

Annex 1. Reported frequencies of occurrence of pathogenic agents in/on dairy products made from raw milk found in specific studies carried out in countries of the European Community

Frequency in % (total number of samples)	Food	Method	Country	Year	References
<b>Salmonella</b>					
0.0 (71)	hard, semi-hard and soft cow milk cheeses, 1 soft goat milk cheese, blue veined sheep milk cheeses, fresh goat milk cheeses (36 varieties)	ISO 6579	Belgium	Not given	De Reu <i>et al.</i> , 2002
- 0.0 (16) - 0.0 (4) - 0.0 (64)	- Cheese - Fresh cheese - Butter	ISO 6579	Belgium	2002	De Reu <i>et al.</i> , 2004
0.0 (334)	Cheese	Not given	Germany	Not given	Hahn <i>et al.</i> , 1999a
0.0 (75)	Cheese	Not given	Ireland	Not given	Coveney <i>et al.</i> , 1994
4.3 (70)	Soft and hard cow, goat and sheep milk cheeses	ISO 6579 Remark: positives found in soft/semi-soft sheep milk cheese	Portugal	2005-2006	Almeida <i>et al.</i> , 2007
0.0 (25)	semi-soft to hard cheeses: - from cow milk (20) - from sheep milk (4) - from goat milk (1) - from organic milk (12) - from non-organic milk (13) - < 3 months ripening (11) - > 3 months ripening (14)	Enrichment, immunomagnetic recovery, selective media	Scotland	Not given	Williams & Withers, 2010
- 0.0 (429) - 0.0 (518)	- Cheese - Butter	BRD-07/11-12/05 Short Protocol	Belgium	2008-2012	Source: DiversiFerm (personal communication)
- 0.0 (57) - 0.0 (52) - 0.0 (11) - 0.0 (45) - 0.0 (1) - 0.0 (57)	- Butter - Semi-hard cheese - Hard cheese - Cream - Processed cheese - Fresh cheese	Detection with PCR	Belgium	2011	Source: FASFC (personal communication)

- 0.0 (58)	- Soft cheese				
- 0.0 (43) - 0.0 (54) - 0.0 (9) - 0.0 (45) - 0.0 (50) - 0.0 (51)	- Butter - Semi-hard cheese - Hard cheese - Cream - Fresh cheese - Soft cheese	Detection with PCR	Belgium	2012	Source: FASFC (personal communication)
- 0.0 (92) - 0.0 (104) - 0.0 (4) - 0.0 (42) - 0.0 (39) - 0.0 (92)	- Butter - Semi-hard cheese - Hard cheese - Cream - Fresh cheese - Soft cheese	Detection with PCR	Belgium	2013	Source: FASFC (personal communication)
<b>VTEC</b>					
5.6 (71)	hard, semi-hard and soft cow milk cheeses, 1 soft goat milk cheese, blue veined sheep milk cheeses, fresh goat milk cheeses (36 varieties)	Enrichment, IMS <sup>1</sup> , selective media, presumptive positive colonies tested by VIDAS O157 assay and O157 PCR ( <i>vtx1</i> , <i>vtx2</i> , <i>eaeA</i> and <i>E-hly</i> genes)	Belgium	Not given	De Reu <i>et al.</i> , 2002
- 0.0 (16) - 0.0 (4) - 0.0 (64)	- Cheese - Fresh cheese - Butter	Enrichment, IMS <sup>1</sup> , selective media, presumptive positive colonies tested by VIDAS O157 assay and O157 PCR ( <i>vtx1</i> , <i>vtx2</i> , <i>eaeA</i> and <i>hlyA</i> genes)	Belgium	2002	De Reu <i>et al.</i> , 2004
0.0 (334)	Cheese	1,5 % VTEC	Germany	Not given	Hahn <i>et al.</i> , 1999a
4.0 (25)	Cheese	Enrichment Remark: 1 <i>stx2</i> -positive strain	Italy	Not given	Volponi <i>et al.</i> , 2012
13.1 (1,039)	399 farm white mould rinded soft cheeses, 184 farm uncooked hard cheeses, 132 farm washed rinded soft cheeses, 324 industrial white mould rinded soft cheeses	Colony hybridization, serotyping, PCR ( <i>vtx1</i> and <i>vtx2</i> genes)	France	Not given	Vernozy-Rozand <i>et al.</i> , 2005
5.7 (1,502)	1,422 semi-hard or hard cheeses, 80 soft cheeses (1,370 from cow milk, 121 from goat milk, 11 from sheep milk)	Enrichment, PCR ( <i>vtx1</i> and <i>vtx2</i> genes) Remark: positives found in 1 hard cheese, 76 semi-hard cheeses and 8 soft cheeses	Switzerland	2006-2008	Zweifel <i>et al.</i> , 2010
0.0 (739)	Scottish blue cheeses, Italian fontino cheeses, Roquefort cheeses	Enrichment, IMS <sup>1</sup> , selective media, , confirmation	Scotland	1997-1999	Coia <i>et al.</i> , 2001
- 8.9 (180)	Unpasteurized cheeses	- Vero cell assay	France	Not	Fach <i>et al.</i> , 2001

