



Review

A review of the microbiological hazards of dairy products made from raw milk



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ABSTRACT

This review concentrates on information concerning microbiological hazards possibly present in raw milk dairy products, in particular cheese, butter, cream and buttermilk. The main microbiological hazards of raw milk cheeses (especially soft and fresh cheeses) are linked to *Listeria monocytogenes*, verocytotoxin-producing *Escherichia coli* (VTEC), *Staphylococcus aureus*, *Salmonella* and *Campylobacter*. *L. monocytogenes*, VTEC and *S. aureus* have been identified as microbiological hazards in raw milk butter and cream albeit to a lesser extent because of a reduced growth potential compared with cheese. In endemic areas, raw milk dairy products may also be contaminated with *Brucella* spp., *Mycobacterium bovis* and the tick-borne encephalitis virus (TBEV). Potential risks due to *Coxiella burnetii* and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) are discussed. Pasteurisation ensures inactivation of vegetative pathogenic microorganisms, which increases the safety of products made thereof compared with dairy products made from raw milk. Several control measures from farm to fork are discussed.

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1. Introduction

Consumers' attitudes show a trend towards increased consumption of foods that are not or only minimally processed such as raw milk and dairy products made from raw milk. Cheeses made from raw cow, sheep and goat milk are the most frequently

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consumed type of dairy products made from raw milk; however, other types of dairy products made from raw milk are also consumed such as mozzarella made from raw buffalo milk, as well as raw milk butter made from cow milk. The consumption of raw milk can hold a risk for the consumer, due to the possible presence of human pathogenic microorganisms. The risks and benefits of the consumption of raw cow milk were described in a review by [Claeys et al. \(2013\)](#) and the nutritional and health benefits of the consumption of raw milk from animal species other than cows were described in a review by [Claeys et al. \(2014\)](#). The microbiological hazards of raw milk from animal species other than cows were described in a review by [Verraes et al. \(2014\)](#). Also EFSA evaluated the public health risk from the consumption of raw drinking milk ([EFSA, 2015](#)).

In the present review, a collation is made of available information in the scientific literature concerning the microbiological hazards of dairy products made from raw milk, in particular cheese, butter, cream and buttermilk. Dairy products made from raw milk from cows, sheep and goats are taken into account. Dairy products made from raw milk from other animal species are less relevant in Europe, with the exception of mozzarella made from raw buffalo milk. Only zoonotic human pathogenic microorganisms and pathogens originating from the environment have been taken into consideration. Pathogenic agents originating from human contamination due to human illness, e.g., *Salmonella* Typhi, *Shigella* spp. and noroviruses are not covered in this review.

2. Occurrence of human pathogenic microorganisms in raw milk and dairy products made from raw milk

In general, pathogenic microorganisms can contaminate raw milk in two ways. The first way is an endogenous contamination where the milk is contaminated by a direct transfer from the blood (systemic infection) to the milk or via an infection in the udder called mastitis. The second way is an exogenous contamination, where the milk is contaminated during or after milking by the faeces, the exterior of the udder and teats, the skin, the environment, etc.

Based on recent review documents ([Claeys et al., 2013](#); [EFSA, 2015](#); [Sci Com, 2011, 2013](#); [Verraes et al., 2014](#)), the following human pathogenic microorganisms are considered as the main microbiological hazards associated with raw milk consumption from cows, goats and sheep: *Bacillus cereus*, *Campylobacter coli* and *Campylobacter jejuni*, enterotoxin-producing *Staphylococcus aureus*, *Helicobacter pylori*, human pathogenic verocytotoxin-producing *Escherichia coli* (VTEC), human pathogenic *Yersinia*, *Leptospira*, *Listeria monocytogenes*, *Salmonella* spp., *Streptococcus agalactiae*, *Streptococcus equi* subsp. *zooepidemicus*, *Clostridium botulinum*, *Brucella* spp., *Mycobacterium bovis*, *Cryptosporidium parvum* and *Toxoplasma gondii*. Some microbiological hazards are indicated as hypothetical because they are still not confirmed, but have the hypothetical potential to be pathogenic for humans (*Mycobacterium avium* subsp. *paratuberculosis*; MAP) or to be foodborne (*Coxiella burnetii*).

Limited systematic data were available on prevalences of human pathogenic microorganisms in dairy products made from raw milk. In a baseline study ([EFSA, 2013b](#)) performed at a European level, 1 sample from 476 raw milk cheese samples exceeded 100 cfu g⁻¹ for *L. monocytogenes*. The EU summary report mentioned that non-compliance of *L. monocytogenes* primarily occurred in soft and semi-soft cheeses made from raw or low heat-treated cows' milk ([EFSA & ECDC, 2015](#)). In addition, studies in the scientific literature describing detection frequencies of human pathogenic microorganisms in dairy products made from raw milk provide an indication of prevalences. Data of such frequencies of occurrence were

collected for Europe and are shown in [Table 1](#), where the detection frequency, the type of food, the method of analysis and the country are given.

Frequencies of occurrence were found for several raw milk cheeses, butter and cream. No frequencies were found for buttermilk made from raw milk as such products are less often produced. No *Salmonella* has been detected in any tested samples of raw milk cheeses, butter and cream with the exception of one publication where the prevalence in cheese was 4.3% (n = 70 samples; [Almeida et al., 2007](#)). Concerning VTEC in raw milk cheeses, *E. coli* strains with virulence genes are detected with frequencies between 0 and 55.3%. [Farrokh et al. \(2013\)](#) stated that the prevalence of *vtx* genes, detected by PCR, does not necessarily reflect the occurrence of a viable *E. coli* isolate containing those genes. VTEC was also detected in butter ([Messelhäuser, Beck, Gallien, Schalch, & Busch, 2008](#)). As illustrated in [Table 1](#), *L. monocytogenes* was detected in raw milk cheeses, butter and cream with frequencies varying between 0 and 41.9%, 3.6 and 29.9%, and 0.7 and 8.3% respectively, although in some cases the numbers were lower than 100 cfu g⁻¹. Of 70 cheese samples, 11.4% were positive for *L. monocytogenes* per 25 g, whereby 1.4% contained counts higher than 200 cfu g⁻¹ and 10.0% lower than 100 cfu g⁻¹ ([Almeida et al., 2007](#)). Of 474 cheese samples and 519 butter samples, respectively, 0.6% and 0.2% of the samples that were taken in Belgium by the sector of small cheese producers in the Walloon region had counts higher than 100 cfu g⁻¹ ([Sci Com, 2015](#)). No *Campylobacter* were detected in 199 tested raw milk cheeses, which contrasts the higher reporting in raw milk (0–12%) ([EFSA, 2015](#); [Verraes et al., 2014](#)). As indicated in [Table 1](#), *S. aureus* was found in raw milk products with frequencies between 5 and 100% in cheeses, and between 1.6 and 20.3% in butter, but the possibility of the strains to produce enterotoxins varies and the share of such strains is difficult to deduce from the available information. [Jørgensen, Mørk, and Rørvik \(2005\)](#) also detected *S. aureus* in sour cream. *B. cereus* was detected in 28% of 25 samples of cheeses made from raw milk of several animal species. All strains were able to produce enterotoxins ([Williams & Withers, 2010](#)). MAP was detected in 4.2 and 20% of raw milk cheese samples using PCR ([Stephan, Schumacher, Tasara, & Grant, 2007](#); [Williams & Withers, 2010](#)). *C. burnetii* was detected with PCR in 57.0% of unpasteurised cheese samples and in one of two cream samples, but the relation of these PCR results with the presence of infectious strains is not clear ([Eldin, Angelakis, Renvoisé, & Raoult, 2013](#)).

