

# Microbiological quality and presence of foodborne pathogens in raw milk cheeses and raw meat products marketed at farm level in Switzerland

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## Abstract

This study investigated the microbiological quality and presence of bacterial foodborne pathogens in 51 raw milk cheeses (mainly semihard and hard cheese) and 53 raw meat products (cured meat products and sausages) marketed at farm level. With regard to Enterobacteriaceae, *Escherichia (E.) coli*, and coagulase-positive staphylococci (CPS), the examined products were generally of a good microbiological quality. Enterobacteriaceae were found in seven cheeses ( $1.0 \times 10^2 - 8.8 \times 10^4$  CFU/g) and one sausage ( $2.0 \times 10^2$  CFU/g). Three of these cheeses were also positive for *E. coli*. CPS results were comparable for cheeses (5.9%;  $1.0-6.0 \times 10^2$  CFU/g) and meat products (3.8%;  $1.0-2.0 \times 10^2$  CFU/g). On the other hand, such raw products may harbor potential health hazards as *Listeria (L.) monocytogenes*, Shiga toxin-producing *E. coli* (STEC), and staphylococcal enterotoxin (SE)-producing *Staphylococcus (S.) aureus*. *L. monocytogenes* were found in one sausage and the isolate belonged to the serotype 1/2c. The two STEC isolates harbored *stx1a* (cheese) or *stx2e* (sausage), but both lacked *eae* and did not belong to the top five-serogroups. Of the five *S. aureus* isolates, the three cheese isolates belonged to the clonal complex (CC) 8, CC22, and CC705, the two sausage isolates belonged to CC7, and all isolates harbored genes for SEs. Thus, to avoid contaminations and to prevent foodborne pathogens from entering the food chain, strict compliance with good hygiene practices during milk and cheese produc-

tion or meat production is of central importance.

## Introduction

In recent years, eating and consumption habits have radically changed under the influence of lifestyle changes and new technologies (Kearney, 2010; Regmi, 2001). Alongside the emergence of convenience and fast food products, natural and unheated foods of animal origin have gained popularity with consumers. In particular products marketed at farm level are thereby considered as healthy and associated with well-treated animals. Hence, many farmers have discovered direct selling of foods as niche market and they offer various products through different channels, e.g. farm shops, farmers markets, or home delivery.

Among foods sold at farm level, cheeses and meat products are of popularity with consumers. Sold cheeses comprise a wide variety of products (e.g. fresh, soft, semihard, or hard cheese) that can be produced from milk of different animal species. Thereby, many cheese varieties throughout Europe are typically made from unpasteurized milk with the natural enzymes and microflora responsible for enhancing desirable flavor characteristics. Sold meat products often comprise cured products as ham or bacon and (fermented) raw sausages as salami, foods usually intended to be eaten raw. However, comprehensive data on the microbiological quality of such raw products marketed at farm level are currently limited. The aim of this study was therefore to generate initial baseline data on general hygienic parameters and selected bacterial foodborne pathogens in raw milk cheeses and raw meat products sold directly from farms.

## Materials and Methods

### Analyzed products

In this study, 51 raw milk cheeses and 53 raw meat products marketed at farm level were analyzed. The 51 raw milk cheeses were grouped in four cat-

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egories: semihard/hard cheese (n=45), fresh cheese (n=4), soft cheese (n=1), and sour cheese (n=1). The semihard/hard cheeses were produced from cow milk (n = 40), goat milk (n=4), and sheep milk (n=1). Thirty of them were produced on mountain pastures. The fresh cheeses were produced from goat milk (n=3) and water buffalo milk (n=1). The soft and sour cheeses were both produced from cow milk. The 53 raw meat products intended to be eaten raw were grouped in two categories: cured meat products (n=14, e.g. ham or bacon) and raw sausages (n=39, e.g. salami). The meat composition was quite variable. Cured meat products were produced from beef (n=7), pork (n=5), mutton (n=1), and water buffalo meat (n=1). Raw sausages were produced from beef and pork (n=18), meat of unknown origin (n=7), beef (n=4), pork (n=2), mutton (n=2), water buffalo meat (n=2), goat meat (n=2), pork and mutton (n=1), and beef and poultry (n=1).