<sup>1</sup> Immunomagnetic separation

- 30.6 (180)		- PCR-ELISA		given	
- 2.4 (41) - 0.0 (24) - 16.7 (18)	Non-cooked and hard or semi-hard Spanish castellano sheep milk cheeses - 2.5 months ripening - 6 months ripening - 12 months ripening	AOAC <sup>2</sup> official method number 997.11: VTEC isolation, PCR: detection of <i>rfbO</i> <sub>157</sub> and <i>fliC</i> <sub>H7</sub> genes Remark: serogroups ONT, O14 and O14, all <i>vtx1</i> -positive, <i>vtx2</i> -, <i>eaeA</i> - and <i>ehxA</i> -negative	Spain	1999	Caro & García-Armesto, 2007
- 30.4 (112) - 27.7 (112) - 2.7 (112)	Soft cow (61) and goat (51) milk cheeses	- PCR-ELISA - RT-PCR ( <i>vtx</i> -IAC <sup>3</sup> ) - Colony hybridization: VTEC isolation	France	2005-2007	Auvray <i>et al.</i> , 2009
- 4.5 (143) - 3.8 (143) - 0.7 (143) - 2.1 (143) - 0.0 (143) - 0.0 (143) 0.0 (143)	Soft (50) and ripened (93) cheeses from cow, goat or sheep milk (Italian Alpine Region)	PCR (average of results from isolated colonies on SMAC and CT-SMAC <sup>4</sup> media): - <i>vtx1</i> gene - <i>vtx2</i> gene - <i>eae</i> gene - <i>ehxA</i> gene - <i>LT</i> gene - <i>VT</i> gene IMS: <i>E. coli</i> O157	Italy	Not given	Civera <i>et al.</i> , 2007
- 0.0 (811) - 0.0 (502)	Dairy products (less than 60 days of ripening, Italian cheese types, ricotta (curd or whey cheese), butter) - Unpasteurized cow dairy products - Unpasteurized sheep dairy products	ISO/DIS 16654:1999: isolation of <i>E. coli</i> O157	Italy	2000-2001	Conedera <i>et al.</i> , 2004
- 4.9 (796) - 41.0 (39)	Soft (52), semi-hard and hard (744) cow, goat and sheep milk cheese	- Enrichment, PCR ( <i>vtx1</i> and <i>vtx2</i> genes) - Colony dot-blot hybridization: non-O157 VTEC strains (1 strain <i>vtx1</i> -positive, 15 strains <i>vtx2</i> -positive, 3 strains <i>E-hlyA</i> -positive, 0 strains <i>eae</i> -positive)	Switzerland	2006-2007	Stephan <i>et al.</i> , 2008
0.0 (70)	Soft and hard cow, goat and sheep milk cheese	ISO 16654: detection of <i>E. coli</i> O157 (IMS <sup>1</sup> , isolation media, confirmation)	Portugal	2005-2006	Almeida <i>et al.</i> , 2007
- 29.8 (400) - 37.3 (400) - 55.3 (400)	Soft cheeses and smear semi-hard uncooked cheeses from cow milk (265) and unpasteurized goat milk cheeses (135)	PCR: - <i>vtx</i> -positive - <i>eae</i> -positive - at least one of O26, O103, O111, O145	France	2009	Madic <i>et al.</i> , 2011