3. Reported human cases and outbreaks due to the consumption of dairy products made from raw milk

The development of a disease after consumption of contaminated dairy products made from raw milk depends on several factors such as the pathogenicity of the strain, the number of ingested microorganisms, the physiological state of the microorganism, the health condition of the consumer at the moment of ingestion, etc. Persons belonging to the YOPI group (young, old, pregnant, immunodeficient) have a higher risk of infection for certain pathogens such as *L. monocytogenes* and healthy persons can also be infected. The likelihood of developing a disease follows a dose–response curve with a higher number of cells increasing the chance of developing a disease. This relationship was categorised by the EFSA BIOHAZ Panel in the framework of a risk ranking exercise of foods of non-animal origin as follows: score 1 stands for a dose–response curve where the pathogen has to grow to high numbers (often higher than 5 log cfu g⁻¹) to produce toxins and cause illness; score 2 stands for a dose–response curve where the pathogen must grow to cause illness; and score 3 stands for a dose–response curve where the pathogen can cause illness in low numbers ([Da Silva et al., 2015](#); [EFSA, 2013a](#)). *Salmonella*,

Table 1
Reported frequencies of occurrence of human pathogenic microorganisms in/on dairy products made from raw milk in Europe.^a

| Dairy product made from raw milk | Frequency of occurrence (%) | Number of samples | Comments | Country | References | |
|--|-----------------------------|--|---|-----------------------------|---|---------------------------|
| <i>Salmonella</i> | | | | | | |
| Cheese | 0.0 | 71 | | Belgium | De Reu, Debeuckelaere, Botteldoorn, De Block, and Herman (2002) | |
| | 0.0 | 20 | | Belgium | De Reu, Grijspeerdt, and Herman (2004) | |
| | 0.0 | 334 | | Germany | Hahn, Walte, Coenen, and Teufel (1999a) | |
| | 0.0 | 75 | | Ireland | Coveney, Fitzgerald, and Daly (1994) | |
| | 4.3 | 70 | | Portugal | Almeida et al. (2007) | |
| | 0.0 | 25 | | Scotland | Williams and Withers (2010) | |
| | 0.0 | 429 | | Belgium | DiversiFerm (pers. comm.) | |
| | 0.0 | 582 | | Belgium | FASFC (pers. comm.) | |
| | Butter | 0.0 | 64 | | Belgium | De Reu et al. (2004) |
| | | 0.0 | 518 | | Belgium | DiversiFerm (pers. comm.) |
| 0.0 | | 192 | | Belgium | FASFC (pers. comm.) | |
| Cream | 0.0 | 132 | | Belgium | FASFC (pers. comm.) | |
| <i>Verocytotoxin-producing Escherichia coli (VTEC)</i> | | | | | | |
| Cheese | 5.6 | 71 | IMS, VIDAS O157 assay and PCR | Belgium | De Reu et al. (2002) | |
| | 0.0 | 20 | IMS, VIDAS O157 assay and PCR | Belgium | De Reu et al. (2004) | |
| | 0.0 | 334 | 1.5% VTEC | Germany | Hahn et al. (1999a) | |
| | 4.0 | 25 | Enrichment; 1 <i>stx2</i> -positive strain | Italy | Volponi et al. (2012) | |
| | 13.1 | 1039 | PCR | France | Vernozy-Rozand, Montet, Berardin, Bavai, and Beutin (2005) | |
| | 5.7 | 1502 | PCR | Switzerland | Zweifel et al. (2010) | |
| | 0.0 | 739 | IMS | Scotland | Coia, Johnston, Steers, and Hanson (2001) | |
| | 8.9 | 180 | - Vero cell assay | France | Fach et al. (2001) | |
| | 30.6 | | - PCR-ELISA | | | |
| | 4.8 | 83 | Isolation | Spain | Caro and García-Armesto (2007) | |
| | 30.4 | 112 | - PCR-ELISA | Italy | Auvray, Lecureuil, Dilasser, Taché, and Derzelle (2009) | |
| | 27.7 | | - RT-PCR | | | |
| | 2.7 | | - Colony hybridisation | | | |
| | 4.5 | 143 | - PCR <i>vtx1</i> gene | Italy | Civera et al. (2007) | |
| | 3.8 | | - PCR <i>vtx2</i> gene | | | |
| | 0.8 | | - PCR <i>eae</i> gene | | | |
| | 2.1 | | - PCR <i>ehxA</i> gene | | | |
| | 0.0 | | - Isolation | | | |
| | 0.0 | 1313 | Isolation of <i>E. coli</i> O157; also butter | Italy | Conedera et al. (2004) | |
| | 4.9 | - 796 | - PCR | Switzerland | Stephan et al. (2008) | |
| | 41.0 | - 39 | - Non-O157 VTEC strains | | | |
| | 0.0 | 70 | IMS | Portugal | Almeida et al. (2007) | |
| | 29.8 | 400 | - PCR <i>vtx</i> gene | France | Madic et al. (2011) | |
| 37.3 | | - PCR <i>eae</i> gene | | | | |
| 55.3 | | - At least one of O26, O103, O111, O145 and O157 genetic markers | | | | |
| 6.5 | | - Combinations of above | | | | |
| 0.0 | 25 | Isolation of <i>E. coli</i> O157 | Scotland | Williams and Withers (2010) | | |
| 10.0 | 10 | Also butter | Germany | Messelhäuser et al. (2008) | | |
| 0.8 | 1042 | PCR | Belgium | FASFC (pers. comm.) | | |
| Butter | 0.0 | 64 | IMS, VIDAS O157 assay and PCR | Belgium | De Reu et al. (2004) | |
| | 0.0 | 366 | PCR | Belgium | FASFC (pers. comm.) | |
| Cream | 0.0 | 131 | PCR | Belgium | FASFC (pers. comm.) | |
| | 0.0 | | | | | |
| <i>Listeria monocytogenes</i> | | | | | | |
| Cheese | 0.0 | 55 | | Sweden | Rosengren et al. (2010) | |
| | 0.8 | 122 | | Norway | Jakobsen, Heggebo, Sunde, and Skjervheim (2011) | |
| | 2.8 | 71 | | Belgium | De Reu et al. (2002) | |
| | 0.0 | 20 | | Belgium | De Reu et al. (2004) | |
| | 2.4 | 334 | | Germany | Hahn et al. (1999a) | |
| | 0.0 | 17 | | Ireland | Coveney et al. (1994) | |
| | 11.4 | 70 | 1.4% > 2.3 log cfu g ⁻¹ ; 10.0% < 2 log cfu g ⁻¹ | Portugal | Almeida et al. (2007) | |
| | 19.0 | 42 | | Italy | Rantsiou, Alessandria, Urso, Dolci, and Cocolin (2008) | |
| | 0.0 | 252 | | Portugal | Kongo, Malcata, Ho, and Wiedmann (2006) | |

