination of mechanical, thermal (38-55 °C water rinses) and chemical (alkaline, and periodically acid, rinses) techniques;
- Thorough cleaning-disinfecting, generally achieved by spraying, of bulk tanks;
- Pre-milking inspection of milk and subsequently discarding the foremilk;
- Avoiding milking of an empty udder to prevent mastitis;
- Storing raw milk at temperatures <6 °C to minimise microbial growth;
- Regular visual inspection of surfaces of milking equipment and bulk tanks for biofilm formation and periodic bacteriological testing [38].

4.1.5 Veterinary drugs and antibiotic resistance

With respect to the use of antibiotics, guidelines have been summarized by the RUMA (responsible use of medicines in agriculture) Alliance in the UK. In summary, antibiotics should be used in accordance with manufacturer's and veterinarian's instructions and they should be administered the full course of treatment. In addition, the farmer should keep an animal medicine record book and also report suspected adverse reactions. The full document can be red at: [116]. Due to increasing concerns about the development of antibiotic resistance of bacteria, antibiotics use is restricted. Cows are, for example, not routinely treated with antibiotics during the dry-off period anymore [117].

4.1.6 Other zoonotic diseases/infections, raw milk

To ensure a proper risk management on the farm, control of a good cattle stock (adhering to Good Farming Practices) is the basis for disease control and preventing the possible transfer of zoonotic pathogens through the food chain. Specific measures to achieve this can be found the following references: [38, 118].

In addition, Table 12 shows control points to reduce the microbiological hazards that can arise from raw milk.

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4.1.7 Summary

To summarize, Table 11 includes major biological hazards (in raw milk as identified by an EFSA panel of experts [22]. In addition to the EFSA selection, *S. aureus* is included in Table 11. Furthermore, as summarized by an EFSA Expert panel: dairy farming practices that are beneficial for both dairy cow's welfare and milk safety particularly include, but are not limited to: effective herd health management including responsible use of antimicrobials; hygienic husbandry including appropriate farm design and effective biosecurity; microbiological quality of feeds (both pasture- and compound feed-based) and water; management for preventing animal stress; hygienic milking; hygienic preparation of animals for slaughter; and management of grazing land with respect to the spread of animal manure [38].

4.2 Implementation of interventions in the primary chain

There are several ways to implement the interventions in the dairy sector. GLOBALG.A.P. is a common standard for farm management practice and provides business-to-business standards for safe and sustainable food production according to the Good Agricultural Practices principle. To date, it is the world's most widely implemented farm certification scheme. At the national level, the quality system Keten Kwaliteit Melk (KKM = Quality Milk Chain is in place. This quality assurance program provides guidelines for good working practices in the primary chain. In the Netherlands Qlip provides certifications and audits to assess dairy farms. Major branded dairies require certification of their farmers.

4.3 Potential interventions in the processing cow milk dairy chain

Raw milk that is contaminated with foodborne pathogens that can cause disease in humans pose a risk when introduced in a dairy processing plant. Pathogens present in the raw milk may end up in final products (e.g. cheese) if used unpasteurized or form a risk for cross contamination via the environment. Therefore, it is of importance to minimise risk of contamination of the milk by measures as described in above sections.

At the processing level, as indicated in section 2.2.2, heat treatment is an important critical control point to meet the food safety standards as part of the HACCP plan. A prerequisite for an effective HACCP plan is also implementation of GMP and GHP (e.g. personal hygiene, training, zoning, cleaning and disinfection strategies for the factory environment). Table 12 summarizes relevant critical control points in the dairy industry. These factors can be more difficult to control for small scale processing of milk and for production processes that may not take place on a daily basis (as in place at the dairy farm).
Industrial processing of milk and dairy products typically involves heat treatment of the raw milk. The applied heating regimes at industrial processing are well controlled but the risk is comparatively higher when milk is pasteurized on the farm according to studies reported in Farrokh et al. [65].There seems to be a growing group of consumers in the EU that prefers to drink raw milk because of assumed health effects that are destroyed upon heating. However,

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a recent study on assumed nutritional and health effects could not confirm benefits associated with raw milk consumption and it is therefore advised to heat milk before consumption [56].

Table 12. Main biological hazards, sources, farm and beyond farm control associated with dairy cow farming. Table adapted from [38].

Biological hazard	***Main sources***	***Main principles of pre-harvest (on farm) control***	***Main principles of harvest- and post-harvest control (beyond farm)***
B. melitensis	Cows	Herd health plans. Mastitis control	Milk pasteurization
Campylobacter spp. (thermophilic)	Cows and also environment, including water, effluents, organic fertilisers	Preventive measures based on hygienic husbandry and management of animal wastes and effluents from dairy farms	Milk pasteurization. Good manufacturing / good hygiene practices
Mycobacterium spp.	Cows	Herd health management	Milk pasteurization
Salmonella spp.	Cows and also effluents, organic fertilisers, water	Preventive measures based on biosecurity, hygienic husbandry and management of animal wastes and effluents from dairy farms	Milk pasteurization. Good manufacturing / good hygiene practices
S. aureus	Cows and humans	Milking hygiene. Mastitis control	Milk pasteurization. Good manufacturing / good hygiene practices
Shiga toxin-producing *E. coli* (STEC) / Vero cytotoxin-producing *E. coli* (VTEC)	Cows and also environment, including water, effluents, organic fertilisers	Preventive measures based on hygienic husbandry and management of animal wastes and effluents from dairy farms	Milk pasteurization. Good manufacturing / good hygiene practices
Tick-borne encephalitis virus (TBEV)	Rodents, ticks, infected cows	Hygienic husbandry. Pest/tick control	Milk pasteurization

Besides heating, physical methods may be applied for removal of microbial contaminants such as filtration techniques in combination with pasteurization to reduce bacterial counts or bactofugation to reduce pathogenic spore formers (*C. botulinum, B. cereus*) can be used [53]. Pulsed electric field (PEF) has been reported in literature as technology to reduce pathogens and

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spoilage microorganism from milk. Although proven successful for treatment of fruit juices, the application for milk poses more challenges because of the higher pH and complexity of the milk. Although some studies show promising results for PEF treatment of milk, there is substantial variation in the outcome of these studies [119] likely caused by variation in equipment design and applied conditions (e.g. process intensity and temperature).

In the dairy processing environment, *L. monocytogenes* has been frequently isolated from the environment albeit with variable incidences [78] that may be explained by variation in the size of the processing plant, manufacturing operations and compliance with GMP. *L. monocytogenes* is able to form biofilms on food contact surfaces typically applied in industrial environments such as stainless steel [120]. Within a biofilm, microorganisms are protected by a matrix of extracellular polymers and display a higher resistance against antimicrobial compounds [121]. Such biofilms persist on equipment or other food contact surfaces. Effective measures to control *L. monocytogenes* in the processing environment is therefore of importance and include hygienic zoning of the processing plant, hygienic design of processing equipment to prevent biofilm formation, and contamination via aerosols [13].
Although limited studies are available in a dairy processing context, micro-organisms other than *L. monocytogenes* have the capacity to form biofilms on materials typically used in a process environment as for example stainless steel. For example, it is known that STEC is able to adhere and form biofilms on stainless steel [121].

Staphylococcal food poisoning is frequently associated with the consumption of milk and milk products such as cheese and in particular raw milk cheese ([63], [62]). Toxins produced by coagulase-positive *S. aureus* are heat stable and remain active after pasteurization, whereas the producing population has declined and may no longer be detectable [62]. Absence of viable *S. aureus* cells is therefore no guarantee for absence of its toxins. Along these lines, European standards for coagulase-positive staphylococci in cheese rely on controlled analyses carried out during the process at time points where numbers are expected to be high (see Table 2). If *S. aureus* numbers exceed maximum counts set for the various types of cheese, *S. aureus* enteroxins should be tested according to European screening methods. Proper storage of raw milk(< 6°C) at the farm level and effective mastitis control programs are reported as measure to reduce the risk for prevalence of staphylococcal enterotoxins in milk intended for processing [62, 63]. Other measures that contribute to prevention of *S. aureus* enterotoxin production in milk products include heat-treatment of milk, salt concentration, fast pH drop, inhibition by using starter cultures, low temperature of processing and storage of cheese and/or minimising the pressing time [63].

Risk management intervention studies for *Cronobacter* ssp.(formerly *Enterobacter sakazakii*) and *Salmonella* ssp. in PIF were evaluated by an EU expert panel [82]. Control of recontamination of PIF with *Cronobacter* ssp. and *Salmonella* ssp. from the processing environment followed by heat

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treatment was considered critical to minimize the risk associated with PIF. Measures reported [82] include:

- Effective implementation of preventive measure (GMP/GHP and HACCP);
- Avoid multiplication of potential contaminants by excluding water from the process environment for example by implementation of a systematic dry cleaning;
- Selection of suppliers of dry mix ingredients according the specific needs;
- Implementation of monitoring and environmental management systems.