<sup>2</sup> Association of Official Analytical Chemists

<sup>3</sup> Internal amplification control

<sup>4</sup> Sorbitol MacConkey agar (supplemented with Cefixime tellurite)

- 6.5 (400)		and O157 genetic markers - combinations of above			
0.0 (25)	semi-soft to hard cheeses: - from cow milk (20) - from sheep milk (4) - from goat milk (1) - from organic milk (12) - from non-organic milk (13) - < 3 months ripening (11) - > 3 months ripening (14)	Enrichment, selective media ( <i>E. coli</i> O157)	Scotland	Not given	Williams & Withers, 2010
10.0 (10)	- Unpasteurized home-made cheese and butter	Enrichment, PCR, colony blot hybridization Remark: positive found in cheese	Germany	2004	Messelhäusser <i>et al.</i> , 2008
- 0.0 (136) - 0.0 (84) - 0.0 (12) - 0.0 (45) - 0.0 (1) - 0.0 (89) - 0.0 (83)	- Butter - Semi-hard cheese - Hard cheese - Cream - Processed cheese - Fresh cheese - Soft cheese	VIDAS UP <i>E. coli</i> O157	Belgium	2011	Source: FASFC (personal communication)
- 0.0 (119) - 1.1 (91) - 7.1 (14) - 0.0 (45) - 0.0 (85) - 0.0 (98)	- Butter - Semi-hard cheese - Hard cheese - Cream - Fresh cheese - Soft cheese	VIDAS UP <i>E. coli</i> O157	Belgium	2012	Source: FASFC (personal communication)
- 0.0 (111) - 0.0 (127) - 0.0 (5) - 0.0 (41) - 0.0 (59) - 0.0 (115)	- Butter - Semi-hard cheese - Hard cheese - Cream - Fresh cheese - Soft cheese	VIDAS UP <i>E. coli</i> O157	Belgium	2013	Source: FASFC (personal communication)
0.0 (74)	Soft cheese	Version draft ISO 13136	Belgium	2012	Source: FASFC (personal communication)
4.8 (105)	Soft cheese	ISO/TS 13136:2012 5 samples presumably ( <i>stx</i> & <i>eae</i> screening): 1x O103, 1x O111, 5x O145, 1x O26, 0x other O serogroup	Belgium	2013	Source: FASFC (personal communication)