Table 1 (continued)

| Dairy product made from raw milk | Frequency of occurrence (%) | Number of samples | Comments | Country | References | |
|--|-----------------------------|-------------------|---|----------------------------|--|----------------------|
| Butter | 0.0 | 51 | | Spain | Arrese and Arroyo-Izaga (2012) | |
| | 4.0 | 25 | | Scotland | Williams and Withers (2010) | |
| | 41.9 | 31 | | Sweden | Loncarevic et al. (1995) | |
| | 0.0 | 230 | | Austria | Schoder et al. (2011) | |
| | 12.2 | 474 | 0.6% > 2 log cfu g ⁻¹ | Belgium | DiversiFerm (pers. comm.) | |
| | 6.0 | 901 | | Belgium | FASFC (pers. comm.) | |
| | 18.7 | 64 | | Belgium | De Reu et al. (2004) | |
| | 29.9 | 519 | 0.2% > 2 log cfu g ⁻¹ | Belgium | DiversiFerm (pers. comm.) | |
| | 12.4 | 259 | | Belgium | De Reu and Herman (2004); De Reu, Herman, De Boosere, and De Ville (2006, 2007); De Reu, Herman, and De Ville (2008) | |
| | Cream | 3.6 | 361 | | Belgium | FASFC (pers. comm.) |
| 5.0 | | 20 | | Belgium | De Reu and Herman (2004) | |
| 8.3 | | 60 | | Croatia | Frece, Markov, Cvek, Kolarec, and Delas (2010) | |
| 4.2 | | 48 | | England & Wales | Greenwood et al. (1991) | |
| <i>Campylobacter</i> Cheese | 0.0 | 66 | | Ireland | Whyte et al. (2004) | |
| | 0.0 | 10 | | Germany | Messelh usser et al. (2008) | |
| | 0.0 | 123 | | Belgium | FASFC (pers. comm.) | |
| <i>Staphylococcus aureus</i> Cheese | 69.0 | 55 | Coagulase-positive staphylococci; 10.9% > 5 log cfu g ⁻¹ ; 0% enterotoxins | Sweden | Rosengren et al. (2010) | |
| | 67.5 | 122 | Average of cheese stages | Norway | Jakobsen et al. (2011) | |
| | 19.7 | 71 | >3 log cfu g ⁻¹ ; 7.1% (14) Enterotoxins | Belgium | De Reu et al. (2002) | |
| | 5.0 | 20 | 0% Enterotoxins | Belgium | De Reu et al. (2004) | |
| | 13.1 | 245 | >4 log cfu g ⁻¹ | Germany | Hahn, Walte, Coenen, and Teufel (1999b) | |
| | 100.0 | 33 | 42.4% Enterotoxins | Italy | Cremonesi et al. (2007) | |
| | 80.4 | 46 | Average of cheese stages; 100% enterotoxins | Italy | Poli et al. (2007) | |
| | 36.0 | 25 | 7 of 18 Examined strains from 2 cheeses able to produce enterotoxin C | Scotland | Williams and Withers (2010) | |
| | Butter | 8.3 | 737 | >5 log cfu g ⁻¹ | Belgium | FASFC (pers. comm.) |
| | | 1.6 | 64 | 0% Enterotoxins | Belgium | De Reu et al. (2004) |
| 20.3 | | 281 | | Belgium | FASFC (pers. comm.) | |
| <i>Bacillus cereus</i> Cheese | 28.0 | 25 | 100% Enterotoxins | Scotland | Williams and Withers (2010) | |
| | 0.0 | 3 | | Belgium | FASFC (pers. comm.) | |
| <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> Cheese | 4.2 | 143 | PCR; no viable cells | Switzerland | Stephan et al. (2007) | |
| | 20.0 | 25 | PCR | Scotland | Williams and Withers (2010) | |
| <i>Coxiella burnetii</i> Cheese | 57.0 | 68 | | France | Eldin et al. (2013) | |
| | 0.0 | Not given | | France | Eldin et al. (2013) | |
| | 50.0 | 2 | | France | Eldin et al. (2013) | |

^a Abbreviations are: IMS, immunomagnetic separation; PCR, polymerase chain reaction; PCR-ELISA, polymerase chain reaction enzyme-linked immunosorbent assay.

Campylobacter, VTEC and the tick-borne encephalitis virus (TBEV) were in the framework of the present study allocated a score 3; *L. monocytogenes*, *Brucella*, *M. bovis* and pathogenic species of *Streptococcus* have to grow to cause illness and have score 2; and *S. aureus* and *B. cereus* have to grow to numbers of several log units to be able to produce sufficient toxins to cause illness and therefore they have a score of 1. For MAP and *C. burnetii* the foodborne and zoonotic potential is not known.

The consumption of dairy products made from raw milk has already caused several human cases and outbreaks. In Table 2, a

non-exhaustive list is shown of reported human cases and outbreaks in Europe, the United States and Canada related to the consumption of dairy products made from raw milk found in scientific literature or reported by official organisations. Only outbreaks due to the consumption of raw milk cheeses were found and one outbreak was ascribed to the consumption of raw milk cream linked to infection with *E. coli* O157:H7 (CDSC, 1998a). Outbreaks due to the consumption of raw milk butter or buttermilk were not found. From a report from EFSA (EFSA & ECDC, 2014), it seems that in 2012 no outbreaks were reported in the EU due to the

Table 2
Reported human cases and outbreaks in Europe, the United States and Canada due to the consumption of dairy products made from raw milk.

| Pathogenic agent | Dairy product made from raw milk | Country | Year | Cases | References |
|---|--|------------------------|-----------|--|---|
| <i>Salmonella enteric</i> Muenster | Cheddar cheese from unpasteurised milk | Canada | 1982 | Not given | D'Aoust, Warburton, and Sewell (1985); van Cauteren et al. (2009) |
| <i>Salmonella</i> Typhimurium PT10 | Cheddar cheese from unpasteurised milk | Canada | 1984 | >1700 confirmed cases | D'Aoust et al. (1985) |
| <i>Salmonella</i> | Farm raw milk cheese | Finland | 1985 | 35 cases | Huchot, Bohnert, Cerf, Farrok, and Lahellec (1993) |
| <i>Salmonella</i> Typhimurium | Raw milk vacherin mont d'or cheese | Switzerland | 1985 | 215 cases | Anonymous (1986) |
| <i>Salmonella</i> Dublin | Soft stilton cheese from unpasteurised milk | England and Wales | 1989 | 42 cases | Maguire et al. (1992) |
| <i>Salmonella</i> Paratyphi B | Raw goat milk cheese | France | 1990 | 277 cases | Grimont and Bouvet (1991) |
| <i>Salmonella</i> Paratyphi B | Raw goat milk cheese | France | 1993 | 273 cases, 1 dead | Desenclos et al. (1996) |
| <i>Salmonella</i> Berta | Farm soft cheese from unpasteurised milk | Canada | 1994 | 35 confirmed cases | Ellis et al. (1998) |
| <i>Salmonella</i> Dublin | Raw cow milk mont d'or cheese | France and Switzerland | 1995 | 25 cases, 12 hospitalised, 5 dead | Vaillant et al. (1996) |
| <i>Salmonella</i> Dublin | Raw cow milk mont d'or cheese | France | 1996 | 14 cases, 1 dead | Infuso, Vaillant, and Desenclos (1997) |
| <i>Salmonella</i> Typhimurium PT12 atypical | Raw milk morbier cheese | France | 1997 | 113 cases | De Valk et al. (2000) |
| <i>Salmonella</i> Typhimurium var Copenhagen phage type DT104 (outbreak 1) and non-var Copenhagen phage type DT 104b (outbreak 2) | Unpasteurised Mexican-style cheese | United States | 1997 | 31 confirmed cases (outbreak 1); 79 cases (outbreak 2) | Cody et al. (1999) |
| <i>Salmonella</i> Typhimurium DT104 | Unpasteurised Mexican-style cheese | United States | 1997 | 54 confirmed cases | Villar et al. (1999) |
| <i>Salmonella</i> Newport | Raw milk cheese | United States | 2001 | 27 cases, 12 hospitalised | CDC (2014) |
| <i>Salmonella</i> Enteritidis phage type 8 | Raw milk cantal cheese | France | 2001 | 215 cases | Haeghebaert et al. (2003) |
| <i>Salmonella</i> Typhimurium | Unpasteurised queso-fresco cheese | United States | 2003 | 50 cases, 7 hospitalised | CDC (2014) |
| <i>Salmonella</i> Typhimurium | Unpasteurised queso-fresco cheese | United States | 2004 | 12 cases, 1 hospitalised and 1 dead | CDC (2014) |
| <i>Salmonella</i> | Unpasteurised queso-fresco cheese | United States | 2005 | 2 cases | CDC (2014) |
| <i>Salmonella</i> Typhimurium | Unpasteurised queso-fresco cheese | United States | 2006 | 20 cases, 2 hospitalised | CDC (2014) |
| <i>Salmonella enterica</i> Newport | Unpasteurised Mexican-style aged cheese | United States | 2006–2007 | 85 cases (but no significant association) | CDC (2008) |
| <i>Salmonella</i> Typhimurium | Raw milk cheese and raw milk | United States | 2007 | 29 cases | CDC (2007) |
| <i>Salmonella enteric</i> Muenster | Unpasteurised goat cheese | France | 2008 | 25 laboratory-confirmed cases, 4 hospitalised | van Cauteren et al. (2009) |
| <i>Escherichia coli</i> O119:B14 (vtx2) | Farm fresh cheese from raw cow and goat milk | France | 1992–1993 | 4 cases, 4 HUS, 1 dead | Casenave et al. (1993) |
| <i>E. coli</i> O157 (PT28, vtx2) | Farm raw milk cheese | Scotland | 1994 | 22 cases, 1 HUS | Ammon (1997) |
| <i>E. coli</i> O103 | Farm fresh cheese from raw goat milk | France | 1994 | 4 cases, 4 HUS | Decludt (1995) |
| <i>E. coli</i> O110:H- | Raw milk cheese | Germany | 1996 | 3 cases, 0 HUS | Bockemühl and Karch (1996) |
| <i>E. coli</i> O157:H7 | Fresh cheddar cheese curds | United States | 1998 | 63 cases, 55 laboratory-confirmed, 24 hospitalised | CDC (2000) |
| <i>E. coli</i> O157:H7 | Unpasteurised cream | England | 1998 | 7 cases | CDSC (1998a) |
| <i>E. coli</i> O157 (PT2, vtx2) | Cheese from unpasteurised milk | England | 1998 | 10 cases, 1 HUS case | CDSC (1998b) |
| <i>E. coli</i> O157 | Unpasteurised farm cheese | Scotland | 1998 | 4 cases | Strachan et al. (2006) |
| <i>E. coli</i> O157 (PT21, PT28) | Homemade raw goat milk cheese | Scotland | 1999 | 27 cases, 1 HUS | Curnow (1999) |
| <i>E. coli</i> O157:H7 | Unpasteurised gouda hard cheese | Canada | 2002–2003 | 13 cases, 2 HUS | Honish et al. (2005) |
| <i>E. coli</i> O157:H7 | Unpasteurised queso-fresco cheese | United States | 2004 | 3 cases, 2 hospitalised | CDC (2014) |
| <i>E. coli</i> O157:H7 | Raw milk cheese | Canada | 2004 | 3 cases | MAPAQ (2004) |
| <i>E. coli</i> O157 (vtx2, eae) | Farm fresh unpasteurised goat cheese | France | 2004 | 3 cases, 2 HUS | Espié et al. (2006) |
| <i>E. coli</i> O26, O80 (vtx2, eae) | Raw milk brie | France | 2005 | 6 HUS | INVS (2007) |
| <i>E. coli</i> O157:H7 | Raw milk cheese | Canada | 2008–2009 | 16 cases | Gaulin et al. (2012) |
| <i>E. coli</i> O157:H7 | Aged raw milk gouda cheese | United States | 2010 | 38 cases, 15 hospitalised | McCullum et al. (2012) |