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Table 13. Processing steps considered critical for the safety of dairy end products.

Product	*Processing step*	*Description of risk*	*Critical limits/preventive measures*	*References*
All dairy products	Milk reception	Milk with high microbial load	Routine control determination pH/acidity (pH 6.4–6.6)	[122]
		Distribution under non-hygienic conditions	Temperature control and vehicle cleaning	[122]
All pasteurized dairy products	Milk pasteurization	Potential pathogen survival	Pasteurization temperature ≥ 72.5°C/15 s (or a similar time/temp profile), negative alkaline phosphatase test	[123]
UHT milk, sterilised cream	Sterilization	Render the product microbiologically sterile	121°C/10 min (25% fat cream) 135°C/1 s (UHT Milk)	[79, 124]
Milk, cream, ice cream	Cooling	Potential pathogen/ spoilage micro-organisms proliferating	Check temp/time profile of cooling post-pasteurization (2–4°C)	[125]
	Filling	Carton sealing Aseptic filling	Correct packaging (squeeze test)	[123]
Pasta filata type cheeses and Halloumi cheese	Scalding/kneading	Potential pathogen survival	Check Scalding temperature ≥90°C for Halloumi ≥77°C for Mozzarella	[126, 127]
All dairy products that require lactic fermentation	Starter culture addition/ripening	Potential pathogen survival cross contamination	Check type/quantity Check ripening time Check for correct storage conditions of starter culture and hygiene status.	[122]
All cheese types	Dry salting/curd salting	Potential pathogen survival	Check amount of salt used	[122]
Brined cheeses	Brining	Potential pathogen survival	Disinfect water or pasteurize whey before adding salt Check salt content in brining solution	[127] [122]

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All cheese types	Maturation	Proliferation of pathogen microflora	Relative humidity	[122]
All dairy products	Cold storage/distribution	Potential pathogen multiplication due to incorrect temperature control	Temperature control < 6 °C at cold storage and during distribution	[122]

Table adapted from [53]. UHT = ultra-high temperature.

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Goats and sheep

Limited information on the processing chain of goat's and sheep's milk is available in the literature. Jimenez-Granado states that adequate sanitary control of herds is the best guarantee to prevent the occurrence of pathogens (mastitis) and to ensure the imperative requirement of food safety of dairy products from small ruminants [128]. However, good methods to detect especially subclinical mastitis are not available for goats and sheep at the moment. This has to do with the finding that, for instance, somatic cell count to monitor udder health (which is often used for dairy cows) depends on many intrinsic and extrinsic factors and not only on the health status of a ruminant [128].

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5 Future perspectives: expected developments in the dairy chain

5.1 Trends in the dairy chain

Based on interviews with scientific experts and experts of dairy industry as well as dairy farmers, the major trends for the coming 10 years have been identified. Furthermore, published reports on the future of the dairy chain/sector have been screened for further substantiation of the expected changes. Future changes foreseen at the various steps in the dairy chain are described below.

Worldwide, the future challenges of the dairy sector are changing consumer demands, growing concern over sustainability and a need for greater efficiency. Various future scenarios can be found in literature [129]. These scenarios are not presented as the absolute truth but often are intended to inspire or give possible directions for future developments. The global demand for dairy products is raising rapidly, driven by the increasing population and purchasing capacity in the developing countries [129]. Other dominant aspects in the dairy industry are the constant strive to increase efficiency and the search for innovative solutions to follow consumer trends and gain additional value. In addition, environmental sustainability, especially those related to the emission of greenhouse-gases becomes increasingly important. Protecting policies of some developed countries are becoming less restricting [129].

It is expected that the ending of the milk quotum per April 2015 is going to build an additional production of 2 billion kg of milk/year in The Netherlands on a current production of 12 billion kg/year [130]. Worldwide production to date is about 700 billion kg/year (FAOSTAT).
The world milk production is expected to have increased with 180 billion kg by 2023 [131].
The majority of this increase will be produced in upcoming countries (e.g. China and Africa) [131]. The Dutch contribution to this increase is with 2 billion kg milk per year relatively minor. Eventually, production growth will be limited by the disposal of manure (phosphate, ammonia) and in the future by the available land area (expert input).

The European market for dairy products will only show a moderate growth and increased production is expected to be exported to Russia, Middle East (in particular China), and north and central Africa (Rabobank, 2015). China is expected to maintain its position as the world's largest importer of dairy commodities, accounting for close to 20% of the world imports by 2024 [132].

A cheap and easy way to stabilize and transport milk is to transform it into milk powder. Currently, over half of the EU dairy products traded are milk powders. By 2024, production of skim milk powder (SMP) is expected to reach 1.6 billion kg [132]. The higher cheese production in the EU is mainly driven by a higher domestic consumption rather than by increased export. By 2024, EU cheese production could reach 11 billion kg which is 1.15 billion kg more compared to 2014 levels [132]. Whey powder is an important ingredient for infant formulas and trade is

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expanding, especially towards China. Whey powder production is expected to increase by 20% to 2.5 billion kg in 2024 [132]. Of this increase, 35% is expected to be exported as whey powder in its original form. The remainder will be used for animal feed (although this market is declining), or will be used as food supplements, sports drinks and predominantly infant formulas [132].

It is expected that the dairy chain will become simpler as traders are increasingly buying factories, which will shorten the dairy chain from farmer to consumer. As a result traceability in the Dutch dairy chain will grow (expert opinion).

5.2 Animal feed

It can be expected that increased dairy production will coincide with increased demand for animal feed. In order to secure the feed supply, more feed materials will be grown within Europe resulting in new or other grain varieties [133] such as lupine, insect proteins and rapeseed, as well as the use of alternative proteins (e.g., from side streams or currently unexploited sources). The Netherlands have a negative nitrogen balance (more imported than exported). It is expected that local production of crops for animal feed and upgrading of agricultural waste streams to animal nutrition will become more important. Full valorisation of side streams in the animal feed sector will be pursued.

Economic factors are important drivers in the formulation of feed, related to fluctuations in the availability of agricultural streams. It is expected that the feed sector will face important changes and rapid changes entail risks (expert opinion). Resulting hazards recognized by experts are more likely in the chemical/ physical risks and less pronounced in microbiological hazards. Domains of current and future research include improving digestibility of dietary components, optimizing ruminal fermentation, improving use of N and P by the animal, reducing environmental pollution, improve health of the digestive tract, and understanding the nutritional requirements of animals [134].

5.3 Dairy farm

The Dutch dairy sector comprises approximately 17,000 dairy farms with on average 90 cows per farm [2]. The number of dairy farms in the Netherlands is expected to decrease to 12,000 in 2020 [130] whereas the average farm size is expected to grow to ca. 250 cows per farm (expert input). Implementation of automation (as for example milking robots) will increase. As a consequence, the farmer will have less direct contact with his cows and will be more dependent on technology to monitor health of the cow and hygiene during milking. The increased use of milking robots is also expected to increase milk yield per cow [132]. The halt on use of preventive antibiotics will increase the risk for infections caused by pathogens like *S. aureus*, and pathogenic *E. coli*. Future alternatives for antibiotics could induce chemical risks (expert input).

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Dairy farms in the Netherlands are typically family business [133], scaling up to mega dairy farms is therefore not expected for the Netherlands (expert opinion). The consulted experts do not foresee specific trends related to goats or sheep farming. Sheep milk is not processed on a large scale in the Netherlands. Before the Q fever crisis, whey from goats and cows were pooled before further processing. The two whey streams are kept separated nowadays.

In total there are 400 smaller dairy farm processers and this number is expected to remain stable over the coming years. Experts especially foresee microbial risks for this relatively small sector. It is difficult for this sector to access (scientific) knowledge as there is no institute to assist them with technical questions as was the case in the past for the Dutch dairy sector. It is foreseen that knowledge infrastructure and data management on the farm will become increasingly important .

There is a large pressure to reduce greenhouse gases emitted by cows. Companies work on the reduction of emissions of methane by cows through additives to the feed. The chemical composition of these additives is not always known. Its use could introduce new hazards for in particular chemical components. Developments in manure and waste management foreseen include increased use of large centralized facilities to recycle farm animal manure and organic wastes with production biogas [129]. Streams from fermenters to farms could contain and spread chemicals or microbes.

5.4 Milk processing

When we look at the product-portfolio of the processed milk in the Netherlands, we see the following trends:

- Increased consumer demands for convenience products, produced from milk of from cows that have outdoor access ("weidemelk"), for organic dairy products, and for fresh and shelf stable products;
- Application of new/ mild processing techniques;
- Process automation in the factory.