***Listeria monocytogenes***

0.0 (55)	3 camembert, 10 chevre, 37 fresh cheese, 5 other: brie, cellar ripened cheese, feta-like cheese, semi-hard cheese, lactique cheese and smear-ripened cheese	Enrichment and selective isolation	Sweden	2005	Rosengren <i>et al.</i> , 2010
- 1.4 (73) - 0.0 (49)	- Cow cheese batch - Goat cheese batch	Enrichment	Norway	Not given	Jakobsen <i>et al.</i> , 2011
2.8 (71)	hard, semi-hard and soft cow milk cheeses, 1 soft goat milk cheese, blue veined sheep milk cheeses, fresh goat milk cheeses (36 varieties)	ISO 11290	Belgium	Not given	De Reu <i>et al.</i> , 2002
- 0.0 (16) - 0.0 (4) - 18.7 (64)	- Cheese - Fresh cheese - Butter	ISO 11290	Belgium	2002	De Reu <i>et al.</i> , 2004
2.4 (334)	Cheese	Not given	Germany	Not given	Hahn <i>et al.</i> , 1999a
0.0 (17)	Soft and semi-soft milk cheese	Not given	Ireland	Not given	Coveney <i>et al.</i> , 1994
- 11.4 (70)  - 1.4 (70) - 10.0 (70)	Soft and hard cow, goat and sheep milk cheese	Detection: VIDAS (+ALOA): positives found in 7 soft/semi-soft and 1 hard/semi-hard cheese Counts: ISO 11290-2: - > 200 cfu/g - < 100 cfu/g	Portugal	2005-2006	Almeida <i>et al.</i> , 2007
- 25.8 (31) - 0.0 (11)	- Fresh cheese - Ripened cheese	Enrichment, PCR	Italy	Not given	Rantsiou <i>et al.</i> , 2008
- 0.0 (90) - 0.0 (96) - 0.0 (66)	- São Jorge curd - Natural whey starter - São Jorge cheese > 4 months ripening	Enrichment, PCR	Portugal	2002-2003	Kongo <i>et al.</i> , 2006
0.0 (51)	Semi-hard idiazabal cheese	ISO 11290-1: detection and enumeration	Spain	Not given	Arrese & Arroyo-Izaga, 2012
4.0 (25) - 5.0 (20) - 0.0 (4) - 0.0 (1) - 8.3 (12) - 0.0 (13) - 0.0 (11) - 7.1 (14)	semi-soft to hard cheeses: - from cow milk - from sheep milk - from goat milk - from organic milk - from non-organic milk - < 3 months ripening - > 3 months ripening	Enrichment, immunomagnetic recovery, selective media	Scotland	Not given	Williams & Withers, 2010

- 41.9 (31)	- raw milk cheese	selective enrichment + quantification	Sweden	189-1993	Loncarevic <i>et al.</i> , 1995
0.0 (230)	raw goat and sheep milk and raw goat and sheep milk cheese	Enrichment	Austria	2009	Schoder <i>et al.</i> , 2011
- 12.2 (474) - 0.6 (474) - 29.9 (519) - 0.2 (519)	- Cheese  - Butter	BRD-07/04-09/98 - presence - > 100 cfu/g - presence - > 100 cfu/g	Belgium	2008-2012	Source: DiversiFerm (personal communication)
5.0 (20)	Farm cream	Presence in 25 g	Belgium	2003-2004	De Reu & Herman, 2004
18.7 (64)	Farm butter	Presence in 25 g	Belgium	2002	De Reu & Herman, 2004
8.0 (25)	Farm butter	Presence in 25 g	Belgium	2003-2004	De Reu & Herman, 2004
9.0 (80)	Farm butter	Presence in 25 g	Belgium	2006	De Reu & Herman, 2006
19.5 (41)	Farm butter	Presence in 25 g	Belgium	2007	De Reu & Herman, 2007
6.0 (49)	Farm butter	Presence in 25 g	Belgium	2008	De Reu & Herman, 2008
8.3 (60)	Domestic cream	Enrichment, PCR	Croatia		Frece <i>et al.</i> , 2010
4.2 (48)	Untreated cream (cow milk)	Enrichment	England and Wales	1988-1989	Greenwood <i>et al.</i> , 1991
- 0.8 (118) - 5.6 (90) - 8.3 (12) - 0.0 (44) - 0.0 (1) - 0.0 (88) - 20.2 (99)	- Butter - Semi-hard cheese - Hard cheese - Cream - Processed cheese - Fresh cheese - Soft cheese	AFNOR BIO VIDAS LMO II (+ALOA): detection ISO 11290-2: counts	Belgium	2011	Source: FASFC (personal communication)
- 6.1 (132) - 1.2 (86) - 0.0 (8) - 0.0 (45) - 5.9 (85) - 11.2 (98)	- Butter - Semi-hard cheese - Hard cheese - Cream - Fresh cheese - Soft cheese	AFNOR BIO VIDAS LMO II (+ALOA): detection ISO 11290-2: counts	Belgium	2012	Source: FASFC (personal communication)
- 3.6 (111) - 4.5 (133) - 0.0 (4) - 2.2 (45)	- Butter - Semi-hard cheese - Hard cheese - Cream	AFNOR BIO VIDAS LMO II (+ALOA): detection ISO 11290-2: counts	Belgium	2013	Source: FASFC (personal communication)