Table 2 (continued)

| Pathogenic agent | Dairy product made from raw milk | Country | Year | Cases | References |
|--|--|---------------|-----------|--|---|
| <i>E. coli</i> O157:H7, <i>E. coli</i> O157:NM(H-) | Unpasteurised cheeses | United States | 2010 | 15 cases | CDC (2014) |
| <i>E. coli</i> O157:H7 and <i>Listeria monocytogenes</i> | Unpasteurised gouda cheese | United States | 2010 | 38 cases, 15 hospitalised, 1 HUS | CDC (2014) |
| <i>Listeria</i> | Vacherin mont d'or cheese from unpasteurised milk | Switzerland | 1983–1987 | 122 cases, 33 dead | Bille (1989) |
| <i>Listeria meningitis</i> | Anari raw goat milk soft cheese | England | 1988 | 1 case | Azadian, Finnerty, and Pearson (1989) |
| <i>Listeria monocytogenes</i> Serovar 4b | Raw milk brie de meauw cheese | France | 1995 | 36 cases, 4 dead, 7 foetal/neonatal cases | Vaillant, Maillot, Charley, and Stainer (1998) |
| <i>Listeria monocytogenes</i> Serovar 4b | Raw milk livarot, pont-l'évêque cheese | France | 1997 | 14 cases | Jacquet, Saint-Clément, Brouille, Catimel, and Rocourt (1998) |
| <i>Listeria monocytogenes</i> | Fresh Mexican-style raw milk cheese | United States | 2000–2001 | 13 cases, 11 pregnant women (5 stillbirths, 3 premature deliveries, 3 infected newborns) | CDC (2001); MacDonald et al. (2005) |
| <i>Listeria monocytogenes</i> | Unpasteurised queso-fresco cheese | United States | 2003 | 12 cases, 12 hospitalised, 1 dead | CDC (2014) |
| <i>Listeria monocytogenes</i> | Unpasteurised queso-fresco cheese | United States | 2005 | 12 cases, 12 hospitalised, 0 dead | CDC (2014) |
| <i>Brucella melitensis</i> | Home-made fresh cottage cheese from unpasteurised milk | Spain | 1996 | 81 cases | Castell Monsalve, Rullán, and CallizoNiet-Sandoval Alcolea (1996) |
| <i>Brucella</i> | Homemade unpasteurised cheese | United States | 2001 | 4 cases, 0 dead | CDC (2014) |
| <i>Brucella melitensis</i> serovar 3 | Unpasteurised raw goat milk cheese | Spain | 2002 | 11 brucellosis cases | Méndez Martínez et al. (2003) |
| <i>Brucella</i> | Unpasteurised queso-fresco cheese | United States | 2005 | 2 cases, 2 hospitalised, 0 dead | CDC (2014) |
| <i>Brucella</i> | Unpasteurised goat cheese | United States | 2006 | 5 cases, 3 hospitalised, 0 dead | CDC (2014) |
| <i>Brucella</i> | Unpasteurised queso-fresco cheese | United States | 2007 | 3 cases, 1 hospitalised, 0 dead | CDC (2014) |
| <i>Brucella abortus</i> | Raw milk cheese | France | 2012 | 1 case (child) | Bronner, Hénaux, Fortané, Hendrikx, and Calavas (2014) |
| <i>Campylobacter jejuni</i> | Homemade unpasteurised cheese | United States | 2000 | 18 cases, 0 hospitalised, 0 dead | CDC (2014) |
| <i>Campylobacter jejuni</i> | Unpasteurised cheese | United States | 2003 | 18 cases, 0 hospitalised, 0 dead | CDC (2014) |
| <i>Campylobacter jejuni</i> | Homemade unpasteurised cheese | United States | 2006 | 58 cases, 2 hospitalised, 0 dead | CDC (2014) |
| <i>Campylobacter jejuni</i> | Fresh raw milk cheese | United States | 2007 | 68 cases | CDC (2009) |
| <i>Campylobacter jejuni</i> | Unpasteurised queso-fresco cheese | United States | 2009 | 10 cases, 0 hospitalised, 0 dead | CDC (2014) |
| <i>Staphylococcus aureus</i> enterotoxins A and D | Farm raw sheep milk cheese | France | 1983 | 20 cases | De Buyser, Janin, and Dilasser (1985) |
| <i>Staphylococcus aureus</i> enterotoxins A | Raw sheep milk cheese | Scotland | 1984 | 27 cases | Bone, Bogie, and Morgan-Jone (1989) |
| <i>Staphylococcus aureus</i> | Stilton cheese from unpasteurised milk | England | 1988 | 155 cases | Maguire et al. (1991) |
| Staphylococcal enterotoxin type E | Soft cheese from unpasteurised milk | France | 2009 | 6 outbreaks, 23 cases | Ostyn et al. (2010) |
| <i>Streptococcus equi</i> , <i>Streptococcus zooepidemicus</i> (group C) | Mexican-style soft raw milk cheese (queso fresco) | United States | 1983 | 16 cases, 5 hospitalised, 3 dead | Altekruse et al. (1998); CDC (1983) |
| Tick-borne encephalitis virus | Cheese from raw cow and goat milk | Austria | 2008 | 6 cases | Holzmann et al. (2009) |

consumption of dairy products made from raw milk and in 2013 one outbreak was reported due to VTEC O157 contamination of unpasteurised cheese (EFSA & ECDC, 2015).