There is an increasing demand for convenience products such as pre-cut slices of cheese, grated cheese. These products are typically vulnerable for contamination, especially for contamination by *L. monocytogenes*. Internet commerce is expanding and sales also include dairy products or food stuffs made thereof. Sampling, control and trader identification poses challenges to authorities [135]. A recent microbiological investigation of cheeses purchased via the internet revealed labelling (raw or pasteurized milk cheese), hygiene and safety of those products as major points of concern [135].
It is expected that there will be no regulation with regard to pasteurization of milk for the manufacturing of cheese, as France will not support such EU-regulation. However, there is increasing pressure from the retail sector to use pasteurized milk for further processing. More and more self-dairy farms apply pasteurization (expert input). A trend that requires specific

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attention is the increasing demand for raw milk and minimally processed products. Raw milk cheese (especially soft cheeses) has been frequently contaminated with *L. monocytogenes*, *Salmonella* ssp. and *E. coli* (see RASFF). These are also the most important pathogens for dairy products.

Traditional thermal inactivation processes can have adverse effects on nutritional and organoleptic properties of the milk. Consequently, alternative techniques have been widely researched and include extreme fast heating, Pulsed Electric Field processing, ultraviolet treatment, ultrasound processing, the addition of carbon dioxide and high pressure processing [129]. Fresh milk with extended cooled shelf life via smart filtration techniques is currently advertised by a Dutch dairy producing company [136]. It remains uncertain to what level these techniques will eventually be implemented as costs may be a limiting factor for implementation on large scale in the dairy sector [129]. Furthermore, novel technologies require an adequate validation of safety before implementation, as shelf life extension may pose a risk towards the microbial safety of dairy products.

In the processing plant, automation will continue further. Via automated Cleaning In Place (CIP) programs, equipment like tanks, pasteurizers and filling machines can be cleaned. If monitoring is correctly applied, product quality and safety will benefit from these systems.

5.5 Other drivers

Climate change is considered an important driver that can have impact on future food safety [137]. Factors of influence reported in literature include effect on microbial ecology including pathogens, effect on maintaining the cold chain when ambient temperatures rise and humidity affecting the production of mycotoxins [137].

Developments in microbial detection methodology, in particular new molecular detections techniques and use of genome information, can be used to improve tracking and tracing systems [137].

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6 Conclusions

Looking at where in the dairy chain pathogens can be introduced, contamination routes reported in literature can be classified in three main categories:

1. contamination via the primary chain for milk production;
2. survival of pathogens during further steps in the milk processing (either due to lack of pasteurization, or inadequate process or storage conditions of milk);
3. contamination from the production environment.

Primary chain

Human pathogens may contaminate milk in the primary chain via milk producing animals. Good farming practice improves milk quality and safety. Animal health, milking equipment, animal feed and water, animal welfare and the farm environment are factors reported in literature that can affect the presence of pathogens in the milk.

A significant microbiological hazard recognized is mastitis which in particular forms a risk for transmission of *Staphylococcus aureus* from the infected udder to the milk **(Figure 13).**

Indirectly, cattle can consume contaminated feed or water and the ingested pathogen can survive/amplify in the cow and contaminate the farm environment via faecal dissemination. This constantly maintained reservoir of foodborne pathogens can reach humans by direct contact with the cattle/environment or via ingestion of raw contaminated milk (products).

Factory processed milk

Industrially processed milk is based on pasteurized milk. Potential microbiological hazards are therefore more likely to result from recontamination from the process environment or via addition of ingredients, provided the pasteurization step has been adequately performed.

Based on a literature study, most important pathogens potentially present in industrially processed milk and environment have been selected and results are depicted in **Figure 14**.

Dairy farm production

Dairy farm processing of milk is more likely to involve raw milk and pathogens potentially present in the raw milk pose a direct risk for contamination especially when raw drinking milk is consumed. An additional factor that may affect microbial hazards is that process control and hygienic zoning may not be optimal at a farm production location.

Based on a literature study, most important pathogens potentially present in processed dairy farm products have been selected and results are depicted in **Figure 15.**

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Disease burden attributed to dairy products

Dairy products contribute to ~8% of the total number of disease incidents involving food as vehicle according to estimates for the Netherlands in the period 2010-2013 for 14 food related pathogens.

The majority of the 55,000 dairy related disease incidents in 2013 are attributed to *S. aureus* toxins (68%) followed by *C. perfringens* toxins (11%) and *Campylobacter* ssp. (7%) based on attribution data from an expert survey.

Expressed in DALYs, dairy as products group ranks, with 410 lost healthy years of life, fifth in comparison to other food groups (2013 data). *Campylobacter* contributes with 127 DALYs to 31% of the total number DALYs attributed by experts to dairy products followed by *T. gondii* (89 DALYs) and *S. aureus* toxin (98 DALYs).

L. monocytogenes accounts for only 14 of total of 55,000 disease incident attributed to dairy by experts. However, expressed in mortality *L. monocytogenes* (20% for the dairy food group in 2013) ranks second after *Campylobacter* (34%) which reflects that although incidence of this pathogen in the dairy food category is relatively low, the impact on disease burden is high.

Intervention measures

Preventive measures should already be taken at the primary dairy chain level and potential interventions (both corrective and preventive) reported in literature are numerous and include taking care of animal health, feed and water, housing, and milking equipment.

At the processing level, besides milk pasteurization, most frequently referred to in literature is the importance of an effective HACCP plan including implementation of GMP and GHP.

Future perspective

The extra milk volume predicted to be produced through the release of the milk quotum is not expected to pose an additional risk, as most of this milk will be converted to milk powder that is intended for export.

In general, future developments foreseen for the dairy farm that reduce the microbiological risk are increase in automation, monitoring and control. Potential risks are foreseen for use in new feed ingredients.

Consumer demands (more fresh, more convenient, longer shelf life, artisanal production) may increase product-related risks.

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For the dairy processing chain in general: more automation, more monitoring and control are expected that may further reduce the risk on microbial contamination. Expected increase in centralization of the supply chain should in theory provide less risks up to the dairy processing step, nevertheless, retail and consumer level (how is the product brought to and used by the consumer) may provide additional risks as regulation, monitoring and control of, e.g., internet-sales has not been established yet.

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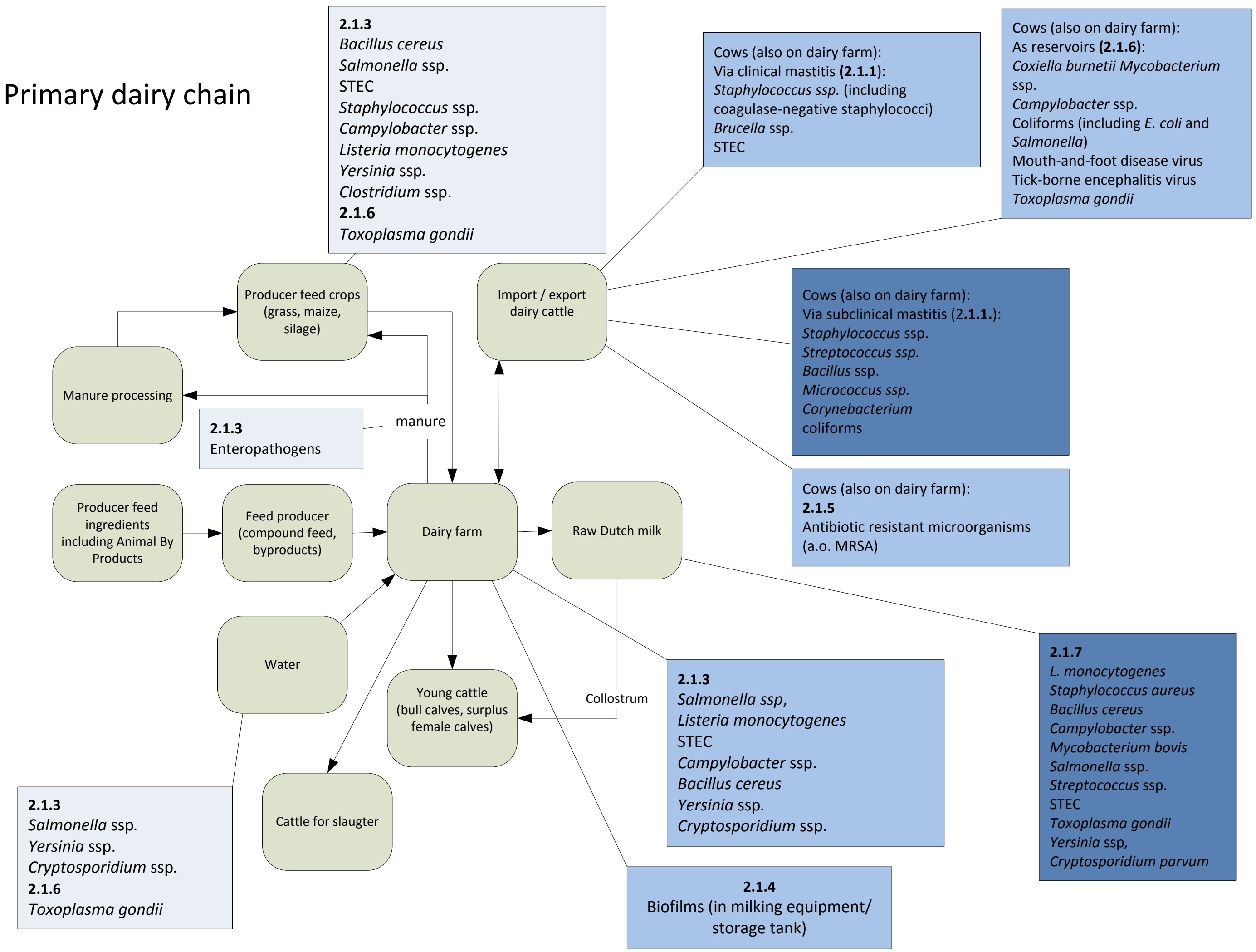

Figure 13: Overview of most important microbial hazards in the primary dairy chain based on available literature data. The colours used indicate the relative importance of a risk for given pathogen in the primary dairy chain and is not based on qualitative numbers. Dark blue represents a relatively higher risk, light blue represents a relatively lower risk. STEC is an abbreviation for Shiga toxin-producing *E. coli*.