### Sample collection and preparation

Samples (raw milk cheeses and raw meat products) were bought at farm level and they originated from 63 farms located in the central, northern, and eastern part of Switzerland. Samples were transported chilled to the laboratory. Before processing, the rind of semihard/hard cheeses was cut away, raw sausages were peeled (if possible), and the bacon rind was removed.

### Total viable counts,

#### *Enterobacteriaceae*, *Escherichia coli*, coagulase-positive staphylococci, and *Listeria monocytogenes*

A subset of each sample (10 g) was homogenized at a 1:10 ratio in 0.85% NaCl and quantitatively analyzed by spreading 0.1 mL. The following agars and conditions were used: plate count agar (Oxoid, Pratteln, Switzerland; 72 h, 30°C) for total viable counts (TVC), violet red bile glucose agar (Becton Dickinson, Allschwil, Switzerland; 48 h, 30°C, anaerobic conditions) for Enterobacteriaceae, Rapid *Escherichia coli* 2 agar (Bio-Rad, Reinach, Switzerland; 24 h, 37°C) for *E. coli*, rabbit plasma fibrinogen agar (Bio-Rad; 48 h, 37°C) for coagulase-positive staphylococci (CPS), and Rapid L.mono agar (Bio-Rad; 48 h, 37°C) for *Listeria (L.) monocytogenes*. For the quantitative analyses mentioned above, the detection limit was 100 CFU/g ( $1.0 \times 10^2$  CFU/g).

### *Salmonella* spp.

For detection of *Salmonella* spp., 25 g of each sample were enriched first at a 1:10 ratio in buffered peptone water (Oxoid; 24 h, 37°C) and subsequently in 10 ml of Rappaport-Vassiliadis broth (Oxoid; 24 h, 41.5°C). The enriched samples were subcultured on xylose-lysine-desoxycholate agar (Bio-Rad; 24 h, 37°C) and mannitol lysine crystal violet brilliant green agar (Oxoid; 24 h, 37°C).

### Shiga toxin-producing *Escherichia coli*

For detection of Shiga toxin genes (*stx*), 10 g of each sample were enriched first at a 1:10 ratio in modified tryptic soy broth (Oxoid) with 1 mg/L acriflavin (24 h, 37°C) and subsequently subcultured (24 h, 37°C) on sheep

blood agar (Difco Columbia blood agar base EH, Becton Dickinson; 5% sheep blood SB055, Oxoid). After washing off the colonies (0.85% NaCl), samples were screened by the Assurance GDS assay for Shiga toxin genes (Bio Control Systems, Bellevue, WA, USA).

For isolation of Shiga toxin-producing *Escherichia coli* (STEC), *stx*-positive enrichment cultures were streaked in triplicate on Rapid *E. coli* 2 agar (Bio-Rad; 24 h, 37°C). Three typical *E. coli* colonies were picked from each plate, subcultured (plate count agar; 24 h, 37°C) and tested for Shiga toxin genes (EU Reference Laboratory, 2013) by real-time PCR (LightCycler, Roche Diagnostics, Rotkreuz, Switzerland) using the QuantiFast Multiplex PCR Kit (Qiagen, Hombrechtikon, Switzerland). Obtained STEC isolates were examined by PCR for *stx1*, *stx2*, and subtypes, *eae* (intimin) and the top-five serogroups O26, O103, O111, O145, and O157 (EU Reference Laboratory, 2013, 2014).

### Methicillin-resistant *Staphylococcus aureus*

For detection of methicillin-resistant *Staphylococcus aureus* (MRSA), 10 g of each sample were enriched first at a 1:10 ratio in Mueller-Hinton broth with 6.5% NaCl (24 h, 37°C) and subsequently in 5 ml tryptone soy broth (TSB; Oxoid) with 75 mg/L aztreonam and 5 mg/L cefoxitin (24 h, 37°C). The enriched samples were subcultured on chromogenic Brilliance MRSA 2 agar (Oxoid; 24 h, 37°C).

### *Staphylococcus aureus*

For confirmation of CPS as *Staphylococcus (S.) aureus* and for further strain characterization, the StaphType DNA microarray assay was used (Alere Technologies, Jena, Germany). This assay covers a variety of target sequences, including *S. aureus* species markers, genes encoding staphylococcal enterotoxins (SEs) and enterotoxin-like proteins, or resistance-associated genes. Resulting DNA microarray profiles were used to assign the *S. aureus* isolates to clonal complexes (Ebner *et al.*, 2013).