The majority of the outbreaks (22 out of 64) due to the consumption of raw milk cheeses was caused by *Salmonella*, followed by VTEC. Consumption of contaminated soft and semi-soft cheeses is often implicated in outbreaks with VTEC, especially when they are made of unpasteurised cow or goat milk. VTEC O157:H7 is

linked to the majority of the VTEC outbreaks, but also other serotypes have been implicated (Farrokh et al., 2013). *C. jejuni* (in the United States), *L. monocytogenes* and *Brucella* spp. were relatively often linked to raw milk cheese outbreaks, respectively 5, 8 and 7 out of 64 outbreaks. Dairy products made from raw milk can be a transmission route of *Brucella* spp. (EFSA & ECDC, 2014). In Mediterranean countries not officially free from brucellosis, it is assumed that the consumption of raw sheep and goat milk as well

as cheese made from raw sheep and goat milk is the main source of contamination (FASFC, WIV, & CODA, 2011). Magwedere et al. (2011) have shown that raw goat milk, home-made goat cheese and coffee served with raw goat milk, as well as contact with goats can be a source of human cases of brucellosis. Enterotoxin-producing *S. aureus*, *Streptococcus* spp. and the TBEV were also linked to the consumption of raw milk cheeses. Three of the four outbreaks of *S. aureus* occurred more than 20 years ago, and two were caused by cheese made from raw sheep milk. Concerning *Streptococcus*, one outbreak occurred in the United States due to Mexican-style soft raw milk cheese in 1983. In 1997–2008, 64 cases of tick-borne encephalitis in patients that consumed unpasteurised milk and cheese made from sheep milk were reported in the Czech Republic (Kříž, Beneš, & Daniel, 2009).

4. Growth and survival of pathogenic microorganisms during production and storage/ripening of dairy products made from raw milk

The circumstances of production and storage of dairy products made from raw milk determine the behaviour (i.e., growth, survival or inactivation) of the microorganisms that are potentially present in the raw milk. The pathogens can grow, survive or be inactivated. Some of these important influencing factors are summarised as follows for raw milk cheeses. The growth of the fermenting bacteria reduces the growth possibilities of the pathogens by, on the one hand, competition and, on the other hand, a decline of the pH. The effect of starter cultures producing bacteriocins and the addition of potassium or sodium nitrate is especially aimed at inhibiting the growth of butyric acid bacteria. The outgrowth of *L. monocytogenes* could also be influenced due to the presence of bacteriocins (Dal Bello et al., 2012) while the outgrowth of *B. cereus* may be ascribed to potassium or sodium nitrate inhibiting spore germination (Avila, Gómez-Torres, Hernández, & Garde, 2014). The coagulation of the milk followed by sineresis of the curd, concentrates the pathogens in the curd. Some herbs used in the cheeses may have a bacteriostatic effect (Leuschner & Ielsch, 2003).

Salting creates conditions that are not optimal for survival or growth due to the decline of the water activity. Depending on the time, temperature, pH, water activity and NaCl concentration, dry matter content and the competing microbiota, growth or inactivation of the pathogens present in the milk will occur during ripening (Farrokh et al., 2013). Plastic coating used on certain cheeses prevents them from drying out and prevents contamination with pathogens and fungi from the environment. The coating may contain antifungal components such as natamycin. Vacuum packaging and paraffin are physical barriers and the vacuum packaging will inhibit the development of fungi and strict aerobic microorganisms. The presence of internal and external microbiota during ripening provides competition with the pathogens. During the ripening period, the physical and chemical characteristics of the cheese often change which can provide a better survival and even enable growth. During ripening a post-contamination can occur. Especially soft and semi-soft cheeses as well as cheeses with a washed crust such as red smear cheeses, are more exposed to surface contamination (Farrokh et al., 2013). Microorganisms will behave differently on the surface or in the crust due to the different conditions such as the pH.

The results documented in specific studies on the survival, growth and inactivation of pathogens in dairy products made from raw milk are summarised in Table 3. Information was only available for cheeses and butter. Concerning cheese, a distinction was made between hard, semi-hard and soft cheeses based on the classification made in the publications. When this classification was not clear, 'non specified' cheeses are mentioned.

It can be concluded from Table 3 that during the production of hard cheeses, some bacteria can survive, such as *Salmonella*, *L. monocytogenes*, *S. aureus* and MAP, albeit without subsequent growth. The number of *E. coli* can increase during the cheese production, which is due to a combination of a concentration effect during the formation of the curd as well as due to real growth of the pathogen (Farrokh et al., 2013). In hard cheeses several pathogens can be inactivated during storage. *Salmonella*, *E. coli*, *L. monocytogenes* and MAP may decrease in numbers, but can still be detected after long ripening periods. During the production of semi-hard cheeses, some bacteria can grow, such as *Salmonella*, *E. coli* and *S. aureus*. *L. monocytogenes* and MAP that survive the production process. During the ripening and storage of semi-hard cheeses, most bacteria will decrease in numbers, but *Salmonella*, *E. coli* and *S. aureus* can still be detected. *L. monocytogenes* and MAP can survive the ripening and storage. During the production of soft cheeses, *E. coli*, *L. monocytogenes* and *Brucella* spp. can grow. During the ripening and storage, *E. coli* and *Brucella* spp. can survive, but *L. monocytogenes* can possibly grow in and on soft cheeses, depending on the ripening stage and the circumstances. The growth of *L. monocytogenes* in butter does not occur or is limited (De Reu & Herman, 2004).

5. Identification of main microbiological hazards in dairy products made from raw milk

The information on the possible presence of human pathogenic microorganisms in raw milk, frequencies of occurrence of human pathogenic microorganisms in dairy products made from raw milk in Europe, outbreaks resulting from consumption of dairy products made from raw milk in Europe, the United States and Canada and the worst case scenario of the behaviour in dairy products made from raw milk is summarised in Table 4.

The main microbiological hazards linked to raw milk cheese are *L. monocytogenes*, human pathogenic VTEC, *Salmonella*, enterotoxin-producing *S. aureus* and *Campylobacter*. These pathogens can be present in raw milk and as there is no heat treatment before cheese production, they will not be inactivated. They have all caused a certain number of outbreaks linked to cheese and *Salmonella*, *L. monocytogenes*, *S. aureus* and VTEC have been detected in cheeses. *Salmonella*, *Campylobacter* and VTEC can cause disease in low numbers (often a few cells) and for *Salmonella* and VTEC there are growth possibilities during production and storage (survival for VTEC during storage). *L. monocytogenes* and *S. aureus* have to grow to cause disease, which is possible as can be seen from Table 4.

Concerning butter, cream and buttermilk, there is less information available in the scientific literature. For butter made from raw milk, the main microbiological hazards are *L. monocytogenes*, VTEC and *S. aureus* because these pathogens have been detected in butter. However, the risk of infection after consumption of raw milk butter is estimated to be relatively lower in comparison with certain cheeses, especially due to the limited growth possibilities of *L. monocytogenes*. For cream made from raw milk, the main microbiological hazards are estimated to be *L. monocytogenes*, *S. aureus* and VTEC because *L. monocytogenes* and *S. aureus* have been detected in cream and VTEC was linked to a cream outbreak. With regard to buttermilk made from raw milk, there is no information available in the scientific literature and the microbiological hazards related to the consumption of this product cannot be identified.