- 1.7 (58) - 2.9 (139)	- Fresh cheese - Soft cheese				
<b><i>Campylobacter</i></b>					
0.0 (66)	Unpasteurized cheese	Enrichment	Ireland	2001-2002	Whyte <i>et al.</i> , 2004
0.0 (10)	Unpasteurized home-made cheese and butter	Enrichment, VIDAS-ELFA-System, PCR	Germany	2004	Messelhäusser <i>et al.</i> , 2008
0.0 (46)	Fresh cheese	ISO 10272-1: detection ISO 10272-2: counts	Belgium	2011	Source: FASFC (personal communication)
0.0 (43)	Fresh cheese	ISO 10272-1: detection ISO 10272-2: counts	Belgium	2012	Source: FASFC (personal communication)
- 0.0 (31) - 0.0 (3)	- Fresh cheese - Soft cheese	ISO 10272-1: detection ISO 10272-2: counts	Belgium	2013	Source: FASFC (personal communication)
<b><i>Staphylococcus aureus</i></b>					
- 69.0 (55) - 10.9 (55) - 0.0 (55)	3 camembert, 10 chevre, 37 fresh cheese, 5 other: brie, cellar ripened cheese, feta-like cheese, semi-hard cheese, lactique cheese and smear-ripened cheese	- Detection of CPS <sup>5</sup> on selective media, classification to <i>Staphylococcus aureus</i> - Counts > 5 log cfu/g - PCR: enterotoxins	Sweden	2005	Rosengren <i>et al.</i> , 2010
- 47.2 (73) - 73.6 (73) - 80.8 (73) - 76.7 (73) - 24.7 (73)  - 91.8 (49) - 91.8 (49) - 93.9 (49) - 95.9 (49) - 42.9 (49)	Cow cheese from 8 different farms: - milk - curd (2-3 hours) - cheese (5-6 hours) - cheese (24 hours) - cheese (30 days) Goat cheese from 9 different farms: - milk - curd (2-3 hours) - cheese (5-6 hours) - cheese (24 hours) - cheese (30 days)	Counts	Norway	Not given	Jakobsen <i>et al.</i> , 2011
- 19.7 (71) - 7.1 (14)	hard, semi-hard and soft cow milk cheeses, 1 soft goat milk cheese, blue veined sheep milk cheeses, fresh goat milk cheeses (36 varieties)	ISO 1999 - Counts > 3 log cfu/g - If counts > 3 log cfu/g: extraction, centrifugation, detection of enterotoxins	Belgium	Not given	De Reu <i>et al.</i> , 2002
- 0.0 (16)	- Cheese	ISO 1999 - Counts > 4 log cfu/g (if counts > 3 log	Belgium	2002	De Reu <i>et al.</i> , 2004