### pH value and water activity ( $a_w$ )

To determine pH and  $a_w$  values, a SevenCompact pH meter (Mettler Toledo, Greifensee, Switzerland) and an AQUALAB water activity meter 3TE (METER Food, Munich, Germany) were used.

## Results and Discussion

### Hygiene parameters

The majority (56.9%) of total viable counts (TVC) from the 51 raw milk cheeses were in the range from  $10^7$  to  $10^8$  CFU/g, followed by counts between  $10^6$  and  $10^7$  CFU/g. The distribution of counts for the different cheese categories is shown in Table 1. For products made from raw milk and often with addition of starter cultures, observed TVC levels were not unusual. For the 53 raw meat products, TVC were mainly (67.9%) in the range from  $10^7$  to  $10^9$  CFU/g (Table 1). In particu-

**Table 1. Total viable counts (TVC) from raw milk cheeses and raw meat products marketed at farm level.**

Products	No of samples	No. (%) of TVC at different ranges (CFU/g)				
		<10 <sup>5</sup>	10 <sup>5</sup> -10 <sup>6</sup>	10 <sup>6</sup> -10 <sup>7</sup>	10 <sup>7</sup> -10 <sup>8</sup>	10 <sup>8</sup> -10 <sup>9</sup>
Raw milk cheeses	51	1 (2.0)	4 (7.8)	9 (17.6)	29 (56.9)	8 (15.7)
Semihard/hard cheese	45	1	4	8	28	4
Soft cheese	1	0	0	0	1	0
Fresh cheese	4	0	0	1	0	3
Sour cheese	1	0	0	0	0	1
Raw meat products	53	4 (7.5)	6 (11.3)	7 (13.2)	23 (43.4)	13 (24.5)
Cured meat products	14	3	3	5	3	0
Raw sausages	39	1	3	2	20	13

lar but as to be expected for fermented products, more than 80% of the raw sausages showed TVC between  $10^7$  and  $10^9$  CFU/g.

Enterobacteriaceae were detected in 13.7% and *E. coli* in 5.9% of the 51 raw milk cheeses sold from farms (Table 2). Counts of the positive cheeses ranged from  $1.0 \times 10^2$  to  $8.8 \times 10^4$  CFU/g for Enterobacteriaceae and from  $4.0 \times 10^2$  to  $8.8 \times 10^4$  CFU/g for *E. coli* (Table 3). When Enterobacteriaceae and *E. coli* were present simultaneously, Enterobacteriaceae were mainly *E. coli*. With regard to cheese category and milk origin (Table 3), four (8.9%; K22, K23, K48, K49) of the 45 semi-hard/hard cheeses were positive for Enterobacteriaceae. They were produced from cow and goat milk and three of them originated from mountain pasture. On the other hand, three (K15, K25, K34) of the four fresh cheeses were positive for Enterobacteriaceae. They were produced from goat and buffalo milk. A goat milk fresh cheese (K15) thereby showed the highest Enterobacteriaceae and *E. coli* counts (almost  $10^5$  CFU/g) found in the present study. Hence, although numbers are

not representative, raw milk cheeses produced on mountain pasture (special hygienic challenges), raw milk cheeses produced from goat and water buffalo milk, and fresh cheeses seem more likely to be contaminated with Enterobacteriaceae (and *E. coli*). Of the 53 raw meat products sold from farms, 1.9% and 0% were positive for Enterobacteriaceae and *E. coli*, respectively (Table 2). The positive meat product (F42) belonged to the category raw sausage, showed an

Enterobacteriaceae count of  $2.0 \times 10^2$  CFU/g, and was produced from pork (Table 3).

Coagulase-positive staphylococci (CPS) were used as indicators for *S. aureus*. CPS were detected in 5.9% of the 53 raw milk cheeses and 3.8% of the 51 raw meat products sold from farms (Table 2). CPS counts thereby ranged from  $1.0 \times 10^2$  to  $6.0 \times 10^2$  CFU/g (Table 3). The three positive cheeses were produced from cow milk and comprised two semihard cheeses (K14,

**Table 2. Enterobacteriaceae, *Escherichia (E.) coli*, coagulase-positive staphylococci (CPS), and *Listeria monocytogenes* results from raw milk cheeses and raw meat products marketed at farm level.**