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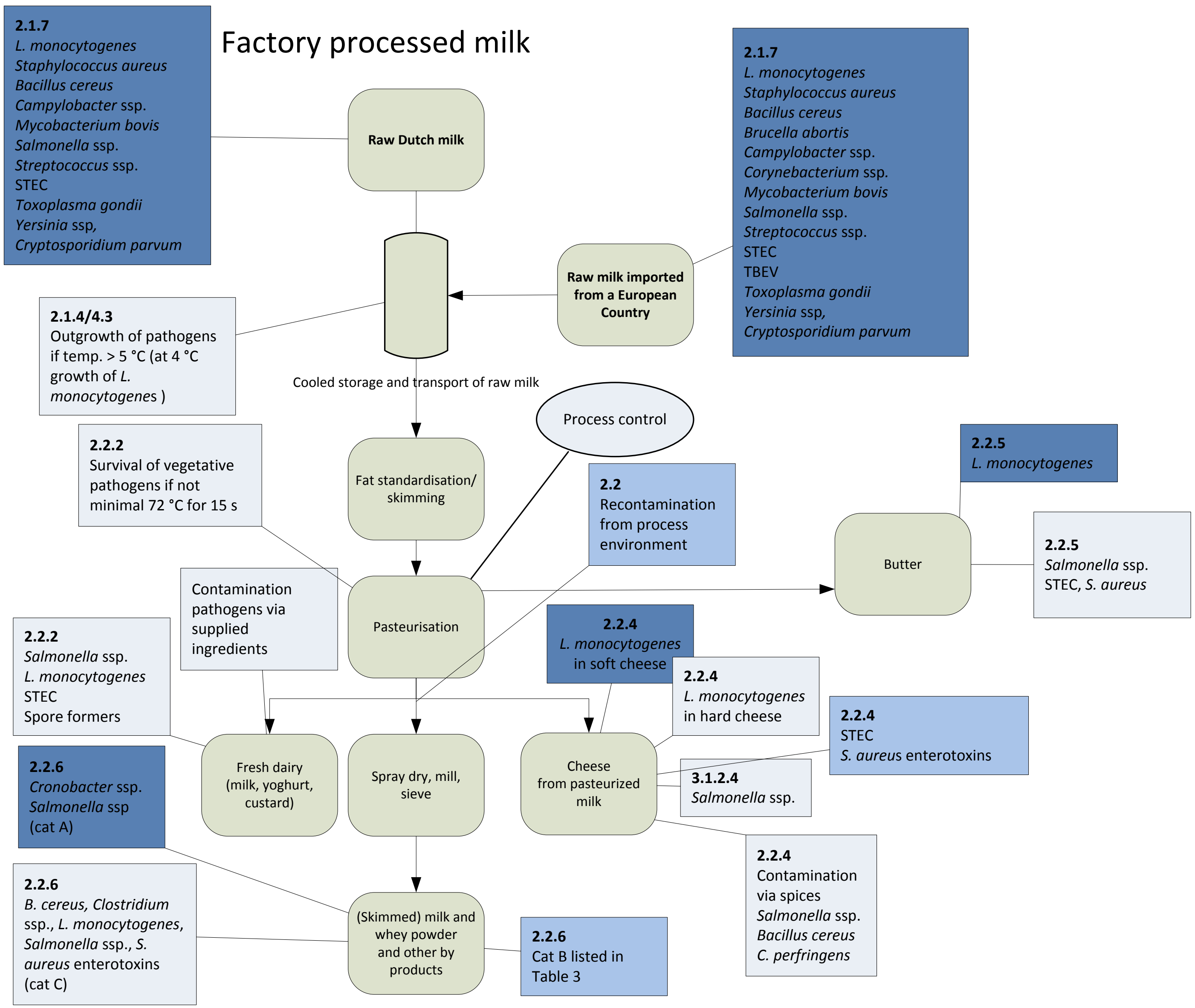

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Figure 14: Overview of most important microbial hazards in factory processed milk based on available literature data. The colours used indicate the relative importance of given pathogen product combination and is not based on qualitative numbers. Dark blue represents a relatively higher risk (based on reported prevalence, outbreak(s) and disease burden for pathogen/product combination). Lighter blue represents a relatively lower risk for given pathogen/product combination. STEC is an abbreviation for Shiga toxin-producing *E. coli* .

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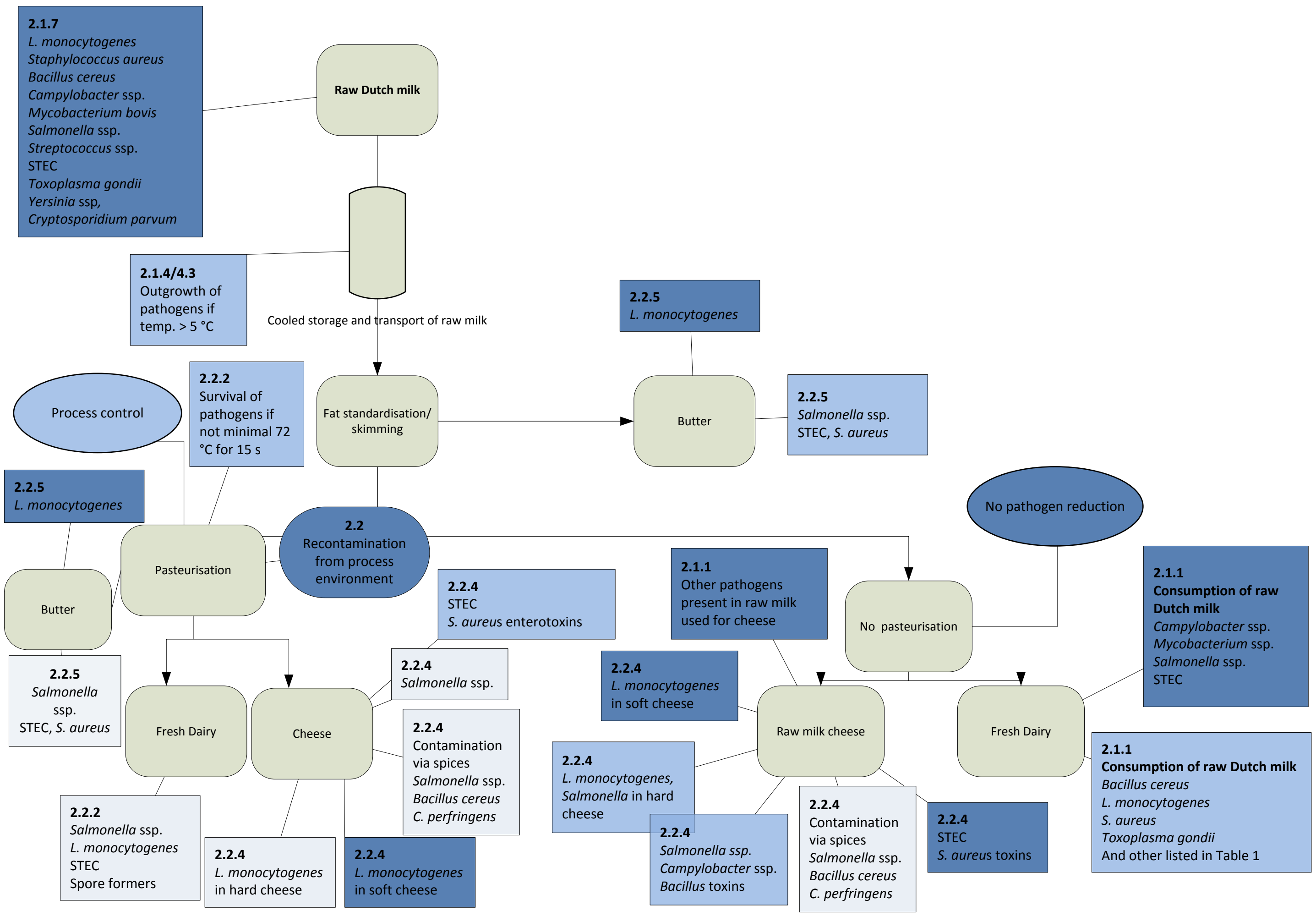

Figure 15. Overview of most important microbial hazards in dairy farm processed milk based on available literature data. The colours used indicate the relative importance of given pathogen product combination and is not based on qualitative numbers. Dark blue represents a relatively higher risk (based on reported prevalence, outbreak(s) and disease burden for pathogen/product combination). Lighter blue represents a relatively lower risk for given pathogen/product combination. STEC is an abbreviation for Shiga toxin-producing *E. coli*.