There are some additional risks linked to the consumption of dairy products made from raw milk, especially when they are produced on farms where animals are frequently infected with certain pathogens. This is the case for *Brucella* spp. Cattle can be

Table 3

Behaviour of human pathogenic microorganisms in different types of cheese made from raw milk during production and storage/ripening.

| Production | Storage and/or ripening | References |
|---|--|---|
| <i>Hard cheese</i> | | |
| Inoculation of 4–6 log cfu mL ⁻¹ of <i>Campylobacter jejuni</i> , <i>Escherichia coli</i> , <i>Listeria monocytogenes</i> , <i>Salmonella</i> Typhimurium, <i>Staphylococcus aureus</i> , <i>Yersinia enterocolitica</i> in milk; 1 d after production: only low numbers of <i>S. aureus</i> | One week after storage at 11–13 °C for 90 d: no detection of any of inoculated pathogens | Bachmann and Spahr (1995) |
| Inoculation of 4–5 log cfu mL ⁻¹ of <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> (MAP) in cow milk | Steady and slow decrease during ripening at 12 °C for 10 d, 22 °C for 60 d or 12 °C for 50 d; viable cells detected at day 120 | Spahr and Schafroth (2001) |
| One of thirty-five cows shedding 2.3 log cfu mL ⁻¹ of <i>Salmonella</i> Muenster in milk; 11 of 181 vats of curd positive; 2 of 11 vats of cheese positive | One of two vats positive after storage at 5 °C for 125 d | Wood, Collins-Thompson, Irvine, and Muhr (1984) |
| Inoculation of 2 log cfu mL ⁻¹ of <i>E. coli</i> K12 in milk; increase during production | Decrease of 1.5–2 log during storage at 4 °C for 120 d | Amornkul and Henning (2007) |
| Inoculation of 1–5 log cfu mL ⁻¹ of <i>E. coli</i> O157:H7 in milk; increase during production | Storage at 7 °C for 1 wk; decrease of less than 1 log cfu g ⁻¹ at day 60; decrease of less than 2 log cfu g ⁻¹ at day 120 | Schlesser et al. (2006) |
| Inoculation of 1.3 log cfu mL ⁻¹ of <i>E. coli</i> O157:H7 in milk; 1.3 log cfu mL ⁻¹ in milk before coagulation, 1.6 log cfu g ⁻¹ in curd after cutting; 1.3 log cfu mL ⁻¹ in whey at draining; 2.4 log cfu g ⁻¹ in curd before salting | Storage at 9 °C; 2.2 log cfu mL ⁻¹ in cheese on day 1; decrease to 1.4 log cfu g ⁻¹ on day 60; below 0.7 log cfu g ⁻¹ after 108 d; Detection after more than 270 d | D'Amico, Druart, and Donnelly (2010) |
| Inoculation of 1.4 log cfu mL ⁻¹ of <i>E. coli</i> O157:H7 in milk; 1.4 log cfu mL ⁻¹ in milk before coagulation, 1.8 log cfu g ⁻¹ in curd after cutting; 0.5 log cfu mL ⁻¹ in whey removed; 0.7 log cfu mL ⁻¹ in whey plus wash water; 2.6 log cfu g ⁻¹ in curd before salting; 3.0 log cfu g ⁻¹ in cheese before brining | Storage at 14 °C for 5 wk, then at 9 °C; 2.2 log cfu mL ⁻¹ in cheese on day 1; decrease to 0.7 log cfu g ⁻¹ on day 60; below 0.7 log cfu g ⁻¹ after 94 d; detection after more than 270 d | D'Amico et al. (2010) |
| Natural contamination of <i>L. monocytogenes</i> in cheese | Never >1.3 log cfu g ⁻¹ ; no detection after 5 months | Dalmaso and Jordan (2014) |
| <i>Semi-hard cheese</i> | | |
| Inoculation of 4–6 log cfu mL ⁻¹ of <i>C. jejuni</i> , <i>E. coli</i> , <i>L. monocytogenes</i> , <i>S. Typhimurium</i> , <i>S. aureus</i> , <i>Y. enterocolitica</i> in milk | After storage at 11–13 °C for 90 d: only <i>L. monocytogenes</i> detected | Bachmann and Spahr (1995) |
| Inoculation of 4–5 log cfu mL ⁻¹ of MAP in cow milk | Steady and slow decrease during ripening at 14–15 °C for 120 d; viable cells detected at day 120 | Spahr and Schafroth (2001) |
| Inoculation of 1 and 3 log cfu mL ⁻¹ of <i>E. coli</i> (5 strains including 3 verocytotoxin-producing <i>Escherichia coli</i> (VTEC)) | Increase of 3.5 log cfu g ⁻¹ at day 1 (concentration effect and growth); slow and continuous decrease during ripening; generic <i>E. coli</i> survived at higher counts; 6 cheeses with low inoculum: >1 log cfu g ⁻¹ at end of ripening; after enrichment: VTEC detection in almost all cheeses | Peng et al. (2013) |
| Inoculation of 2.4 log cfu mL ⁻¹ <i>S. aureus</i> in milk; 3.3 log cfu mL ⁻¹ in gel of early coagulation; 2.3 log cfu mL ⁻¹ in whey. 4.2 log cfu g ⁻¹ in curd at pressing | 3.8 log cfu g ⁻¹ in cheese at day 7; 0 cfu g ⁻¹ in cheese at week 10 | Jørgensen et al. (2005) |
| Inoculation of 4 log cfu g ⁻¹ of <i>L. monocytogenes</i> in sheep milk | Decrease after 6 wk; log reduction at 4 °C: 2.10 at day 91, 3.19 at day 101, presence at day 111, absent at day 114; log reduction at 12 °C: 1.74 at day 77, 2.90 at day 91, presence at day 98, absent at day 104; log reduction at 22 °C: 1.76 at day 54, 2.45 at day 67, 4.02 at day 70, presence at day 74, absent at day 77 | Valero et al. (2014) |
| <i>Soft cheese</i> | | |
| Inoculation of 4 log cfu mL ⁻¹ of <i>E. coli</i> O157:H7 and <i>L. monocytogenes</i> ; growth of both pathogens during production | Storage at 2 °C for 75 d; <i>E. coli</i> O157:H7: highest count at day 10, decrease after 10 d, count at day 75 higher than inoculum; <i>L. monocytogenes</i> : survival, count at day 75 higher than inoculum | Ramsaran, Chen, Brunke, Hill, and Griffiths (1998) |
| Inoculation of 4 log cfu mL ⁻¹ of <i>E. coli</i> O157:H7 and <i>L. monocytogenes</i> ; growth of both pathogens during production | Storage at 2 °C for 65 d; <i>E. coli</i> O157:H7: highest count after 24 h, decrease after 24 h, count at day 65 higher than inoculum; <i>L. monocytogenes</i> : survival, count at day 65 higher than inoculum | Ramsaran et al. (1998) |
| Inoculation of 3 log cfu mL ⁻¹ of VTEC; increase of 1–2 log cfu g ⁻¹ during production; stabilisation during salting/drying | Storage at 4 °C; decrease during early stages of ripening | Montet et al. (2009) |
| Inoculation of 3.6 log cfu mL ⁻¹ of <i>Brucella abortus</i> in milk; 4.2 log cfu g ⁻¹ in curd; 4.3 log cfu g ⁻¹ in curd out of mould | Storage at 12 °C; 3.0 log cfu g ⁻¹ in cheese at day 5, 1.5 log cfu g ⁻¹ in cheese at day 8, 1.3 log cfu g ⁻¹ in cheese at day 12, 1.1 log cfu g ⁻¹ in cheese at day 15, 0.4 log cfu g ⁻¹ in cheese at day 18, 0 cfu g ⁻¹ in cheese at day 22 | Plommet et al. (1988) |
| Inoculation of –0.7 and 0.3 log cfu cm ⁻² of <i>L. monocytogenes</i> | Storage at 4 °C; increase after day 28; increase to 2.96 log cfu g ⁻¹ at day 60 (low inoculum); increase to 4.55 log cfu g ⁻¹ at day 60 (high inoculum) | D'Amico et al. (2008) |
| Natural contamination of 3.8 log cfu mL ⁻¹ of <i>L. monocytogenes</i> in sheep milk | 2.3–2.8 log cfu g ⁻¹ 7 d after storage at 4 °C for 7 d | Schoder, Winter, Kareem, Baumgartner, and Wagner (2003) |
| <i>Mozzarella</i> | | |
| Inoculation of 5 log cfu mL ⁻¹ of <i>E. coli</i> O157:H7 in cow milk; curd stretching at 80 °C for 5 min: loss of viability; curd stretching at 70 °C for 5 min: 1 log reduction | Storage at 4 °C for 7 d; no detection | Spano et al. (2003) |
| Inoculation of 7.1–8.7 log cfu g ⁻¹ of VTEC O157 and O26 of curd; 4D reduction during heating curd | | Trevisani, Mancusi, and Valero (2014) |