<sup>5</sup> Coagulase-positive staphylococci

- 25.0 (4)	- Fresh cheese	cfu/g: extraction, centrifugation: no detection of enterotoxins) - Counts > 2 log cfu/g (if counts > 2 log cfu/g: extraction, centrifugation: no detection of enterotoxins)			
- 1.6 (64)	- Butter	cfu/g: extraction, centrifugation: no detection of enterotoxins) - Counts > 3 log cfu/g (if counts > 2 log cfu/g: extraction, centrifugation: no detection of enterotoxins)			
13.1 (245)	Semi-hard and hard milk cheese	> 4 log cfu/g	Germany	Not given	Hahn <i>et al.</i> , 1999b
- 100.0 (33) - 42.4 (33)	Fresh, soft, semi-hard and hard cow (23) and goat (10) milk cheese	- ISO 6888: counts - if counts > 5 log cfu/g: PCR for enterotoxin detection	Italy	Not given	Cremonesi <i>et al.</i> , 2007
- 95.2 (21) - 68.8 (16) - 66.7 (9)	Monte veronese cow milk cheese: - Mature curd - 1 month ripening - 3 months ripening	PCR (detection limit 2 log cfu/g) Remark: 100 % contained enterotoxins	Italy	2005-2006	Poli <i>et al.</i> , 2007
36.0 (25) - 40.0 (20) - 25.0 (4) - 0.0 (1) - 58.3 (12) - 15.4 (13) - 36.4 (11) - 35.7 (14)	semi-soft to hard cheeses: - from cow milk - from sheep milk - from goat milk - from organic milk - from non-organic milk - < 3 months ripening - > 3 months ripening	Selective media Remark: 7 of 18 examined strains from 2 cheeses able to produce enterotoxin C	Scotland	Not given	Williams & Withers, 2010
- 22.7 (88) - 11.1 (54) - 6.3 (16) - 0.0 (1) - 14.9 (67) - 0.0 (55)	- Butter - Semi-hard cheese - Hard cheese - Processed cheese - Fresh cheese - Soft cheese	Conform if counts < 5 log cfu/g TEMPO ISO 6888-2	Belgium	2011	Source: FASFC (personal communication)
- 24.7 (81) - 19.0 (63) - 0.0 (9) - 0.0 (51) - 10.7 (56)	- Butter - Semi-hard cheese - Hard cheese - Fresh cheese - Soft cheese	Conform if counts < 5 log cfu/g TEMPO	Belgium	2012	Source: FASFC (personal communication)
- 15.2 (112) - 0.9 (110)	- Butter - Semi-hard cheese	Conform if counts < 5 log cfu/g TEMPO	Belgium	2013	Source: FASFC (personal communication)

- 0.0 (4) - 0.0 (39) - 8.0 (100)	- Hard cheese - Fresh cheese - Soft cheese				
<b><i>Bacillus cereus</i></b>					
28.0 (25) - 30.0 (20) - 50.0 (4) - 0.0 (1) - 16.7 (12) - 38.5 (13) - 45.5 (11) - 21.4 (14)	semi-soft to hard cheeses: - from cow milk - from sheep milk - from goat milk - from organic milk - from non-organic milk - < 3 months ripening - > 3 months ripening	Selective media Remark: all isolates were able to produce enterotoxin	Scotland	Not given	Williams & Withers, 2010
0.0 (3)	Soft cheese	ISO 7932	Belgium	2013	Source: FASFC (personal communication)
<b><i>Mycobacterium avium subsp. paratuberculosis</i></b>					
- 0.0 (143) - 4.2 (143)	Soft (9), semi-hard (133) and hard (1) cheeses	- Immunomagnetic capture, culture - PCR	Switzerland	Not given	Stephan <i>et al.</i> , 2007
20.0 (25) - 10.0 (20) - 75.0 (4) - 0.0 (1) - 16.7 (12) - 23.1 (13) - 0.0 (11) - 35.7 (14)	semi-soft to hard cheeses: - from cow milk - from sheep milk - from goat milk - from organic milk - from non-organic milk - < 3 months ripening - > 3 months ripening	Selective media, immunomagnetic recovery, detection with PCR	Scotland	Not given	Williams & Withers, 2010
<b><i>Coxiella burnetii</i></b>					
- 57.0 (68) - 0.0 - 50.0 (2)	- Unpasteurized cheese - Unpasteurized butter - Unpasteurized cream	PCR Remark: by culture 100 % negative	France	Not given	Eldin <i>et al.</i> , 2013