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Acknowledgments

The authors would like to thank Hein van Valenberg (WU) for critically reading this report.

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Appendix 1

Dutch Dairy factories. Source: Wikepedia.

Bedrijf	Plaats	Product	Capaciteit melk
FrieslandCampina Butter	's-Hertogenbosch	Boter (ca. 65.000 ton)	650.000 ton
FrieslandCampina Butter	Lochem	Boter (ca. 52.000 ton boter, 64.000 ton melkpoeder en 4.000 ton melkprisma's)	1.000.000 ton
FrieslandCampina Butter	Noordwijk (Groningen)	Boter (ca. 55.000 ton)	550.000 ton
VIV Buisman	Zelhem	Boter en boterolieproducten	
FrieslandCampina (CCF)	Leeuwarden	gecondenseerde melk (verwerkt in	1.000.000 ton melk, groeit naar 1.400.000[
Hochwald Nederland	Bolsward	gesuikerde gecondenseerde melk	100.000 ton (2006)
Arla	Nijkerk	dagverse zuivel (daarnaast levering zuivelproducten andere delen Arla concern)	
A-Ware Fresh Dairy	Coevorden	yoghurt, melk, room (was tot 2013 Katshaar Zuivel)	
Den Eelder	Well	Dagvers van koe- en geitenmelk	10.000 ton
De Zuivelmakers	Benschop	Yoghurt, hangop en toetjes (tot 2014 onder de naam Natuurhoeve)	
Farm Dairy	Lelystad	Dagverse zuivel	200.000 ton[7]
FrieslandCampina CP	Maasdam	Dagvers + distributie	
FrieslandCampina CP	Rotterdam	Dagvers en schoolmelk	
FrieslandCampina Ecomel	Limmen	Dagverse biologische zuivel	
FrieslandCampina Ecomel	Drachten	Dagverse biologische zuivel (voorheen Friese Ecologische Zuivel)	
Vecozuivel	Zeewolde	Dagverse biologische zuivel: melk, yoghurt, vla , boter	
Weerribben Zuivel	Nederland (Overijssel)	Dagverse biologische zuivel	2.400 ton (2014)
FrieslandCampina Kievit	Meppel	Geëncapsuleerde producten voor de wereldwijde voedingsindustrie	
FrieslandCampina Professional	Nuenen	slagroom, room en producten op basis van room voor de bakkersindustrie	
Globemilk	Boxmeer	halffabrikaten uit melk	150.000 ton[11]
A-Ware Food Group	Heerenveen	Kaas (geopend december 2014, capaciteit 100.000 ton)	1.000.000 ton
Bel Leerdammer	Schoonrewoerd	Leerdammer kaas (samen met Dalfsen en Wageningen in 2014: 70.000 ton)	340.000 ton
Bel Leerdammer	Dalfsen	Leerdammer Kaas in folieverpakking, capaciteit 42.500 ton [8]	425.000 ton
Bel Leerdammer	Wageningen	Leerdammer kaas	
CONO Kaasmakers	Middenbeemster	Beemster kaas + weipoeder (in 2014: 30.000 ton kaas)	300.000 ton
CZ Rouveen	Rouveen	Kaasspecialiteiten, eigen merk Bastiaansen + private labels (in 2014: 15.000 ton)	150.000 ton
De Graafstroom (Deltamilk)	Bleskensgraaf	Goudse kaas (2014: 40.000 ton)	400.000 ton
DOC Kaas	Hoogeveen (2x)	Kaas, 'Dutch Original Cheese' en private labels (in 2013: 125.000 ton)	1.250.000 ton
FrieslandCampina Butter / Cheese	Born	Kaas en boter	
FrieslandCampina Cheese	Balkbrug	Kaas	
FrieslandCampina Cheese	Dronrijp	Kaasspecialiteiten	
FrieslandCampina Cheese	Gerkesklooster	Kaas	
FrieslandCampina Cheese	Lutjewinkel	Kaas	450.000 ton[10]
FrieslandCampina Cheese	Marum	Kaas	
FrieslandCampina Cheese	Rijkevoort	Kaas	
FrieslandCampina Cheese	Steenderen	Kaas	
FrieslandCampina Cheese	Wolvega	Kaas	
FrieslandCampina Cheese	Workum	Kaas	
FrieslandCampina Cheese	Leerdam	Kaas	
Henry Willig	Heerenveen	kaas van koe, schaap en geit (cap. samen met Katwoude in 2014: 5000 ton kaas)	50.000 ton
Henry Willig	Katwoude	kaas van koe, schaap en geit (cap. samen met Heerenveen in 2014: 5000 ton kaas)	"0" zie Heerenveen
Özgazi	Etten-Leur	Witte kaas (Feta) uit koemelk (80%) en schapen- en geitenmelk (2014 ca. 40.000 ton v	75.000 ton (80% koe)
Ausnutria Hyproca	Heerenveen	Babyvoeding (in aanbouw sinds 2014)	
Hyproca Dairy (Ausnutria Hyproca)	Ommen	(biologische) koemelkpoeder, geitenmelkpoeder en (biologische) boter	
Lyempf (Ausnutria Hyproca)	Kampen	melkontvangst, drogen, half-fabricage flesvoeding	
Lypack (Ausnutria Hyproca)	Leeuwarden	verwerkt en verpakt poeders van Lyemph te Kampen tot o.m. flesvoeding	
FrieslandCampina DMV	Veghel	o.m. Melkpoeder, melksuiker, caseïnaten.	1.500.000 ton[9]
FrieslandCampina Domo	Beilen	Melkpoeder, zuigelingenvoeding	
FrieslandCampina Domo	Borculo	Kindervoeding, medische voeding en celvoeding	
FrieslandCampina Domo / Cheese	Bedum	Kaas, weipoeder	
Nestlé Nederland	Nunspeet	Babyvoeding voor eigen merken NAN, NIDINA, ALFARÉ, AL110	
Nutricia Nederland (Danone)	Zoetermeer	Zuigelingenvoeding	
Nutricia Cuijk (Danone)	Cuijk	Zuigelingenvoeding	
Phoenix (Vreugdenhil)	Scharsterbrug	Melkpoeder (samen met Gorinchem in 2014 ca. 65.000 ton)	250.000 ton (helft)
Promelca (Vreugdenhil)	Gorinchem	Melkpoeder (samen met Scharsterbrug in 2014 ca. 65.000 ton)	250.000 ton (helft)
Kaasfabriek Eyssen (Kaptein Groep)	Oosthuizen	Smeerkaas, rookkaas, blokkaas en industriële kaas	
Koninklijke ERU	Woerden	Smeerkaas	
Lebo Kaas	Lopik	Verse roomkaas, smeerkaas en kruidenboter (verwerkt melk van ca. 10 boeren)	
Vika BV	Ede	Smeltkaas (verwerkt voornamelijk kaas van derden)	
Yakult	Almere	Probiotische zuiveldrank	
Zuivelhoeve	Hengelo	Vla, yoghurt, pap (verwerkt melk van 5 boeren)	5.000 ton (2014)
Boermarke	Enschede	Vla, pap, yoghurt, roomijs (bereiding uit melkpoeder, geen verse melk)	

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Appendix 2

Studies reporting on the occurrence STEC in cheese and other dairy products. Source: [65].

Table 4
Selected studies reporting on the occurrence of STEC and prevalence of *stx* genes in cheeses and other dairy products.