(continued on next page)

Table 3 (continued)

| Production | Storage and/or ripening | References |
|--|---|---|
| <i>Non specified cheese</i> | | |
| Inoculation of <i>E. coli</i> O157:H7, O26:H11, O103:H2 and O145:H28 in cow, goat or sheep milk; during first 24 h of production: growth of 2–3 log cfu g ⁻¹ , no growth when acid coagulation | Decrease during 6 months of ripening; detectable at end of storage/ripening | Miszczucha et al. (2013) |
| Inoculation of <i>E. coli</i> O157:H7 and O26:H11 in cow milk; during first 24 h of production: growth from 2 to 4 log cfu g ⁻¹ | Stable around 4 log cfu g ⁻¹ during ripening and storage | Miszczucha et al. (2014) |
| Inoculation of 1.52 log cfu mL ⁻¹ of <i>E. coli</i> O157:H7 in milk | Storage a 6 °C; increase to 3.4 log cfu g ⁻¹ at day 1; decrease during ripening to <0 cfu g ⁻¹ in rind at day 21; decrease during ripening to <1 log cfu g ⁻¹ in core at day 21; detection of viable cells at day 90 | Maher, Jordan, Upton, and Coffey (2001) |
| Inoculation of 3.56 log cfu g ⁻¹ dry weight of <i>L. monocytogenes</i> in cow milk; no growth during production | Storage at 13 °C for 14 d and at 8 °C for 14 d; increase of 2 log cfu g ⁻¹ dry weight during first 4 d of ripening; survival/decrease until end of ripening | Schwartzman et al. (2011) |
| Inoculation of 6 log cfu mL ⁻¹ of <i>E. coli</i> , <i>Listeria innocua</i> and <i>S. aureus</i> in milk; during production; increase of <i>E. coli</i> , survival of <i>L. innocua</i> and <i>S. aureus</i> | Storage at 8 °C for 56 d; <i>E. coli</i> highest count at day 7, decrease after day 7; <i>Listeria innocua</i> and <i>Staphylococcus aureus</i> no growth | Masoud et al. (2012) |
| Average counts of <i>S. aureus</i> : 1.26 log cfu mL ⁻¹ in cow/goat milk, 2.80 log cfu mL ⁻¹ in curd | Average counts of <i>S. aureus</i> : 3.50 log cfu g ⁻¹ in cheese (5–6 h), 3.13 log cfu mL ⁻¹ in cheese (24 h), 1.04 log cfu g ⁻¹ in cheese (30 d) | Jakobsen et al. (2011) |
| Inoculation of 9.7 log cfu g ⁻¹ of <i>Brucella melitensis</i> in goat milk; decrease during production; 6.3 log cfu mL ⁻¹ in whey at curd cutting; 6 log cfu mL ⁻¹ at draining | 3.0 log cfu mL ⁻¹ during storage at 4 °C for 13–30 d; 4 log cfu mL ⁻¹ during storage at 24 °C for 6–15 d; no detection during storage at 24 °C for days 16–30 | Méndez-González et al. (2011) |
| <i>Butter</i> | | |
| Inoculation of <i>L. monocytogenes</i> and <i>L. innocua</i> in cream | No significant growth during storage at 4, 10 or 20 °C for 4 wk | De Reu and Herman (2004) |
| Natural contamination of –0.6 to 1.2 log cfu g ⁻¹ of <i>L. monocytogenes</i> | One log reduction during storage at 4 or 10 °C for 4 or 5 wk | De Reu and Herman (2004) |

infected with *Brucella abortus* and sheep can be infected with *Brucella melitensis* in certain areas of Southern Europe. The same holds for *M. bovis* in regions where this pathogen is endemic, for example among cattle. Goats can be parasitised by the tick *Ixodes ricinus* that often carry the TBEV. The infection of goats is asymptomatic and the TBEV can enter the bloodstream through the tick followed by an excretion in the milk during the viraemic phase. In risk areas in Eastern Europe the TBEV can be endemic and therefore this is also an important microbiological hazard linked to dairy products made from raw milk.

For some microorganisms, the foodborne potential is not known or limited documentation is available. Cattle, goats and sheep are

the main reservoirs of *C. burnetii* (Rahimi, Doosti, Ameri, Kabiri, & Sharifian, 2008). In general, people get infected by inhalation of contaminated dust and aerosols mainly formed during birth of animals, via manure or via contact with infected animals (Sci Com, 2010a, 2010b). Infection via the food chain occurs less frequently (Berri et al., 2005). Infectious *C. burnetii* can persist for long periods in milk and milk products. From scientific literature, it appears that dairy products made from unpasteurised milk may contain viable *C. burnetii*. Concerning MAP, the information in the scientific literature is limited and up to now, there is uncertainty whether this bacterium is zoonotic or to be considered as a human foodborne pathogen. Nevertheless, MAP and *C. burnetii* could be considered as

Table 4
Summary of information on microbiological hazards.

| Organism | Possibly present in raw milk | Detected in dairy products made from raw milk (minimum – maximum frequency of occurrence in %) in Europe | Reported human cases and outbreaks in Europe, the US and Canada due to cheese consumption | Worst case behaviour in raw milk cheese | | Score according to EFSA (2013) |
|---|------------------------------|--|---|---|------------------------------|--------------------------------|
| | | | | Production | Storage/ripening | |
| <i>Listeria monocytogenes</i> | Yes | 0.0–41.9 (cheese, butter, cream) | 8 | Growth survival ^b | Growth survival ^b | 2 |
| <i>Escherichia coli</i> (human pathogenic) | Yes | 0.0–55.3 (cheese) | 18 ^a | Growth | Survival | 3 |
| <i>Staphylococcus aureus</i> | Yes | 0.0–100.0 (cheese, butter) | 4 | Growth | Growth | 1 |
| <i>Salmonella</i> | Yes | 0.0–4.3 (cheese) | 22 | Growth | Growth | 3 |
| <i>Campylobacter</i> | Yes | 0.0 (cheese) | 5 | No survival | No survival | 3 |
| <i>Brucella</i> | Yes | – ^c | 7 | Growth | Survival | 3 |
| <i>Bacillus cereus</i> | Yes | 0.0–28.0 (cheese) | 0 | – | – | 1 |
| <i>Mycobacterium bovis</i> | Yes | – | 0 | – | – | 3 |
| Tick-borne encephalitis virus | Yes | – | 1 | – | – | 3 |
| <i>Coxiella burnetii</i> | Yes | 0.0–57.0 (cheese, cream) | 0 | – | – | – ^d |
| <i>Streptococcus</i> | Yes | – | 1 | – | – | 3 ^e |
| <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> | Yes | 0.0–20.0 (cheese) | 0 | Survival | Survival | – ^f |

^a One due to cream consumption.