Products, microorganisms	No. (%) of results $\geq$ detection limit	No. of results at different ranges (CFU/g)		
		$10^2$ – $10^3$	$10^3$ – $10^4$	$10^4$ – $10^5$
Raw milk cheeses (n=51)				
Enterobacteriaceae	7 (13.7)	3	2	2
<i>E. coli</i>	3 (5.9)	1	1	1
CPS	3 (5.9)	3	0	0
<i>L. monocytogenes</i>	0 (0.0)	0	0	0
Raw meat products (n=53)				
Enterobacteriaceae	1 (1.9)	1	0	0
<i>E. coli</i>	0 (0.0)	0	0	0
CPS	2 (3.8)	2	0	0
<i>L. monocytogenes</i>	1 (1.9)	1	0	0

**Table 3. Characteristics of raw milk cheeses and raw meat products with Enterobacteriaceae, *Escherichia (E.) coli*, coagulase-positive staphylococci (CPS), or *Listeria monocytogenes* results above the detection limit ( $1.0 \times 10^2$  CFU/g).**

Sample	Raw material	Microorganisms and corresponding counts		pH value	Water activity
Raw milk cheeses					
Semihard/hard cheese					
K14	Cow milk	CPS	$3.0 \times 10^2$ CFU/g	6.1	0.95
K22	Cow milk <sup>b</sup>	Enterobacteriaceae	$4.0 \times 10^2$ CFU/g		
		<i>E. coli</i>	$4.0 \times 10^2$ CFU/g		
		CPS	$1.0 \times 10^2$ CFU/g	5.6	0.94
K23	Goat milk	Enterobacteriaceae	$1.0 \times 10^2$ CFU/g	nd <sup>c</sup>	0.97
K48	Goat milk <sup>b</sup>	Enterobacteriaceae	$1.2 \times 10^4$ CFU/g	5.7	0.96
K49	Cow milk <sup>b</sup>	Enterobacteriaceae	$4.2 \times 10^3$ CFU/g	5.8	0.97
Soft cheese					
K38	Cow milk	CPS	$6.0 \times 10^2$ CFU/g	6.9	0.98
Fresh Cheese					
K15	Goat milk	Enterobacteriaceae	$8.8 \times 10^4$ CFU/g		
		<i>E. coli</i>	$8.8 \times 10^4$ CFU/g	4.8	0.96
K25	Buffalo milk	Enterobacteriaceae	$2.4 \times 10^3$ CFU/g		
		<i>E. coli</i>	$2.0 \times 10^3$ CFU/g	4.9	0.97
K34 <sup>a</sup>	Goat milk	Enterobacteriaceae	$5.0 \times 10^2$ CFU/g	4.7	0.99
Raw meat products					
Raw sausages					
F3	Beef	CPS	$1.0 \times 10^2$ CFU/g		
		<i>L. monocytogenes</i>	$1.0 \times 10^2$ CFU/g	5.4	0.82
F11	Unknown	CPS	$2.0 \times 10^2$ CFU/g	5.4	0.87
F42	Pork	Enterobacteriaceae	$2.0 \times 10^2$ CFU/g	5.3	0.95

<sup>a</sup>In addition, an STEC isolate (*stx1a*) was obtained from K34. <sup>b</sup>Cheeses produced on mountain pastures. <sup>c</sup>Not determined.

K22) and one soft cheese (K38). The two positive meat products belonged to the category raw sausage and were produced from beef (F3) or meat of unknown origin (F11).

### Foodborne pathogens

All 51 raw milk cheeses and 53 raw meat products sold from farms tested negative for *Salmonella* spp. (not detectable in 25 g). *Salmonella* spp. are still a major cause of foodborne diseases, but according to the EU zoonoses report the proportion of meat products intended to be eaten raw or of cheeses not complying with the EU *Salmonella* criteria was low (EC, 2005; EFSA/ECDC, 2017).