Region/country	Sample analysed	STEC[a] isolate occurrence (%) and/or *stx* gene prevalence (%) (N = number of samples)	Reference
America			
Peru	Soft cheese made from raw milk	7.8% (N = 102) *E. coli* O157	Mora et al. (2007)
Venezuela	Telita cheese	2% (N = 100) *E. coli* O157	Márquez and García (2007)
Asia			
Turkey	White pickled cheese made from raw milk	4% (N = 50) *E. coli* O157	Oksuz et al. (2004)
Europe			
EU	Cheese made from raw milk	0.2% (N = 2876) STEC in 2005 2.4% (N = 1064) STEC in 2006 0.5% (N = 1961) STEC in 2007 1.8% (N - 700) STEC in 2008	EFSA (2010)
Belgium	Cheese made from raw cow's milk	5.6% (N = 71) *E. coli* O157:H7	De Reu et al. (2002)
	Cheese made from raw goat's and sheep's milk	0% (N = 222) *E. coli* O157	Imberechts et al. (2007)
	Butter and cream made from raw milk	0% (N = 181) *E. coli* O157	
	Butter, yoghurt, cheese, ice cream and fresh cheese made from raw milk	0% (N = 64, N = 9, N = 16, N = 7, N = 4 resp.)	De Reu et al. (2004)
Italy	Cheese made from raw milk	0% (N = 143) *E. coli* O157	Civera et al. (2007)
	Cheese made from pasteurised milk	0% (N = 60) *E. coli* O157	
	Mozzarella cheese made from raw buffalo's milk	0% (N = 93) *E. coli* O157	Martucciello et al. (2008)
	Dairy products from pasteurised cow's milk	0% (N = 657) *E. coli* O157	Conedera et al. (2004)
	Dairy products from raw cow's milk	0% (N = 811) *E. coli* O157	
	Dairy products from pasteurised sheep's milk	0% (N = 477) *E. coli* O157	
	Dairy products made from raw sheep's milk	0% (N = 502) *E. coli* O157	
	Mozzarella cheese made from buffalo milk	0% (N = 501) *E. coli* O157	
Portugal	Cheese made from raw cow's, sheep's and goat's milk	0% (N = 70) *E. coli* O157	Almeida et al. (2007)
Spain	Dairy products from raw ovine and caprine milk (milk, cheese curd, cheese)	1.8% (N = 502) STEC (45 *stx* + samples)	Rey et al. (2006).
	Fresh cheese curds (ovine/caprine)	0% (N = 103) STEC (4 *stx* + samples)	
	Cheese	0% (N = 39) STEC (2 *stx* + samples)	
	Raw ewe's Castellano cheese (long-ripened, hard cheese)	2.4% (N = 84) STEC	Caro and Garcia-Armesto (2007)
Scotland	Cheese made from raw cow's milk	0% (N = 739) *E. coli* O157	Coia et al. (2001)
Switzerland	Cheese (semihard, hard and soft) made from raw cow's and goat's milk	2% (N = 796) STEC (4.9% stx + samples)	Stephan et al. (2008)
	Cheese (semihard, hard and soft) made from raw cow's, goat's and ewe's milk	1.9% (N = 1502) STEC (5.7% stx + samples)	Zweifel et al. (2010)
France	Cheese made from raw milk	11.7% (N = 180) STEC(30.5% stx + samples)	Fach et al. (2001)
	Cheese (soft, hard, unripened, blue mold) made from raw milk	13.1% (N = 1039) STEC	Vernozy-Rozand et al. (2005a)
	Cheese	1% (N = 603) STEC (10% *stx* + samples)	Pradel et al. (2000)
	Cheese made from raw milk	27.7% (N = 112) *stx* + samples	Auvray et al. (2009)
	Cheese made from raw milk	5.5% (N = 400) STEC, including 1.8% STEC O26:H11 (29.8% *stx* + samples)	Madic et al. (2011)
Germany	Cheese made from raw cow's milk	0.48% (N = 209) STEC	Messelhäusser et al. (2008)

[a] Culture positive sample with strain harbouring *stx1* and/or *stx2* unless otherwise commented.

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Appendix 3:

Table A. Comparison of estimated disease incidents, DALYs (discounted) and deaths attributed to food groups.

Sources: [105-108].

	2009			*2010*			*2011*			*2012*			*2013*		
Productgroep	Incidentie	Sterfte	DALY	Incidentie	Sterfte	DALY	Incidentie	Sterfte	DALY	Incidentie	Sterfte	DALY	Incidentie	Sterfte	DALY
Overig voedsel	123000	5.3	419	125440	5.9	437	122,065	5	426	121,685	5	421	121300	5	450
Rund en lam	107000	9.1	760	109255	9.9	785	107,290	9	768	106,568	9	747	105900	8	910
Kippenvlees	59000	14	914	63731	14.5	1009	62,724	12	999	61,050	12	954	59800	16	1060
Vis en schelpdieren	58000	6.6	328	63760	8.5	367	57,397	6	343	80,281	7	829	55400	7	370
Zuivel	56000	5.7	373	57490	7.5	405	55,790	5	392	55,376	6	367	54900	6	410
Varken	44000	9.3	924	45985	10	948	45,943	9	927	45,494	9	914	44700	9	1250
Graanproducten	40000	3	158	42578	3.6	172	41,732	3	162	41,207	3	167	41100	3	180
Verse groente	44000	6	310	47416	7	339	41,778	6	314	39,871	6	303	40000	6	360
Ei en eiproducten	22000	5.9	216	23136	6.3	242	22,597	5	224	22,388	5	221	21200	5	225
Dranken	16000	2	88	17362	2.3	97	16,378	2	90	16,017	2	86	15900	2	90
Totaal	569,000	67	4,490	596,153	76	4,801	573,694	62	4,645	589,937	63	5,009	560200	67	5305

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Appendix 4: RASFF Notification for milk and dairy products between 2009 and 2014.

Search criteria | **Notified from** 01/11/2009 | **Notified till** 01/11/2014 | **Product category** milk and milk products | **Hazard category** pathogenic micro-organisms

	Classification	Date of case	Last change	Reference	Country	Type	Product Category	Subject
1	alert	27/10/2014	01/12/2014	2014.1447	France	food	milk and milk products	shigatoxin-producing Escherichia coli (O26H11 serotype with eae and stx1 genes) in raw milk camembert from France
2	alert	08/10/2014	10/11/2014	2014.1373	Denmark	food	milk and milk products	Salmonella Dublin (presence /25g) in raw milk cheese from France
3	alert	08/10/2014	10/11/2014	2014.1372	Denmark	food	milk and milk products	Listeria monocytogenes in organic soft white cheese from Denmark
4	alert	07/10/2014	10/10/2014	2014.1366	Belgium	food	milk and milk products	Listeria monocytogenes (presence /25g) in raw milk soft cheese from Belgium
5	alert	03/10/2014	03/11/2014	2014.1349	Denmark	food	milk and milk products	Listeria monocytogenes (presence /25g) in soft white brie cheese made from goat milk from Denmark
6	alert	01/10/2014	09/10/2014	2014.1337	Denmark	food	milk and milk products	Listeria monocytogenes (presence /25g) in camembert cheese from Denmark
7	information for attention	30/09/2014	03/10/2014	2014.1325	Switzerland	food	milk and milk products	Listeria monocytogenes (800 CFU/100g) in gorgonzola from Italy
8	information for follow-up	12/09/2014	03/10/2014	2014.1272	France	food	milk and milk products	Listeria monocytogenes (
9	alert	22/08/2014	10/10/2014	2014.118	France	food	milk and milk products	shigatoxin-producing Escherichia coli (O-26H-11 stx+ eae+) in raw milk cheese from France
10	alert	15/08/2014	18/12/2014	2014.115	United Kingdom	food	milk and milk products	Bacillus subtilis (>3000 CFU/g) in flavoured milk from Germany
11	alert	14/08/2014	15/09/2014	2014.1145	France	food	milk and milk products	shigatoxin-producing Escherichia coli (O26H11 stx+, eae+) in raw goat milk cheese from France
12	alert	12/08/2014	21/11/2014	2014.113	Germany	food	milk and milk products	shigatoxin-producing Escherichia coli (stx1) in roquefort cheese from raw sheep's milk from France
13	information for attention	08/08/2014	08/09/2014	2014.1122	United Kingdom	food	milk and milk products	Bacillus cereus (between 240 and 1*10E6 CFU/g) in cream from the United Kingdom
14	alert	08/08/2014	08/09/2014	2014.1114	France	food	milk and milk products	shigatoxin-producing Escherichia coli (O26-H11 eae+ stx+) in goat cheese made from raw milk from France