^b In butter.

^c A dash (–) indicates no information was available unless otherwise indicated.

^d Foodborne potential not known.

^e Pathogenic species not frequent.

^f Zoonotic potential not known.

a potential microbiological hazard present in dairy products made from raw milk.

6. Discussion

The contamination levels of several pathogens such as *L. monocytogenes* and *S. aureus* usually encountered in raw milk are mostly lower than the level they have to reach to cause disease. It is assumed (based on the authors' expert opinion) that numbers of *L. monocytogenes* in raw cow milk are usually lower or at the level of ca. 1 log cfu mL⁻¹. In the case of a subclinical mastitis of the milk producing animal, high initial numbers of *L. monocytogenes* or *S. aureus* could be encountered in the raw milk (Pintado et al., 2009; Sahebkhari et al., 2011). Although this is rare and such milk is diluted in the normal industrial milk supply, it may occur, e.g., in a short supply chain. For low initial numbers of these pathogens that have to grow to produce disease, the growth possibilities during cheese production and storage will influence the risk of infection. When present in the raw milk, the growth of most pathogens is restricted by storage at refrigerator temperatures although, depending on the storage time and temperature, some pathogens such as *L. monocytogenes* could develop.

From the scientific literature, it seems that prevalences or concentrations of certain pathogens such as VTEC, *L. monocytogenes*, *S. aureus* and *C. burnetii* are mostly lower in dairy products made from pasteurised or heat treated milk than in dairy products made from raw milk (Civera et al., 2007; Eldin et al., 2013; Fach, Perelle, Dilasser, & Grout, 2001; Greenwood, Roberts, & Burden, 1991; Loncarevic, Danielsson-Tham, & Tham, 1995; Rosengren, Fabricius, Guss, Sylvén, & Lindqvist, 2010). Concerning outbreaks, it seems that cheese outbreaks are more linked to cheese made from unpasteurised milk than from cheese made from pasteurised milk (Altekruse, Timbo, Mowbray, Bean, & Potter, 1998; De Buyser, Dufour, Maire, & Lafarge, 2001; Gould, Mungai, & Behraves, 2014). Moreover, the study of Gould et al. (2014) showed that raw milk cheese outbreaks were mostly related to contamination of the raw product or the ingredients by pathogens from animals or the environment, whereas pasteurised cheese outbreaks were more related to health and hygiene of the workers in contact with the cheese.

Regarding the behaviour of the pathogens during production and storage of cheese, there are differences depending on the use of raw milk or pasteurised milk. For example, *L. monocytogenes* showed in an inoculation experiment growth during the cheese production process when made from pasteurised milk whereas no growth was observed when made from raw milk, most probably due to the presence of background flora. However, during cheese ripening, growth of this pathogen occurred in raw milk cheese, but inactivation occurred in pasteurised cheese due to the physicochemical conditions of the cheeses (Schvartzman et al., 2011). Another study found no difference in the growth potential of *L. monocytogenes* inoculated on the surface of cheeses made from raw or pasteurised milk during storage (D'Amico, Druart, & Donnelly, 2008). In addition, the natural microbiota can influence the behaviour of pathogens in cheese. Delcenserie et al. (2014) found that cheese made from raw and pasteurised milk had different microbiota in the crust of the cheeses. Overall, pasteurisation ensures the inactivation of vegetative pathogenic microorganisms, which increases the safety of products made thereof in comparison with dairy products made from raw milk. However, it should be noted that dairy products made from pasteurised milk could be susceptible to post-contamination. Therefore, application of a good Hazard Analysis and Critical Control Points (HACCP) system during production is essential.

To limit the exposure to pathogens due to consumption of dairy products made from raw milk several control measures can be taken from farm to fork. At farm level, the pathogenic microorganisms that contaminate the raw milk can come from the milk-producing animal or from the environment. Mastitis or udder infection is the most common disease in cattle (Halasa, Huijps, Osteras, & Hogeveen, 2007). The intramammary infection is mostly caused by bacteria that penetrate the udder through the teat canal. In the holistic approach of prevention and control of mastitis, it is of utmost importance to keep the infection pressure in the stable as low as possible by means of good hygiene management and optimal comfort of the cows to obtain a good udder health and optimal milk quality (Barkema et al., 1998; Elbers et al., 1998; van Gastelen, Westerlaan, Houwers, & van Eerdenburg, 2011; Schukken, Grommers, Vandegheer, Erb, & Brand, 1990). However, contamination of the milk during milking cannot completely be avoided, not even with elaborate cleaning and disinfection (Magnusson, Christiansson, Svensson, & Kolstrup, 2006). Between the milking and the processing of dairy products, time should be restricted and temperatures kept as low as possible to prevent the pathogens that are possibly present from growing. During milk processing, a quick fermentation with efficient starter cultures should be applied to restrict the growth of the pathogens. After the production of cheese, a restriction of the shelf life and adherence to the cold chain from retail to consumer is important to ensure the safety of such products. The control of the procedures of cleaning and disinfection of the material that is used during the production process is also important from the milking to the sales of the products. Finally, both producers and consumers, in particular persons belonging to the YOPI group, should be aware of the risks pertaining to dairy products made from raw milk.

There are some limitations linked to the analysis of the information collected in this work. The frequencies of occurrence of pathogenic microorganisms in dairy products made from raw milk originate from ad hoc convenience sampling and the modalities conditions of the sampling plan (number of samples taken, method of analysis and corresponding limit of detection) as well as the method of analysis and detection limit may vary among these surveys and consequently it was not always easy to compare the outcome of these publications. Also, it is not known if the detected pathogens are able to cause disease. In particular, in the case of the detection of potential pathogenic *E. coli* serotypes, it is not known if all positive results actually refer to *E. coli* strains that carry virulence genes. Concerning the outbreaks due to the consumption of dairy products made from raw milk, only reported outbreaks were included. There can be underreporting due to, e.g., a long incubation period. Regarding the behaviour of the pathogens during the production process and the storage or ripening period of dairy products made from raw milk, it is evident that there is a variety of cheeses with their proper production process, intrinsic physicochemical characteristics, indigenous microbiota and actual storage conditions, which will impact on the growth potential of the pathogens.

7. Conclusion

Overall, the available information on some pathogens or dairy products made from raw milk is limited, but if everything is taken into consideration the microbiological hazards and dairy products made from raw milk of main concern are linked to raw milk cheeses and *L. monocytogenes*, pathogenic VTEC, enterotoxin-producing *S. aureus*, *Salmonella* and *C. jejuni/coli*. Next, *L. monocytogenes*, VTEC and *S. aureus* have been identified as microbiological hazards in raw milk butter and cream, albeit to a lesser extent because of a reduced growth potential of these pathogens in the latter products

compared with cheese. In endemic areas, dairy products made from raw milk may also be contaminated with *Brucella* spp., *M. bovis* or the tick-borne encephalitis virus and need particular attention in monitoring and surveillance. Finally, *C. burnetii* and MAP are identified as potential hazards. For *C. burnetii* the role of food (dairy products made from raw milk) seems to be limited and for MAP no sufficient knowledge is available.

Due to the possible exposure of the consumer to the above mentioned pathogenic microorganisms in dairy products made from raw milk, appropriate risk communication on the consumption of these products in particular to a vulnerable population, is recommended.

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