As a foodborne pathogen, *L. monocytogenes* has the potential to cause serious and life-threatening conditions (including septicemia, meningitis, and abortion) in persons with reduced immunity (Allerberger and Wagner, 2010). Human clinical cases are thereby frequently associated with strains of serotypes 1/2a, 1/2b, and 4b (Gianfranceschi *et al.*, 2009; Lopez-Valladares *et al.*, 2014). In the present study, *L. monocytogenes* were detected in 0% of the 51 raw milk cheeses and 1.9% of the 53 raw meat products sold from farms. Our results are in agreement with a previous study examining semihard raw milk cheese during production (Zweifel *et al.*, 2006), but it must be considered that cheese surfaces/rinds (semihard/hard cheese) were not examined and *L. monocytogenes* are more likely to be found in association with soft cheese. The *L. monocytogenes*-positive meat product (F3) belonged to the category raw sausage, was produced from beef, and showed a *L. monocytogenes* count of  $1.0 \times 10^2$  CFU/g (Table 3). The *L. monocytogenes* isolate obtained from the beef sausage belonged to the serotype 1/2c. With regard to EU legislation (EC, 2005), the respective beef sausage was in compliance with the EU criterion for ready-to-eat products not supporting growth of *L. monocytogenes*. On the other hand, some of the products showed  $a_w$  and pH values (see below) probably supporting the growth of *L. monocytogenes* (EC, 2005). Thus, samples were additionally analyzed

qualitatively for *L. monocytogenes* (two step enrichment: Half-Fraser broth (Oxoid; 30°C, 24 h), Fraser broth (Oxoid; 30°C, 24 h); Rapid-*L.mono* agar plate (Bio-Rad; 37 °C, 24 h)). However, none of the examined products tested positive in the qualitative assay.

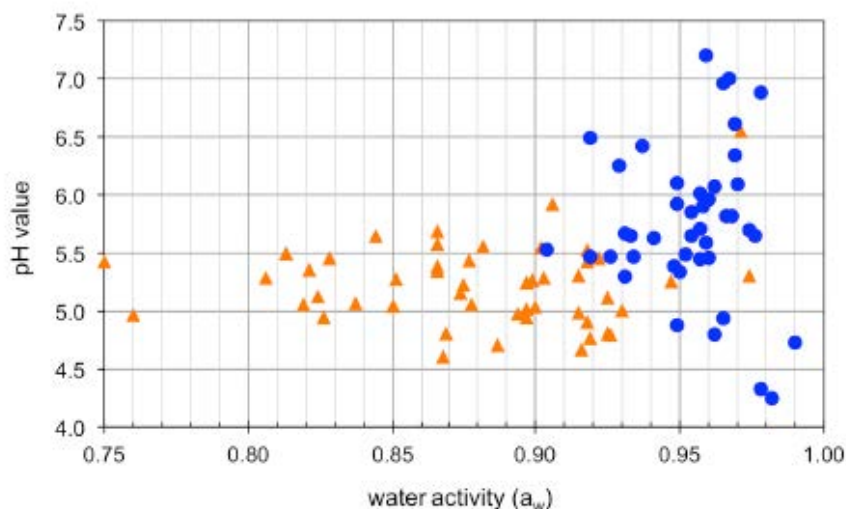
Shiga toxin-producing *E. coli* (STEC) can cause foodborne gastrointestinal illnesses in humans and conditions may be complicated by neurological and renal sequelae, including the life-threatening hemolytic uremic syndrome (Karch *et al.*, 2005; Fruth *et al.*, 2015). STEC are characterized by the production of Shiga toxins (Stx1, Stx2, and subtypes). STEC pathogenic for humans tend to feature Stx2 (in particular Stx2a and/or Stx2c) and other virulence traits as the adhesion factor intimin. In the present study, Shiga toxin genes were detected by the Assurance GDS assay in three (2.9%) of the 104 raw products sold from farms. STEC isolates were obtained from a goat milk fresh cheese (K34) and a beef/pork sausage (F45). Previous studies have shown that raw milk cheeses and raw sausages can be potential sources for transmission of STEC pathogenic to humans (Currie *et al.*, 2018; Ethelberg *et al.*, 2009; MacDonald *et al.*, 2004; Schimmer *et al.*, 2008; Zweifel *et al.*, 2010). The importance of reducing STEC contamination of raw milk or meat and of maintaining process hygiene must therefore be emphasized. The two STEC isolates found in the present study harbored *stx1a* (K34) or *stx2e* (F45), but both lacked *eae* (intimin) and did not belong to the top-five serogroups (O26, O103, O111, O145, O157). Isolates harboring *stx2e* are typically found among pigs and isolates from pigs and humans differ in their virulence profiles (Fratamico *et al.*, 2004; Sonntag *et al.*, 2005; Zweifel *et al.*, 2006). Hence, STEC from the examined raw products are unlikely to cause severe human disease, but such isolates might acquire virulence factors by horizontal gene transfer.