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15	alert	01/08/2014	04/09/2014	2014.1078	France	food	milk and milk products	Listeria monocytogenes (40 CFU/g) in sweet gorgonzola from Italy
16	alert	28/07/2014	29/10/2014	2014.1037	France	food	milk and milk products	shigatoxin-producing Escherichia coli (O26 H11 eae+ stx1+) in cow's milk cheese made with raw milk from France
17	alert	28/07/2014	01/09/2014	2014.1034	France	food	milk and milk products	Listeria monocytogenes (1400 CFU/g) in gorgonzola from Italy
18	alert	24/07/2014	30/07/2014	2014.1019	Germany	food	milk and milk products	Salmonella in "white beer" cheese from Germany
19	information for attention	18/07/2014	01/10/2014	2014.0986	Italy	food	milk and milk products	Bacillus cereus (22000 CFU/g) in chilled ricotta from Italy
20	alert	18/07/2014	13/08/2014	2014.0988	Netherlands	food	milk and milk products	Salmonella Brandenburg (presence /25g) in processed cheese powder from the Netherlands
21	alert	17/07/2014	23/07/2014	2014.0984	France	food	milk and milk products	Listeria monocytogenes (> 10 CFU/g) in gorgonzola from Italy
22	alert	01/07/2014	25/08/2014	2014.0898	France	food	milk and milk products	enteropathogenic Escherichia coli (O26 H11; eae positive) in raw goat milk cheese from France
23	alert	27/06/2014	19/09/2014	2014.0887	France	food	milk and milk products	Salmonella kedougou in raw milk cheese Reblochon from France
24	information for attention	20/06/2014	22/07/2014	2014.0848	France	food	milk and milk products	Listeria monocytogenes (between 1100 and 12000 CFU/g) in milkshakes produced in France from sterilised milk preparation from Belgium
25	alert	19/06/2014	08/08/2014	2014.0835	Germany	food	milk and milk products	Listeria monocytogenes (> 6000 CFU/g) in raw milk soft cheese from France
26	alert	02/05/2014	22/05/2014	2014.0602	France	food	milk and milk products	Listeria monocytogenes (500 CFU/g) in raw milk cheese from France
27	alert	30/04/2014	11/06/2014	2014.0592	France	food	milk and milk products	Listeria monocytogenes (< 100 CFU/g) in raw goat's milk cheese from France
28	information for follow-up	29/04/2014	17/06/2014	2014.0579	France	food	milk and milk products	high count of Escherichia coli (2000000 CFU/g) in cheese from France
29	alert	28/04/2014	20/05/2014	2014.0571	Germany	food	milk and milk products	Salmonella spp. (presence) in skimmed milk powder from Poland, via the Netherlands
30	information for attention	17/04/2014	12/06/2014	2014.0526	Austria	food	milk and milk products	Listeria monocytogenes (180 CFU/g) in mascarpone gorgonzola from Italy
31	information for follow-up	17/04/2014	07/05/2014	2014.0531	Finland	food	milk and milk products	high count of Escherichia coli (2600 CFU/g) in cheese from Hungary
32	alert	15/04/2014	16/04/2014	2014.0509	France	food	milk and milk products	Listeria monocytogenes (< 100 CFU/g) in cheese from France

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33	alert	04/04/2014	04/04/2014	2014.0453	Belgium	food	milk and milk products	shigatoxin-producing Escherichia coli (stx+ ; eae+ /25g) in raw milk from Belgium
34	alert	28/03/2014	10/04/2014	2014.0415	France	food	milk and milk products	Listeria monocytogenes (140000 CFU/g) in cheese from France
35	alert	24/03/2014	19/09/2014	2014.039	Spain	food	milk and milk products	Listeria monocytogenes (150000; 230000 CFU/g) in gorgonzola cheese from Italy
36	alert	21/03/2014	07/07/2014	2014.0383	France	food	milk and milk products	Listeria monocytogenes (10 CFU/100g) in raw milk cheese from France
37	alert	18/03/2014	29/04/2014	2014.036	Germany	food	milk and milk products	Listeria monocytogenes (16000; 31000 CFU/g) and Listeria spp (1000000; 197000 CFU/g) in farmhouse cheese with red culture from Germany
38	alert	13/03/2014	18/03/2014	2014.0345	Germany	food	milk and milk products	Listeria monocytogenes (brie: 1200, 1810, 156000, 10000; camembert: 4700, 237000, 4000, 519000 CFU/g) in frozen brie and camembert from Germany
39	alert	11/03/2014	02/07/2014	2014.0323	France	food	milk and milk products	Listeria monocytogenes (80 CFU/g) in chilled pasteurized sheep's milk cheese from France
40	alert	11/03/2014	03/06/2014	2014.0327	Portugal	food	milk and milk products	Listeria monocytogenes (presence /25g) in sheep's milk from Spain
41	alert	03/03/2014	10/04/2014	2014.0285	Austria	food	milk and milk products	Listeria monocytogenes (510 CFU/g) in raw milk cheese from Italy
42	alert	14/02/2014	26/03/2014	2014.0225	France	food	milk and milk products	Listeria monocytogenes (>15000 CFU/g) in raw milk cheese coated with grape marc from France
43	alert	13/02/2014	03/06/2014	2014.0222	Belgium	food	milk and milk products	shigatoxin-producing Escherichia coli (presence /25g) in Roquefort cheese from France
44	alert	27/01/2014	06/02/2014	2014.0117	Switzerland	food	milk and milk products	Listeria monocytogenes (4100 CFU/g) in gorgonzola cheese from Italy
45	alert	22/01/2014	18/07/2014	2014.0091	France	food	milk and milk products	Listeria monocytogenes (120 CFU/g) in goat cheese from France
46	information for attention	02/01/2014	02/01/2014	2014.0003	France	food	milk and milk products	shigatoxin-producing Escherichia coli (in 2 samples /25g) in raw milk cheese from France
47	alert	30/12/2013	29/01/2014	2013.1741	Germany	food	milk and milk products	Listeria monocytogenes (presence /25g) in soft cheese from France
48	alert	20/12/2013	20/12/2013	2013.1708	Germany	food	milk and milk products	Listeria monocytogenes in raw milk sheep's cheese from France
49	alert	10/12/2013	19/03/2014	2013.1637	Luxembourg	food	milk and milk products	Listeria monocytogenes in sheep's cheese from France
50	alert	21/11/2013	11/04/2014	2013.153	France	food	milk and milk products	Listeria monocytogenes (300 CFU/g) in raw cow's milk cheese from France
51	alert	18/11/2013	19/11/2013	2013.1515	Denmark	food	milk and milk	Listeria monocytogenes (230;

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							products	
52	information for attention	13/11/2013	21/11/2013	2013.1494	Italy	food	milk and milk products	Pseudomonas fluorescens (7.2 x 10E7 CFU/g) in mozzarella cheese from Germany
53	information for attention	11/11/2013	21/11/2013	2013.1481	Germany	food	milk and milk products	Listeria monocytogenes (
54	alert	16/10/2013	29/11/2013	2013.1372	Poland	food	milk and milk products	Salmonella enteritidis (presence /25g) in cheese from the Czech Republic, with raw material from Poland
55	alert	15/10/2013	20/12/2013	2013.1366	France	food	milk and milk products	Salmonella spp. (presence /25g) in raw milk sheep cheese from France
56	alert	11/10/2013	22/11/2013	2013.1356	France	food	milk and milk products	Listeria monocytogenes (
57	alert	06/09/2013	30/10/2014	2013.1224	France	food	milk and milk products	Listeria monocytogenes (
58	information for attention	05/09/2013	07/09/2013	2013.1216	France	food	milk and milk products	Listeria monocytogenes (
80	alert	30/10/2012	13/12/2012	2012.1513	Belgium	food	milk and milk products	Listeria monocytogenes (460 CFU/g) in cheese from Belgium
81	information for attention	29/10/2012	31/10/2012	2012.1504	Italy	food	milk and milk products	Listeria monocytogenes (presence /25g) in chilled ricotta cheese from Italy
82	alert	23/10/2012	08/04/2013	2012.1478	France	food	milk and milk products	Listeria monocytogenes (1800 CFU/g) in mozzarella from Spain, with raw material from Lithuania
83	alert	03/10/2012	01/11/2012	2012.1395	Italy	food	milk and milk products	Listeria monocytogenes (presence /25g) in ricotta cheese from Italy
84	alert	12/09/2012	03/07/2013	2012.1309	Spain	food	milk and milk products	Listeria monocytogenes (1400; 3100 CFU/g) in fresh cheese from Portugal
85	alert	11/09/2012	07/11/2012	2012.1302	France	food	milk and milk products	foodborne outbreak suspected (Salmonella Dublin) to be caused by raw milk cheese from France
86	information for attention	08/08/2012	24/09/2012	2012.1143	France	food	milk and milk products	Listeria monocytogenes (600 CFU/g) in raw milk cheese from France
87	alert	25/07/2012	27/08/2012	2012.1069	France	food	milk and milk products	shigatoxin-producing Escherichia coli (O103:H2 eae+, stx1+) in chilled Roquefort cheese from France
88	alert	13/07/2012	19/11/2012	2012.0994	France	food	milk and milk products	shigatoxin-producing Escherichia coli (O26:H10; stx1+; eae+) in roquefort cheese from France
89	alert	02/07/2012	06/09/2012	2012.0906	Italy	food	milk and milk products	Listeria monocytogenes (presence /25g) in cheese from Italy
90	alert	22/06/2012	03/07/2012	2012.0863	France	food	milk and milk products	Listeria monocytogenes (presence < 10 CFU/g CFU/g) in chilled raw milk cheese from France

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91	alert	18/06/2012	28/06/2012	2012.084	Denmark	food	milk and milk products	Listeria monocytogenes (presence /25g) in organic camembert from Denmark
92	alert	07/06/2012	27/06/2012	2012.0781	Germany	food	milk and milk products	Listeria monocytogenes (4700 CFU/g) in soft cheese preparation made with gorgonzola and cream from Italy
93	alert	06/06/2012	23/07/2012	2012.0772	France	food	milk and milk products	Listeria monocytogenes (1 out of 5 samples /25g) in raw milk cheese from France
94	alert	01/06/2012	13/06/2012	2012.0755	Belgium	food	milk and milk products	Listeria monocytogenes (presence /25g) in goat cheese from Belgium
95	alert	16/05/2012	25/07/2012	2012.0664	Germany	food	milk and milk products	Listeria monocytogenes (1900 CFU/g) in Manouri sheep's cheese from Greece
96	information for attention	09/05/2012	24/05/2012	2012.063	Sweden	food	milk and milk products	Listeria monocytogenes (
97	information for follow-up	30/04/2012	16/01/2014	2012.06	Germany	food	milk and milk products	Mycobacterium tuberculosis (suspicion) in chilled raw milk from Germany
98	alert	25/04/2012	07/02/2013	2012.0586	France	food	milk and milk products	Listeria monocytogenes (210 CFU/g) in raw milk sheep cheese coated with herbs from France
99	information for attention	16/04/2012	25/04/2012	2012.0541	France	food	milk and milk products	Brucella in raw cow's milk cheese from France
100	information for attention	13/04/2012	16/04/2012	2012.0534	Italy	food	milk and milk products	Salmonella spp. (presence /25g) in buffalo mozzarella from Italy
101	alert	06/04/2012	04/05/2012	2012.0507	Italy	food	milk and milk products	food poisoning suspected to be caused by semi-skimmed milk from Germany
102	information for attention	24/02/2012	06/06/2012	2012.0288	Germany	food	milk and milk products	shigatoxin-producing Escherichia coli (O17:H18 stx1+, stx2-, eae- /25g) in cheese from France
103	information for attention	10/02/2012	03/08/2012	2012.0222	Belgium	food	milk and milk products	Salmonella spp. and Cronobacter sakazakii in dried milk formulae from Belgium
104	alert	26/01/2012	31/01/2012	2012.014	Ireland	food	milk and milk products	Listeria monocytogenes (4,300 CFU/g) in blue cheese from Ireland
105	alert	17/01/2012	09/02/2012	2012.0094	Belgium	food	milk and milk products	foodborne outbreak (Salmonella Oranienburg) caused by dried milk formula from Belgium
106	alert	30/12/2011	26/01/2012	2011.1955	France	food	milk and milk products	Salmonella typhimurium (presence /25g) in raw milk brie and camembert from France
107	alert	05/12/2011	05/01/2012	2011.1775	Austria	food	milk and milk products	Listeria monocytogenes (presence /25g) in cheese from Austria
108	information for follow-up	02/12/2011	12/12/2011	2011.1771	Slovakia	food	milk and milk products	Listeria monocytogenes (presence /25g) in cheese from Slovakia
109	alert	21/11/2011	22/11/2011	2011.1683	Italy	food	milk and milk products	Listeria monocytogenes (4400 CFU/g) in chilled gorgonzola from Italy

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110	alert	27/10/2011	20/12/2011	2011.151	France	food	milk and milk products	Listeria monocytogenes (6500 CFU/g) in munster cheese from France
111	alert	21/10/2011	22/02/2012	2011.1491	France	food	milk and milk products	Listeria monocytogenes (>330000 CFU/g) in cheese from France
112	alert	21/10/2011	22/12/2011	2011.1489	France	food	milk and milk products	Listeria monocytogenes (150 CFU/g) in chilled gorgonzola from Italy
113	information for attention	14/10/2011	14/10/2011	2011.1427	Austria	food	milk and milk products	Listeria monocytogenes (41000 CFU/g) in chilled gorgonzola cheese from Italy
114	alert	13/10/2011	08/11/2011	2011.1421	France	food	milk and milk products	Listeria monocytogenes (
137	information	10/11/2010	10/11/2010	2010.1539	France	food	milk and milk products	Listeria monocytogenes (6100; 990 CFU/g) in cheese from France
138	alert	08/11/2010	24/01/2011	2010.1522	Belgium	food	milk and milk products	Listeria monocytogenes (presence /25g) in organic goat's cheese with bacon from Belgium
139	information	03/11/2010	03/11/2010	2010.1498	Germany	food	milk and milk products	verotoxin producing Escherichia coli (presence /25g) in cheese made from raw milk from France
140	information	20/10/2010	26/11/2010	2010.1423	Italy	food	milk and milk products	Pseudomonas aeruginosa (in 3 samples) and Pseudomonas fluorescens (in 2 samples) in cow's milk mozzarella from Poland
141	alert	06/09/2010	29/10/2010	2010.1205	Italy	food	milk and milk products	Salmonella Blockley (serotype C2 /25g) in mozzarella light cheese made from cow's milk from Germany
142	information	31/08/2010	20/09/2010	2010.119	Italy	food	milk and milk products	Pseudomonas aeruginosa (1.7*6; 1*6;1.1*4 CFU/g) in mozzarella from Poland
143	information	09/08/2010	19/10/2010	2010.1096	France	food	milk and milk products	Listeria monocytogenes (3600 CFU/g) in gorgonzola cheese from Italy
144	information	05/08/2010	14/03/2011	2010.1077	Greece	food	milk and milk products	high count of Escherichia coli (between 1500000 CFU/g) in mozzarella from Italy
145	information	04/08/2010	04/08/2010	2010.1074	Czech Republic	food	milk and milk products	Listeria monocytogenes (presence in 1 out of 5 samples /25g) in cheese from the Czech Republic
146	alert	30/07/2010	30/07/2010	2010.1059	Germany	food	milk and milk products	Salmonella Dublin (presence /25g) in cheeses made from raw cow's milk from Germany
147	information	15/07/2010	18/08/2010	2010.0973	Austria	food	milk and milk products	Escherichia coli (13000000; 4300000; 86000000; 64000000; 15000000 CFU/g) and Listeria monocytogenes in cheese made from raw milk from Austria
148	information	09/07/2010	09/07/2010	2010.0941	Italy	food	milk and milk products	Pseudomonas fluorescens (40 CFU/g) in soft cheese from Germany

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149	alert	08/07/2010	09/07/2010	2010.0918	Ireland	food	milk and milk products	Listeria monocytogenes (290 CFU/g) in Lavistown cheese from Ireland
150	alert	06/07/2010	09/07/2010	2010.0897	Italy	food	milk and milk products	Bacillus cereus (27xE6 CFU/g) in ricotta from Italy
151	alert	29/06/2010	12/08/2010	2010.0863	France	food	milk and milk products	Salmonella Mbandaka (presence /25g) in cow's milk camembert and brie from France
152	information	21/06/2010	22/06/2010	2010.0816	Italy	food	milk and milk products	altered organoleptic characteristics (blue coloured: 1 mm) of and Pseudomonas fluorescens (3000000 * 1000 CFU/g) in mozzarella cheese from Germany
153	information	18/06/2010	05/07/2010	2010.0807	Italy	food	milk and milk products	altered organoleptic characteristics (blue) of and Pseudomonas fluorescens (5100000 CFU/g) in mozzarella cheese from Germany
154	alert	09/06/2010	17/08/2010	2010.0746	Italy	food	milk and milk products	Bacillus cereus (300 CFU/g) in and altered organoleptic characteristics (blue coloured) of mozzarella from Germany
155	alert	09/06/2010	09/06/2010	2010.074	Poland	food	milk and milk products	Listeria monocytogenes (presence /25g) in gouda cheese from Poland
156	information	28/05/2010	13/10/2010	2010.0675	Austria	food	milk and milk products	Listeria monocytogenes (500 CFU/g) in peppers filled with goat cheese from Austria
157	alert	27/05/2010	23/06/2010	2010.0662	Romania	food	milk and milk products	Escherichia coli (between 95 and 1400 CFU/g) in cheese from Bulgaria
158	information	14/05/2010	03/06/2010	2010.0595	Ireland	food	milk and milk products	Listeria monocytogenes (
159	alert	04/03/2010	19/04/2010	2010.0271	Germany	food	milk and milk products	Salmonella enteritidis (presence /25g) in raw milk cheese from France
160	alert	22/01/2010	23/06/2010	2010.0071	France	food	milk and milk products	Listeria monocytogenes (
161	alert	22/01/2010	24/03/2010	2010.0073	Austria	food	milk and milk products	Listeria monocytogenes (< 10 CFU/g) in syrečky cheese (Quargel Käse) from Austria
162	alert	07/01/2010	07/01/2010	2010.0019	France	food	milk and milk products	Listeria monocytogenes (3600 CFU/g) in cheese tray from France
163	alert	07/12/2009	13/01/2010	2009.1692	Poland	food	milk and milk products	Listeria monocytogenes (presence in 3 out of 5 samples /25g) in cheese products from Poland
164	information	20/11/2009	17/02/2012	2009.1611	Italy	food	milk and milk products	Salmonella spp. in buffalo milk mozzarella cheese from Italy
165	alert	06/11/2009	06/11/2009	2009.1525	Netherlands	food	milk and milk products	Salmonella in dried dairy powder from the Netherlands

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