In addition of being a commensal colonizer and being involved in various symptoms, *S. aureus* can also cause

staphylococcal food poisoning (SFP) in humans (Johler and Stephan 2010; Le Loir *et al.*, 2003). SFP results from ingestion of heat-stable staphylococcal enterotoxins (SEs), in particular SEA–SEE, and SFP is characterized by nausea, emesis, abdominal cramps, and diarrhea. Of the five *S. aureus* isolates characterized by DNA microarray profiling, the three cheese isolates (K14, K22, K38) belonged to the clonal complex (CC) 8, CC22, and CC705 and harbored genes for various newly described SEs and enterotoxin-like proteins (e.g. the enterotoxin gene cluster, *egc*). The two sausage isolates (F3, F11) belonged to the CC7 and were positive for the gene encoding SEA. Recently, an SFP outbreak due to soft cheese contaminated with CC8 *S. aureus* producing SEA and SED has occurred at a Swiss boarding school (Johler *et al.*, 2015b). Moreover, there is evidence for cheese-associated SFP outbreaks caused by *egc*-encoded enterotoxins (Johler *et al.*, 2015a). Of the various resistance-associated genes tested, a cheese *S. aureus* isolate (K14) harbored *blaZ/R/I* ( $\beta$ -lactam resistance), *sdrM* (putative transport protein), and *fosB* (putative fosfomycin-bleomycin resistance gene). The other *S. aureus* isolates were positive only for *sdrM* (K38, F3, F11) or lacked all tested genes (K22). With regard to antibiotic resistance, MRSA are a problem involving not only the health care system but also the general community, the environment, animals, and food products (Otter and French, 2010; Vanderhaeghen *et al.*, 2010). In our study, applying selective methods, no MRSA were detected among the 104 examined raw products.

### pH value and water activity ( $a_w$ )

Analyses of pH and  $a_w$  values were performed to assess potential bacterial growth. The pH-range for growth of many bacteria is between 4.5 and 9.0, whereas the minimal  $a_w$  value is 0.95 for most gram-negative bacteria (e.g. Enterobacteriaceae) and 0.86 for *S. aureus* (Krämer and Prange, 2011). However, for products having a natural bacterial flora or being produced using starter cultures, the competitive effect is crucial when assessing potential



**Figure 1.** pH values and water activity ( $a_w$ ) of raw milk cheeses (O) and raw meat products ( $\Delta$ ) marketed at farm level (eight cheeses and four meat products excluded due to incomplete data).

growth. The pH and  $a_w$  values of the examined raw milk cheeses and raw meat products are shown in Figure 1. For the raw products positive for Enterobacteriaceae, *E. coli*, CPS, or *L. monocytogenes*, pH values ranged from 4.7 to 6.9 for cheeses and 5.3 to 5.4 for sausages, whereas  $a_w$  values were mainly  $\geq 0.95$  for cheeses and between 0.82 and 0.95 for sausages (Table 3).

## Conclusions

This study generated initial baseline data on the microbiological quality and the presence of bacterial foodborne pathogens in raw milk cheeses (semi-hard/hard cheese, fresh cheese, soft cheese, sour cheese) and raw meat products (cured meat products, raw sausages) marketed at farm level in Switzerland. With regard to the detection and counts of Enterobacteriaceae, *E. coli*, and CPS, the examined products were generally of a good microbiological quality. However, raw milk cheeses produced on mountain pasture, raw milk cheeses produced from goat (and water buffalo) milk, and fresh cheeses seem more likely to be contaminated with Enterobacteriaceae (and *E. coli*). On the other hand, it was shown that such raw products may harbor potential health hazards as *L. monocy-*

*togenes*, STEC, and SE-producing *S. aureus*. However, the *L. monocytogenes* isolate (beef sausage) belonged to a serotype not typically associated with human listeriosis, due to their virulence factors the STEC isolates (goat milk fresh cheese; beef/pork sausage) are unlikely to cause severe human disease, and growth of SE-producing *S. aureus* is not to be expected in products with competitive natural bacterial floras or starter cultures. Nevertheless, to avoid contaminations and to prevent foodborne pathogens from entering the food chain, strict compliance with good hygiene practices during any step of milk and cheese production or meat production is of central importance.

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